Ceratophyllum demersum L. as Phytoindicator and Potential Phytoremediator of Lead Under Hydroponic Conditions

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***Abstract*— The contamination of water by heavy metals like Pb is a huge problem for the environment. In this paper, we test *Ceratophyllum demersum L.* plants as phytoindicator. These were exposed to different concentrations of Pb for 1–21 days, under hydroponic conditions, where they exhibited both adsorption and absorption efficiency. These efficiencies influenced in concentration and duration in a dependent manner. For the three initial treatments 0.125, 0.250, 0.500 µg/ml, the values of regression coefficients described the occurred variance on the rapid decrease in the Pb concentration in the hydroponic media, reflecting highest removal efficiency by *C. demersum*. *Significant variation (*P< 0.05) was recorded between the concentration of Pb accumulated in *C. demersum* at 0.125 and 0.250 µg/ml, while a highly significant value (P< 0.01) was recorded between them at 0.500 µg/ml. The regression coefficient denotes the pronounced impact of treatment concentration on the accumulation rate (R^2 = 0.9987). The adsorption efficiency of *C. demersum* appeared to be influenced by the Pb hydroponic media concentration, where after 21 days, the higher Pb adsorption was recorded at 0.125 µg/ml and the lowest one was obtained at 0.500 µg/ml. Results suggest that plants responded positively to the increase of Pb concentrations and they accumulated a high amount of metal. Due to metal removal coupled with detoxification potential, the plant appears to have potential for its use as phytoremediator species in aquatic environments.**

Keywords-lead; Biomonitoring; Bioremoval; biosorption; macrophytes;

I. INTRODUCTION

Pb is a very persistent element, which has been used in the past and is still used nowadays. This is the cause that is one of the most frequent inorganic contaminants in water [1]. It is potentially toxic even at low concentrations and above 15 µg/ L in drinking water, is considered as a risk for human health [2]. Many industries, such as the petrochemical, painting and coating, newsprint, smelting, metal electroplating, mining, plumbing and battery industries, discharge lead (Pb) into the environment without adequate purification in some cases [3].

The presence of lead in wastewater is dangerous for the aquatic flora and fauna even in relatively low concentration and stringent environmental regulations attract the attention of chemists and environmental engineers for its control [4]. While the focus has turned towards remediation with regards to preventing human exposure, much is still needed in the way of determining appropriate measures to monitor and protect the aquatic environment, particularly with regards to point source pollution [5].

Macrophytes (Aquatic plants) confirm very well too many of the criteria listed for an "ideal" biomonitor organism: they are sedentary, visible to the naked eye, easy to collect, simple to handle and easy to identify in the field. Also, they concentrate metals and nutrients in their tissues and reflect the environmental contamination [6]. Some species have the expressive ability of bioconcentration, and therefore, increased accumulation, of nutrients and heavy metals [7]. Further, the high concentration of some elements in plant tissues may be the result of the substantial availability of those elements in the surrounding environment. In this way, macrophytes can be used as bioindicators. Some of those species can also be used to remove, degrade or transform harmful hazardous materials, present in the aquatic environment. This application of plants, as phytoremediators, highly depends on factors, which define the absorption, accumulation and organic production of each taxon. In the case of *Ceratophyllum demersum, it grows* in water heavily polluted either by domestic sewage or by agricultural wastes [8]. It is known that the high accumulation of heavy metals like Cd, Cu, Cr, Pb, and Hg, are very usual in this kind of wastes [9][10].

Recently, substantial accumulation and high tolerance of Pb has been reported in *C. demersum* [11] when it is exposed to various concentrations of Pb, exhibited both phytotoxic and tolerance responses. Accordingly, the aim of this research was to determine the Pb removal efficiency by *C. demersum*, through investigating its absorption and adsorption capability, under hydroponic conditions.

The rest of the paper is structured as follows. Section II presents the materials and methods that were used in the study. Section III the results of our experiment are presented. In section IV the discussion is shown. Finally, In Section V the conclusions and future works are explained.

II. MATERIAL AND METHODS

In this section, we explain the material used in the experiment and the methods. In the first section, we present the apparatus used, in section b present the grown condition, sequently we show the preparation of lead as a heavy metal contaminant, and finally, we present the protocol of the experiment.

