

Uptake of Some Elements with Aquatic Plants Exposed to the Effluent of Wastewater Treatment Plant

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ABSTRACT: In this study, in the removal of macro (P, S, Na, K, Ca, Mg) and micro (Sb, Ba, Co, Cu, Fe, Pb, Mn, Hg, Mo, Se, Ag, Zn) elements in the effluent of Elazig Wastewater Treatment Plant, the efficiency of *Lemna minor* L. and *Lemna gibba* L. has been studied comparatively. For this aim, fronds of these plants have been adapted to the effluent of the treatment plant that feeds pilot scale reactors. The concentrations of elements in fronds harvested during the working period were analyzed by ICP/MS. The analytical results show that *Lemna minor* L. has a high accumulation potential for P, Sb, Ba, Co, Fe, Pb, Mn, Hg, Ag and Zn while *Lemna gibba* L. has a high accumulation potential for S, Na, Ca, Mg, Cu, Mo and Se. The results show that *Lemna minor* L. and *Lemna gibba* L. can be used as phytoremediators of wastewater.

Keywords: Accumulation, *Lemna gibba* L., *Lemna minor* L., phytoremediation, wastewater

INTRODUCTION

Researchers detected organic and inorganic (macro and micronutrient elements) substances in high concentrations in the wastewater treatment effluents discharged into the surface waters such as lakes, rivers and streams (Chen et al., 2005; Upadhyay et al., 2007; Azarpira et al., 2014). Unlike many organic substances in the ecosystem, heavy metals cannot be degraded by biological means. For this reason, heavy metals accumulate in water, food and sediment and reaches up to human by way of food chain. These element ions, which accumulate in biological systems, inhibit biological activity

by damaging cell functions after a certain threshold value. For this reason, it has been reported that their high concentrations must be removed from the discharge waters before entering the receiving environment (Lone et al., 2008; Mishra & Tripathi 2008; Singh et al., 2012; Azarpira et al., 2014). Many physico-chemical methods such as chemical precipitation, ultrafiltration, chemical oxidation and reduction, electrochemical purification, reverse osmosis, coagulation-flocculation, ion exchange is commonly used to remove these chemicals from the water. These methods, which have high investment and operating costs and high energy requirements, offer different efficiencies for

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different metals. When the metal concentration in water is in the range of 10-100 mg/L they can be ineffective or excessively expensive (Miretzky et al., 2004; Hou et al., 2007). In recent years, phytoremediation techniques have been used as an environmentally friendly method, which is more effective and efficient, has lower investment and operating costs, requires less human power, and has less energy consumption than these methods (Singh et al., 2012; Dhir & Srivastava, 2013; Chaudhuri et al., 2014; Ren et al., 2017). It is known that *Lemna minor* L. and *Lemna gibba* L., which are small and free floating duckweed (*Lemnaceae* family) species, which grow in shallow water bodies has the ability to remove macro and micro elements such as P, Ca, Mg, Zn, Ni, Fe, Cd, Cr, Fe, Cu, Mn, Pb from aqueous solutions (Rakhshae et al., 2009; Khellaf & Zerdaoui, 2010). They have been suggested to be used as a test organism in polluted water studies because of their specific properties as described above.

Duckweed (family *Lemnaceae*) is a small, fragile, free-floating aquatic plant that flourishes in quiescent, shallow water bodies) (Öbek, 2009; Chaudhuri et al., 2011; Singh et al., 2012; Lu et al., 2011; Priya et al., 2012; Şaşmaz & Öbek, 2012; Favas et al., 2016). Many of these studies involve data on laboratory conditions that do not fully reflect natural conditions, or on the removal of one or more elements in dilute wastewater or synthetically prepared aqueous solutions. However, under natural conditions, aquatic macrophytes and other living things are not in contact with one another but with a mixture of different levels of one or more elements. An element in the mixture may act as a synergistic, antagonistic, or negligible mild enhancer of the other elements in the mixture (Paustenbach, 2000). Therefore, research data are needed to elucidate the absorption/accumulation efficiency or metabolic diversity of aquatic macrophyte

