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Accumulation of chromium in *Lemna minor* under the effect of pH and EDTA variation and assessment of the treatment impact on the Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

Water pollution with chromium (Cr) is a major threat to the environment and human health. Therefore this study was conducted to evaluate the efficiency of aquatic plant *Lemna minor* as a natural biological tool in the accumulation of Cr from polluted water under the effect of pH and Ethylene Diamine Tetra Acetic Acid (EDTA) and assess the impact of this treatment on the growth of Nile tilapia. *L. minor* fronds were exposed to different concentrations of Cr (0, 2, 4, 6, 8 and 10 mg L^{-1}) with pH (5, 6, 7, 8 and 9) for periods of 5, 10, 15 and 20 days. The highest accumulation of Cr was (9563.81±222.47 µg g^{-1} dry wt) at 10 mg L^{-1} with pH 5 after 10 days of treatment. While in the groups that enriched with EDTA (25, 50, 75 and 100 µM) for periods of 5, 10, 15 and 20 days, maximum accumulation of Cr was (9926±235.84 µg g^{-1} dry wt) at 10 mg L^{-1} with pH 5 and 25 µM of EDTA after 10 days of treatment. The highest removal efficiency of Cr by *L. minor* was 99.26 % at 10 mg L^{-1} with pH 5 and 25 µM of EDTA after 10 days of treatment. This study indicated that pH and EDTA influenced chromium accumulation in *L. minor*. The impact of the treated water by *L. minor* on the growth of Nile tilapia indicated that fish muscles had no chromium accumulation and the treated water became acceptable and safe. Meanwhile; a specific growth rate (SGR) was (1.2±0.01 %/day) at the end of exposure period which lasted 30 days the same as control group. So, we can conclude that Nile tilapia played an important role in monitoring chromium in the treated water by *L. minor* under the effect of pH and EDTA.

INTRODUCTION

In contaminated aquatic environments several toxic metals occur, often creating undesirable living conditions for many plants and animals (Divya *et al*., 2012). Among animal species, fishes are the inhabitants that cannot escape from the detrimental effects of heavy metals (Olaifa *et al.*, 2004). One category of toxic contaminations accumulated by fishes is heavy metals such as lead (Pb), mercury (Hg), cadmium (Cd), chromium (Cr) and arsenic (As). Any of these heavy metals can destroy life when they concentrate in the

body above acceptable levels (Nwabunike, 2016). Chromium contamination in water is a major concern, as various anthropogenic activities. Chromium toxicity in plants depends on its valence state (Oliveira, 2012). Chromium (Cr) has two stable forms Cr (VI) and Cr (III) amongst which Cr (VI) is more mobile and toxic than Cr (III) (Shanker *et al*., 2005). Chromium enter the aquatic ecosystem through effluents discharged from leather tanneries, textiles, electroplating, metal finishing, mining, dyeing and printing industries, ceramic, photographic and pharmaceutical industries etc. (Abbas and Ali, 2007). Fish assimilate Cr by ingestion or by the gill uptake tract and accumulation in fish tissues, mainly liver, occurs at higher concentrations than those found in the environment (Ahmed *et al*., 2013). Contaminated fish enter the human body through consumption and it causes health hazards (Nwabunike, 2016). Chronic chromium toxicity causes cancer in the respiratory tract and lungs (Thayaparan *et al*., 2015).

There are several methods for removing heavy metals from bodies of water. They can be ineffective and expensive. Therefore, there is an urgent need to adopt technology with optimum efficacy and low capital investment and can be acceptable for wide range of metal contamination (Kurniawan *et al*., 2006). Aquatic plants are considered to be simple, ecofriendly technology applicable for the removal of heavy metals from the aquatic medium (Perumal *et al*., 2010). Some aquatic plants have been investigated for their potential to improve wastewater quality because of their ability to grow in water polluted by heavy metals (Jafari and Akhavan, 2011). Aquatic plants play an important role in maintaining the purification capability of water and the entire aquatic ecosystem (Wang *et al*., 2008). Due to the high growth rate and large uptake metal potential, members of genus *Lemna* have been appeared as potential candidates for designing a duckweed-based heavy metal phytoremediation set-up (Rashmi and Surindra, 2015). In the field of ecotoxicology, *Lemna* spp. has been used for the removal of heavy metals from wastewater and constructed wetlands (Uysal and Taner, 2009). Duckweeds are able to remove and accumulate large amounts of heavy metals, principally through the fronds (Chaudhary and Sharma, 2014).

