Potential role of *Ceratophyllum demersum* in bioaccumulation and tolerance of some heavy metals

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ARTICLE INFO

ABSTRACT

Contamination of aquatic ecosystems by heavy metals is considered one of the most major threats of the aquatic life. So, the present work aims to investigate the accumulation and tolerance of *Ceratophyllum demersum* plant to different concentrations of Pb^{+2}, Al^{+3} and Cd^{+2} during four treatment periods for 16 days. Results indicated that the highest tolerance percentage of *C. demersum* was (96.26 %) of Pb at 10 mg L^{-1} after 4 days; whereas the highest tolerance percentage values were (95.89 % and 80.84 %) at 3 mgL^{-1} of Al and at 0.1 mg L^{-1} of Cd after 8 days, respectively. *C. demersum* accumulated the highest amount of Pb (17029.19 μg g^{-1}) at 50 mg L^{-1} after 12 days, followed by Al amount which was (2893.04 μg g^{-1}) at 9 mg L^{-1} after 8 days and the highest accumulation value of Cd was (521.28 μg g^{-1}) at 0.5 mg L^{-1} after 4 days. Metal accumulation in *C. demersum* showed at a descending order as follow; Pb >Al >Cd and it was significantly increased with increasing the concentration and treatment period. In addition, results showed that the maximum BCF value of Cd in *C. demersum* at low concentration was (1295.37) after 12 days of the treatment. Therefore, *C. demersum* is considered a hyperaccumulator species for cadmium.

INTRODUCTION

Aquatic ecosystems act as the receptacle for various contaminants from natural and anthropogenic sources such as effluents from mines, smelters, industries, excessive use of fertilizers and pesticides, agricultural runoff and partially from aerial deposition (Adriano, 2001). Heavy metals are a major of water pollutants concern because of their persistent and tendency to be concentrated in aquatic organisms (Chang *et al*., 2009; Yadav *et al*., 2009). The most commonly heavy metals found in wastewater arsenic, cadmium, chromium, copper, lead, nickel and zinc, all of which cause risks for human health and the environment (Järup, 2003; Kastratović *et al*., 2016).

According to the Environmental Protection Agency (EPA), lead is considered a carcinogen. Lead has major effects on different parts of the body (Monisha *et al*., 2014). Aluminium showed adverse effects on the nervous system and resulted in loss of memory, problems with balance and loss of coordination (Krewski *et al.*, 2007). Cadmium and its compounds are classified as Group 1 carcinogens for humans by the International Agency for Research on Cancer (Henson and Chedrese, 2004).

The uses of conventional technologies, such as ion exchange, chemical precipitation, reverse osmosis and evaporative recovery for this purpose is often inefficient and /or very expensive (Kinare and Shingadia, 2014).

Recently, phytoremediation has appeared as an alternative process in the treatment of contaminated water, sediments and soils. This sustainable technique used plants to remove toxic or undesirable heavy metals from polluted mediums (Ravera, 2001; Susarla *et al.*, 2002; Rai, 2009; Singh *et al.*, 2012). Aquatic macrophytes are unchangeable biological filters and they carry out purification of the water bodies by accumulating dissolved metals and toxins in their tissue; also relatively inexpensive and eco-friendliness (Ravera, 2001; Prasad *et al.*, 2010; Singh *et al.*, 2012).

Selection of plant species for metal removal is based on the plants which should have high tolerance to the contaminant and high capacity accumulation rate for the element and capability to accumulate several metals (Rajkumar *et al.*, 2009). *Ceratophyllum demersum* is a submerged aquatic macrophyte belonging to Ceratophyllaceae family (Arber, 2010). It has been used to remove heavy metals and can be biofilter for heavy metals (Mishra *et al.*, 2006; Mishra *et al.*, 2008; Fawzy *et al.*, 2012; Matache *et al.*, 2013). *C. demersum* grows fast in shallow, muddy, quiescent water bodies at low light intensities. Moreover, it has unique features, which make it ideal for laboratory toxicity bioassays (Aravind and Prasad, 2005).

The present investigation aims to determine the efficiency of *C. demersum* to accumulate and tolerate toxic concentrations of lead, aluminium and cadmium through their impact on growth and chlorophyll content. Moreover, the tolerance index and bioconcentration factor of this plant were also determined to assess its phytoremediation property.

