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BIOLOGICAL ASPECTS OF CADMIUM AND LEAD UPTAKE BY *PHRAGMITES AUSTRALIS* (CAV. TRIN EX STEUDEL) IN NATURAL WATER ECOSYSTEMS

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Abstract

In natural environment plants are exposed to many different stress factors, including heavy metals, whose elevated concentration causes oxidative stress, connected with formation of reactive oxygen species (ROS). Therefore, plants have developed defence systems, including enzymatic antioxidant system, able to remove ROS.

The work concerns the accumulation of two heavy metals, cadmium (Cd) and lead (Pb), as well as the phenomenon of oxidative stress caused by increased concentration of these metals in common reed (*Phragmites australis*), a dominant species in the littoral zone of many water reservoirs. The plants were obtained from four water bodies situated in Poznan: Kierskie Lake, Rusałka Lake, Strzeszyńskie Lake and Sołacki Pond.

The aim of the study was to examined the accumulation of heavy metals and the relation between activity of antioxidant enzymes in rhizome, stem and reed leaves during the vegetative period. Three antioxidant enzymes were analyzed: ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and superoxide dismutase (SOD).

The statistical analysis was done to determine the influence of the heavy metals on the activity of the antioxidant enzymes, involved in limiting and removing results of oxidative stress.

Heavy metals were accumulated in common reed in all the four water reservoirs, but the activity of enzymes was variable during the observation period. Statistical analyses suggest that there are some correlations among concentration of metals and the activity of antioxidative enzymes. However, the results do not provide an unambiguous determination of the effect of heavy metals on enzymatic activity. Summing up, the contamination

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of the water ecosystems caused by heavy metals was so low that it did not influence the activity of the analysed enzymes.

Key words: cadmium, lead, antioxidant enzymes, water reservoirs, bottom sediments, common reed *Phragmites australis*

BIOLOGICZNE ASPEKTY POBORU KADMU I OŁOWIU PRZEZ *PHRAGMITES AUSTRALIS* (CAV. TRIN EX STEUDEL) W NATURALNYCH ZBIORNIKACH WODNYCH

Abstrakt

W środowisku naturalnym rośliny są narażone na działanie metali ciężkich. Nadmierne ich stężenia w roślinach powodują stres oksydacyjny, wywoływany powstawaniem reaktywnych form tlenu (ROS). W celach obronnych organizmy roślinne wykształciły wiele systemów antyoksydacyjnych, w tym enzymatyczne umożliwiające usuwanie ROS.

Praca dotyczy akumulacji dwóch metali ciężkich – kadmu (Cd) i ołowiu (Pb) oraz zjawiska stresu oksydacyjnego w roślinach powodowanego przez nadmierne stężenia jonów metali ciężkich w środowisku oraz w organach trzciny pospolitej (*Phragmites australis*) – gatunku występującego i dominującego w strefie litoralnej wielu zbiorników. Materiał roślinny pochodził z czterech zbiorników wodnych zlokalizowanych w obrębie miasta Poznania: Jeziora Kierskiego, jeziora Rusałka, Jeziora Strzeszyńskiego i Stawu Sołackiego.

Celem badań było określenie zależności między zawartością metali ciężkich a aktywnością enzymów antyoksydacyjnych w kłączach, łodydze i liściach trzciny na przestrzeni okresu wegetacyjnego. Analizowano aktywność trzech enzymów antyoksydacyjnych: peroksydazy askorbinianowej (APX) i gwajakolowej (GPX) oraz dysmutazy ponadtlenkowej (SOD).

Przeprowadzono analizy statystyczne celem określenia wpływu czynników stresu oksydacyjnego wywołanego przez metale ciężkie na aktywność enzymów antyoksydacyjnych, które są zaangażowane w ograniczanie i usuwanie skutków tego zjawiska.

