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ASSESSMENT OF THE PHYTOREMEDIATION EFFICACY OF BORON-CONTAMINATED WATERS BY *Salvinia natans*

Local excesses of acceptable concentrations of boron in the aquatic environment may have negative impact on aquatic ecosystems, thus posing potential health hazards to humans and animals. The chemical methods commonly used for the treatment of surface waters may be successfully replaced by phytoremediation processes. Phytoremediation consists in accumulation of the contaminants in biomass, thus bringing the aquatic environment to conditions acceptable from the standpoint of legal regulations. *Salvinia natans* is an established bioaccumulator of metals from aquatic environments. The goal of this study was to verify the efficacy of the test species (*Salvinia natans*) in bioaccumulation of boron in Polish climate conditions. The experiment was conducted in waters contaminated with 1, 3, 6 and 8 mg B/dm^3 for five days. Boron mass balance and biomass accumulation were evaluated at the final stage of the experiment. Biochemical analysis of the organic matter revealed changes in plants occurring as the result of boron accumulation.

1. INTRODUCTION

Current monitoring of environmental conditions in Poland shows that boron is present in excessive amounts (1.34 to 4.63 mg B/dm^3) in the surface waters at Potok Goławiecki measuring point (at the outlet into the Mała Wisła river) [1]. High boron contamination of the multilayer aquiferous system was observed in the vicinity of the liquidated chemical plant "Tarnowskie Góry". The measured boron concentrations are 240 mg B/dm^3 in Quaternary layers and 116 mg B/dm^3 in Triassic layers (shell limestone) [2]. Boron concentration in copper industry wastewaters (KGHM Legnica) after conventional treatment methods was as high as 3.58 mg B/dm^3 [3]. Phytoremediation may be excellent supplementation of common chemical methods in final purification of aquatic environments [4]–[6]. The rate and efficacy of the process depend on the natural

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bioaccumulation capabilities of plants and on various environmental factors such as chemical speciation of a particular toxin, pH, environmental presence of organic chelating agents, other organic substances, metals and non-metals, salinity, ionic strength, temperature, light intensity and oxygen levels [7]. *Lemna* sp. and *Salvinia natans* are Poland's native pleustophytes with enormous bioaccumulation capabilities. *Lemna* spp. are known for their capability to remove nutrients and organic substances [8], [9], suspensions [10], [11] and trace elements [7], [12]–[14]. *Salvinia* spp., compared to other pleustophytic species, are invaluable in bioaccumulation of heavy metals from contaminated waters [7], [15]–[20]. *Lemna minor* [21], [22] and *Lemna gibba* [23], [24] confirmed their efficacy in the treatment of boron-contaminated water. WECHTEROWICZ et al. [25] showed that phytoremediation by *Lemna minor* was efficient in relation to metals (Cd, Pb, Zn, Cu and Ni) present in excessive amounts in the industrialized regions of Lower Silesia. Unfortunately, there is no information regarding the efficacy of phytoremediation by *Salvinia natans* as pertains the removal of boron from the contaminated waters. This research attempted to quantify boron balance before and after phytoremediation in order to determine the bioaccumulation capability of *Salvinia natans*. Physiological condition of the tested species following exposure to boron was also subject to preliminary assessment. Quantitative changes in chlorophyll *a* and *b*, as well as in the total protein content, were observed in plant tissues, affecting the biomass growth rates and bioaccumulation of boron in the organic matter.

2. EXPERIMENTAL

The preliminary research focused on the determination of optimum autotrophic organism culturing conditions consistent with those observed in Polish climate zone during the vegetative period. Organisms of the specified species (*Salvinia natans*) selected in the process of preliminary morphological evaluation were used for the investigation. Following a one-month adaptation period, specimens characterized by good ontogenetic condition were transferred into separate parent growth cells. Plants were cultured in Hoagland medium [26]. Comparable amounts of the biological material (inoculates) per wet mass were introduced into reactors containing water contaminated with boron at concentrations of 1, 3, 6 and 8 mg B/dm^3 and the control sample (uncontaminated medium). The experiment was conducted in five-day cycles in three repetitions for each of the concentrations. The reactors were continuously fed (average flow rate of 1 cm^3/min) with the medium contaminated with boron at the appropriate concentration. Plants were successively removed from the reactor after each day of the experiment and submitted to physicochemical analysis and biochemical assessment.

