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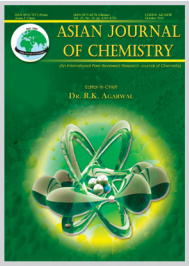

Determination of Growth Rate Change and Accumulation Efficiency of *Lemna minor* Exposed to Cadmium and Lead Ions

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The purpose of this study is to investigate the effects of lead and cadmium ions, which are present together in the same aquatic medium, on the growth of an aquatic plant, *Lemna minor* and to determine the accumulation potential of these metals by *L. minor*. Results showed that Cd ions caused a slight increase in the growth rate when they were added at a rate of 0.1 mg/L to Pb containing medium, but an increase of the Cd concentration to 5 mg/L decreased the growth rate of plants by 80 % for 0.1 mg Pb/L and 82 % for 10 mg Pb/L. Lead ions caused a slight decrease in the growth rates of plants and the results showed that observed toxic effects in plants resulted from Cd ions. Lead concentration in plants showed an increase in both metal ions containing medium in comparison to results obtained in only Pb containing medium. However, Cd accumulation of plants was not significantly affected by the presence of Pb in the medium. The concentrations of these metals in plant biomass were higher than both the mediums containing Pb and Cd ions alone.

Key Words: Cadmium, Duckweed, Lead, *Lemna minor*, Metal accumulation, Phytoremediation.

INTRODUCTION

In recent years, the ability of plants to accumulate pollutants has received significant attention and given rise to a new technology called phytoremediation[1]. The idea of using metal ion accumulating plants to remove heavy metal ions and other compounds was first introduced in 1983, but the concept has been actually implemented during the past 300 years[2]. This process involves rising of plants hydroponically and transplanting them into metal-polluted waters where plants absorb and concentrate the metal ions in their roots and shoots. As they become saturated with the metal contaminants, roots or whole plants are harvested for disposal[3]. This technology can be applied to both organic and inorganic pollutants present in soil (solid substrate), water (liquid substrate) or air[4,5]. In phytoremediation technologies, plants are used to stabilize or even to remove metals from water and soil through five mechanisms *i.e.*, phytoaccumulation, phytovolatilization, phytodegradation, rhizodegradation and phytostabilization[6]. Aquatic plants such as the floating *Eichhornia crassipes* (water hyacinth), *Lemna minor* (duckweed) and *Azolla pinnata* (water velvet) are known to accumulate metal ions from their environment[7,8]. The hyper accumulative capacities of the aquatic plants are beneficial for the removal of heavy metal ions. In addition to this, the aquatic macrophytes used in any treatment system should meet several criteria *viz.*, rapid growth, easy spreading, high pollutant uptake capacity and easy harvesting[9].

Lemna minor is small and green vascular plant growing rapidly under favorable conditions. Ease of culture and possibility of manipulation in aseptic laboratory conditions makes it a suitable organism to study on metal ion uptake capability as well as on phytotoxic effects of metal ions[10-12]. Since duckweeds exhibit rapid growth, they are effective in trapping mineral components from water, including heavy metal ions, but the intensity of such uptake varies significantly, depending on environmental conditions and *Lemna* species[13].

The uptake of metal ions by aquatic plants is influenced by several factors, such as temperature, pH, light[14] and the presence of other metal ions in water, all of which alter the uptake of heavy metal ions into the tissues[15]. Also, the rate of phytoremediation is directly proportional to plant growth rate and the total amount of phytoremediation is correlated with total plant biomass. Thus, a typical test end point is to change of growth rate for plants exposed to heavy metal ions. Many studies have been carried out to remove toxic metal ions from artificially prepared aquatic mediums. Most of these studies were primarily aimed on investigating the effects of individual metal ions. However, the uptake and accumulation of some

metal ions by aquatic plants can be affected by other metal ions, which are present in the same medium. For example, copper ions reduce the accumulation of other metal ions by duckweed[16]. In addition to this, mixtures of heavy metals are usually found and their uptake by plants is considerably influenced by synergistic or antagonistic interactions among these elements in aquatic ecosystems[17].