A. Experimental apparatus

Ceratophyllum demersum L. was grown in a growth chamber equipped with holding tanks, lighting systems, aeration system, and temperature- monitoring system. Hold tanks used in this design have consisted of three boxes, each box for each pollutant concentration. Each box (25 x 100 x 30 cm) was divided into four compartments: one compartment was used as a control, while the others received the different pollutant concentrations. Moreover, each compartment (25 x 25 x 30 cm) was filled with 10 L of Hoagland nutrient solution; where it was previously used to improve cucumber grow [12]. The light intensity of 1950 lux provided by the artificial lighting system was used to be similar to that of natural lighting required for aquatic plants. Also, an aeration unit was installed for each compartment to provide oxygen for aquatic plants.

The air flows from the main laboratory supply to a manifold with four outlets. Each outlet was connected to the aerator which was located in each compartment. Further, the dissolved oxygen daily monitored and was ranged between 7-7.5 mg/l. The water and the ambient air temperature were monitored during the experimental period. Besides, the thermometer was used to measure the ambient air temperature above the water surface in each box and also to measure the water temperature in each compartment.

B. Plant material and growth conditions

Ceratophyllum demersum L. (Coontail or hornwort, family Ceratophyllaceae) is a submerged macrophyte of broad geographical distribution and is widely used in wastewater treatments. It is selected because it grows quickly, has high biomass, and has been found to accumulate metals in its leaves and stem. It is a completely submersed plant and commonly seen in ponds, lakes, ditches, and quiet streams with moderate to high nutrient levels [13]. It does not produce roots, instead, it absorbs all the nutrients it requires from the surrounding water. Also, it grows rapidly in shallow, muddy, quiescent water bodies at low light intensities. If it is growing near the lake bottom, it will form modified leaves, which it uses to anchor to the sediment. However, it can float free in the water column and sometimes forms dense mats just below the surface. The plant reproduces by fragments breaking from the parent plant.

Samples were taken from plants growing in the mainstream of the River Nile bank at Sohag Governorate, south Egypt. The plants were grown in a growth chamber, where they were grown hydroponically. Before the start of the experiment, whole plants of *Ceratophyllum* were thoroughly cleaned under running tap water to remove debris and other foreign particles and then rinsed in redistilled water. The plants were transferred to the system and kept for 2 weeks in Hoagland nutrient solution (Cleland and Briggs Formulation) to acclimatize before starting the experiment and adding the contaminant. The nutrient solution was aerated continuously to achieve constant stirring and buffered to pH 5.8 with NaOH or HCL.

C. Preparation of lead as a heavy metal contaminant

Three concentrations of lead were investigated in this study (125, 250, and 500 µg/l). The lead element was used as Lead nitrate Pb $(NO_3)^2$. All the concentrations were calculated based on the individual element versus their compound form. The reagents were dissolved in bidistilled water to achieve the appropriate contamination level. After the adaptation period, the contaminants were added to the three compartments receiving three treatments.

D. Experimental protocol

One box (four compartments) was used for each treatment. Each compartment received 10 L of water and the recommended amount of nutrient solution. About 100 g healthy fresh weight of *C. demersum* was placed in each of the four compartments in the holding tank assigned for the different treatments: A control in each box, with modified Hoagland nutrient solution and three Pb treatments: 125, 250, and 500 µg/l of Pb. Each treatment was replicated three times. A constant volume of water was maintained in the aquaria by the addition of redistilled water. Moreover, the lighting system was turned on and was controlled with a timer, which was adjusted to achieve 11 h light and 13 h dark.

The plants were left for 2 weeks to adapt to the new environment. After the acclimatization period, the desired amounts of lead were added to the treatment compartment of each holding tank. Temperature readings were recorded every day in order to monitor the change in the water (21 ± 2 ^{0}C during the light period and 20 ± 2 ^{0}C during dark periods) and the ambient air temperatures (25 ± 2 ^{0}C) during the experimental period. The growth of the individual plant was also observed on a daily basis. The entire experiment was reported. The plants were treated under the above-mentioned laboratory conditions for a period of 21 days.

E. Sampling and analysis

Water samples of 15 ml were collected from all compartments at 1, 10, and 21 days intervals for lead analyses. A polarography and voltammetry 797 VA Computrace Ω Metrohm Ltd CH-9101 Herisau, Switzerland was used for Pb analyses. Lead concentration values are expressed as µg / L (Ppp). Batch adsorption experiments were performed for all compartments at 1, 10, and 21 days intervals for lead analyses by using 0.2 g F.W of plant material (leaves or stems) with 10 ml of freshly prepared solution of $MgCl^2$ containing 100g/L was added.

