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Phytoremediation of Chromium and Copper from Aqueous Solutions Using *Hydrilla verticillata*

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Abstract

The current study included testing the ability of plant *Hydrilla verticillata* (L. F.) on the accumulation of two heavy metals in its tissues, and use the plant in phytoremediation. The plant was exposure to different concentrations of chromium and copper metals (2.5, 5, 10, 15, 20) ppm, for a period of fourteen days, for each solution.The results showed that Hydrilla was more efficient in the removal of chromium, where the amount of the remaining concentration of chromium at the last day of the experiment was (0.20 ± 0.014- 0.66 ± 0.114- 0.99 ± 0.176- 0.79 ± 0.073- 1.80 ± 0.131) ppm, while for copper was (0.33 ± 0.06- 1.13 ± 0.39- 1.66 ± 0.05- 1.96 ± 0.043- 2.33 ± 0.0497) ppm at the last day of the experiment, respectively.

Keywords: *Hydrilla verticillata*, accumulation, phytoremediation, chromium, copper

المعالجة النباتية للكروم والنحاس من المحاليل المائية بأستخدام نبات *Hydrilla verticillata*

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الخلاصة

تضمنت الدراسة الحالية أختبار مقدرة نبات الهايدريلا *Hydrilla verticillata* (L.F)على مراكمة بعض العناصر الثقيلة في انسجتة، واستخدام النبات في المعالجة النباتيه عرض النبات الى تراكيز مختلفة من عنصري الكروم والنحاس (2.5، 5، 10، 15، 20) جزء بالمليون ، ولمدة اربعة عشر يوماً لكلا المحلولين. وقد بينت النتائج ان نبات الهايدريلا كان اكثر كفاءة في ازالة عنصر الكروم من المحلول ،حيث كانت كمية عنصر الكروم المتبقي في اخر يوم من التجربة (0.20 ± 0.014، 0.66 ± 0.114، 0.99 ± 0.176، 0.79 ± 0.073، 1.80 ± 0.131) جزء بالمليون، في حين كانت كمية عنصر النحاس المتبقي (0.33 ± 0.06، 1.13 ± 0.39، 1.66 ± 0.05، 1.96 ± 0.043، 2.33 ± 0.0497) جزء بالمليون، على التوالي.

Introduction

Heavy metals are released into aquatic systems through waste water 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 increase 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 [2]. Phytoremediation is utilizing plants to treat contaminated sites. It takes the

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advantage of plants natural ability to extract chemicals from water, soil, and air. Furthermore, phytoremediation has been used to treat a variety of pollutants including metals, petroleum, solvents, explosives, polycyclic aromatic hydrocarbons, and other organic contaminants [3]. The use of plants to clean up contaminated aquatic environments is not a new method, since it began 300 years ago in the polluted water treatment processes [4].

Phytoremediation describes the treatment of environmental problems through the use of plants that mitigate the environmental problem without the need to excavate the contaminant material and dispose of it elsewhere. [5-6]. The most important of phytoremediation Advantages are the variety of organic and inorganic compounds .Phytoremediation can be used either as an in situ or ex situ application. In situ applications are frequently considered because minimizes disturbance of the soil and surrounding environment and reduce the spread of contamination via air and waterborne wastes. [7]. It is a green technology and environmentally friendly and aesthetically pleasing to the public [8]. Several studies have described the performance of heavy metals uptake by plants. It is reported that phytoremediation technology is an alternative to treat heavy metal contaminated side which will be more admitted in order to remediate the environment. In this context, [9] were suggested *Marsilea minuta* and *Hydrilla verticillata* can be used for phytoremediation of Cr and Pb from the contaminated water bodies.

H. verticillata showed as an efficient aquatic plant for phytoremediation of waste water as well as variable tendency in their growth and of generation of biomass [10]. The plant has a high potential for the removal of lead and cadmium from water as well as its ability to absorb large amounts of iron and manganese from the water [11].

