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Combined Effects of Lead and Different pH Levels on *Lemna minor* L., a Free-Floating Aquatic Macrophyte

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Abstract: In the present study, effects of pH at 5.0, 7.0 and 9.0 levels on uptake of lead, content of chlorophyll and nitrogen were investigated in *Lemna minor*. After 12-days Pb^{2+} treatment, total chlorophyll and nitrogen contents in *L. minor* were adversely affected from Pb concentrations dose dependently at each pH levels. The plant was adversely affected by pH 5.0, more than by pH 9.0. However, the lowest toxic effects of Pb^{2+} was found at pH 7.0. Lead accumulations in the plant tissues increased with increasing Pb^{2+} concentrations. Lower Pb^{2+} accumulations were measured at pH 7.0 and 9.0 compared to pH 5.0. In conclusion, that uptake rate of Pb^{2+} and its toxicity on chlorophyll and nitrogen contents in the macrophyte were dependent upon the pH value of the solutions.

Key words: Lead · pH · *Lemna minor* · Chlorophyll and nitrogen

INTRODUCTION

Lead is one of the hazardous heavy metal pollutants of the environment that originates from various sources like mining and smelting of lead-ores, burning of coal, effluents from storage battery industries, automobile exhausts, metal plating and finishing operations, fertilizers, pesticides and from additives in pigments and gasoline [1]. Lead has not been shown to be essential in plant metabolism, although it occurs naturally in all plants [2]. Responses of plants to Pb^{2+} exposure include decrease in root elongation and biomass [3], inhibition of chlorophyll biosynthesis [4] and induction or inhibition of several enzymes [5].

The pH of water has a major influence on the physical and chemical forms of metals and metal compounds in the aquatic environment because it controls the solubility and concentrations of major metal species. Increasing the acidity of a solution increases the concentrations of free metal ion in that solution. This is due to the competition between H^+ and metal ions for binding sites on inorganic and organic ligands. Because of the relationship between pH and concentration of free metal ions, it has been assumed that metals are more likely to be toxic to biota in acidic than in neutral waters [6].

Duckweed is distributed world-wide in wetlands. These plants are free-floating, fast growing, adapt easily to various aquatic conditions and are easy to culture in laboratory. They are a convenient plant for ecotoxicological investigations and play an important role in the extraction and accumulation of metals from waters [7].

The objective of this study was to determine the effects of pH on the accumulation and toxicity of Pb^{2+} in wetland species *Lemna minor* L. under acidic, neutral and basic pH conditions.

MATERIALS AND METHODS

The widespread and often prolific occurence of *Lemna minor* L. (duckweed) in Cukurova region fresh water has made it an ideal choice as a test organism for pollution studies. Duckweed plants were collected from the local streams of Cukurova in Adana, Turkey. These were acclimatized in 10 % nutrient solution [8], a day temperature of 22±2°C and a night temperature of 18±2°C and with a daily photoperiod of 16 h of light (6000±200 lux). Duckweed plants were cultured for 12 days in beakers containing 500 ml solution. The concentrations of lead in the polluted waters source are in the range of 1-100 $\mu g\ mL^{-1}$. These concentrations are, however, frequently reduced during treatment, prior to discharge to the receiving waters [9]. Thus, the plants (2 g fw) were exposed to Pb as $Pb(CH_3COO)_2$. $3H_2O$ (Merck) at 1, 5, 10, 25, 50 and 100 $\mu g\ mL^{-1}$ concentrations which were added

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to nutrient solution. Plants without added trace elements served as controls. Three replicate groups of plants were exposed to Pb^{2+} and different pH treatments for 12 d. The pH of solutions were adjusted to 5.0 (±0.04), 7.0 (±0.04) and 9.0 (±0.04) via HANNA HI 9025 using 0.1 N NaOH (Merck) and 0.1 N HCl (Merck). The test media were changed every second day, the Pb concentrations were replenished and pH's of solution were adjusted.

