

Katarzyna MALINOWSKA, Małgorzata MIKICIUK,  
Jacek WRÓBEL and Ewa CZEŻYK<sup>1</sup>

**INFLUENCE OF CADMIUM  
ON PHYSIOLOGICAL PARAMETERS  
OF CLONE JORR OF BASKET WILLOW (*Salix viminalis* L.)  
FROM AQUATIC CULTURES**

**WPLYW KADMU NA PARAMETRY FIZJOLOGICZNE  
KLONU JORR WIERZBY WICIOWEJ (*Salix viminalis* L.)  
Z KULTUR WODNYCH**

**Abstract:** In the experiment the effect of differentiated doses of cadmium (0; 1.4; 28; 280 mg · dm<sup>-3</sup>), applied in the form of CdCl<sub>2</sub> on selected physiological parameters of clone Jorr *Salix viminalis*, cultivated in water cultures with Hoagland's medium was determined. The statistical analysis of the results showed a significant influence of cadmium on the examined physiological parameters of clone Jorr. The addition of CdCl<sub>2</sub> to the medium in a dose of 280 mg · dm<sup>-3</sup> caused a decrease in intensity of CO<sub>2</sub> assimilation of the examined clone by 70 % and transpiration by nearly 83 % in relation to the intensity of these processes in control leaves. A positive significant correlation between assimilation of CO<sub>2</sub> and transpiration was recorded. At all the dates of studies decreased contents of both chlorophyll and carotenoids in leaves were obtained after the application of three doses of cadmium chloride. The increase in the concentration of cadmium salt caused a decrease in the relative water content index (RWC) and an increase in the water saturation deficit (WSD) in the leaves of clone Jorr.

**Keywords:** *Salix viminalis*, cadmium, CO<sub>2</sub> assimilation, transpiration, assimilation dyes, water balance

The increase in the level of cadmium in the environment results from economic and industrial activity of human beings. The characteristic feature of this chemical element is the largest value of concentration index both in soil and in plant, as compared with other metals [1]. This phenomenon is attributed to the geochemical nature of cadmium, determining its large mobility in environment and easy uptake by plants [1–4]. The plants reaction to stress caused by an excessive amount of cadmium in the environment is reflected, among other things, by changes undergoing in the assimilation apparatus

<sup>1</sup> Department of Plant Physiology, West Pomeranian University of Technology in Szczecin, ul. J. Słowackiego 17, 71–434 Szczecin, Poland, phone: +48 91 449 63 82, email: Katarzyna.Malinowska@zut.edu.pl

and physiological processes [3, 5–7]. The resistance of plants to toxic properties of cadmium is very differentiated and it depends not only on genetic properties of plants but also physiological ones.

For the reclamation of areas anthropogenically degraded plants are more and more frequently used [8–9]. Basket willow (*Salix viminalis* L.) and particularly its hybrid forms can play here a significant role, as due to its capability of removing impurities hard to degrade biologically, it is commonly used in protection and reclamation of soils [10–12]. To evaluate usefulness of plants for the reclamation of degraded areas a lot of anatomical and physiological features of plants are used.

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

## Material and methods

Material for the studies was clone Jorr of basket willow (*Salix viminalis* L.) [13]. 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. The experiment was carried out in laboratory conditions in 2007–2008. During the period from April to June basket willow breeding was carried out in water cultures (of volume 1 dm<sup>3</sup>) filled with a 1.5-fold concentrated full Hoagland's medium of pH = 5.8 and with appropriate doses of cadmium. Cadmium was introduced to the medium in the form of CdCl<sub>2</sub>. 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 Cd (1.4 mg · dm<sup>-3</sup>); 3 – a full medium + II concentration Cd (28 mg · dm<sup>-3</sup>); 4 – a full medium + III concentration Cd (280 mg · dm<sup>-3</sup>). During the experiment the composition of individual media was changed every 5 days in order to maintain a stable level. Each set water culture contained 4 willow cuttings of 22 cm in length. After the cuttings had rooted and the shoots had formed, differentiated doses of CdCl<sub>2</sub> were added according to the experimental combination. The determination of physiological parameters was carried out on three dates: on the 26<sup>th</sup> (1<sup>st</sup> date), 36<sup>th</sup> (2<sup>nd</sup> date) and 46<sup>th</sup> (3<sup>rd</sup> date) day after the setting of the experiment. The content of assimilation dyes (chlorophyll *a*, *b*, total and carotenoids) leaves was determined by means of the Lichtenthaler and Welburn method [14]. Water balance was defined by the RWC index (*relative water content*) and WSD (*water saturation deficit*) [15]. Intensity of photosynthesis and transpiration was measured (repeating the measurements four times) only in 2008 using a mobile gas analyzer TPS-2 manufactured by PP Systems (UK), at stable lighting of 2053 μmol · m<sup>-2</sup> · s<sup>-1</sup>. On the basis of the obtained results of intensity of assimilation and transpiration the photosynthetic efficiency of water use was calculated ( $\omega_F$ ). The obtained results were worked out by means of a two factor variance method using Tukey's test at the level of significance LSD<sub>0.05</sub>. Using the coefficient of correlation (*r*) the relation between intensity of CO<sub>2</sub> assimilation and intensity of transpiration was presented.

