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BIOACCUMULATION CAPACITIES OF COPPER(II) IONS IN *SALVINIA NATANS*

Capacities of the pleustophytes in the purification of the aquatic environments in Polish climatic zone have been evaluated. Studies of bioaccumulation of copper(II) from culture media contaminated with 5, 10, 15 and 20 mg Cu/dm^3 by the plants of *Salvinia natans* species were performed. The process was carried out for 14 days with the use of artificial plant-breeding species. The balance of the content of copper(II) in dry matter of plants and in the culture media was investigated. Capabilities of *Salvinia natans* to bioaccumulate copper(II) have been confirmed in all contaminated culture media.

1. INTRODUCTION

The ions of copper(II) at acceptable concentrations in the environment are essential to the development and growth of the plant cell. Particularly interesting is the problem of the excessive copper content in the environment. It causes water contamination, which further forms a stress factor for the organism, resulting in the formation of physiological disorders. Current investigations are focused on the accumulative capacity of extremely large amounts of toxic metals in the plant tissues and ability of plants to adapt to life in the contaminated ecosystems.

Due to a high degree of development and industrialization of the region of Lower Silesia (metalliferous dust emissions from mining and metallurgical industries and combustion processes), the natural concentrations of toxic metals in the aquatic environment exceed the limit values. It is estimated that the natural content of copper in the river water, now rarely encountered, is 1–2 μg/dm^3. The average concentration of copper in the unpolluted river waters is 102 μg/dm^3, while the contaminated water can reach values ranging from 30 to 602 μg/dm^3. The highest concentrations of copper were observed in the areas of the KGHM Company – above 500 mg/dm^3 [1]. In the

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Odra river, the main river of the Lower Silesia region, exceeding the normative copper(II) ions concentration was also noted [2]. Overall, the copper ions discharged into the Baltic Sea originate mainly from human activities (82%). The sources of natural origin of the copper are the natural runoff – 310 tons/year and the natural precipitation – 130 tons/year [3].

There has been an increase in importance of the biological methods which use the plant activity based on absorption and degradation of xenobiotic pollutants. The phytoremediation technology is based primarily on the natural ability to collect, store and biodegrade substances regarded as contaminants within plant tissues. The effectiveness of phytoremediation is strongly correlated with the selection of appropriate plant species. The availability of metal for a plant is influenced by many environmental factors such as pH, organic matter content, deficiency or excess of other elements, the activity of the microbial flora, redox potential and aeration of the environment.

Pleustofity of the genus *Salvinia* sp. are known hyperaccumulators with high capacity for bioaccumulation of toxic metals [4–9]. They are characterized by high tolerance to vegetative conditions that prevail in Poland. *Salvinia natans* (the species of *Spirodelo-Salvinietum natantis*) is present in about 200 locations, primarily focused in the Odra and Vistula valleys [10].

Research with the use of the *Salvinia natans* species was conducted in the activated sludge containing 0.001, 0.01, 0.1 and 1 mg Cu/dm^3 [11]. In the control sample (plant breeding in the pure Hoagland culture medium), the initial number of leaves had doubled by the 8th day. In the case of the culture media contaminated with copper(II) ions it has been observed that the process of the growth of *Salvinia natans* was inhibited. Changes were evaluated based on an indicator expressing the ratio between the number of leaves in the control sample (pure Hoagland culture medium) and the number of leaves in the copper contaminated culture medium on the 8th day of the experiment. The indicator confirming the loss of biomass was 7.4 for the 0.01 mg Cu/dm^3 after eight days of the experiment.

The research with the use of *Salvinia minima* confirms effectiveness of the plant in the sorption of copper ions from culture media with copper concentrations of 1.0, 2.0, 2.5 and 3.0 mg/dm^3 corresponding to the admissible limit of copper concentrations in drinking water [12]. Both after 7 days of *Salvinia minima*'s exposure to Cu(II) ions and after 14 days of the experiment, gradual loss of the plant fresh mass was observed. The maximum loss of the biomass occurred in the culture media with 2.5 and 3 mg Cu/dm^3. It has also been observed that the higher the initial concentration of copper in the culture medium, the higher the content of the element in the biomass during the first days of the process was. *Salvinia minima* can exist in the aquatic environments in which the copper concentrations are 100-fold higher than the levels occurring in natural environment.

