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Effect of Lead and pH on Lead Uptake, Chlorophyll and Nitrogen Content of *Typha latifolia* L. and *Ceratophyllum demersum* L.

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ABSTRACT

In the present study, effects of pH (5.0, 7.0 and 9.0) and lead (@1,5,10,20,50 and 100 µg mL^{-1}) on uptake of lead, N and content of chlorophyll and nitrogen were investigated in *Typha latifolia* L. and *Ceratophyllum demersum* L. Total chlorophyll and nitrogen contents in *T. latifolia* and *C. demersum* were adversely affected from Pb^{2+} concentrations dose dependently at each pH. Toxicity of Pb^{2+} on both macrophyes was pH dependent. The plants were adversely affected by pH 5.0 or more than 9.0. However, the lowest toxic effects of Pb^{2+} was found at pH 7.0. According to the parts of *T. latifolia*, Pb^{2+} amounts were found in all tested pH levels and the metal concentrations in the following order: root>leaf. Pb^{2+} concentrations in plant tissues in relation to pH were generally found for *T. latifolia* in following order in the roots: 9.0> 7.0> 5.0; in the leaves: 7.0> 9.0> 5.0, and in whole *C. demersum* tissues: 7.0> 9.0> 5.0.

Key Words: Lead; pH; *Typha latifolia* L.; *Ceratophyllum demersum* L.

INTRODUCTION

In contaminated aquatic environments several toxic metals occur, often creating undesirable living conditions for many plants and animals (Palmer & Wittbrot, 1991). Aquatic macrophytes are known to have great importance, forming a substantial component of the primary production in many aquatic habitats (Pip, 1990). Vascular aquatic macrophytes may accumulate considerable amounts of heavy metals in their tissues (Kovacks *et al*., 1984). In the recent past, several of the submerged, emergent and free-floating aquatic macrophytes are reported to bioconcentrate heavy metals in natural waters as well as after exposure to wastewaters (Greger, 1999). Some aquatic or semi aquatic plants such as *Eichornia crassipes* (Dierberg *et al*., 1987), *Lemna minor* (Mo *et al*., 1989), *Spirogyra fluviatilis* (Saygıdeğer, 1998), *Veronica anagallis-aquatica* and *Ranunculus aquatilis* (Saygıdeğer, 2000) can take up heavy metals from contaminated solutions.

Lead has not been shown to be essential in plant metabolism, although it occurs naturally in all plants (Kabata-Pendias & Pendias, 1984). 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 (Eick *et al*., 1999). Excess Pb^{2+} content in plants interferes with and inhibits various physiological processes (Balsberg Pahlsson, 1989). Responses of plants to Pb^{2+} exposure include decrease in root elongation and biomass (Fargasova, 1994), inhibition of chlorophyll biosynthesis (Miranda & Ilangovan, 1996), induction or inhibition of several enzymes (Van Assche & Clijsters, 1990).

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 (Lewis, 1995; Lewander *et al*., 1996). Metal toxicity is often dependent on pH in freshwater and soil (Campbell & Stokes, 1985). The objective of the present study was to determine the effects of pH on the accumulation and toxicity of Pb in *Typha latifolia* L. (cattail, a emergent macrophyte) and *Ceratophyllum demersum* L. (coontail, a rootless submerged macrophyte).

MATERIALS AND METHODS

Typha latifolia L. and *Ceratophyllum demersum* L. are commonly available aquatic macrophytes in Adana, Turkey. The macrophytes were collected from the local water bodies. These were acclimatized in 10% Arnon and Hoagland (1940) nutrient solution at 26-27^{o}C , 16 h light (6000 lux) and 8 h dark periods. The concentrations of lead in polluted sources waters lie typically in the range 1-100 µg mL^{-1}. These concentrations are, however, frequently reduced during treatment, prior to discharge to the receiving waters (Harrison & Laxen, 1981). According to this, the macrophytes were treated with Pb as $Pb(CH_3COO)_2 \cdot 3H_2O$ (MERCK) at 1, 5, 10, 25, 50 and 100 µg mL^{-1}. A sample of 10% nutrient medium was used as control. Macrophytes were placed in 3000 mL plastic pods and this process repeated three times. Solution pH was adjusted to 5.0 (±0.04), 7.0 (±0.04) and 9.0 (±0.04) via HANNA HI 9025 using N/10 H_2SO_4 or N/10 NaOH. The test media were changed every second day and replenish Pb and adjust to pH's of solution. The macrophytes were harvested after 12-days of treatment and analyzed for lead, chlorophyll and nitrogen content.