species at the site conditions. For the above reasons, in this study, it was aimed to determine the P, S, Na, K, Ca, Mg, Sb, Ba, Co, Cu, Fe, Pb, Mn, Hg, Mo, Se, Ag and Zn elements accumulation capacity of *Lemna minor* L. and *Lemna gibba* L. fronds from the effluent of the Elazığ Municipal Wastewater Treatment Plant (EMWWTP).

MATERIAL AND METHODS

The details of the practice and analysis in this study are carried out as given in Tatar & Öbek, 2014. *Lemna minor* L. and *Lemna gibba* L. plants were selected as material because of their properties as tolerance to environmental conditions, their prevalence and potentials to remove pollutants which are proved. These aquatic macrophytes were obtained from natural water ponds.

Four conical reactors (diameter of the top 50 cm, diameter of the bottom 40 cm, water column height 12 cm) which were feed with effluent of EMWWTP were operated with flow of 0.02 L/min. *Lemna gibba* L. and *Lemna minor* L. fronds were monocultured (Fig.1). 200 g *Lemna gibba* L. fronds were placed in two reactors while 200 g *Lemna minor* L. fronds were placed in the other two reactors. One of the reactors was used for daily analysis while the other one was used for plant replacement. Thus, plant was transferred from the second reactor to the first reactor which the plant samples are taken. 10 g of plant fronds and 50 mL of composite water samples were daily taken for element analysis. Water sample taken was filtered from the filter with pore size of 0.45 µm, and then placed in the tubes with 0.1% HNO₃. Furthermore, 100 mL of composite water samples were placed in tubes and let at 4 °C.

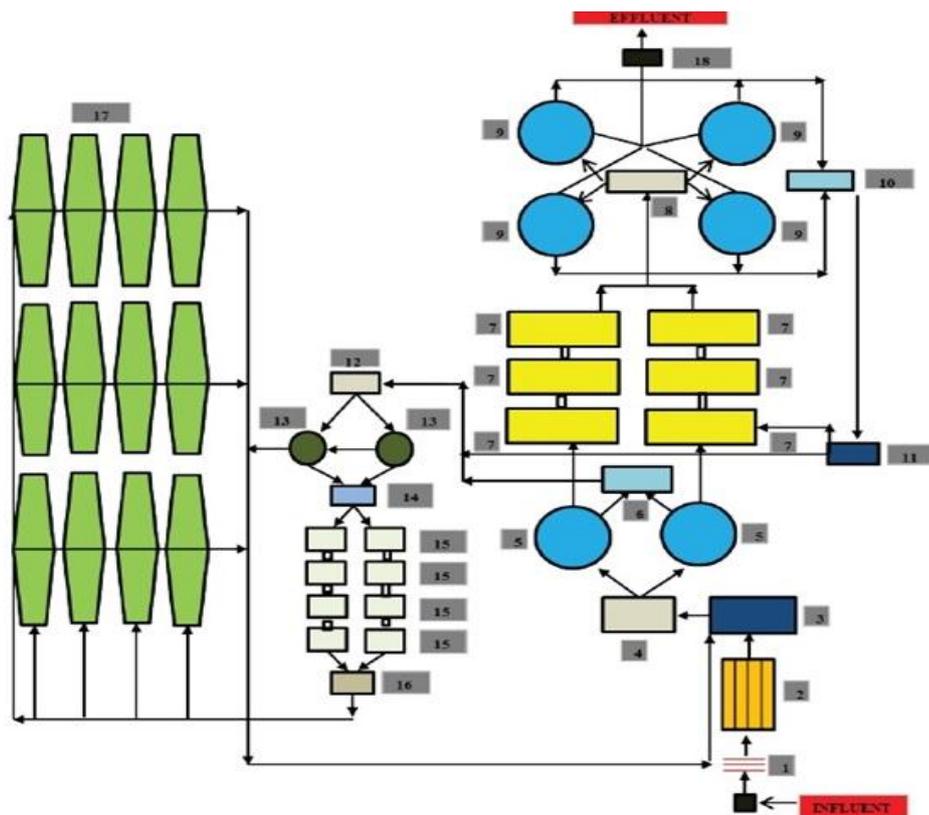
The WWTP is located in Elazığ city, Turkey. The WWTP was shown in Fig. 2. Elazığ Municipal Wastewater Treatment Plant consists of conventional activated-sludge system. In the EMWWTP, wastewater flows of about 36.000 and 54.000 m³/d (varies seasonally) are treated in a series of