The biosorption of the copper ions by plants of the *Salvinia* sp. genus from culture media with higher concentrations (from 5 to 25 mg Cu/dm^3) is possible [5], which

allows their use in purification of aquatic environment as well. The highest effectiveness of the phytoremediation process carried out by *Salvinia* sp. in the culture media contaminated with 5, 10, 15, 20 and 25 mg Cu/dm^3 was observed between the 6th and 9th day of the experiment. In the culture medium with 5 mg Cu/dm^3, the effectiveness of the biosorption of the metal reached 96% on the 6th day of the research. In the culture media with 10 and 15 mg Cu/dm^3, the effectiveness of the process was highest on the 8th day of the experiment and reached 79 and 71% respectively, whereas for the culture media with 20 and 25 mg Cu/dm^3 the biosorption of mass reached 65 and 55% on the 9th day of the experiment. Extending the time of the plants exposure to the copper ions resulted in yellowing up to partial discoloration of their leaves. Morphological changes occurred in the second week of the experiment (8th day) in the culture media with 20 and 25 mg Cu/dm^3.

The ability of *Salvinia natans* to bioaccumulate copper(II) ions was investigated also for concentrations of 1, 10, 20, 50 and 100 mg/dm^3 in the culture media [13]. The results were collected during the first, third and fifth day of the experiment. The content of copper in the plants after the process of phytoremediation of the culture media contaminated with 1 and 10 mg Cu/dm^3 is similar in both culture media in the following days of the experiment and amounts to 0.066 and 0.65 mg Cu/dm^3 of fresh matter (3rd day), respectively. It corresponds to the decrease of Cu in the culture media caused by plants by 98.0 and 97.6%, respectively. With the initial concentration of 20 mg Cu/dm^3, the content of the element in the plant equaled 1.24 (first day) to 1.33 mg Cu/g of fresh matter on the third day of the experiment. It corresponds to the decrease of Cu in the culture medium caused by plants by 92.8 and 99.4%, respectively. Plants exposed to concentrations of 50 mg Cu/dm^3 did not survive through the 5th day of the experiment. The accumulation of the element in the biomass equaled 2.75 and 3.10 mg Cu/g of fresh matter. It corresponds to the decrease of Cu in the culture medium caused by plants by 82.5 and 92.8%, respectively. In the culture medium contaminated with 100 mg Cu/dm^3, the plants survived only the first day, accumulating 4.48 mg Cu/g of fresh matter which corresponds to 67.4% removal of Cu by plants. It confirms the data encountered in the literature that the higher accumulation of copper in plants during the first days of the experiment is observed in the culture media containing higher initial concentrations of metal.

The result of the work is an evaluation of the ability of the native species (*Salvinia natans*) to remove copper(II) ions from aqueous solutions in our climatic zone. The balance of the copper contents confirmed the loss of the element from contaminated culture media and the accumulation of copper(II) ions in the plant material. The development of environmentally friendly technologies for the purification of polluted water from industrial areas is an opportunity to reach a state acceptable by the regulatory framework for surface waters.

2. MATERIALS AND METHODS

The preliminary research was aimed to optimize the culture conditions for autotrophic organisms characteristic of the Polish climate zone in the vegetative season (the temperature of the air 22±1 °C during the day/15±1 °C at night, the air humidity 40±2% in the day/night cycle 12h/12h). During the studies, the artificial plant breeding species of *Salvinia natans* was used, selected based on preliminary morphological evaluation. After one month, the organisms with good individual condition were sieved to separate reactors. The plants were cultured in the Hoagland culture medium with following composition: KNO_3 – 1.02 g/dm^3, $Ca(NO_3)_2 \times 4H_2O$ – 0.71 g/dm^3, $NH_4H_2PO_4$ – 0.23 g/dm^3, $MgSO_4 \times 7H_2O$ – 0.49 g/dm^3, $MnCl_2 \times 4H_2O$ – 1.81 mg/dm^3, H_3BO_3 – 2.86 mg/dm^3, $CuSO_4 \times 5H_2O$ – 0.08 mg/dm^3, $ZnSO_4 \times 7H_2O$ – 0.22 mg/dm^3, MoO_3 – 0.09 mg/dm^3, $FeSO_4 \times 7H_2O$ (0.5%) – 0.60 mg/dm^3 [14].

