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Effects of lead and cadmium ions on water balance parameters and content of photosynthetic pigments of prairie cordgrass (*Spartina pectinata* Bosk ex Link.)

Wpływ jonów ołowiu oraz kadmu na parametry bilansu wodnego i zawartość barwników fotosyntetycznych u spartiny preriowej (*Spartina pectinata* Bosk ex Link.)

Summary. The aim of the work was to assess the impact of a varied level of soil contamination with lead and cadmium ions on selected physiological parameters of prairie cordgrass. The content of photosynthetic pigments in leaves (chlorophyll a, b, total chlorophyll and carotenoids) and water balance of plants on the basis of two indicators (RWC – relative water content in tissues and WSD – water saturation deficit) were determined. Pot-vegetative experiments were performed using a complete randomization method in a one-factor system. The factor in the first experiment was the level of soil contamination with lead (28.15, 56.30, 112.60 mg Pb · kg soil^{-1}), in the second experiment – the level of soil contamination with cadmium (4.60, 10.00, 18.39 mg Cd · kg soil^{-1}). The levels of soil contamination with lead did not influence the content of chlorophyll a, b and total chlorophyll in prairie cordgrass leaves. In the case of carotenoids, an increase in their content was demonstrated after introducing lead into the soil at the dose of 28.15 mg Pb · kg soil^{-1} compared to the control. Soil contamination with cadmium did not affect the content of chlorophyll a, total chlorophyll and carotenoids in the leaves of prairie cordgrass. The highest level of soil contamination with lead contributed to the reduction of chlorophyll b. Lead at doses of 56.30 and 112.60 mg · kg soil^{-1} caused deterioration in the water balance parameters of the prairie cordgrass. In the case of soil contamination with cadmium, this relationship was demonstrated only for the dose of 10.00 mg Cd · kg soil^{-1}.

Key words: cadmium, lead, *Spartina pectinata* Bosk ex Link., photosynthetic pigments, water balance

INTRODUCTION

Due to the growing level of industrialization, heavy metals are increasingly introduced into the soil, which accumulating in the environment, are characterized by high toxicity to humans, animals and plants [Korzeniowska and Stanisławska-Glubiak 2015, Hou et al. 2015, Yin et al. 2016, Gill et al. 2017, Rehman et al. 2017]. The high toxicity to plants is demonstrated, among others, by cadmium and lead ions [Seregin and Ivanoc 2001]. These metals appear mainly in the vicinity of roads with intensified traffic [Sayed 1997, Ali et al. 2013]. Cadmium can additionally come from industrial and agricultural sources [Karantev et al. 2008, Järup and Åkesson 2009]. Lead originates from the gasoline combustion processes, from the mining industry, the waste incineration plants [Duzgoren-Aydin 2007]. These metals inhibit plant growth negatively affecting their water and mineral status, photosynthesis and protein synthesis [Karantev et al. 2008, Pourrut et al. 2011, Arena et al. 2017]. The permissible concentration of cadmium in the soil should not exceed 4 mg · kg of soil^{-1}, whereas lead – 100 mg · kg of soil^{-1} [Dziubanek et al. 2012].

Prairie cordgrass (*Spartina pectinata* Bosk ex Link.) is a plant cultivated in Poland mainly for energy purposes [Pogrzeba et al. 2010]. Praire cordgrass is known as a plant tolerant to high concentration of heavy metals in soil [Montemayor et al. 2008, Kowalczyk-Juśko 2013, Redondo-Gómez 2013, Li et al. 2014].

A number of studies have been conducted referring to the effects of various soil contaminants (mainly heavy metals) on the growth and yield of prairie cordgrass [Weiss et al. 2006, Cambrolle et al. 2011, Kim et al. 2012, Nalla et al. 2012, Curado et al. 2014, Helios et al. 2014, Guo et al. 2015, Helios et al. 2015, Korzeniowska and Stanisławska-Glubiak 2015, Zhang et al. 2015, Ociepa et al. 2017]. Due to the lack of studies on the effects of cadmium and lead compounds, the aim of the conducted research was to assess the impact of a varied level of soil contamination with these elements on water balance parameters and content of photosynthetic pigments of prairie cordgrass.

