

## AQUATIC PLANT (*HYDRILLA VERTICILLATA*) ROLES IN BIOACCUMULATION OF HEAVY METALS

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### ABSTRACT

The current study was aimed to test the ability of *Hydrilla verticillata* plant ability to accumulated of cadmium and lead in its tissues. The plant was treated with different concentrations of cadmium (1,2,4,8) ppm and lead (0.5,5,10,20) ppm for 21 days'. The concentration of metals was measured in plant and water, Bioaccumulation factor and removal ratio were also measured during days (1,7,14,21), as well as measured biomass and length of the plant were measured at the end of the experiment. The results were shown that the highest cadmium and lead removal rate were (77, 88) % at concentration (4, 10) ppm during (21, 7) days, respectively, also the bioaccumulation factor (BCF) of the two metals were (21639 and 45467) at concentrations (4 and 0.5) ppm during the 21<sup>st</sup> day of the experiment respectively. The biomass of plant in treated aquarium with different concentrations of cadmium showed a decrease during the experimental days, while increased in biomass of plant treated aquarium with different concentrations of lead. The length rate of the plant was significantly reduced in the plant that treated with cadmium but significant increase in plant treated with lead compared to the control.

**KEW WORD:** accumulation, biomass, cadmium, heavy metal, lead, phytoremediation.

الزرفي والطبباني

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دور النبات المائي *Hydrilla verticillata* في التراكم الاحيائي للمعادن

هدى حسين محمد الطبباني

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باحث

أستاذ

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المستخلص

هدفت الدراسة الحالية الى اختبار قابلية نبات *Hydrilla verticillata* على تراكم عنصري الكاديوم والرصاص في أنسجته. حيث عومل النبات بتراكيز مختلفة من الكاديوم (1,2,4,8) جزء في المليون وتراكيز مختلفة من الرصاص (0.5,5,10,20) جزء في المليون لمدة 21 يوماً. تم قياس تركيز المعادن في الماء والنبات ، وقياس عامل التراكم الحيوي ونسبة ازالة الملوثات (الكاديوم والرصاص ) خلال (1,7,14,21) يوم، كما تم قياس الكتلة الحيوية وطول النبات في نهاية التجربة. بينت النتائج ان أعلى نسبة لإزالة للكاديوم والرصاص (77 ، 88) % عند التركيز (4 ، 10) جزء في المليون خلال (21 ، 7) ايام على التوالي ، كما بينت النتائج ان عامل التراكم الحيوي للمعدنين (21639 و 45467) عند التراكيز (4 و 0.5) جزء في المليون خلال اليوم 21 من التجربة لكل من الكاديوم والرصاص على التوالي. كما أظهرت الكتلة الحية للنبات في الاحواض المعاملة بتراكيز مختلفة من الكاديوم انخفاضاً خلال أيام التجربة، في حين زاد في الاحواض المعاملة بتراكيز مختلفة من الرصاص، كذلك لوحظ انخفاض في معدل طول النبات في الاحواض المعاملة بالكاديوم ولكنه زاد في الاحواض المعاملة بالرصاص مقارنة بمعامل السيطرة.