Metale ciężkie akumulowane w roślinach we wszystkich ocenianych zbiornikach wodnych wykazywały tendencję wzrostową, natomiast aktywność enzymów w roślinach była silnie zróżnicowana w całym okresie obserwacji. Analizy statystyczne sugerują wprawdzie istnienie pewnych korelacji między stężeniem metali w roślinach a aktywnością enzymów antyoksydacyjnych, jednakże nie pozwala to na jednoznaczne określenie wpływu badanych metali na zmienność w aktywności tych enzymów. Podsumowując, stwierdzone skażenie ekosystemów wodnych metalami ciężkimi było na tyle niskie, że nie wpływało modyfikująco na aktywność badanych enzymów.

Słowa kluczowe: kadm, ołów, enzymy antyoksydacyjne, zbiorniki wodne, osady denne, trzcina pospolita (*Phragmites australis*).

INTRODUCTION

Water reservoirs are very attractive components of open and urban landscapes, but in order to serve their functions well they need to have good water quality. The quality of water in surface water bodies is largely determined by the concentration of heavy metals. Stability of heavy metals in aquatic environment and their accumulation in successive links of the food chain make it absolutely necessary to search for efficient and safe methods to remove these toxic elements from individual elements of the ecosystem, especially sediments and plants inhabiting water bodies.

Although the total content of heavy metals in the environment is very low, less than 1%, and while some of the metals are vital for living organisms, cadmium and lead are examples of toxic elements. Heavy metals enter natural ecosystems with discharges from industry and urban sewage or through atmospheric deposition, which increases their concentration in nature. Most of them remain insoluble in soil and water ecosystems or, as dissolved elements, become available for plant uptake and cumulate in their tissues (GRZYBOWSKI et al. 2000). Plant uptake directly reduces the overflow of metals into adjacent waters (LIANG et al. 2006). The accumulation in plants and the direction and intensity of transport depend on biological factors and on the chemical properties of metals.

Cadmium and lead belong to pollutants present in areas with heavy road traffic and near cities, which are taken by plants but at different bioaccumulation indexes. Generally, the highest Pb concentrations have been observed in roots, while only small amounts are transported to other parts of plants (VERMA, DUBEY 2003). In contrast, cadmium belongs to the elements capable of being translocated through plants and accumulated in leaves. However, in the experiments conducted by Fediuc and Erdei (2002) on young *Phragmites australis* grown hydroponically, most of the Cd content was collected in the shoots.

Some plants, called hyperaccumulators, such as *Thlaspi caerulenscens* and *Brassica juncea*, demonstrate a particularly high ability to accumulate metals in unusually high concentrations (SALT et al. 1998). Also common reed (*Phragmites australis*), which is a widely distributed species worldwide, proves useful in cleaning eutrophic lakes and waste waters (YE et al. 1997).

High concentrations of cadmium or lead can also be stress factors for plants (IANELLI et al. 2002, VERMA, DUBEY 2003). They can disrupt cellular homeostasis and enhance the generation of reactive oxygen intermediates (ROIs), which cause oxidative stress. To avoid ROIs, plants create mechanisms called ROI-scavenging mechanisms, which are responsible for removing reactive oxygen intermediates (e.g. singlet oxygen ($O_2{}^1$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) (MITTLER 2002). The scavenging systems include antioxidant enzymes: catalase (EC 1.11.1.6), peroxidases (EC 1.11.1.7), superoxide dismutases (EC 1.15.1.1), and low molecular antioxidants such as ascorbic acid, glutathione or a-tocopherol (MITTLER 2002, VERMA, DUBEY 2003).

The aim of the study was to examine the relation between the level of heavy metals in sediments of natural water ecosystems (four lakes near the city of Poznan, Poland) and their accumulation in *Phragmites australis* plants growing in those reservoirs. The study focused on cadmium and lead bioaccumulation and the interrelations between those contaminants in aquatic plants and the activity of antioxidant defence enzymes (which can be indicators of stress caused by toxic metals), such as ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and superoxide dismutase (SOD), including the effect of seasons.

