The Capacity of *Lemna Minor* L. to Accumulate Heavy Metals (Zinc, Copper, Nickel)

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The assessment of the storage capacity of heavy metals (Zn, Cu, Ni) in Lemna minor L., was carried out on wastewater from galvanizing plants. Purification yield decreases with increasing metal concentration in water. The rate of bioaccumulation is high in the first three days, then decreases over the next four days.

Keywords: galvanization, Lemna minor L., heavy metals

Metals and metalloids, due to their extensive use, represent an important fraction of pollutants released into the air, soil and water; they seem to be even omnipresent [1]. Due to their properties, heavy metals can cause adverse effects on environmental components. These elements do not decompose, but accumulate in plants, animals, and the environment, and subsequently they enter the human body through the food chain after high enrichment in the propagation bodies [2, 3].

Mining and tailings management is one of the major sources of heavy metals disposal in the environment. In many regions of the world, the concentration of heavy metals in drinking water resources, in urban areas, is alarmingly increased due to the elimination of untreated or partially treated industrial wastewater [4]. In recent years, the removal of pollutants from water is a priority, because heavy metal ions, among other potential harmful chemical compound [5, 6] can cause risks to human health and ecosystems [7, 8]. Water is the most important, limited and vulnerable natural resource. At present, water quality assessment is also concentrated on heavy metals because they are non-degradable, toxic and persistent in nature [9]. However, water resources are open to pollution due to population growth, technological development and industrial activity growth [10].

Duckweed (*Lemna minor* L.) is a widespread aquatic plant located in lakes, rivers, ponds and other bodies of water. Due to its small size, simple structure and morphology, rapid growth rate, short life span and environmental pollutant sensitivity, duckweed was commonly used as a hydrophilic model in ecotoxicology studies. In particular, *Lemna minor* L. has been reported to accumulate toxic metals and is therefore used as an experimental model to investigate the accumulation of heavy metals (Cd, Cu, Zn, Ni and Co) and their toxicity [11].

The objective of the study was to investigate the potential of *Lemna minor* L. plants in the accumulation of heavy metals in wastewater. For this, on the one hand, was evaluated the quality of the wastewater before and after the treatment with plants, on the other hand was evaluated the metal storage capacity in the plants. Experimental studies used actual wastewater, resulting from galvanizing installations of the zinc, copper and nickel processes.

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Experimental part

Plant material and growth conditions

The plant material (*Lemna minor* L.) was collected from a natural environment and it was multiplied in the laboratory for 7 days in Hoagland's modified culture medium, described by Cowgill & Milazzo in 1989 [12]. Hoagland's modified culture medium is described in table 1 and table 2.

Table 1
HOAGLAND'S MODIFIED MEDIUM CULTURE PREPARATION

Composition	Preparation stock solution	Quantity used
1. $MgSO_4 \cdot 7H_2O$	24.6 g/100 mL	1.0 ml/L
2. $Ca(NO_3)_2 \cdot 4H_2O$	23.6 g/100 mL	2.3 ml/L
3. KH_2PO_4	13.6 g/100 mL	0.5 ml/L
4. KNO_3	10.1 g/100 mL	2.5 ml/L
5. Micronutrients	See below (table 2)	0.5 ml/L

Table 2
PREPARATION OF MICRONUTRIENTS SOLUTION

H_3BO_3	2.86 g/L
$MnCl_2 \cdot 4H_2O$	1.82 g/L
$ZnSO_4 \cdot 7H_2O$	0.22 g/L
$Na_2MoO_4 \cdot 2H_2O$	0.09 g/L
$CuSO_4 \cdot 5H_2O$	0.09 g/L

Experimental conditions

All experiments data, presented in the present paper represent the average of three samples. *Lemna minor* L. plants were placed in plastic containers (size Lxlxh = 20x16x6 cm), containing various concentrations of zinc (15, 30, 50, 75 mg/L), copper (5, 10, 20, 30 mg/L) and nickel (5, 15, 30, 50 mg/L) from galvanizing plants wastewater. The amount of plants used was about 10 g of wet vegetal material, sufficient to cover the entire surface of the container in a single layer of leaves. The experimental duration was 7 days at an ambient temperature of between 19 and 25°C and the heavy metal quantification in the plants and in the waters was carried out on days 0, 3 and 7.

