PHYTOREMEDIATION OF DANUBE WATER, AS A SUPPLY SOURCE FOR A RECIRCULATING AQUACULTURE SYSTEM, THROUGH THE USE OF *LEMNA MINOR*

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Abstract

In order to use the Danube water as a supply source for a recirculating system, an attempt was made to use L. minor for phytoremediation by bioaccumulation of lead, a heavy metal that is toxic to aquatic life. The experiment was designed to achieve a gradient of concentrations to determine the most effective conditions for removing lead. In the first experimental variant, the Danube water was used undiluted as a growth medium, in the second experimental variant the Danube water represented 75% of the growth medium and in the last experimental variant the Danube water represented 50% of the growth medium, the experiment being performed in triplicate. The best percentage of lead removal was achieved in the aquarium with 100% Danube water, obtaining a reduction of lead in the water volume by 97.86±0.1%. The species L. minor can be used successfully for phytoremediation of Danube water, in order to use it as a water source for a recirculating aquaculture system.

Key words: *Lemna minor*, lead bioaccumulation, recirculating system

INTRODUCTION

For the environment and human health protection as well as for the mitigation of heavy metal toxicity that threatens aquatic ecosystems, it is important to remove or recover heavy metals from wastewater.

There are various techniques that can be used to remove toxic metal compounds from water, such as: evaporation, galvanization, adsorption and ion exchange. The high technological costs and the possibility of using these techniques only in waters that contain high concentrations of metal ions, make their implementation difficult.

The bioaccumulation capacity of heavy metals differs depending on the plant species. Duckweed (*Lemna minor*) is known for its high bioaccumulation capacity and tolerance to higher concentrations of heavy metals, compared to other macrophyte species.

Conventional methods of lead removal are reverse osmosis, ion exchange, chemical precipitation, electrodialysis [1].

MATERIAL AND METHOD

The plants were sterilized by bathing in a 1% hydrogen peroxide solution for 5 minutes to remove organisms (bacteria, algae, invertebrates) that could interfere with bioaccumulation, as Pb can also accumulate in phytoplankton or zooplankton tissues. After sterilization, the plant was distributed in equal amounts in the experimental aquariums, respectively 0.1 g in each.

The water used as a culture medium represents the water from the Danube River, a potential supply source for the recirculating system, belonging to ICDEAPA Galați, where different species of fish are reared.

The aquariums were placed so as to receive natural light, in conditions similar to the environment from which the plants were taken, namely the surface of Brateș Lake, in Galați County.

The experiment was mounted in 10 glass aquariums measuring 100×38×34 cm and

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lasted 21 days. An aquarium was considered for control in which only plant-free Danube water was added. In experimental aquariums, 0.1 g of *L. minor* was used for each. The volume of water used (38 litres) was 10 cm high, enough to allow the roots to extract nutrients. The water lost by evaporation was replaced with dechlorinated drinking water so as not to alter the dilution of lead. In 3 aquariums the Danube water was used as such, in other 3 the Danube water was diluted with 3:1 dechlorinated drinking water, and in the last 3 Danube water was diluted with 1:1 dechlorinated drinking water.

The pH of the water used in the experiment was monitored every 3 days using the portable multi-parameter with the pH probe from HACH.

To accurately determine the Pb concentrations in water and plant samples, the ANALYTIK JENA ContrAA 700® atomic absorption spectrophotometer was used, which allows the sequential analysis of metallic and non-metallic traces in liquid and dissolved samples.

To determine the development of plants and the effects of lead on them, a number of indices were calculated:

- The relative growth rate of *L. minor* plants was calculated using Hunt's equation:

  $R = (\ln W2 - \ln W1) / (T2 - T1)$,
  where:

- R is the relative growth rate measured as g/(g*day),
- W1 and W2 represent the initial and final dry weights,
- the difference T2 - T1 represents the duration of the experiment [4].

- The FBC bioconcentration factor was calculated as follows:

  FBC = (Pb in plant biomass mg/kg) / (Pb in solution mg/L) [8].

The Pb concentration was determined both in the volume of water in each aquarium and in the plant samples at the beginning and end of the experiment. In order to perform the analyses of the Pb content from the studied plant biomass, the digestion of the samples is performed beforehand, using the TOPWAVE ANALYTIKJENA GERMANY digestion furnace. The analysis of the samples was performed in duplicate by reference to the calibration curve and the final value of each concentration represents the average of the 2 readings.