كلمات مفتاحية: النبات المائي ، التراكم الاحيائي ، المعادن، الكاديوم، الرصاص

## INTRODUCTION

Many plants have the ability to absorb pollutant from the soil, water and air and accumulate in their tissues in a manner that does not cause harm to them and thus play a role in the treatment of environmental pollution in the sites where they grow (24,25). The accumulation of heavy elements in the accumulated plants has received considerable attention from researchers because of their important applications in plant treatments. These plants can be exploited and used to extract heavy elements from soil and water (21,36). Bioaccumulation can, therefore, be defined as the concentration of chemical compounds within the body of an organism. these compounds may include heavy metals and pesticides (19). The bioaccumulation of heavy elements in the tissues of aquatic plants may pose a threat to wildlife in the water system (31). Heavy metals are released into aquatic systems through wastewater emanating from increased industrial and domestic activities increased concentrations of heavy metals in water bodies, especially rivers and ponds shown adverse effects on aquatic flora and fauna (1). When increasing the level of heavy metals within the plant tissue, the plant either collected the metals in special locations in the root or stem or converts them to other non-toxic forms that may be distributed and used again in the metabolic processes (20). These elements poison depend on their quantity of the object, the absorbed dose and the duration of exposure (34). Hydrilla is a submerged, herbaceous, perennial aquatic plant. It is capable of living in many different freshwater habitats. It will grow in springs, lakes, marshes, ditches, rivers, or anywhere there are a few inches of water. Hydrilla can tolerate low nutrient and high nutrient conditions as well as salinity of up to 7%. Another adaptation Hydrilla possesses, that enable it to out-compete for native plants, is the ability to grow in low light conditions. It is able to grow at deeper depths and can begin to photosynthesize earlier in the morning than most other aquatic plants. Hydrilla can reproduce in The different ways, fragmentation, tubers, turions, and seed (26). Confirmed (13) *H. verticillata* is the dominant species in the aquatic environments in which it

grows to be highly efficient in growth. It thrives in high and low organic deposits as well as growth in sandy and rocky substrates. For the purpose of developing the use of plant treatments technology to repair the aquatic environment (51) studied the absorption and transport of heavy metals in the wetland system through submerged aquatic plants. *Hydrilla verticillata* plant was used in accumulating copper ions were accumulated in the walls of plant tissue cells. (8) studied Hydrilla and Ceratophyllum plants in the laboratory by exposing them to different concentrations (10,20,30,50) as they proved the efficiency of the Hydrilla in the withdrawal and accumulation of heavy metals more than the other plant. Hydrilla plant proved highly efficient to remove (2). Phukan et al.,(39) concluded that the cadmium element gates its highest concentration ( 3mg/l) in the roots and stems together during their studies of racist and chesty on the Hydrilla plant. (34) were to assessed three types of water plants (Hydrilla ,Salvinia, Aldea ) to remove three of the heavy iron elements, iron, inspiration and nickel, these plants have made very good performance in the removal of iron, copper and nickel from their solution and were able to remove mail to 98%iron 95%copper and 90%nickel within 10 days (45). The use of the Hydrilla plant and the wrap of the wine of the vineyard of the water solution and succeeded with the vineyard of absorption. (46) study the effect of the cd and Pb on the plant. Accumulation of lead in Hydrilla plant was found to be more than cadmium decrease the plant growth and missed self. So (11) were investigated the anatomical and physiological effects of cadmium in *Hydrilla verticillata* and plant response when exposed to cadmium metals and found the high concentration of the cadmium was caused by the dissolution of plant cells and plant death. The hypothesis of study that *Hydrilla verticillata* could accumulate heavy metals in their biomass. The aim of this study, was investigate the ability of *Hydrilla verticillata* aquatic plant accumulated of cadmium and lead in its tissues.

## MATERIALS AND METHODS

### Collection of plant

Plant samples, *H. verticillata* collected from Euphrates River ( Al-Zarqa region at Kufa

city, Najaf, Iraq) located between Longitude °32 99' 861''N and Latitude °44 29' 00''E.(Map1). The experiment was carried out in the culture room that included with constant light irratically (500 Lux.); photoperiod 12/12 light/dark (h/h.) and temperature 28C (10) during the period from October 2018 to January (2019) at the Faculty of science, University of Kufa(Najaf, Iraq).



**Map 1. Sampling sites from Euphrates River**

### Plant treatments

*Hydrilla verticillata* were cut of terminal shoots into 10 cm length fragments. The existing branches (roots and flower) were eliminated. The fragments of shoot were planted in plastic containers all were equal dimensions (40cm length x 25cm width x 25cm height) filled with 15 liters of water per container and water level was in each container maintained at a same level throughout the experiment. The experiment was designed in 30 containers (plastic tank) and these containers divided into 2 groups, each group contain 15 containers after acclimation for 14 day in tap water ,the first group treated with cadmium at 5 containers (1,2,4 ,8 and control) ppm also the second group treated with lead at 5 containers (0.5,5,10 ,20 and control) ppm each tank

contain 40 shoot and each treated triplicate. 'Table 1'. Then concentrations of the cadmium and lead in plant and water, bioaccumulation factor and removal ratio) were recorded after (1, 7, 14 and 21) day and length of plant and biomass measured in end of the experiment.