Exposure to high levels of toxic metals has been linked to adverse effects on human health and wildlife[18]. Cadmium and Pb were chosen as toxic metal ions in this study. Cadmium(II) is toxic to living organisms and it is often present in the environment. Industrial wastewaters such as electroplating, pigments, stabilizers, batteries alloy, *etc*. contain this metal ion. Lead is also found in several industrial wastewater and exhaust gases. The widespread use of such element becomes the main cause for Pb contamination in the environment. Heavy metal ions such as Cu, Zn, Mn, Fe and Ni are essential micronutrients for plant metabolisms, but Cd and Pb are non-essential metal ions. In plants, the exposure to toxic concentrations of Cd and Pb to single treatment causes oxidative stress, growth inhibition, chlorosis, browning of root tips and disturbance of photosynthesis, reduction of water and nutrient uptake and inhibition of enzymatic activities[19] and finally, death[17]. Investigating the effects of another kind of metal ion existing in the medium, on the accumulation of a metal ion by a plant biomass is quite important because metal ions often exist together in natural and wastewaters. For this reason, the goals of the present study are (a) to compare the toxic effects of Cd and Pb in single and combined treatment on *L. minor* growth, (b) to determine the removal of Cd and Pb from single and binary mixtures and their accumulation by *L. minor*.

EXPERIMENTAL

The plants were collected from quiescent water canals in Mersin, Turkey. Plants taken to laboratory were first washed carefully with tap water several times to remove dirt, sludge and other debris. Then, the plants were set into culture pots with a capacity of 1 L and filled with artificial Duckweed nutrient solution (DNS)[20]. They were grown in this culture solution and allowed to acclimate to the laboratory conditions for a month before addition of metal ion solutions. All stock and experimental cultures were maintained in a growth chamber at 25 ± 3 ºC with a 16 h photoperiod at a photosynthetic photon density of 2100 lux.

Experiments have been carried out in flasks for 168 h, in triplicate and the mean values were reported. At the beginning of each run, 40 healthy, light green fronds of the same size were set in each flask of 250 mL containing 200 mL of DNS. Fresh mass of plants was determined gravimetrically at the beginning of each experiment.

The nutrient medium DNS was prepared by using the following salts in stock solution: $NaNO_3$, 25.500 g L^{-1}; $NaHCO_3$, 15.000 gL^{-1}; K_2HPO_4, 1.040 gL^{-1}; $CaCl_2.2H_2O$, 4.410 gL^{-1}; $MgCl_2$, 5.700 gL^{-1}; $FeCl_3$, 0.096 gL^{-1}; $MnCl_2$, 0.264 gL^{-1}; $MgSO_4.7H_2O$, 14.700 gL^{-1}; H_3BO_3, 0.186 gL^{-1}; $Na_2MoO_4{\cdot}2H_2O$, 7.260 mg L^{-1}; $ZnCl_2$, 3.270 mg L^{-1}; $CoCl_2$, 0.780 mg L^{-1}; and $CuCl_2$, 0.009 mg L^{-1}. This stock solution was diluted with deionized water at 1:100 ratios to make the plant nutrient medium.

Metal ion solutions were added directly to the nutrient medium. Cadmium stock solutions were prepared with cadmium(II) sulphate (Merck, analytical reagent grade) and lead stock solutions were prepared with lead(II) nitrate (Merck, analytical reagent grade). The initial pH of solution was adjusted with HCI and NaOH solutions at pH 5 and the pH change of each experimental set was daily monitored. The control samples without metal ions were used to determine the effect of metal ions on the plant growth. The frond numbers of plants were daily counted to calculate the growth rate constants and compared with mediums with and without metal ions. The medium temperature was taken as the laboratory medium temperature (22-28 ºC). In order to explore Cd and Pb removal activity of plants, metal ions were added at concentrations of ranging from 0.1-10.0 mg Pb(II)/L and 0.1-5.0 mg Cd(II)/L. In order to determine the amount of Cd and Pb accumulated in the plants, the plant samples were collected from the medium by filtration at the end of the study period. All plants of each replicate and control were then washed with deionized water and smoothly treated with tissue paper. The final fresh masses of plants were determined gravimetrically.

Plant samples were dried at 70 ºC to determine the dry mass, X_m. The dried samples were digested with mixture of HNO_3 and H_2SO_4 solution according to the process of Sen and Mondal[21]. The metal content of all digested water and plant samples was determined by graphite furnace atomic absorption spectrophotometer (GFAAS). A reagent blank was also digested in the same manner. The culture solutions having no plants were used as controls to determine if there was any adsorption involving the flask wall. The growth rate changes of *L. minor* at the different conditions were studied by a batch technique[22].

