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ТОКСИЧНО ВЪЗДЕЙСТВИЕ НА ОЛОВОТО ВЪРХУ ФИЗИОЛОГИЧНИТЕ ПАРАМЕТРИ НА ВЪРБАТА (*SALIX VIMINALIS* L.)

THE TOXIC IMPACT OF LEAD ON THE PHYSIOLOGICAL PARAMETERS OF BASKET WILLOW (*SALIX VIMINALIS* L.)

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Резюме

Проучена беше физиологичната реакция на два клона върба (Bjor и Tora) на нарастващи концентрации от олово (15-1000 mg.dm^{-3}). Отчетени бяха съдържанието на фотосинтетичните пигменти, интензивността на CO_2 асимилацията, транспирацията и водния баланс. Доказано беше, че приложените дози олово значително намаляват съдържанието на хлорофил *a*, *b* и каротиноидите, намаляват интензивността на CO_2 асимилацията и транспирацията при изследваните клонинги. Показано беше, че е налице значителна корелация между концентрацията на олово и интензивността на CO_2 асимилацията, съдържанието на хлорофил *a* + *b* в листата на изследваните клонинги върба. Клонингът Bjor беше посочен като по-устойчив на стрес, причинен от високо съдържание на олово.

Abstract

The effect of lead on the physiological reaction of two clones - Bjor and Tora of basket willow was studied within the concentrations of 15-1000 mg.dm^{-3}. The content of photosynthetic pigments, the intensity of CO_2 assimilation and transpiration and water balance were reported. It was shown that the doses of lead significantly decreased the content of chlorophyll a, b and carotenoids, reduced the intensity of CO_2 assimilation and transpiration of the examined clones. It was shown that there was a significant correlation between the concentration of lead and the intensity of CO_2 assimilation, content of chlorophyll a + b in the leaves of the examined willow clones.The clone more resistant to the stress caused by the high lead content in the medium was Bjor.

Ключови думи: фотосинтетични пигменти, CO_2 асимилация, транспирация, воден баланс, олово, *Salix viminalis* L.
Key words: photosynthetic pigments, CO_2 assimilation, transpiration, water balance, lead, *Salix viminalis* L.

INTRODUCTION

Basket willow (*Salix viminalis* L.) is used in phytoremediation and in phytoextraction of soils contaminated with heavy metals and also with other toxic compounds. Due to its high abilities to accumulate harmful substances and their degradation, it is used for protecting plantings around industrial plants, landfills and along motorways (Deng et al., 2006; Eltrop et al., 1991; Hermle et al., 2006; Љottnikovб et al., 2003; Wrzosek et al., 2008). According to Jensen et al. (2009) willow has the features which classify it as an ideal plant for environmental protection purposes, namely, resistance to temperature changes, tolerance to water deficit and a deep root system.

Lead, as one of the most harmful heavy metals, causes disturbances in physiological processes of plants. It inhibits synthesis of photosynthetic dyes and creation of reactive forms of oxygen (Chen, Kreeb, 1990; Pacha, Galimska-Stypa, 1984; Stiborova et al., 1986; Verma and Dubey, 2003).

The aim of the studies was to determine the physiological reaction of clone Bjor and Tora of basket willow (*Salix viminalis* L.) under the conditions of a medium contaminated with lead and to define usefulness of this form for bringing anthropogenically degraded areas into cultivation.

MATERIALS AND METHODS

Material for the studies was clones of basket willow (*Salix viminalis* L.). With its three clones, i.e. 'Bjor', 'Jorr' and 'Tora', being covered by examination. Willow cuttings used in the experiment were taken from the plantation of the Department of Physiology of Plants, Westpomeranian Technological University in Szczecin. Whereas maternal material was from a plantation in Denmark, possessing a health certificate.

During the period from April to June 2009 basket willow breeding was carried out in water cultures (of volume 1 dm^3) filled with a 1.5-fold concentrated full Hoaland's medium of pH = 5.8 and with appropriate doses of lead. Lead was introduced to the medium in a form of $Pb(NO_3)_2$. In the experiment, set in 3 replications, the following combinations were taken into consideration: 1 – control (a full medium according to Hoagland); 2 – a full medium + I concentration Pb (15 $mg.dm^{-3}$); 3 – a full medium + II concentration Pb (100 $mg.dm^{-3}$); 4 – a full medium + III concentration Pb (1000 $mg.dm^{-3}$). After the cuttings had rooted and the shoots had formed, differentiated doses of $Pb(NO_3)_2$ were added according to the experimental combination. The determination of physiological parameters was carried out at three dates: on the 26th (1st date), 36th (2nd date) and 46th (3rd date) day after the setting of the experiment.