MATERIAL AND METHODS

Biological material used for the study was prairie cordgrass (*Spartina pectinata* Bosk ex Link.). The pot experiment was carried out in the vegetation hall of the Faculty of Environmental Management and Agriculture of the West Pomeranian University of Technology in Szczecin (53^{o}25'N, 14^{o}32'E, 25 m above sea level), outside the greenhouse, in the roofed part. The method of complete randomization in a one-factor system, in three replications was applied (single repetition consisted of one pot). For experiments, Kick's pots with a capacity of 10 dm^3 were used. These pots were filled with soil substrate (8 dm^3) from the upper-medium soil level (0–30 cm) in the Agricultural Experimental Station of West Pomeranian University of Technology located in the town of Lipnik, near Stargard. The soil had a mechanical composition of loamy, light and dusty sand. In the middle of April, cordgrass seedlings were put into the pots in the amount of 15 rhizomes/pot. Mineral fertilization in the amount corresponding to 60 and 75 kg PK · ha^{-1}, respectively, was carried out before placing plants in pots in the form of potassium sulfate and triple superphosphate. During the growing season, all pots were watered with 0.5 dm^3 · pot^{-1}, with soil contact tensiometers readings, which were placed at a depth of 20 cm, equal to ψ_w = –0.04 MPa.

In the first experiment, the experimental factor was the level of soil contamination with lead, in the second – cadmium. Lead was introduced into the soil in the form of $Pb(NO_3)_2$ solution twice during the growing season, each time at doses of: 28.15, 56.30, 112.60 mg Pb · kg^{-1} soil (35.10, 70.38, 140.76 mg Pb · dm^{-3} soil). Cadmium was introduced into the soil in the form of $CdCl_2 \cdot 2.5\ H_2O$ solution at the following doses: 4.60, 10.00, 18.39 mg Cd · kg^{-1} soil (5.75, 11.50, 23.00 mg Cd · dm^{-3} soil). The control plants were watered with distilled water. The first date for the introduction of lead and cadmium salts was the beginning of June, the second – the beginning of July. At the end of July, during the time of plant full vegetation, the content of photosynthetic pigments in leaves (chlorophyll a, b, total chlorophyll and carotenoids) and the water balance of studied species were determined. Determination of the content of photosynthetic pigments [mg · g^{-1} of fresh mass (f.m.)] was carried out by collecting plant material – leaves, from three representative plants, from each variant of the experiment. From the prepared material, sample of 0.05 g fresh mass for extraction of the pigments, was taken. The samples were ground in a mortar adding 10 ml of 80% acetone. The solutions were placed into test tubes and then transferred to a centrifuge to separate the liquid phase from the solid phase. Centrifugation was carried out for 10 minutes at 1500 rpm. Optical density of samples was determined using the Marcel Mini spectrophotometer. The determinations were carried out at 440, 645 and 663 nm wavelengths. The content of chlorophyll was calculated applying the method of Arnon et al. [1956] based on the formulas:

$$\text{mg of chlorophyll a} \cdot g^{-1}\ \text{f.m.}\ [12{,}7\ (E\ 663) - 2{,}69\ (E\ 645)] \cdot V/W;$$

$$\text{mg of chlorophyll b} \cdot g^{-1}\ \text{f.m.}\ [22{,}9\ (E\ 645) - 4{,}68\ (E\ 663)] \cdot V/W;$$

$$\text{mg of total chlorophyll} \cdot g^{-1}\ \text{f.m.}\ [20{,}2\ (E\ 645) + 8{,}02\ (E\ 663)] \cdot V/W;$$

The content of carotenoids was calculated by method of Hager and Mayer--Bartenrath [1966], from the formula:

$$\text{mg of carotenoids} \cdot g^{-1}\ \text{f.m.}\ [4{,}16\ (E\ 440) - 0{,}89\ (E\ 663)] \cdot V/W;$$

where:

E – extinction at a specific wavelength;
V – amount of 80% acetone used for extraction;
W – mass of fresh sample in grams.

