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EFFECT OF A MIXTURE OF CADMIUM AND LEAD ON NITRATE AND PHOSPHATE REMOVAL BY THE DUCKWEED *LEMNA GIBBA*

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Abstract – The purpose of the present study is to examine the effect of binary mixtures of the heavy metals, cadmium (Cd) and lead (Pb), on the assimilation of nitrate (NO_3^-) and orthophosphate (PO_4^{3-}) from N and P-rich culture medium of the duckweed *Lemna gibba*. In binary mixtures, varied Cd concentrations (0.01, 0.1 and 1mg/L) were tested with 0.1 and 1 mgPb/L respectively. Experiments were run for 2, 4, 6 and 8 days. Results showed that in all treatments (Cd+Pb), orthophosphates concentration decreased markedly within the two days of initiating experiments. The highest phosphate removal efficiency is 89.39%, in the solutions containing a binary mixture of Pb at 0.1 mg/L combined with Cd mg/L on the fourth day and about 83% in a binary mixture of Pb and Cd at 1mg/L each metal. Nevertheless, nitrate removal was weak with time. The co-contamination of the plant culture medium with varied Cd + Pb concentrations has no effect on phosphate removal, as compared to the control. Additionally, Cd and Pb were removed from the medium and accumulated on *Lemna gibba*.

Keywords: heavy metals stress, nutrients, phytoremediation, aquatic plant, eutrophication, binary mixtures.

Introduction

Human activities and industrialization loaded aquatic environment by nutrients (N and P) accelerating the eutrophication of water and released heavy metals, the main group of inorganic contaminants (Yang and al, 2005). The most problematic heavy metals are cadmium (Cd) and lead (Pb). Heavy metals such as Cd and Pb are of interest because of their toxicity even at low levels. They are considered as priority pollutants (PP) (Annibaldi et al., 2015).

Cadmium (Cd) is a potentially toxic element, hazardous to humans and all other living organisms (Hattab et al., 2014; Sarwar et al., 2010). Cadmium originates from natural and essentially, anthropogenic sources (Schützendübel et al., 2002). Major emissions to the environment are through industrial waste from processes such as mining, paint pigments, power stations, metalworking industries and waste incineration (Suzuki et al., 2001).

The most important sources of lead pollution in wastewater include batteries, pigments; paints, petrol, cables, steels, alloys, and plastic industries (Ali et al., 2013). Cadmium and lead are strongly phytotoxic (Salim et al., 2008). High levels of these pollutants can have lethal effects on plant growth.

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Phosphorus is an important nutrient. Orthophosphate the predominant form is one of the most common elements on earth and an essential nutrient to all living organisms (Kamika & Momba, 2015). However, an excess of phosphorus in the aquatic environment leads to a rapid algal growth, which results in eutrophication (Kamika & Momba, 2015; Akpor et al., 2008). Similar to phosphorus (phosphate), nitrogen is one of the essential elements for any form of life (Camargo & Alonso, 2006). The most common variety of chemical forms present and used in the aquatic environment are ammonium (NH_4^+), nitrite(NO_2^-) and nitrate (NO_3^-) (Rabalais et al., 2009) Excessive concentrations of nitrate in groundwater and surface water can cause health problems (Akpor et al., 2008). There have been many environmental and health problems associated with high amounts of nitrogen in water. High concentration of nitrates in drinking water can cause "blue baby" syndrome in infants.

Due to several health and environmental hazards caused by phosphates and nitrates, several conventional methods have been developed for the removal of nitrogen and phosphorus (Kamika & Momba, 2015; Akpor et al., 2008). Most of the studies on N and P removal from wastewater were based on biological processes and different combinations of anaerobic, aerobic, and anoxic zones (Kargi & Uygur, 2003; Obaja et al., 2005; Zeng et al., 2013) However, these biological processes have numerous disadvantages, including costliness, instability, and the generation of secondary pollution of groundwater, among others (Zeng et al., 2013).

Phytoremediation is a cost-effective, non-intrusive, environmentally friendly, and safe alternative to conventional clean-up techniques (Eugenia, 2003; Zhang et al., 2007). Plant-based cleanup strategies have more advantages compared with traditional clean-up methods and other bioremediation technologies (Sharm & Sahi, 2005). Because of high biomass yields and extraordinary nutrient absorption capabilities, some aquatic plants are attracting growing attention and considered promising to be used in nutrient removal from wastewaters (Iamchaturapatr et al., 2007; Yi et al., 2009; Akinbile & Yusoff, 2012).

Duckweed can absorb large amounts of nutrients such as nitrogen (N) and phosphorus (P). It also often used as a phytoremediation agent for pollutant removal because of its capability of assimilating nutrients, metals ions and organic pollutants.

Regardless of our knowledge, little experimental information exists on the effect of heavy metal co-contamination on nitrate and phosphate assimilation by duckweed. Hence, the purpose of this study was to evaluate the potential of duckweed for the removal of nitrate and phosphate from the culture medium of *Lemna gibba* under different levels and durations of Cd+Pb stresses. Relative growth of *Lemna gibba* was greatly increased over time in all treatments.