**Table 1. The terminal shoots of *H. verticillata* used per container in all treatments**

Treatment	Concentrations (ppm)				
	T1	T2	T3	T4	T5
<i>Cd</i>	1	2	4	8	0
<i>Pb</i>	0.5	5	10	20	0

### Measurement the concentrations of Heavy Metals

Digested of plant to measure heavy metals after rinsed thoroughly with distilled water, then dried, grounded and sieved using a 63µm and put (1g) into the teflon beaker with 5 ml nitric acid than 2 ml Perchloric acid, heated at 70C until all the materials were dissolved. Therefore, escalation for 30 min and the temperature of 200 to become a solution is clear (23). To determine the disposal of the materials of the stuff in the solution, the sample was released during the filtering of 0.45 µm washed with water and acid and dry in advance (48). and the samples were diluted with deionized water to a volume of 50 ml and filtered. Samples were stored in tight stopper polyethylene vials to be ready for analysis, using Atomic Absorption Spectrophotometer (15).

### Statistical Analysis

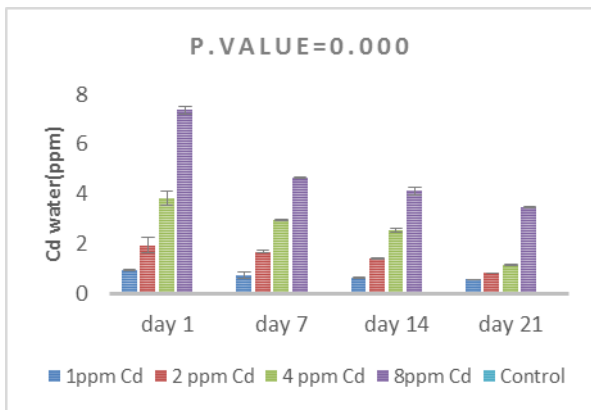
The experimental were arranged in a Randomized Design Completely; data were analyzed by using SPSS statistical software (version 16).

## RESULTS AND DISCUSSION

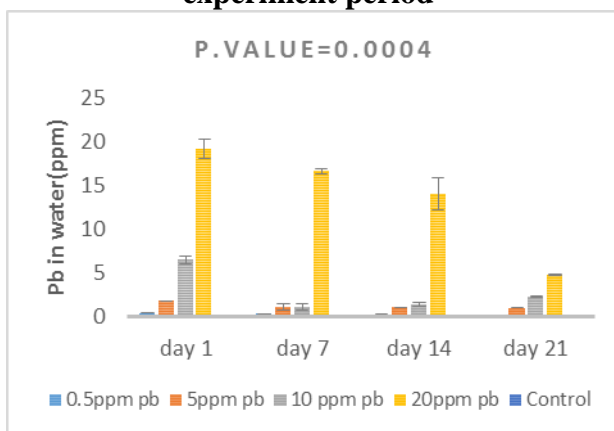
### 4.1 Heavy metals in water

The results of water in aquariums that exposed to different concentrations of cadmium ( $Cd^{+2}$ ) element have been recorded gradual decrease during the experiment period, arranged between (0.56-7.35) ppm; the highest value was 7.35 ppm in 8ppm treatment during first day of the experiment and lowest value was 0.56 ppm in 1ppm treatment during 21<sup>st</sup> day '(Figure 1)'. Statically analysis under probability ( $p < 0.01$ ) showed there are significant differences between all interactions. While in aquariums that exposed

to different concentrations of lead ( $Pb^{+2}$ ) element have been recorded gradual decrease during the experiment period, arranged between (0.26-19.12) ppm; the highest value was 19.12 ppm in 20 ppm treatment during first day of the experiment and lowest value was 0.26 ppm in 0.5 ppm treatment during 14<sup>th</sup> and 21<sup>st</sup> day of experiment '(Figure 2)'. Statically analysis under probability ( $p < 0.01$ ) showed there are significant differences between all interactions.