MATERIALS AND METHODS

Study area and plant material

Four recreational water reservoirs located in the city of Poznań were selected for the study: Lake Kierskie (referred to as KL), Lake Strzeszyńskie (SL), Lake Rusałka (RL) and Sołacki Pond (SP). The experiments were performed in 2005 and 2006. Four heavy metals, i.e. zinc, cadmium, lead and copper, were determined in the bottom deposits and in whole plants of common reed (*Phragmites australis*, Cav. Trin. ex Steudel). Independently, cadmium and lead concentrations and the antioxidant enzymes were analysed separately in leaves, stems and rhizomes (without adventitious roots). All analyses were carried out three times during the vegetation period, i.e. in May, August, and November. Plants, after being transferred to the laboratory, were weighed and frozen in liquid nitrogen.

Heavy metal analyses

Plant samples (1 g) and sediments (3 g) were dried at 105°C for 48 h and mineralised in a Star 6 microwave oven (CEM) for 40 minutes, adding 25 cm^3 HNO_3 and 5 cm^3 H_2O_2. Metal contents were estimated by electrothermal atomic absorption spectroscopy using an AAS spectrometer (Varian Spectra AA 200 Plus).

Antioxidant enzyme analyses

For ascorbate and guaiacol peroxidase (APX and GPX) frozen samples (0.2 g) were homogenized for 30 seconds in a chilled mortar with 50 mM phosphate potassium-buffer (pH 7.0) and 2% Polyclar AT. Homogenates were centrifuged at 15 000 g for 30 minutes at 4°C. APX activity was determined according to Nakano and Asada (1981). The reaction mixture contained 50 mM phosphate-potassium buffer, 0.5 mM L-ascorbate, 0.1 mM H_2O_2 and enzyme extract. Absorbance was measured at λ_{max} = 290 nm using an UV/VIS Spectrophotometer Lambda 11 (Perkin Elmer) and was expressed as absorbance increment per 1 minute per 1 mg protein (ΔE min^{-1} mg^{-1}).

GPX activity was measured according to HAMMERSCHMIT et al. (1982). Enzyme assays contained 25 mM phosphate-potassium buffer (pH 7.0), 0.2 mM guaiacol, 0.09 mM H_2O_2 and enzyme extract. Absorbance was recorded at λ_{max} = 480 nm and was expressed as absorbance increment per 1 minute per 1 mg protein (ΔE min^{-1} mg^{-1}).

Superoxide dismutase (SOD) was assayed according to Beauchamp and Fridovich (1971). Frozen tissues (0.2 g) were homogenized in a chilled mortar with 50 mM phosphate-potassium buffer (pH 7.8), containing 1% polyvinylpolypyrrolidone (PVP), 1.0 mM EDTA-Na and 0.5 M NaCl. Extracts were centrifuged at 15 000 g for 25 min at 4°C. The incubation mixture contained 50 mM phosphate-potassium buffer (pH 7.8), 0.01 mM EDTA-Na, 4 mM methionine, 0.1 mM nitro blue tetrazolium (NBT) and crude extract. Finally, 2.4 mM riboflavin was added and afterwards samples were placed under an UV lamp for 10 minutes. At the same time a blank sample was prepared. Absorbance was measured, in relation to the blank test, at λ_{max} = 560 nm using an UV/VIS Spectrophotometer Lambda 11 (Perkin Elmer). One unit of SOD activity was defined as the quantity of the enzyme required to reduce absorbance by 50% in comparison to the blank sample per one mg of protein. The level of proteins was measured according to Bradford (1976) using serum albumin (Sigma) as a standard.

Statistical analysis

All preparations, for each year, were done as independent triplicates. Standard deviations for means were calculated and correlations and linear regressions were determined using Statistica and GraphPad Prim software.

Multivariate analysis of variance for factorial experiments was applied in order to determine spatial and time variability in metal contents (zinc, cadmium, lead and copper) in sediments of analyzed reservoirs and in *Phragmites australis* plants.

RESULTS

Estimation of heavy metal contamination in water reservoirs

The analysis of canonical variables, used to assess the variation and similarity of analyzed reservoirs in terms of heavy metal accumulation in sediments, showed that the biggest differences in the contents of analyzed metals in sediments were found between Sołacki Pond (SP) and the other reservoirs (Figure 1*a*). Mahalanobis distances between this reservoir and the other water bodies were 230.95, 186.46 and 207.64 for KL, SL and RL, respectively, and they were on average 4 times bigger than that e.g. between KL and SL, amounting to 54.27. Thus, a markedly different distribution of metals was recorded in the SP sediment, which may be related to the location of this reservoir closest to the city centre of Poznań.