Materials and Methods

Plant material and metals

Duckweed (*Lemna gibba*), an aquatic angiosperm of the lemnacea family was collected from a natural pond in the north of Algeria. Before initiating the experiments, colonies were disinfected by immersion in 0.5% (v/v) ethanol for few seconds, in a solution of sodium hypochlorite at 0.5% for 3 min and then rinsed in sterile distilled water. Cultures of *L.gibba* were maintained in 1L glass beakers containing autoclaved growth medium at least 4 weeks under axenic conditions prior to their use. The nutrient medium consisted of (mg/L): $Ca(NO_3)_2{\cdot}4H_2O$, 118; KNO_3, 5.055; $MgSO_4{\cdot}7H_2O$, 4.932; KH_2PO_4, 0.68; Fe-EDTA, 0.1; H_3BO_3, 0.286; $MnSO_4{\cdot}7H_2O$, 0.155; $ZnSO_4$, 0.022; $CuSO_4$, 0.0079; $NiSO_4{\cdot}7H_2O$, 0.00478.

The pH was adjusted with the drop-wise addition of NaOH at 0.1 N or HCl at 0.1 N to be between 6 and 6.5. The medium was changed every 7 days. The incubation conditions are as follows: temperature 27±1°C, 4000 lux light intensity, 16h/8h light and dark cycle.

To assess the duckweed ability in metal accumulation, the nutrient medium was supplemented with varied Cd concentrations prepared from $CdSO_4$ (0.01, 0.1 and 1 mg/L) combined to Pb prepared from $PbCl_2$ (0.1 or 1 mg/L).

The final pH of the solutions was adjusted to 6.0 ± 0.5.

Thirty (30) uniform and healthy colonies (each colony with two fronds) of *Lemna gibba* have been selected and inoculated into flasks containing 100 mL of nutrient medium. The duckweed was exposed to binary mixtures of Cd (0.01, 0.1 and 1 mg/L) and Pb (0.1 and 1 mg/L). *Lemna gibba* cultured in the growth medium without heavy metals were considered as controls. All experiments lasted 8 days and were performed in triplicate. The frond number measurements have been monitored on days 0, 2, 4, and 8.

The growth was determined using Eq.(1) (Megateli et al., 2009):

$$RG\%=\frac{Nt-N0}{N0}\times 100 \qquad (1)$$

Where N_t is the average number of fronds at time t,

N_0 is the average number of fronds at the beginning of the experiments (N_0=60).

Analysis of heavy metals in plant samples

Atomic absorption spectrophotometry (AAS) was employed to analyze the metals in the plants and in the culture medium, using an optical emission spectrophotometer Perkin Elmer (Optima 700 DV). Plants samples were dried for 48 h at 80°C.

After the drying, the fronds were weighed and then grinded and digested with ($HNO_3/HClO_4$) 3/1(v/v). Afterward, each sample was dried and then dissolved in deionized water.

Analysis of phosphate and nitrate in the culture medium

The phosphate and nitrate concentrations of the growth medium were measured colorimetrically using the ascorbic acid and salicylate methods, respectively, as described in standard methods (APHA, 2001) performed by a spectrophotometer HACH (DR/2000). The percentage removal efficiency of phosphate and nitrate in the medium was calculated following Eq. (2):

$$\% = \frac{Co - Ct}{Co} \times 100 \qquad (2)$$

Where C_t is the concentration of $[PO_4^{3-}]$ or $[NO_3^-]$ in the culture medium at time t and C_0 is the initial concentration of $[PO_4^{3-}]$ or $[NO_3^-]$ in the culture medium at time t = 0

Statistical treatment of data

Statistical analysis was performed using SigmaPlot.11. All data were analyzed to one way Analysis of Variance, using Bonferroni t-test.

RESULTS

Phosphate removal

The variation of orthophosphate concentration in culture medium containing a mixture of Cd and Pb at different initial Cd concentrations combined with 0.1 mg/L Pb are shown in Figure 1. Initial phosphate concentrations were 0.25 ± 0.01, 0.66 ± 0.01, 0.56 ± 0.01 and 0.51 ± 0.01 in the control and in the mixtures treated with 0.1 mg/L of Pb combined to 0.01, 0.1 and 1 mg/L of Cd respectively.

The orthophosphate concentration in all treatments and in the control decreased markedly within 4 days of initiating the experiments.

The phosphate removal efficiencies from the medium with *Lemna gibba,* monitored on days 2, 4, 6 and 8 of the experiments are presented in Table 1.

The maximum removal efficiencies on day 4, were 80%, 89.39%, 87.50% and 88.88% in the control and in the solutions containing binary mixtures of Pb (0.1 mg/L) combined with Cd at 0.01, 0.1 and 1mg/L respectively.

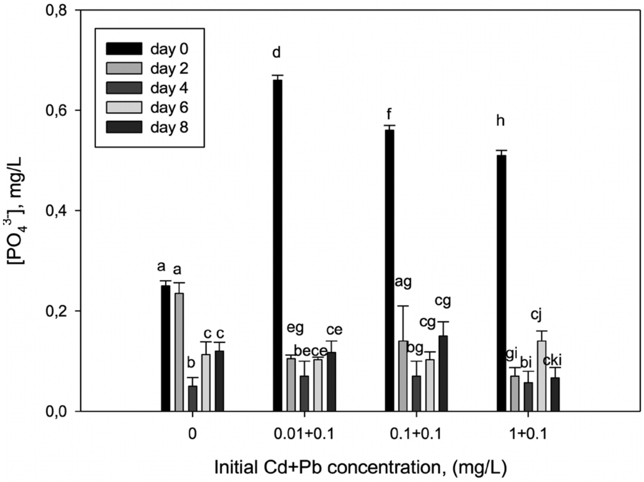

Figure 1. Effect of Cd + Pb on orthophosphate concentration at 0.1mg/L Pb. Values represent mea n± S.D. Different letters indicate significant differences (P< 0.05) according to the ANOVA test.