pre-treatment (screens, a grit chamber), primary clarifier and cyclic activated sludge system, followed by a secondary clarifier, before being discharged into a surface water (Kehli stream) and eventually into Keban Dam Lake, which is an important source for

city to supply water (drinking, usage and irrigation water) from it (Topal & Arslan Topal, 2011; Topal et al., 2015). Physicochemical properties of effluent of the EMWWTP are given in Table 1.



Fig. 1. Experimental setup



(1) Bar screen, (2) Grit chamber, (3) Centrifuge pump, (4) Distribution building, (5) Primary clarifiers, (6) Telescopic valve, (7) Aeration tanks, (8) Distribution building, (9) Thickened sludge pump, (10) Telescopic valve, (11) Centrifuge pump, (12) Distribution building, (13) Sludge thickening tanks, (14) Thickened sludge pump chamber, (15) Sludge digestion tanks, (16) Digested sludge pump chamber, (17) Sludge drying beds, (18) UV disinfection, (Belt press unit is exist, but it does not operated)

Fig. 2. Elazig Municipal Wastewater Treatment Plant (Topal et al., 2014)

Table 1. Physicochemical properties of effluent of the EMWWTP

Parameters Unit	P ppb	S ppm	K ppm	Na ppm	Ca ppm	Mg ppm	Sb ppb	Ba ppb	Co ppb
Detection Limit	0.001	0.01	0.001	0.01	0.01	0.001	0.02	0.1	0.01
Wastewater (n=3)(±SD)	3266±105	30±2	18.33±0.2	112±6	79.54±2.3	30.13±0.1	0.34±0.05	39.38±3.3	0.29±0.2
Parameters Unit	Cu ppb	Fe ppb	Pb ppb	Mn ppb	Hg ppb	Mo ppb	Se ppb	Ag ppb	Zn ppb
Detection Limit	0.01	0.001	0.01	1	1	0.01	0.1	2	0.1
Wastewater (n=3)	3.1±0.3	466±32	15.6±0.2	37.4±0.2	<0.1	3.1±0.3	1.9±0.3	0.31±0.1	28.8±0.3

10 g plant fronds taken daily were dried under atmospheric conditions and then placed in porcelain crucible. The samples dried at 70 °C during 24 hours in incubator were weighted and net dry weights of the fronds were determined. All frond samples were ignited at 250 °C during 24 hours in muffle furnace. The water samples and ash samples were sent to Canada (ACME) for the analysis of elements (P, S, Na, K, Ca, Mg, Sb, Ba, Co, Cu, Fe, Pb, Mn, Hg, Mo, Se, Ag and Zn). The samples were analyzed by inductive coupled plasma mass spectroscopy (ICP/MS-Perkin-Elmer ELAN 9000) technique. Detection limit (DL) which several metals determined are as follows: P: 0.001, S: 0.01, Na: 0.001, K: 0.01, Ca: 0.01, Mg: 0.001, Sb: 0.02, Ba: 0.1, Co: 0.01, Cu: 0.01, Fe: 0.001, Pb: 0.01, Mn: 1, Hg: 1, Mo: 0.01, Se: 0.1, Ag: 2 and Zn: 0.1. Some of the results of the elements was previously presented at the International Energy and Conference held in Gaziantep (Turkey) (Tatar et al., 2017a; Tatar et al., 2017b). The analysis results done for plants were calculated as dry weight basis. All analysis was done triplicate. The results were evaluated by comparing the values of control group and the treated ones. Physicochemical analysis of water samples was done according to Standard Methods (APHA, 1995).