H. verticillata has ability to tolerate low light levels which give it a longer growing season than other submerged species and make it capable of outcompeting other submerged plants [12]. In Iraq, *H. verticillata* is considered as new species, when it was recorded for the first time in Abu-Zirig marsh, southern Iraq at 2004 after restoration process of Iraqi marshes [13].

Material and Methods

Collection, identification and acclimatization of plant samples

The plants were collected from different sites of AL-Adhamiya Corniche channel during October 2013 to March 2014 as shown in Figure-2. And put in plastic backs contain water taken from the channel and then transferred to the lab. The collected plants were washed well by tap water and a small brush to remove sediment and algae while maintaining the root hairs, in the lab and the plants were acclimated (for about 10 days) in basins measured (30 X 25 X 20) cm containing tap water, left for three days to get rid of chlorine, estimated at 10 liters, with temperature range 25 ±2 °C, ventilation the basin using a small air pump and was exposed to light 10 hours daily by using fluorescent tubes at 30 cm high from the surface of water.

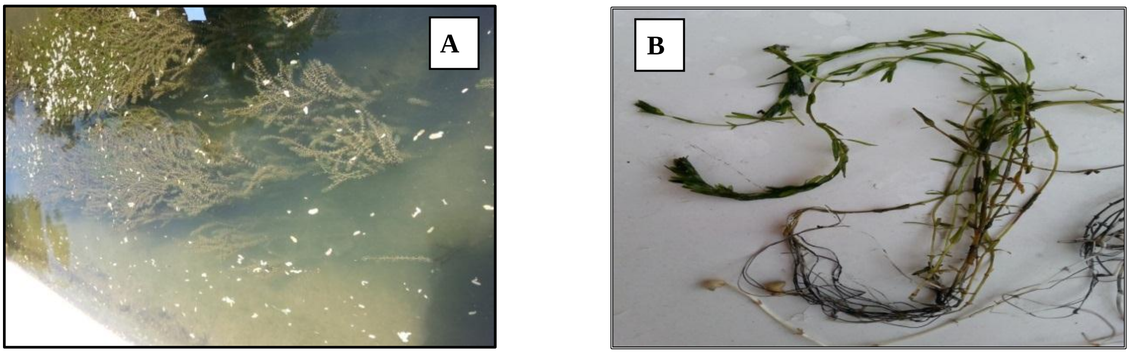

Figure 1- *Hydrilla verticillata* A: The plant in the site of the collection, B: Whole plant with tuber