Methods of analysis

Plants: about 3-4 g of wet vegetal material was thoroughly washed with distilled water and oven-dried at 80°C for one and a half hours. The dry tissue was weighed (0.1-0.3 g) and calcined at 550°C. The resulting ash was treated with aqua regia (HCl: HNO3 = 3: 1), and the crucible being placed on the sand bath to bring the amount to dry. The crucible was washed three times with 3 mL of mixture of HCl and distilated water in the report 1 to 1. The solutions were collected in a 25 mL volumetric flask after the remaining residue was washed and filtered through Sartorius filter papers 2-206 FT. The solutions were filled to the mark with mixture of HCl and distilated water in the report 1 to 1.

*Water*the detection of heavy metals in waters was performed according to SR ISO 8288: 2001 standard. Metal detection and quantification was performed using a GBC Avanta Atomic Absorption Spectrometer.

Bioaccumulation factor

The concentration of a chemical substance by aquatic organisms is generally expressed as a bioaccumulation factor (BCF). This factor is expressed as the ratio between the final concentration of metal ions in the plant and the initial concentration of metal in water (equation 1). It is an indicator of plant metal storage capacity at metal concentration in the environment and provides a comparison of results [13].

$$BCF = C_{Me\text{-}plant} / C_{Me\text{-}water} \qquad (1)$$

where $C_{Me\text{-}plant}$ is the final metal concentration in *Lemna minor* L., expreseed in mg Me/kg and $C_{Me\text{-}water}$ is the initial metal concentration in water, expressed in mg/L Me.

Results and discussions

Purification efficiency using the Lemna minor L. plant

The following figures show the concentration of metal in wastewater containing zinc, copper and nickel at the initial moment and then at day 3 and day 7. For each pollutant, different initial concentrations were used to set the limits of the concentrations in which high purification efficiencies were obtained.

Overall, the results showed that for all metal, their concentrations in the wastewater diminished during the time, especially on the day 7. The initial concentration of Zn (15 mg/L) decreased in wastewater is about 72 percent (from 15 mg/L to 4.20 mg/L) on day 7. For the concentration of 30 mg/L Zn, the percentage decrease is 56% (from 30 mg/L to 13.2 mg/L) and for the water with a concentration of 50 mg/L Zn we have a percentage decrease of 36% (from 50 mg/L to 32.2 mg/L). The lowest decrease in metal concentration of water is seen with 75 mg/L Zn, this being 20% (from 75 mg/L to 59.7 mg/L) (fig. 1).

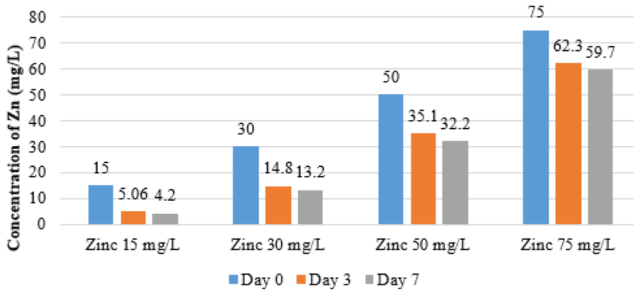

Fig. 1. Concentrations of Zn in wastewater (day 0, day 3 and day 7)

The percentages of Cu removal increased by the end of the day 7 and in the figure 2, they were shown in descending order, such as: the best results were for the water sample with a concentration of 5 mg/L Cu, with a decrease percentage of 59% (from 5 mg/L to 2.04 mg/L), followed by a 30 mg/L sample with a decrease of 44% (from 30 mg/L to 16.9 mg/L). For the other two concentrations of 10 and 20 mg/L Cu, the percentage

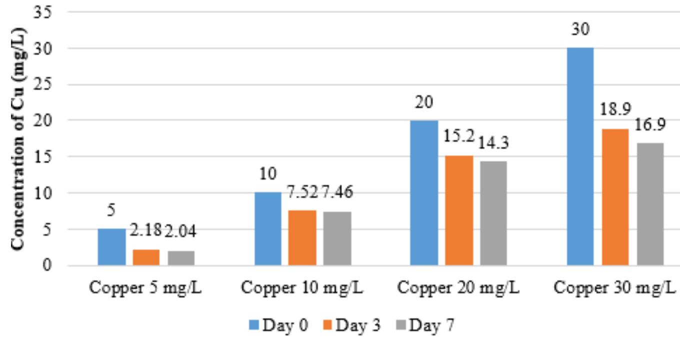

Fig. 2. Concentrations of Cu in wastewater (day 0, day 3 and day 7)

decrease was 30% (from 10 mg/L to 7.46 mg/L), respectively 26% (from 20 mg/L to 14.3 mg/L).