However, a mixture with the highest Cd concentration (1 mg/L) and 0.1 mg/L of Pb decreased phosphate concentration in the nutrient medium to 70% and 40% on days 2 and 8 of the control value, respectively. While a mixture with the lowest Cd concentration and 0.1 mg/L Pb decreased phosphate concentration by 55% of control value at day 2. No significant changes in phosphate concentrations were recorded among the other treatments as compared to control values.

Phosphate concentration was significantly decreased after 2 days of metal treatments with binary mixtures of varied Cd concentrations and 1 mg/L Pb (Figure 2). Initial phosphate concentrations were 0.28 ± 0.01, 0.18 ± 0.01, 0.24 ± 0.01 and 0.24 ± 0.01 in the control and in the mixtures treated with 1 mg/L of Pb combined to 0.01, 0.1 and 1 mg/L of Cd respectively. The maximum decrease in phosphate concentration (83.46%) was recorded in the medium treated with the combination of Cd and Pb at 1 mg/L each metal (Table 1).

Results of the ANOVA analysis showed that each of the three metals mixture concentrations had no significant effect on phosphate removal as compared to control groups on day 2 and 4 while a significant decrease of phosphate concentration of 39% and 37% was observed on day 8 when Cd concentrations were 0.01 and 1 mg/L in the mixture. On day 6, removal efficiencies did not exceed 26%.

Nitrate removal

Initial nitrate concentrations were 592.33 ± 0.01, 681.00 ± 0.01, 561.33 ± 0.01 and 641.66 ± 0.01 in the control and in the mixtures treated with 0.1 mg/L of Pb combined to 0.01, 0.1 and 1 mg/L of Cd respectively.

As shown in Figure 3, nitrate concentration decreased significantly (39%) in the solution containing a mixture of 1 mg/L Cd and 0.1 mg/L Pb, on day 8 as compared to day

Table 1 Effect of Cd+Pb on removal efficiencies (%) of phosphate.

Removal efficiencies (%)									
Time (days)	Initial **Cd+Pb** concentrations(mg/L)				Time (days)	Initial Cd+ **Pb** concentrations(mg/L)			
	0	0.01+0.1	0.1+0.1	1+0.1		0	0.01+1	0.1+1	1+1
2	6.00	84.09	75.00	86.27	2	80.94	67.90	77.17	83.46
4	80.00	89.39	87.50	88.88	4	78.15	56.21	82.50	76.51
6	54.80	84.39	81.61	72.54	6	70.27	65.21	71.59	73.87
8	52.00	82.27	73.21	86.92	8	61.32	63.60	59.83	71.23

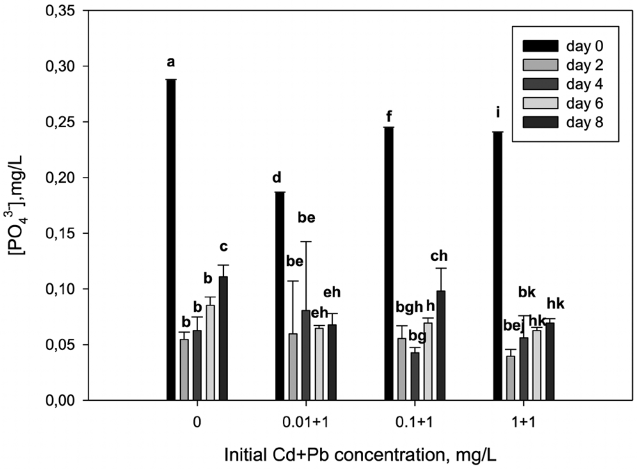

Figure 2. Effect of Cd + Pb on orthophosphate concentration at 1mgPb/L. Values represent mean ± S.D. Different letters indicate significant differences (P< 0.05) according to the ANOVA test.

0. While a nonsignificant removal efficiency was recorded in the control and the other treatments over time (Table 2). Additionally, the presence of Cd and Pb in solutions had no significant effect on nitrate removal on days 2, 4 and 6 as compared to control but nitrate concentration decreased by 30% from control value on day 8, in the mixture combining 1mg/L Cd to 0.1 mg/L Pb.

Figure 4 showed nitrate concentration changes over time in medium containing binary mixtures of varied Cd concentrations (0.01, 0.1 and 1 mg/L) and 1 mg/L Pb. Initial nitrate concentrations were 63 ± 1, 61 ± 0.01, 57 ± 1 and 56 ± 1 in the control and in the mixtures treated with 1 mg/L of Pb combined to 0.01, 0.1 and 1 mg/L of Cd respectively. In general, nitrate concentration did not vary much during the course of the experiment and also when compared to control values, resulting in weak removal efficiencies (Table 2).

