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THE EFFECT OF LEAD (PB) ON THE GROWTH RATES OF TWO AQUATIC MACROPHYTE SPECIES; *LIMNOBIUM LAEVIGATUM* (HUMB & BONPL. EX WILLD) HEINE AND *EGERIA DENSA* PLANCH. GROWN IN DIFFERENT EXPERIMENTAL MEDIA

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ABSTRACT. Anthropogenic causes contribute to toxic pollutants in aquatic environments and heavy metal pollution. As a heavy metal, Lead (Pb), is one of the most common causes of pollution in water. Heavy metals must be removed from the aquatic environment because they adversely affect health and all living things in each environment. In this study we aimed to determine the effects of lead (Pb) exposure on the growth rates and biomass of two aquatic macrophyte species, *E. densa* and *L. laevigatum*. Plants grown in in two different experimental media. For this purpose, both plants were exposed to 3 different concentrations of lead (1 ppm, 5 ppm, 15 ppm). Samples were measured on the 1st, 4th and 7th days, and the first and last weights of the plants were compared. Bioexperiments were run in triplicate. Positive values were observed in the growth rates of both plants, except for the negative growth rates observed on the 1st day at 1 ppm and 5 ppm lead concentrations in the pond water environment. Both plants showed positive growth in 25% Hoagland medium at all concentrations and days, except for the 1 ppm lead concentration, being observed for *E. densa*. As a result of our study, lead exposure did not significantly alter the growth rates of *E. densa* and *L. laevigatum* in the experimental media used for short-term (up tp 7 days) durations.

1. INTRODUCTION

Water is an essential resource needed for life on earth. Therefore, access to safe water is vital for humans and other living organisms in the ecosystem. Water quality is adversely affected by population growth, industrialization, urbanization, and eventually pollution of water sources [1-2]. Potential major sources of water pollution are toxic compounds such as pesticides and heavy metals, sewage and household wastes, plastics, nanoparticles, industrial and agricultural wastes. [3]. Heavy metals are one of the most common pollutants discharged into natural environment [4].

Heavy metals have an atomic weight between 63.5 and 200.6 (Da) and a density greater than 5 g/cm^3. Heavy metals including arsenic (As), cadmium (Cd), lead

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(Pb), and mercury (Hg) are toxic even at lower concentrations. Lead (Pb) is a non-essential metal for living organisms and toxic to biota even at a very low dose. It is mainly found in the soil within a range of 15-40 ppm and not considered as a serious threat to living organisms as long as it does not exceed 150 ppm. However, if the lead concentration is >300 ppm in the environment, it poses a serious health concern [5-6-7].

In order to eliminate the potential hazards of heavy metals to the environment and living organisms, physical, chemical, physicochemical and biological techniques can be used to remove or minimize the toxic effects of heavy metals [8]. Since conventional methodologies including chemical precipitation, ion exchange, and electrochemical removal methods have many disadvantages, such as high energy requirements, incomplete removal and toxic waste generation after treatment, the phytoremediation technique have become a prominent technique, which is considered a cost-effective, environment-friendly and easy method [8-9].

Phytoremediation is a natural process being carried out by plants and those absorb pollutants such as pesticides, nanoparticles and heavy metals from the environment [10]. Several plant species including aquatic macrophytes can uptake and store heavy metals in high concentrations thus, they are used to remediate polluted areas or remove pollutants from polluted water bodies [9]. Phytoremediation capacity of each plant species depends on the tolerance of the plant to pollutant, the growth rate and efficiency, and the depth of the root systems [9]. Therefore, it is essential to investigate the optimum growth conditions, tolerance of plant species to specific pollutants and their removal capacities. Although, several macrophyte species are shown to be tolerant to pollutants, there is limited information on the growth performances *of L. laevigatum* and *E. densa* under heavy metal stress.

In this study we aimed to determine the effects of lead (Pb) exposure on the growth rates and biomass increment of two aquatic macrophyte species; *E. densa* and *L. laevigatum*. Plants were exposed to 3 different concentrations of lead (1 ppm, 5 ppm, 15 ppm) in pond water and 25% Hoagland solutions. Bioexperiments lasted for 7 days and plants were harvested on the 1st, 4th and 7th days and the weights of the plants were measured and compared among groups. Pond water was used to imitate a natural environment to reveal the effects of Pb in natural environment, thus providing a deeper understanding on the effects of lead on the growth performance of those aquatic macrophytes which is a critical factor for an effective removal process.

