METALS AND ORGANIC POLLUTANTS REMOVAL FROM WASTEWATER BY LOCAL *LEMNA MINOR* GENOTYPE

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Abstract. Lemna minor was collected from a local pond from the Floresti area, Cluj county. The plants were left for vegetative multiplication, and then analyzed in order to investigate their ability to reduce the pollutants from wastewater. The untreated wastewater contained high concentrations of As, Cd, Cr, Co, Cu, Mn, Ni, Pb, Zn, Al, Ca, Fe, Mg, Na, low concentrations of Ba, Be, Ca, Fe, Hg, Li, Rb, Se, Sr, Sn, Ti, V and high levels of organic pollutants. The results showed that the Lemna minor genotype has a high potential to reduce the totally oxidizable organic pollutants from the wastewater with 65%. Lemna minor achieved high removal efficiency of Mn and Pb from wastewater. Also, the results demonstrate the impressive ability of the Lemna minor genotype to accumulate high concentrations of Na, Al, Hg and Fe compared to that of the control plants. Simultaneously, an antagonistic effect was observed in case of the Cu, Ni and Zn after the accumulation metals from the wastewater.

Keywords: *Lemna minor*, wastewater, COD, metals, phytoremediation

INTRODUCTION

Clean, affordable water is one of the 21st century's biggest goals. Only 0.003% of the total global water is available as freshwater to support life on Earth (U.S. Geological Survey, 2016). Therefore, water recycling plays an important role to reduce the overuse of freshwater in the world (McLaughlin et al., 2020). Moreover, the reuse of treated wastewater is considered to be a critical element of sustainable water management (Michael et al., 2015).

Many studies were conducted to test the efficiency of various natural or synthetic materials and several methods were applied for decontamination of wastewaters. (Vijayaraghavan et Balasubramanian, 2015; Anastopoulos et al., 2019; Barkshia et al., 2020; Mansoori et al., 2020).

The applied decontamination process needs to effectively recover the pollutants in order to obtain high quality of water after treatment. The decontamination process needs to offer some facilities such as to reduce pollutants, to have renewable perspectives for a better water quality, to have lower operation costs and to have reliable operation possibilities (Nakkasunchi et al., 2021).

Phytoremediation has emerged as a low cost and environmentally-friendly decontamination process (Rosen and Morling, 1998). Lemna *minor spp. (L. minor),* a free-floating and small aquatic plant from the *Lemnaceae* family, is considered to be a native and invasive species across Europe (Ceschin et al., 2018; Paolacii et al., 2018; Ekperusi et al., 2019). The present aquatic plant has a high adaptability and sensitivity in various growth media. *L. minor* achieved high efficiency for organic and inorganic pollutants removal from agricultural, industrial and municipal wastewaters. (Ceschin et al., 2019, Li et al., 20291). The used biomass, plant or algal species are recommended to be native species in order to avoid maladministration (Hu et al., 2020). The use of native plant and algal species is recommended due to their ability to be non-vulnerable to extinction. The non-native species introduced into a new habitat can not adapt to the environment conditions or can exhibit slow growth because of habitat loss (Hu et al., 2020).

In the present study, the potential of local *L minor* genotypes for heavy metal removal and accumulation of total chemically oxidizable organic pollutants from wastewater was investigated.

MATERIAL AND METHODS

1. Plants growth

L. minor was collected from a local pond from the Floresti area, Cluj county (46°44'19.5"N 23°30'36.8"E). The collected healthy plants were washed with distilled water and sterilized with 5% v/v solution of sodium hypochlorite in order to remove the microorganisms from the plant's surface. The sterilized fronds were rinsed with distilled water and were inoculated in ½ Hoagland nutrient solution for vegetative multiplication and further uses (Hoagland et Arnon, 1950). The inoculum fronds were cultivated at a temperature of 20±2°C, with a 14/10 h photoperiod.

2. Experimental design

2 g of healthy plant were maintained, for an acclimatization period, in Hoagland solution for 3 days. After the acclimatization period, the plants were placed in 250 ml of wastewater containing high concentration of various metals, such as As, Cd, Cr, Co, Cu, Mn, Ni, Pb and Zn (target metals) along with various concentrations of Al, Ca, Ba, Be, Ca, Fe, Hg, Li, Mg, Na, Rb, Se, Sr, Sn, Ti, V. An additional control pot (plant in Hoagland solution) was grown alongside with the phytoextraction experiments in order to determine the plant's heavy metal accumulation capacity. The experiments were carried out for 7 days. The experiments were carried out in triplicate under the same conditions.