Growth of *Lemna gibba*

Growth results of the binary metal mixtures are given in Figure 5 and Figure 6, where Pb concentration was kept at 0.1 mg/L or 1 mg/L while Cd concentration was set at 0.01, 0.1 and 1 mg/L. According to ANOVA analysis, all duckweed samples showed a significant increase in the relative growth with time in all treatment groups (p<0.05). The relative growth (RG) values obtained at the end of the experiment, were 243.3%, 256.11%, 194.44% and 187.77% in the control and at varied Cd concentrations from the lowest to the greatest concentration, respectively.

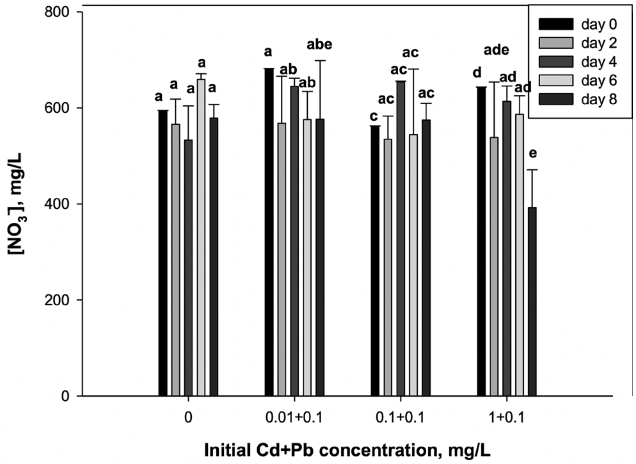

Figure 3. Effect of Cd + Pb on nitrate concentration at 0.1mgPb/L**.** Values represent mean± S.D. Different letters indicate significant differences (P< 0.05) according to the ANOVA test.

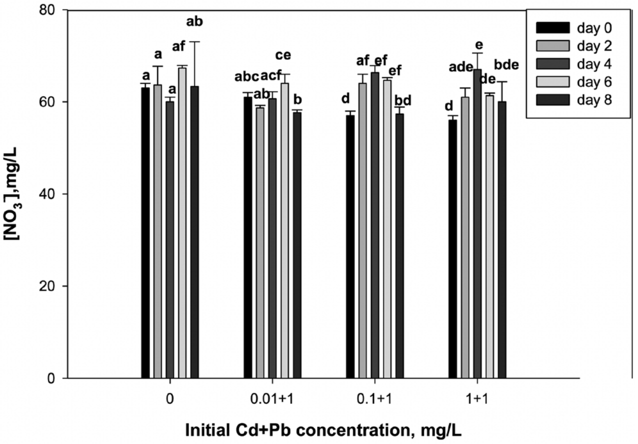

Figure 4. Effect of Cd + Pb on nitrate concentration at 1mgPb/L**.** Values represent mean± S.D. Different letters indicate significant differences (P< 0.05) according to the ANOVA test.

Table 2 Effect of Cd and Pb on removal efficiencies (%) of nitrate.

Removal efficiencies (%)									
Time (days)	Initial **Cd+Pb** concentrations (mg/L)				Time (days)	Initial Cd+ **Pb** concentrations (mg/L)			
	0	0.01+0.1	0.1+0.1	1+0.1		0	0.01+1	0.1+1	1+1
2	4.50	16.64	4.75	16.08	2	0	3.82	0	0
4	10.07	5.33	0	4.37	4	4.75	0.54	0	0
6	0	15.46	3.08	8.57	6	0	0	0	0
8	2.30	15.37	0	38.85	8	0	5.46	0	0

Similarly, as shown in Figure 6, relative growth of *Lemna gibba* greatly increased with time, at 1 mg/L Pb when combined with varied Cd concentrations (0.01, 0.1 and 1 mg/L) and obtained the maximum values of 149.99%, 142.77% , 139.44% and 104.44% at the end of the experiment (day 8).

However, the relative growth of plants exposed to a mixture of Cd and Pb at 1 mg/L and 0.1 mg/L respectively, decreased significantly (p<0.05) by 30% as compared to the control on day 6 and at 1 mg/L of each metal on day 8. There were no significant differences among the other treatments group with respect to the control .

Lemna gibba did not show any visual symptoms in the binary mixtures. Fronds looked green and healthy at the concentrations of metals to which *L. gibba* was exposed.

Cd and Pb accumulation

The amount of Cd accumulated in the tissues of *Lemna gibba* over time and at varied Cd concentrations combined with 0.1 mg/L of Pb are shown in Figure 7, A.

The maximum Cd accumulation was found to be 1.30 ± 0.15 mg/g in the presence of a binary combination of 1mg/L Cd and 0.1 mg/L Pb, whereas the highest Cd content in plant tissues co-contaminated by a mixture of Cd and Pb at 0.1 mg/L of each metal was 0.39 ± 0.03 mg/g. Additionally, no significant differences were found in Cd contents among the treatments in these mixtures, over time. In the mixture combining the lowest Cd concentration (0.01 mg/L) and 0.1 mg/L Pb, plants contained 0.213 ± 0.03 mg/g. Also, a significant increase of Cd accumulation was observed with the combination of Cd and Pb at 1 mg/L and 0.1 mg/L as compared to the control.

