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INFLUENCE OF SELECTED HEAVY METALS ON THE CONTENT OF PHOTOSYNTHETIC PIGMENTS IN FERN *AZOLLA CAROLINIANA*

An aquatic fern *Azolla carolinina* Willd. (*Azollaceae*) showed the ability to adapt itself to the presence of Pb(II), Cd(II) and Hg(II) ions in medium, each in the following concentrations: 0.1, 0.5 and 1.0 mg dm^{-3}. Pb(II) and Cd(II) ions in the concentrations of 0.5 and 1.0 mg decreased pigment contents by ca. 50%. Simultaneously their lowest doses stimulated pigment synthesis. Hg^{2+} ions, however, strongly stimulated this synthesis at all concentrations tested (332% increase in pigment content).

1. INTRODUCTION

Small water ferns (1–5 cm) from the *Azolla* genus live in symbiosis with cyanobacterium *Anabaena azolle* Strasb. (*Nostoceae*), which can fix an atmospheric nitrogen. These ferns occur in tropical and temperate climate on the surface of such warm, still waters as ponds and rice fields. Recently *Azolla filiculoides* was found also in Poland [9].

Because photosynthetic pigments are indispensable for photosynthesis, the changes in their contents caused by accumulation of heavy metals may disturb this process. Our aim was to examine how heavy metals (Cd, Pb, Hg) influence production of photosynthetic pigments in *A. caroliniana*. These studies were carried out within the framework of the programme aimed at applying *Azolla* sp. to phytoremediation of waters polluted with heavy metals. In our earlier investigations, we determined the influence of selected metals on medium aeration and the growth of fern biomass [3], [4].

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2. MATERIAL AND METHODS

2.1. EXPERIMENT PREPARATION

The experiment was conducted in ten glass aquariums containing 3 dm^3 of IRRI medium solution [4]. This mixture was enriched by selected heavy metals in the form of salts ($PbCl_2$, $HgCl_2$, $CdCl_2 \cdot 2½H_2O$, POCH, Poland) each in the following doses: 0.1, 0.5 and 1.0 mg dm^{-3}. One of the aquariums, which contained only IRRI solution, was used as a control.

2.2. CULTIVATION

20 g of *A. caroliniana* from the Warsaw Botanical Garden were rinsed with deionized water and then introduced to each aquarium. A lower part of each aquarium from the water surface to the bottom was shadowed in order to provide optimal conditions for the growth of plant's roots. Fluorescent lamps (Philips TLD36W/89 Aqua Relle, The Netherlands) provided 16-hour photoperiod.

2.3. MEASUREMENTS

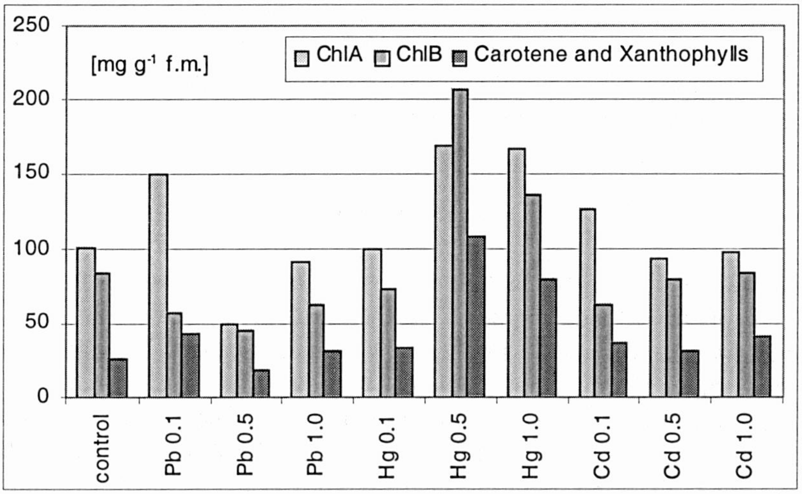
After 12 days of fern cultivation the concentrations of photosynthetic pigments (chlorophyll *a*, *b*, xanthophylls and carotenes) were determined in *A. caroliniana* biomass using Lichternthaler's and Wellburn's method with acetone extract [7]. These measurements were performed with UV/VIS spectrophotometer (Hitachi U-2001) at the wavelengths λ equal to 470, 646 and 663 nm. These assays were done in 3 replications and the results obtained were expressed in mg per g of fresh biomass.

During *A. caroliniana* cultivation 50 cm^3 of solution were taken from each aquarium in order to determine the contents of heavy metals using Flame Atomic Absorption Spectrophotometry (FAAS) method, by means of Hitachi Z-8200 spectrophotometer. After completing cultivation, plant material was dried at 80 °C to a constant weight and then mineralized in a nitric acid at a closed cycle (minrealizer MSP-1000, CEM). The solutions obtained were analyzed by the Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) method with Varian Liberty Series II spectrometer. Mercury content was determined by means of a direct decay technique (mercury analyzer AMA-254, ALTEC).