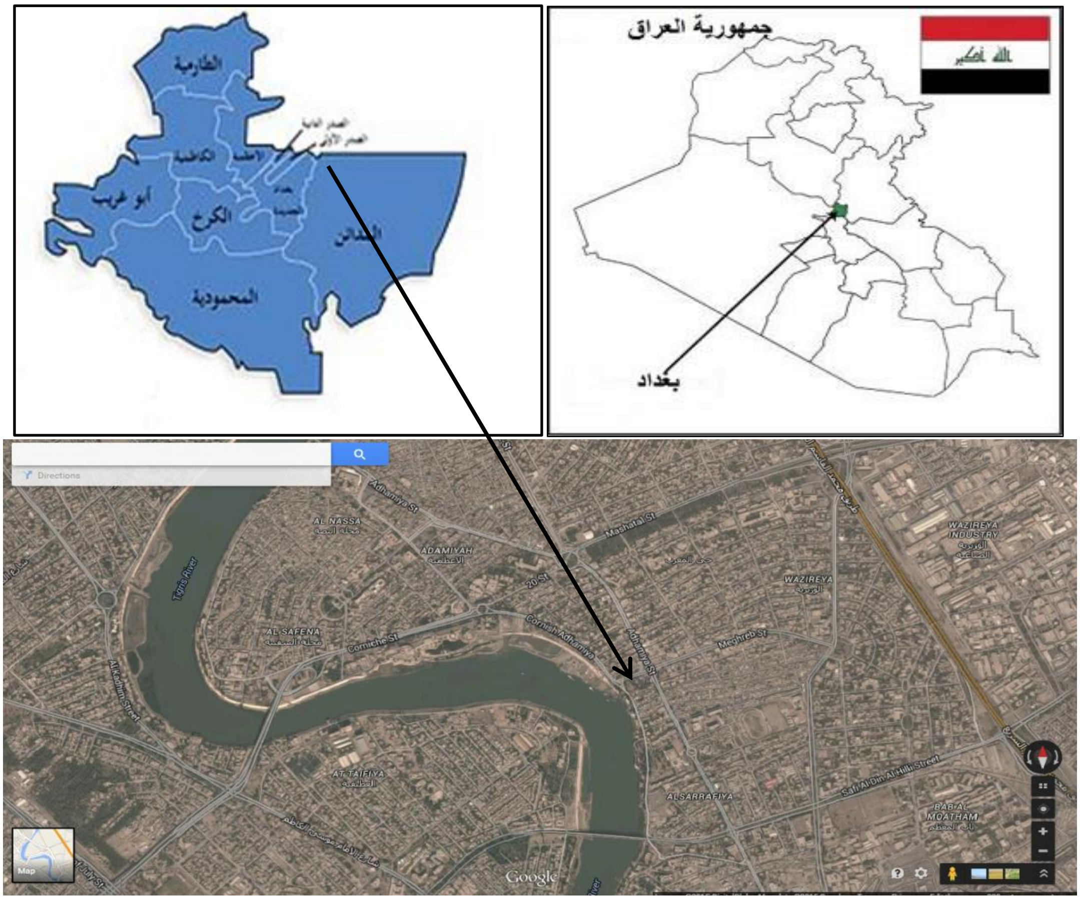

Figure 2- Map of Iraq, Baghdad city and Tigris River; the location of samples collection from AL-Adhamiya Corniche (google map)

Then the plants were distributed to ten basins with three replicates of each basin containing the tap water and heavy metals with different concentration, and control without heavy metals. All the tests include measurement of the metal concentrations in both water and plant for each basin.

Preparation of chromium and copper standard solution:

In order to prepare standard solution of 1000 ppm concentration, 5.124 gm of chromic chloride ($CrCl_3.6H_2O$) and 3.801 gm of copper nitrate ($Cu (NO_3)_2.3H_20$ were dissolved in 100 ml of Deionized water (DW). With constant stirring by Magnetic stirrer for 30 minutes and then complete the volume to 1000 ml. This solution was sterilized by filtration through 0.45 µm membrane filter, and served as a stock solution for further preparations [14]. Preparation the concentrations of elements by diluting the stock solution with distilled water using the equation:

$\mathbf{C_1V_1 = C_2V_2}$,

Measure the concentration of heavy metals of channel water:

One liter of water sample was filtered using 0.45 Mm filter paper, 1.5 ml of Nitric acid HNO_3 was added to the filtrate. Then measuring the concentration of (Cr, Cu) using Atomic Absorption Spectrophotometer [15].

1. Measurement of the heavy metals concentrations in basin water:

One liter of basin water was taken throw the days of experiment and filtered using 0.45 µm filter paper, 1.5 ml of nitric acid HNO_3 was added to the filtrate. Then measuring the concentration of (Cr, Cu) using Atomic Absorption Spectrophotometer [16].

2. Measurement of the of heavy metals concentrations in the plant:

The level of heavy metals in the plant was estimated by:

Putted the plant in an electric oven under 105℃ until the drought. Then the dried plant was grinded, one g was tacked and 20 ml of Nitric acid concentration of 70% was added for digestion process and lifted it covered in acid for 24 hours by watch glass. The samples then heated using hot plate under 60℃ until complete the digestion, with constant stirring. The sample was cooled then 1.5 ml of Perchloric acid concentration of 60% $HClO_4$ have been added, heating the sample at a lower temperature 40°C until the stage before the drought. The sample was lifted from the hot plate to cool it; added 1.5 ml of hydrochloric acid HCl and 1.5 ml of distilled water, the sample was heated at 40 °C for melting sediment. The sample was filtered by 0.45 µm filter paper, the solutions putted in volumetric flask of 50 ml capacity; the volume was completed with distilled water. The concentrations of metals have been estimated by flame atomic absorption spectrum device [16].