Metal bioaccumulation depends upon plant species, its organ and numerous abiotic factors like temperature, pH, transportation of metal contaminated particles and dissolved ions in water (Divya *et al*., 2012). Metal toxicity is often dependent on pH in freshwater and soil. In metal uptake and chemical kinetic process, the role of initial metal load and pH of medium are very critical factors. Meanwhile, pH is deemed to offer a very decisive role in bioremediation process (Rashmi and Surindra, 2015). The chelator ethylene diamine tetra acetic acid (EDTA) is often included in nutrient media that are used to grow aquatic macrophytes. EDTA was often found to be the most effective (Grčman *et al*., 2001). When culturing duckweeds, the presence of a chelator is necessary for optimal growth (Landolt and Kandeler, 1987) and consistency between replicates (Hughes, 1991). EDTA may mobilize metal ions from river sediments and the resulting complexes may increase the uptake of metals such as cadmium, mercury, copper and chromium (Venier *et al*., 1987). Fish are widely used to evaluate the health of aquatic ecosystems because pollutants build up in the food chain and are responsible for adverse effects and death in the aquatic systems (Vinodhini and Narayanan, 2008). Various fish species are widely used as bioindicators of metal contamination (Svobodova *et al*., 2004).

The objective of this study was to evaluate the efficiency of the aquatic macrophyte *L. minor* in accumulation of different concentrations of chromium from polluted water

under greenhouse conditions. So, we conducted a study with *Lemna*, 1) to determine the growth responses and accumulation of Cr in *L. minor* under effect of various levels of pH and different treatment periods; 2) to determine the growth responses and accumulation of Cr in *L. minor* under effect of various concentrations of EDTA and different treatment periods and 3) to assess the treatment impact of polluted water with chromium by *L. minor* under the effect of pH and EDTA on the growth of economically important organism (Nile tilapia).

MATERIALS AND METHODS

2.1. Plant material and growth conditions

The freshwater *L. minor* was collected from Soils & Water and Environment Research Institute, Agricultural Research Center. The plant material was washed carefully to remove dirt, sludge and other adhesive debris from it. To avoid any contamination, the second generation of *L. minor* was obtained by culturing original individual in 1/10 diluted Hoagland's nutrient solution for 10 days as per standard methodology described by Eliasson (1978). Hoagland's nutrient solution was prepared according to Hoagland and Arnon (1950).

2.2. Effect of pH on chromium accumulation in *L. minor* (*Exp. 1*)

In this study, analytical grade of potassium dichromate salt ($K_2Cr_2O_7$) was used for the accumulation studies of Cr in *L. minor* fronds. The stock solution was prepared by dissolving 2.829 g of $K_2Cr_20_7$ in 1000 ml of distilled water. Before the start of the experiment, the prominent and healthy plants were collected and rinsed with distilled water. One gram of plant material was placed in plastic pots contained 1000 ml of 1/10 Hoagland's nutrient solution for each treatment. Chromium concentrations were tested at (0, 2, 4, 6, 8 and 10 mg L^{-1}). To investigate the effect of pH on Cr accumulation in *L. minor*, test solutions were adjusted with pH (5, 6, 7, 8 and 9) either with 1 N NaOH or with 1 N HCl at the corresponding Cr concentrations. The initial pH of tested solutions was 5.8. Pots without Cr grown alongside the experimental groups served as control which were necessary to compare the results. All treatments were carried out in triplicate and exposed to natural sunlight in a greenhouse at a temperature of 37°C ± 2 for four different periods (5, 10, 15 and 20 days). The evaporation loss was compensated weekly.

2.3. Effect of EDTA on chromium accumulation in *L. minor* at pH 5 (*Exp. 2*)

Different concentrations of Cr were prepared at (0, 2, 4, 6, 8 and 10 mg L^{-1}) and different concentrations of Na_2- EDTA were separately added at (25, 50, 75 and 100 μM). All solutions were adjusted with pH 5. One gram of plant material was placed in plastic pots contained 1000 ml of 1/10 Hoagland's nutrient solution for each treatment. Experimental set-ups with zero Cr concentration served as control. All treatments were carried out in triplicate at a temperature of 35°C±2 under greenhouse conditions for four different periods (5, 10, 15 and 20 days). The evaporation loss was compensated weekly.

