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ACCUMULATION OF MERCURY IN THE BIOMASS OF SELECTED PLEUSTOPHYTES

Application of phytoremediation process for the purification of the environment, particularly of surface water (running or standing) may be a future alternative to existing standard purification methods, in particular for heavy metal removal. Numerous hyperaccumulators are known which can be used for the purification of water, soil or sediment such as *Salvinia* or *Lemna* lemnids, occurring in temperate climates, and thus on the territory of Poland. Ability of mercury accumulation by *Salvinia natans* and *Lemna minor* was analyzed. The phytoremediation ability of lemnids was compared depending on their origin – commercial (artificial) cultures and those obtained from the natural environment. Phytoremediation process was carried out for a period of 14 days for the concentration of 0.15 mg Hg/dm^3 and 0.2 mg Hg/dm^3. It was found that both *Salvinia natans* and *Lemna minor* show a significant increase in biomass in the presence of mercury, even up to 76% for *Lemna minor* and 40% for *Salvinia natans*. The result of this increase was incorporation a considerable amount of mercury in the tissues of plant. The quantity of mercury in plants biomass was in the range of 41.16–115.28 mg Hg/kg DM, while in the control samples – only 0.2–0.6 mg Hg/kg DM.

1. INTRODUCTION

Civilization progress results in a continuous increase in the amount of exhaust gas, waste, as well as excessive use of various types of chemicals applied in industry and agriculture. This leads to environmental pollution, not only locally, but more and more often on a global scale [1–4]. Contamination of ecosystems adversely affects metabolism of plants and animals, but also human health [5–7]. Particular attention should be paid to aquatic ecosystems. Water, due to its significant human consumption and continuous use in the household (washing dishes, cooking meals, livestock watering, etc.), despite the increasingly sophisticated methods of treatment, continues to be the main

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path for organic and inorganic pollutants entering the human body. A large part of impurities is also collected by plants forming the basis of the trophic chain. Through their intermediary, harmful substances, in particular heavy metals, are transferred to the human body; indirectly, through various links of the trophic chain, or directly. Their toxicity is primarily due to the ability to form bonds with the functional groups of enzymes and structural proteins. These properties result in an increase in bioaccumulation and inhibiting their excretion from the body [6, 7].

Among the most dangerous metallic elements, mercury, widely used in pumps, pressure sensors, light bulbs, batteries, etc. plays a special role. Combustion of growing amounts of fossil fuels and the use of mercury compounds as stabilizers in many processes (for example, in the polymerization of vinyl chloride) increases its concentration in ecosystems [8, 9].

Mercury has a high affinity for thiol groups in proteins, impairing functions of enzymes, haemoglobin and serum albumin. Organomercury compounds are soluble in fats, they also have a significant vapor pressure, which results in a high degree of absorption and ease of distribution in biological systems. Methylmercury similarly as elemental mercury breaks the blood brain barrier, causing neurotoxic effects. It also shows teratogenic effects [6].

Because of such high risk to human health, its admissible concentration in drinking water is only 0.0005 mg Hg/dm^3 for first class quality surface water and 0.001 mg Hg/dm^3 for groundwater [10]. Treatment of aquatic environmental from mercury is therefore a necessary ecological measure. Phytoremediation may turn out to be an alternative to traditional methods of treatment of the aquatic environment from heavy metals, using among other natural abilities of some plants to accumulate metals in their biomass [11–15]. To hyperaccumulators there belong, among others, lemnids such as *Lemna minor* (hyperaccumulator for Mn, Pb, Ba, B, Cd, Cu, Cr, Ni, Se, Zn, Fe) and *Salvinia natans* (hyperaccumulator for Pb, Cr) floating fern [16].

2. EXPERIMENTAL

Ability of two species of lemnids *Salvinia natans*, *Lemna minor* to accumulate mercury(II) compounds was examined. The plants were collected from their natural environment and from commercial cultures, enabling comparison of their accumulation capacity depending on the origin. In the case of *Salvinia natans,* plants from the natural environment were collected according to the decision obtained from the Regional Director for Environmental Protection in Wrocław from the Oława river, whereas *Lemna minor* – from a pond located in the Wrocław Zoo. The study was conducted on plants collected in the summer 2012. Two mercury concentrations were used in the studies: 0.15 mg Hg/dm^3 and 0.20 mg Hg/dm^3 in Hoagland medium, using mercury(II) nitrate. The control samples were plants grown on Hoagland medium without mercury.