The growth medium and plants in each aquarium were gently shaken for about 5 minutes each day to ensure homogeneity of the culture medium.

All analyses were performed in triplicate. Statistical analysis was performed using Excel tools. Mean values are reported along with standard deviations. Statistical interpretation of the data was performed according to a significance threshold of $P<0.05$.

RESULTS AND DISCUSSIONS

The amount of lead measured in samples taken from the Danube water (4.21 μg/l) is below the limit of the average quantity of 7.2 μg/l, registered by Directive 2008/105 / EC of the European Parliament on environmental quality standards in aquatic field [3].

In the experimental aquariums with 100% Danube water, the amount of Pb in the water was reduced by 97.86 ± 0.1%. In aquariums with 75% Danube water, the amount of Pb was reduced by 83.93 ± 0.1%. In aquariums with 50% Danube water, the amount of Pb was reduced by 76.38 ± 0.1%. In the control aquarium the lead concentration did not change significantly ($p>0.05$). Small differences can most likely be attributed to the deposition or precipitation of lead compounds at the bottom of the aquarium. Figure 1 shows the changes in the amount of lead in the 3 experimental variants. The percentage of lead accumulated in the present experiment (97.86%, 83.93% and 76.38%) using duckweed, is comparatively higher than that obtained by Axtell et al. for Pb (76%) [2] and then those obtained by Parra et al., for duckweed grown at pH=7 [7].

The largest amount of lead removed by duckweed was in the experimental version with 100% Danube water as a growth medium. This phenomenon can be explained by the higher bioavailability of the metal in the aquatic environment.

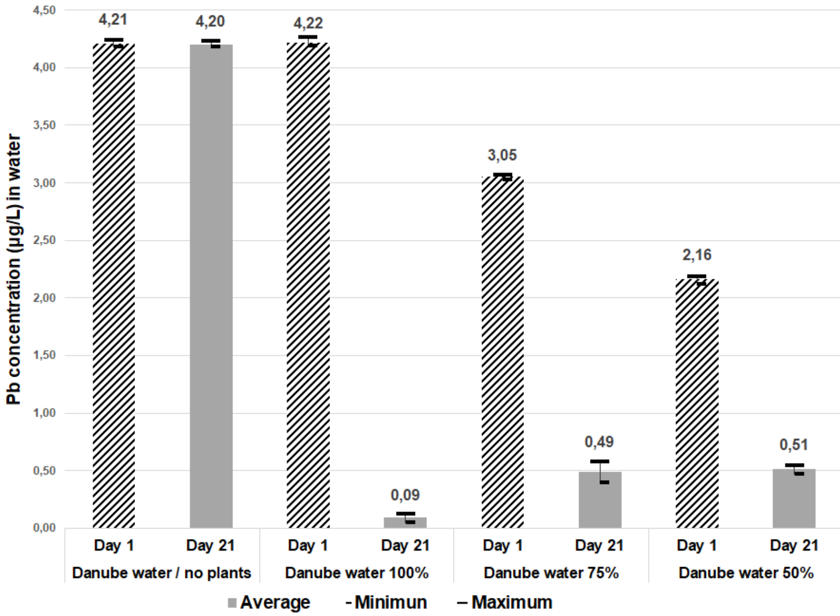

Fig. 1 Pb quantity found in Danube water, from the 3 experimental variants, at the beginning and end of the experiment, after 21 days

In figure 2, the amount of lead that was accumulated by plants in its tissues, is presented as the average value of the 3 aquariums in each experimental variant.

At the beginning of the experiment, traces of lead ions (0.01 µg/g) were detected in the plants, most likely due to the presence of lead in the water from which the plants were collected.

pH is considered the most important physicochemical water parameter that influences the bioaccumulation capacity of heavy metals by *L. minor* species. Figure 3 shows the evolution of pH over the 21 days of the experiment.

The bioconcentration factor reached the values 2077±4.5 for plants grown with 100% Danube water, 236.32±1.9 for plants grown with 75% Danube water and 147.25±1.1 for plants grown with 50% Danube water. These values are comparable to those obtained by Uysal et al. in 2009, at similar water pH values [9].