2.4. Plant growth parameters

After completion of each treatment period, the plant samples were harvested, washed thoroughly three times with distilled water to remove any chromium on the plant surface and then kept on filter paper for few seconds to remove excess liquid. Fresh weight (f.wt) was recorded immediately. The plant samples were dried at 100°C for 10 min, then at 70°C for 24 hr until completely dry and the dry weight (d.wt) was recorded. Fresh and dry weights of *L. minor* were given as g m^{-2} (El- Berashi, 2008).

2.5. Analysis of chromium accumulation in *L. minor* tissues

Two hundred milligrams of the plant samples were first digested with HNO_3 followed by $HClO_4$ (3:1) on hot plate and during that temperature was raised to about 95°C. The temperature was maintained until nitrous gas evolution stopped and the digest was clear (Kara, 2004). The digest was then made up to a final volume of 10 ml in polythene tubes with dilution. Determination of Cr concentrations in plant tissue was carried out by inductively coupled plasma optical emission spectroscopy (ICP-OES) according to Duman *et al*. (2009). Chromium accumulation in *L. minor* tissues was calculated on dry weights basis and expressed as μg g^{-1} dry wt.

2.6. Estimation of bioconcentration factor

Bioconcentration factor (BCF) which is a useful parameter (Lu *et al*., 2004) to evaluate the potential of plants for accumulating metals. BCF for Cr was calculated by dividing metal concentration in plant's tissue (μg g^{-1} dry wt) with the initial concentration of the metal in nutrient solution.

2.7. Removal efficiency of chromium by *L. minor*

In order to investigate the removal efficiency of Cr at 2, 4, 6, 8 and 10 mg L^{-1} by *L. minor*, the residual concentration of Cr was determined in nutrient solutions after 5, 10, 15 and 20 days with pH 5 and 25 μM of EDTA. Water samples were collected under plant mat in polyethylene bottles from each pot to measure Cr concentrations. The water samples were digested according to (Kara, 2004). The residual concentration of Cr in the solution was quantified using (ICP). The percentage metal efficiency was calculated according to Tanhan *et al*., (2007).

$$\% \text{ efficiency} = (C_0\text{-}C_1)/C_0 \times 100$$

C_0 and C_1 are initial and residual concentrations of metal in medium (mg L^{-1}).

2.8. Effect of the treated water on Nile tilapia growth (*Exp. 3*)

2.8.1. Fish collection and growth conditions

Fishes were purchased from a commercial fish farm. Fishes were immediately transferred to glass aquaria and randomly distributed at a stocking density of 10 fish per aquarium under laboratory conditions for two weeks. The experiment was carried out in experimental aquaria of the dimensions; 75 cm (length), 40 cm (width) and 50 cm (height) with a total volume of 40 L of dechlorinated tap water. Experimental aquaria were supplied with well-aerated in a recirculating system at a temperature of 27°C. Aquaria were continously cleaned and the water exchange including fish feces and remaining food. The fishes were fed with standard powdered feed twice daily and were starved for 24 hr prior to the experiment.

2.8.2. Experimental design

This experiment was conducted to assess the treatment impact by *L. minor* on Nile tilapia growth. The treatment was carried out in aquaria which described as previous, then filled with 40 L of 1/10 Hoagland's nutrient solution and one gram of plant material was exposed to 10 mg L^{-1} of Cr per litre with pH 5 and 25 μM of EDTA. Aquaria supplied with plant and without Cr served as control. The treatments were carried out in triplicate under greenhouse conditions at a temperature of 35°C ± 2 for 10 days.

2.8.3. Exposure of Nile tilapia to the treated water

The treated water was collected from each aquarium and transferred to other clean aquaria. Healthy fishes were collected and weighed for the next experiment. Average

initial weight and length of fish were (30.5±1.61 gm and 21.6±1.0 cm) respectively. Five individuals of fish were exposed to the treated water per each aquarium under laboratory conditions for 30 days with a continuous aeration at a temperature of 27°C. For comparison, similar set of aquaria was also kept wherein fish was grown in dechlorinated tap water served as control. The fishes were fed with powdered feed twice daily.