MATERIALS AND METHODS

Plant collection and cultivation:

Ceratophyllum demersum was collected from the River Nile around Gizert El Warrak, Giza, Egypt. In the laboratory, *C. demersum* was washed gently in distilled water for several times and grew in 30 L plastic containers filled with half-strength Hoagland's nutrient solution which pH = 7.0 and at temperature (29°C $\pm$ 2) about four weeks and replaced after each 3 days (Marin and Oron, 2007).

Experimental Setup:

Preliminary tests showed that this plant can tolerate 50 mg L^{-1} of Pb but is completely destroyed by the same concentration of Al and Cd. Hence the concentrations of Al and Cd in this study were lower than that of Pb.

Plant treatments divided into four categories as follow:

First category: plant exposed to (10, 30 and 50 mg L^{-1}) of Pb (No$_3$)$_2$.

Second category: plant exposed to (3, 6 and 9 mg L^{-1}) of Al (No$_3$)$_3$.9H$_2$O.

Third category: plant exposed to (0.1, 0.3 and 0.5 mg L^{-1}) of Cd (NO$_3$)$_2$.4H$_2$O.

Fourth category: The control (plant without metal).

For each concentration, heavy metal salts were dissolved in 1000 ml of distilled water in glass jars, each test were performed triplicates; five grams fresh weight of plants was added and treatments were exposed to 13 hr of daylight for four periods 4, 8, 12 and 16 days at a temperature 35°C $\pm$ 2 under laboratory conditions. Every day during all experiments the evaporated volume was replaced with distilled water when it was necessary. After each period, plant samples were washed three times with distilled water then, taken for determination of fresh weight and chlorophyll content. Plant samples were dried to a constant weight in an oven at 70

ºC for 24 hr. in order to measure dry biomass and heavy metals accumulation. The fresh weight and dry biomass were expressed as g m^{-2} (Chorom *et al*., 2012).

Estimation of chlorophyll content:

After 16 days of treatment, one gram fresh weight of *C. demersum* was homogenized with 10 ml of 95 % ethanol in a porcelain mortar and centrifuged at 3000 rpm for 10 minutes for estimation the total chlorophyll content (Ritchie, 2008). Absorbance of the supernatant extracts of each treatment was measured spectrophotometrically using spectronic 601(Milton Roy, USA) (Shimadzu UV/VIS 1240, Kyoto, Japan) at 664 nm and 647 nm according to a formula of Jeffrey and Humphrey (1975).

Determination of tolerance index:

The tolerance of the plant to different concentrations of heavy metals was determined by Wilkins (1978).

$$\text{Tolerance index} = \frac{\text{Dry biomass of treated plants}}{\text{Dry biomass of control plants}} \times 100$$

Heavy metal analysis:

The concentration of heavy metals (Pb^{+2}, Al^{+3} and Cd^{+2}) in the plant tissue was determined for each interval of the treatment periods by digesting 1 gram of plant dry powder with 10 ml of concentrated nitric acid and followed by perchloric acid, then the digest were made up to 50 ml with distilled water (Jones, 1984). Metal concentration of samples was determined using Inductively Coupled Plasma Spectrometry ICP (Matache *et al.*, 2013; Patricia *et al*., 2006).

Estimation of bioconcentration factor:

The bioconcentration factor (BCF) was calculated by dividing the metal concentration in plant tissue (μg g^{-1}) by the initial concentration (mg L^{-1}) of the metals in the nutrient solution (Zayed *et al*., 1998).

Statistical analysis:

Total chlorophyll, fresh weight and dry biomass results for control and treatments, in addition to the metal accumulation results for each treatment period and each concentration were statistically analyzed by one-way ANOVA at *P<0.05* using Tukey test.