After the termination of experiment, the macrophytes were washed three times with distilled water. Then, they were dried to a constant weight at 80°C in electric furnace (NUVE FN 300) and pulverized using mortar and pestle. The samples were dissolved in 14 M HNO_3 and residues were dissolved in 1 M HCl. After mineralization, the metal was determined using an atomic absorption spectrophotometer (Perkin Elmer Model 3100). Control samples were also treated by the same way. Values of uptake were obtained by deducting metal contents of control macrophytes. Bioconcentration factor (BCF) was estimated from ratio of lead concentrations in plants to lead in test medium [10]. Chlorophyll content of fronds was determined following Arnon [11]. The extraction of pigment was done in acetone (80% v/v, Merck). Then, the extraction solutions were filtered and the filtrate was recorded at a spectrophotometer (UNICAM UV/VIS). Extraction solution was used as blank. Total Nitrogen was analyzed by an ammonia distillation process using a Micro-Kjeldahl methods [12]. Lead concentrations, nitrogen and chlorophyll amounts in *L. minor* are means of the three replicates.

For statistical analyses we chose the analysis of variance (ANOVA) in Statistical Analysis System (SPSS 11.0 for windows). The significance of differences among mean values were determined by a multiple range test (LSD; Least Significant Difference). For this reason, alpha (á) was preferred to be 0.05.

RESULTS AND DISCUSSION

Every plant has an optimal pH for its growth. In the medium without Pb^{2+}, the growth of *L. minor* was affected adversely at pH 5.0 respective to pH 7.0 and 9.0. As medium pH range of the macrophytes collected were 6.4-7.2, they showed the best development at pH 7.0. According to Miranda and Ilangovan [4], reduced frond size and chlorosis were observed on *Lemna gibba* at the concentrations of 500 μg Pb mL^{-1} than at 30 and 50 μg Pb mL^{-1}. In the present study, significantly reduced frond size was observed in the medium of 50 and 100 μg Pb mL^{-1} at all tested pH levels, especially at pH 5.0.

Chlorophyll content is a parameter that is sensitive to heavy metal toxicity. It is reported by various investigators that Pb^{2+} inhibits chlorophyll synthesis and consequently leads to decrease in chlorophyll content in duckweed [4,13]. At all tested pH levels, a dose dependent reduction was found in chlorophyll content of *L. minor* fronds (Figure 1). While, a decrease in chlorophyll content in the *L. minor* exposed to 1 and 5 μg Pb mL^{-1} at all tested pH levels was found to be insignificant, it was statistically significant at 100 μg Pb mL^{-1} concentration at all pH levels compared to controls ($p<0.05$). The highest chlorophyll content was determined in the control of macrophytes grown at pH 7.0. The minimum chlorophyll contents were found in plants exposed to 100 μg mL^{-1} Pb at pH 5.0, 7.0 and 9.0 and reductions in chlorophyll relative to their control were 39.7, 33.3 and 43.1 %, respectively. In many cases, heavy metal toxicity is reduced at low pH, with often increased metal availability, possibly owing to decreased uptake [14]. In consideration of our results, chlorophyll content at pH 5.0 was lower than that of observed at other pH values, which possibly occurs as pH 5.0 has more adverse effects than that of pH 7.0 and 9.0 as well as effects of the metal. The substitution of the Mg^{2+} in the chlorophyll molecule by certain toxic heavy metals such as Cu, Zn, Cd or Hg has been shown to occur during heavy metal stress in higher plants, resulting in a breakdown of photosynthesis [15]. On the other hand, Van Assche and Clijters [5] reported that the reduction in chlorophyll content in the presence of the Pb^{2+} may be due to an inhibition of chlorophyll biosynthesis.