Removal efficiency (RE %) of heavy metals

The percentage to remove metals from the water account, according to the following equation referred to [17]: $C_\circ$

Removal efficiency (RE %) = $[C_\circ - C_F / C_\circ] \times 100$

$C_\circ$= initial concentration before treatment

C_F= final concentration after treatment

Statistical analysis:

All experiments data were subjected to various statistical tests for the significant differences such as; analysis of variance (ANOVA), (F test) and least significant differences test (LSD test) [18].

Results and Discussion

Cu, Cr Remained in the water Solution

Table-1 showed a gradual decrease in mean concentrations remaining in water with time when the plant exposure to different concentration of Cu and Cr; it's clear that the concentration of Cu deceases from the highest value 1.25 ± 0.23 ppm in first day to the lowest value 0.33 ± 0.06 ppm after 14 days of treatment in the concentration of 2.5 ppm, and from (14.91 ± 0.224 to 2.33 ± 0.0497) ppm of the initial concentration of 20 ppm. The concentration of Cr remaining in the water was decreased from (1.17 ± 0.078 to 0.20 ± 0.014) ppm in case of 2.5 ppm and from (14.09 ± 0.107 to 1.80 ± 0.131) ppm for 20 ppm.The results also showed that the remaining concentrations for chromium in the last day of treatment were less than the remaining concentrations for copper in all concentration which were (0.20 ± 0.014- 0.66 ± 0.114, 0.99 ± 0.176, 0.79 ± 0.073, 1.80 ± 0.131) ppm for Cr and (0.33 ± 0.06, 1.13 ± 0.39, 1.66 ± 0.05, 1.96 ± 0.043, 2.33 ± 0.0497) ppm for Cu, which mean that *Hydrilla verticillata* have the beast removal efficiency for chromium than for copper. The lowest percentage of removal efficiency for copper was 1.2% after one day of 15 ppm, and for chromium was 26% after on day of 15 ppm, the highest percentage of removal efficiency for copper was 88% after fourteen days of 20 ppm, and for chromium was 94% after fourteen days of 15 ppm. As shown in Table-1, 2 and Figure-2 and 3.

Table 1- Mean ± SD of each copper and chromium concentrations remained after plant biosorption at different concentration and various periods.

Period	Biosorbed metal	Mean remaining concentration ± standard deviation					
		control	2.5 ppm	5 ppm	10 ppm	15 ppm	20 ppm
One day	Copper	0	1.25 ± 0.23	2.94 ± 0.12	7.01 ± 0.07	14.81 ± 0.14	14.91 ± 0.224
four days		0	0.88 ± 0.08	2.22 ± 0.09	4.21 ± 0.03	9.38 ± 0.059	13.10 ± 0.128
seven days		0	0.74 ± 0.03	1.23 ± 0.25	3.45 ± 0.11	7.51 ± 0.128	9.23 ± 0.059
Ten days		0	0.41 ± 0.08	1.11 ± 0.07	2.32 ± 0.25	3.25 ± 0.227	4.10 ± 0.137
fourteen days		0	0.33 ± 0.06	1.13 ± 0.39	1.66 ± 0.05	1.96 ± 0.043	2.33 ± 0.0497
One day	Chromium	0	1.17 ± 0.078	2.78 ± 0.193	6.46 ± 0.05	10.96 ± 0.102	14.09 ± 0.107
four days		0	0.77 ± 0.15	1.47 ± 0.057	4.11 ± 0.09	8.52 ± 0.172	10.93 ± 0.098
seven days		0	0.66 ± 0.114	1.12 ± 0.118	2.59 ± 0.209	5.42 ± 0.359	5.06 ± 0.209
Ten days		0	0.36 ± 0.089	1.08 ± 0.085	1.85 ± 0.481	2.95 ± 0.622	3.71 ± 0.284
fourteen days		0	0.20 ± 0.014	0.66 ± 0.114	0.99 ± 0.176	0.79 ± 0.073	1.80 ± 0.131

L.S.D. concentration ($P \leq 0.05$) = 0.216

Table 2- Removal efficiency (RE %) of copper and chromium after plant biosorption at different concentration and various periods.