RESULTS

Lead, aluminium and cadmium heavy metals are well known for their toxic effects on plant growth, but toxicity levels are variable. Generally, *C. demersum* growth density was inversely proportional to Pb^{+2}, Al^{+3} and Cd^{+2} concentrations and treatment periods, as shown in Fig. (1). Fresh weight and dry biomass of *C. demersum* for all treatments were lower than that of the control, with significant difference at (*P<0.05*) during all the treatment periods. Results showed that maximum growth density of *C. demersum* was observed at 10 mg L^{-1} of Pb (790.23 g m^{-2} Fresh weight and 55.32 g m^{-2} dry biomass) after 4 days, however, this increase was insignificant at (*P>0.05*) with the control group. Followed by a gradual significant decrease in growth density was recorded after 8, 12 and 16 days of the treatment.

It was cleared that the maximum growth density of *C. demersum* was observed at 3 mg L^{-1} of Al (950.27 g m^{-2} Fresh weight and 61.90 g m^{-2} dry biomass) after 8 days; whereas inhibitory effects were most pronounced in plant growth after 12 and

16 days of the treatment at the same concentration. There were significant decreases of fresh weight and dry biomass when the treatment periods and metal concentrations increased compared to control. The same pattern was observed at 0.1 mg L^{-1} of Cd, where the growth density of *C. demersum* (801.08 g m^{-2} Fresh weight and 56.08 g m^{-2} dry biomass) was higher after 8 days than that of 12 and 16 days. Comparatively, the toxicity effect of Al and Cd on *C. demersum* growth density was more pronounced than that of Pb after 4 days of the treatment (Fig. 1).
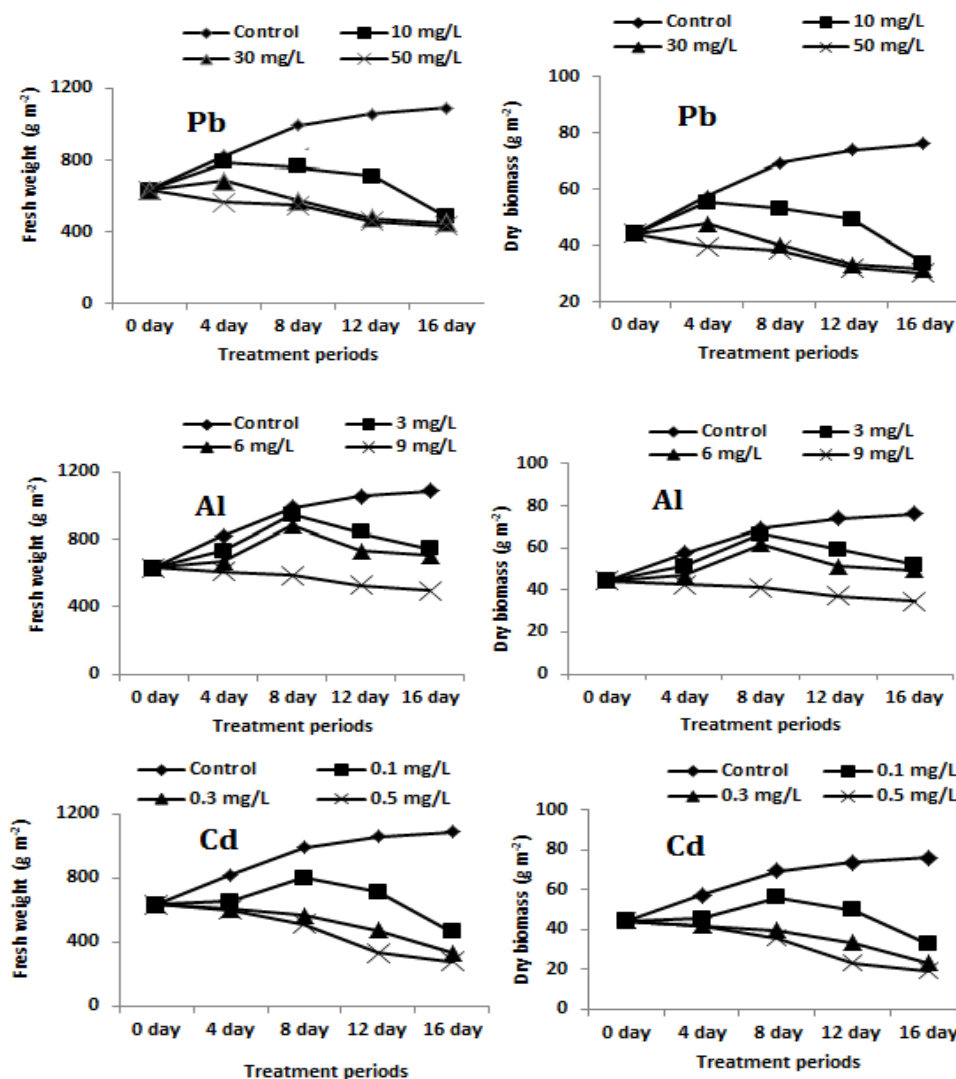

Fig. 1: Effect of Pb^{+2}, Al^{+3} and Cd^{+2} concentrations on growth density of C. demersum at different treatment periods.