Chlorophyll content was determined following Arnon (1949). The extraction of pigment was done in acetone (80% v/v, MERCK). For extraction, 100 mg of macrophyte leaves were used. Then, the extraction solution was filtered and the filtrate was recorded at spectrophotometer (UNICAM UV/VIS). Micro-Kjeldahl method was used to determine of total nitrogen in the macrophytes (Kacar, 1972).

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 (NÜVE FN 300). Dried samples were dissolved in 14 M HNO_3 and, residues solved in 1 M HCl. After mineralization the metal was determined by Atomic Absorption Spectrophotometer (Perkin Elmer 3100). Control samples were also treated by the same way. Values of uptake were given after deducting metal contents of control macrophytes.

For statistical analyses we chose the analysis of variance (ANOVA) in Statistical Analysis System (SPSS 11.0 for windows). The significance of differences between mean values were determined by a multiple range test (LSD; Least Significant Difference). For this reason, alpha (α) was preferred to be 0.05, which corresponds to a confidence level of 95%.

RESULTS AND DISCUSSION

Every plant has an optimal pH for its growth. In order to survive, all plant cells must maintain near neutral pH in the cytoplasm. In the medium without Pb^{2+}, both *T. latifolia* and *C. demersum* were affected more adversely by the pH of 5.0 than that of at pH 7.0 and 9.0. As medium pH range of the macrophytes collected was 8.1-8.6. They showed the best development at pH 9.0. In addition, during daily measurement, the macrophytes at pH 5.0 appeared to increase the medium pH to basic levels, which suggests that the plants tend to change the medium pH to a convenient level to provide an optimum living conditions for themselves. During the experiments, macrophytes seemed to be affected in an adverse way from the increase of the metal concentrations at each observed pH, especially at the concentrations of 50 and 100 µg Pb mL^{-1}. During the experiment, *C. demersum* appeared to be affected in a more adverse way than other plants at pH 5.0 as its tissues softened, especially with the increase of the metal concentration.

Chlorophyll content is a parameter that is sensitive to heavy metal toxicity (Gupta & Chandra, 1996). Inhibition in chlorophyll level by Pb^{2+} has been observed in aquatic plants (Jana & Chaudhary, 1984). At all tested pH levels, a dose dependent reduction was found in chlorophyll content of *T. latifolia* leaves (Fig 1). While decrease in chlorophyll content in the *T. latifolia* exposed to 1 µg Pb mL^{-1} at all tested pH levels were found to be insignificant ($P>0.05$), it was statistically significant compared to control with plants exposed 50 and 100 µg Pb mL^{-1} at all tested pH levels ($P<0.05$). The highest chlorophyll content was determined in control of macrophyte grown at pH 9.0. The lowest chlorophyll contents were found in plants exposed to 100 µg

Table I. Pb^{2+} amounts (µg g^{-1} dry weight) at different Pb concentrations (µg mL^{-1}) and pH levels in *T. latifolia* root tissues

Pb in medium (mg mL^{-1})	pH		
	5.0	7.0	9.0
0	44±40a	43±60a	46±60a
1	182±16ab	336±21ab	237±17a
5	244±23ab	838±38bc	1040±15b
10	405±31ab	1158±85c	2140±21c
25	441±20ab	2347±16d	3029±57d
50	699±43bc	3953±23e	5670±25e
100	1261±11c	6249±39f	12328±41f

Values are mean ± SE; Means with different letters are significantly different from one another ($P<0.05$); (n=4)

Table II. Pb^{2+} amounts (µg g^{-1} dry weight) at different Pb concentrations (µg mL^{-1}) and pH levels in *T. atifolia* leaf tissues

Pb in medium (mg mL^{-1})	pH		
	5.0	7.0	9.0
0	9±1a	8±1a	10±3a
1	24±2ab	41±5b	36±3b
5	32±4abc	45±6bc	55±6ab
10	42±6bc	63±9bc	59±8bc
25	53±8c	67±8c	68±8c
50	63±10cd	81±12c	94±11d
100	85±12d	145±14d	124±14e

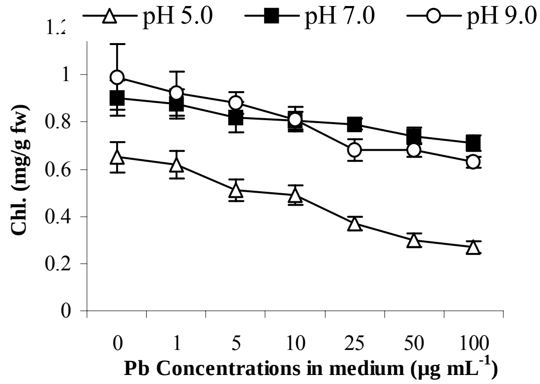
Values are mean±SE. Means with different letters are significantly different from one another ($P<0.05$). (n=4)