The yields of Ni removal from wastewater decreased with increasing of the initial Ni concentration in wastewater (fig. 3). In the case of the 5 mg/L Ni variant, the percentage decrease was 36% (from 5 mg/L to 3.19 mg/L), for 15 mg/L Ni is 33% (from 15 mg/L to 10.1 mg/L), for 30 mg/L Ni was 21% (from 30 mg/L to 23.8 mg/L) and for 50 mg/L Ni the percentage decrease was 15% (from 50 mg/L to 42.5 mg/L).

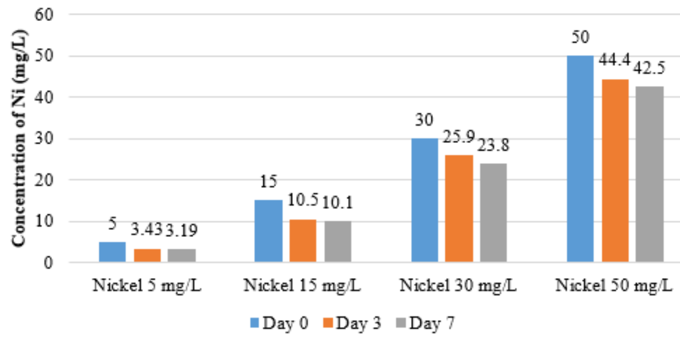

Fig. 3. Concentrations of Ni in wastewater (day 0, day 3 and day 7)

In figure 4, was a synthetic presentation of the purging water purification yields for day 7. In general, purification yield decreased with the increasing metal concentration in wastewater. The best purification yields were obtained at the lowest initial concentrations: 72% for zinc wastewater, 59% for copper wastewater and 36% for wastewater containing nickel.

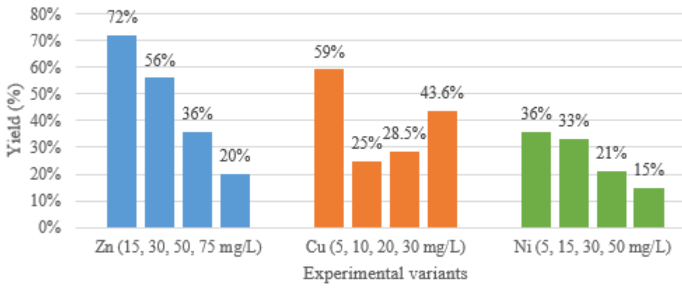

Fig. 4. Percentage of purification yield

The amount of metal accumulated by Lemna minor L.

Tables 3, 4 and 5 showed the accumulation of heavy metals by the plant after day 3 and day 7.

For all the studied metals, a small difference between day 3 and day 7 was observed in the bioaccumulation of metals by the plant, meaning that the specific bioaccumulation processes occurred at high speed in the first 3 days, followed by a decrease in the next 4 days, due to the maximum accumulation of metal in plant cells.

Plant death was characterized by a massive discoloration of the leaves, from dark green to brown and even completely white. It was observed a zinc accumulations in plant of 2810 mg/kg d.m. for the initial

Table 3
BIOACCUMULATION OF ZINC IN THE PLANTS

Concentration of Zn in wastewater 15 mg/L		
C_{Zn} in plant- day 0	**C_{Zn} in plant- day 3**	**C_{Zn} in plant- day 7**
504 mg/kg d.m.	1166 mg/kg d.m.	1292 mg/kg d.m.
Concentration of Zn in wastewater 30 mg/L		
C_{Zn} in plant- day 0	**C_{Zn} in plant- day 3**	**C_{Zn} in plant- day 7**
504 mg/kg d.m.	1982 mg/kg d.m.	2202 mg/kg d.m.
Concentration of Zn in wastewater 50 mg/L		
C_{Zn} in plant- day 0	**C_{Zn} in plant- day 3**	**C_{Zn} in plant- day 7**
504 mg/kg d.m.	2142 mg/kg d.m.	2430 mg/kg d.m.
Concentration of Zn in wastewater 75 mg/L		
C_{Zn} in plant- day 0	**C_{Zn} in plant- day 3**	**C_{Zn} in plant- day 7**
504 mg/kg d.m.	2520 mg/kg d.m.	2810 mg/kg d.m. *Obs: all the plants died*

