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Bioaccumulation and phytoremediation of some heavy metals (Mn, Cu, Zn and Pb) by bladderwort and duckweed

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Abstract. *Azeez NM. 2021. Bioaccumulation and phytoremediation of some heavy metals (Mn, Cu, Zn and Pb) by bladderwort and duckweed. Biodiversitas 22: 3093-3098.* This paper discussed the changes in some heavy metals (Mn, Cu, Zn, and Pb) in *Utricularia vulgaris* L. (Bladderwort) and *Lemna minor* L. (Duckweed) in the sewage treatment unit at the University of Basrah Campus, Iraq. For all examined heavy metals, the better accumulator was *U. vulgaris* than *L. minor*. This study proved that both plants significantly reduced the concentrations of heavy metals in wastewater. The efficiency of removing heavy metals was more than 68%. For both species, the bioconcentration factor (BCF) values of the tested metals were <1, and the BCFvalues decreased in the following order in both species: Pb>Mn>Cu>Zn. Reduction rates of pH, TSS, TDS, and conductivity were significant at the end of the experiment. The results showed that the tested macrophytes exhibit a good characteristic as bioaccumulators and could be effectively used in phytoremediation techniques.

Keywords: Accumulation, bioconcentration factor, *Lemna minor*, metals contamination, *Utricularia vulgaris*

INTRODUCTION

Heavy metals are typical components of Earth's crust, but certain heavy metals' concentrations reached toxic levels because of anthropogenic activities' consequences in many ecosystems. In recent years, pollution of the environment with heavy metals has become a significant global problem of modern society with characteristic cumulative effects (Tchounwou et al. 2012; Nouri and Haddioui 2016). Almost every human activity result in the production of waste. In water use, this is more so, as nearly 80% of water ends up as wastewater (Emenike et al. 2015). The management of this wastewater can, if not well handled, be a problem. One way to reaching environmental sustainability is via effective wastewater treatment. The treatment of wastewater is a critical community practice for both environmental and public health reasons. Heavy metals in wastewater testing are carried out to determine their consistency, potential changes, make adjustments to the measures if required, and assess drainage systems' functionality.

Exposure of aquatic plants to toxic levels of heavy metals starts a broad spectrum of physiological and metabolic changes (Jaishankar et al. 2014; Ashfaque et al. 2016; Sarma and Prakash 2020), which include the following: decreasing in plant's growth, changing in cell metabolism, damage of cell membranes, proteins changing and inhibition of photosynthesis, which leads to senescence and, in the end, to plants death (Paunov et al. 2018; Varma 2021). Some aquatic plants have a good ability to tolerate increased levels of heavy metals and accumulate them up to unusually high concentrations, causing accumulative effects (Pant and Tripathi 2014; Solomon et al. 2015; Al-Abbawy et al. 2021). Plants growing in polluted environments are often under stress because of bioaccumulation through direct uptake by root, stem, or sprouts (Babović et al. 2010).

Current technologies available for mitigation of pollution are costly, and in most cases, they represent a cost for industry (Rai 2009). Aquatic plants are used as bioindicators of water quality and phytosanitary of water pools more often (Ceschin et al. 2010; 2012). Various studies (Azeez and Sabbar 2012; Taha et al. 2019; Ali et al. 2020) showed that aquatic macrophytes concentrate certain metals to various degrees; hence, critical levels could vary among species. Phytoremediation is a relatively new approach to the cost-effective treatment of wastewater, groundwater, and soils contaminated by organic, heavy metals, and radionuclides (Singh et al. 2017; Prieto 2018).

Phytoremediation has been described as an advanced bioremediation method that uses flowering plants and associated microbes to extract, pass, stabilize, metabolize, degrade, or volatilize pollutants in soil and water for environmental cleanup (Carvalho and Martin 2001; Mahmoud and Hamza 2017). Plants used in phytoremediation should be ideal for the setting and can withstand stress induced by pollutants (Njoku et al. 2009). Phytoremediation is a non-destructive and cost-effective technology that uses plants to extract toxins from soil and water.