Table III. Pb^{2+} amounts (µgg^{-1} dry weight) at different Pb concentrations (µg mL^{-1}) and pH levels in whole *C. demersum*

Pb in medium (mg mL^{-1})	pH		
	5.0	7.0	9.0
0	25±40a	26±40a	26±50a
1	168±18ab	205±23ab	273±27a
5	236±25abc	284±24ab	325±39ab
10	316±29abc	513±39b	638±57bc
25	423±34bc	1146±16c	862±65c
50	512±39c	1392±14c	1445±18d
100	878±53d	2916±24d	2550±31e

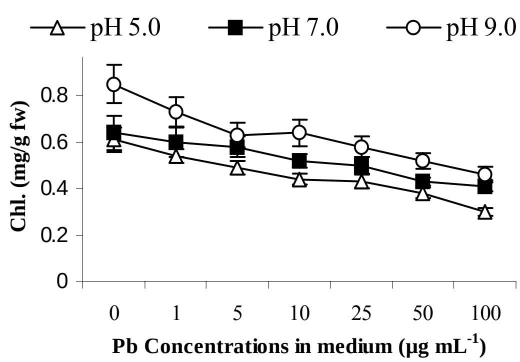
Values are mean±SE; Means with different letters are significantly different from one another ($P<0.05$); (n=4)

Fig. 1. Total chlorophyll content of *T. latifolia* leaves at different Pb concentration and pH levels



Pb mL^{-1} at pH 5.0, 7.0 and 9.0 and reduction rates in chlorophyll to their control were estimated by 58.5, 21.1 and 36.4%, respectively. Similarly, a dose dependent reduction was found in chlorophyll content of *C. demersum* (Fig 2). While decrease in chlorophyll content in the *C. demersum* exposed to 1 µg Pb mL^{-1} at all tested pH levels were found to be insignificant (P>0.05), it was statistically significant compared to control with plants exposed 25, 50 and 100 µg

Fig. 2. Total chlorophyll content of *C. demersum* leaves at different Pb concentration and pH levels



Pb mL^{-1} at all tested pH levels (P<0.05). The minimum chlorophyll contents were found in the macrophyte exposed to 100 µg Pb mL^{-1} at pH 5.0, 7.0 and 9.0 and reduction rates in chlorophyll to their control were estimated by 50.8, 35.9 and 45.9%, respectively. It is reported by various investigators that Pb^{2+} inhibits chlorophyll synthesis and consequently leads to decrease in chlorophyll content (Miranda & Ilangovan 1996; Mohan & Hosetti 1997). Van Assche and Clijters (1990) reported that the reduction in chlorophyll content in the presence of the Pb^{2+} may be due to an inhibition of chlorophyll biosynthesis. One of important enzyme of chlorophyll biosynthesis is aminolevulinic acid (ALAD) which catalyses the formation of porphobilinogen. It is explained that lead inhibits ALAD activity by binding with –SH group of the enzyme and overall chlorophyll biosynthesis through Mg^{2+} (Singh, 1995). In many cases, heavy metal toxicity reduced for acidic values, with often increased metal availability, possibly owing to decreased uptake (Huang *et al*., 1988). 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 low pH 5.0 have more adverse effects than that of pH 7.0 and 9.0 as well as effects of the metal.

In plants, with less existing in inorganic forms, nitrogen is mostly found in organic forms. Nitrogen (N) content of plants varies depends on factors such as plant species, plant age and the parts which samples are excised from plants. Generally, total N in plants ranges from 0.2% and 6.0% (Millar, 1959). In this study total nitrogen content of controls of *T. latifolia* were estimated 2.11, 2.69 and 2.77% at pH 5.0, 7.0 and 9.0, respectively. At all tested pH levels, there was a decrease in total N content of *T. latifolia* in parallel to increased Pb concentration as shown in Fig III. At pH levels of 5.0 and 7.0 in the macrophyte reduction of N was found to be significant in all applied concentration except 1 µg Pb mL^{-1}. But at pH 9.0 reduction of N was found to be significant in the medium of 25, 50 and 100 µg Pb mL^{-1} for the macrophyte. The lowest N contents were found in *T. latifolia* exposed to 100 µg Pb mL^{-1} at pH 5.0, 7.0 and 9.0 and reduction rates in N to their control were estimated by 51.2, 44.6 and 43.7%, respectively. A dose dependent reduction was found in N content of *C. demersum* (Fig. 4). It was estimated that reductions of total N in pH 7.0 solutions were not statistically significant comparing the control (P>0.05). The most significant decreases in the macrophyte were estimated by 45.2, 12.6 and 44.3% in the medium including 100 µg Pb mL^{-1} at pH levels of 5.0, 7.0 and 9.0, respectively. A study have revealed that lead reduces the uptake and transportation of some nutrients in plants (Godbold & Kettner, 1991). Particularly at pH 5.0 and 9.0, it was shown that reduction in the uptake and transportation of nitrogen to plants related