The study was conducted over a period of 14 days in reactors containing a modified Hoagland medium (composition: KNO_3 – 1.02 g/dm^3, $Ca(NO_3)_2{\cdot}4H_2O$ – 0.71 g/dm^3, $NH_4H_2PO_4$ – 0.23 g/dm^3, $MgSO_4{\cdot}7H_2O$ – 0.49 g/dm^3, $MnCl_2{\cdot}4H_2O$ – 1.81 mg/dm^3, H_3BO_3 – 2.86 mg/dm^3, $CuSO_4{\cdot}5H_2O$ – 0.08 mg/dm^3, $ZnSO_4{\cdot}7H_2O$ – 0.22 mg/dm^3, $FeSO_4{\cdot}7H_2O$ – 0.60 mg/dm^3). Plant weigh gain and mercury analyses were conducted on the 7th and 14th day. In order to maintain constant physical conditions during the experiment, incubation was conducted in a Biosell's FD 147 Inox phytotron equipped with Biolux 18W/965 lamps produced by OSRAM company, in the day/night cycle (12 h/12 h); the air humidity was 40% and temperature – 22 °C/15 °C.

Plant weight gain significantly affects the volume of accumulation, therefore a study was conducted to determine its size in the presence of toxins. For this purpose, the mass weighing was performed before and after the experiment, using an analytical balance WAA 160/C/1 by Radwag company. Analyses of mercury concentration in the dry matter were performed using Altem's AMA 254 [17]. The weighed sample of plants was placed in a nickel boat of analyzer. The sample analyzer was transferred to a combustion furnace where it was dried (for 35 s) and decomposed in a stream of oxygen (at 850 °C). The combustion products were then passed through a catalytic furnace (700 °C, for 200 s) and mercury was trapped on a gold amalgamator. The amalgamator was then heated for a short time, thereby mercury was released and transported to two measuring cells, where its concentration was determined based on absorbance of the radiation emitted by the mercury lamp of the photometer system.

3. RESULTS

3.1. PLANT WEIGHT GAIN

Lemna minor obtained from the natural environment and *Lemna minor* and *Salvinia natans* obtained from commercial cultures showed some weight gain during the experiment for both the control samples and the two concentrations of mercury used in the study. *Salvinia natans* obtained from the environment showed a significant increase in the case of control samples, but the increase in the presence of mercury remained stable over time for the both concentrations.

For *Lemna minor* obtained from commercial cultures, a significant increase in biomass was observed: 48% after 7 days of the experiment, and even up to 121% after 14 days. Exposure to mercury caused stimulation of plant growth for both concentrations. At the concentration of 0.15 mg Hg/dm^3 it was 59%, and at 0.20 mg Hg/dm^3 – even 61% after 7 days of the experiment. On the 14th day, the increase was still significant, however, in comparison to the initial values it was 99% for the concentration of 0.15 mg Hg /dm^3 and 78% for 0.20 mg Hg/dm^3.

After exposure to mercury the growth of plants was higher after 7 days and lower after 14 days with respect to control. On the 7th day it was higher by 11% for of 0.15 mg Hg/dm^3 and 13% for 0.2 mg Hg/dm^3, while for 0.15 mg Hg/dm^3 the increase in biomass was lower by 22% and for 0.2 mg Hg/dm^3 by 43% on the 14th day (Fig. 1).

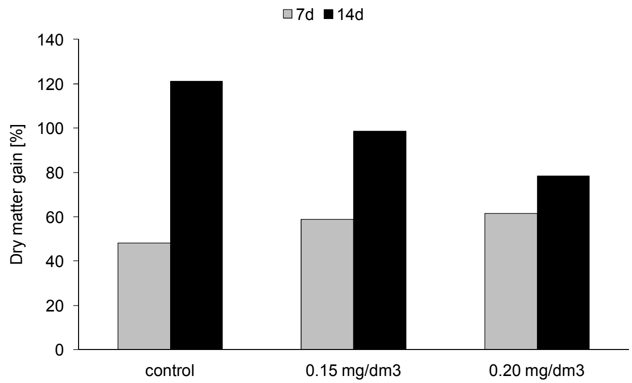

Fig. 1. Percentage weight gain in *Lemna minor* commercial cultures (per dry matter)

Lemna minor obtained from the natural environment showed on the 7th day a significant 114% weight gain in the control samples. Transfer from its natural environment resulted in sustained physiological stress, manifested in a smaller increase on the 14th day (90% compared to the initial value) as compared to the 7th day. However, the effect of mercury concentration on the activity of plants was observed because the growth on the 7th day for the concentration of 0.15 mg Hg/dm^3 was only 13%, while on the 14th day it was already 87%. For 0.20 mg Hg/dm^3, the weight gain did not exceed 3% on the 7th day, and on the 14th it increased to 36%.