One-way variance analysis (ANOVA), Tukey test ($p < 0.05$), independent sample t test in SPSS (SPSS 15.0 for Windows) package program was used in the statistical

evaluation of the data obtained. Data were given as the mean of three independent analysis (\pm SD). Furthermore, correlations between the parameters were evaluated by Pearson correlation test.

RESULTS AND DISCUSSION

The concentration of some elements (P, S, Na, K, Ca, Mg, Sb, Ba, Co, Cu, Fe, Pb, Mn, Hg, Mo, Se, Ag and Zn) detected in the fronds of *Lemna gibba* L. and *Lemna minor* L. before and after the study were given in Figure 3.

Lemna minor L. fronds in the reactors with effluent accumulated elements from water as follows, except Ca and K: P by 770% (at day 7), S by 36% (at day 7), Na by 101% (at day 7), Mg by 9% (at day 1), Sb by 80% (at day 6), Ba by 39% (at day 7), Co by 95% (at day 5), Cu by 17% (at day 6), Fe by 101% (at day 4), Pb by 81% (at day 6), Mn by 1638% (at day 7), Hg by 310% (at day 6), Mo by 49% (at day 6), Se by 90% (at day 6), Ag by 469% (at day 6), Zn by 122% (at day 7) ($P < 0.05$). *Lemna gibba* L. fronds accumulated elements as follows, except K: P by 303% (at day 7), S by 44% (at day 4), Na by 119% (at day 7), Ca by 16% (at day 3), Mg by 12% (at day 2), Sb by 62% (at day 6), Ba by 18% (at day 4), Co by 51% (at day 7), Cu by 131% (at day 4), Fe by 32% (at day 4), Pb by 68% (at day 6), Mn by 14% (at day 4), Hg by 158% (at day 4), Mo by 142% (at day 7), Se by 130% (at day 4), Ag by 350% (at day 4), Zn by 21% (at day 4) ($P < 0.05$).