Boron content was monitored by boron mass balance including changes in concentrations of aqueous solutions and bioabsorption capabilities of the phytoremediating plants. Samples of contaminated waters were periodically collected and analyzed for boron content during the process. Boron concentrations at the reactor inlet and outlet were determined by means of molecular spectroscopy using a Hach Lange DR2800 spectrophotometer (absorbance wavelength of 605 nm).

The efficacy of boron bioaccumulation by *Salvinia natans* in the phytoremediation process was determined from the total plant boron content. In order to determine the boron content, plant samples were collected before and after phytoremediation and subsequently processed to dry mass at about 103 °C, according to the standard procedure [24]. Dry plant matter was reconstituted in 65% nitric acid(V) solution using Milestone START D microwave mineralizer. The determination of boron content in plant mineralizates was performed by means of graphite furnace atomic absorption spectroscopy (AAS-GF) using a GBC apparatus.

Physiological condition of plants, i.e. changes occurring in plants after exposure to boron, was also assessed. Photosynthetic pigments (chlorophyll *a* and *b*) and total protein levels were quantified during the process of phytoremediation of boron-contaminated waters.

In order to determine the chlorophyll *a* and *b* content [28], fresh biomass was homogenized in 90% acetone solution using the IKA Ultra-Turrax dispenser (6000 rpm/min for 5 minutes). The pigment extraction process lasted about 22 h at 2–8 °C.

The determination of total protein content was carried out by the Lowry method in fresh plant matter hydrolyzates on sample collection days [29]. Hydrolyzate preparation procedure was analogous to that employed for photosynthesis pigments, except that the hydrolyzate for protein determination was prepared using 1 M NaOH solution. Determination was performed in the filtrates after protein denaturation at 100 °C for 10 minutes.

The filtrates of the extracts and the hydrolyzates were analyzed by spectrophotometry on a T80+ UV/VIS spectrophotometer manufactured by PG Instruments, Ltd. Absorbance was measured at the wavelength of 663 for chlorophyll *a*, 645 nm for chlorophyll *b*, and 750 nm for total protein. The levels of pigments and total protein were determined from reference curves.

The assessment of the toxic effect of boron on the morphology of the tested species was conducted at 24-hour intervals on the basis of visual evaluation of changes in leaf pigmentation.

Biomass toxicity was investigated by the reference method in Californian earthworm (*Eisenia fetida*) [30].

Chemical analyses were performed in the certified Toxicology and Environmental Research Lab of the Institute of Environment Protection Engineering, Wrocław University of Technology.

3. RESULTS OF INVESTIGATIONS

The dry biomass matter growth rates in water contaminated with boron at concentration of 1 mg B/dm^3 were comparable to that in the control sample (uncontaminated

Hoagland medium culture). The difference was only 1% in favour of the culture exposed to 1 mg B/dm^3 in water (figure 1). Loss in dry matter content was observed at higher boron concentrations, reaching the maximum value of 3.58% on the fourth day of exposure to 8 mg B/dm^3.

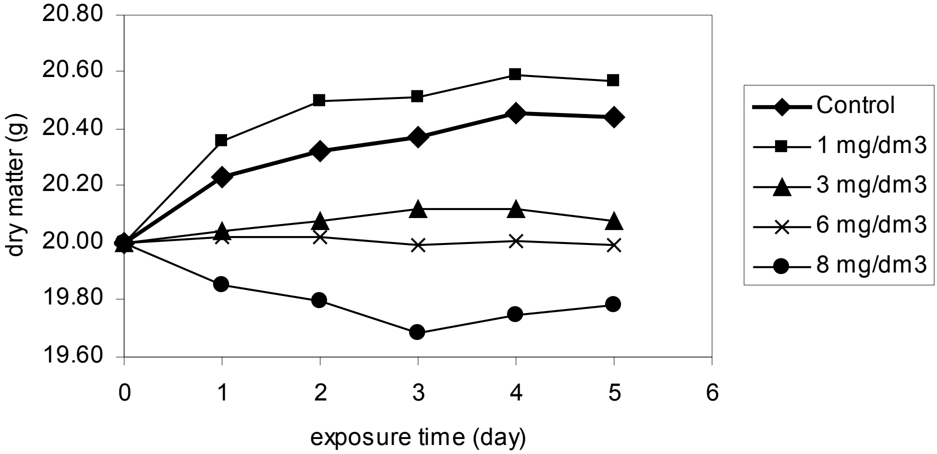

Fig. 1. Changes in the dry matter content during the phytoremediation process

The analysis of dry matter content for individual concentrations showed the maximum increase of 0.59 g (2.94%) compared to baseline (day 0) in the culture exposed to boron at concentration of 1 mg B/dm^3 after the fourth day of exposure (table). The maximum dry matter loss of 0.32 g (1.58%) was observed on the third day of exposure to boron at 8 mg B/dm^3.