Graphic analysis of similarities and differences in metal contents in sediments in terms of sampling dates showed the dissimilarity of the third date, i.e. autumn (November) – Figure 1*b*. Metal contents at that time were

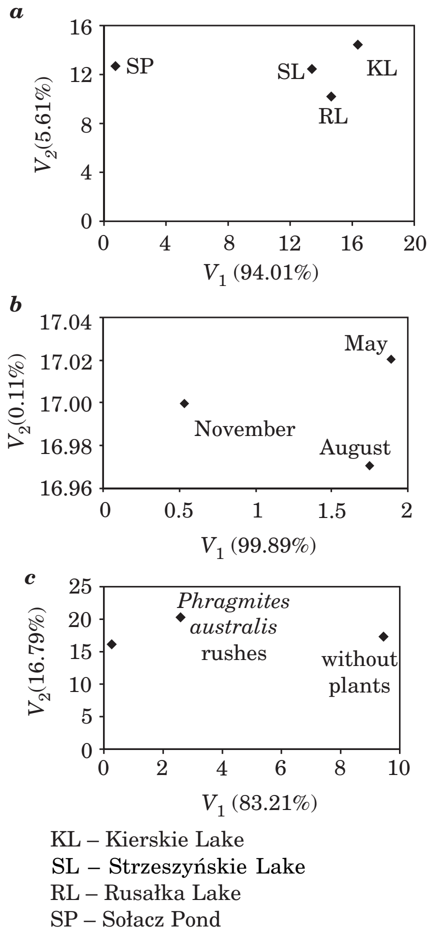

Fig. 1 . Similarities of heavy metal accumulation in sediments analyzed in years 2005-2006: *a* – between four reservoirs (KL, SL, RL and SP), *b* – between periods of analyses, *c* – rushes compare to area out of plants

higher than on the other two dates, which is confirmed by the calculated Mahalanobis distance, which for May and November was 10 times higher than for May and August.

When analyzing rushes, i.e. sites overgrown with shore plants, in terms of the accumulation of heavy metals in sediments, differences were found between sampling sites in the case of samples free from plants and sites where macrophytes were found, which indicates that heavy metals are accumulated in aquatic macrophytes (Figure 1*c*). The discussed differences are reflected in the calculated Mahalanobis distance, which amounted to 70.04 for *Phragmites australis* rushes and a site free from vegetation.

Cadmium and lead accumulation in common reed

Annual mean cadmium content in common reed organs was 2-fold higher in 2006 than in 2005 (Figure 2*a*). The lowest concentration was recorded in plants collected from Lake Kierskie (KL), a slightly higher level was observed in those from Lake Strzeszynskie (SL) and the highest – in plants from Rusałka (RL) and Sołacki Pond (SP). Similar relations were observed in both years.

The average lead concentration was 5-10-fold higher than that of cadmium and similar in both years. The lowest accumulation was recorded in plants collected from Lake Strzeszyńskie (SL). In the remaining reservoirs concentrations of this metal were similar.

When analyzing similarities of plant collection dates in the years 2005-2006 in terms of the accumulation of all the analyzed metals (Zn, Cd, Pb and Cu), a considerable difference was found between the first (May) and the third date (November) (Figure 2*b*).