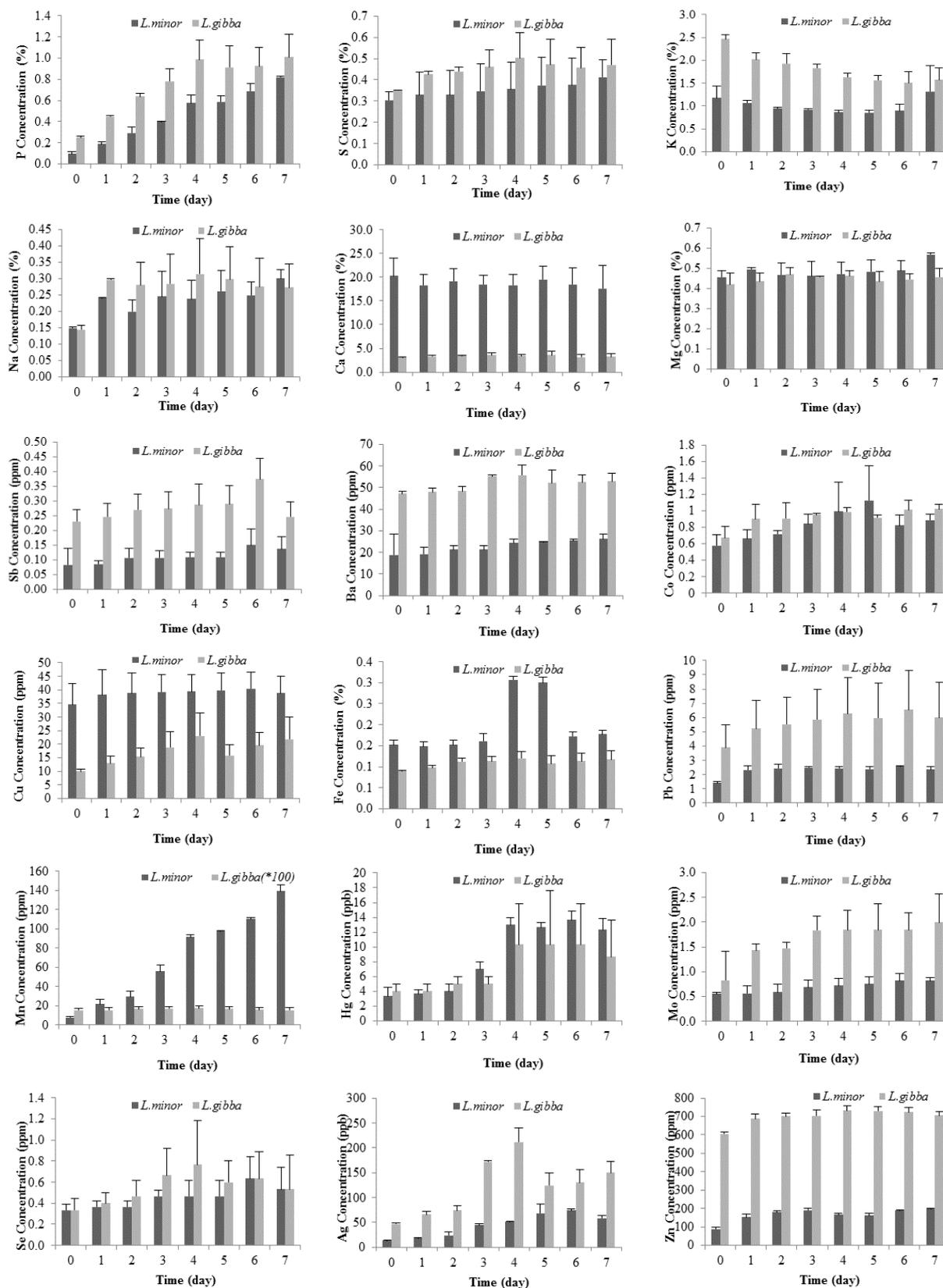


Fig. 3. The concentrations of some elements in the fronds of *Lemna gibba* L. and *Lemna minor* L. before and after the study

The analytical results showed that two different of the same genus growths in same conditions were accumulated macro and micro elements at various levels according to incubation period. According to the percentage of the mean of the accumulation levels in the fronds, the order of presence of *Lemna minor* L. to accumulate the elements is as follows: Mn> P> Ag> Hg> Fe> Zn> Co> Pb> Na> Se> Sb> Mo> Ba> S> Cu> Mg while the order of preference of *Lemna gibba* L. is as follows: Ag> P> Hg> Cu> Se> Mo> Na> Pb> Co> S> Fe> Sb> Zn> Ba> Mn> Ca> Mg. Furthermore, *Lemna minor* L. accumulated P, Sb, Ba, Co, Fe, Pb, Mn, Hg, Ag and Zn more efficiently than *Lemna gibba* L. *Lemna gibba* L. accumulated S, Na, Ca, Mg, Cu, Mo and Se more efficiently than *Lemna minor* L. ($p<0.05$). The results of this study were compared with the studies of Öbek (2009) and Şaşmaz & Öbek (2012) those were similar to the conditions of this study. Öbek (2009) and Şaşmaz & Öbek (2012) studied the accumulation of elements in *Lemna gibba* L. plant adapted to the waters of the secondary clarifier of the wastewater treatment plant. In the study of Öbek (2009), concentrations of P, Ca, Mg, Cd, Al, Fe, Zn, Ni, Pb, Cr were 0.66 (%), 3.66 (%), 0.38 (%), 0.046 ppm, 167 µg/g, 0.03 (%), 8 ppm, 7.2 ppm, 0.31 ppm, 0.9 ppm, in the plant structure before the study. In the next days of the study, particular increases were seen and the highest concentrations were reported as 0.74 (%) (at day 5), 4.3 (%) (at day 2), 0.38 (%), 0.07 ppm (at day 6), 767 µg/g (at day 2), 0.13 (%) (at day 5), 37.66 ppm (at day 6), 7.63 ppm (at day 5) ve 1.19 ppm (at day 5), 6 ppm (at day 5). Şaşmaz & Öbek (2012) reported that Ag and As concentrations in the fronds of *Lemna gibba* L. were 11 ppb and 26 ppb before the study and the maximum concentrations were 264.3 ppb (at day 6) and 64.33 ppb (at day 5). When we compare our study with these studies,