Photosynthetic pigments (chlorophyll a, b and carotenoids) were measured in fresh leaf samples before harvesting. The concentration of pigments was determined in fresh leaf tissue through extraction in 80% acetone. Pigment concentrations were calculated from the absorbance of extract at 663, 645 and 440 nm using the formula of Lichtenthaler (1987). Water balance was defined by the RWC index (relative water content) and WSD (water saturation deficit). Intensity of photosynthesis and transpiration was measured (repeating the measurements four times) using a mobile gas analyzer TPS-2 manufactured by PP Systems (UK), at stable lighting of 2053 $\mu mol.m^{-2}.s^{-1}$. On the basis of the obtained results of intensity of assimilation and transpiration the photosynthetic efficiency of water use was calculated (ωF). The results were worked out by means of the method of two-factor variance using Duncan's test at a significance level $NIR_{0.05}$. Using Pearson's linear correlation coefficient (r), correlation between the concentration of lead in leaves and the examined physiological features of wheat was shown.

RESULTS AND DISCUSSION

The studies showed significant changes in determined physiological parameters of basket willow under the conditions of the medium contaminated with lead.

Таблица 1. Токсично въздействие на оловото върху съдържанието на фотосинтетични пигменти [$mg.g^{-1}$ св. тегло], CO_2 асимилацията [$\mu mol.m^{-2}.s^{-1}$], транспирацията [$mmol.m^{-2}.s^{-1}$], ефективността на използване на водата във фотосинтезата (ω_f) в изследваните клонинги върба

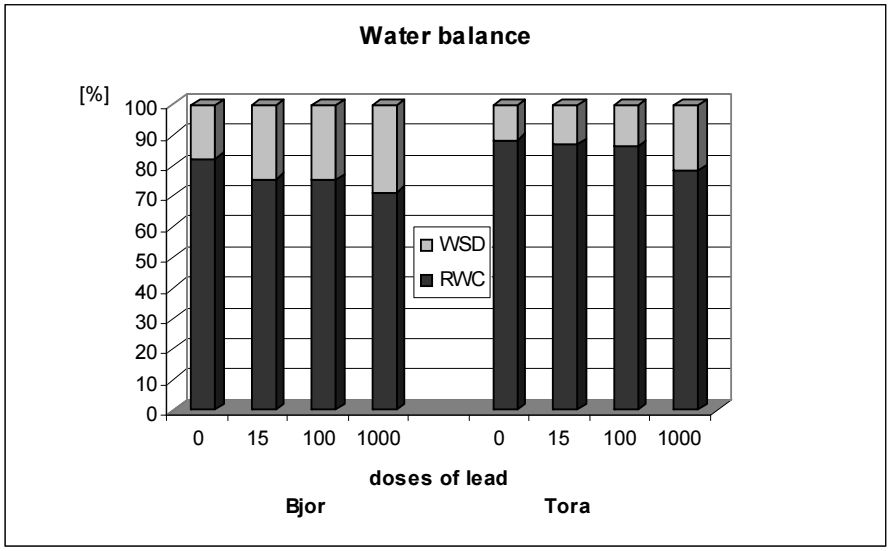
Table 1. The toxic impact of lead on the content of photosynthetic pigments [$mg.g^{-1}$ FW], CO_2 assimilation [$\mu mol.m^{-2}.s^{-1}$], transpiration [$mmol.m^{-2}.s^{-1}$] and water use photosynthetic efficiency (ω_f) in the examined clones of basket willow