The higher bioabsorption capacity of duckweed at acidic pH was also observed by Kaur et al., who obtained the highest lead bioaccumulation at pH 5 and the lowest at pH 10 [6].

The acidification of the culture medium was directly proportional to the percentage of Danube water used. In the experimental variant with 100% Danube water, the pH reached 6.8±0.1 at the end of the experiment compared to the pH=8.1 in the control aquarium. This change is due to the metabolic stress caused on plants by the presence of lead ions in larger quantities, effects also confirmed by Jayasri et al. [5].

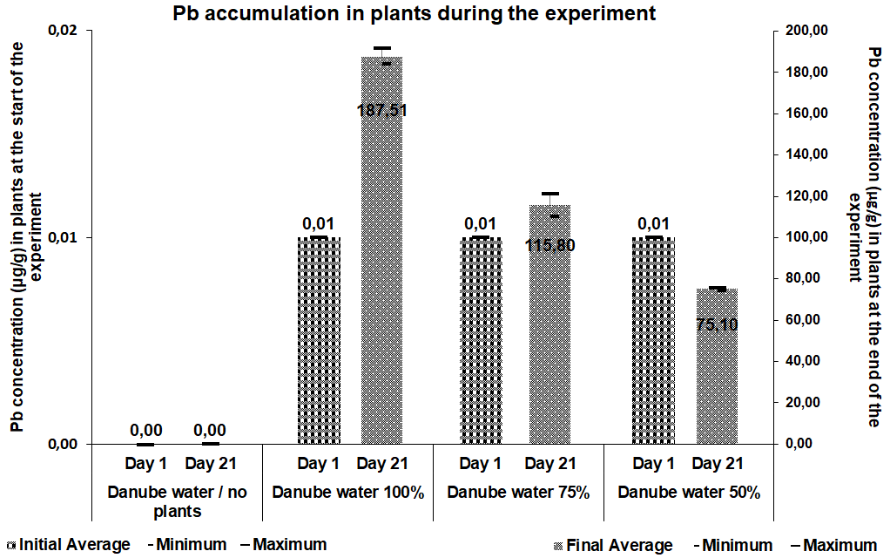

Fig. 2 Pb quantity found in plants, from the 3 experimental variants, at the beginning and end of the experiment, after 21 days

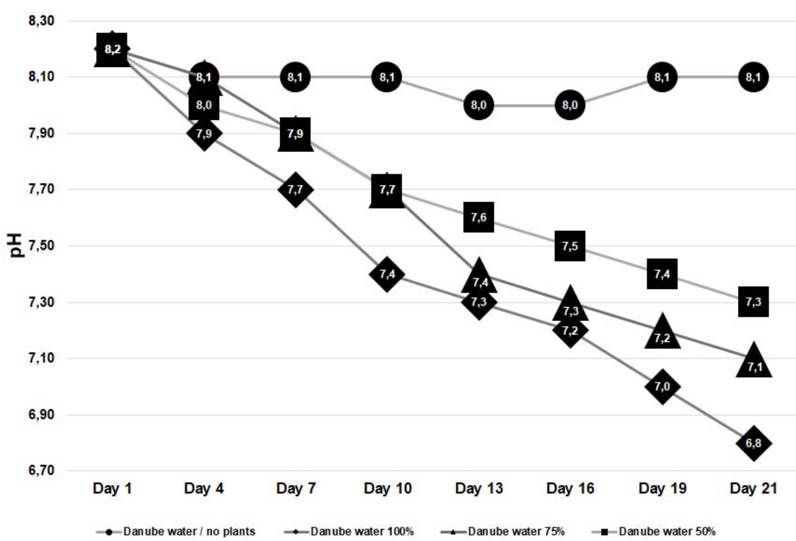

Fig. 3 Variation of water pH during the experiment

Foliar development of plants was influenced by the amount of lead and its ability to inhibit plant growth. Figure 4 graphically represented the plant biomass from each experimental variant, both at the beginning and at the end of the experiment.

The quantity of plants found in the 3 experimental variants at the end of the experiment was between 12.04±0.7 g and 12.62±0.3 g, which suggests a similar inhibitory effect of lead ions on plants at concentrations present in the water. The best development of biomass took place in the experimental variant with 50% of the culture medium represented by the Danube water.