**Figure 1. Mean of Cd element concentration in aquariums that exposed to different concentrations of  $Cd^{+2}$  during the experiment period**



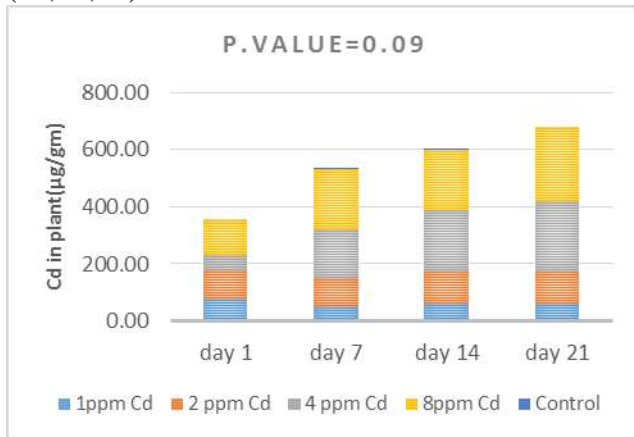
**Figure 2. Mean of Pb element concentration in aquariums after exposed to different concentrations of  $Pb^{+2}$  during the experiment period**

#### 4.2 Heavy metals in plant

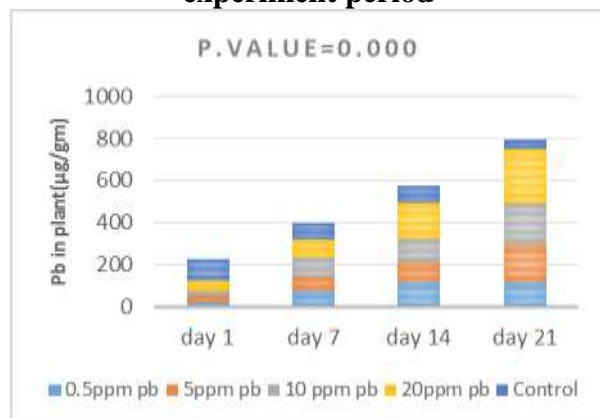
Figure (3)' shows that the concentrations of the cadmium in plant material that exposed to different concentrations of cadmium ( $Cd^{+2}$ ) have been recorded gradual increased during the experiment period, arranged between (55.2-260.26)  $\mu g/gm$ ; the highest value was 260.26  $\mu g/gm$  in 8 ppm treatment during 21<sup>st</sup> day of the experiment and lowest value was 52.2  $\mu g/gm$  in 4ppm treatment during first day. Statically analysis under probability ( $p < 0.01$ )

showed there are no significant differences between all interactions. Either in plant material that exposed to different concentrations of lead ( $Pb^{+2}$ ) element have been recorded gradual increased during the experiment period, arranged between (21.7-261.1)  $\mu g/gm$ ; the highest value was 261.1  $\mu g/gm$  in 20 ppm treatment during 21<sup>st</sup> day of the experiment and lowest value was 21.7  $\mu g/gm$  in 0.5 ppm treatment during first day. Statically analysis under probability ( $p < 0.01$ ) showed there are significant differences between all interactions these clear in figure 4. The results showed that the content of the *H. verticillata* plant recorded a gradual increase in cadmium and lead values at high concentrations during the experiment period. This is due to the fact that aquatic plants are efficient in removing pollutants and extracting heavy elements and accumulating in their bodies because it possesses resistance mechanism, such as protein-binding elements as metallothionine or peptides with low molecular weight called plant claws (29). The concentration of heavy elements in plant bodies is controlled by several factors, including pH, presence of other elements and ions, temperature, salinity, light intensity, amount of oxygen, organic matter and humic substances (18). *H. verticillata* plant effects in the high concentrations of cadmium despite of its high efficiency in the reduction at low concentrations (1 and 2) mg / l. While it proved its efficiency in reduction of the lead element and carry to higher concentrations than cadmium, and possibly due to the availability of surrounding conditions such as pH, plant age and plant physiological characteristics (33). And Possibly to the absorption of the lead element by plant a higher of cadmium. The increase in the accumulation of cadmium in the 4 mg / l concentrations of plant is probably due to the heavy tolerance of plant to this concentration and the balance of all enzymatic and molecular antioxidants as well as the increased secretion of cellular metabolism (9). For plant growth and tolerance. Srivastava et al.,(47) were carried of aquatic plants to different concentrations of the elements and the continued growth was a result of a balance in the levels of enzymatic and molecular