However, for Pb accumulation (Figure 7, B), the analysis of a one-way ANOVA showed that the differences in all the treatments were no significant (P > 0.05). Additionally, the ability of Pb uptake was weak as compared to Cd.

The elements were detected in plant tissue of the control groups but not in the culture medium.

As shown in Figure 8, A, in the presence of a binary combination of Cd and 1 mg/L Pb, it was observed that Cd accumulation occurs only at 1mg/L Cd. The amounts of Cd were 0.13 ± 0.04 mg/g, 0.37 ± 0.04 mg/g and 0.54 ± 0.11 mg/g and 0.46 ± 0.05 mg/g on days 2, 4, 6 and 8 respectively.

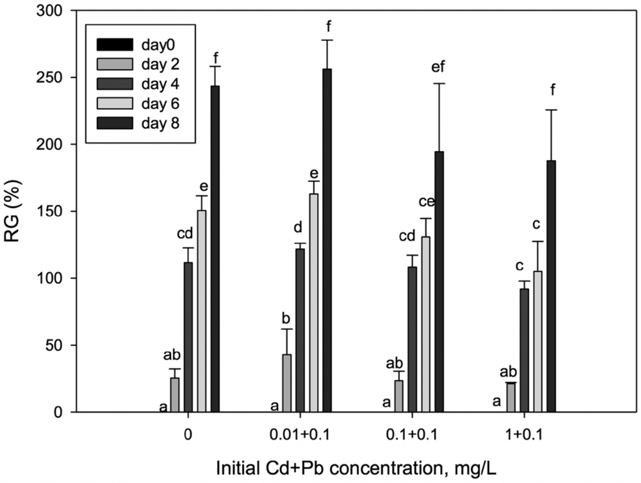

Figure 5. Effect of Cd + Pb on the growth of *Lemna gibba* at different concentrations and exposure times. Values represent mean± S.D. Different letters indicate significant differences (P< 0.05) according to the ANOVA test.

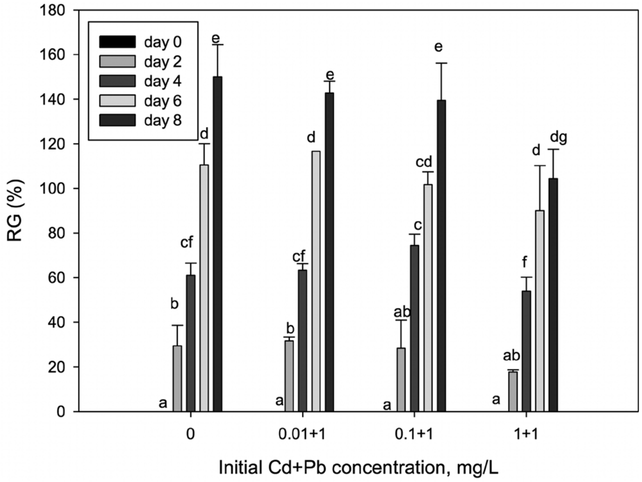

Figure 6. Effect of Cd + Pb on the growth of *Lemna gibba* at different concentrations and exposure times. Values represent mean ± S.D. Different letters indicate significant differences (P< 0.05) according to the ANOVA test.

According to Figure 8, B, the highest Pb uptake by the plant was found when Cd and Pb concentrations in the mixture were 0.01 mg/L and 1 mg/L respectively. Pb accumulations

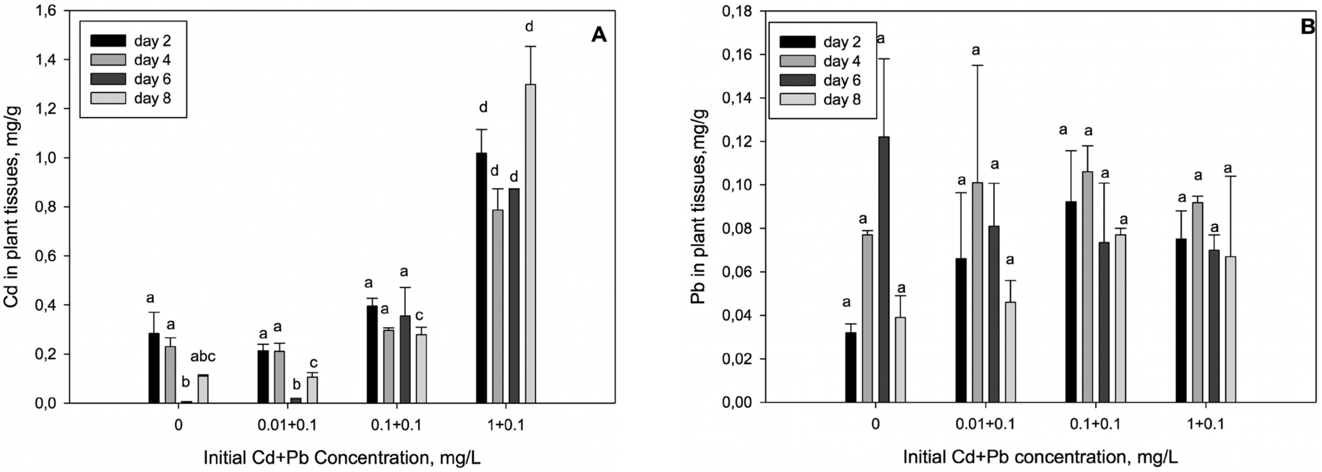

Figure 7. The accumulation of Cd (A) and Pb (B) by *Lemna gibba* at different concentrations and exposure times. Values represent mean ± S.D. Different letters indicate significant differences (P< 0.05) according to the ANOVA test.