A similar amount of biological material (inoculates) calculated per fresh matter (ca. 30 g) was introduced into the reactors containing 2.5 dm^3 of culture medium contaminated with copper(II) salt at concentrations of 5, 10, 15 and 20 mg Cu/dm^3 and into the control sample (the uncontaminated Hoagland medium). The contaminated Hoagland culture medium was made using redistilled water and copper salt $CuSO_4 \times 5H_2O$. pH of pure Hoagland culture medium and copper(II) contaminated culture media were of 4.5–4.8.

The experiments were carried out in 14 day cycles for each concentration of copper. They were performed at least three to six times. The plant biomass was introduced into a separate reactor (stationary culture) each day of the experiment, and the plants were entirely recovered from reactor and used for relevant physicochemical and biochemical analyses. Each designation was performed in duplicate.

The control of the metal content before and after the process of phytoremediation was inspected by monitoring the changes of copper(II) concentration in the culture media and in the plants. Water samples were collected on consecutive days from respective reactors and analyzed for the copper content by atomic absorption spectroscopy (AAS) with flame and flameless atomization on the iCE 3500 Solar apparatus manufactured by Thermo Scientific. The dry matter of plants was digested in the process of microwave mineralization in 65% HNO_3 (supra pure) using Milestone Start D device. Before digestion, the plants were treated until reaching a constant dry weight, with drying temperature of about 105 °C, following the procedure described in the document [15].

The chemical analysis was performed in a certified Laboratory of Toxicology and Environmental Research in the Faculty of Environmental Engineering at the Wrocław University of Technology.

3. RESULTS AND DISCUSSION

Increase of dry matter of *Salvinia natans* (the artificial plant-breeding) in culture media contaminated with 5, 10, 15 and 20 mg Cu/dm^3 is minor to the control – the uncontaminated Hoagland culture medium (Fig. 1). In the contaminated culture media, the dry matter change is closely related to the amount of copper ions that influence the plants' growth. In the culture media with 5 and 10 mg Cu/dm^3 the increase of dry matter was observed, in contrast to plant breeding with higher concentrations of copper (15 and 20 mg Cu/dm^3).

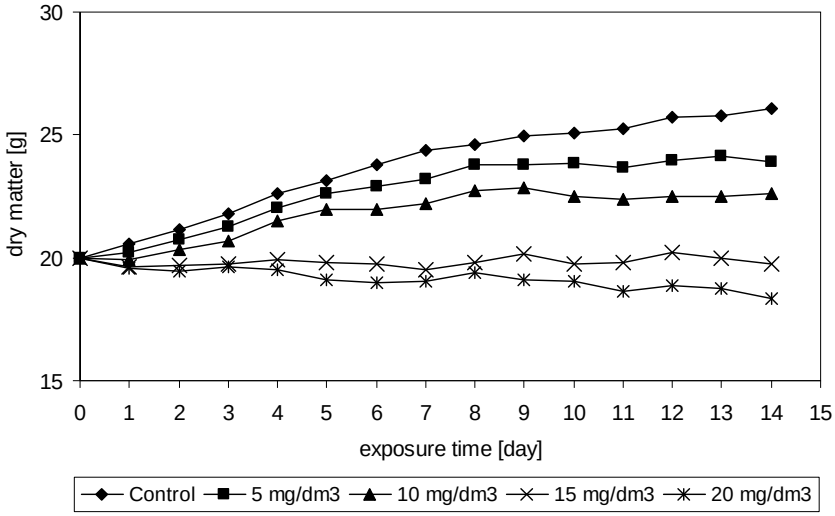

Fig. 1. Time dependence of the dry matter content during the phytoremediation process

The maximum decrease of dry matter compared to the control sample approximately at around 42% was observed on the 14th day of the experiment in the culture medium with 20 mg Cu/dm^3. The minimum decrease of dry matter – ca. 9% – was observed for culture medium with 5 mg Cu/dm^3 on the 14th day compared to the control. The research shows that the growth of plants depends on the time they are exposed to the metal and the initial dose of the element in the culture medium. At lower concentrations (5 and 10 mg Cu/dm^3), only deceleration of the plants' growth was observed compared to the control sample. It may confirm the influence of copper at given concentrations on the plant's organism. It also shows that *Salvinia natans* can survive in aquatic environments contaminated with copper in the investigated period. At higher concentrations of metal (15 and 20 mg Cu/dm^3), the lack of growth of the biomass was observed, which confirms the damaging influence of the high concentrations of copper on the plants. This fact was also confirmed by the fragmentation of the

plant bodies at the end of the second week and the occurrence of the symptoms of chlorosis.