Data in Table (1) shows a significant reduction at ($P<0.05$) in the total chlorophyll content of *C. demersum* after 16 days of treatment with Pb^{+2}, Al^{+3} and Cd^{+2} concentrations comparing to the control group. The lowest total chlorophyll content was 280.65 mg g^{-1} at 50 mg L^{-1} of Pb, 163.49 mg g^{-1} at 9 mg L^{-1} of Al and 154.44 mg g^{-1} at 0.5 mg L^{-1} of Cd compared to the control (694.38 mg g^{-1}). It is important to note that plant showed reduced fresh weight, dry biomass and chlorophyll content when grown in contaminated nutrient solution with high concentrations of Pb, moderate concentrations of Al and low concentrations of Cd compared with control, but did not die from phytotoxicity during all the periods of treatment.

Table 1: Effect of Pb^{+2}, Al^{+3} and Cd^{+2} concentrations on the total cholorophyll content of *C. demersum* after 16 days of treatment.

Metal	Metal concentration in solution (mg L^{-1})	Total chlorophyll content (mg g^{-1} w.wt)
	Control	694.38±16.80
Pb	10	335.34* ±13.30
	30	287.11* ±10.64
	50	280.65* ±11.76
Al	3	242.70* ±11.53
	6	213.93* ±9.28
	9	163.49* ±9.04
Cd	0.1	594.02* ±21.17
	0.3	473.42* ±15.31
	0.5	154.44* ±6.77
F- value		5.84.41
P-value		0.000

Data are presented as mean of three samples ± S.D, *F-value*: the difference among groups (one-way ANOVA). * Refer to significant results between each treatment and control group at *P<0.05*.

Regarding the tolerance ability of *C. demersum* that exposed to different concentrations of Pb^{+2}, Al^{+3} and Cd^{+2} during various treatment periods was shown in Fig. (2). Indices of metal tolerance based on dry biomass of treated plants relative to the control. The highest tolerance percentage of *C. demersum* (96.26 %) was observed at low concentration of Pb after 4 days, whereas the highest tolerance (95.89 % and 80.84 %) were recorded at low concentration of Al and Cd after 8 days, respectively.
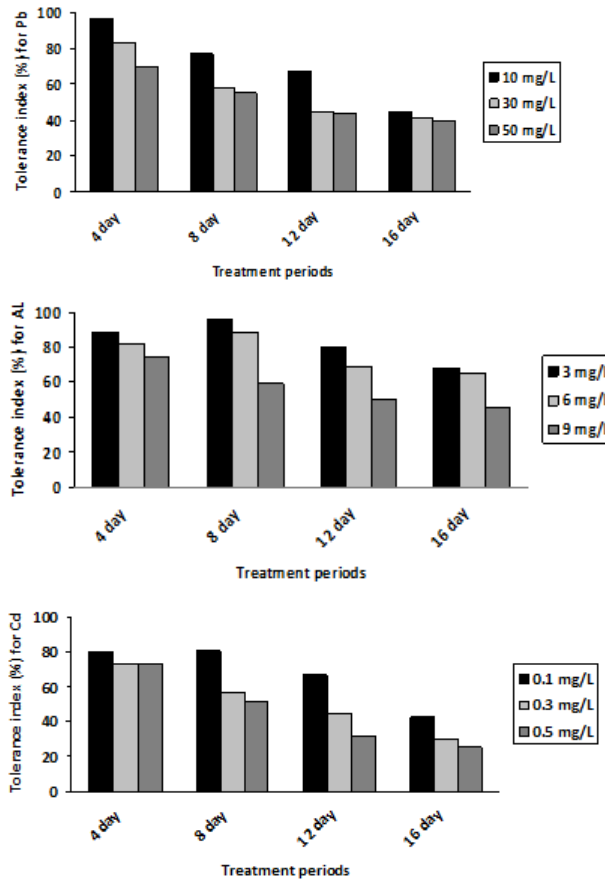

Fig. 2: Tolerance index of *C. demersum* exposed to different concentrations of Pb^{+2}, Al^{+3} and Cd^{+2} at various treatment periods.