Weight gain of plants exposed to mercury at the concentration of 0.15 mg Hg/dm^3 was about 101% on the 7th day, and 3% lower on 14th day as compared to controls. Growth of plants exposed to mercury at 0.2 mg Hg/dm^3 was still lower by as much as 111% on the 7th day and 54% on the 14th day (Fig. 2). In the initial period of exposure, the toxicity effect was observed, manifested by a small increase in weight. But with time running, adaptive processes of the body might start, which manifests itself in the significant increase in biomass. Plants, in the presence of heavy metals at increased may start processes of tolerance, among them we distinguish building heavy metals in the plant cells walls, active transport of ions into the vacuole and chelation [13, 18–20].

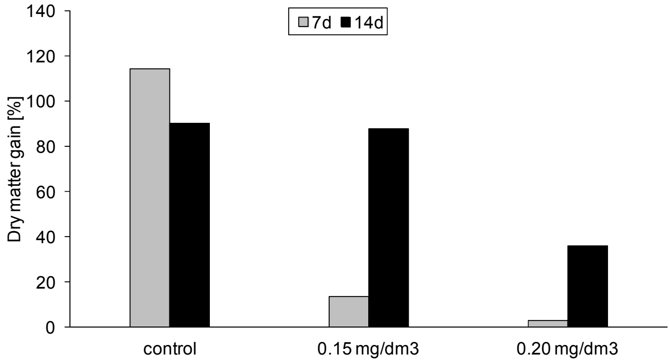

Fig. 2. Percentage weight gain in *Lemna minor* obtained from the natural environment (per dry matter)

Tests were also conducted on *Salvinia natans* originating, similarly as *Lemna minor*, from commercial cultures and from natural environment. Amount of mercury in the control samples did not exceed 0.45 mg Hg/kg DM.

In the case of commercial plants, growth of biomass during the experiment was observed both for control samples and the samples with mercury. The largest growth after both 7 (22%) and 14 days (63%) was observed for concentrations of 0.15 mg Hg/dm^3. However, for 0.20 mg Hg/dm^3, smaller increase was observed with respect to the control samples: only 7% on the 7th day and 14% on the 14th day. This suggests that at the concentration of 0.20 mg Hg/dm^3 toxic effects to *Salvinia natans* commercial plants occur, manifested by a significant decrease in the intensity of metabolic processes and, consequently, reduced biomass and plant death. It was found that for the concentration of 0.15 mg Hg/dm^3, the weight gain after 7 days was about 11% higher with respect to control, and after 14 days about 35% higher while for samples with 0.2 mg Hg/dm^3, the weight gain was lower by 4% after 7 days and 14% after 14 days (Fig. 3).

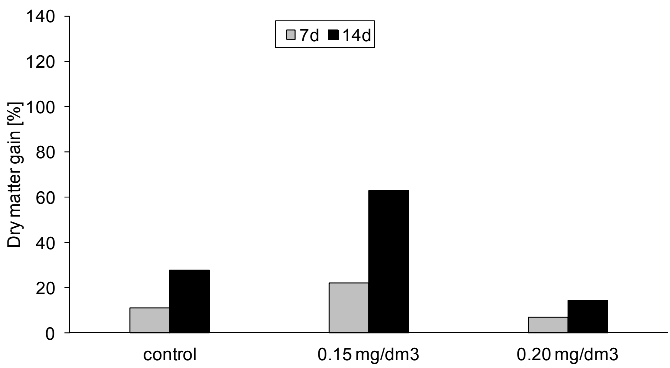

Fig. 3. Percentage weight gain of commercial *Salvinia natans* (per dry matter)

Weight gain for control samples of *Salvinia natans* taken from the environment, was equal to 13% on the 7th day and 79% on the 14th day, representing the five-fold increase. For 0.15 mg Hg/dm^3, the gain remained at a similar level of 23% on the 7th and 14th day. For 0.20 mg Hg/dm^3, it was 30% on the 7th day, whereas on the 14th day it was already lower (23%).