Period	Biosorbed metal	Removal efficiency %				
		2.5 ppm	5 ppm	10 ppm	15 ppm	20 ppm
One day	Copper	50	41	29	1.2	25
four days		64	55	57	37	34
seven days		70	75	65	49	53
Ten days		83	77	76	78	79
fourteen days		86	77	83	86	88
One day	Chromium	53	44	35	26	29
four days		69	70	58	43	45
seven days		73	77	74	63	74
Ten days		85	78	81	80	81
fourteen days		92	86	90	94	91

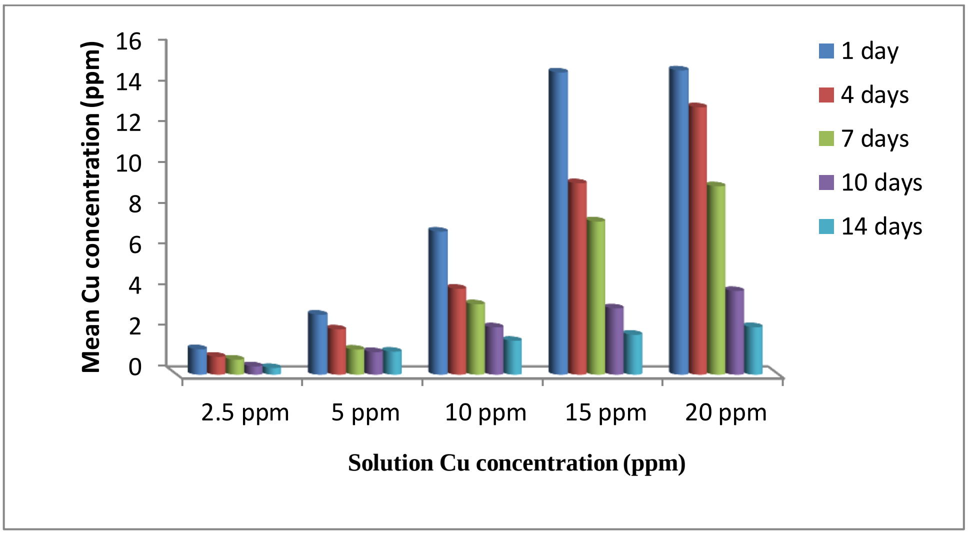

Figure 2- Mean copper concentration (ppm) remained after fresh biosorbed plants from copper solution.

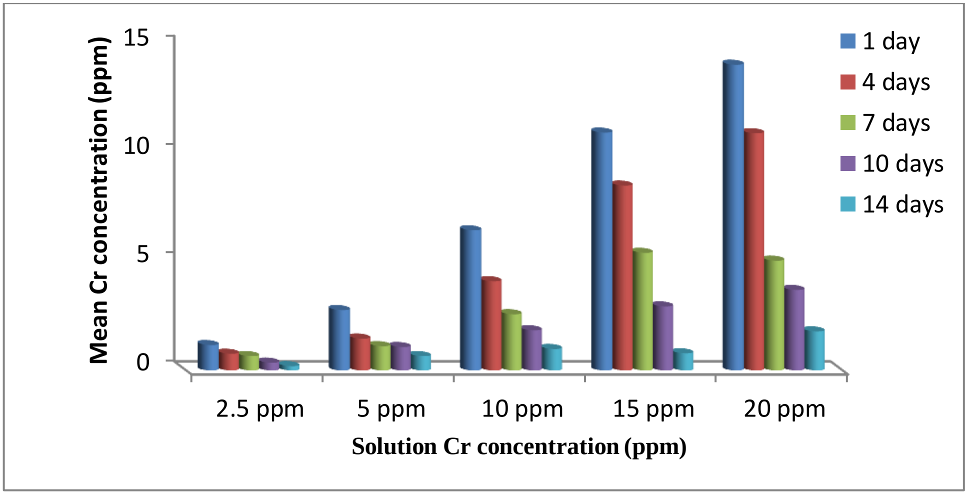

Figure3- Mean chromium concentration (ppm) remained after fresh biosorbed plants from chromium solution.