3. Plant digestion and metal analysis

After the phytoextraction period, the plants were harvested and dried at 65°C for 24h. The dried biomass was grind and passed through a 4 mm sieve. A quantity of 2 g of dried plant was digested with 15 mL of 69% HNO_3 and 6 mL 30% H_2O_2 and finally diluted to 25 mL. The concentrations of Mg, Al, Ca, Fe, Mn, Na, Cd, Cr, Co, Pb, Ba, Se, Li, Ti, V, Sr, Sn, As and Hg were determined using an inductively coupled plasma Elan DRC II, quadrupole mass spectrometer (ICP-MS, Perkin Elmer, Canada) and the results were expressed in mg/kg dry weight (DW).

For the metal analysis, a volume of 40 mL of wastewater was digested with 21 mL of 37% HCl and 7 mL of69% HNO_3 and diluted to 50 mL before the analysis. The metal concentrations of wastewater before and after phytoextraction experiments were determined. The ELAN DRC II Spectrometer was calibrated against external standard solutions prepared by a serial dilution of 10 mg/L multi-element calibration standard 3 (Perkin Elmer). All chemicals were of analytical grade purchase from Merck and all the dilutions were made with deionized water.

4. Analysis of the chemical oxygen demand (COD)

In order to determine the *L minor*. efficiency to remove the oxidizable organic pollutants from wastewater, the chemical oxygen demand (COD) was measured before and after the phytoextraction period. The COD values were determined using a titrimetric method. A strong chemical oxidizing agent (potassium dichromate) was used to oxidize the organic material from the wastewater samples, under appropriate conditions (heat and strong acid) in the presence of mercury to reduce the interference from oxidation of chloride ions and silver compound and to enhance the oxidation of certain organic compounds. 0.4 g of mercury sulphate and 10 ml of potassium dichromate (0.25 N) were added to 20 ml of wastewater sample and heated in a reactor (VelpScientifica) at 150 °C for 2h. After cooling, the excess dichromate was titrated with ferrous ammonium sulfate (0.125 N) using orthophenanthroline ferrous complex as indicator.

5. Heavy metal adsorption efficiency and capacity

The adsorption capacity or accumulation capacity was calculated using equation (1), while the removal efficiency was calculated by equation (2):

$$q_e = \frac{(C_0 - C_e)}{m} \cdot \frac{V}{1000} \tag{1}$$

$$E(\%) = \frac{(C_0 - C_e)}{C_0} \cdot 100 \tag{2}$$

where, q_e is the amount of heavy metal adsorbed per gram of adsorbent after 7 days (mg/g dry weight plant, DW), V is the volume (mL), m is the weight of the adsorbent (g), E is the removal efficiency (%), C_o and C_e are the initial and final heavy metal content (mg/L). The adsorption capacity and the removal efficiency were computed using the Eq 1 and 2, respectively for As, Cd, Cr, Co, Mn and Pb (Mudyawabikwa et al., 2017).

RESULTS AND DISCUSSION

1. Wastewater quality improvement

The COD analysis was performed in order to determine the total chemically oxidizable material in the wastewater samples, before and after the phytoremediation period using local *L. minor* genotype. The COD value showed that the *L. minor* has a beneficial impact on the wastewater quality, obtaining a 3.3-fold smaller COD value after the 7 day of treatment with *L. minor* genotype (Figure 1). The results showed a 69.4% decrease in COD compared to the control value. The obtained results are in concordance with other research reports (Amare et al. 2018). Amare et al. 2018 reported notable low COD value after 28 days of wastewater treatment using *L. minor* (Amare et al., 2018). Based on the obtained results and previous reports it can be concluded that the *L. minor* presence enhances the degradation/removal of organic materials from wastewater and can be recommended as a suitable biomass for removal of organic matters in secondary or tertiary treatment of wastewater (Körneret al., 2003).