2.8.4. Estimation of growth parameters

At the end of exposure period which lasted 30 days, fishes were caught from each aquarium and then washed thoroughly with distilled water. The average final weight and length of exposed fish and control group were measured. Specific growth rate of fish was calculated according to Khattab (1996).

Specific growth rate (SGR, % /day) = (In W_t - In W_0)/ T X 100 (In = natural logarithm; W_0 = initial weight; W_t = final weight; and T = time in day).

2.8.5. Analysis of chromium accumulation in fish muscles

Fishes were dissected to extract a 10 gm sample of the fish muscles and were dried in oven at 100°C ±1 for 3 hr. Two grams of the dried samples were put in a 250 ml conical flask and then subjected to acid digestion with nitric acid and perchloric acid (4:1). The flasks were then cooled at room temperature and the residues were dissolved in 10 ml of dilute nitric acid and filtered. The filtrate was diluted to 50 ml with distilled water (Frank, 1984). Chromium analysis was estimated using ICP and the results were expressed as μg g^{-1} dry wt.

2.9. Statistical analysis

The mean and standard deviation (S.D) of three replicates for each treatment and control group were calculated. The results of the effect of pH and EDTA on fresh, dry weights of *L. minor* and accumulation of Cr by *L. minor* for each concentration at all treatment periods in all experimental set-ups were statistically analyzed by one-way ANOVA at $P<0.05$ using Tukey test.

RESULTS

3.1. Effect of different concentrations of Cr and pH on growth of *L. minor*

Results of fresh and dry weights of *L. minor* were taken after 5, 10, 15 and 20 days of treatment to assess the effect of different concentrations of Cr (0, 2, 4, 6, 8 and 10 mg L^{-1}) and pH (5, 6, 7, 8 and 9) (Fig. 1). Fresh and dry weights of *L. minor* gradually increased with increasing Cr concentrations as compared to control after 5 and 10 days of treatment. Also, fresh and dry weights were positively affected by pH 5> pH 6> pH 7> pH 8> pH 9 during all treatment periods. However, the highest fresh and dry weight of *L. minor* was (1053.29±25.21 g m^{-2} f.wt and 73.73±3.53 g m^{-2} d.wt) at 10 mg L^{-1} of Cr with pH 5 after 10 days of treatment. Fresh and dry weights were dependent on the concentrations of Cr and pH. Furthermore, the results of fresh and dry weights with pH 5 were significantly different compared to pH (6, 7, 8 and 9) at $P<0.05$.

3.2. Effect of pH on accumulation of Cr in *L. minor*

Similarly, chromium accumulation in *L. minor* was also gradually increased with increasing Cr concentrations with pH (5, 6, 7, 8 and 9) after 5 and 10 days of treatment (Table 1). All experimental set-ups with different pH range can be arranged as in terms of accumulation efficacy: pH 5> pH 6> pH 7> pH 8> pH 9 during all treatment periods. Overall, the highest accumulation of Cr was (9563.81±222.47 μg g^{-1} dry wt) at 10 mg L^{-1} with pH 5 after 10 days of treatment. The accumulation of Cr in *L. minor* with pH 5 was significantly different compared to pH (6, 7, 8 and 9) at $P<0.05$.