Analysis of variance of these data shows the differences ($P \leq 0.05$) for mean Cu and Cr ions remaining after fresh plant biosorption with different concentration and days. Least significant difference ($LSD_{0.05}$) test was 0.216 ppm.

As shown in Table-1 the values of copper and chromium remaining in the water was gradually beginning to decline with the increase of contact time of the experiment, which shows the possibility of using *Hydrilla verticillata* in the biological treatment of contaminated water with copper and chromium ion.

Results can be explained that copper is an essential micronutrient for normal plant metabolism; it has been reported to be toxic at high concentration [19].

However, better biosorption performance was found in the case of chromium ions than for copper ions, because the excessive accumulation of Cu in plant tissue can be toxic affecting several physiological and biochemical processes and growth. Cu treatment brings changes in nitrogen metabolism with a reduction in total nitrogen [20]. It results in an increase of free amino acid [21]. While chromium involves in direct and indirect metabolic reactions inside the plants. The reduction in chromium concentration due to binding of the softer Cr (III) with thiol (-SH) part of the protein in root via soft-soft interaction [22].

The result agrees with [23], which said that the concentrations of copper also gradually decline when *Hydrilla* exposed to (10, 15, 20, 30, 50) ppm where it was (1.06, 1.71, 2.99, 5.23, 27.45) ppm, respectively, remaining in water after 18th days of the experiment.[24] mentioned that the remaining concentrations of copper were low when use of *Eichhornia crassipes* for removal of (1, 3, 6, 10, 12, 15, 20, 30) ppm of copper which were (0.03, 0.12, 0.3, 0.32, 0.37, 0.6, 0.8) ppm, respectively after 10 days of exposure. Copper when present in the nutrient solution at concentrations ≤ 0.2 $mg.L^{-1}$ was an essential element for the development of *Lemna* fronds because of its important role in cellular metabolism. At a concentration higher than 0.4 mg/L, Cu caused the photosystem alteration by reducing electron transport. This effect was explained by a rapid development of chlorosis [25].

Although Cr is a non-essential element and its compounds is highly toxic and detrimental to the growth and development of the plants [26] but, it is easily absorbed by roots and then transported via the vascular system [27]. [21] Reported that the use of Hydrilla sp. could remove up to 99.70% of chromium and Chara sp. remove 91.70% at a concentration of (2 mg/L) after 7 days of treatment which revealed their potential as good metal bioabsorbant.

Biosorbent plant material:

Table-2 show that *Hydrilla verticillata* has the ability to accumulate heavy metals in water; which the concentration in plant increase with contact time. The initial concentration (2.5, 5, 10, 15, 20) ppm of copper were at the first day of exposure (1.31 ± 0.248- 2.10 ± 0.221- 3.01 ± 0.135-1.09 ± 0.123- 5.12 ± 0.112) ppm; after 14 days of exposure were (2.19 ± 0.093- 4.20 ± 0.131- 8.67 ± 0.18- 13.21 ± 0.18- 17.57 ± 0.206) ppm, respectively.

For chromium the concentrations were (1.28 ± 0.204 - 3.31 ± 0.227- 3.29 ± 0.078- 3.08 ± 0.171- 6.01 ± 0.149) ppm at the first day and (2.24 ± 0.203 - 4.35 ± 0.108- 8.88 ± 0.135- 14.51 ± 0.165- 17.75 ± 0.184) ppm at the end of the exposure. The lowest percentage of removal efficiency for copper was 11% after fourteen days of 15 ppm, and for chromium was 3% after fourteen days of 15 ppm, the highest percentage of removal efficiency for copper was 92% after one day of 15 ppm, and for chromium was 79% after one day of 15 ppm. As show in Table-3, 4 and Figure-4, 5

Table 3- Mean ± SD of each copper and chromium concentrations in plants after plant biosorption at different concentration and various periods.