Table 4
BIOACCUMULATION OF COPPER IN THE PLANTS

Concentration of Cu in wastewater 5 mg/L		
C_{Cu} in plant- day 0	**C_{Cu} in plant- day 3**	**C_{Cu} in plant- day 7**
15.4 mg/kg d.m.	278 mg/kg d.m.	327 mg/kg d.m.
Concentration of Cu in wastewater 10 mg/L		
C_{Cu} in plant- day 0	**C_{Cu} in plant- day 3**	**C_{Cu} in plant- day 7**
15.4 mg/kg d.m.	245 mg/kg d.m.	276 mg/kg d.m.
Concentration of Cu in wastewater 20 mg/L		
C_{Cu} in plant- day 0	**C_{Cu} in plant- day 3**	**C_{Cu} in plant- day 7**
15.4 mg/kg d.m.	367 mg/kg d.m.	396 mg/kg d.m.
Concentration of Cu in wastewater 30 mg/L		
C_{Cu} in plant- day 0	**C_{Cu} in plant- day 3**	**C_{Cu} in plant- day 7**
15.4 mg/kg d.m.	775 mg/kg d.m.	823 mg/kg d.m. *Obs: all the plants died*

Table 5
BIOACCUMULATION OF NICKEL IN THE PLANTS

Concentration of Ni in wastewater 5 mg/L		
C_{Ni} in plant- day 0	**C_{Ni} in plant- day 3**	**C_{Ni} in plant- day 7**
5.8 mg/kg d.m.	125 mg/kg d.m.	168 mg/kg d.m.
Concentration of Ni in wastewater 15 mg/L		
C_{Ni} in plant- day 0	**C_{Ni} in plant- day 3**	**C_{Ni} in plant- day 7**
5.8 mg/kg d.m.	350 mg/kg d.m.	364 mg/kg d.m.
Concentration of Ni in wastewater 30 mg/L		
C_{Ni} in plant- day 0	**C_{Ni} in plant- day 3**	**C_{Ni} in plant- day 7**
5.8 mg/kg d.m.	382 mg/kg d.m.	396 mg/kg d.m. *Obs: all the plants died*
Concentration of Ni in wastewater 50 mg/L		
C_{Ni} in plant- day 0	**C_{Ni} in plant- day 3**	**C_{Ni} in plant- day 7**
5.8 mg/kg d.m.	421 mg/kg d.m. *Obs: all the plants died*	-

metal concentration in waste water of 75 mg/L Zn. For copper, the cellular senescence was highlighted at a plant concentration of 823 mg/kg d.m. at 30 mg/L Cu initial metal concentration in wastewater. In the case of nickel, the symptoms characteristic of cell death, can be noticed at plant accumulations of approx. 400 mg/kg d.m., in wastewater with an initial metal concentration of 30 mg/L Ni. Cellular discoloration was due to the diminution of photosynthetic pigments and especially chlorophyll a and b.

This decline in chlorophyll in leaves was related to the nature of plants and tolerance to heavy metals Zn, Cu and Ni, which influenced the accumulated amount. Thus, decreased in the amount of photosynthetic pigments of *Lemna minor* L. can be correlated with the accumulated amounts of heavy metals causing inhibitory effects in plant photosynthesis. The results obtained were consistent with those in the literature. Hegazy et al. in 2009 observed phytotoxicity manifestations in the *Lemna gibba* L. aquatic plant by leaf discoloration and significant decrease of photosynthetic pigments during the bioaccumulation of Cu and Zn metals [14]. Torok Anamaria Iulia in 2015 reported a decrease in *Lemna minor* L. of the Chl *a* by 48% and 42% of the Chl *b*, in multielement solution with Zn, Cu and Cd [15].

A synthetic presentation of the performance of the bioaccumulation process for wastewater was presented in figure 5. Only values that were determined in plants until they show symptoms of toxicity were considered.

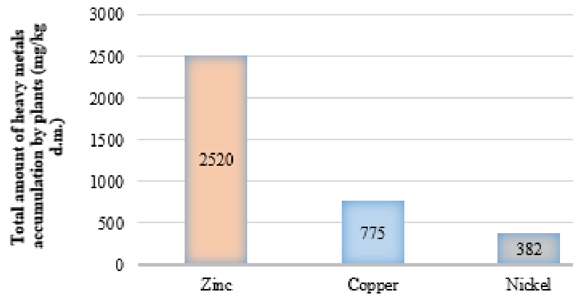

Fig. 5. The maximum amount of metal accumulated in plants

The results presented in figure 5 for three metals studied individually (Zn, Cu and Ni) indicate that the affinity of the *Lemna minor* L. for metals grows in the order: Ni <Cu <Zn.

BCF calculation

Based on the results obtained and described above, the bioaccumulation factor for each metal was calculated. This factor, allowed a comparison of the metal accumulation results in the plant body in accordance with the initial metal concentration in the waste water.