## Results and discussion

Increasing doses of cadmium in the medium significantly inhibited intensity of CO<sub>2</sub> assimilation and transpiration in clone Jorr basket willow. A negative effect of the length of the period of the influence of an increased concentration of cadmium in the medium on intensity of these processes was also observed. The largest decrease in intensity of the examined physiological processes was observed when the maximum dose of cadmium was applied at all the dates of studies. Intensity of the photosynthesis at a dose of 280 mg · dm<sup>-3</sup> decreased by 70 %, while that of transpiration by nearly 83 % as compared with the control (Table 1).

Table 1

Intensity of CO<sub>2</sub> assimilation and transpiration and water use photosynthetic efficiency ( $\omega_t$ ) of clone Jorr

CO <sub>2</sub> assimilation [ $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ]				
Date of studies	Dose CdCl <sub>2</sub> [ $\text{mg} \cdot \text{dm}^{-3}$ ]			
	0	1.4	28	280
I	2.75	1.65	1.33	0.90
II	2.65	1.40	1.10	0.75
III	1.88	0.97	0.82	0.50
The average	2.43	1.34	1.08	0.72
LSD <sub>0.05</sub> for: dose – 0.98; date – n.s.				
Transpiration [ $\text{mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ]				
I	1.27	0.87	0.48	0.22
II	1.11	0.59	0.36	0.19
III	0.74	0.48	0.16	0.14
The average	1.04	0.65	0.33	0.18
LSD <sub>0.05</sub> for: dose – 0.36; date – 0.19				
Water use photosynthetic efficiency [ $\omega_t$ ]				
I	2.16	1.89	2.77	4.09
II	2.39	2.37	3.05	3.95
III	2.54	2.02	5.12	3.57
The average	2.36	2.09	3.64	3.87

Photosynthetic effectiveness of water use is often a decisive indicator of productivity of plants under stressful conditions [16, 17]. The calculated index was differentiated depending on applied doses and dates of studies. A decrease in the effectiveness of this parameter by 11.5 % was recorded when a dose of 1.4 mg · dm<sup>-3</sup> was applied, as compared with the control plants. Whereas the remaining doses caused a 1.5 fold increase in this index, as compared with the control. A high value of this parameter results first of all from low intensity of transpiration. Similar reactions of plants to the