antioxidants such as (Proxidase, proline and total phenols) and the possibility of increasing the secretion of cellular metabolism products such as Cysteine and glutamine. The study agrees with the study (6) and the study (49,30,41).



**Figure 3. Mean of Cd element concentration in *H. verticillata* plant after exposed to different concentrations of Cd<sup>2+</sup> during the experiment period**



**Figure 4. Mean of Pb element concentration in *H. verticillata* plant after exposed to different concentrations of Pb<sup>2+</sup> during the experiment period**

#### 4.3. Bioaccumulation factor of heavy metals. (BAF)

The results shows in Table (2) that the highest recorded BAF of the cadmium in the experimental aquariums of the *H. verticillata* at (4 ppm) treatment during the 21<sup>st</sup> day was

(21639) and the lowest value at (4 ppm) was (1444) during the first day, while the BAF of the lead element recorded the highest value at the treatment of 0.5 ppm during the 21<sup>st</sup> day was (45467) and the lowest recorded value is (264) at the treatment of 20 ppm during the first day (Table 3). Values of the bioaccumulation factor are indicators of plant susceptibility to the aggregation of elements in their tissues (35). And the plants are differed in their ability to accumulate heavy metals (28,37); Also the results showed that the highest recorded BAF of the cadmium in the experimental aquarium of the *H. verticillata* at (4 ppm) treatment during the 21<sup>st</sup> day and the lowest value at 4 ppm during the first day, while the BCF of the lead element recorded the highest value at the treatment of 0.5 ppm during the 21<sup>st</sup> day and the lowest recorded value at the treatment of 20 ppm during the first day. The high values of element ability to concentrate and accumulated the elements within its tissues (41). Or perhaps the plant's ability to absorb the cadmium elements at its preferred concentration (4 ppm) while the concentration of 0.5 ppm of lead is preferred for the *H. verticillata* plant during the experiment period without damage to the plant. Differences in the accumulation of heavy metals within plant tissues may be due to the different cellular mechanisms of aggregation and the biological concentration of necessary and unnecessary elements in plants (40). The process of reduction and selection of pollutants depends on the plant's the ability of plant to uptake the element depend on the transfers proteins and the plasma membrane properties. The transport process depends on the transport of ion and how it is linked and transported by the plasma membrane to another cell (7)



**Table 2. Biological accumulation factor (BAF) of Cd element in *H. verticillata* during the experiment period**

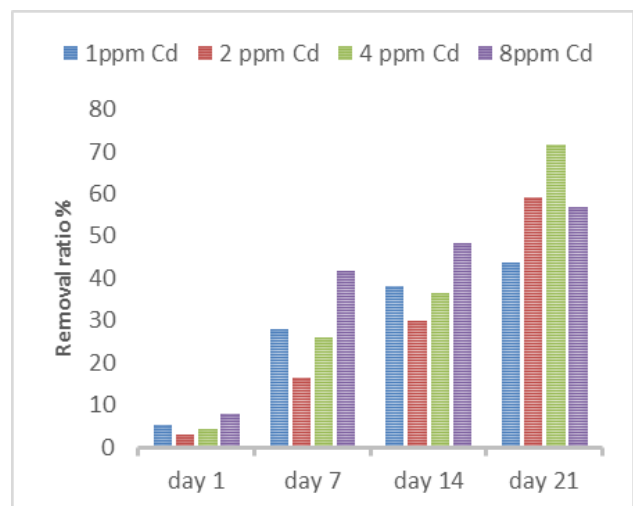
Cd concentration in plant	Days	1ppm	2 ppm	4 ppm	8ppm
	day 1	77.46	98.15	55.20	126.07
	day 7	52.39	99.80	169.79	209.37
	day 14	59.35	111.31	217.15	210.68
	day 21	60.54	112.57	244.52	260.26
Cd concentration in water	day 1	0.94	1.94	3.82	7.35
	day 7	0.72	1.67	2.95	4.64
	day 14	0.62	1.4	2.53	4.11
	day 21	0.56	0.82	1.13	3.45
BAF	day 1	8183	5068	1444	1714
	day 7	7277	5988	5755	4512
	day 14	9624	7951	8572	5122
	day 21	10811	13784	21639	7551