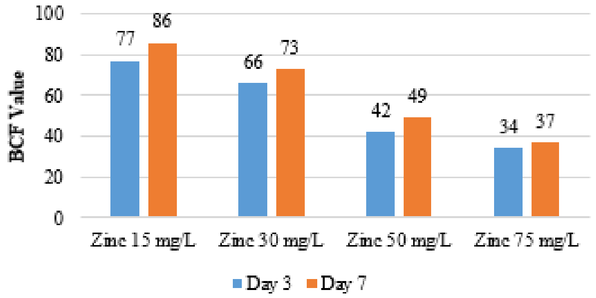

Fig. 6. Variation of BCF in day 3 and day 7 for Zinc

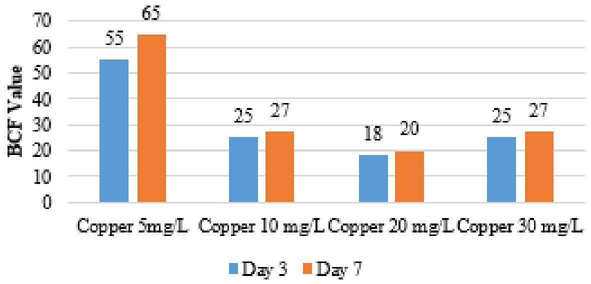

Fig. 7. Variation of BCF in day 3 and day 7 for Copper

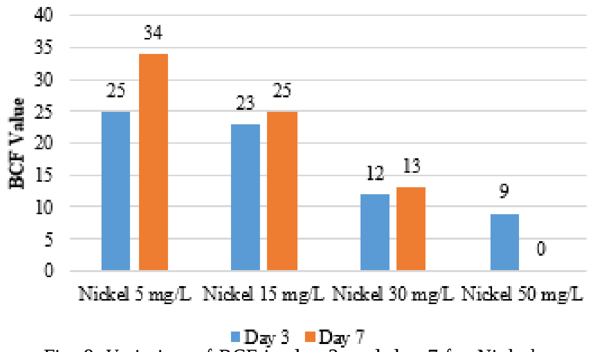

Fig. 8. Variation of BCF in day 3 and day 7 for Nickel

Figures 6, 7 and 8 show the bioaccumulation factors (for days 3 and 7) for Zn, Cu and Ni in the case of wastewater resulting from the galvanizing process.

In the case of zinc, the highest bioaccumulation metal in the plant, in accordance with the initial concentration of metal in the wastewater, was found at a concentration of 15 mg/L Zn both on day 3 and day 7, with a maximum for day 3 of 77 and for day 7 of 86. For the other concentrations, a gradual decrease of the values, up to 34 (day 3) in zinc water with a concentration of 75 mg/L and 37 on the 7th day, in galvanizing water of the same concentration.

For copper, the highest value for BCF on day 7 is at the initial metal concentration in water of 5 mg/L Cu (BCF= 65) and the lowest at a concentration of 20 mg/L Cu (BCF= 20); for day 3, the same conditions are maintained where the highest values are at concentrations of 5 mg/L Cu (BCF= 55) and the smallest values are observed at a concentration of 20 mg/L Cu (BCF= 18). The other two concentrations (10 and 30 mg/L) show similar values on day 3 and day 7 (25 and 27 respectively).

Nickel-loaded wastewater offers the best bioaccumulation on day 3 and day 7 at the initial concentration of 5 mg/L Ni (BCF= 25 and 34 respectively). The lowest bio-concentration is observed for day 3 at the initial concentration of 50 mg/L Ni (BCF= 9) and for day 7 at the concentration of 30 mg/L Ni (BCF= 13).

Conclusions

The study showed that the *Lemna minor* L. accumulated considerable amounts of zinc, copper and nickel after 3 days, without any symptoms of toxicity. Leaf discoloration and cell death occur at plant concentrations above 2810 mg/kg d.m. in the case of zinc, 823 mg/kg d.m. in the case of copper and approximately 400 mg/kg d.m. in the case of nickel. If a comparison was made between the quantities of the three metals accumulated by the plant, it was observed that it has been an increased affinity for zinc, followed by copper and then nickel. The best purification yields were obtained at the lowest initial concentrations, namely: 72% at the initial concentration of 15 mg/L Zn, 59% at the initial concentration of 5 mg/L Cu and 36% at the initial concentration of 5 mg/L Ni. Regarding the speed of the accumulation processes, it was noticeable that they were greatly increased in the first three days, then decreased over the next four days. From this it can be concluded that for an efficient treatment of wastewater loaded with heavy metals, especially those loaded with zinc, copper and nickel, the experimental duration should be between three and four days, where the accumulation intensity is the highest.

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