Period	Biosorbed metal	Mean remaining concentration ± standard deviation				
		2.5 ppm	5 ppm	10 ppm	15 ppm	20 ppm
One day	Copper	1.31± 0.248	2.10± 0.221	3.01± 0.135	1.09 ± 0.123	5.12 ± 0.112
four days		1.59± 0.177	2.81± 0.127	5.69± 0.276	5.72 ± 0.179	7.10 ± 0.115
seven days		1.71± 0.241	3.82± 0.222	6.57± 0.213	7.71 ± 0.107	11.18± 0.154
Ten days		2.11± 0.177	3.91± 0.177	7.81± 0.118	12.01 ± 0.2	15.88± 0.269
fourteen days		2.19± 0.093	4.20± 0.131	8.67 ± 0.18	13.21 ± 0.18	17.57± 0.206
One day	chromium	1.28± 0.204	3.31± 0.227	3.29± 0.078	3.08 ± 0.171	6.01 ± 0.149
four days		1.88± 0.062	3.92± 0.102	5.91± 0.135	6.51 ± 0.165	8.98 ± 0.170
seven days		1.91± 0.142	4.17± 0.802	7.42± 0.128	9.40 ± 0.160	14.83± 0.136
Ten days		2.09± 0.149	4.02± 0.131	8.53± 0.082	12.43± 0.096	16.01± 0.192
fourteen days		2.24± 0.203	4.35± 0.108	8.88± 0.135	14.51± 0.165	17.75± 0.184

L.S.D. concentration (P≤0.05) = 0.345

Table 4- Removal efficiency (RE %) of copper and chromium in plant tissue at different concentration and various periods.

Period	Biosorbed metal	Removal efficiency %				
		2.5 ppm	5 ppm	10 ppm	15 ppm	20 ppm
One day	Copper	47	58	69	92	74
four days		36	43	43	61	64
seven days		31	23	34	48	44
Ten days		15	21	21	19	20
fourteen days		12	16	13	11	12
One day	Chromium	48	33	67	79	69
four days		24	21	40	56	55
seven days		23	16	25	37	25
Ten days		16	19	14	17	19
fourteen days		10	13	11	3	11

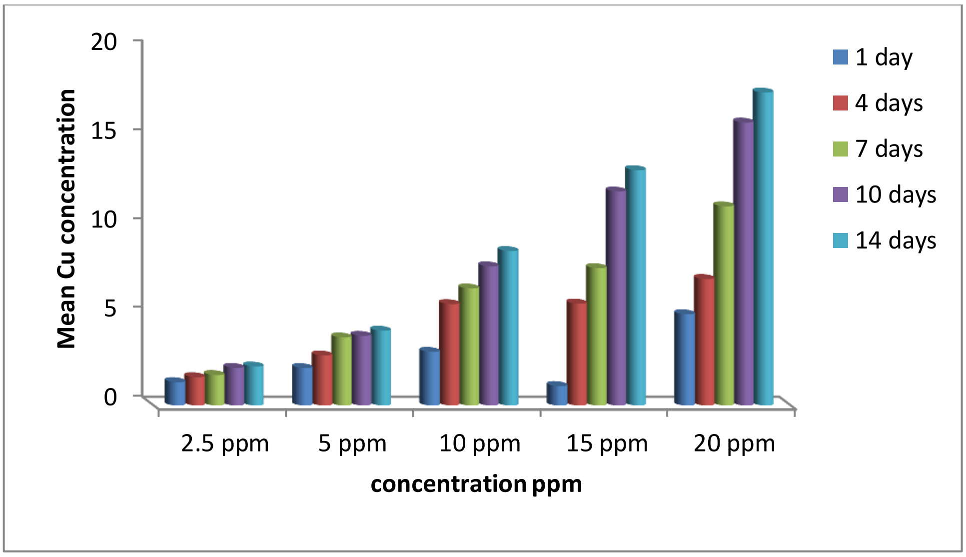

Figure 4- Mean copper concentration (mg/g) in biosorbed plant (*Hydrilla verticillata*) at different concentration and period values.