Depending on the copper content in the culture medium, variable influence of the culture on the plant growth was observed (Figs. 2 and 3). The studies of changes of dry matter of *Salvinia natans* for each culture medium contaminated with copper ions (Fig. 2) showed the maximum increase of dry matter up to 300 mg/g of dry matter in control (the uncontaminated Hoagland culture medium). It corresponds to the 30% increase of dry matter on the 14th day of culture in relation to 0 day (Fig. 3).

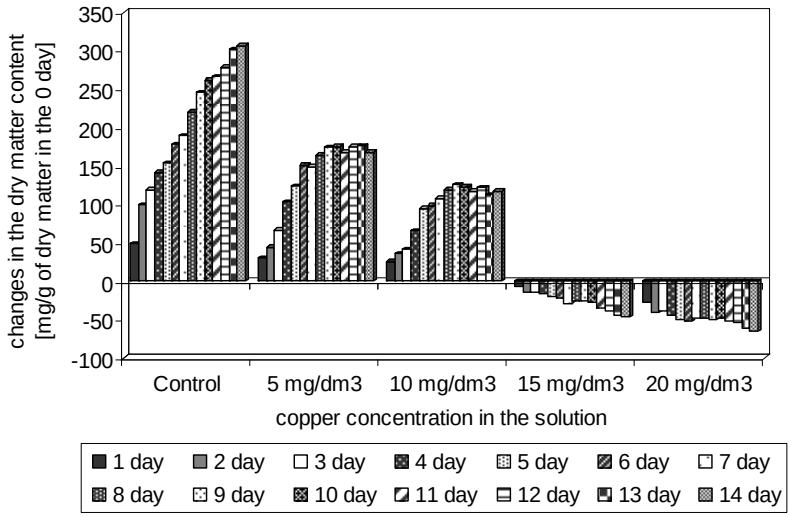

Fig. 2. Time dependences of the dry matter content in the phytoremediation process

This process was less effective in the culture media with 5 and 10 mg Cu/dm^3. The respective increase of the biomass by approximately 20% (176 mg/g of dry matter) and 12% (126 mg/g of dry matter) in relation to the 0 day was observed (Figs. 2, 3). In the culture medium contaminated with 15 mg Cu/dm^3 the dry matter change was not significant (loss of 2%). The maximum decrease of dry matter was 65 mg/g of dry matter observed on the last day of culture contaminated with 20 mg Cu/dm^3 (Fig. 2), corresponding to 9% of dry matter decrease as compared to 0 day (Fig. 3).

The weekly assessment of cultures of *Salvinia natans* after the 7th and 14th day shows a sharp increase in dry matter in the culture medium with lower concentrations of copper (5 and 10 mg Cu/dm^3) and in the control sample in the first week of culture with respect to the second week. After 7 days of the process, the dry matter level was stabilized. In the culture medium with 15 mg Cu/dm^3, the change of dry matter was constant (2%), whereas in the culture medium contaminated with 20 mg Cu/dm^3, a continuous decrease of dry matter was observed.

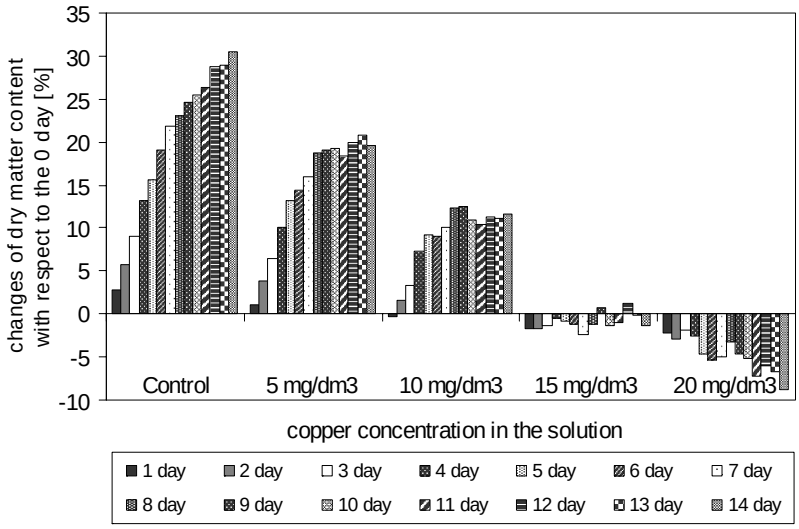

Fig. 3. Dry matter content with respect to the baseline (the 0 day) in the phytoremediation process [%]