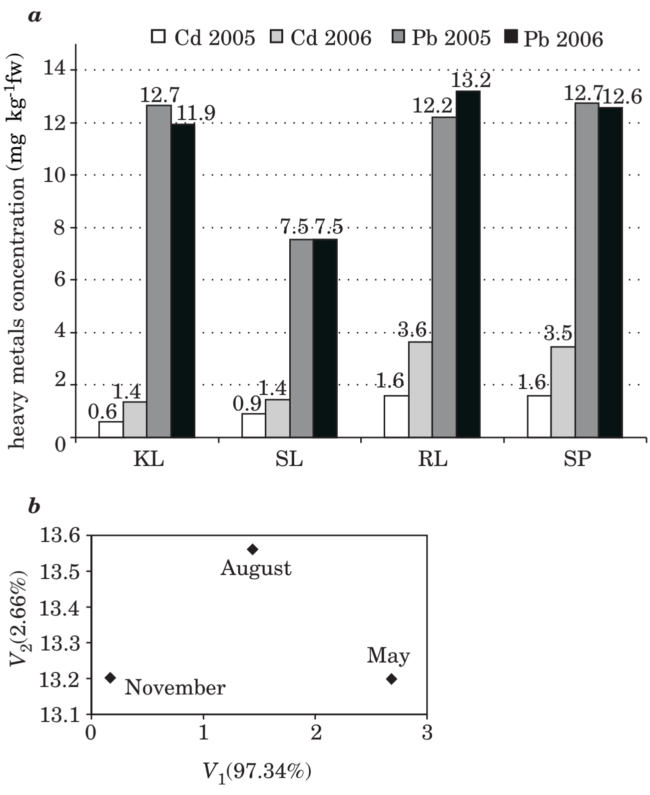

Fig. 2. Heavy metal accumulation in common reed (*Phragmites australis*) grown in four natural water ecosystems (KL, SL, RL and SP – explanations see Fig. 1) analyzed in 2005-2006: *a* – cadmium and lead concentration, *b* – similarity between periods of analyses (means for zinc, cadmium, lead and copper)

Moreover, in 2005 the accumulation of both metals was analysed in individual organs, i.e. in leaves, stems and rhizomes. In all plant parts the lowest content of cadmium (Figure 3) was recorded in May, at the beginning of the vegetative period, and the highest – at the end of vegetation (November), so the Cd content was increasing during the growth process. There was a noticeable disproportion in its accumulation depending on the examined lakes: slight in KL and SL, while considerable in RL and SP plants.

The level of lead contamination measured in leaves was similar in all the lakes and changed little during the vegetation season (Figure 4). In shoots its content was twice as high in KL as in the other lakes and

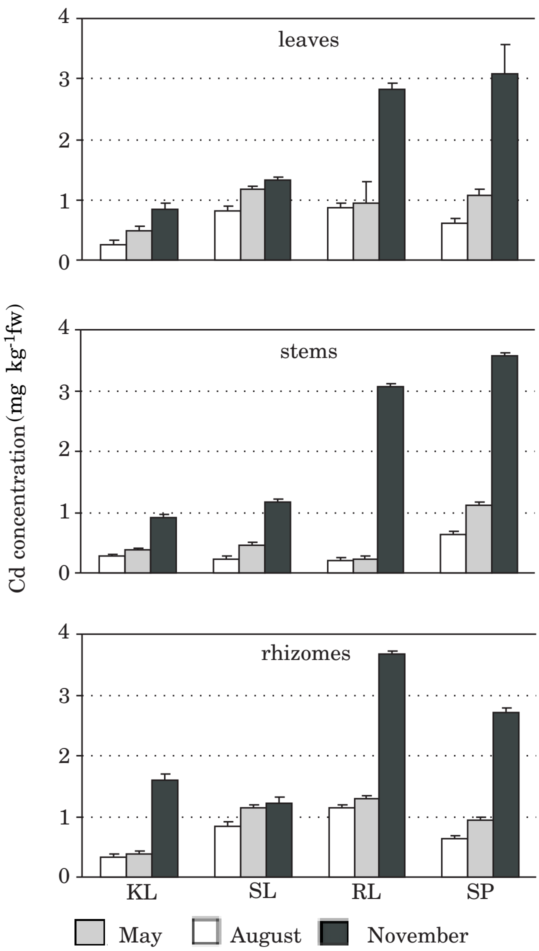

Fig. 3. Cadmium concentration in common reed leaves, stems and rhizomes grown in natural water ecosystems (KL, SL, RL, SP – explanations see Fig. 1), measured in May, August, and November