the accumulation values were lower than ours. This situation could be the result of the physicochemical properties of the water that the plant fronds were adopted. It was reported that some elements (P, S, Na, K, Ca, Co, Cu, Fe, Mg, Mn, Mo, Se and Zn) are essential while some do not (Sb, Ba, Pb, Ag and Hg) for several biochemical and physiological functions in biological systems (Tchounwou et al., 2012). It was reported that aquatic plants accumulate macro and micro element ions via their root and other organs higher than the concentrations detected in the water whether necessary or not for their lives. Also, it was reported that accumulation amounts were changed between kinds and the difference sourced from the biological characteristics (Demirezen & Aksoy 2004; Deng et al. 2004; Miretzky et al. 2004; Aksoy et al., 2005; Upadhyay et al. 2007; Bragato et al., 2009; Öbek 2009; Şaşmaz and Öbek 2012; Azarpira et al., 2014; Parzych et al., 2015). Parzych et al. (2015) reported that the absorbance and accumulation of the compounds by plants are especially as a result of the physiological request. For example, Mg is an important essential macronutrient for plant system and plays several important functions (Rehman et al. 2018), with some 75% of leaf Mg involved in protein synthesis and 15–20% of total Mg associated with chlorophyll pigments, acting mainly as a cofactor of a series of enzymes involved in photosynthetic carbon fixation and metabolism (Guo et al., 2016). The macronutrients (N, P, K, Ca, Mg) are required by plants in relatively large quantities (>0.1% of dry mass) (Maathuis, 2009; Munyati, 2018). Upadhyay et al. (2007) reported that the difference between the plant species reflects the metabolism activities and difference between their growths. This also showed the capacity difference on the element removal. It was reported that metal uptake could decrease

by time and accumulation because of the toxic effects (Duman et al., 2009; Azarpira et al., 2014) and the main aim of this mechanism. Some decreases at some days were seen in amounts of some elements accumulated in this study. Some of the amounts of the accumulated elements in *Lemna minor* L. fronds were as follows: Mg (at day 2), Al (at day 4), Sb (at day 7), As (at day 2) Co (at day 6), Fe (at day 5), Pb (at day 3), Hg (at day 7), Ag (at day 7), Zn (at day 4) and *Lemna gibba* L. S (at day 4), Na (at days 2, and 5-7), Ca (at day 3), Mg (at days 3-5), Sb (at day 7), As (at day 4), Ba (at day 4), Co (at day 5), Cu (at day 5), Fe (at day 5), Pb (at day 5), Hg (at day 7), Se (at day 5), Ag (at day 5), Zn (at day 7) (Figure 3).