The container flasks were caped and shaken at 200 rpm at room temperature for one hour. After thorough mixing the solution was centrifuged at 4000 rpm for 5 min, Supernatant was collected for Pb concentration analyses. Plant samples (5 g each) were collected at the start, at 10 days, and at the end of the experiment and analyzed for the presence of lead. Harvested plants were thoroughly washed in distilled water and then separated among stems and leaves. The plant samples were dried in a convection oven for 24 h at 48 ^oC to determine the accumulation of contaminants. After drying, the plants were ground to a fine powder.

For analysis, dry plant material was digested according to the wet digestion procedure involving concentrated nitric acid [14]. 10 ml concentrated nitric acid (HNO_3) and 0.5 ml hydrofluoric acid (HF) were added to 0.5 g of dry plant sample in a closed Teflon vessel, was designed for the purpose, at a temperature of 130 ^oC for 24h. Digestion in solution continues until clear. The resultant liquid was diluted up to 25 ml with distilled water then stored for analysis. Then the Pb was determined by Perkin–Elmer 2380 atomic absorption spectrophotometer.

III. RESULTS

In this section we presented he results. The different data were analyzed statistically by ANOVA and regression investigated using the basic statistic module of Statistical software (StatSoft Inc., ’99 edition, kernel release 5.5 A). Homogeneity of variances and normality of variables tests were performed

A. Lead removal from nutrient media

Results revealed an increasing trend of Pb removal with time in all the experimental sets. The changes in Pb concentration in the nutrient medium over the duration of the experiment are shown in Figure 1. Removal of lead at its low initial treatment 0.125 µg/ml over time is shown in Figure 1, it reduced to 0.0833µg/ml within the 1^{st} day. The Pb removal continued over the remaining days of the experiment and decreased to 0.056 µg/ml after 10 days and to 0.0437 µg/ml by the end of the experiment. The decrease of Pb concentration in the nutrient medium with an initial value of 0.250 µg/ml is shown in Figure 1. the concentration of Pb was sharply decreased to 0.1848 µg/ml after the 1^{st} day of the experiment. Meanwhile, the concentration of Pb was reduced to 0.1277 µg/ml and 0.1099 µg/ml after 10 and 21 days, respectively. The removal of lead at its high initial value of 0.500 µg/ml is shown in Figure 1. It appears that there is an increasing trend in lead removal all over the experimental period. Where, Pb concentration decreased to 0.4108, 0.3211, and 0.2102 µg/ml after 1, 10, 21 days, respectively.

The highest removal was recorded on the last day in all the experimental sets. For all the three initial treatment 0.125, 0.250, 0.500 µg/ml, the values of regression coefficients using exponential model are R^2:0.8475, 0.8342 and 0.9696. This described the occurred variance of the rapid decrease in the Pb concentration. The exponential model is represented in the equation 1, 2 and 3 for the treatment of 0.125, 0.250, 0.500 µg/ml respectively.

All treatments achieved the highest removal efficiency (Table 1) at the end of the experiment, while the lowest treatment 0.125 µg/ml recorded the highest removal efficiency (65%) than the others.

$$Concentration\ (\frac{\mu g}{ml}) = 0.1006e^{-0.043*time\ (days)} \quad (1)$$

$$Concentration\ (\frac{\mu g}{ml}) = 0.2106e^{-0.035*time\ (days)} \quad (2)$$

$$Concentration\ (\frac{\mu g}{ml}) = 0.4639e^{-0.038*time\ (days)} \quad (3)$$

TABLE I. THE REMOVAL EFFICIENCY OF LEAD BY *C. DEMERSUM* GREW IN DIFFERENT LEAD TREATMENTS THROUGHOUT THE EXPERIMENT.