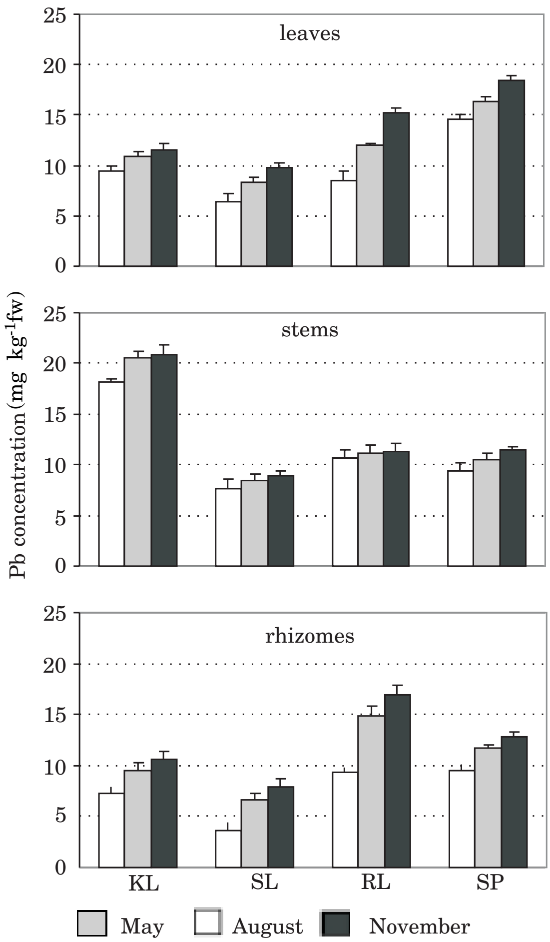

Fig. 4. Lead concentration in common reed leaves, stems and rhizomes grown in natural water ecosystems (KL, SL, RL, SP– explanations see Fig. 1), measured in May, August, and November

during the vegetation period the changes were noticeable. In rhizomes the Pb content increased between May and November and was relatively similar in plants from all the lakes.

Table 1 presents bioaccumulation indexes of elements, expressed in the ratios of contents in leaves and rhizomes to the concentrations of this metal in the bottom sediment of the reservoir. The value of this index recorded for cadmium was 2- to 4-fold higher than that of lead. When comparing the bioaccumulation index of both analyzed metals in aerial and underground parts of common reed plants, higher bioaccumulation of Cd and Pb was found in rhizomes.

Table 1

Bioaccumulation of cadmium and lead in *Phragmites australis* measured in leaves and rhizomes in relation to sediment (mean for 2005 and 2006)

Metal	Plant organ	Bioaccumulation index			
		KL	SL	RL	SP
Cd	leaves	2.91	0.91	1.13	1.26
	rhizomes	4.62	1.90	1.28	0.96
Pb	leaves	1.01	0.93	0.81	0.36
	rhizomes	1.28	1.31	1.03	0.30

KL, SL, RL, SP – see Fig 1.

In conclusion, Pb content in common reed tissues exceeded the level of Cd and its dynamics of changes in individual plant organs and bioaccumulation indexes were different for the examined lakes.

Antioxidant enzyme activity

The constitutive level of ascorbate peroxidase (APX) activity was the highest in leaves and the lowest in common reed stems (Figure 5*a*). During the experimental season the changes were negligible in leaves and stems. In rhizomes the APX level was very low in May and then successively increased, irrespective of the analyzed water reservoir.

Guaiacol peroxidase (GPX) activity was relatively high in common reed stems as compared to leaves and rhizomes (Figure 5*b*). The changes were not dependent on the investigated water ecosystem.

Superoxide dismutase (SOD) activity was the highest at the beginning of plant growth, especially in leaves (Figure 5*c*). In stems and rhizomes the highest activities were observed in the middle of the summer (August). Generally, no differences were found between the analysed lakes.

Statistical analysis

In order to compare heavy metal accumulation and antioxidant enzyme activities, correlation coefficients were calculated using the Statistica software. In 2005 all data were subjected to analyses jointly, without division into individual plant organs and lakes. A positive correlation was found between lead concentration and the activity of ascorbate (r=0.423 at p<0.05) and guaiacol peroxidases (r=0.42 at p<0.05). A negative correlation was observed between the concentration of cadmium and the activity of superoxide dismutase (Table 2).