Data in Table (2) summarized the accumulation of Pb, Al and Cd in the whole parts of *C. demersum* plant during different treatment periods. However, the accumulation of Pb^{+2}, Al^{+3} and Cd^{+2} in *C. demersum* increased with increasing initial concentration in the contaminated Hoagland's nutrient solution during all the treatment periods. The highest amount of Pb (17029.19 µg g^{-1}) was accumulated in *C. demersum* when the medium was supplied with 50 mg L^{-1} after 12 days, followed by Al amount was (2893.04 µg g^{-1}) at 9 mg L^{-1} after 8 days and the highest accumulation value of Cd was (521.28 µg g^{-1}) at 0.5 mg L^{-1} after 4 days. In this manner, results showed that metal accumulation in a descending order as follow Pb> Al > Cd.

Table 2: The accumulation of Pb^{+2}, Al^{+3} and Cd^{+2} in *C. demersum* exposed to different concentrations and treatment periods.

Metal	Metal concentration in solution (mg L^{-1})	Metal accumulation (µg g^{-1} dry wt) after different treatment periods				*F-value*	*P-value*
		4 days	**8 days**	**12 days**	**16 days**		
Pb	Control	0	0	0	0	-	-
	10 mg/l	1813.30[a]± 24.36	4360.60[b]± 94.80	5145.48[c]± 112.20	2703.16[d]± 79.06	975.14	0.000
	30 mg/l	4874.52[a]± 97.68	8660.61[b]± 129.34	10659.83[c]± 132.35	8381.65[bd]± 125.55	1164.94	0.000
	50 mg/l	12687.88[a]± 206.86	13122.83[a]± 243.60	17029.19[b]± 291.12	10716.80[c]± 137.07	407.52	0.000
Al	3 mg/l	301.29[a]± 10.85	414.14[b]± 12.46	725.51[c]± 14.08	190.17[d]± 5.16	1283.66	0.000
	6 mg/l	359.14[a]± 12.15	1652.88[b]± 31.62	882.02[c]± 18.46	604.65[d]± 16.15	2160.36	0.000
	9 mg/l	949.97[a]± 19.33	2893.04[b]± 82.02	2359.85[c]± 63.32	2179.73[d]± 84.68	443.19	0.000
Cd	0.1 mg/l	29.48[a]± 1.49	75.99[b]± 3.29	129.54[c]± 5.41	111.54[d]± 4.62	367.14	0.000
	0.3 mg/l	281.51[a]± 9.41	209.33[b]± 8.33	198.92[bc]± 9.71	178.33[dc]± 8.13	75.93	0.000
	0.5 mg/l	521.28[a]± 15.65	413.66[b]± 10.97	343.64[c]± 12.16	266.33[d]± 10.50	225.73	0.000

Data are presented as mean of three samples ± S.D, *F-value*: the difference among treatment periods at each concentration (one-way ANOVA). The same letters refer to insignificant results and the different letters refer to significant results at *P < 0.05*.