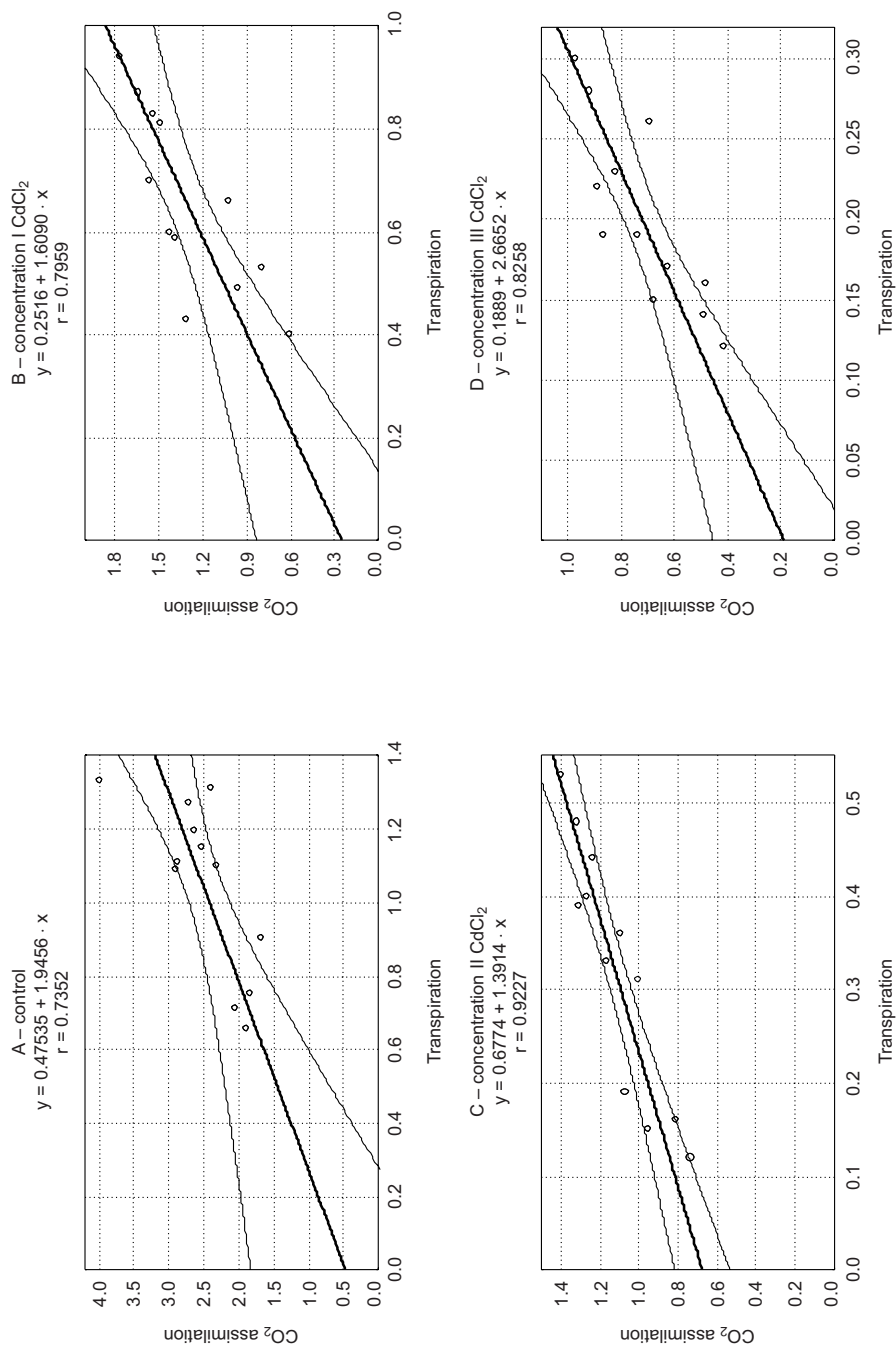


Fig. 1. Dependence of intensity of assimilation of CO<sub>2</sub> [ $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ] on transpiration [ $\text{mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ] in clone Jorr

effects of heavy metals were obtained by Malinowska and Smolik [18], Smolik and Malinowska [19], Jasiewicz et al [20]. Malinowska [21] recorded in her studies a negative significant correlation between intensity of photosynthesis and the content of cadmium in leaves of Norway maple. A decrease in intensity of photosynthesis and transpiration can be related to disturbances in the functioning of the photosynthetic apparatus of plants, a decrease in turgor, inhibition of transport of electrons in the process of photosynthesis and a decrease in activity carboxylase RuBP [5, 22, 23]. On the basis of the value of the correlation coefficient significant relationships were observed between assimilation and transpiration of clone Jorr, both under the control and stressful conditions (Fig. 1ABCD). The relationship between these parameters increased with an increase in the concentration of cadmium chloride in the medium. The highest value of the correlation coefficient:  $r = 0.9227^*$  was observed at concentration II in the medium (Fig. 1C). High values of the correlation coefficient prove a close relationship between the examined features.