In 2006 analyses were carried out for individual organs and some effect was observed only for leaves. Correlations between the concentration of cadmium and GPX activity (r=-0.35 at p<0.05) and lead and both enzymes (r=-0.31 and r=-0.45) were negative.

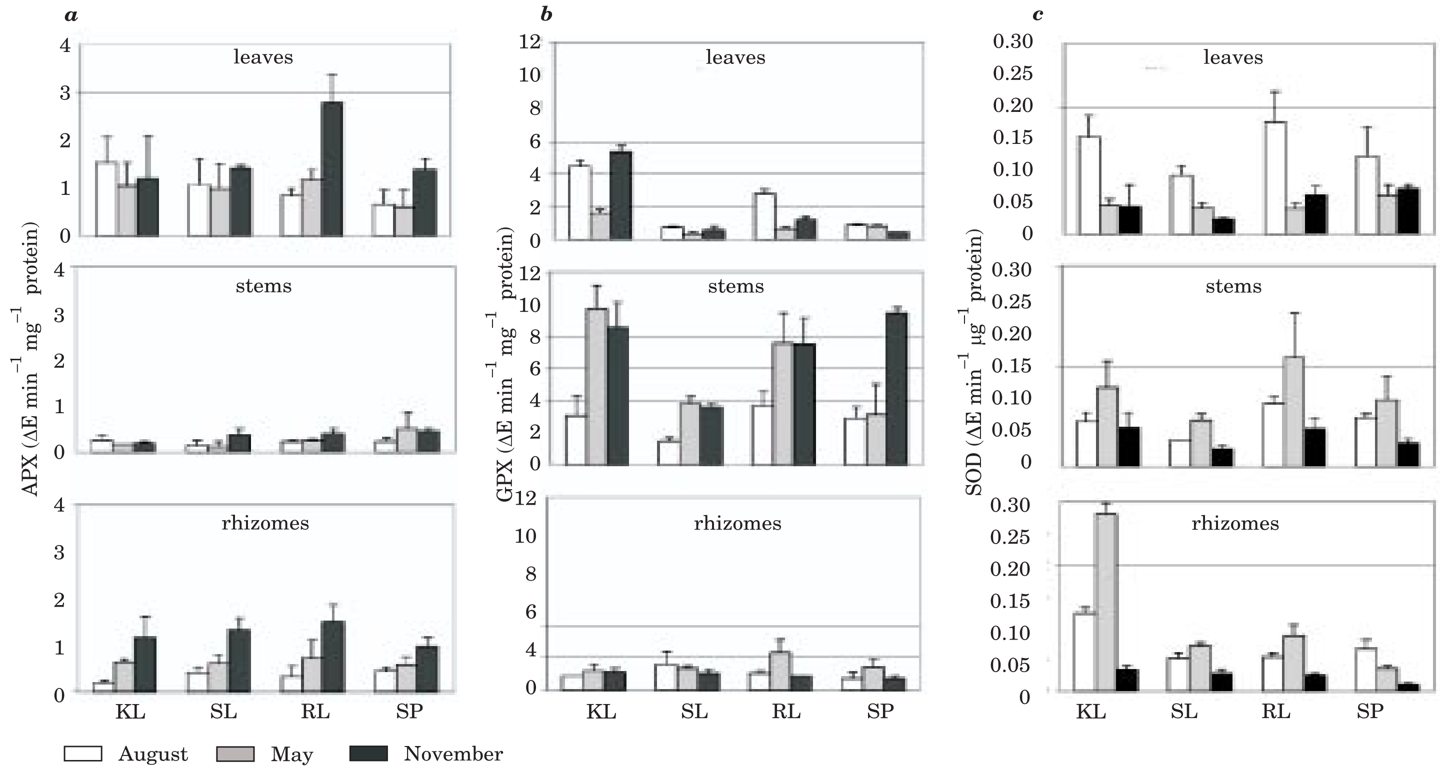

Fig. 5. Activity of antioxidant enzymes APX, GPX and SOD in common reed leaves, stems and rhizomes grown in natural water ecosystems (KL, SL, RL, SP), measured in May, August and November