in *Lemna gibba* were 0.45 ± 0.09 mg/g, 0.45 ± 0.03 mg/g, 0.54 ± 0.04 mg/g and 0.65 ± 0.02 mg/g on days 2, 4, 6 and 8 respectively. A significant decrease of Pb content in plants exposed to Cd and Pb at 0.1 mg/L and 1 mg/L and also in the mixture of Cd and Pb at 1 mg/L of each metal was observed. The elements were not present in plant tissue of the control group nor in the medium.

Removal of Cd and Pb

The change of Cd and Pb concentration in the culture medium co-contaminated by Cd and 0.1 mg/L Pb is illustrated in Figure 9A and Figure 9B respectively. Concentrations of metals decreased significantly ($p<0.05$) in the first two days after their introduction. The percentage of Cd and Pb removed from binary mixture solutions by *Lemna gibba* is shown in Table 3. As can be seen, the Cd removal in duckweed system ranged between 6-100% among different experiments. The maximum removal as compared to initial concentration was recorded 100% at 0.01 mg/L Cd followed by 41% at 0.1 mg/L and 16% at 1mg/L Cd. Pb removal (%) was in the ranges of 66-100%. The maximum removal of Pb was 83% at 0.01 mg/L Cd and 100% at 0.1 and 1 mg/L Cd (Table 4).

The variation of metals concentrations in the mixtures of Cd (0.01, 0.1 and 1mg/L) when combined to 1 mg/L of Pb, is shown in Figure 10.

Results revealed a weak removal of Cd (Figure 10A). The maximum removal as compared to initial Cd concentration

Table 3 Effect of Cd+Pb on removal efficiencies (%) of Cd from nutrient solution.

Removal efficiencies (%)									
Time (days)	Initial **Cd+Pb** concentrations(mg/L)				Time (days)	Initial Cd+ **Pb** concentrations(mg/L)			
	0	0.01+0.1	0.1+0.1	1+0.1		0	0.01+1	0.1+1	1+1
2	0	100	33	12	2	0	0	5	23
4	0	100	19	6	4	0	0	3	27
6	0	100	16	16	6	0	0	6	24
8	0	100	41	13	8	0	0	0	14

Table 4 Effect of Cd+Pb on removal efficiencies(%) of Pb from nutrient solution.

Removal efficiencies (%)									
Time (days)	Initial **Cd+Pb** concentrations(mg/L)				Time (days)	Initial Cd+ **Pb** concentrations(mg/L)			
	0	0.01+0.1	0.1+0.1	1+0.1		0	0.01+1	0.1+1	1+1
2	0	81	100	100	2	0	nd	64	67
4	0	83	90	100	4	0	nd	61	73
6	0	68	74	83	6	0	nd	55	62
8	0	66	71	96	8	0	nd	40	60

was 27% obtained at the highest Cd concentration while the plants removed 0% and 6% when Cd concentration in the mixture was 0.01 and 0.1 mg/L. Nevertheless, Pb removal was higher (64% and 73% at 0.1 and 1 mg/L of Cd respectively). Pb removal at the lowest Cd concentration was not determined. A significant decrease of Pb concentration in the medium was achieved between day 0 and day 2 (Figure 10B).

Discussion

Phosphorus (P) and nitrogen (N) are the major plant nutrients. Orthophosphate, the predominant inorganic form of P, is the more easily removed of the three type of phosphorus (Ozengin & Elmaci, 2007). N can be removed through plant uptake of ammonium and nitrate. It is well recognized that duckweeds can remove efficiently nitrate and phosphate. Duckweeds act as a “nutrient pump” in wastewater treatment absorbing nutrients like nitrate and phosphate (Gupta & Prakash, 2014). Korner & Vermaat (1998) reported removal efficiencies of 14 - 92.2% for when using *L. gibba*. According to Vermaat & Hanif (1998) a removal of 77% of total phosphorus was reached with lemnaceae. In the study conducted by Aggoun et al. (2018), phosphate removal (%) was in the ranges of 75.50-97.53%, whereas nitrate removal efficiencies with *Lemna gibba* monitored on days 2, 4 and 6 of the experiments ranged between 48.59 % and 78.63 %, in presence of Cd and Pb respectively.

During the biological treatment of wastewater, heavy metals are capable to affect the removal of nitrate and phosphate (Balamane-Zizi & Ait-Amar, 2012). Leblici and Aksoy (2011) found that nutrient removal (nitrate, phosphate and sulfate) was reduced when *Lemna minor* and *S. polyrrhiza* were exposed to Pb. In contrast, Aggoun et al. (2018) concluded that Cd and Pb did not have negative effect on nitrate and phosphate removal with *Lemna gibba*.

However, to our knowledge, no study has been conducted about the effect of a binary mixture of heavy metals stress on the ability of a duckweed *Lemna gibba* on the removal of phosphate and nitrate.