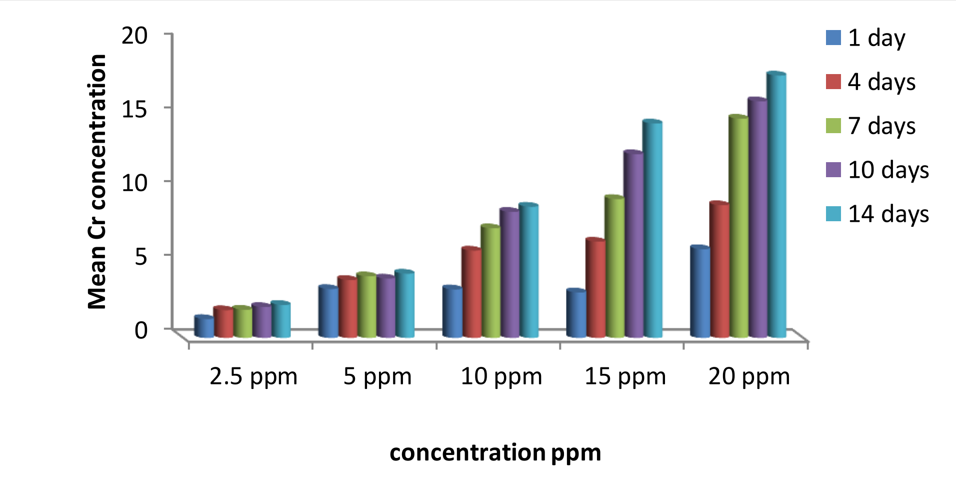

Figure 5- Mean chromium concentration (mg/g) in biosorbed plant (*Hydrilla verticillata*) at different concentration and period values.

Results of statistical analysis and less significant difference LSD at the possibility $P \leq 0.05$ shows the existence of a significant effect of the factors involved in the study (time and concentration) in the level of copper and chromium absorption by plant.

Analysis of variance of these data shows significant ($P \leq 0.05$) effects of contact time on plant accumulation of both heavy metal ions. The value of least significant differences ($LSD_{0.05}$) was 0.345 ppm. [28] Reported that *Salvinia natans* has effective biosorption capacity for copper reached 96% on the 6th day of the research. In the culture media with 10 and 15 mg Cu/dm^3. It took less time than *H. verticillata* to accumulate copper on this study. [29] prove the efficiency of *Centella asiatica* and *Eichhornia crassipes* in copper removal where their findings from the two plants high-capacity in the copper removal as the percentage of the removal of 99.6% for the plant *C. asiatica* of the concentration of 2.5 ppm and 97.30% for *E. crassipes* of the concentration of 1.5 ppm and during21 days of the experiment, and in the current study the efficiency of *H. verticillata* approach to the efficiency of the two plants.[23] reported that *H. verticillata* removed copper after 30th days of experiment which were (9.86, 14.66, 19.24, 28.29, 15.2) ppm for initial concentration of (10, 15, 20, 15, 20) ppm respectively, which took the longest time may be due to the differences in the experimental conditions. [30] studied chromium, lead and zinc uptake in *H. verticillata* and observed that this macrophyte uptake the metals on a dose response basis. They also showed that *H. verticillata* decreased chromium, lead and zinc concentrations by 72-80, 68-86 and 60-80% after 4 weeks, respectively. But the plants die at higher concentrations (> 20 mg kg-1) and longer period of exposure. [31] study the effect of heavy metals on *Salvinia natans* after 48 hours of exposure, where the plant possesses capacity to accumulate Cr, Fe, Cu, and Cd which was (8.72, 9.72, 7.26, 6.48) mgg^{-1} dry wt. respectively of 35 ppm initial concentration. They suggest that *Salvinia* possesses efficient photosynthetic machinery to with stand heavy metal stress.

Conclusions

According to the results of this experiment, the percentage of copper and chromium remaining in the water was gradually beginning to decline with the increase of contact time of the experiment. The removal of the chromium component ratios was higher than the percentages for the removal of copper. The results proved the efficiency of the plant in phytoremediation even in high concentrations of heavy metals, which shows the possibility of using *H. verticillata* in the biological treatment of contaminated water with copper and chromium ion, because of the abundance of plant in Iraqi water.

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