Assessing the effectiveness of the phytoremediation by *Salvinia natans*, it is important to determine the ability of plants to retain copper in the body as a result of bioaccumulation or sorption of this element. In this study the changes in copper content in plants and its amount in contaminated Hoagland culture medium were monitored.

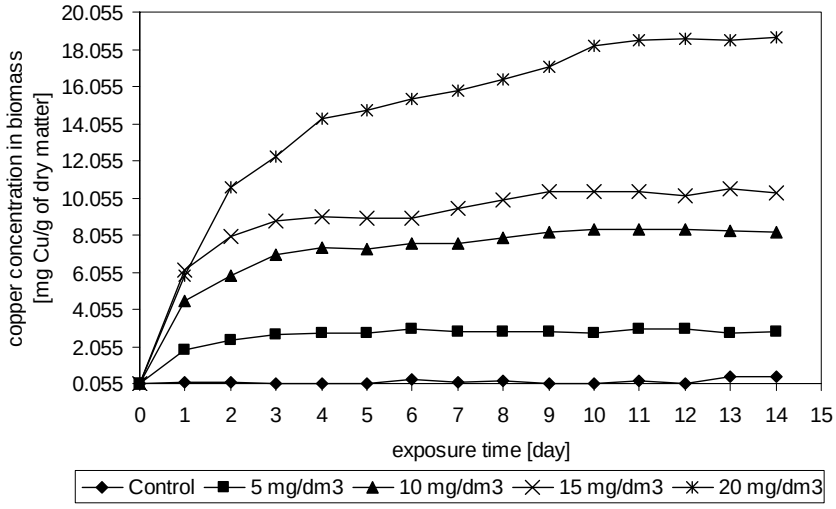

Fig. 4. Bioaccumulation of copper in plants during phytoremediation

The bioaccumulation of copper by *Salvinia natans* occurred in all culture media but with varying intensity (Fig. 4). Before the experiment, the copper concentration in plants was 0.055 mg Cu/g of dry matter.

The bioaccumulation process was most effective in the first 2–3 days of the experiment for concentrations of 5, 10 and 15 mg Cu/dm^3, whereupon the metal level in the dry matter of the plant was stabilizing (Fig. 4). In the culture media contaminated with 5, 10 i 15 mg Cu/dm^3, the content of copper in *Salvinia natans* amounted to 3, 8, 10 mg Cu/g of dry matter, respectively. In the culture medium with the highest concentration of metal (20 mg Cu/dm^3), the bioaccumulation process was still continued until 10–12 day of the experiment, despite the observed decrease in biomass (Fig. 2). On the last day of the plant culture, the copper concentration was about 19 mg Cu/g of dry matter in *Salvinia natans*. It corresponds to the 40-fold increase in the copper content (14th day) compared to the control sample.

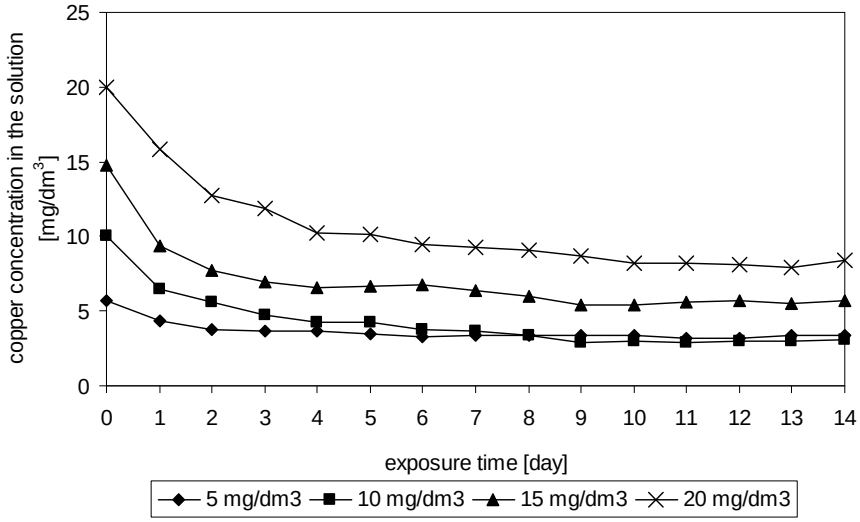

Fig. 5. Time dependences of the copper concentration in solutions in the phytoremediation process