These decreases could be result of the dropping back of the live to prevent the toxicity of the metals. Metal accumulation changed by time and concentrate at first days. These data show us that accumulated metals by *Lemna minor* L. and *Lemna gibba* L. are leaved to the water in some periods. To prevent this situation, the plants should be regularly harvested at certain times. Plant biomass containing accumulated heavy metals can be

combusted to get energy and the remaining ash is considered as “bio-ore”. This bio-ore can be processed for the recovery or extraction of the heavy metals. An advantage of phyto-mining is the sale of energy from combustion of the biomass (Anderson et al., 1999). Sood et al. (2012) reported that the accumulation difference between the different plant species or some plant kind and/or species was because of the tolerance level of them. They also reported that this difference was result of phytoremediation potential, chemical species and initial concentration of metals, temperature, pH redox potential, salinity and interaction between the heavy metals. Some elements increase the uptake of the others while the others decrease (Kacar & İnal, 2008). An element in the growth environment could effect of the absorption by the plant via enhancing of the effects of the other elements in the environment (synergistic), reducing (antagonistic) or milder at neglect level (simple interaction) effect (Paustenbach, 2000). In this study, the correlation results between elements in the fronds of *Lemna minor* L. and *Lemna gibba* L. under the effect of the effluent is given in Table 2.

Table 2. The correlation results between elements in the fronds of *Lemna minor* L. and *Lemna gibba* L. under the effect of the effluent

	P	S	K	Na	Mg	Ca	Ba	Mn	Ag	Hg	Fe	Zn	Co	Pb	Se	Sb	Mo	Cu
P		0.918	-0.961	0.689	0.592	0.298	0.838	0.461	0.834	0.874	0.917	0.879	0.887	0.926	0.785	0.567	0.951	0.920
S	0.977		-0.917	0.906	0.686	0.537	0.810	0.634	0.834	0.721	0.890	0.964	0.926	0.938	0.858	0.491	0.943	0.879
K	-0.630	0.041		-0.776	-0.504	-0.324	-0.731	-0.390	-0.712	-0.860	-0.838	-0.939	-0.922	-0.966	-0.718	-0.673	-0.957	-0.828
Na	0.825	0.914	0.010		0.619	0.693	0.562	0.608	0.589	0.459	0.685	0.927	0.850	0.829	0.703	0.405	0.811	0.635
Mg	0.645	0.757	0.624	0.747		0.539	0.551	0.670	0.604	0.219	0.849	0.612	0.690	0.625	0.651	0.193	0.609	0.716
Ca	-0.610	-0.703	-0.128	-0.805	-0.665		0.326	0.797	0.319	-0.007	0.371	0.569	0.357	0.376	0.527	-0.021	0.434	0.192
Ba	0.098	0.929	-0.157	0.745	0.556	-0.471		0.612	0.984	0.654	0.812	0.697	0.710	0.769	0.918	0.426	0.820	0.882
Mn	0.995	0.975	-0.017	0.827	0.668	-0.590	0.974		0.637	0.248	0.606	0.608	0.387	0.496	0.836	0.223	0.455	0.464
Ag	0.909	0.856	-0.368	0.718	0.349	-0.388	0.928	0.906		0.655	0.838	0.699	0.716	0.755	0.923	0.365	0.799	0.915
Hg	0.920	0.849	-0.317	0.695	0.386	-0.429	0.944	0.927	0.958		0.685	0.722	0.652	0.767	0.609	0.649	0.731	0.730
Fe	0.443	0.337	-0.525	0.292	-0.069	0.013	0.542	0.464	0.548	0.671		0.826	0.876	0.884	0.826	0.501	0.869	0.947
Zn	0.708	0.756	-0.242	0.786	0.457	-0.779	0.638	0.646	0.615	0.523	0.061		0.909	0.957	0.801	0.626	0.916	0.765
Co	0.731	0.680	-0.480	0.654	0.208	-0.251	0.784	0.734	0.812	0.823	0.858	0.473		0.956	0.700	0.563	0.950	0.876
Pb	0.595	0.623	-0.531	0.714	0.236	-0.679	0.566	0.532	0.603	0.535	0.249	0.922	0.554		0.804	0.733	0.945	0.859
Se	0.854	0.826	-0.435	0.756	0.316	-0.609	0.827	0.827	0.923	0.868	0.368	0.793	0.670	0.795		0.522	0.765	0.810
Sb	0.887	0.871	-0.059	0.687	0.566	-0.602	0.854	0.853	0.810	0.753	0.073	0.785	0.426	0.643	0.859		0.517	0.457
Mo	0.980	0.946	-0.111	0.776	0.560	-0.533	0.962	0.979	0.954	0.937	0.413	0.657	0.708	0.548	0.891	0.896		0.875
Cu	0.719	0.718	-0.584	0.754	0.245	-0.594	0.716	0.671	0.786	0.721	0.404	0.866	0.699	0.960	0.900	0.720	0.703	