Water balance was determined on the basis of relative water content indicators in leaf tissues (RWC) and water saturation deficit of leaf tissues (WSD). The necessary data was obtained after weighing on the analytical balance the three leaves randomly selected from each of the experimental variants. The fresh weight (FW) of leaves was determined immediately after harvest. The full saturation weight (SW) with water was determined after 24-hour wetting in distilled water and drying with tissue. The dry weight (DW) was determined after drying the leaves to constant weight at 105°C in the dryer. The RWC and WSD parameters were calculated as described by Turner [1986] and Yamasaki and Dillenburg [1999], from the formulas:

$$RWC\ (\%) = (FW - DW)/(SW - DW) \cdot 100$$

$$WSD\ (\%) = 100 - RWC$$

Results of the studies on the content of photosynthetic pigments were statistically analyzed using the one-way analysis of variations method, in a complete randomization system, in triplicate. Significance of differences between means were determined using the Duncan test at the significance level $\alpha = 0.05$.

RESULTS AND DISCUSSION

Heavy metals inhibit chlorophyll and carotenoid biosynthesis and inhibit their functions in photosystems [Vazquez et al. 1987]. Statistical analysis did not show any significant difference among the contents of chlorophyll a, b and total chlorophyll in leaves of prairie cordgrass growing under conditions of varying levels of soil contamination with lead. The soil contamination with lead at a dose of 28.15 mg Pb · kg^{-1} soil (35.10 mg Pb · dm^{-3} soil) led to an increase in the content of carotenoids relative to control plants (Fig. 1).

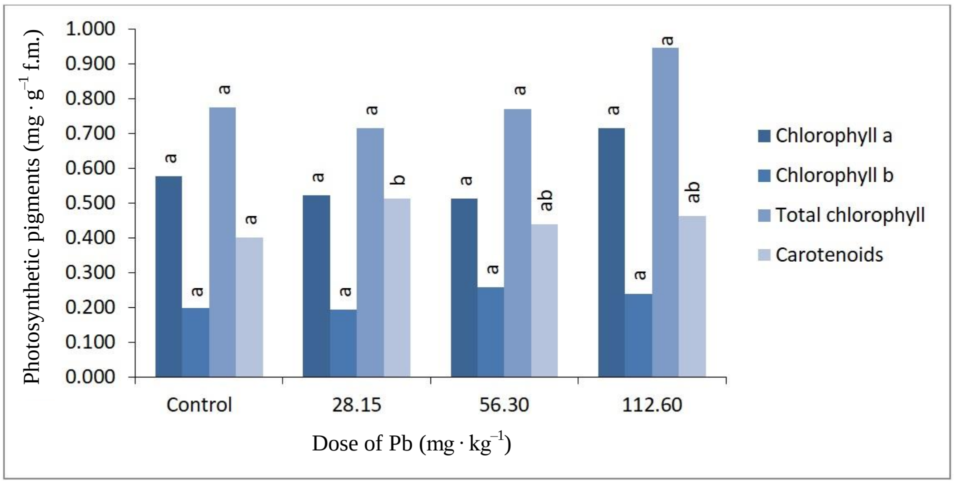

Fig. 1. The content of photosynthetic pigments in the leaves of prairie cordgrass growing under conditions of varying levels of soil contamination with lead

In the research on maize (*Zea mays* L.), an increased dose of lead ions (25–75 mg · kg^{-1}) caused an increase in the content of photosynthetic pigments in the leaves [Aliu et al. 2013], which is confirmed by our own research.

Different results have been received by Akinci et al. [2010] in research on tomato (*Solanum lycopersicum* L.), in which it was shown that increasing doses of lead ions (75, 150, 300 mg Pb · dm^{-3}) caused decreasing content of chlorophyll a, b, total chlorophyll in leaves. Similar results were obtained by Sayed [1999] in research on safflower. In his research, the content of chlorophyll a and b decreased progressively with increasing Pb concentration (1, 10, 50, 100 mg Pb · dm^{-3}).