Babović et al. (2010) and Tanwir et al. (2020) found that some macrophytes, including *Typha latifolia, Phragmites australis, Ceratophyllum demersum, and Salvinia natans*, are good indicators of some heavy metal contamination and may have remedial properties.

Some studies of aquatic plants were performed in laboratory conditions. Weiss et al. (2006) highlighted in a laboratory study on heavy metal phytoremediation by three wetland macrophytes that all tested species accumulated high concentrations of the four examined heavy metals compared with its media. Abdallah (2012) investigated two aquatic macrophytes, *Ceratophyllum demersum* and *Lemna gibba*, to remove two toxic metals (Pb and Cr) from an aqueous solution.

This study aimed to examine aquatic plants' ability of two different aquatic macrophytes, *Utricularia vulgaris* and *Lemna minor*, to bioaccumulate heavy metals in laboratory conditions and the possibility of their use in the phytoremediation of some heavy metals in the main wastewater unit of the University of Basrah campus, Iraq.

MATERIALS AND METHODS

Study area

Wastewater samples (five liters) were collected from the main wastewater treatment unit in the University of Basrah, Qarmat Ali campus, Iraq located at 30º 33' 21.3" N, 47º 44'56.7" E. Besides the teacher residence camp and Student accommodation, many scientific laboratory activities in the University are the main source of wastewater collected by the treatment unit. The wastewater passes through large tanks then discharges to Shatt Al-Arab River directly. The wastewater collected from the campus passes a primary treatment in large tanks so the sludge can settle. This step produces a homogenous liquid capable of being treated by some aquatic plants.

Wastewater and aquatic plant samples

Samples were the wastewater that had been primarily treated and discharged collected in sterilized 1000 mL polyethylene bottles and transported to Basrah University's laboratory. Physical parameters such as Total dissolved solids (TDS), pH, conductivity, total suspended solids (TSS), and temperature were tested in-situ using a handheld Hanna multimeter (model HI9813-6).

Plants samples (1 kg) were collected in shallow parts of Huwaiza marsh (South of Iraq). The collection of fresh aquatic plants, the bladderwort *Utricularia vulgaris*, and the duckweed *Lemna minor* during the vegetation season was carried out.

Sample analysis

In the laboratory, plant material was sorted, rinsed in tap and distilled water, dried in an oven at 105 °C, and was prepared for further chemical analysis applied for water and aquatic plants (APHA, 2005). The wastewater samples were tested for chemical contaminants such as manganese (Mn), lead (Pb), copper (Cu), and zinc (Zn). All sample analyses were done by following standard methods prescribed by APHA, AWWA, WEF (2012). All tests were carried out in triplicate, and the average value was recorded.

Experimental setup

The experimental setup followed the procedure of Daud et al. (2018). One collection of containers for experimentation was arranged to have three tubs. Each container of investigation 10 L of wastewater and 100 g of fresh bladderwort and duckweed separately. Three replicates of the experiment were performed to achieve the plant's efficiency. The temperature was 23 ± 5°C and a 12-hour photoperiod during the investigation. The average humidity at the experimental site was 65 ± 15 percent. The testing period for the physicochemical characteristic of wastewater and removing heavy metals was 0, 3, 6, 9, 12,15, 18, and 21 days.

Estimation of heavy metals in wastewater and plant samples

Plant samples were thoroughly washed until they were dried at 70 ° C in the oven. Plant samples after complete drying were crushed and sieved < 1 mm. Samples from plants (0.25 g each) were gradually digested with diacids (HNO_3-$HClO_4$) as used by Uddin et al. (2016) with increasing temperature. Following complete digestion, distilled water was added to the sample to make the final volume up to 50 mL.