The bioconcentration factor (BCF) values of Pb^{+2}, Al^{+3} and Cd^{+2} in *C. demersum* at different concentrations and treatment periods are shown in Fig. (3). The BCFs of Pb increased when the treatment periods were increased from 4 to 12 days at all concentrations. The maximum BCF values of Pb and Cd in *C. demersum* were (514.55 and 1295.37) respectively at low concentration after 12 days of treatment. Meanwhile, the maximum BCF value of Al in *C. demersum* was (321.45) at 9 mg L^{-1} after 8 days. Furthermore the higher value of BCF indicates the ability of plant to concentrate metals in their tissues.
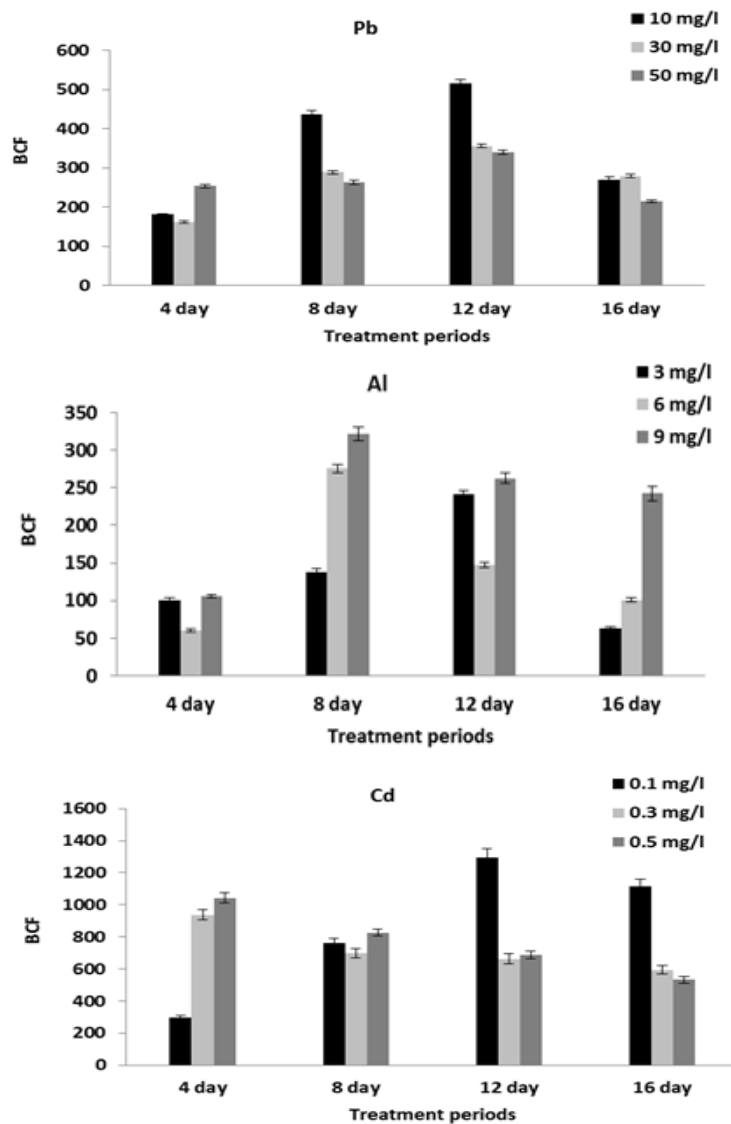
Fig. 3: Bioconcentration factor (BCF) of Pb^{+2}, Al^{+3} and Cd^{+2} in *C. demersum* at different concentrations and treatment periods.

DISCUSSION

Environmental exposure to toxic heavy metals is one of the main critical issues on environmental and public health. Heavy metals are common pollutants in aquatic ecosystems. These ecosystems are particularly susceptible and often final receptor of heavy metals (Kanoun *et al*., 2009). The metals are deposited in the bottom sediments and from there may be transferred to plants and the aquatic food chain (Rai, 2009; Mazej *et al*., 2010). Their common property is that even at relatively low concentrations their effects are toxic and therefore they fall under the category of very dangerous environmental pollutants (Kastratović *et al*., 2016).

Phytotechnology is an emerging technology that has a potential to treat a wide range of contaminants for a lower cost than traditional technologies (Chorom *et al*., 2012). Many macrophytes found to be the potential scavengers of heavy metals from aquatic environment and are being used in wastewater renovation systems (Nirmal *et*

al., 2006; Abida, 2009). *Ceratophyllum demersum* L. is a free floating perennial macrophyte, growing in slow flow and nutrients-rich water bodies. It represents an important food source for invertebrates, fish and herbivorous aquatic birds (Ostroumov and Shestakova, 2009).

On the other hand, metal toxicity produces adverse biological effects on an organism's survival, activity, growth, metabolism, or reproduction. Metals can be lethal or harm the organism without killing it directly (Wright and Welbourn, 2002) this is confirmed in the present study. It is noticed that *C. demersum* plant showed reduction in growth and chlorophyll content when grown in contaminated nutrient solution with high concentrations of Pb, moderate concentrations of Al and low concentrations of Cd but did not die from phytotoxicity during all the treatment periods.