The content of assimilation dyes in plants is a characteristic feature of species and varieties. The influence of many environmental and anthropogenic factors considerably modifies their amount in plants [5, 6, 24, 25]. The application of a dose of cadmium significantly decreased their content in clone Jorr. At all the dates of studies decreased amounts of both chlorophyll and carotenoids were obtained in leaves after three doses of cadmium chloride had been used. An average amount of chlorophyll  $a + b$  in leaves of control plants was about  $3.50 \text{ mg} \cdot \text{g}^{-1} \text{ f.m.}$  The highest applied concentration –  $280 \text{ mg} \cdot \text{dm}^{-3}$ , caused in 2007 a decrease in concentration of total chlorophyll by 50 % on the 1<sup>st</sup> date and by 75 % on the 2<sup>nd</sup> and 3<sup>rd</sup> dates of studies, whereas in 2008, by 80 % on all the dates, as compared with its content in the control leaves (Table 2).

Table 2

Content of assimilation dyes [ $\text{mg} \cdot \text{g}^{-1} \text{ f.m.}$ ] in leaves of clone Jorr

Dose $\text{CdCl}_2$ [ $\text{mg} \cdot \text{dm}^{-3}$ ]	2007			2008		
	I	II	III	I	II	III
Content of chlorophyll $a$ [ $\text{mg} \cdot \text{g}^{-1} \text{ f.m.}$ ]						
0	3.43	3.02	1.92	2.59	2.40	2.22
1.4	2.49	1.52	1.73	0.74	0.60	0.46
28	1.75	0.95	0.65	0.73	0.58	0.43
280	1.63	0.73	0.43	0.48	0.37	0.27
LSD <sub>0.05</sub> for:	dose – 0.45; date – n.s.			dose – 0.85; date – n.s.		
Content of chlorophyll $b$ [ $\text{mg} \cdot \text{g}^{-1} \text{ f.m.}$ ]						
0	0.62	1.01	0.64	1.09	1.01	0.92
1.4	0.48	0.53	0.55	0.31	0.26	0.21
28	0.51	0.28	0.32	0.33	0.24	0.19
280	0.42	0.25	0.22	0.21	0.19	0.16
LSD <sub>0.05</sub> for:	dose – 0.24; date – n.s.			dose – 0.38; date – n.s.		

Table 2 contd.

Dose CdCl <sub>2</sub> [mg · dm <sup>-3</sup> ]	2007			2008		
	I	II	III	I	II	III
Content of total chlorophyll [mg · g <sup>-1</sup> f.m.]						
0	4.05	4.03	2.56	3.68	3.41	3.14
1.4	2.97	2.05	2.28	1.05	0.86	0.67
28	2.26	1.23	0.97	1.06	0.82	0.62
280	2.05	0.98	0.65	0.69	0.56	0.43
LSD <sub>0.05</sub> for:	dose – 0.67; date – n.s.			dose – 1.22; date – n.s.		
Content of carotenoids [mg · g <sup>-1</sup> f.m.]						
0	0.89	1.22	0.85	1.39	1.38	1.29
1.4	0.58	0.68	0.77	0.38	0.27	0.30
28	0.66	0.44	0.36	0.39	0.24	0.22
280	0.48	0.30	0.17	0.27	0.25	0.20
LSD <sub>0.05</sub> for:	dose – 0.19; date – n.s.			dose – 0.68; date – n.s.		

The addition of cadmium salt to the medium also caused a decrease in the content of carotenoids. The largest decrease of this dye was observed on the 46<sup>th</sup> day of the experiment and at the highest dose applied – by 80 % in 2007 and by 84.5 % in 2008 (Table 2). The inhibition of chlorophyll and carotenoids synthesis is a reflection of the activity of different heavy metals [5, 18, 20, 26]. Chen and Kreeb [6] recorded a more than twofold decrease in chlorophyll in maize under the influence of heavy metals, as compared with the control. Lukasik et al [24] and Malinowska [27] observed in their studies a negative significant correlation between the content of cadmium and chlorophyll *a + b* in leaves.