Pb concentration in the nutrient media (µg/mL)	**Days**		
	1st day (%)	***10 days (%)***	***21 days (%)***
0.125	33.36	55.20	65.04
0.250	26.08	48.92	56.04
0.500	17.84	35.78	57.96

B. Lead absorption

An increase in lead absorption by *C. demersum* was detected with the different applied treatments Figure 2. The initial concentration of Pb in the plant biomass was 1.0704 µg/g at 0.125 µg/ml. After one day, the lead content increased up to 1.5442 µg/g. With an increase in duration, a sharp increase in metal accumulation rate was recorded, since lead content was 4.4224 µg/g after 10 days and 8.5289 µg/g after 21 days. The same trend in accumulation pattern was recorded for the applied initial treatments 0.250 µg/ml Figure 2. Where the initial concentration of Pb was 1.3766 µg/g. After one day treatment, the concentration reached 3.1625 µg/g but after 10 days the accumulation increased up to 9.3569 µg/g. By the end of the experiment, it was 13.5341 µg/g. The initial concentration of Pb in the higher treatment (0.500 µg/ml of Pb) was 0.6647 µg/g then increased to 3.4918 µg/g after the 1st day and 13.0933 µg/g after 10days. By the end of the experiment, the amount of Pb absorbed by *C. demersum* reached up to 21.5744 µg/g. The experiment showed that the lead uptake by *C. demersum* occurs very rapidly over time with different types of treatments. The values of R^2 were 0.9987, 0.9649, and 0.9831 respectively (with a linear model). The linear model is represented for equation 4, 5 and 6 for the treatment of 0.125, 0.250, 0.500 µg/ml respectively.

$$Concentration\left(\frac{\mu g}{ml}\right) = 0.3509 * time(day) + 1.0846 \quad (4)$$

$$Concentration\left(\frac{\mu g}{ml}\right) = 0.5647 * time(day) + 2.3399 \quad (5)$$

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$$Concentration\left(\frac{\mu g}{ml}\right) = 0.9682 * time(day) + 1.9608 \quad (6)$$

Significant variation ($P< 0.05$) was recorded between the concentration of Pb accumulated in *C. demersum* growing at 0.125 and 0.250 µg/ml, while a highly significant value ($P< 0.01$) was recorded between them at 0.500µg/ml. The concentration of Pb at the end of the experiment (after 21 days of treatment) demonstrated that the uptake of Pb by C. *demersum* increased with increasing metal concentration.

After 21 days, the higher Pb accumulation was recorded at 0.500 µg/ml treatment and the lowest with those of 0.125 µg/ml. The regression coefficient denotes the pronounced impact of treatment concentration on the accumulation rate (R = 99.8%).

C. *Lead adsorption (exchangeable fraction)*

The effect of contact time on the extent of adsorption of lead on *C. demersum* over the duration of the experiment is illustrated in Figure 3. The initial concentration of Pb, in the lower treatment (0.125 µg/ml), adsorbed on the tested plant was 0.3099 µg/g and it reached up to 0.331 µg/g after one day, 1.6574 µg/g after 10 days and 1.6792 µg/g at the end of the experiment. At 0.250 µg/ml Pb treatment, the concentration of the adsorbed Pb increased from 0.443 µg/g to 0.7429 µg/g within one day and reached up to 1.8806 µg/g after 10 days.

At the end of the experiment (after 21 days), the adsorption of lead on *C. demersum* was diminished to 1.4157 µg/g. The initial concentration of Pb adsorbed on the plant was 0.4876 µg/g at 0.500 µg/ml. After one day the adsorbed Pb fraction was 1.3084 µg/g and 3.0178 µg/g after 10 days. With an increase in duration, a sharp decrease in metal adsorption rate was recorded, where the adsorbed Pb fraction was 0.897 µg/g after 21 days. For all the three initial treatment 0.125, 0.250, 0.500 µg/ml, the values of R are 0.9784, 0.9974 and 0.9909, respectively. The polynomic model of 2 degrees is represented in equations 7, 8 and 9.

$$Concentration\left(\frac{\mu g}{ml}\right) = -0.0065 * time(day)^2 + 0.2067 * time(day) + 0.2273 \quad (7)$$

$$Concentration\left(\frac{\mu g}{ml}\right) = -0.0088 * time(day)^2 + 0.2284 * time(day) + 0.4801 \quad (8)$$

$$Concentration\left(\frac{\mu g}{ml}\right) = -0.0207 * time(day)^2 + 0.4461 * time(day) + 0.6701 \quad (9)$$

The final adsorption of lead on *C. demersum* after 21 days treatment is shown in Figure 3. The adsorption of Pb by *C. demersum* decreased with increasing metal concentration. After 21 days, the higher Pb adsorption was recorded at 0.125 µg/ml treatment and the lowest one was recorded with 0.500 µg/ml, the regression coefficient denotes the important impact of treatment concentration on the adsorption rate.

