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THE INFLUENCE OF HEAVY METALS ON THE GROWTH OF *AZOLLA* DURING PURIFICATION OF MUNICIPAL WASTEWATER

Azolla caroliniana Willd. is a floating water fern living in symbiosis with blue-green algae *Anabaena azollae* Stasb. that fixes atmospheric nitrogen.

This fern was cultivated for 12 days under laboratory conditions in the medium enriched with 1.0 mg Zn(II) dm^{-3} . This medium resulted in an increase in a total nitrogen content in a plant biomass and a reduced growth of biomass. The plants grown in the medium supplemented with 0.1, 0.5 mg Zn(II) dm^{-3} did not differ significantly in total nitrogen concentration from the control plants. The addition of 0.1, 0.5, 1.0 mg dm^{-3} Pb(II) to the nutrient solution increased a total nitrogen content and biomass growth which were higher than in the control plants.

The concentrations of both metals in the plant biomass increased considerably.

1. INTRODUCTION

Azolla sp. is a small (1–5 cm) floating water fern, which is widely spread all over the world in a tropical climate. It occurs on surface of eutrophic, warm, still waters [7] and lives in symbiosis with nitrogen-fixing blue-algae *Anabaena azollae* [5], [6].

Azolla plants have been used for centuries in Asia as a nitrogen biofertilizer, because the fern in optimal conditions can double its biomass every 2–3 days [1], and the blue-algae fixes atmospheric nitrogen in the quantity of 38–93 kg N ha^{-1} for 30–46 days [12].

Recently, many tests on the fern have been conducted in order to apply it in wastewater purification. *A. caroliniana* can grow in polluted environement and is able to remove nitrogen and phosphorous compounds [9], sulfonamides [8] and some heavy metals [2], [3] from it.

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The aim of this paper was to answer the question as to whether or not *Azolla caroliniana* (*Azollaceae*) Willd. displays the ability to grow and develop in polluted water, which contains the selected heavy metals (zinc and lead). During the laboratory experiments the fern usability for phytoremediation, e.g. for wastewater cleaning, was investigated.

2. MATERIALS AND METHODS

The responses of *A. caroliniana* to zinc and lead were investigated. The selected heavy metals in the form of $ZnSO_4 \cdot 7H_2O$ and $PbCl_2$ were introduced into nutrient solutions in the concentrations of 0.1, 0.5, 1.0 mg dm^{-3}.

Glass aquariums were filled with 3 dm^{-3} of nutrient solution, which was prepared according to recommendations of International Rice Research Institute (IRRI) [3] and enriched with heavy metals. One of the aquariums, containing only nutrient medium was used as a control. At the beginning of the experiment the medium in each aquarium was inoculated with 20 g of *A. Caroliniana.* The investigations were performed under laboratory conditions at photoperiod of 16\8, atmospheric air temperature of 24–25 °C and water temperature of 23–25 C.

After 12 days of cultivation the biomass of the fern was collected, weighed and dried at 80 °C until a constant weight was obtained. The metal concentrations in the biomass were determined by ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometry) method. A total nitrogen content in plants was determined by the Kjeldahl method [11].

3. RESULTS AND DISCUSSION

After completing the experiment the concentrations of zinc and lead in the biomass increased. The ferns grown in the medium with the concentrations of both metals of 0.1, 0.5 and 1.0 mg dm^{-3} accumulated 111.5, 253.8, 411.8 mg Zn kg^{-1} d.m. and 53.0, 245.1, 412.2 mg Pb(II) kg^{-1} d.m., respectively.

The addition of zinc salt limited the growth of *A. caroliniana* which was reduced by 27.0, 26.2 and 31.1% in comparison to the control plants (table 1). However, the fern did not show any morphological modifications, was still dark green and properly developed.

The plants grown in the nutrient solutions enriched with lead developed better than these in the control medium. The plants in the medium containing 0.1 and 1.0 mg of lead dm^{-3} increased their biomass by about 17.3 and 12.3%, respectively, while the biomass of the plants grown at the concentration of 0.5 mg of lead dm^{-3} was comparable to that of the control (lesser by about 0.5%) (figure 1).

Table

An increase in the biomass and per cent of nitrogen in plant material

Concentrations of Zn and Pb ($mg \cdot dm^{-3}$)	Fresh biomass (g)	Increase in biomass (g/12 days)	% H_2O	% N
Control	89.900	69.900	96.60	1.51
Zn 0.1	71.183	51.183	96.18	1.45
Zn 0.5	71.553	51.553	95.92	1.55
Zn 1.0	89.901	48.901	96.00	2.91
Control	56.982	36.982	96.48	1.65
Pb 0.1	63.387	43.387	96.44	2.01
Pb 0.5	56.785	36.785	96.50	1.72
Pb 1.0	61.525	41.525	96.50	1.99