Concentrations of heavy metals were well-defined by atomic absorption spectrophotometry (AAS) directly from the basic liquid. Samples were recorded on atomic absorption spectrometer Phoenix 986(UK), using a flame technique, standard method EPA 7000B.

Calculation

The uptake of heavy metals by the plant was calculated using the dilution factor as follows:

Dilution factor = total volume of sample (mL)/ weight of the plant (g)

Percentage efficiency was calculated by determining the initial (Ci) and final concentration (Cf) of metals in the sample.

Removal (%) = Ci − Cf/Ci∗ 10

Where; Ci and Cf are the initial and final metal concentrations in the medium ($mg \cdot L^{-1}$).

The bioconcentration factor was calculated as the ratio of heavy metals concentration in plants' tissue relative to its water content (Mkumbo et al. 2012). High BCF values involve a higher ability of the plant to bioaccumulate a particular heavy metal.

BCF = metal concentration in plant ($mg \cdot kg^{-1}$)/ metal concentration in medium ($mg \cdot L^{-1}$)

Statistical analysis

The results were statistically processed using the SPSS program (Statistical Package for the Social Sciences) v.23. All the analyses were performed in three independent repetitions. The Shapiro-Wilk and Levene tests were carried out to assess data normality and homogeneity, respectively. The analyzed parameters were processed using the variance method (ANOVA) followed by Tukey's test to get the significant difference between different mean values.

RESULTS AND DISCUSSION

Physico-chemical characteristics wastewater

Table 1 shows the results of the potential of the phytoremediation test on *U. vulgaris* and *L. minor*. By the end of the 21-days experiment, the pH of wastewater decreased from its initial value of 7.60 to the final value of 7.13 in *U. vulgaris*. Under a wide range of pH (Iqbal and Baig 2016), Duckweed (*L. minor*) can thrive. The values are within that recommended pH standard limits (6.0 to 9.0) of WHO (2017).

The TSS and TDS values in wastewater were 58.13 $mg{\cdot}L^{-1}$ and 3320 $mg{\cdot}L^{-1}$, respectively.TSS concentration decreased over time, reaching a minimum level of 27.66 $mg{\cdot}L^{-1}$ at the end of the experiment of *U. vulgaris*. The reduction of TSS was consistent with the previous studies (Aziz et al. 2020). Meanwhile, after the 21-day experimental duration, the least TDS value of 2493 $mg{\cdot}L^{-1}$ was reported. The TDS and conductivity were decreased during the experiment due to plants' ability to absorb inorganic and organic ions. Conductivity is a measure for the mineralization and calculating the amount of chemical reagents or treatment chemicals to be added to the water. Electrical Conductivity results from total dissolved ions in the aquatic ecosystem, and according to Walakira and Okot-Okumu (2011), it is revealing of some influence from human activities in the catchments. However, this study is mostly attributed to the release of chemical salts and high TDS from the campus effluent. This is reflected in the high TDS recorded in wastewater.

A high concentration of dissolved solid elements affects the density of water, influences osmoregulation of freshwater organisms, and decreases the solubility of gases (like oxygen) and possibility of water being used for drinking, irrigational, and industrial purposes (Singare et al. 2012).

Four heavy metals (Mn, Pb, Cu, and Zn) have been analyzed in the present study. The levels of these pollutants in the considered wastewater samples are shown in Table 1. The concentration of Zn in the wastewater decreased from 2.29 $mg.L^{-1}$ to 0.69 $mg.L^{-1}$ in the presence of *U. vulgaris*, while it reached 0.78 $mg.L^{-1}$ in the presence of *L. minor*. The other heavy metals took the same trend in decreasing at the end of the experiment. Because of their metal uptake mechanism, the tested plants can act as an accumulator. The heavy metal in the wastewater will accumulate in the leaves tissues of the plants, where they are biodegraded or biotransformed into active forms (Sinha et al. 2004).

Different activities such as wastewater from the university campus were discharged into a river. This has resulted in severe pollution problems in the aquatic environment and may cause harmful effects to the ecosystem and human life.