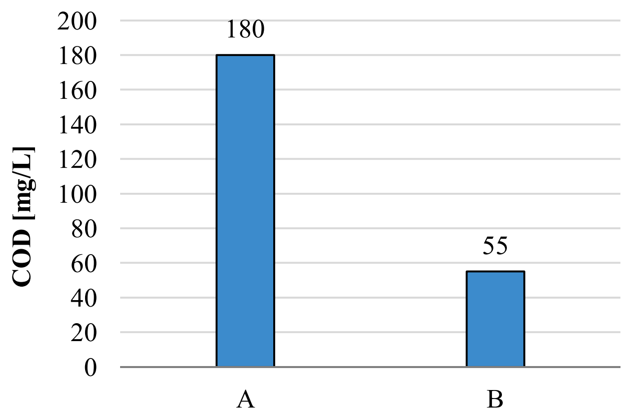

Fig.1. COD values of the wastewater before (A) and after (B) phytoremediation using *L. minor*

2. Removal of heavy metals from wastewater using L. minor

The phytoextraction potential of *L. minor* was assessed in terms of the removal efficiency and adsorption capacity. All the investigated heavy metals (As, Cd, Pb, Zn, Cu, Ni) present in the wastewater subjected to treatment were higher than the permissible limits, according to the Romanian legislation (Norm 002/2002). Generally, the As, Cd, Cr, Co, Mn, and Pb removal ranged from 96.9 to 99.4%. The highest removal efficiency was observed in the case of Cr and Co. No changes or a small decrease in the Cu, Ni and Zn concentration were observed after the phytoextraction experiments.

Bokhari et al. 2015 recommended to apply primary treatment to wastewaters, to reduce the organic contaminants, in order to enhance the *L. minor* metal (Cd, Cu, Ni and Pb) accumulation capacity (Bokhari et al., 2015). High concentrations of macronutrients (Ca, K, Mg, Na, P, S, and Si), micronutrients (Fe, Zn, Cu, and Mn), and heavy metals (Ag, Cd, Co, Ni, and Pb) can disturb the nutrient uptake by the plants, can inhibit photosynthesis, and can produce several limitations in the metabolites biosynthesis of the plant or in the physiological processes (Hajihashemi et al., 2020).

Based on the obtained results, it can be concluded that the presence of various metal and organic pollutants had an antagonistic effect on accumulation of Cu, Ni and Zn by the *L. minor* genotype. These results also suggest that the *L. minor* metabolite undergoes some modifications during the phytoremediation experiments, which can include the activation of the defence mechanism at heavy metal stress. The obtained results revealed that *L. minor* was able to accumulate high amounts of Mn and Pb and low amounts of Cd (Figure 2). This can be due to high concentration of Mn in the wastewater (160 times higher than the Cd concentration in the untreated wastewater). Similar results were obtained by Velichkova 2019, who reported a metal downtrend in the following order Mn>Fe>Zn>Cu>Ni>Cr>Pb>Cd accumulated by the *L. minor* during wastewater treatment (Velichkova et al., 2019).

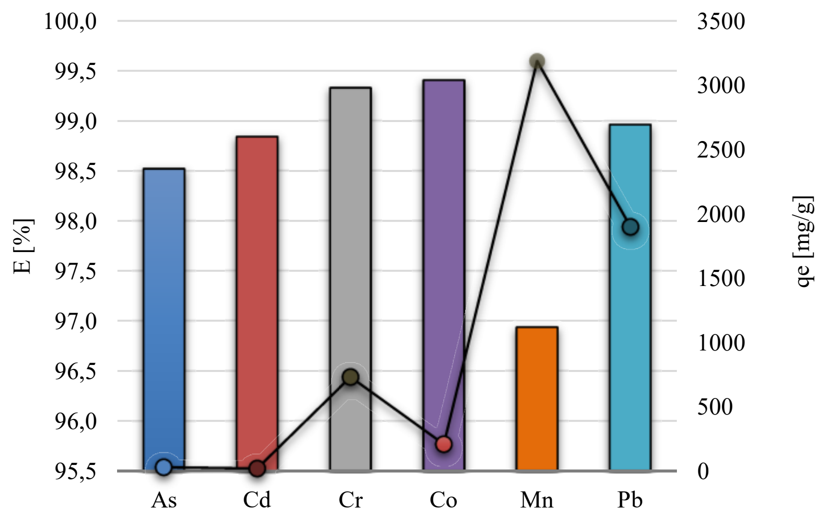

Fig. 2. Removal efficiency and adsorption capacity of heavy metals onto *L. minor*

Several changes were observed in the *L. minor*'s macro and micro-element content after 7 days of phytoextraction (Figure 3). The *L. minor* accumulated high concentrations of Na, Al, Li and Hg, from the wastewater, with 31-, 28-, 25- and 13-fold higher than the control. Moreover, *L. minor* accumulated concentrations of Sc, Sr, Ba, Rb, Ti, Fe, Sn, Mg, Ca, Se, Be, and V from wastewater, with 3.5 to 1.1 times higher compared to the control. The results suggest that the metal accumulation potential of *L. minor* genotype is strongly influenced by the initial concentration of the elements. The *L. minor* easily accumulated Na and Al, which were found in higher concentration in the wastewater than other elements, such as Sc, Sr or V.