IV. DISCUSSION

The results obtained from the experiment indicated that *C. demersum* can accumulate and adsorb a high amount of Pb in concentration and duration dependent manner. With an increase in duration, metal accumulation increased while adsorption decreases after 10 days. The maximum rate of metal accumulation was found after 21 days when about (21.57µg/ g at 0.500 µg/ml) of the total metal was taken up by the plant. Being without roots and having forked leaves with thin cuticle, *C. demersum* can efficiently uptake metals from aquatic bodies through its large surface area with no complication of root– shoot metal partitioning. These features thus contributed to adequate Pb accumulation observed in this study.

A comparison between initial and final metal concentrations within the plant had shown that the final concentrations were more than 7, 12, 21 folds of the initial metal concentration, in the nutrient media, at 0.125, 0.250, and 0.500 µg/ml, respectively. Biosorption is the first stage of metal accumulation. It involved adsorption of metal onto the cell wall of microorganisms, algae, and aquatic macrophytes, independently of metabolism [15]. Adsorption processes on the biosurface involve the release of hydrogen ions or the other cations and the adsorption or surface complexing of metal ions. Adsorption fraction represents very loosely bound elements and may regulate and/or reflect the composition of surface water [16].

From the work presented here, *C. demersum* could be an effective biosorbent for lead under dilute metal conditions. This plant adsorbed an appreciable amount of Pb from the water up to 1.3 Fold at 0.125, 1.4 fold at 0.250, and 2.6 fold at 0.500µg/ml after 10 days. However, the adsorbed fraction increased after the 10^{th} day. This suggests saturation of a finite number of binding sites on the plant cell surface after exposure to Pb, and possibly the advent of metabolism dependent transport of metal to the inner cell mass [17], [18]. This decrease in the rate of adsorption could also be explained by the decrease of Pb concentration in the water, which causes a reduced concentration gradient.

According to Guilizzoni [19] and Keskinkan [20], there is, however, scarce research about heavy metal uptake by submerged aquatic plants. The use of submerged aquatic macrophytes for wastewater treatment is therefore still at an experimental stage, with species like *Potamogeton* spp. [21],[22],[23], *Ceratophyllum demersum* [7] and *Myriophyllum spicatum* [20] being tested. Bader and Fawzy [24], reported that *C. demersum* proved to be an effective biosorbent and bioaccumulator for Pb rending the species of interest for use in phytoremediation and bio-monitoring of polluted waters.

The rootless submerged plant *C. demersum* was found capable of removing comparatively higher amounts of Pb from pond water. This agrees with the earlier report [25] that submerged plants had higher metal uptake ability due to more surface/biomass ratio.

V. CONCLUSIONS

In the course of this study, it was concluded that: *C. demersum* proved to be an effective biosorbent and bio-accumulator for Pb. This plant was found to accumulate 7, 12, 21 folds of the initial metal concentration, in the nutrient media, at 0.125, 0.250, and 0.500 µg/ml, respectively after 21 days. Also, it can absorb an appreciable amount of Pb from the water up to 1.3 Fold at 0.125, 1.4 fold at 0.250, and 2.6 fold at 0.500µg/ml after 10 days. Being widely distributed and fast-growing submerged plants, *C. demersum* can be utilized as a safe and cost-effective tool for the removal of Pb from low to medium strength wastewater.

The use of submerged aquatic macrophytes for wastewater treatment is still at an experimental stage. Studies with regard to aquatic macrophytes combinations to be used in treatment ponds and the period of macrophyte replacement should be seriously undertaken for developing a more efficient, natural and economic integrated macrophyte based system, most advantageous for heavy metal removal.

In future works the performance of *C. demersum* and other aquatic plants will be investigated using multi-elements solution instead of mono-element one to test their potentials to remediate polluted waste water containing various toxic metals