2. MATERIALS AND METHODS

2.1 Plant Material

Plants, *L. laevigatum* and *E. densa* were collected from a local shop and were identified [11] from fresh specimens. Plants were acclimatized to laboratory conditions for 4 weeks prior to use. The stock plant cultures were kept in 150 L aquaria supplied with 25% Hoagland solution [12]. Half of the water from the bottom of the stock culture tanks was removed and renewed every second day. No internal aquarium heaters or filters were used. The temperature was maintained at 19±3.2°C, under a 12:12 h light:dark cycle.

2.2 Bioexperiments

Experiments were carried out in 1 L glass beakers containing either 25% Hoagland solution or pond water. Pond water was used to imitate a natural waterbody and it was obtained from an open pond in Ankara University, Faculty of Science with 5L stainless steel containers. Pond water was transferred to the laboratory and allowed to reach ambient temperature and filtered through 0.2 mm stainless steel sieves remove large suspended particles.

Only green and healthy plants were used in bioexperiments. Plant fragments (2 fragments per beaker) of a similar size (approximately 10 cm long) were used for experiments carried out with *E. densa*. A different approach was followed for *L. laevigatum*; 5-8 individual plants in similar size with a total of 30-35 leaves were placed in each beaker. Plants were gently washed with distilled water twice to remove stock culture solution. Initial wet weight of the plants was weighed (Precisa-BJ 100M) carefully after gently touching with filter papers to remove excess water.

Each plant was exposed to 3 different lead concentrations (1ppm, 5ppm, and 15ppm) in each media in separate beakers. The tested lead concentrations were adopted from relevant studies [14-15]. Lead nitrate ($Pb(NO_3)_2$, (Merck) was used as a lead source. Bioexperiments were run in triplicate and were carried out in 25% Hoagland solution or pond water containing required amount of lead stock solutions. Beakers were covered with transparent stretch film to reduce evaporation. Experiments lasted 7 days and plants were harvested and weighed on 1^{st}, 4^{th} and 7^{th} days. Relative growth (%) of the plants were calculated using the formulae given below [13].

$RGR = (\ln W2 - \ln W1)/(t2 - t1)$ W=weight, t=time

All of the glass material used in the experiments were washed prior to use, with a mixture of 1:3 nitric acid (HNO_3 65% Merck) and hydrochloric acid (HCl 35% Merck) and rinsed 3 times with distilled water.

2.3. Statistical Analysis

The data presented as the mean ± standard deviation (SD) of the triplicates for each group. The data were tested for goodness of fit to a normal distribution prior to the analysis using Shapiro Wilk-W test. Since the normality was violated, non-parametric Kruskal-Wallis tests were performed to determine significant differences among the growth rates of each group. All of the statistical analyses were performed using SPSS 26.0.

1. RESULTS

3.1 Growth Rates in Pond Water

The weight measurement of the plants and growth rates (%) at 1st, 4th and 7th days for each plant exposed to three different lead concentrations in pond water are shown in Table 1.

TABLE 1. The relative growth rates of the plants exposed to Pb in pond water.

Medium	Plant	Day	1ppm			5ppm			15ppm		
			Intitial	Final	Percent %	Intitial	Final	Percent %	Intitial	Final	Percent %
Pond Water	*L. laevigatum*	1	1.63 ± 0.21	1.58 ±0.13	-3.21	1.17 ±0.22	1.15 ±0.26	-2.51	2.13 ±0.82	2.23 ±0.8	4.67
		4	1.60 ± 0.34	1.69 ± 0.30	5.21	1.35 ±0.27	1.41± 0.28	4.21	2.45 ±0.95	2.57 ±0.93	4.58
		7	1.50 ± 0.30	1.58 ±0.25	5.02	1.55 ±0.25	1.68 ±0.39	8.19	1.65 ±0.60	1.79 ±0.59	8.16
	E. densa	1	1.16 ± 0.26	1.10 ±0.22	-5.50	1.28 ±0.26	1.24 ±0.25	-2.73	1.31 ±0.23	1.33 ±0.27	1.30
		4	1.16 ± 0.39	1.28 ±0.40	9.17	1.15 ±0.34	1.16 ±0.36	0.46	1.28 ±0.29	1.36 ±0.34	5.36
		7	1.22 ± 0.37	1.33 ±0.43	9.36	1.32 ±0.29	1.41 ±0.40	6.14	1.31 ±0.22	1.34 ±0.29	2.27

The difference between the weight values of *L. laevigatum* at 1 ppm, 5ppm and 15ppm concentration was statistically significant ($P<0.05$), however, there was no statistical difference for *E. densa* according to the Kruskal Wallis test. Although there was an increase in weight values for both plants on the 1st, 4th and 7th days, the difference between these values was not statistically significant ($p>0.05$).