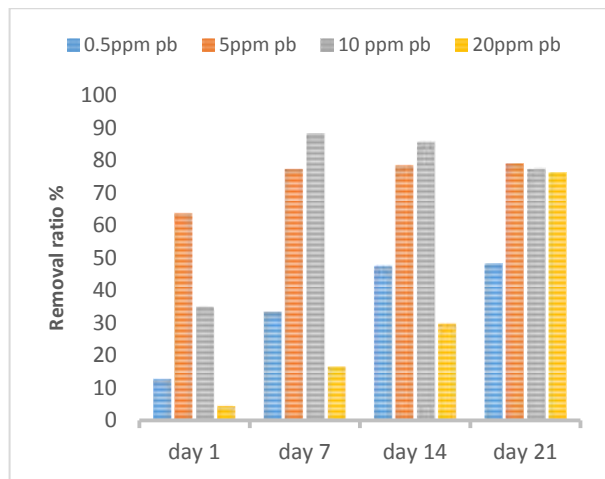
**Table 3. Biological accumulation factor (BAF) of Pb element in *H. verticillata* during the experiment period**

Pb concentration in plant	Days	0.5ppm	5 ppm	10 ppm	20 ppm
	day 1	21.67	30.13	23.41	50.53
	day 7	78.45	63.93	93.91	80.37
	day 14	116.29	95.31	112.27	170.76
	day 21	118.21	175.36	194.84	261.13
pb concentration in water	day 1	0.44	1.82	6.51	19.12
	day 7	0.33	1.14	1.20	16.65
	day 14	0.26	1.075	1.46	14.03
	day 21	0.26	1.052	2.27	4.77
BAF	day 1	4963	1659	360	264
	day 7	23535	5616	7848	483
	day 14	44162	8866	7708	1217
	day 21	45467	16670	8583	5474

#### 4.4. Removals Efficiency of Heavy Metals

Figure (5) shows the removal ratio of cadmium by *H. verticillata* plant during the experiment period. The results showed that the highest percentage recorded for cadmium removal was (72 %) during the 21<sup>st</sup> day at the concentration of 4 ppm and the lowest (3 %) at the concentration of 2 ppm during the first day. While in the aquarium that exposed to different concentrations of lead ( $Pb^{+2}$ ) element were recorded higher values of removal ratio by *H. verticillata* (88% ) at 10 ppm during 7<sup>th</sup> day of the experiment and lowest was 4.4% at 20 ppm treatment during first day '(figure 6)'. These results because of its ability to withstand different environmental conditions. Metal uptake was higher at medium concentration (4ppm for Cd and 10 ppm for pb) and decreased thereafter with increase in metal concentration. This is probably due to toxicity of concentration. Similar results were obtained in various other aquatic plant species (12,32).

**Figure 5. Removal ratio of *H. verticillata* in aquariums that exposed to different concentrations of Cd<sup>2+</sup> during the experiment period**



**Figure 6. Removal ratio of *H. verticillata* in aquariums that exposed to different concentrations of Pb<sup>2+</sup> during the experiment period**