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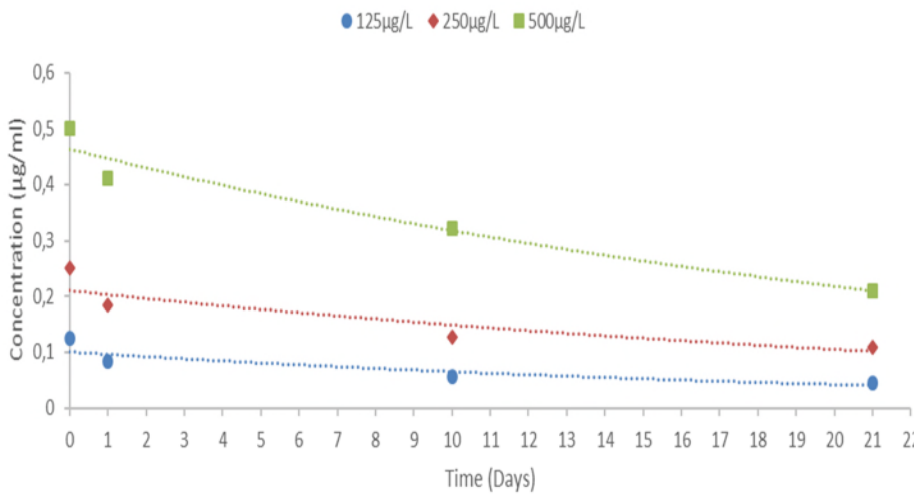

Figure 1. Average concentration of pb removal from the nutrient media at different pb treatments; 0.125; 0.250; 0.500 µg/ml.

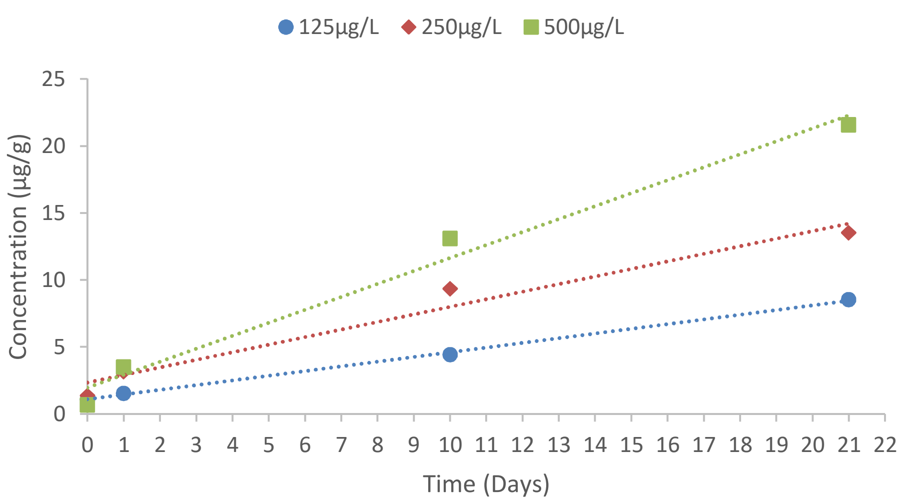

Figure 2. The average concentration of pb absorbed by c. Demersum growing in nutrient media with different pb treatments.

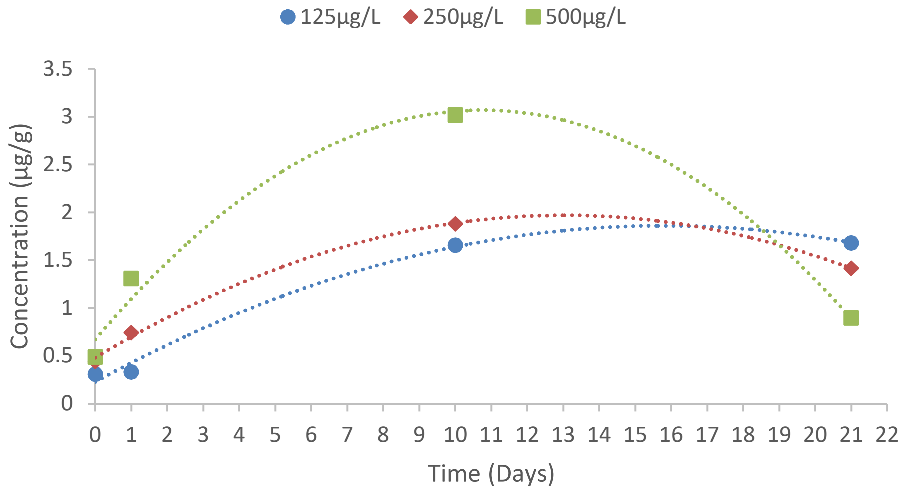

Figure 3. Average Concentration Of Pb Adsorbed (Exchangeable Fraction) on c. demersum growing in nutrient media with different pb treatments; 0.125; 0.250; 0.500 µg/ml, during the experiment duration.