Dose of Pb [$mg \cdot dm^{-3}$]	Chlorophyll a (% of control)	Chlorophyll b (% of control)	Carotenoids (% of control)	CO_2 assimilation (% of control)	Transpiration (% of control)	Water use photosynthetic efficiency (ω_f)
Clone Bjor						
0	1.81±0.11 (100)	0.72±0.12 (100)	1.09±0.19 (100)	2.87±0.22 (100)	0.66±0.05 (100)	4.35
15	1.58±0.18 (87.3)	0.53±0.09 (73.6)	0.94±0.14 (86.2)	2.19±0.09 (76.3)	0.60±0.02 (90.1)	3.65
100	1.52±0.17 (83.9)	0.51±0.06 (70.8)	0.72±0.21 (66.1)	1.86±0.11 (64.8)	0.39±0.04 (59.1)	4.76
1000	1.29±0.13 (71.3)	0.41±0.10 (56.9)	0.76±0.15 (69.7)	1.25±0.06 (43.6)	0.22±0.06 (33.3)	5.68
Clone Tora						
0	2.15±0.25 (100)	0.96±0.09 (100)	1.39±0.11 (100)	5.50±0.24 (100)	0.65±0.10 (100)	8.46
15	2.08±0.19 (96.7)	0.76±0.15 (79.2)	1.12±0.07 (80.6)	3.70±0.17 (67.3)	0.52±0.09 (80.0)	7.11
100	1.54±0.09 (71.6)	0.64±0.08 (66.7)	0.97±0.10 (69.8)	2.32±0.10 (42.2)	0.33±0.08 (50.7)	7.03
1000	0.97±0.07 (44.6)	0.43±0.03 (44.8)	0.62±0.04 (44.6)	1.40±0.12 (25.5)	0.21±0.09 (32.3)	6.67

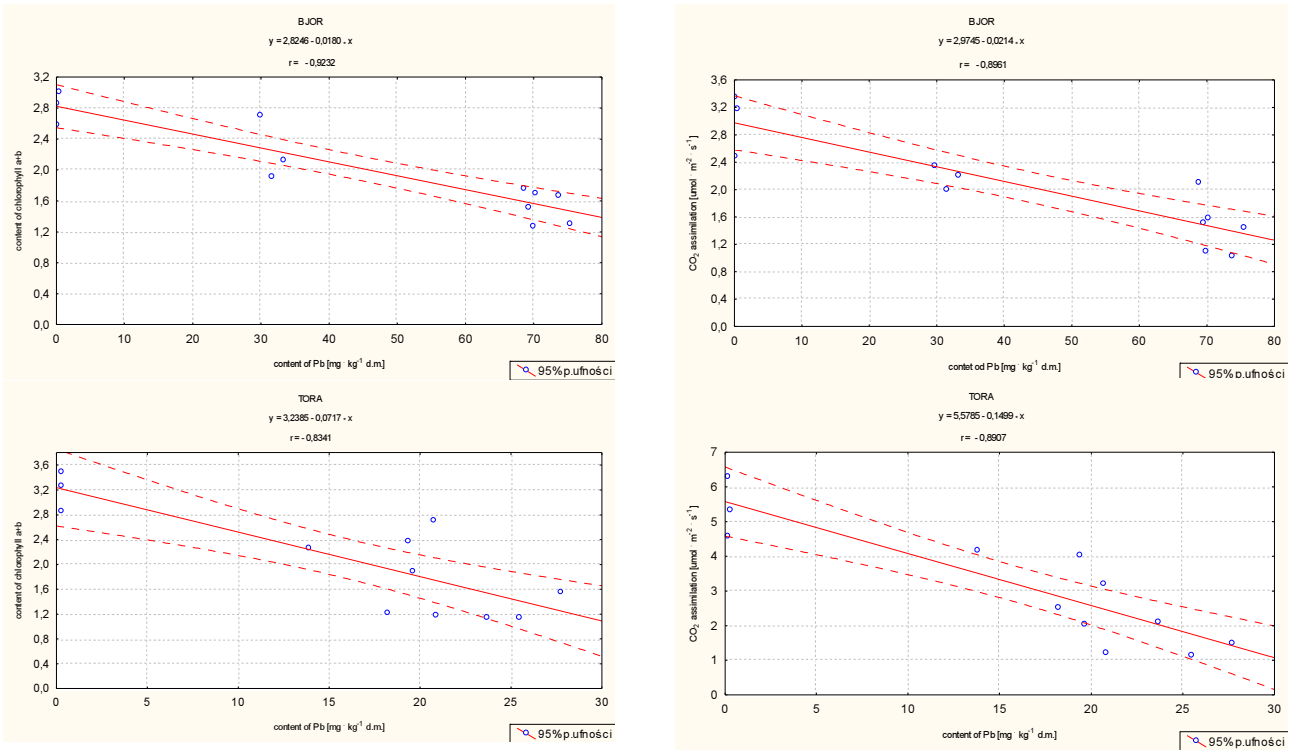
The applied doses of lead resulted in a decrease in the content of photosynthetic pigments in leaves of the examined clones of basket willow. An unfavourable effect of the length of actiivity period of lead doses added to the medium on the physiological parameters was observed. The addition of Pb at the rate of 1000 mg.dm^{-3} to the medium caused a decrease in the concentration of chlorophyll a in Bjor clone by 28.7%, whereas in Tora clone by 55.4% (Table 1). The content of chlorophyll b in leaves of Bjor clone and Tora clone in the presence of the highest rate of lead constituted respectively 56.9% and 44.8% in relation to the control plant (Table 1). The contamination of the medium with lead also caused a significant decrease in the content of carotenoids in leaves of the studied clones. The largest decrease in the amount of this dye in Bjor clone was observed after addition of 100 mg.dm^{-3} of lead to the medium, whereas the largest decrease in the content of this dye in Tora clone was noticed at the highest rate of lead (Table 1). The inhibition of chlorophyll synthesis results from the activity of different heavy metals. Stronger inhibition of chlorophyll b synthesis as compared to chlorophyll a in leaves of barley was recorded by Stiborova et al. (1986). Chen and Kreeb (1990) observed an over twofold decrease in chlorophyll in maize under the influence of heavy metals activity, as compared to the control. A decreased amount of chlorophyll a, in spring wheat by 29.4% and chlorophyll b by 50% after a dose of 1035 mg.kg^{-1} of soil had been applied, was reported by Malinowska and Smolik (2006). The research works carried out by Becerril et al. (1988) showed in lucerne and clover a decrease both in chlorophyll a and b and in carotenoids after the application of lead.