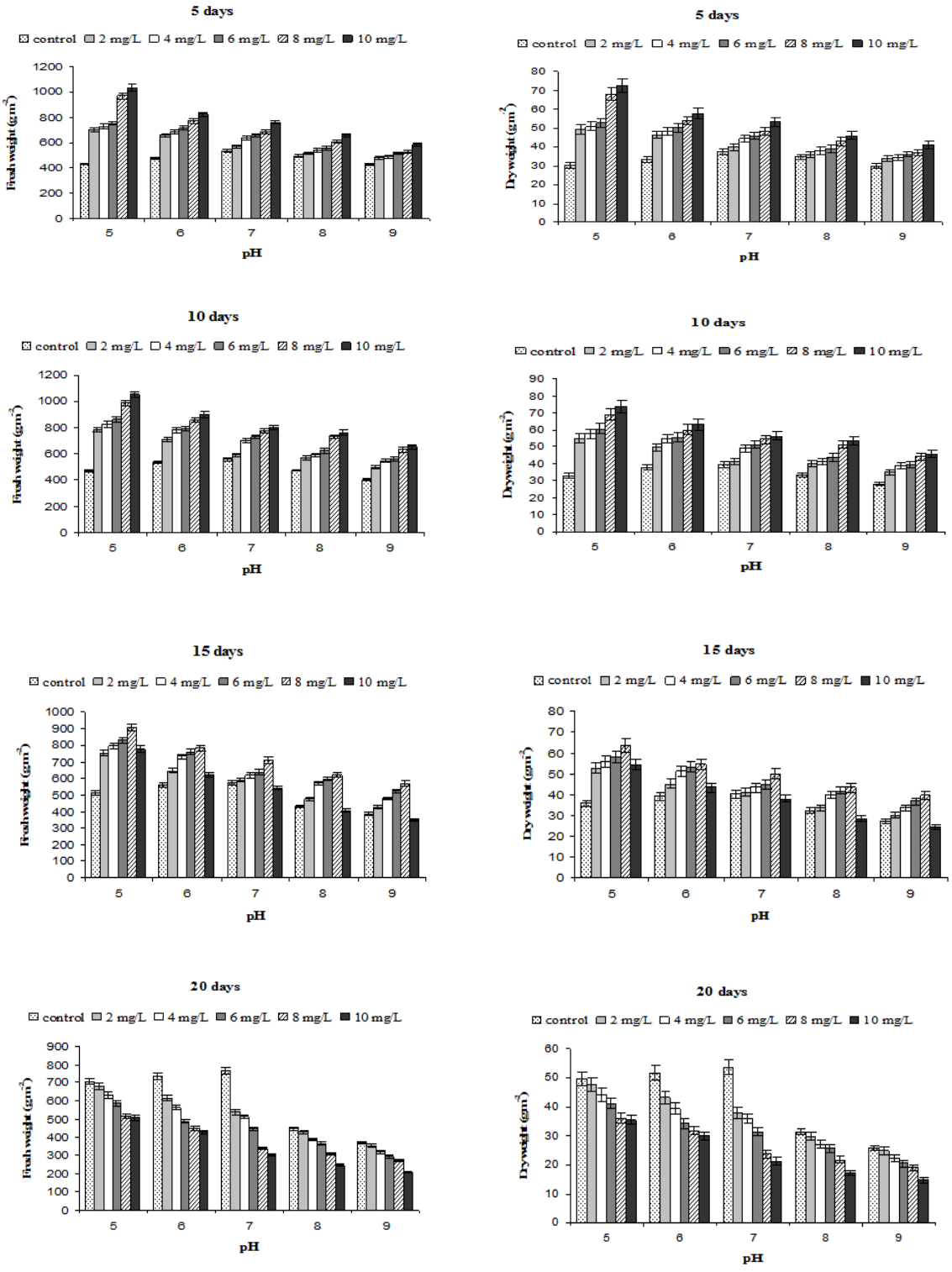

Fig. (1): Effect of different concentrations of Cr and pH on fresh and dry weights of *L. minor* after different treatment periods.

Table (1): Effect of pH on accumulation of Cr in *L. minor* after different treatment periods.