There was a decrease (-5.5%) in the final weight of *E. densa* when exposed to 1 ppm Pb in pond water. The maximum weight for *E. densa* was recorded on 4th day of the experiment when exposed to 5 and 15 ppm Pb. The growth rate on the 1st day was recorded as the minimum at the 1ppm concentration (-3.21%), while the highest rate was observed at the 15 ppm concentration (4.67%) for *L. laevigatum*. (Figure 1).

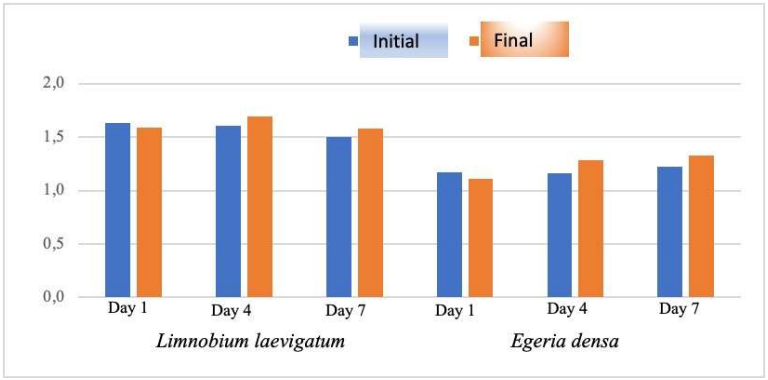

FIGURE 1. Initial and final weights of the plants exposed to 1 ppm Pb in pond water.

The final weights of both plants decreased when exposed to 5 ppm Pb at 1st day (%-2,-2,5), however showed a gradual increase after 4th day of the experiments reaching up to 6-8%. These values were similar to the plants exposed to 1 ppm Pb (Figure 2).

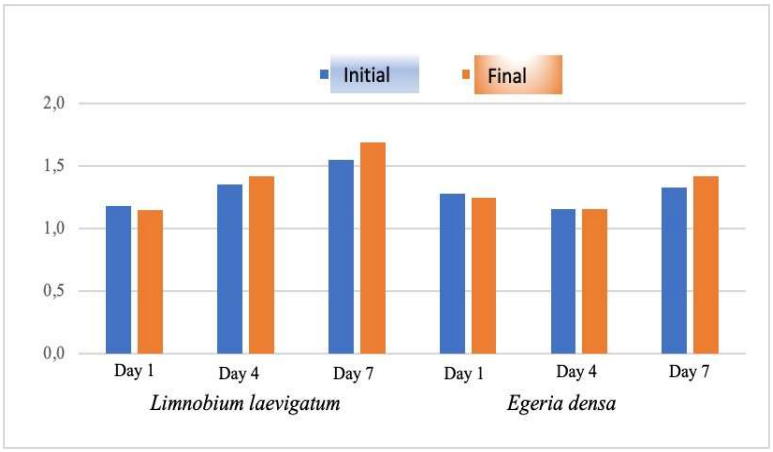

FIGURE 2. Initial and final weights of the plants exposed to 5 ppm Pb in pond water.

The final weights of both plants showed a slight increase when exposed to 15 ppm Pb in the pond water, this increase rate was observed similar on the 4th day (Figure 3).

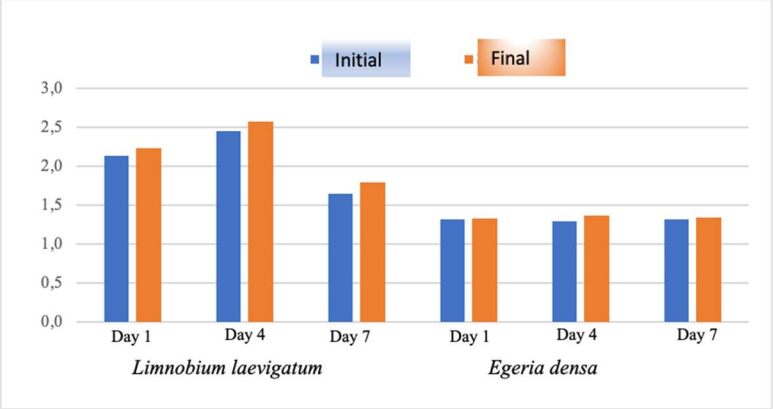

FIGURE 3. Initial and final weights of the plants exposed to 15 ppm Pb in pond water.

3.2. Growth Rates in 25% Hoagland Medium

The weight measurements and growth rates of plants exposed to 3 different concentrations of Pb in 25% Hoagland solution at 1st, 4th and 7th days are shown in Table 2.