Table

Dynamics of dry matter changes (increase/loss) compared to baseline (day 0) in %

Exposure time	Control	1 mg B/dm^3	3 mg B/dm^3	6 mg B/dm^3	8 mg B/dm^3
1st day	1.16	1.79	0.21	0.11	–0.75
2nd day	1.63	2.51	0.40	0.09	–1.01
3rd day	1.86	2.57	0.58	–0.04	–1.58
4th day	2.27	2.94	0.58	0.03	–1.27
5th day	2.21	2.85	0.37	–0.04	–1.08

Boron was accumulated by *Salvinia natans* in all boron-contaminated waters, albeit with different intensities. The process was most effective on the first days of the experiment in all cultures. No further quantitative changes were observed in plants on the successive days and the boron content in biomass was maintained at a comparable level.

An over two-fold increase in *Salvinia natans* boron bioaccumulation compared to the control (0.06 mg B/g dry matter) was observed in waters at concentrations of 1 and 3 mg B/dm^3 (figure 2).

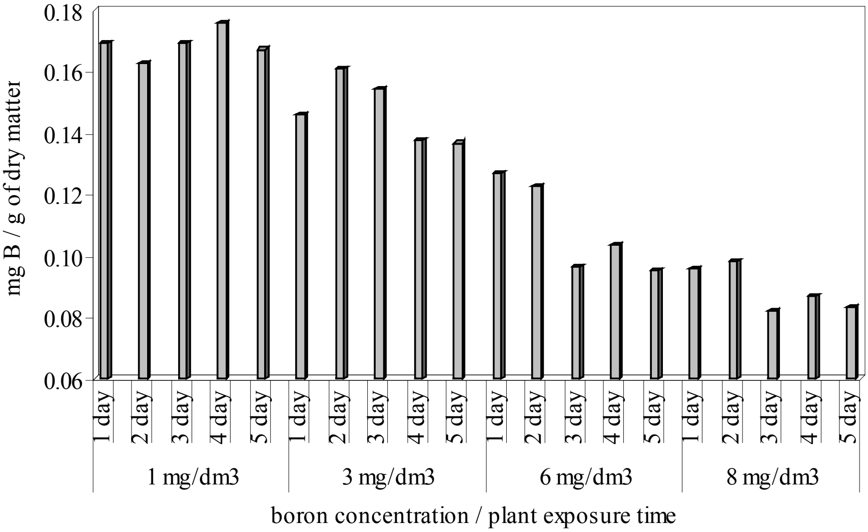

Fig. 2. Accumulation of boron in plants during phytoremediation compared to control (0.06 mg B/g dry matter)

Maximum biosorption of 0.18 mg B/g dry matter was achieved on the fourth day of plant exposure to the concentration of 1 mg B/dm^3 water, corresponding to a three-fold increase in the biomass boron content. The initial doses of 6 and 8 mg B/dm^3 water lead to lower accumulation of boron in plants.

Visual evaluation of plants during the phytoremediation process showed no significant changes, except for isolated cases of chlorosis in cultures exposed to higher boron concentrations (6 and 8 mg B/dm^3). The acute toxicity test in *Eisenia fetida* at average biomass concentration of 0.15 mg B/g dry matter confirmed that the tested species were insensitive to the levels of boron accumulated in *Salvinia natans*.

The assessment of the physiological condition of plants based on the changes occurring during phytoremediation of boron-contaminated waters was performed by means of quantitative determination of photosynthesis pigments and total protein levels. The analysis of chlorophyll *a* and *b* levels in fresh plant matter following exposure to boron at concentration of 1 mg B/dm^3 showed that *Salvinia natans* was insensitive to the presence of boron in water, particularly in the case of short exposure times.