Table 2

Correlation coefficient for linear regression between cadmium and lead accumulation and the enzymatic activity

Metal accumulation	Enzyme activity		
	APX	POD	SOD
Results of 2005 (whole plants)			
Cd	0.088 –	0.004 –	- 0.538 +
Pb	0.423 +	0.420 +	- 0.069 –
Results of 2006 (leaves):			
Cd	0.17 –	- 0.35 +	
Pb	- 0.31 +	- 0.45 +	

\+ linear dependence
– linear independence

DISCUSSION

The examined water reservoirs are classified as typical urban reservoirs, in which the quality of water is affected by the neighbouring Poznan agglomeration. When analyzing sediments from the water reservoirs, similar contents of the four analyzed heavy metals were determined in threelakes: KL, SL and RL. Moreover, contamination of bottom sediments was statistically higher in the autumn period (November) than in the spring-summer period. In turn, the uptake of metals by *Ph. australis* plants was most intensive in the spring-summer period, which was probably caused both by intensive transpiration and the demand for functional minerals. Heavy metals are also absorbed with nutrients. In the late autumn, especially in 2005, low temperature promoting the transition of plant to the state of dormancy reduced metal uptake, even at their highest content in sediments. This dependence was not observed in the case of accumulation of cadmium and lead.

Contents of these contaminants, especially toxic metals, increase from May until November, as has been reported by YE (1997) and VERMA and DUBEY (2003).

The data concerning bioaccumulation of cadmium and lead are not conclusive. However, cadmium is considered to be an element of a much higher mobility than lead, although its content in the environment is much lower, which was also confirmed in our study. In the analyzed water reservoirs, Cd content was at least several-fold lower than that of Pb and varied in successive years of the study. In terms of the metal content in the analyzed organs, accumulation of both Cd and Pb adjusted to the fresh weight basis was similar. In model studies using small *Ph. australis* plants generated from embryonic callus tissue, roots accumulated more cadmium than shoots, which would suggest limited mobility of Cd in this species (FEDIUC, ERDEI 2002). SIMILARLY, YE et al. (1997) stated that the highest content of cadmium was accumulated in reed roots. These data, similarly as the results of our investigations, indicate that this is characteristic for this aquatic plant. Another species, *Typha latifolia*, under identical experimental conditions accumulated three times more cadmium than *Ph. australis* and higher concentrations were recorded in shoots when compared to common reed.

The fact that lead was accumulated mostly in the underground parts of common reed is consistent with the commonly accepted view and observations on different plant species (WINDHAM 2001). However, the content of heavy metals in plants is dependent on their concentration in the nutrition solution, in soil or in water ecosystems, etc. (YE 1997, WINDHAM 2001, VERMA DUBEY 2003).

An important part of this study was an attempt to assess the dependence between the degree of reservoir contamination, bioaccumulation of elements, especially cadmium and lead, and the activity of antioxidant enzymes. A positive correlation between antioxidant enzyme activities and heavy metals was recorded only between guaiacol and ascorbate peroxidases and lead, although changes in the content of this element were slight. Such correlations are known from literature data, but pertain to cadmium (FEDIUC et al. 2001, IANNELI et al. 2002, RULEY et al. 2004, PACZKOWSKA et al. 2007). A negative correlation was recorded between superoxide dismutase and cadmium, which was very different from results available in published data, e.g. SHAH and KUMAR (2001) presented opposite results for rice.

Different results and few statistically proven relations may have been caused by the fact that plants were taken directly from their natural environment, where concentrations of cadmium and lead were considerably lower than in plants used in laboratory experiments. Furthermore, various environmental factors, such as temperature or solar exposure, etc., had a considerable effect on the activity of antioxidant enzymes.

CONCLUSION

Very low concentrations of heavy metals in the natural ecosystems were determined in the present study, with variations occurring between the reservoirs and between the sampling dates (the spring and autumn periods).

Although marked cadmium bioaccumulation was recorded, it did not have an effect on the activity of antioxidant enzymes. Enzymatic activities could have been stimulated by other environmental factors. At such a low heavy metal contamination, the tolerance of *Ph. australis* is probably sufficient for homeostasis.

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