The form of molecules present in solution can also be effected by pH. For example at a low pH, ammonia is ionized to ammonium due to a large number of hydrogen ions, while in solutions with pH above 7.2, ammonium ions (NH_4^+) are deionised back to ammonia [16]. On the other hand, lead reduces the uptake and transportation of some nutrients in plants [17]. In the present study, total nitrogen (N) content in control plants were estimated to be 3.27, 3.60 and 3.36 % at pH 5.0, 7.0 and 9.0, respectively (Figure 2). At all Pb^{2+} concentrations and pH levels total N content decreased compared to their controls. There were decreases in total N content of *L. minor* in parallel to increased Pb^{2+} concentrations. In all tested pH levels in the macrophyte reduction of N was found to be insignificant in 1, 5 and 10 μg Pb mL^{-1} with respect to their control. But, the decline of N was found to be significant in the medium of 50 and 100 μg Pb ml^{-1} for the plant at all pH levels and significant reductions were also observed in the 25 μg Pb mL^{-1} concentration at

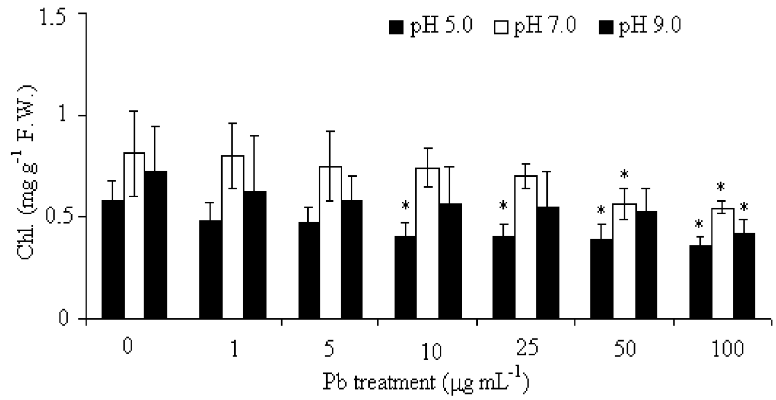

Fig. 1: Total chlorophyll content of *L. minor* at different Pb^{2+} concentration and pH levels. Error bars represent the standard deviation about the mean of three replicates. The asterisks (*) denotes differences compared to the control group (p<0.05).

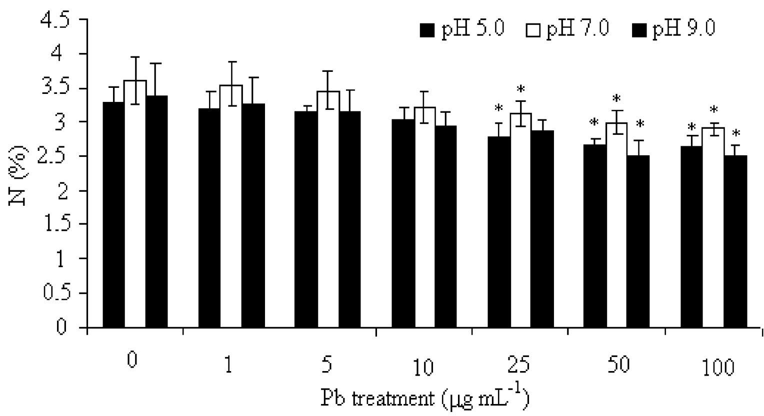

Fig. 2: Total nitrogen content of *L.minor* at different Pb^{2+} concentration and pH levels. Error bars represent the standard deviation about the mean of three replicates. The asterisks (*) denotes differences compared to the control group (p<0.05)

pH 5.0 and 7.0 (p<0.05). The minimum N contents were found in *L. minor* exposed to 100 µg Pb mL^{-1} at pH 5.0, 7.0 and 9.0 and reduction rates in N relative to their controls were estimated by 19.6, 19.7 and 25.6%, respectively. Particularly at pH 5.0 and 9.0, it was shown that reduction in the uptake and transportation of nitrogen to plants related with increasing Pb^{2+} concentration. The multiplication factor, 6.25, used for the conversion of nitrogen into protein. When the factor is considered, the reduction in nitrogen content indicates decreased protein content in the duckweed.