Heavy metals are conservative pollutants, so they accumulate in the body and, over time, begin to fester, leading to several symptoms of diseases (Khan and Malik 2014). Thus, untreated or incompletely treated wastewater from the treatment unit can be harmful to aquatic life by adversely affecting the natural ecosystem and long-term health effects. Adequate wastewater treatment such as conventional and phytoremediation methods before their final discharge is one way to driving water pollution prevention.

Concentrations of heavy metals in aquatic plants

Submerged and floating species store metals in leaves and contribute significantly to the extraction of heavy metals from water basins (Murat et al. 2014; Ali et al. 2020). Different concentrations of Mn (Figure 2) amongst submerged and floating species were found; this was also pointed by other researchers (Branković et al. 2009; Babović et al. 2010; Polechońska et al. 2019).

Data on plants' ability to accumulate different metal types could be critical in selecting species in the phytoremediation technique. In the tissue of *U. vulgaris*, the highest Mn concentrations were measured, while there was an increase in concentrations but a significant decrease in the Mn content observed in *L. minor* after 12 days. Research by Branković et al. (2009) showed that submerged plants had a smaller Mn accumulation capacity than floating plants, but not following our findings. In contrast, the Mn content in the tissue of *U. vulgaris* varied more compared to *L. minor.*

Cu contents in the tissue of *U. vulgaris* varied from 0.11 to 0.15 $mg.kg^{-1}$ (Figure 3), whereas higher contents were measured in this plant. Other authors also showed that Cu accumulated better in submerged tissue than floating plants (Stanković et al. 2000; Babović et al. 2010; Maksimović et al. 2020).

Table 1. Physiochemical characteristic of wastewater and phytoremediation at the end of the experiment (21 days)

Parameter	Unit	Phytoremediation (% reduction)				
		Before (wastewater)	After (with plants)			
		U. vulgaris	**% Reduction**	***L. minor***	**% Reduction**	
pH		7.6	7.13	6.184	7.32	3.684
TSS	$mg.L^{-1}$	58.13	27.66	52.417	34.12	41.303
TDS	$mg.L^{-1}$	3320	2493	24.909	2524	23.975
EC	mS.cm	5.22	3.92	24.904	3.97	23.946
Zn	$mg.L^{-1}$	2.92	0.69	76.369	0.78	73.287
Pb	$mg.L^{-1}$	0.73	0.23	68.493	0.31	57.534
Cu	$mg.L^{-1}$	0.29	0.08	72.413	0.11	62.068
Mn	$mg.L^{-1}$	0.41	0.11	73.170	0.17	58.536

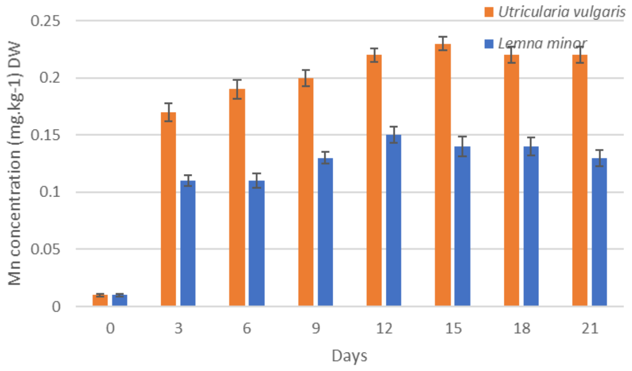

Figure 2. The concentration of Mn in *Utricularia vulgaris* and *Lemna minor* during experimental time (±SD)