In the case of the medium contaminated with boron at concentration of 3 mg B/dm^3, a drop in chlorophyll *a* levels was observed in the plants starting from the third day of the experiment (figure 3). For boron concentrations of 6 and 8 mg B/dm^3, the loss of chlorophyll *a*, and the proportional loss of chlorophyll *b*, in fresh plant matter increased with every day of exposure, reaching an over 50% loss of both chlorophyll types after five days of exposure (8 mg B/dm^3).

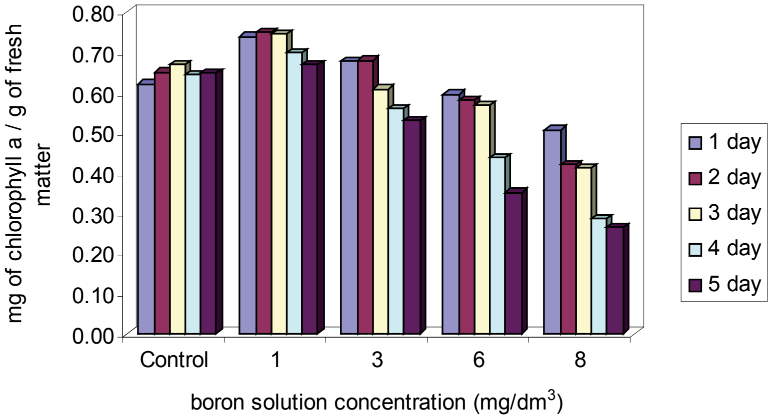

Fig. 3. Changes in plant chlorophyll *a* content

The changes in total protein content during the experiment confirm that boron concentration of 1 mg B/dm^3 had no negative effect on the plants (figure 4).

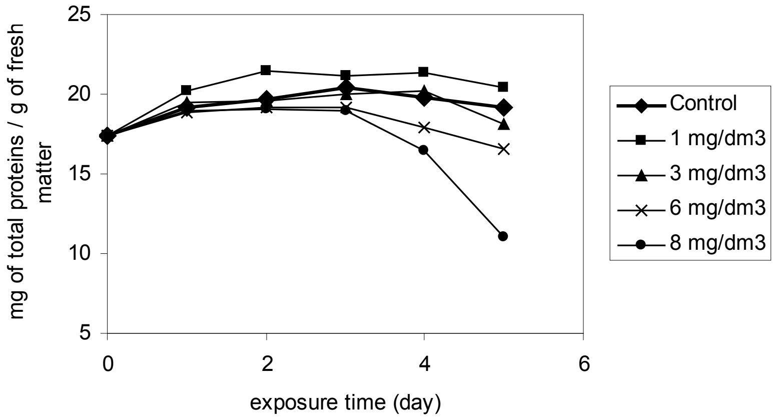

Fig. 4. Changes in total plant protein content

Plant sensitivity to boron concentration of 3 mg B/dm^3 was observed at the final stage of the experiment and in plant cultures exposed to higher boron doses (6 and 8 mg B/dm^3). About 40% loss of protein content was observed in the plant mass following a five-day exposure to the medium with boron concentration of 8 mg B/dm^3.

4. CONCLUSIONS

Boron concentration of 1 mg B/dm^3 of water had no negative effect on the tested species (*Salvinia natans*). The dry mass content increased by 1% compared to control. Loss of dry matter mass was observed on the final days of the experiment in cultures contaminated with 6 and 8 mg B/dm^3.

Salvinia natans confirmed its boron bioaccumulation capabilities. In cultures exposed to boron at concentrations of 1 and 3 mg B/dm^3, bioaccumulation was even three times higher than that in the control culture. For concentrations of 6 and 8 mg B/dm^3, the absorptive capabilities were limited to 50% on the day 0.

Biochemical tests conducted in waters contaminated with boron at concentrations of 6 and 8 mg B/dm^3 showed a negative effect of this element on the physiological condition of plants (50% reduction in photosynthesis pigment levels and 40% reduction in total protein levels compared to the control sample), as confirmed by changes in pigmentation of the tested specimens (early stage of chlorosis) in the final days of the experiment.

The acute toxicity test in the reference species *Eisenia fetida* showed no negative effects of boron-containing biomass on living organisms.

Elongation of boron exposure time would lead to better understanding of the bioaccumulation processes in *Salvinia natans* and the resulting changes in the biological material.

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