In this study, the results showed that in treatments of the aquatic plant by a mixture of Cd (0.01, 0.1 and 1 mg/L) and 0.1 mg/L Pb, orthophosphate concentration decreased markedly within four days of initiating the experiments. The maximum removal efficiencies on day 4 were not less than 80%. In the binary mixture with Cd and 1 mg/L Pb, phosphate concentration was significantly declined after two days of metal treatments, leading to maximum efficiencies ranging between 63 - 87%. Also, the initial concentration of the mixture of Cd + Pb has no negative effect on phosphate removal. The relative growth of *Lemna gibba* increased over time leading to higher phosphate removal from the medium. In general, nitrate removal efficiencies were weak as compared to phosphate in all treatments. According to Fang et al. (2007), duckweeds prefer to take up NH_4^+ than NO_3^- by both roots and fronds. Additionally, Cd and Pb concentration in the medium declined significantly within the first days (2 days). Thus, *L. gibba* is capable of rapid and effective bioremediation. Our finding is in accordance with Üçüncü et al. (2013). They found that the bioremediation of a mixture of Cr, Cu and Pb is largely completed within the first 24 h.

Cd removal percentage of 100% was obtained at the lowest Cd concentration (0.01 mg/L) combined to 0.1 mg/L Pb, whereas lower to weak removal efficiencies were realized at the other

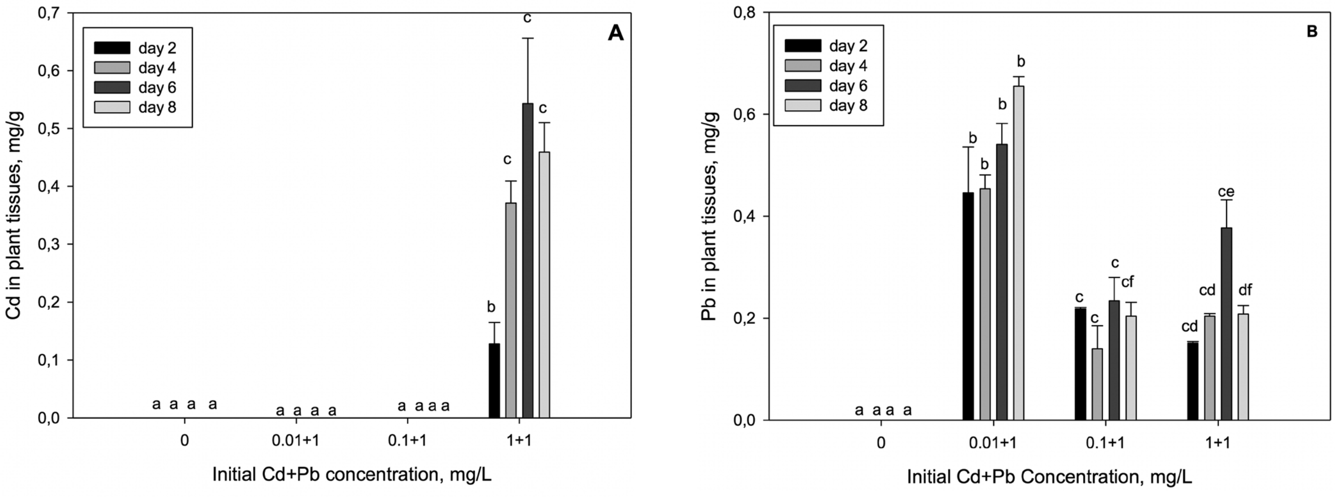

Figure 8. The accumulation of Cd (A) and Pb (B) by *Lemna gibba* at different concentrations and exposure times. Values represent mean ± S.D. Different letters indicate significant differences (P< 0.05) according to the ANOVA test.

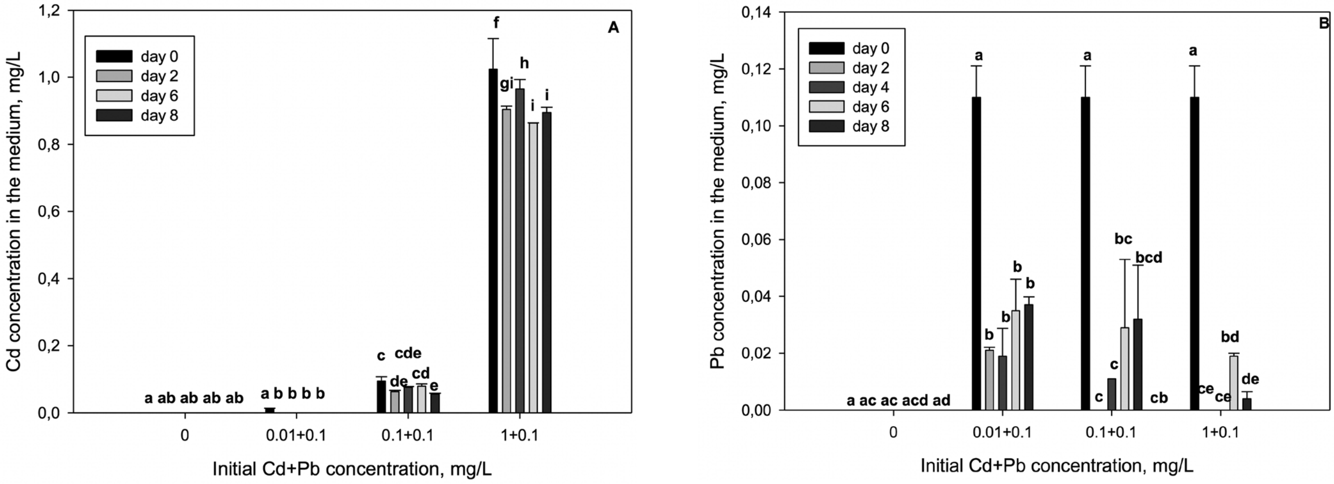

Figure 9. Cd (A) and Pb (B) concentration in the culture medium at different concentrations and exposure times. Values represent mean ± S.D. Different letters indicate significant differences (P< 0.05) according to the ANOVA test.