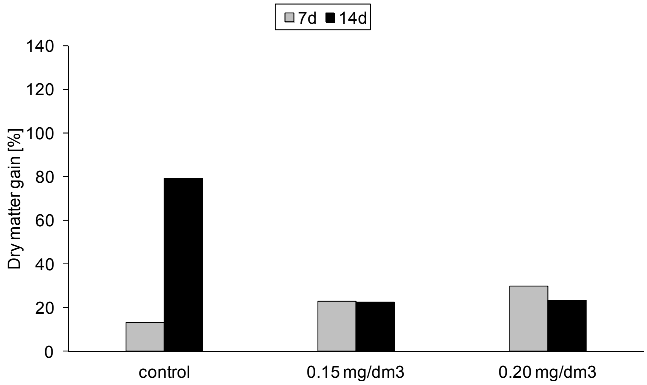

Fig. 4. Percentage weight gain of *Salvinia natans* obtained from the environment (per dry matter)

Comparing the growth of plants in the presence of mercury to their growth in cultures without mercury, it was found after 7 days it was higher for both 0.15 mg Hg/dm^3 (10%) and 0.2 mg Hg/dm^3 (17%). After 14 days, the plant growth in the presence of mercury decreased significantly (about 57%) compared to controls (Fig. 4).

3.2. MERCURY ACCUMULATION

In the control samples, commercial samples, and those taken from the *Lemna minor* environment, mercury content during the experiment was within the range of 0.27–0.45 mg Hg/kg DM (dry matter). For both concentrations of mercury, there was a decrease in the mercury content in the dry matter after 14th day as compared to 7th day, regardless of the origin of the plant material. At the concentration of 0.15 mg Hg/dm^3 in the first days of the experiment, commercial plants accumulated 67.05 mg Hg/kg DM, but after 14 days, decrease in mercury content to 41.16 mg Hg/kg DM was discovered, which represented a decrease by 39%. Similar changes were observed for the concentration of 0.20 mg Hg/dm^3. On the 7th day, mercury content in plants was 102.66 mg Hg/kg DM, whereas on the 14th day it dropped by 28% and was equal to only 73.95 mg Hg/kg DM. In the case of *Lemna minor* obtained from the natural environment, similar tendency was observed, however decrease in the mercury content in plants after 14 days in relation to 7 days did not exceed 15% for the mercury concentration in the medium of 0.15 mg Hg/dm^3 and 5% for 0.20 mg Hg/dm^3 (Fig. 5).

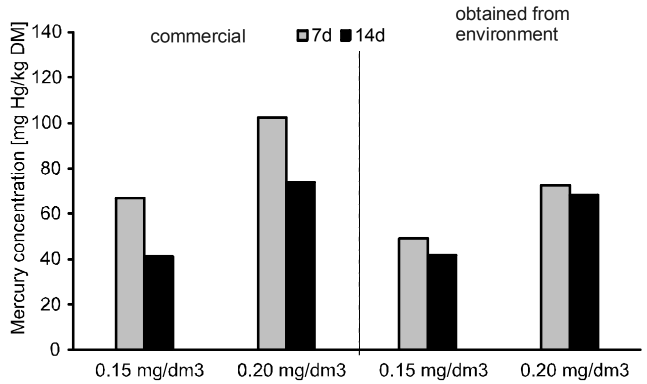

Fig. 5. Mercury content in dry matter of *Lemna minor*

This may suggest that the plants examined have enzymatic equipment allowing them to carry out the process of mercury volatilization in the case of its excessive accumulation in the tissues by passing it into the environment in a volatile non-toxic form [14]. Intensity of changes may depend on the effectiveness of the body detoxification process and its physiological condition.

It was also observed that the plants originating from commercial cultures accumulate greater amounts of mercury, even up to 30 mg Hg/kg DM more than those from the natural environment. This could result in a much greater increase in weight of the commercial plants and hence the higher accumulation of mercury.