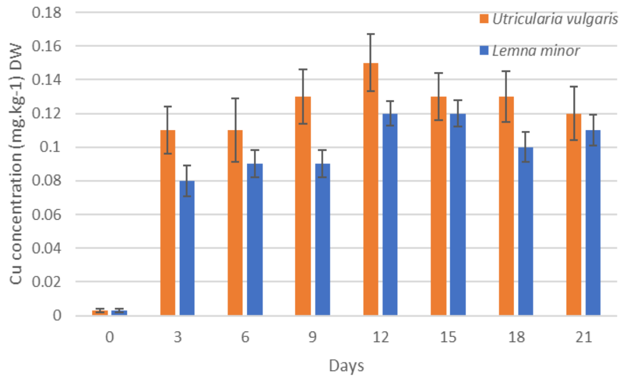

Figure 3. The concentration of Cu in *Utricularia vulgaris* and *Lemna minor* during experimental time (±SD)

The results in Figure 4 showed that the Zn contents for investigated species were highest at the start of the experimental period and then decreased, which was in line with the previous results (Maksimović et al. 2014). Significant differences ($P<0.05$) in Zn contents (accumulation capacity) were recorded between studied species: submerged *U. vulgaris* expressed the highest accumulation capacity compared to floating *L. minor* species. Zn contents during the experimental days in *U. vulgaris* tissue vary from 0.63 to 0.79mg.kg^{-1}. Zn content in the wastewater decreased by 76.3% by the end of the experiment. The results gained in this study show that Zn contents were higher in submerged leaves than floating leaves, which had been by the results of Stanković et al. (2000).

Lead contents in *U. vulgaris* and *L. minor* tissue during the research period were reached 0.37 and 0.27 mg.kg^{-1} respectively (Figure 5), which was significantly lower than previous research (Leblebici and Aksoy 2011; Štrbac et al. 2014; Maksimović et al. 2020).

The heavy metal uptake varied considerably with the metal and the plant species; the metals removed by *U. vulgaris* were higher than *L. minor* and ranged between 68.49% and 76.36% in 21 days experimental period (Figure 6). The removal efficiency was above 57.53% for all studied metals in *L. minor*, while it was above 68.49% in *U. vulgaris.*

For *U. vulgaris*, significant increases ($P<0.05$) in uptake were observed for all metals. The highest removal percentage was shown by *U. vulgaris* for both Zn and Mn, whereas this was the lowest for Pb in both aquatic plants.

Phytoremediation potential of *Utricularia vulgaris* and *Lemna minor*

Bioaccumulation results from the difference between the concentration of metals that the plant adopts and its concentration in water (Tangahu et al. 2011; Bonanno et al. 2017; Moogouei et al. 2017).

The plant accumulates heavy metals independently of their water concentration, which is evident from the results obtained. Differences between tested plants in bioaccumulation can result from the plants' phytoextraction of heavy metals because the bioaccumulation capacity of plants' tissue for certain metals is different. According to Koleli et al. (2015), a plant is considered a good bioaccumulator if the BCF exceeds 1. Results of BCF (Table 2) showed that the leaves of *Utricularia vulgaris* and *Lemna minor*, indicated that the accumulation of heavy metals decreases in the following order: Pb> Mn >Cu>Zn. *U. vulgaris* has a higher BCF for all metals than *Lemna minor*. The highest value of BCF in *U. vugaris* was 0.561 for Mn, while it was 0.356 in *L. minor* for Pb.

Table 2. Bioconcentration factor for *Utricularia vulgaris* for heavy metals from wastewater during experimental days

Metal	Experimental time (days)						
	3	6	9	12	15	18	21
Utricularia vulgaris							
Zn	0.267	0.271	0.233	0.229	0.223	0.216	0.216
Pb	0.425	0.493	0.479	0.479	0.493	0.452	0.438
Cu	0.379	0.379	0.448	0.517	0.448	0.448	0.414
Mn	0.415	0.463	0.488	0.537	0.561	0.537	0.537
Lemna minor							
Zn	0.003	0.236	0.243	0.226	0.219	0.219	0.209
Pb	0.014	0.315	0.301	0.356	0.342	0.370	0.356
Cu	0.010	0.276	0.310	0.310	0.414	0.414	0.345
Mn	0.024	0.268	0.268	0.317	0.366	0.341	0.341