Treatment periods (day)	Cr concentration (mg L^{-1})	Cr accumulation (µg g^{-1} dry wt)					*F-value*	*P-value*
		pH 5	pH 6	pH 7	pH 8	pH 9		
5	2	1621.70^{a}±66.37	1527.02^{b}±64.10	1143.54^{c}±53.99	1021.23^{c}±56.61	866.61^{cd}±39.83	162.00	0.000
	4	3300.53^{a}±93.71	2633.22^{b}±65.33	1329.30^{c}±55.54	1239.30^{c}±54.84	931.23^{d}±40.11	144.18	0.000
	6	5314.55^{a}±109.21	2750.85^{b}±75.88	1742.40^{c}±52.88	1721.61^{c}±55.93	1609.65^{c}±53.20	176.09	0.000
	8	7371.44^{a}±123.84	4450.59^{b}±94.74	2272.47^{c}±71.73	2077.02^{c}±73.11	1893.50^{cd}±60.07	178.94	0.000
	10	9169.12^{a}±223.92	6040.62^{b}±131.76	3503.25^{c}±80.65	3140.91^{c}±78.02	2385.09^{d}±72.56	160.77	0.000
10	2	1716.29^{a}±93.97	1674.33^{b}±77.23	1338.95^{c}±63.63	1262.75^{d}±53.64	1104.72^{e}±51.67	108.4	0.000
	4	3662.49^{a}±118.95	3112.53^{b}±98.64	2499.30^{c}±70.96	2199.33^{d}±62.96	2154.33^{d}±58.90	732.22	0.000
	6	5477.61^{a}±163.50	5116.32^{b}±121.86	4154.04^{c}±90.47	3103.92^{d}±79.35	2573.19^{d}±82.72	97.25	0.000
	8	7597.61^{a}±213.70	6492.96^{b}±154.89	5080.08^{c}±111.53	3298.95^{d}±82.36	3113.19^{d}±88.98	818.59	0.000
	10	9563.81^{a}±222.47	7283.34^{b}±171.35	6138.72^{c}±146.82	3895.56^{d}±99.58	3556.26^{d}±89.63	908.55	0.000
15	2	1578.22^{a}±90.34	1345.09^{b}±75.63	1199.27^{c}±55.77	1040.09^{d}±54.93	1024.65^{e}±49.80	704.75	0.000
	4	3107.08^{a}±113.51	2847.78^{b}±72.06	1981.92^{c}±65.29	1590.03^{d}±58.14	1398.51^{d}±54.70	101.20	0.000
	6	5444.01^{a}±137.34	3897.81^{b}±84.71	2440.44^{c}±63.57	2282.40^{c}±63.20	1860.03^{d}±56.21	879.87	0.000
	8	7112.64^{a}±190.71	5741.73^{b}±155.27	3897.81^{c}±107.88	2593.98^{d}±72.69	2113.92^{e}±70.15	979.92	0.000
	10	5052.87^{a}±121.18	3513.60^{b}±90.60	3118.95^{c}±82.04	1992.78^{d}±64.18	1624.68^{e}±45.82	772.13	0.000
20	2	1337.02^{a}±81.70	1292.02^{b}±67.33	1148.68^{c}±59.13	1090.13^{d}±53.74	838.89^{e}±45.26	742.55	0.000
	4	1292.4^{a}±75.52	1229.3^{b}±54.90	1124.54^{c}±53.44	1003.44^{d}±52.62	778.86^{e}±46.76	782.82	0.000
	6	1188.92^{a}±67.13	1110.23^{b}±57.56	1011.2^{c}±48.20	821.61^{d}±45.55	747.72^{d}±38.36	832.47	0.000
	8	1107.75^{a}±67.20	1005.5^{b}±49.99	938.51^{c}±47.85	700.38^{d}±36.98	613.89^{d}±35.92	948.92	0.000
	10	998.51^{a}±60.14	935.03^{b}±47.85	811.17^{c}±44.90	437.31^{d}±26.80	371.52^{d}±25.22	112.23	0.000

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

3.3. Effect of pH on bioconcentration factor (BCF) for Cr

The results of bioconcentration factor (BCF) for Cr with different levels of pH after different treatment periods are shown in Fig. 2. It is clear that *L. minor* showed comparatively high BCF values for Cr in experimental set-up with pH 5. In terms of pH of media, the set-ups for BCF of Cr can be arranged in the order: pH 5 >pH 6 >pH 7 >pH 8> pH 9. The highest value of BCF for Cr was (956.38±23.69) at 10 mg L^{-1} with pH 5 after 10 days of treatment. However, the higher value of BCF indicates the ability of *L. minor* to accumulate higher concentrations of chromium in its tissues.