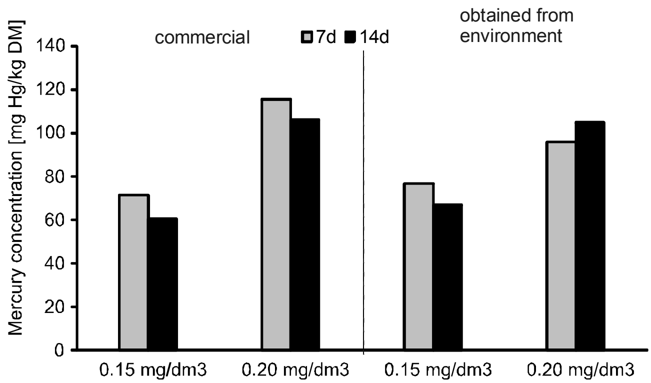

Fig. 6. Mercury content in dry matter of *Salvinia natans*

Accumulation of mercury in *Salvinia natans* from the environment showed high instability, which could indirectly be caused by weight gain. While for the concentration of 0.15 mg Hg/dm^3, mercury content in dry matter on the 7th day of the experiment was 76.66 mg Hg/kg DM, on the 14th it was 66.86 mg Hg/kg DM, which represented a decrease of 13%. This trend, however, did not occur for the concentration of

0.20 mg Hg/dm^3. On the 7th day, the dry matter contained mercury in the amount of 96.01 mg Hg/kg DM, and on the 14th day of the experiment it was already 104.72, so a growth of 9% was observed. (Fig. 6)

The size of biomass growth significantly affects the amount of mercury accumulated, which was confirmed by the study carried out. In commercial plants, whose increase was even more than 30% greater than of those taken from the environment, the amount of accumulated mercury increased by 6–19 mg Hg/kg DM.

Comparing cultured *Salvinia natans* and *Lemna minor* it was found that *S. natans* accumulates more mercury and its loss on the 14th day is smaller. While on the 14th day, the amount of mercury in the dry matter of *Lemna minor* at the concentration of 0.15 mg Hg/dm^3 was 41.16 mg Hg/kg DM, for *Salvinia natans* it was 60.36 mg Hg/dm^3. The loss of mercury in the dry matter on the 14th day compared to the 7th day for *Salvinia natans* was approximately 15% at the concentration of 0.15 mg Hg/dm^3 and only 8% at the concentration of 0.20 mg Hg/dm^3.

4. DISCUSSION

The phytoremediation process consists of such unit processes as phytoextraction, phytofiltration, phytostabilization, phytovolatilization, phytodegradation, rhizodegradation and phytodesalination . The greatest important processes in cleaning the aquatic environment from heavy metals is phytoextraction and phytovolatilization, in the less extent is rhizodegradation [11, 13, 15].

Hyperaccumulator in accordance with the definition it is the plant that can accumulate in their biomass more than 1000 mg/kg of the test substance, and in the case of strong accumulators more than 100 mg/kg of the test substance [21]. In this study, mercury accumulated in the biomass of *Lemna minor* remained at an average level of 64.51 mg Hg/kg DM. The average content of mercury in the biomass of *Salvinia natans* was 87.12 mg Hg/kg DM, of which approximately 40% were samples containing mercury in quantities exceeding 100 mg Hg/kg DM. Increase of mercury content in the Hoagland medium caused increase of mercury accumulated in the biomass. The accumulation of mercury in *Salvinia natans* was higher than that in *Lemna minor*, despite its smaller percentage increase. The quantity of mercury in plants decreased in the 7th day in comparison with the 14th day, which may indicate the start of detoxification of plants through metabolic forms of mercury (phytovolatilization) [19, 20].

Due to the low cost of phytoremediation methods compared to traditional methods of cleaning the environment with heavy metals, more and more attention is paid to its improvement. One way is to use genetically modified plants or placing rhizosphere bacteria capable of carrying out biochemical transformations of metal compounds [12, 22–24].

A side effect of the process is formation of large amounts of biomass. However, we can use biomass in combustion process and get energy [25].

5. CONCLUSIONS

- Commercial *Lemna minor* grows to a greater extent in both the control as well as in solutions containing mercury than plants of this species obtained from the environment.
- *Salvinia natans* obtained from the environment shows a similar weight gain of 22–30% for both concentrations of mercury on the 7th and 14th day of the experiment.
- Commercial *Lemna minor* and *Salvinia natans* and *Lemna minor* obtained from the environment showed downward trend in mercury content in dry matter along with prolongation of their exposure to the element.
- Mercury content in dry matter of *Salvinia natans* and *Lemna minor* ranged from 41–120 mg Hg/kg DM.

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