Increasing doses of lead in the medium significantly inhibited intensity of CO_2 assimilation and transpiration in the studied clones of basket willow. The largest decrease in the intensity of the studied physiological processes was observed when a maximum dose was applied at all the times of the experiments. The intensity of the photosynthesis process at the rate of 1000 mg.dm^{-3} decreased by 56.4% in clone Bjor and by 74.5% in clone Tora, whereas the intensity of transpiration in the investigated clones of willow decreased by 67% as compared to the control (Table 1). A decrease in the index of effectiveness of the use of water in photosynthesis was observed in Tora clone at all the rates as compared to the control plants. Whereas the addition of lead to the medium at the rate of 100 and 1000 mg.dm^{-3} in Bjor clone caused an increase in this index as compared to the control. A high value of this parameter results first of all from low intensity of transpiration. The decrease in the intensity of photosynthesis caused by lead was shown, among others, by Becerril et al. (1989), Malinowska and Smolik (2006), Poskuta et al. (1987). On the basis of the value of the coefficient of correlation a significant negative relationship between the concentration of lead in leaves of the studied clones of willow and the content of chlorophyll a+b and CO_2 assimilation (Fig. 2) was stated.

The indices of water balance changes are, among others, RWC and WSD. The increasing doses of lead resulted in a decrease in the water content in leaves of the examined clones of basket willow. The largest decrease in the index of relative content of water by 11.1% in Bjor and by 10.2% in Tora was observed after the application of the



Фиг. 1. *Воден индекс (%) при върбата (Bjor and Tora) в зависимост от дозата на оловото*
Fig. 1. *Water indices (%) of basket willow – of clone Bjor and Tora in relation to a dose of the lead*



Фиг. 2. *Корелация между съдържанието на фотосинтетични пигменти [mg.g^{-1} св. тегло], CO_2 асимилацията [μmol.m^{-2}.s^{-1}] и съдържанието на олово в листата от върба*

Fig. 2. *Correlation between the content of photosynthetic pigments [mg.g^{-1} FW], CO_2 assimilation [μmol.m^{-2}.s^{-1}] and the content of lead in the leaves of basket willow*

highest rate of lead as compared to the control plants (Fig. 1). The observed changes of intensity of the studied physiological parameters can be caused, under unfavourable conditions, by both stress and repair mechanisms (Starck, 2002).

The obtained results of the studied physiological parameters can be useful for the assessment of the resistance of the studied clones of willow to stress caused by lead and their usefulness for reclamation of the areas anthropogenically degraded. Clone Bjor of basket willow was characterised by higher values of the physiological parameters defined under stress and that suggests its higher tolerance to stress resulted from a high content of lead in the medium.

CONCLUSIONS

1. The applied doses of lead reduced the intensity of assimilation of CO_2 and transpiration and the content of photosynthetic pigments in leaves of the studied clones of basket willow.
2. The addition of lead to the medium caused a decrease in the index of the relative content of water and an increase in the deficit of water saturation in leaves of the studied clones of basket willow.
3. Clone Bjor of basket willow showed higher tolerance to the stress caused by a high content of lead in the medium.

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