TABLE 2. The relative growth rates of the plants exposed to Pb in 25% Hoagland solution.

Medium	Plant	Day	1ppm			5ppm			15ppm		
			Intitial	Final	Percent %	Initial	Final	Percent %	Intitial	Final	Percent %
25% Hoagland	*L. laevigatum*	1	4.31 ± 0.93	4.59 ±1.11	6.12	4.83 ±0.52	4.95 ±0.33	2.47	3.67 ±0.39	3.75 ±0.60	2.27
		4	4.48 ± 0.57	4.81 ±0.52	6.87	4.55 ±0.61	4.80 ±0.56	5.17	4.70 ±1.16	5.07± 1.13	7.16
		7	4.95 ± 0.94	5.33 ±1.25	8.40	4.74 ±0.98	4.79 ±1.04	0.88	6.78 ±1.67	6.95 ±1.5	2.45
	E. densa	1	0.91 ± 0.22	0.84 ±0.20	-8.06	1.06 ±0.27	1.16 ±0.19	8.27	0.83 ±0.14	0.89 ±0.18	6.34
		4	0.98 ± 0.33	0.98 ±0.36	0.19	0.85 ±0.21	0.91 ±0.20	11.64	0.87 ±0.11	0.93 ±0.12	9.91
		7	0.69 ± 0.15	0.73 ±0.15	5.94	0.64 ±0.08	0.71 ±0.23	7.64	0.83 ±0.12	0.89 ±0.17	5.95

The difference between the weight values of the *L. laevigatum* exposed to 3 different concentrations of Pb was not statistically significant ($P>0.05$) in the Hoagland solution. However, there was a significant difference in the weight values of *E. densa* at 1ppm and 5 ppm concentrations ($P<0.05$). When the data were compared among to experimental duration, we found a significant difference between the measured weights values for the both plants in the 25% Hoagland medium on all days ($P<0.05$).

The final weight values for *E. densa* were observed at the highest on the 4th day and a decrease was observed in the weight on the 7th day at 1 ppm Pb concentration (Figure 4).

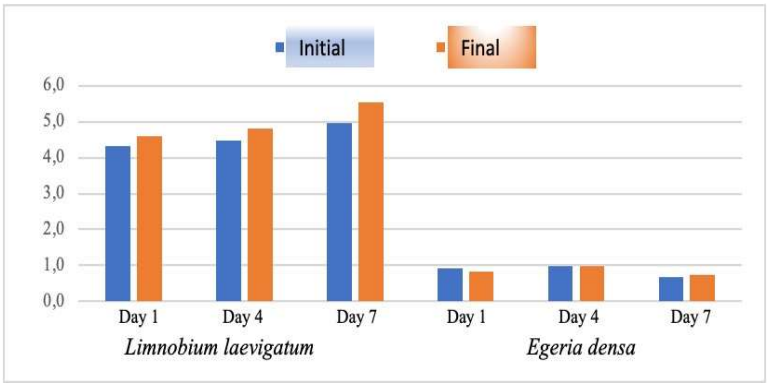

FIGURE 4. Initial and final weights of the plants exposed to 1 ppm Pb in 25% Hoagland solution.

The highest final weight for both plants were observed on the 4th day (5.17-11.64%) and showed a decrease on the 7th day at 5 ppm concentration (0.88-7.6%) (Figure 5).

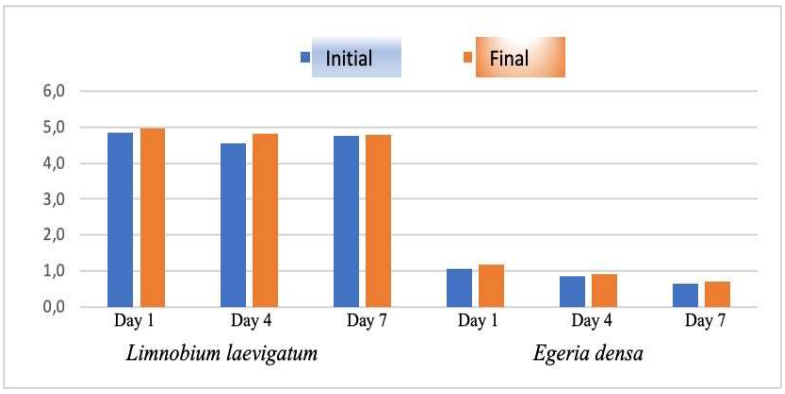

FIGURE 5. Initial and final weights of the plants exposed to 5 ppm Pb in 25% Hoagland solution

The highest final weight of the plants exposed to 15 ppm Pb was observed on the 4th day (7.16-12.91%) for *E. densa*, and it showed a decrease at the end of the 7th day (2.45-5.95%) (Figure 6).