treatments. Pb removal efficiencies from the medium were at least 66% and 63% at 0.1 mg/L and 1 mg/L of Pb respectively. It is obvious that Pb is preferentially removed by *L. gibba*. Our results agree with Alonso-Castro et al.; (2014) when they used plants of *T. latifolia*. Cd and Pb removal percentages were 38% and 87% respectively from a binary mixture of 5 mg/L Cd combined to 5mg/L Pb. When these elements were combined at concentrations of 7.5 mg/L each metal, 32% of Cd and 80% were eliminated. Also, Nga & Ha (2016), reported Cd and Pb removal efficiencies of 69.5% and 92% in a mixture of five heavy metals containing 0.1 mg/L of Cd and 0.6 mg/L of Pb when treated with *Phragmite australis*.

Several studies reported that the presence of one metal influenced the uptake of others metals (Peralta-Videa et al., 2002, An et al., 2004, John et al., 2008).

CONCLUSIONS

Over the last century, the increased industrialization has led to dramatic elevated releases of anthropogenic pollution into the aquatic environment. Eutrophication is also a global problem. To minimize the impact of this pollution, it was important to develop innovative technologies to purify the contaminated sites waters. Phytoremediation becomes a cost-efficient and effective strategy in situ removal of prevalent contaminants. The use of aquatic plants for wastewaters treatment has gained popularity. The use of duckweed in water body restoration and in heavy metals removal is a good issue because of the advantages of duckweed and the low cost of this alternative. Concern about heavy metals effect

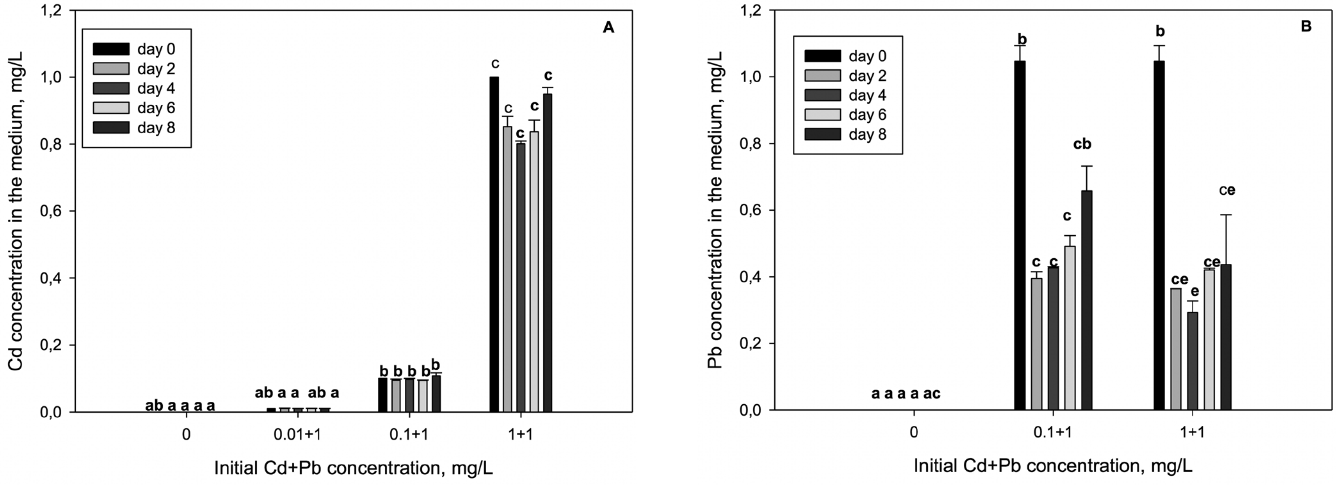

Figure 10. Cd (A) and Pb (B) concentration in the culture medium at different concentrations and exposure times. Values represent mean ± S.D. Different letters indicate significant differences (P< 0.05) according to the ANOVA test.

on aquatic environment, turns toward the impact of mixtures. Our study indicated that a mixture of cadmium and lead can be taken up by the duckweed *Lemna gibba* leading to high removal efficiencies from the medium. Furthermore, orthophosphate was the most efficiently removed from the culture solution as compared to nitrate. At the concentrations of binary mixtures of Cd and Pb to which *Lemna gibba* was exposed, no negative effect was observed neither on phosphate removal nor on the plant growth. Nevertheless, the removal of nitrate was very weak.

Thus, in a medium containing a mixture of Cd and Pb, *Lemna gibba* removed successfully Cd, Pb and orthophosphate.

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