The present work has cleared that maximum growth was observed in *C. demersum* exposed to 3 mg L^{-1} of Al, followed by plant treated with 0.1 mg L^{-1} of Cd after 8 days and the elevation in growth was mentioned with 10 mg L^{-1} of Pb after 4 days, whereas the inhibitory effects were most pronounced in plant growth after 12 and 16 days of treatment. This confirmed by Umebese and Motajo (2008) who stated that zinc, copper and aluminium are well known for their toxic effects on plant growth and metabolism but toxicity thresholds are highly variable. *C. demersum* is fairly sensitive to Zn (3 and 9 mg L^{-1}) and Al (3 and 9 mg L^{-1}) but highly sensitive to Cu (2.5 and 7 mg L^{-1}). All concentrations of Cu, Zn and Al caused significant reductions in growth rate of this plant.

The effects of Pb^{+2}, Al^{+3} and Cd^{+2} on the total cholorophyll of *C. demersum* are exhibited in the results revealed that a significant reduction in total chlorophyll content when *C. demersum* grown in high concentrations of Pb, Al and Cd comparing to the control. The total chlorophyll content in the control was three or five folds than plant treated with 50 mg L^{-1} of Pb (280.65 mg ± 11.76); 9 mg L^{-1} of Al (163.49 mg ± 9.04) and 0.5 mg L^{-1} of Cd (154.44 mg ± 6.77) after 16 days of treatment. These results are in agree with Umebese and Motajo (2008) who explained that, excess of Cu, Zn and Al cause numerous toxic effects in plants. All three metals caused reduction in chlorophyll content and the rate of photosynthesis; while Al and Cu inhibit respiration in some plants. In addition, Doncheva *et al*. (2001) declared that heavy metals accumulation in the tissue of different plants resulted in a decrease of biomass and chlorophyll content in the leaves/stems.

As presented in this study, the tolerance indices of *C. demersum* exposed to different concentrations of Pb^{+2}, Al^{+3} and Cd^{+2} during four periods are explored that high tolerance of *C. demersum* with low concentration of Pb, Al and Cd recorded (96.26%) with Pb and (95.89 % and 80.84%) with Al and Cd recpectively. These results are parallel with those of Umebese and Motajo (2008), they found that tolerance index of *C. demersum* plant was low for Cu (21.62 and 13.43% at low and high doses, respectively) and moderate for Zn (63.74 and 54.85%) and Al (72.83 and 68.79%). According to Sabreen and Sugiyama (2008), tolerant populations can be characterized by a lower metal accumulation than the sensitive one.

C. demersum has the capacity to remove lead from aqueous solutions where the highest amount of Pb accumulated was (17029.19 µg g^{-1}) at 50 mg L^{-1} after 12 days, followed by Al amount was (2893.04 µg g^{-1}) at 9 mg L^{-1} after 8 days and the highest accumulation value of Cd was (521.28 µg g^{-1}) at 0.5 mg L^{-1} after 4 days of treatment. Results showed that accumulation of metals in *C. demersum* was at a descending order as follow Pb> Al > Cd. This result was in agree with Chen *et al.* (2015) who reported that *C. demersum* appears to possess a high potential for lead

bioaccumulation, where the maximum amount of lead accumulated in the plants reached 4016.4 mg kg^{-1}. Moreover, *C. demersum* has been reported to exhibit strong tolerance to lead (El-Khatib *et al.,* 2014; Mishra *et al.,* 2006). In addition, some studies improved that *C. demersum* L. have the ability to remove the bullets Lead, Nickel and Cadmium more than the rest of Iron, Manganese and Zinc (Zimmels *et al.,* 2009).

Dogan *et al.* (2015) observed that the reduction rates at 0.1, 1 and 10 mg/l of Cd concentrations were estimated to be 15.8%, 12.7% and 34.9% for *C. Demersum*. Moreover, Gafta and Mountford (2008) decleared that *C. demersum* has the ability to cumulative Cadmium in contaminated water up to 1000 ppm.