#### 4.5. Biomass and Rate of plant length

The results in aquariums that exposed to different concentrations of cadmium (Cd<sup>2+</sup>) element have been recorded decrease in biomass during the experiment days the highest value in control treatment was 20.45 gm and the lowest value in 4 ppm treatments was (9.91) gm (Figure 7), the statically analysis under probability ( $p < 0.01$ ) showed there are significant differences between all interactions. While in aquariums that exposed to different concentrations of lead (Pb<sup>2+</sup>) element, the biomass increased at 10 ppm treatment was 20.86 gm and the lowest value was 16.07 gm in 5 ppm treatment and recorded the statically analysis under probability ( $p < 0.01$ ) showed there are significant differences between all interactions these clear in Figure 8. The results in Figure (9) shows that the decreased rate length of *H. verticillata* plant in the aquarium that exposed to different concentrations of the cadmium at all treatments except 1 ppm treatment that recorded increase rate in length of plant (3cm) and the lowest value of decreased rate at 8 ppm was (-3.5 cm) during experiment period (plate 1); statically analysis under probability ( $p < 0.01$ ) showed there are significant differences between all interactions. While in the aquarium that exposed to different concentrations of the lead(pb) the rate of plant length increased compare control treatment the highest value at the treatment of 20 ppm was (8 cm) and the lowest recorded value is (2.2) at the control treatment (Figure 10) (plate 2);

statically analysis under probability ( $p < 0.01$ ) showed there are significant differences between all interactions. The biomass of selected plant for phytoremediation technology is very important to ensure high removal rate of toxic heavy metals. Some plant species can accumulate a high contents of heavy metals in their tissues; however, produce little biomass and are slow-growing plants, which makes it unfeasible to use these species in phytoremediation. For example, (44) revealed a decline in biomass production of *B.napus* grown on Cd contaminated soil. As mentioned above, the biomass of selected plant for phytoremediation technology is very important to ensure high removal rate of toxic heavy metals. The *H. verticillata* plant was chosen in this study based on their high biomass, fast growth rate and its ability to remove heavy metals from contaminated water (22,14). Plant appeared healthy in the control and aquarium exposing to low metals concentration, whereas plant grown on the higher Pb and Cd contaminated water showed yellowing to browning of the leaves. In this experiment, there were higher differences between the same plant in, weight and length of plant grown in aquariums amended with different concentrations of different metals. It seems that cadmium (Cd) was more effective for decreasing plant biomass and shoots length of the growing plants compared to lead (Pb). Growth and metabolism of *H. verticillata* were affected variably by the stress of heavy metals such as Cd. High concentrations of Cd resulted in stunted growth, reduced biomass production and produced characteristic visible effects similar to those described by other workers in different plant species (50,52,17). The reduction in biomass was significantly high in Cd excess followed by Pb to lesser extend in control treatment. This may represent the cumulative effect of damaged or inhibited physiological function under stress condition. The observed decrease in dry matter production as a result of metal stress is in agreement with that reported earlier in plants other than *H. verticillata* (38,42,5). The exposure of excess concentration of metals to plants inhibits physiologically active enzymes (16), inactivates photosystems (43), and destruct mineral metabolism (27).

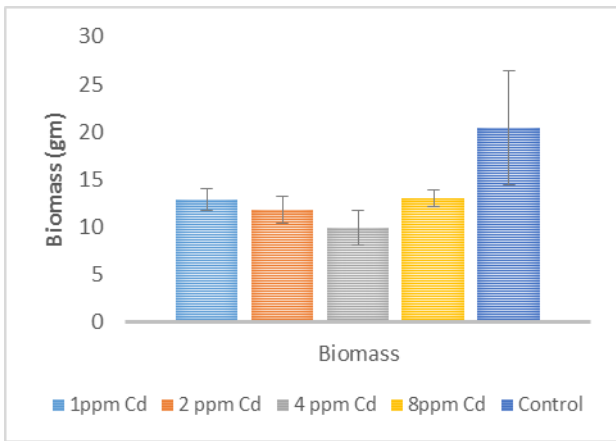


Figure 7. Mean of Biomass of *H. verticillata* that exposed to different concentrations of Cd<sup>+2</sup> during the experiment period with SD



Plate 1. Length rate of *H. verticillata* plant after exposing to cadmium at 8 ppm during 21<sup>st</sup> day of the experiment