Metal bioaccumulation depends upon plant species, its organ and numerous abiotic factors like temperature, pH, transportation of metal contaminated particles and dissolved ions in water [18,19]. Few studies have considered the role of pH in determining metal bioavailability to aquatic plants. Metal levels in *Utricularia purpurea* have been shown to be related more to water column pH than to total metal [20]. A similar water column pH effect has been demonstrated for *Nuphar lutea*, which obtains most of its nutrition though leaves and *Phragmites australis*, which obtains its nutrients primarily from the sediments. These macrophytes showed little response to water column pH in that metal accumulation [21]. *L. minor* is a free-floating aquatic macrophyte, absorbs heavy metals in water and was used to determine Pb^{2+} accumulation capability related to different pH levels in our study (Table 1). Lead accumulations in the plant tissues increased with increasing Pb concentrations. The plants, exposed at pH 5.0 accumulated more lead, compared to other pH levels. On the otherhand, low Pb^{2+} accumulation was measured at pH 9.0. The maximum Pb^{2+} concentrations were measured in the medium of 100 µg Pb mL^{-1}as 4405 (±762), 2721 (±633) and 1102 (±232) at 5.0, 7.0 and 9.0 pH levels, respectively. These are about

Table 1: Pb^{2+} accumulations (μg g^{-1} dw) and BCFs at different nominal Pb^{2+} concentrations and pH levels in *L. minor* after 12-days Pb application

	Pb^{2+} accumulations and BCFs at different pH levels					
Pb treatment (μg mL^{-1})	5.0		7.0		9.0	
0	$19\pm6^{a,x}$	BCF	$23\pm9^{a,x}$	BCF	$21\pm7^{a,x}$	BCF
1	$341\pm17^{abc,x}$	341	$183\pm38^{ab,x}$	183	$95\pm20^{ab,x}$	95
5	$709\pm157^{bcd,x}$	142	$250\pm41^{ab,y}$	50	$156\pm29^{ab,y}$	31
10	$845\pm192^{cd,x}$	85	$387\pm102^{ab,y}$	39	$268\pm65^{bc,y}$	27
25	$1187\pm206^{de,x}$	47	$691\pm155^{b,y}$	28	$378\pm57^{c,y}$	15
50	$1752\pm313^{e,x}$	35	$1654\pm383^{c,x}$	33	$870\pm172^{d,y}$	17
100	$4405\pm762^{f,x}$	44	$2721\pm633^{d,y}$	27	$1102\pm232^{e,z}$	11

Values expressed as mean±SD. Letters *a, b, c, d, e* and *f* show the differences among exposure concentrations; letters *x, y* and *z* show the differences among pH levels ($p<0.05$)

231.8, 118.3 and 52.5 times greater than those of the control macrophytes at the respective pH levels. Lower Pb^{2+} accumulations were measured at pH 7.0 and 9.0 compared to pH 5.0. It is known that lead is precipitated as $Pb(OH)_2$ at pH levels >7.0 [22]. Based on this reason, uptake of Pb^{2+} by plant was limited because of metal precipitation, especially at pH 9.0. Pb^{2+} is mobilized at pH levels under pH 5.0 and thus Pb ion is available to plants.

BCF, which indicates the efficiency of the plant in accumulating metals, generally decreased with increased Pb^{2+} concentration in tissues of *L. minor* (Table 1). The highest BCF, 341.6, was estimated in *L. minor* treated with 1 μg Pb ml^{-1} at pH 5.0. The lowest BCF, 15.1, was observed in the plant exposed to 25 μg Pb ml^{-1} at pH 9.0. Maximum BCF's were observed in the solutions of pH 5.0. This indicates that *L. minor* has high Pb^{+2} accumulation capability at the lower pH level.

In conclusion, it can be easily said that *L. minor* is a good accumulator of Pb. Thus, duckweed may be useful from a phytoremediation standpoint. Most trace metals are more toxic to plants at lower pH levels (Lepp, 1981). At high external Pb^{2+} concentrations and all tested pH levels nitrogen and chlorophyll contents were decreased in the species particularly at pH 5.0. Besides Pb^{2+} toxicity, low levels of pH was also an inconvenient media for the duckweed. The present study indicated that uptake rate of Pb^{2+} and its toxicity on chlorophyll and nitrogen contents in the macrophyte were dependent upon the pH value of the solutions. Results obtained in the present study can be a source for further investigation dealing with similar subjects.

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