Calculations: The simplest method to calculate the growth rate is to count the visible fronds with time. In this study, plant growth rate was explained depending on the increase of frond numbers of *Lemna* with time and calculated according to Landolt and Kandeler[23], eqn. (1). The bioremoval potential of *Lemna* was expressed as the percentages of metal uptake (PMU) and calculated according to eqn. (2).

$$R = \frac{dF}{dt} = kF \quad k = \frac{(\ln F_{t2} - \ln F_{t1})}{t_2 - t_1} \tag{1}$$

k: growth rate constant (1/d); R: growth rate (frond number/d); F_{t1}: the frond number at t_1; F_{t2}: the frond number at t_2; t_2-t_1: study period, (d).

$$\text{Metal uptake (\%)} = \frac{\text{mg metal in Lemna (t = 168 h)}}{\text{mg metal in medium (t = 0)}} \times 100 \tag{2}$$

Bioconcentration factors (BCF) were also calculated for *Lemna* grown in the medium containing both the ions. Bioconcentration factor is expressed as the ratio of the final metal ion concentration in the biomass to the initial metal ion concentration in the culture medium and defined by eqn. 3. This factor is an indicator of the metal accumulation ability of plants in respect of metal concentration in the medium and allows a comparison of the results between species. The bioconcentration factors of *Lemna* were calculated for Pb(II) and Cd(II) coexisting in medium[24] according to eqn. 3.

TABLE-1
GROWTH RATE CONSTANTS (k) OF *LEMNA MINOR* GROWN IN CULTURE MEDIUM CONTAINING BOTH Cd AND Pb IONS (25 ± 3 ºC, pH 5.0)

Metal ion-conc.	k (d^{-1})				
	Cd (mg/L)				
Pb (mg/L)	0.0	0.1	0.5	1.0	5.0
0.0	0,160 ± 0.011	0.145 ± 0.012	0.095 ± 0.008	0.090 ± 0.010	0.058 ± 0.012
0.1	0.097 ± 0.012	0.116 ± 0.010	0.084 ± 0.011	0.065 ± 0.006	0.020 ± 0.005
0.5	0.088 ± 0.009	0.088 ± 0.009	0.079 ± 0.008	0.060 ± 0.012	0.023 ± 0.008
1.0	0.080 ± 0.005	0.094 ± 0.005	0.065 ± 0.014	0.058 ± 0.010	0.023 ± 0.006
2.5	0.086 ± 0.004	0.092 ± 0.007	0.067 ± 0.011	0.058 ± 0.008	0.023 ± 0.003
5.0	0.080 ± 0.003	0.104 ± 0.016	0.048 ± 0.012	0.053 ± 0.015	0.026 ± 0.006
10.0	0.076 ± 0.004	0.078 ± 0.013	0.043 ± 0.009	0.053 ± 0.007	0.014 ± 0.005

$$\text{Bioconcentration factor (BCF)} = \frac{[Me]_P}{[Me_0]_S} \quad (3)$$

where, $[Me]_P$ is the metal concentration (mg/kg) in the plant tissues at harvest (end of the experiment) and $[Me_0]$ is the initial concentration (mg/L) of the metal in the culture solution.

RESULTS AND DISCUSSION

Results of plant growth: The effect of Cd(II) was evaluated with respect to the change of growth rate of plants in different concentrations of Cd(II) ranging from 0.1-5.0 mg/L in the culture medium containing Pb(II) of 0.1-10.0 mg/L. Growth rate constants before and after Cd addition are listed in Table-1. It is important to describe that growth rate constants (Table-1) were calculated for only plants presented in growth flasks and they were not calculated for healthy plants. Because the number of healthy fronds per flasks considerably decreased in all culture flasks.

Metal ions reduced the growth rates of plants compared to control samples. It can be shown from the growth rate results for only one metal ion containing medium. For example, Pb ions caused a decrease in growth rate of plants compared with control when they existed alone in culture medium. Growth rate constant of plants decreased by 40 % in 0.1 mg Pb/L. When concentration was increased by 100-fold (for 10 mg Pb/L), the growth rate decreased by only 53 %. Similarly, Cd ions also reduced the growth rate of plants compared to the control sample when they were added alone to the medium. While the growth rate of plants decreased only about 9 % in 0.1 mg Cd/L, it decreased 64 % in 5 mg Cd/L. Namely, growth rates decreased about 7-fold, when concentration increased 50-fold in medium. However, at low concentrations, Pb ions caused a decrease of the growth rates comparable with Cd(II) in the same concentration of 0.1 mg/L.