According to Umebese and Motajo (2008), results found that accumulation of Al, Zn and Cu by *C. demersum* was three fold at higher doses compared with the lower doses. In this respect, Keskinkan *et al*. (2004) showed that *C. demersum* plant can be successfully used for heavy metals (Pb, Zn and Cu) removal under dilute metal concentration.

The uptake rate of metals by plants from their surrounding environment is better described by the bioconcentration factor for the floating species *C. demersum* L. (Thiebaut *et al*., 2010). BCF measures the ability of a plant to bioconcentrate an element in its tissue taking into account the concentration of that element in the substrate. In the present investigation, the maximum BCF values of Cd and Pb in *C. demersum* were (1295.37 and 514.55) respectively, at low concentration after 12 days of treatment. The BCF values over 1000 are considered as evidence of a useful plant for phytoremediation. These results were harmony with Matache *et al*. (2013) who indicated that, *C. demersum* was identified as a hyperaccumulator species for cadmium. But low BCF values with different concentrations of Al confirmed that *C. demersum* was not a hyperaccumulator of this metal. The recorded results are in the same line with Umebese and Motajo (2008) who reported that bioconcentration factor (BCF) was very low indicating that this plant is not a hyper- accumulator of Al.

CONCLUSION

C. demersum could tolerate and accumulate different concentrations of Pb^{+2}, Al^{+3} and Cd^{+2} during different treatment periods. The present results indicated that *C. demersum* is fairly sensitive to Pb but highly sensitive to Al and Cd. Moreover, the capacity of *C.demersum* to hyperaccumulate cadmium makes it as an excellent choice for removal of this metal. *C. demersum* is a promising plant for heavy metals accumulation and could be used to clean aquatic bodies. Furthermore, it has proven to be an ideal for laboratory toxicity bioassays, which makes it a suitable for phytoremediation process.

Conflict of interest: The authors declare that they have no conflict of interest.

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ARABIC SUMMARY

الدور المحتمل لنبات نخشوش الحوت فى التراكم الحيوى و تحمل بعض العناصر الثقيلة

قدرية محمود على محمود ، حسناء أحمد محمود ، ساره سيد محمود سيد
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تلوث البيئة المائية بالعناصر الثقيلة يعتبر من أهم التهديدات للحياة المائية. لأجل هذا استهدف العمل الحالى دراسة قدرة تحمل وتراكم نبات نخشوش الحوت للتركيزات المختلفة لكلا من عنصر الرصاص و الالومنيوم والكادميوم خلال اربع فترات من المعالجة البيولوجية لمدة 16 يومًا. وأوضحت النتائج أن أعلى نسبة تحمل للنبات كانت (96.26٪) عند 10 ملليجرام / لتر من الرصاص بعد 4 أيام ، بينما كانت أعلى نسبة تحمل (95.89٪ و 80.84٪) عند 3 ملليجرام / لتر من الالومنيوم و 0.1 ملليجرام / لتر من الكادميوم بعد 8 أيام ، على التوالي. وأشارت النتائج الحالية الى ان أعلى تراكم لعنصر الرصاص فى نبات نخشوش الحوت كان 17029.19 ميكروجرام عند تركيز 50 ملليجرام / لتر بعد 12 يومًا ، يليه عنصر الالومنيوم 2893.04 ميكروجرام عند تركيز 9 ملليجرام / لتر بعد 8 أيام. بينما كان أعلى تراكم لعنصر الكادميوم فى نبات نخشوش هو 521.28 ميكروجرام عند 0.5 ملليجرام / لتر بعد 4 أيام. وأظهرت نتائج تراكم العناصر الثقيلة فى نبات نخشوش الحوت ترتيبا تنازليا على النحو التالي: الرصاص > الالومنيوم > الكادميوم ، حيث تناسبت نسبة التراكم طرديا مع زيادة التركيز وفترة المعالجة. بينما سُجلت أعلى قيمة لمعامل التركيز الحيوى (BCF) (1295.37) عند تركيز منخفض من عنصر الكادميوم بعد 12 يومًا من المعالجة ، مما يدل على قدرة نبات نخشوش الحوت للتراكم الحيوي لعنصر الكادميوم عند التركيزات المنخفضة.