In view of the accumulation of copper(II) ions in the biomass, the loss of this element in the contaminated Hoagland culture medium was tested (Fig. 5). The highest decrease in the copper content in the medium was observed on the 2–3 day of the experiment, being confirmed by the balance of metal in the biomass of *Salvinia natans* (Figs. 2, 3). In the subsequent days of the experiment, the level of copper in the medium is maintained at a similar level. After the 14th day of the experiment, the content of the metal in the culture media with 5, 10, 15 and 20 mg Cu/dm^3 (in the 0 day) is 3.1, 3.3, 5.5 and 8.4 mg Cu/dm^3 (Fig. 5), respectively. It corresponds to the decrease of the element in the culture media by 40, 70, 62 and 58%, respectively.

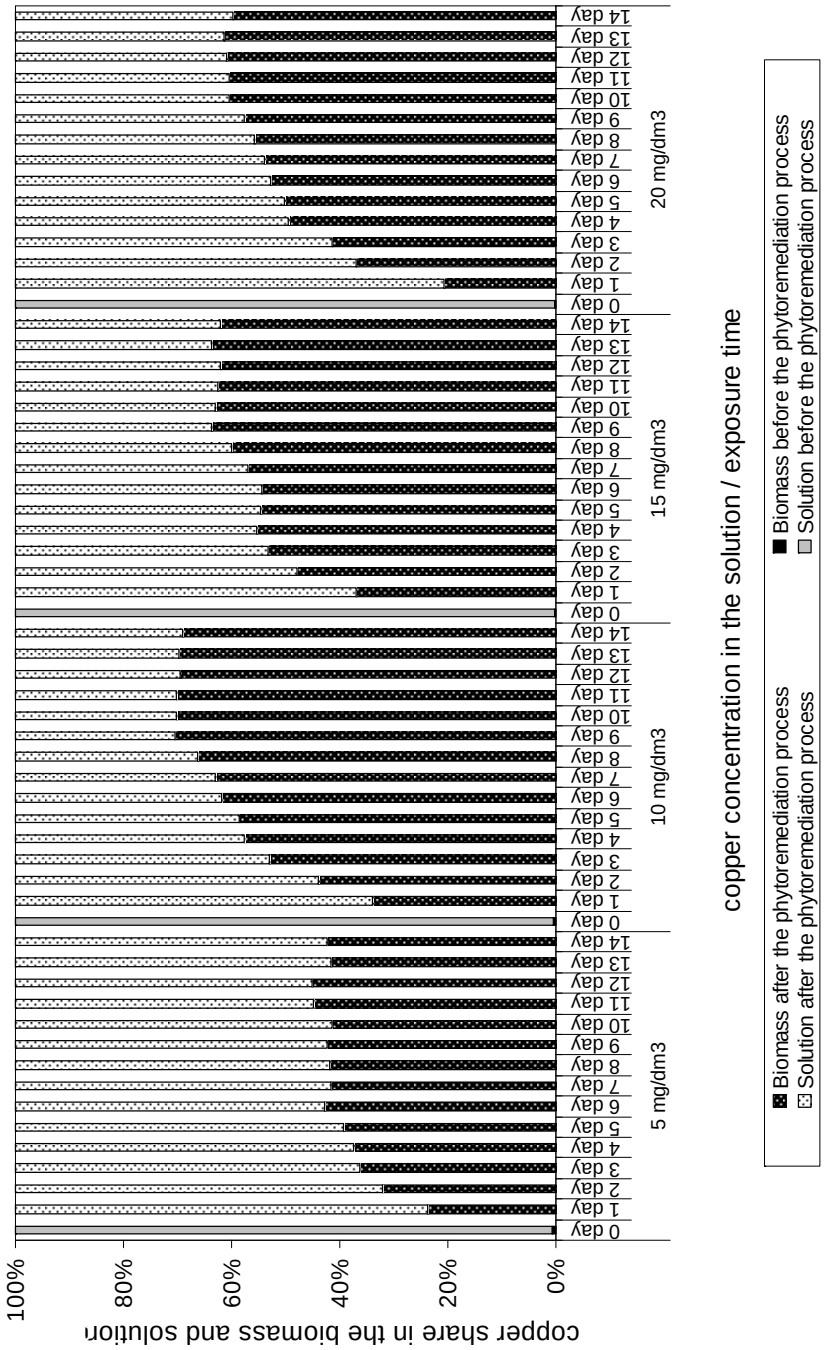

Fig. 6. The balance of copper in the process of phytoremediation

The maximum 72% loss of copper was observed in the culture medium with the initial concentration of 10 mg Cu/dm^3. In the culture medium containing 15 mg Cu/dm^3, the maximum loss amounted to 64%, and in the culture containing 20 mg Cu/dm^3 the loss of metal reached 60%. Similar figures were noted in the literature for the culture media with 10, 15 i 20 mg Cu/dm^3 [5, 13]. The decrease of copper in the culture medium after the biosorption by *Salvinia* sp. amounted to 79, 71 and 65%, respectively [5]. Sen and Mondal [13] report that they have achieved nearly 100% loss of the element from culture media with 10 and 20 mg Cu/dm^3. The differences in the effectiveness in removing metal from contaminated culture medium may stem from dissimilar specimen attributes of the *Salvinia* species which was used. Our experiment was carried out on a small scale due to the *Salvinia natans* being protected under the environmental law in Poland (an organism from artificial plant breeding was used). The results showing the effectiveness of the bioaccumulation process were compared with the results from literature achieved by using organisms living in favorable vegetative conditions in a warm climatic zone and perhaps acquired directly from natural habitat.