In previous study, various toxicity symptoms were observed in plants, which were grown in Cd (0.05-10 mg/L) or Pb (0.1-10 mg/L) containing culture medium. These symptoms were more apparent especially in Cd(II) containing medium. For example, it was observed that the plants were pale and transparent and did not grow too much in the concentration range of 0.5-10 mg/L. Although the total and produced frond number increased with time, the number of healthy plants (light green, bright) decreased significantly. New fronds remained small and frond deformations were observed after 48 h. It was observed that, paling of fronds occurred especially as band form in the center of the fronds. In high Cd(II) concentrations, such as 10 mg/L, these toxicity effects were observed immediately after 24 h[22].

Similar toxicity symptoms, such as partly fading of fronds and breaking off roots, were also observed in plants growing in ≥ 2.5 mg/L of Pb containing medium. These symptoms were observed at low initial pH of 4.5, especially. It was supposed that this might have resulted from chlorosis mechanism. The growth rate constants decreased from 63 % (in 0.1 mg/L) to 39 % (in 100 mg/L) when Pb ion concentration in the medium increased. Although the frond number of plants decreased with an increase of Pb concentration, the relative growth rate did not change significantly at 50-100 mg Pb/L range. The results of this study showed that Pb ion concentrations higher than 10 mg/L did not affect plant growth significantly and plants were not influenced by an increase of Pb ions in the culture medium[25].

When Pb and Cd ions coexisted in the culture medium, their toxic effects increased comparing to existing alone. For example, in 0.1 mg Cd/L containing flasks, frond deformation, small frond formation and chlorosis were observed in flasks added 2.5 and 5 mg Pb/L. These symptoms were observed in the first 24 h for 0.5-5 mg Cd/L containing medium after addition of Pb ions.

The increase of Cd concentration in culture medium caused a decrease in the production of fronds and growth rates during the experimental period. However, increasing Cd concentration to 5 mg/L decreased the growth rate of plants by 80 % for 0.1 mg Pb/L and 82 % for 10 mgPb/L. Namely when Pb(II) concentration was increased from 0.1 mg/L to 10 mg/L (100-fold), growth rate of plants decreased by 22 %; while Cd(II) addition to each solutions caused a decrease in the growth rates at the same percentage. This result showed that observed toxic effects in plants resulted from Cd ions.

In this study Pb ions slightly caused a decrease in growth rates of plants. Addition of Pb ions to Cd (II) containing medium of 0.1 mg/L decreased the growth rate by 20 %, while this value increased to 46 % for 10 mg/L. When Cd(II) concentration was increased to 5 mg/L, decrease of growth rate was 60 %, while this value was only 28 % with adding of 5 mg Pb(II) /L. When two metal ions were added to growth medium at the highest concentrations (5 mg Cd(II)/L and 10 mg Pb(II)/L), growth rate of plants decreased by 91 % compared to the control sample. The results and observations showed that intensity of toxic effects on plants increased and plant's growth nearly stopped growing in this medium.

Heavy metal cations, such as Cd(II) and Pb(II), may be present at high concentrations in plant's environment and cause toxic effects on the metabolic functions and growth of these organisms. Some of these toxic effects are the reduction of chlorophyll contents, the degeneration of chloroplasts and the decrease of photosynthetic activity[26]. Although Cd(II), like Pb(II), is not an essential for plant growth, it is readily taken up by roots and translocated into the leaves in many plants. It depresses plant's growth by affecting photosynthesis, chlorophyll fluorescence and nutrient uptake[27].

Megateli *et al.*[28] reported that Cd inhibition on the ratio of chlorophyll to phaeophytin (D665/D665a) in *Lemna gibba* was more important than that observed for growth, especially at 0.1 mg/L. They also reported that D665/D665a seemed to be a more sensitive end point that could be proposed as a biomarker of Cd pollution. The same visual symptoms had been observed in their experiments, together with root loss in several colonies. Same authors[28] reported that Cd is the most toxic metal for *L. gibba*, followed by copper and finally zinc ions.

Results of metal ions accumulation: Accumulated Cd and Pb concentrations in plant biomass grown in culture solution containing Cd and Pb ions were shown in Figs. 1 and 2. The Cd and Pb bioremoval capacity of *Lemna*, denoted as percentages of metal uptake (PMU) was calculated for each metal and medium. The change of percentages of metal uptake values with concentration for Pb and Cd were shown in Figs. 3 and 4, respectively.