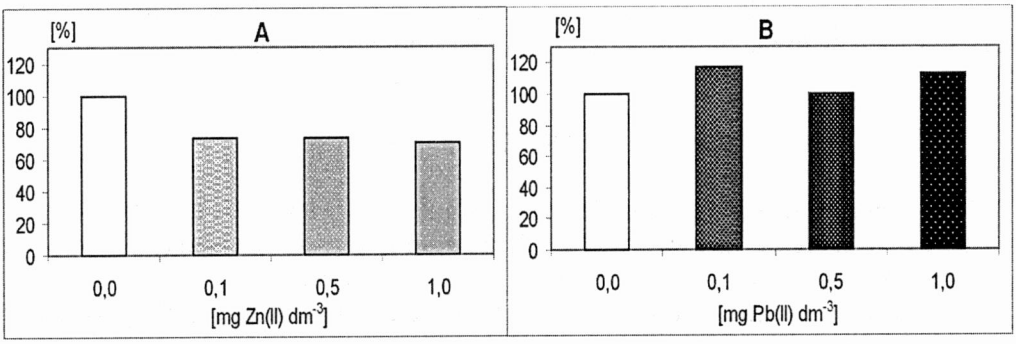

Fig. 1. Increase in the biomass of *A. caroliniana* grown in the medium enriched with Zn(II) (A) and Pb(II) (B)

A total nitrogen content in the plants grown in the medium containing 0.1 and 0.5 mg Zn(II) dm^{-3} was similar to that in the control (1.45 and 1.55%), whereas in the fern grown at 1.0 mg Zn (II) dm^{-3} its content in dry mass was almost twice as high.

A total nitrogen content in the biomass of plants collected from the nutrient solutions enriched with 0.1, 0.5 and 1.0 mg of Pb(II) dm^{-3} increased by about 2.0, 1.7, 1.99%, respectively, while in the control plants it was higher by 1.65% (figure 2).

The results obtained showed a negative influence of zinc in the concentrations tested on the *A. caroliniana* development. The inhibition of plant growth and an increase in total nitrogen content in the fern biomass at 1.0 mg Zn(II) dm^{-3} in the medium testify to this statement. The plant response was probably caused by defence mechanism activation and synthesis of aminoacids or proteins, which are responsible for metals binding and detoxification [4].

During the experiment the ability of *A. caroliniana* to accumulate zinc and lead was confirmed, but no morphological differences in plants were observed.

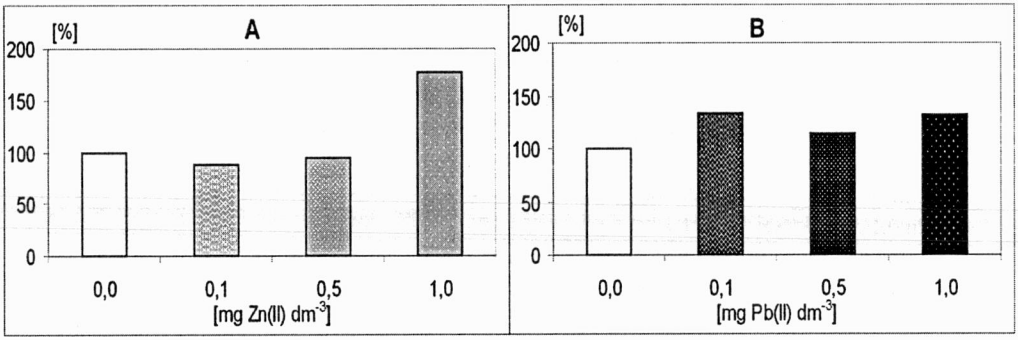

Fig. 2. The changes in nitrogen concentration in plants grown in the medium enriched with Zn(II) (A) and Pb(II) (B)

Contrary to zinc, lead stimulated the growth of *A. caroliniana*, which was connected with a slight increase in a total nitrogen content in the biomass.

4. CONCLUSIONS

Based on a 12-day experiment the following conclusions were formulated:

1. A high increase in Zn(II) and Pb(II) concentrations in the plant biomass suggests that *A. caroliniana* is able to accumulate heavy metals.

2. *A. caroliniana* can be used in the phytoremediation method, e.g. for purification of municipal wastewaters.

3. The ferns cultured in polluted waters should not be applied as green manure, because they can contain accumulated zinc and lead.

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WPŁYW METALI CIĘŻKICH NA PRZYROST BIOMASY *AZOLLA* PODCZAS DOCZYSZCZANIA ŚCIEKÓW MIEJSKICH

Azolla caroliniana Willd. jest paprocią wodną żyjącą w symbiozie z wiążącą azot atmosferyczny sinicą *Anabaena azollae* Stasb. Podczas 12-dniowej hodowli paproci w warunkach laboratoryjnych na podłożu zawierającym Zn(II) stwierdzono, że zawartość azotu całkowitego w materiale roślinnym rośnie dla stężenia 1,0 mg Zn(II) dm^{-3} (dla stężeń 0,1; 0,5 mg Zn(II) dm^{-3} zawartość azotu w roślinach niewiele różniła się od zawartości w roślinach kontrolnych) oraz że przyrost biomasy jest ograniczony. Dodanie do pożywki 0,1; 0,5; 1,0 mg Pb(II) dm^{-3} spowodowało wzrost zawartości azotu oraz większy przyrost biomasy niż na podłożu kontrolnym. Stwierdzono znaczny wzrost stężenia obu metali w materiale roślinnym.