Varied level of soil contamination with cadmium did not affect the content of chlorophyll a, total chlorophyll and carotenoids in the leaves of the test species. The lowest content of chlorophyll b, significantly different from the content in leaves of plants growing in soil with the addition of cadmium at a dose of 4.60 and 10.00 mg · kg^{-1} (45.99 and 91.98 mg · dm^{-3}), was found in spartina growing in conditions of the highest level (23.00 mg · kg^{-1}) of soil contamination with this heavy metal (Fig. 2). In the studies of Malinowska et al. [2010], increasing concentration of cadmium in the nutrient solution (1.4–280 mg · dm^{-3}) resulted in a reduction in the content of all photosynthetic pigments in the leaves of another species used in phytoremediation, i.e. basket willow (*Salix viminalis* L.). Similar relationship was also demonstrated by Aliu et al. [2013] for maize and by De Maria et al. [2013] for sunflower. The lack of reduction in chlorophyll and carotenoid content in leaves under the soil contamination with Pb, which is a specific response of the plant to metal stress, may indicate that these concentrations of Pb did not cause defensive reactions in the test plants [Shu et al. 2012].

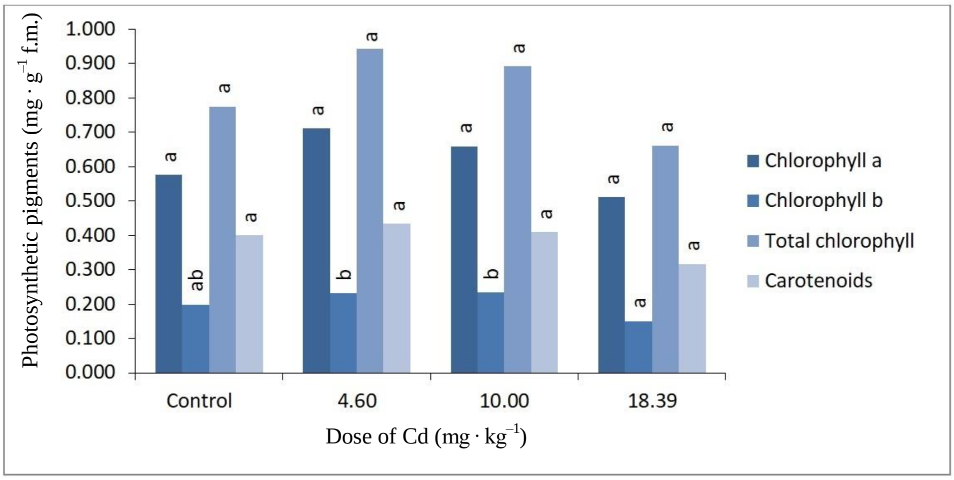

Fig. 2. The content of photosynthetic pigments in the leaves of prairie cordgrass growing under conditions of varying levels of soil contamination with cadmium

Indicators of relative water content in leaves (RWC) and water saturation deficit (WSD) are one of the most important parameters commonly used to assess the plant water management. Plants characterized by low RWC index are usually characterized by low photosynthetic activity [Tezara et al. 2002]. The addition of lead at a dose of 28.15 mg · kg^{-1} (35.10 mg · dm^{-3}) caused an increase in the RWC index in the cordgrass leaf tissues compared to the control. Similar results were also obtained for beans [Barceló et al. 1986]. Sayed [1999] shown that the RWC measured in safflower leaves decreased progressively when the concentration of lead was increasing (1, 10, 50, 100 mg · dm^{-3}).

Soil contamination with cadmium at various doses did not affect the water balance parameters. Only cadmium at a dose of 10.00 mg · kg^{-1} caused a decrease in RWC as compared to the control by 5%. Remaining variants caused an increase in RWC compared to controls from 68% (control) to 77% (4.60 mg · kg^{-1}) and 75% (18.39 mg · kg^{-1}) (Tab. 1).

Table 1. Indicators of relative water content in leaves (RWC) and water saturation deficit (WSD) in prairie cordgrass growing under diversified conditions of soil contamination with cadmium or lead

Index	Dose of Pb (mg· kg^{-1})				Dose of Cd (mg· kg^{-1})			
	control	28.15	56.30	112.60	control	4.60	10.00	18.39
RWC (%)	68	77	57	58	68	77	63	75
WSD (%)	32	23	43	42	32	23	37	25