The flow of copper ions between the contaminated Hoagland culture medium and the biomass of *Salvinia natans* in the following days of the phytoremediation process is shown in Fig. 6. The initial copper content in plants (0.055 mg Cu/g of dry matter) prior to the experiment was approximately 0.5% of the metal in dry matter. Thus, before the phytoremediation process (the 0 day) nearly total amount of copper ions was stored in the culture medium. The copper share in biomass increased daily during the phytoremediation process, which corresponded to the decrease of the metal in the culture medium. In the Hoagland culture medium contaminated with 5 mg Cu/dm^3 the accumulation process in the *Salvinia natans* tissues and/or sorption process has affected 45% of the copper. In the remaining copper concentrations in the culture media (10, 15, 20 mg Cu/dm^3) the share of the metal in plants amounted to, respectively, 70, 64 and 60%.

The results confirmed the cumulative properties of *Salvinia natans* and its ability to remove copper ions from aquatic environment.

4. CONCLUSIONS

- The increase of dry matter (*Salvinia natans*) in all of copper contaminated culture media is lower than in the control sample (pure Hoagland culture medium).
- The minimum decrease of dry matter was 9% on the 14th day with 5 mg Cu/dm^3 in the culture medium compared to the control sample. The maximum decrease of dry matter (42%) was observed on the last day in the medium contaminated with 20 mg Cu/dm^3.
- In the case of *Salvinia natans* cultures contaminated with 5 and 10 mg Cu/dm^3, 20 and 12% increase in dry matter in comparison with the 0 day was observed, respectively, while in cultures with higher concentrations of Cu(II) (15 and 20 mg Cu/dm^3), 2 and 9% loss of dry matter was observed, respectively.

• Capability of *Salvinia natans* to bioaccumulate copper(II) ions in all contaminated culture media has been confirmed. The maximum concentration of copper after 14 days of the phytoremediation process in the culture medium contaminated with 20 mg Cu/dm^3 was 40-fold higher with respect to the control sample (the 0 day) and amounted to 19 mg Cu/g of dry matter.

• The decrease of copper(II) ions from culture medium and their simultaneous accumulation in biomass confirm the bioaccumulative capacity of *Salvinia natans* in the phytoremediation process of aquatic environment contaminated with copper(II) ions. The maximum loss of Cu, reaching 72%, was observed in the culture medium contaminated with 10 mg Cu/dm^3.

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