Lemna minor L. (shown in grey color), *Lemna gibba* L. (shown in blue color)

The correlation expression is change between +1 and -1. The expressions from 0.5 to 1 shows high correlations, the expressions from 0.3 to 0.5 shows medium correlations, the expressions from 0.0 to 0.3 shows weak and valueless correlations.

When the correlations between the elements are investigated, it is seen that the relationship between K and Ca and the other elements in the frond of *Lemna minor* L. and the relationship between K and Ca and the other elements in the frond of *Lemna gibba* L. was negative ($p < 0.05$). The relationship between the other elements was positive ($p < 0.05$). There was a negative relationship between concentrations of Ca and concentrations of Hg and Sb in the frond of *Lemna gibba* L. ($p < 0.05$). Şaşmaz et al (2015) reported that there was negative relationship (antagonism) between elements as follows: Fe relationship with Na, P, S, Mn and Na and P relationship with Cu, Pb, Zn, As and S relationship with Cu, Pb and Mn relationship with Cu, Pb, As. Contrary to this, in this study, there was a positive relationship between these elements. Synergistic and antagonistic effects are also reported in the study of Khan et al. (2016). They were conducted a study to evaluate the effects of Cd, Pb and Cd–Pb mix on bioaccumulation of different nutrients in three edible plant species. Their results indicated significant variations in elemental concentrations. The different responses at various metal treatments are due to synergistic and antagonistic effects, as the same metal responded differently at variable concentration. Similarly, the effects also varied among the plant species which may be linked with their uptake mechanisms (Khan et al., 2016). In our study, the difference could be result of the properties of the waters. The obtained results showed that there was a synergic effect in the uptake capacity of the plants grown in the environments rich in macro and micro element ions and plants increased the accumulation capacities of these element ions.

CONCLUSION

There were high, low and weak correlations in the positive direction between K and Ca in *Lemna minor* L., and elements in *Lemna gibba* L. except K. When the contents of the studied micro elements in *Lemna minor* L. frond are examined, high positive correlation for P-S, P-Na, P-Na, P-Mg, P-Mn, P-Ag, P-Hg, P-Zn, P-Co, P-Pb, P-Se, P-Sb, P-Mo, P-Cu, moderate positive correlation for P-Fe, low positive correlation for P-Ba, and highly negative correlation for Ca and K were found. Among the elements in the *Lemna gibba* L. frond, high positive correlation was found for P-S, P-Na, P-Mg, P-Ba, P-Ag, P-Hg, P-Fe, P-Zn, P-Co, P-Pb, P-Se, P-Sb, P-Mo, P-Cu, medium positive correlation was found for P-Mn, low positive correlation was found for P-Ca and highly negative correlation for K. The results of this study can be said as follows: 1) the elements in the effluent may cause a food chain hazard due to the accumulation levels in both plant fronds, 2) these plants can be used for the improvement of domestic and agricultural wastewater, which is rich in macro and micro elements, and water bodies that have undergone eutrophication, 3) these macrophytes, which are easy to collect, can be used as an indicator plant transport in order to understand the pollution situation in aquatic environments.

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