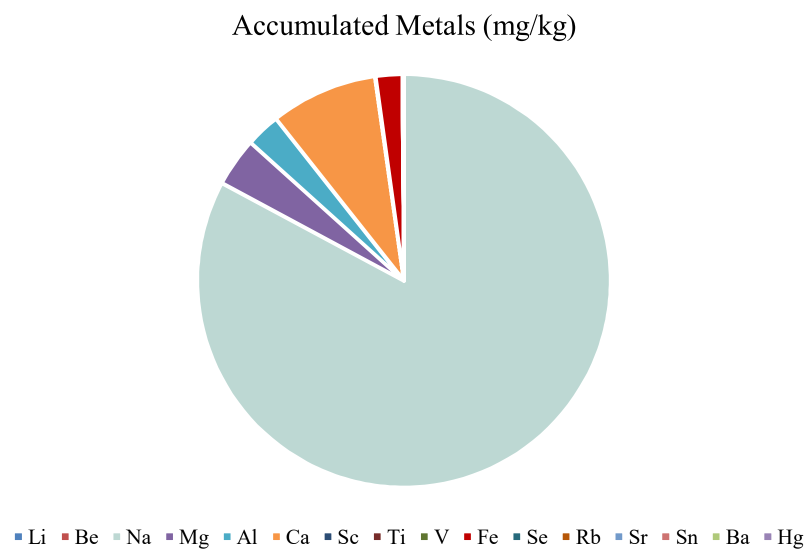

Fig. 3. Accumulated metals (mg/kg DW) after the phytoextraction period

CONCLUSIONS

The wastewater with a high content of various metals and organic pollutants had various effects on the plant's macro- and micro-element content. The results indicated an antagonistic effect after the accumulation of Cu, Ni and Zn. The Cu, Ni and Zn are essential micronutrients and are known to have pivotal roles in cellular metabolism and in the defence mechanisms against oxidative stress. The decreased concentration of these elements may suggest the activation of the plant's defence mechanism as a response to the heavy metal stress. Thus, synergistic effects were observed in the accumulation of high amounts of Mn, Pb, Cr, Na, Al, and Hg. The COD value indicates that the *L. minor* genotype has a great ability to reduce the organic pollutants from the wastewater with 69.4%. The obtained results suggest that the local *L. minor* genotype showed a great capability to reduce metal and organic pollutants from wastewaters with high removal efficiencies. The present study highlights the phytoextraction potential of the *L. minor* genotype. Further investigations to identify the plant's defence mechanism in order to design a highly efficient decontamination process are necessary.

ACKNOWLEDGMENT. This work was funded by the Core Program, under the support of ANCS, project no. PN 19-18.01.01 (contract no. 18N/08.02.2019). The funder had no role in the design of the study; in the collection, analysis and interpretation of data; in the writing of the manuscript, and in the decision to submit the article for publication.