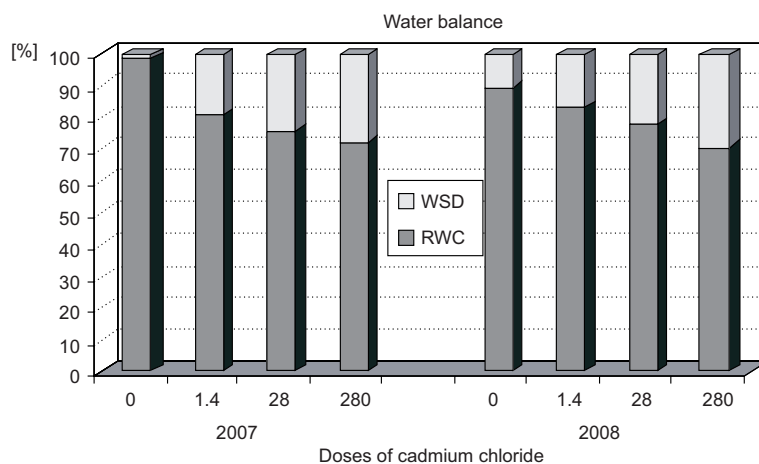


Fig. 2. Water indices [%] of basket willow – of clone Jorr in relation to a dose of cadmium chloride

An indicator of the changes in water balance in a plant are, among other things, RWC and WSD. The increasing doses of cadmium salt caused an increase in the content of the water in leaves of clone Jorr in both years of studies. The largest decrease in the index of relative water content (by 27 % in 2007 and by 21 % in 2008) was noticed after the application of the highest concentration of cadmium chloride in relation to the control plants (Fig. 2). The observed changes in intensity of the examined physiological parameters can be, under unfavourable conditions, the result of both stress and repair mechanisms [28]. The obtained results of the examined physiological parameters can be useful for evaluation of the resistance of clone Jorr to stress evoked by cadmium and the usefulness for reclamation of anthropogenically degraded areas.

## Conclusions

1. The applied concentrations of cadmium chloride decreased significantly the intensity of CO<sub>2</sub> assimilation and transpiration and the content of assimilation dyes in leaves of clone Jorr.

2. A significant correlation between assimilation of CO<sub>2</sub> and transpiration in the studied form of willow was observed. The value of the correlation coefficient was the highest at the dose of 28 mg Cd · dm<sup>-3</sup> in the medium.

3. The increase in the concentration of salt caused a decrease in the index of the relative water content and an increase in the water saturation deficit in leaves of clone Jorr.

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**WPLYW KADMU NA PARAMETRY FIZJOLOGICZNE  
KLONU JORR WIERZBY WICIOWEJ (*Salix viminalis* L.)  
Z KULTUR WODNYCH**

Zakład Fizjologii Roślin  
Zachodniopomorski Uniwersytet Technologiczny w Szczecinie

**Abstract:** W przeprowadzonym doświadczeniu określano wpływ zróżnicowanych dawek kadmu (0; 1,4; 28; 280 mg · dm<sup>-3</sup>), zastosowanych w formie CdCl<sub>2</sub> na wybrane parametry fizjologiczne klonu Jorr *Salix viminalis* L., uprawianej w kulturach wodnych z pożywką Hoaglanda.

Analiza statystyczna wyników wykazała istotny wpływ kadmu na badane parametry fizjologiczne klonu Jorr. Dodatek do pożywki CdCl<sub>2</sub> w dawce 280 mg · dm<sup>-3</sup> spowodował obniżenie intensywności asymilacji CO<sub>2</sub> badanego klonu o 70 %, a transpiracji o prawie 83 % w stosunku do intensywności tych procesów w liściach kontrolnych. Stwierdzono statystycznie istotną dodatnią korelację między asymilacją CO<sub>2</sub> a transpiracją. We wszystkich terminach badań uzyskano obniżone zawartości zarówno chlorofilu, jak i karotenoidów w liściach po zastosowaniu trzech dawek chlorku kadmu. Wzrost stężenia soli kadmu spowodował spadek wskaźnika *względnej zawartości wody* (RWC) oraz wzrost *deficytu wysycenia wodą* (WSD) w liściach klonu Jorr.

**Słowa kluczowe:** *Salix viminalis*, kadm, asymilacja CO<sub>2</sub>, transpiracja, barwniki asymilacyjne, bilans wodny.