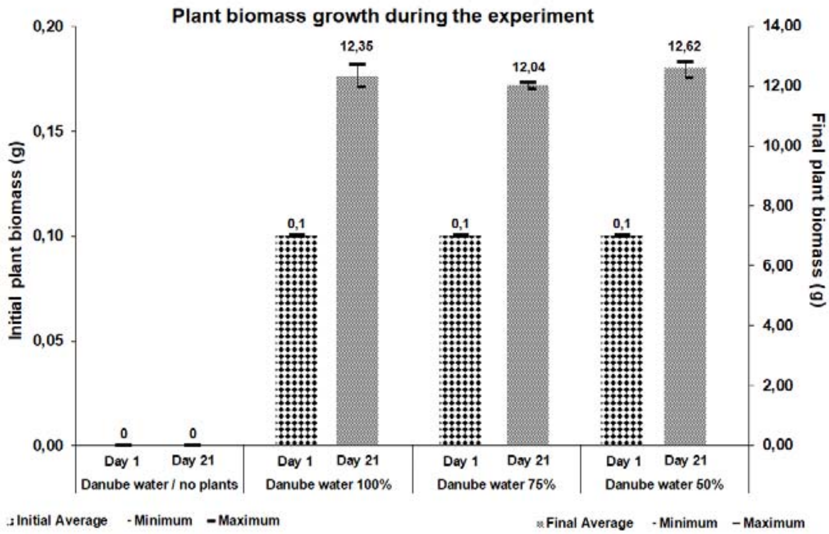

Fig. 4 The evolution of *Lemna minor* biomass in the 3 experimental variants at the beginning and end of the experiment

CONCLUSIONS

The ability of *Lemna minor* to remove lead from the aquatic environment is affected by the capacity to increase plant biomass and the kinetics of lead in the volume of water, which alters its bioavailability to the plant.

There is an upward trend in the removal of lead from water by plants, as the gradient of Pb in the water increases.

The species *Lemna minor* can be used successfully for phytoremediation of Danube water, in order to use it as a supply source for a recirculating aquaculture system.

REFERENCES

[1] Akpor O.B., Muchie M., 2010. Remediation of heavy metals in drinking water and wastewater treatment systems: Processes and applications. International Journal of the Physical Sciences, 5(12), pp. 1807-1817.

[2] Axtell N.R., Sternberg S.P.K., Claussen K., 2003. Lead and nickel removal using Microspora and Lemna minor. Bioresource Technology 89, pp. 41-48.

[3] Directiva 2008/105/CE a Parlamentului European și a Consiliului din 16 decembrie 2008 privind standardele de calitate a mediului în domeniul apei, de modificare și de abrogare a Directivelor 82/176/CEE, 83/513/CEE, 84/156/CEE, 84/491/CEE, 86/280/CEE ale Consiliului și de modificare a Directivei 2000/60/CE, Jurnalul Oficial al Uniunii Europene.

[4] Hunt R., 1978, Plant growth analysis. Studies in biology. London: Edward Arnold, pp. 67.

[5] Jayasri M.A., Suthindhiran K., 2017. Effect of zinc and lead on the physiological and biochemical properties of aquatic plant Lemna minor: its potential role in phytoremediation. Appl. Water Sci. 7, pp. 1247-1253.

[6] Kaur L., Gadgil K., Sharma S., 2012. Role Of Ph In The Accumulation Of Lead And Nickel By Common Duckweed (Lemna Minor). International Journal of Bioassays, 01 (12), pp.191-195.

[7] Parra L.M.M., Torres G., Arenas A.D., Sánchez E., Rodríguez K., Phytoremediation of Low Levels of Heavy Metals Using Duckweed (Lemna minor), In book: Abiotic stress responses in plants: Metabolism, Productivity and sustainability (pp.451-463) Edition: 2012, Chapter: 24, Publisher: Springer, Editors: P. Ahmad, M.N Prasad

[8] Rahmani G.N.H., Stenberg S.P.K., 1999, Bioremoval of lead from water using Lemna minor, Bioresource technology, vol. 70, pp. 225-230.

[9] Uysal Y., Taner F., 2009. Effect of pH, Temperature, and Lead Concentration on the Bioremoval of Lead from Water Using Lemna Minor, International Journal of Phytoremediation, 11:7, pp. 591-608.