Lowering the RWC index under the influence of cadmium has been observed, among others, at sunflower [Kastori et al. 1992]. In studies carried out by Malinowska et al. [2010], an increase in the dose of cadmium introduced into hydroponics from the value of 1.4 to 280.0 mg · dm^{-3} resulted in the reduction of the RWC index determined for two genotypes of basket willow. The most negative impact of this plant on the water balance was demonstrated in the case of the highest level of cadmium contamination. Also during hydroponic cultivation, the addition of 50 μM Cd^{2+} had no effect on the RWC index of *Arabidopsis thaliana* L. leaves [Perfus-Barbeoch et al. 2002]. For safflower (*Carthamus tinctorius*) plants, however, increased doses of cadmium (0–30 μM) caused a gradual decrease in the RWC index (75.54–65.31%). De Maria et al. [2013] in the research on sunflower shown that Cd at doses of 2.2–15 mg · kg^{-1} did not cause significant decrease of RWC.

Changes observed in values of the physiological traits studied under unfavorable conditions may be the effect of both stress and plant repair mechanisms to maintain homeostasis [Starck 2002].

CONCLUSIONS

1. Lead dose applied had an effect on the content of photosynthetic pigments in the prairie cordgrass leaves. The soil contamination with lead at a dose of 28.15 mg · kg^{-1} led to an increase in the content of carotenoids relative to control plants. There was no significant effect of lead at doses of 28.15, 56.30 and 112.60 mg · kg^{-1} on the content of chlorophyll a, b and total chlorophyll in the leaves of prairie cordgrass.

2. Cadmium dose applied had an effect on the content of photosynthetic pigments in the prairie cordgrass leaves. The soil contamination with cadmium at the doses of 4.60 and 10.00 mg · kg^{-1} led to an increase in the content of chlorophyll b. There was no significant effect of cadmium at doses of 4.60, 10.00 and 18.39 mg · kg^{-1} on the content of chlorophyll a, total chlorophyll and carotenoids in the leaves of prairie cordgrass.

3. The applied doses of lead and cadmium affected the change in the water balance parameters in the prairie cordgrass. Lead at a dose of 25.15 and cadmium at doses of 4.60 and 18.39 caused an increase in RWC compared to the control. Lead at doses of 56.30 and 112.60 and cadmium at a dose of 10.00 caused a decrease in RWC compared to the control.

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Streszczenie. Celem pracy była ocena oddziaływania zróżnicowanego poziomu zanieczyszczenia gleby jonami ołowiu oraz kadmu na wybrane parametry fizjologiczne spartiny preriowej. Oznaczono zawartość barwników fotosyntetycznych w liściach (chlorofilu a, b, całkowitego oraz karotenoidów) oraz bilans wodny roślin na podstawie dwóch wskaźników (RWC – wskaźnik względnej zawartości wody w tkankach i WSD – wskaźnik deficytu wysycenia tkanek wodą). Wazonowe doświadczenia wegetacyjne przeprowadzono metodą kompletnej randomizacji w układzie jednoczynnikowym. Czynnikiem w pierwszym doświadczeniu był poziom skażenia gleby ołowiem (28,15, 56,30, 112,60 mg Pb · kg gleby^{-1}), w drugim – poziom skażenia gleby kadmem (4,60, 10,00, 18,39 mg Cd · kg gleby^{-1}). Zastosowane poziomy zanieczyszczenia gleby ołowiem nie wpłynęły na zawartość chlorofilu a, b oraz chlorofilu całkowitego w liściach spartiny preriowej. W przypadku karotenoidów wykazano wzrost ich zawartości w porównaniu z kontrolą po wprowadzeniu ołowiu do gleby w dawce 28,15 mg Pb · kg gleby^{-1}. Zanieczyszczenie gleby kadmem nie wpłynęło na zawartość chlorofilu a, chlorofilu całkowitego oraz karotenoidów w liściach spartiny preriowej. Najwyższy z zastosowanych poziom skażenia gleby tym pierwiastkiem skutkował zmniejszeniem zawartości chlorofilu b. Ołów w dawkach 56,30 oraz 112,60 mg · kg gleby^{-1} spowodował pogorszenie parametrów bilansu wodnego spartiny preriowej. W przypadku skażenia gleby kadmem zależność taką wykazano jedynie w przypadku dawki 10,00 mg Cd · kg gleby^{-1}.

Słowa kluczowe: kadm, ołów, *Spartina pectinata* Bosk ex Link., barwniki fotosyntetyczne, bilans wodny

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