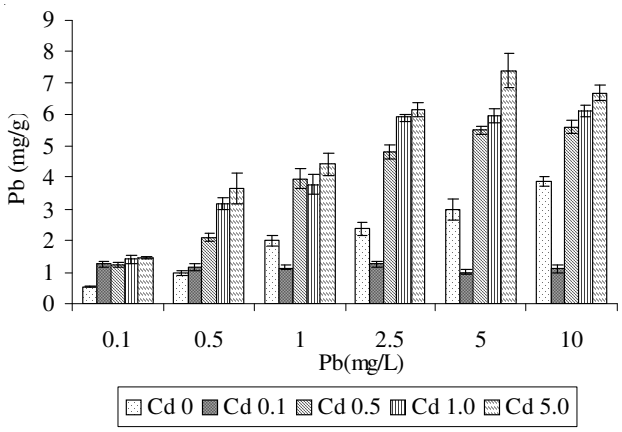

Fig. 1. Concentration of Pb in plant biomass with concentration of Pb in medium having different concentrations of Cd

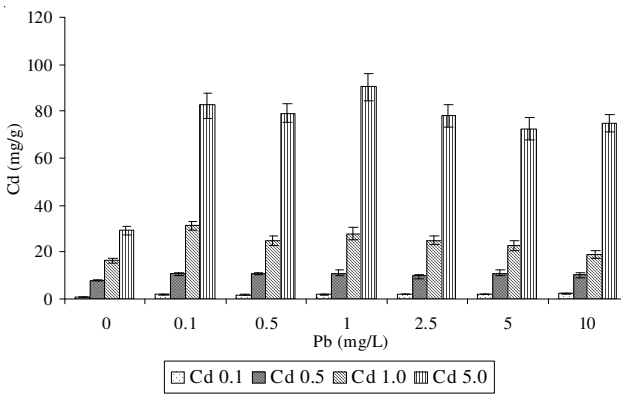

Fig. 2. Concentration of Cd in plant biomass with concentration of Pb in medium

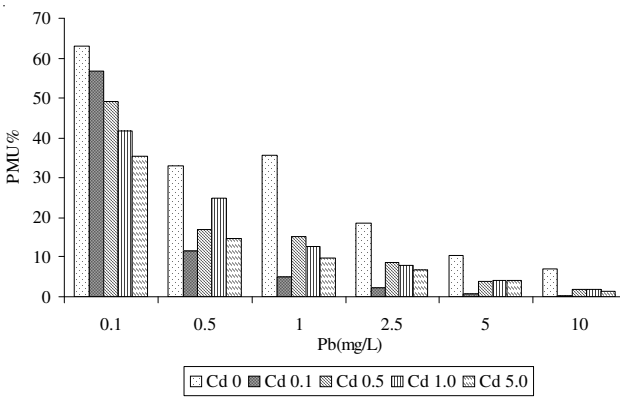

Fig. 3. Percentages of metal uptake by plants for Pb in medium containing Cd ions in different concentrations

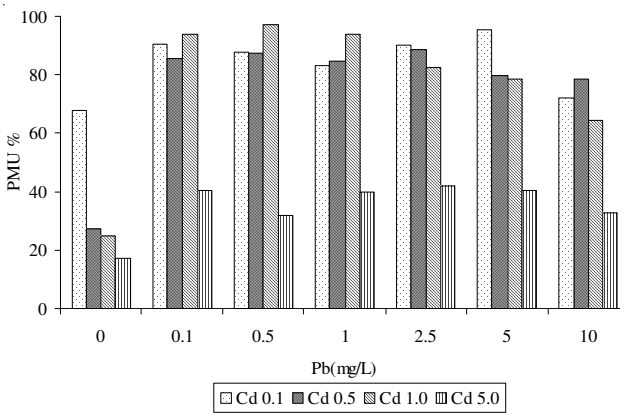

Fig. 4. Percentages of metal uptake by plants for Cd in medium having different Pb concentration

As could be seen from Fig. 1, the increase of Pb concentration in medium generally caused an increase in the accumulated Pb concentration in the biomass. Although accumulated Pb concentrations in plant biomass for Cd containing medium of 0.1 mg Cd(II)/L were lower than those in medium without Cd ions, in the 0.5-5.0 mg Cd(II)/L range they showed an increase. In other words, the accumulated Pb concentration showed an increase in the medium containing both metal ions compared to the results obtained from the medium containing Pb(II) alone. These results showed that the plants preferred Cd uptake when Cd(II) concentration was low in the medium, but they tended to Pb uptake with increasing Pb(II) concentration of the medium.