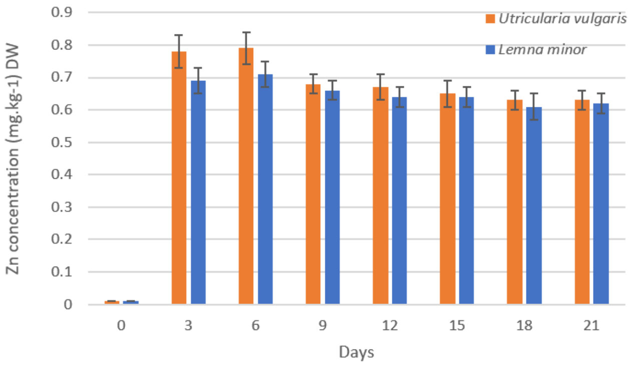

Figure 4. The concentration of Zn in *Utricularia vulgaris* and *Lemna minor* during the experimental time (±SD)

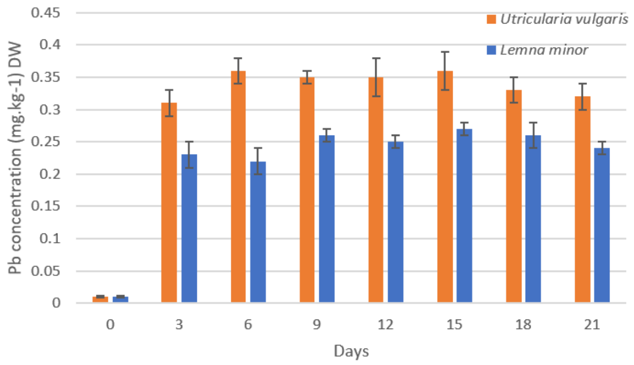

Figure 5. The concentration of Pb in *Utricularia vulgaris* and *Lemna minor* during the experimental time (±SD)

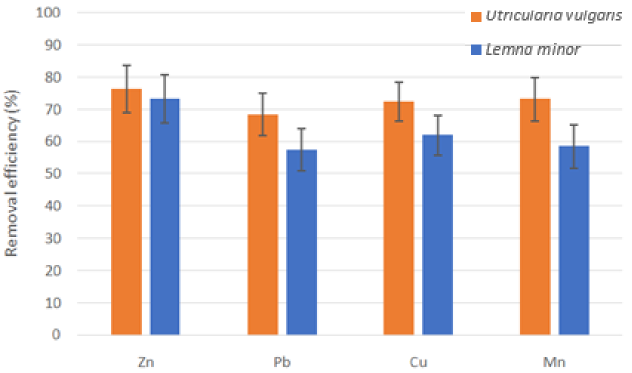

Figure 6. Removal efficiency (%) for wastewater by *Utricularia vulgaris* and *Lemna minor*at 21 days of the experimental time(±SD)

This shows the potential of *Utricularia* in the field of heavy metal accumulation and phytoremediation (Wani et al. 2017). The *Lemna* species has also been shown as the most used plant for phytoremediation than the other aquatic plants. It has been used widely for the phytoremediation of heavy metals (Soni and Kaur 2018).

Reduction of wastewater physiochemical characteristics was apparent using *U. vulgaris* and *L. minor* as phytoremediators. Based on the measured heavy metals concentrations (Mn, Cu, Zn, and Pb) in water, we can conclude that investigated localities are not overloaded by these pollutants, dangerous for the ecosystem. Considering all the results obtained, we can conclude that *U. vulgaris* and *Lemna minor* accumulate Mn and Zn to a high degree with the superiority of *U. vulgaris*. The results acquired in this study in the wastewater area imply that bioaccumulation of heavy metals and their availability to the plants had not reached a critical level. Hence, it is important to set constant monitoring to include monitoring seasonal and spatial variations in the accumulation of heavy metals to estimate researched species' capacity in phytoremediation techniques.

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