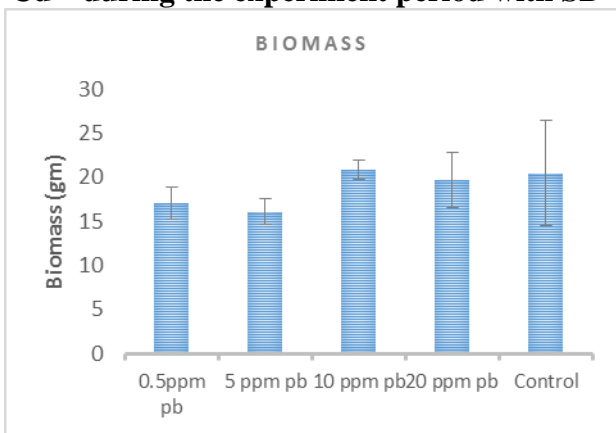


Figure 8. Mean of Biomass of *H. verticillata* that exposed to different concentrations of Pb<sup>+2</sup> during the experiment period with SD

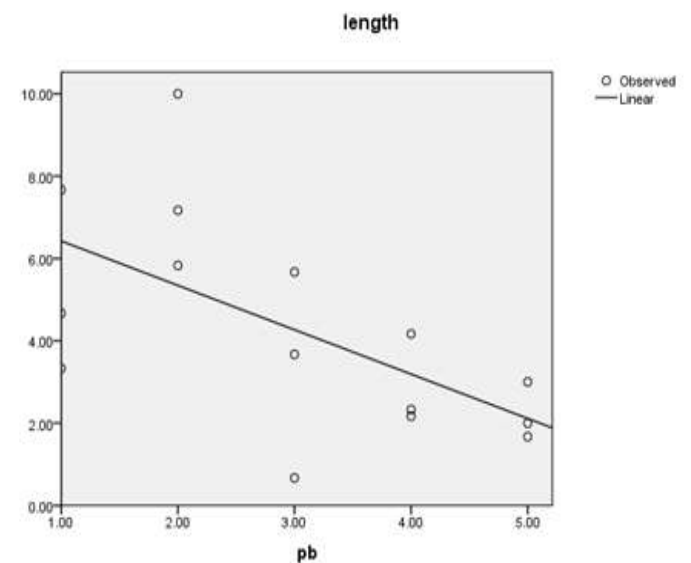


Figure 10. Mean of length of *H. verticillata* that exposed to different concentrations of Pb<sup>+2</sup> during the experiment period

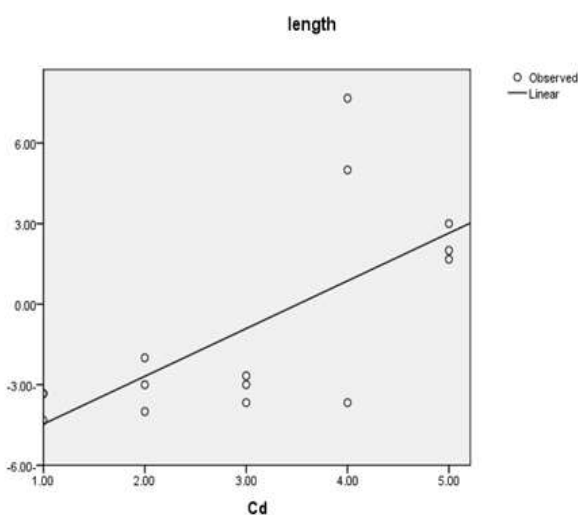


Figure 9. Mean of length of *H. verticillata* that exposed to different concentrations of Cd<sup>+2</sup> during the experiment period



Plate 2. Length rate of *H. verticillata* plant after exposing to Lead at 20 ppm during 21<sup>st</sup> day of the experiment



**CONCLUSION**

The current study was proved that the *H. verticillata* had the ability to remove cadmium and lead elements from contaminated water; and by measuring the various parameters, it can be said that uptake of both the metals increased with metal concentration increased. However, uptake of Pb was more as compared to uptake of Cd. Thus, it may serve as the good bioaccumulation of Pb as compared to Cd under metal concentrations. The lead metal was limited because the experiment concluded under limited time (21 day). Futures studies must recommend the effect of heavy metals on *Hydrilla verticillata* for a longer time (more than 21days).

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