3. RESULTS AND DISCUSSION

In the control aquarium, the concentrations of chlorophyll and carotenes + xantophyllys were ca. 100 and 25 mg per 1 g of biomass, respectively. The presence of heavy metals changed these values, depending on the dose and the metal introduced (the figure).

The content of chlorophyll *a* (+49%), carotenes and xanthophylls (+69%) increased considerably compared to the control value at a 0.1 mg Pb(II) $\cdot$ dm^{-3} dose. The concentrations of chlorophyll *b*, however, decreased by ca. 31%. At higher doses of Pb(II) the concentrations of all pigments decreased maximally to 45%. The content of carotenes increased by about 23% at the Pb(II) dose of 0.5 mg dm^{-3}. These tendencies were also reported by DEVARS et al. [5].



Photosynthetic pigment contents at the end of the experiment
in a fresh biomass of *A. caroliniana*

A slight reduction in the content of chlorophylls *a* and *b* (by 2 and 13%, respectively) and an increase in the content of carotenes and xanthophylls (by about 32%) were measured at the Hg dose of 0.1 mg dm^{-3}. Higher doses of Hg(II) (0.5 and 1.0 mg·dm^{-3}) caused a rapid increase in pigment content: that of chlorophyll *a* by more than 60%, these of chlorophyll *b* by ca. 150 and 63%, respectively, and these of carotenes by ca. 332 and 218%, respectively. These changes were also confirmed by Devars experiment [5].

Cadmium stimulated synthesis of carotenes and xanthophylls (the increase by 50, 65, 26% at the doses of 0.1, 0.5 and 1.0 mg dm^{-3}, respectively). Only at the lowest dose of Cd(II), the content of chlorophyll *a* was higher than that of the control (by 24%) and at other doses it approached that of the control. The content of chlorophyll *b* was the lowest at the cadmium dose of 0.1 mg per dm^3 (a decrease by 25%) and at other doses it approached the control values. These results were confirmed by Devars' experiment [5]. Other experiments [8] also revealed a reduction in the content of chlorophyll by 27–75% with the increase in the concentration of Cd(II). We obtained similar results at the cadmium doses of 0.5 and 1.0 mg per 1 dm^3. Cadmium in soil decreased the content of pigments by about 38% (chlorophylls) and by 43% (carotenes) in *Brassica napus* [2]. Cadmium ions have a destructive effect on a photosynthetic apparatus of *Hordeum vulgare* [6] decreasing the content of chlorophylls by ca. 60%.

The concentraions of heavy metals in the media and biomass of *A. caroliniana* have proved that this fern demonstrates the ability to take up and accumulate them. The utake of heavy metals exceeded 90% and their accumulation ranged from 53 to 578 mg kg^{-1}. Lead ions only slightly stimulated the fern growth (a biomass increase by 17% at the lowest metal dose). At the dose of 0.5 mg of lead per 1 dm^3 the reduction in biomass of *A. caroliniana* reached 1%, and cadmium and mercury inhibited the fern growth, which manifested itself as a 30–60% decrease in its biomass.

4. CONCLUSIONS

The experiment demonstrated that lead, mercury and cadmium change the concentrations of photosynthetic pigments. In the opinion of numerous scientists, this is due to some disturbances in the synthesis of pigments caused by inhibition of some enzymes and structural damage of whole chloroplast or its structural modification [1], [2], [6], [8]. The results obtained confirmed that selected heavy metals in the concentrations of 0.1–1.0 mg dm^{-3} are toxic to *A. carolinina*. Photosynthetic disturbances are not such drastic as these reported by other researchers [2], [5], [6], [8]; only mercury is responsible for considerable changes in pigment contents. This allows the conclusion that *A. caroliniana* has the ability to withstand the stress caused by heavy metals and may be used for water purification of Hg(II), Pb(II) and Cd(II), which was proved in some experiments [3], [4].

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WPŁYW WYBRANYCH METALI CIĘŻKICH NA ZAWARTOŚĆ BARWNIKÓW FOTOSYNTETYCZNYCH PAPROCI *AZOLLA CAROLINIANA*

Wodna paproć *Azolla caroliniana* Willd. (*Azollaceae*), rosnąc na podłożu zawierającym Pb(II), Hg(II) oraz Cd(II) w stężeniach 0,1; 0,5 i 1,0 mg dm^{-3}, wykazała się zdolnością adaptacji do ich obecności w pożywce. Obecność jonów Pb(II) i Cd(II) spowodowała obniżenie zawartości barwników sięgające 50% wartości kontrolnej, gdy ich stężenie wynosiło 0,5 i 1,0 mg dm^{-3}. Jednocześnie przy najniższych dawkach tych metali obserwowano stymulację syntezy barwników. Natomiast obecność jonów Hg^{2+} działała silnie stymulująco na syntezę barwników we wszystkich stężeniach, powodując nawet 332% przyrost ich zawartości.