Percentages of metal uptake values for Pb calculated for medium containing Cd ions are shown in Fig. 3. Contrary to accumulation results, the percentages of metal uptake values for Pb were higher in the media without Cd content and they were found as 62.9, 32.8, 35.7, 18.7, 10.4 and 6.9 % for concentrations of 0.1, 0.5, 1.0, 2.5, 5.0 and 10.0 mg Pb(II)/L, respectively. Results showed that percentages of metal uptake values of *Lemna* for Pb decreased with Cd ion existence. Although an increase of Pb content of medium caused an increase in the Pb content of plants, percentages of metal uptake values decreased with increase of Pb concentration. The reason of the decrease of percentages of metal uptake values in both Pb and Cd containing medium results from probably the combined higher toxicity of metals. Thus, the amount of produced biomass decreased in these media.

TABLE-2
AMOUNT OF DRY BIOMASS, ACCUMULATED METAL CONCENTRATIONS AND BCF OF *Lemna* GROWN IN CULTURE MEDIUM CONTAINING BOTH Cd AND Pb IONS (pH 5.0, 25 ± 3 ºC)

Concentration		Cd			Pb		
mg Pb/L	mg Cd/L	X_m (g dm/L)	mg Cd/g dm	BCF	X_m (g dm/L)	mg Pb/g dm	BCF
0.0	0.1	0.057	1.200 ± 0.060	12 000	-	-	-
0.1	0.1	0.048	1.880 ± 0.110	18 800	0.045	1.260 ± 0.073	12 600
0.5	0.1	0.051	1.740 ± 0.093	17 400	0.051	1.145 ± 0.106	2 290
1.0	0.1	0.045	1.850 ± 0.098	18 500	0.045	1.142 ± 0.078	1 142
2.5	0.1	0.045	2.000 ± 0.078	20 000	0.045	1.261 ± 0.113	504
5.0	0.1	0.046	2.180 ± 0.125	21 800	0.046	1.000 ± 0.086	200
10.0	0.1	0.030	2.400 ± 0.146	24 000	0.030	1.100 ± 0.124	110
0.0	0.5	0.018	7.810 ± 0.210	15 620	-	-	-
0.1	0.5	0.040	10.680 ± 0.600	21 360	0.040	1.230 ± 0.062	12 300
0.5	0.5	0.040	10.900 ± 0.718	21 800	0.040	2.100 ± 0.130	4 200
1.0	0.5	0.039	11.000 ± 1.160	22 000	0.039	3.951 ± 0.326	3 951
2.5	0.5	0.046	9.600 ± 0.756	19 200	0.046	4.800 ± 0.253	1 920
5.0	0.5	0.037	10.930 ± 1.230	21 860	0.037	5.498 ± 0.135	1 100
10.0	0.5	0.038	10.460 ± 1.150	20 920	0.038	5.610 ± 0.210	561
0.0	1.0	0.015	16.690 ± 1.050	16 690	-	-	-
0.1	1.0	0.030	31.300 ± 1.634	31 300	0.030	1.390 ± 0.145	13 900
0.5	1.0	0.039	24.940 ± 2.087	24 920	0.039	3.185 ± 0.195	6 370
1.0	1.0	0.034	28.000 ± 2.415	28 000	0.034	3.775 ± 0.321	3 775
2.5	1.0	0.033	24.990 ± 1.965	24 990	0.033	5.900 ± 0.123	2 360
5.0	1.0	0.035	22.770 ± 1.880	22 770	0.035	5.970 ± 0.216	1 194
10.0	1.0	0.034	18.960 ± 1.556	18 960	0.034	6.120 ± 0.184	612
0.0	5.0	0.029	29.240 ± 1.599	5 848	-	-	-
0.1	5.0	0.025	82.570 ± 5.512	16 514	0.025	1.450 ± 0.043	14 500
0.5	5.0	0.020	79.170 ± 3.866	15 834	0.020	3.650 ± 0.478	7 300
1.0	5.0	0.022	90.490 ± 5.955	18 098	0.022	4.420 ± 0.343	4 420
2.5	5.0	0.027	78.100 ± 4.460	15 620	0.027	6.160 ± 0.216	2 464
5.0	5.0	0.028	72.440 ± 4.890	14 488	0.028	7.380 ± 0.536	1 476
10.0	5.0	0.022	75.200 ± 3.756	15 040	0.022	6.680 ± 0.250	668