Fig. 3. Total nitrogen (N%) content of *T. latifolia* leaves at different Pb concentration and pH levels

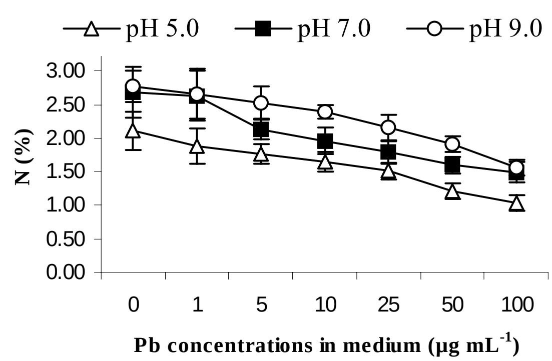
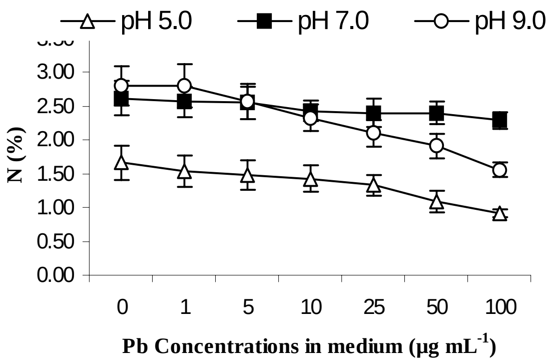


Fig. 4. Total nitrogen (N,%) content of *C. demersum* leaves at different Pb concentration and pH levels



with increasing Pb^{2+} concentration. When the coefficient of 6.25 (or multiplication factor) is considered, the reduction in nitrogen content indicates decreased protein content in both macrophytes.

Table I and II show the concentrations of Pb^{2+} found in the root and leaf tissues of *T. latifolia* depending on different metal concentrations and pH levels. According to our findings, it was determined that there were Pb concentrations difference among the parts of *T. latifolia*. There was an increase in the metal concentrations of root tissues depend on rise in Pb^{2+} in all pH levels. The highest metal concentrations were measured at pH 9.0 in the tissue, although the lowest Pb^{2+} concentrations were found at pH 5.0 in root tissues. On contrary to Pb quantities in root tissues, the metal concentrations in leaf tissues were measured the highest at pH 7.0, which showed that the pH was more suitable environment for the plant and more convenient for the plant to accumulate the metal. Metal concentrations in plant tissue are generally a function of the metal concentration in the growth solution or in the soil, but the relationship differs according to plant species and tissue (Kabatta-Pendias & Pendias 1984). Absorbed trace elements are not uniformly distributed throughout the plant, so that different organs may vary in their ability to concentrate heavy metals. In aquatic plants, roots frequently reported to contain higher concentrations of most metals than above-ground parts (Outridge & Noller, 1991). Taylor and Crowder (1983) reported *T. latifolia* roots showed higher concentrations of Ni and Cu than rhizomes and above-ground tissues. The authors concluded that the macrophyte can exclude toxic levels of metals above-ground parts. Pb quantities were shown in Table III for whole *C. demersum*. There was an increase in the Pb^{2+} concentrations of whole the macrophyte tissues depend on rise in the metal in all pH levels. The highest metal concentrations were measured at pH 9.0 in the macrophyte tissues. The lowest Pb^{2+} values were found at pH 5.0. This may be due to competition with H^+ on the cell membran surface.

While solubility of Pb^{2+} increased at pH 5.0, the metal uptake by the macrophytes decreased probably due to negative effect of low pH. The minimum Pb concentrations were determined in *T. latifolia* leaves all treated pH levels. The maximum Pb^{2+} concentrations were measured in whole *C. demersum* in pH 7.0 levels. The present study indicated that uptake rate of Pb^{2+} by macrophytes was dependent upon pH value of the solutions.

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