REFERENCES

1. Amare E., Kebede F., Mulat W. (2018). Wastewater treatment by *Lemna minor* and *Azollafiliculoides* in tropical semi-arid regions of Ethiopia, Ecological Engineering, 120, 464-473.
2. Anastopoulos I., Pashalidis I., Hosseini-Bandegharaei A., Giannakoudakis D. A., Robalds A., Usman M., Escudero L.B., Zhou Y., Colmenares J.C., Nunez-Delgado A., Lima, É. C. (2019). Agricultural biomass/waste as adsorbents for toxic metal decontamination of aqueous solutions, Journal of Molecular Liquids, 295, 111684.
3. Bakshia P.S., Selvakumara D., Kadirvelub K., Kumara N.S. (2020). Chitosan as an environment friendly biomaterial – A review on recent modifications and applications, International Journal of Biological Macromolecules, 150, 1072-1083.
4. Bokhari S. H., Ahmad I., Mahmood-Ul-Hassan M., Mohammad A. (2015). Phytoremediation potential of *Lemna minor L.* for heavy metals, International Journal of Phytoremediation, 18(1), 25-32.
5. Ceschin S., Abati S., Ellwood N. T. W., Zuccarello V. (2018). Riding invasion waves: Spatial and temporal patterns of the invasive *Lemna minuta* from its arrival to its spread across Europe, Aquatic Botany, 150, 1-8.
6. Ceschin S., Sgambato V., Ellwood N. T. W., Zuccarello V. (2019). Phytoremediation performance of *Lemna* communities in a constructed wetland system for wastewater treatment, Environmental and Experimental Botany, 162, 67-71.
7. Ekperusi A.O., Sikoki F.D., Nwachukwu E.O. (2019). Application of common duckweed (*Lemna minor*) in phytoremediation of chemicals in the environment: State and future perspective, Chemosphere, 223, 285-309.

8. Hajihashemi S., Mbarki S., Skalicky M.,. Noedoost F, Raeisi M., Brestic M. (2020). Effect of Wastewater Irrigation on Photosynthesis, Growth, and Anatomical Features of Two Wheat Cultivars (Triticum aestivum L.), Water, 12.

9. Hoagland D.R. and Arnon D.I. (1950). The water-culture method for growing plants without soil, Circular. California Agricultural Experiment Station, 347(2), 32.

10. Hu H., Li X., Wu S., Yang C. (2020). Sustainable livestock wastewater treatment via phytoremediation: Current status and future perspectives, Bioresource Technology, 213, 123809.

11. Körner S., Vermaat J. E., Veenstra S. (2003). The Capacity of Duckweed to Treat Wastewater, Journal of Environment Quality, 32(5), 1583-1590.

12. Li X., Wu S., Yang C., Zeng G. (2020). Microalgal and Duckweed Based Constructed Wetlands for Swine Wastewater Treatment: A Review*,* Bioresource Technology, 123858.

13. Mansoori S., Davarnejad R., Matsuura T., Ismail A.F. (2020). Membranes based on non-synthetic (natural) polymers for wastewater treatment, Polymer Testing, 84, 106381.

14. McLaughlin M.C., Borch T., McDevitt B., Warner N.R., Blotevoge J. (2020). Water quality assessment downstream of oil and gas produced water discharges intended for beneficial reuse in arid regions, Science Total Environmental, 713, 136607.

15. Michael I., Michael C., Duan X., He X., Dionysiou D.D., Mills M.A., Fatta-Kassinos D. (2015). Dissolved effluent organic matter: Characteristics and potential implications in wastewater treatment and reuse applications, Water Research, 77, 213-248.

16. Mudyawabikwa B., Mungondori H.H., Tichagwa L., Katwire D.M. (2017). Methylene blue removal using a low-cost activated carbon adsorbent from tobacco stems: kinetic and equilibrium studies, Water Science Technology, 75(10), 2390-2402.

17. Nakkasunchi S., Hewitt N. J., Zoppi C., Brandoni C. (2021). A review of energy optimization modelling tools for the decarbonisation of wastewater treatment plants, Journal of Cleaner Production, 279, 123811.

18. Norm regarding the conditions of wastewater discharge in the sewerage networks of the localities and directly in the treatment plants, NTPA-002/2002 of 28.02.2002.

19. Paolacci S., Jansen M. A. K., Harrison S. (2018). Competition Between *Lemna minuta*, *Lemna minor*, and *Azollafiliculoides*, Growing Fast or Being Steadfast?, Frontiers in Chemistry, 6.

20. Rosen B. and Morling S. (1998). A systematic approach to optimal upgrading of water and waste water treatment plants, Water Science and Technology, 37, 9 - 16.

21. U.S. Geological Survey. National Water Information System. http://waterdata.usgs.gov/nwis (accessed 20 October 2020).

22. Velichkova K. (2019). Bioconcentration efficiency of *Lemna minor L.* and *Lemnagibba L.* for trace metals in three southeastern Bulgarian water reservoirs, Anales de Biología, 41, 5-10.

23. Vijayaraghavan K. and Balasubramanian R. (2015). Is biosorption suitable for decontamination of metal-bearing wastewaters? A critical review on the state-of-the-art of biosorption processes and future directions, Journal of Environmental Management, 160, 283-296.