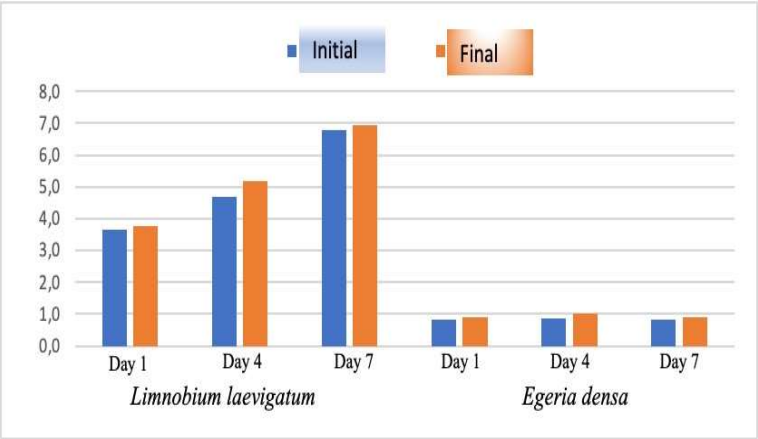

FIGURE 6. Initial and final weights of the plants exposed to 15 ppm Pb in 25% Hoagland solution

4. DISCUSSION

In this study we aimed to investigate the effects of Pb exposure on the growth rates of *L. laevigatum* and *E. densa* in two different experimental media. We found that, the stress caused by lead exposure did not suppress the growth rates of plants, in general. During the experiment, the plants generally showed no physical wear or deterioration. However, in some experiments we found growth rates were reduced followed by wilting and destruction of the leaves.

In general, the weight of *L. laevigatum* showed a gradual increase during the experiment, except for the 1st day, when it was exposed to 1 and 5 ppm Pb in pond water. The weight increment reached up to 9% in the following days of the experiment (Table 1). The final weight of the plants (*E. densa*) reached the highest level (11,64%) on the 4th day in 25% Hoagland solution and showed a gradual decrease at 7th days (Table 2). In a study where *Pistia stratiotes* L. was exposed to Pb for 7 days a decrease in the growth rates at the 7th day was observed [16]. In another study where *E. densa* was exposed to 3 different concentrations of vanadium, it was found that the average weight of the plant (*E. densa*) reached to its maximum level when exposed to highest V concentration (1.8 ppm) [17]. No significant changes were observed in the wet weight of the plant (*Scripus grossus* L. f.) in the first week when exposed to lead, but an increase of 25-50% was observed in the 2nd and 3rd weeks [18]. Interestingly [19], the growth rates for *P. stratiotes* decreased only at concentrations of 50 ppm and above when exposed to various Zn-Cd concentrations for 9 days.

In general, our results demonstrated that the plant growth was higher in Hoagland solutions indicating the necessity of macro and micronutrients to obtain a high

growth rate and therefore remediation capacity. In a similar study[20], it was shown that *Lemna* sp. L. and *P. stratiotes* L. showed the highest growth rates (14% and 12%) when grown in Hoagland solution. The decrease in the growth rates observed in the first day for both plants in both media might indicate the acclimation potential of plant in a very a short time (4 days or above).

When the responses of the two plants to different lead concentrations in the aquatic medium are examined (Tables 1 and 2), the growth rates of *E. densa* showed more variation than *L. laevigatum*. This change was more visible in *E. densa* in both directions (+,-). This might indicate that *E. densa* is less tolerant to Pb pollution when compared to *L. laevigatum*. A similar finding was also reported by Aran et. al., [14] stating that *L. laevigatum* was resistant to high lead and zinc concentrations. They also reported that the chlorophyll concentration of the plant showed only a slight decrease without exhibiting any morphological abnormalities in the leaves [15]. Higher growth rates were observed in *L. laevigatum* for %25 Hoagland media in 1 ppm Pb than *E. densa* during the 1st, 4th and 7th days.

This is the first study reporting the growth performance of *L. laevigatum* and *E. densa* exposed to lead (Pb). According to the results of this study, lead exposure did not significantly alter the growth rates of *E. densa* and *L. laevigatum* in the experimental media used for short term durations (up to 7 days). The results of this study may provide a better understanding on the responses of those two plant species under heavy metal stress.

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Author Contribution Statements. MBE-supervising, resources, conceptualization, writing–review & editing. FD-Conceptualization, Investigation, Data curation, Formal analysis, Writing. VCS-experimental analysis. All authors have read and approved the manuscript.

Declaration of Competing Interests The authors declare no conflict of interest

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