The Cd uptake efficiencies of plants were also investigated in the medium containing both Cd and Pb and are shown in Fig. 2. The Cd concentration in plants increased with an increase in Cd concentration in medium. When the results were compared with Pb containing and any Pb containing medium, it was shown that Cd accumulation of plants was not significantly affected by the presence of Pb(II) in the medium. The Cd contents in plants did not change with increasing Pb content of medium except for solutions with a concentrations of 1 and 5 mg Cd(II)/L. In these mediums, the presence of Pb ions caused much more Cd(II) uptake of plants. This finding shows that Cd uptake by plants increases independently from high Pb(II) concentration of medium.

The percentages of metal uptake values for Cd were calculated for several Pb ions concentrations and were shown in Fig. 4. It was determined that the percentages of metal uptake for Cd increased in the Pb containing medium. However, these values did not change significantly with Pb concentration. Therefore, the percentages of Cd uptake were not affected by increasing Pb concentration, while decreasing with the increase of Cd concentration in medium.

The bioconcentration factors values calculated for Pb and Cd for each culture medium and the amount of dry biomass obtained in these mediums are given in Table-2. According to the results, the bioconcentration factors for Cd increased with Cd supply increased from 0.1 to 1 mg Cd(II)/L and then decreased again in 5 mg Cd(II)/L. This was also observed if the medium did not contain any Pb(II). However, it was determined that the change of bioconcentration factors for Cd(II) did not change significantly with increasing Pb(II) concentration.

The bioconcentration factors for Pb decreased when Pb increased from 0.1 to 10 mg/L. The highest bioconcentration factor was 14500 in 0.1 mg/L solution, but the lowest was 110 in 10 mg/L solution. This result was expected, because in previous studies related with lead and cadmium accumulation by *Lemna* it was observed that an increase in the metal concentration in medium caused a decrease in the bioconcentration factors, while increasing in the accumulated metal concentration of biomass[29,30]. An interesting result was that plants tended to more Pb accumulation with addition of Cd to culture medium. Namely, the uptake of Cd was not affected by the existence of Pb, while plants were encouraged to uptake Pb in the presence of Cd. When bioconcentration factors were compared with each other for Pb and Cd, it was seen that bioconcentration factors for Cd were higher than bioconcentration factors for Pb. Although Cd is more toxic than Pb for *Lemna*, bioconcentration factors for Cd showed that *Lemna* is a good accumulator for Cd.

In this study, the findings obtained from the medium containing both metal ions showed that the concentrations of these metal ions in plant biomass was higher than those in medium containing metal ions separately. It was considered that this probably resulted from free metal ion activity. Pena-Castro *et al.*[31] studied the Cd and Cu uptake performance of a microalgae species *Scenedesmus incrassatulus* and reported

that a charged double positive free metal ion activity increased with the presence of another charged double positive metal ion. The authors found that the percentage of Cd uptake was 24.1 % when only Cd ions were present in medium, while it increased to 59.9 % when both ions were contained in the medium.

Conclusion

According to present study, it was shown that accumulated Cd and Pb ion concentrations in plant biomass grown in a medium containing both of metals were higher than those of a medium containing a single metal ion. This probably resulted from increased free metal ion activity in the media containing two metal ions together. From the results it was also shown that Cd uptake by plants was quite higher than Pb ions when two metal ions were present together. Percentages of metal uptake for Pb decreased with existence of Cd while, percentages of metal uptake for Cd increased with existence of Pb in the medium. However, Cd accumulation was not affected significantly with an increase in the concentration of Pb(II). These results showed that these plants have a higher accumulation affinity for Cd than for Pb ions. Plants preferred Cd ions to Pb ions, so that the presence of Pb(II) ions in medium did not significantly change the Cd(II) uptake because of complete filling of bonding sites with Cd(II) ions. This may probably be explained by the fact that two metal ions such as Pb(II) and Cd(II) have the same charges and attracting forces, since Cd ions have smaller ion diameter than Pb ions, they can more easily enter into equal cavities in the organic molecules.

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