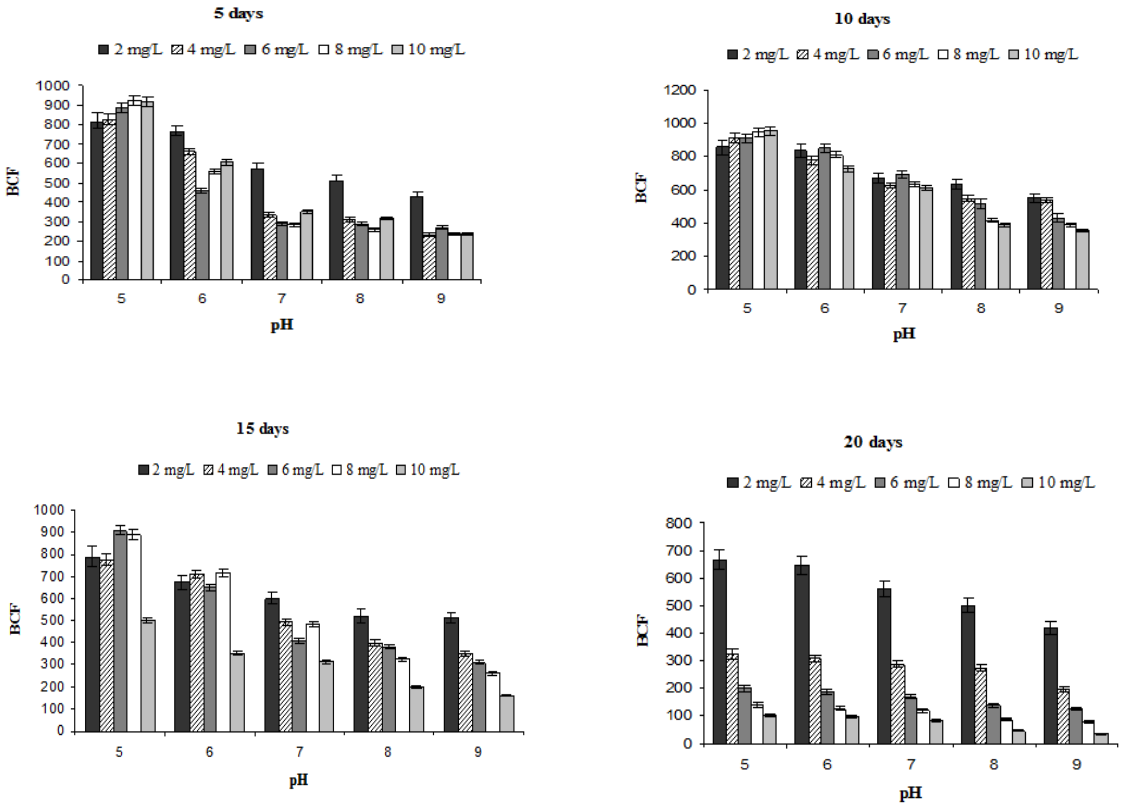

Fig. (2): Effect of pH on bioconcentration factor (BCF) for Cr after different treatment periods.

3.4. Effect of different concentrations of Cr and EDTA on growth of *L. minor* at pH 5

The previous results indicated that *L. minor* showed better growth and Cr accumulation at 10 mg L^{-1} of Cr with pH 5. In test sets supplemented with EDTA concentrations (25, 50, 75 and 100 µM), growth of *L. minor* was affected by different concentrations of Cr in medium with pH 5 after 5, 10, 15 and 20 days of treatment (Fig. 3). The highest fresh and dry weight of *L. minor* was (1267.54±29.63 g m^{-2} f.wt and 88.73±4.15 g m^{-2} d.wt) at 10 mg L^{-1} of Cr with pH 5 and 25 µM of EDTA after 10 days of treatment. The effect was more pronounced in treatments EDTA 25> 50> 75> 100 µM during all treatment periods. Meanwhile, the results of fresh and dry weights with 25 µM of EDTA were significant compared to EDTA (50, 75 and 100 µM) at $P<0.05$ after 10 days of treatment.

3.5. Effect of EDTA on accumulation of Cr in *L. minor* at pH 5

The results of this experiment indicated that *L. minor* had the capacity to accumulate large quantities of Cr in solutions containing different concentrations of EDTA. Chromium is chelated by different concentrations of EDTA with pH 5 (Table 2). Maximum accumulation of Cr was (9926±235.84 µg g^{-1} dry wt) at 10 mg L^{-1} with 25 µM of EDTA after 10 days of treatment. The results revealed that Cr accumulation increased significantly ($P<0.05$) at 10 mg L^{-1} with 25 µM of EDTA compared to EDTA (50, 75 and 100 µM) after 10 days of treatment.

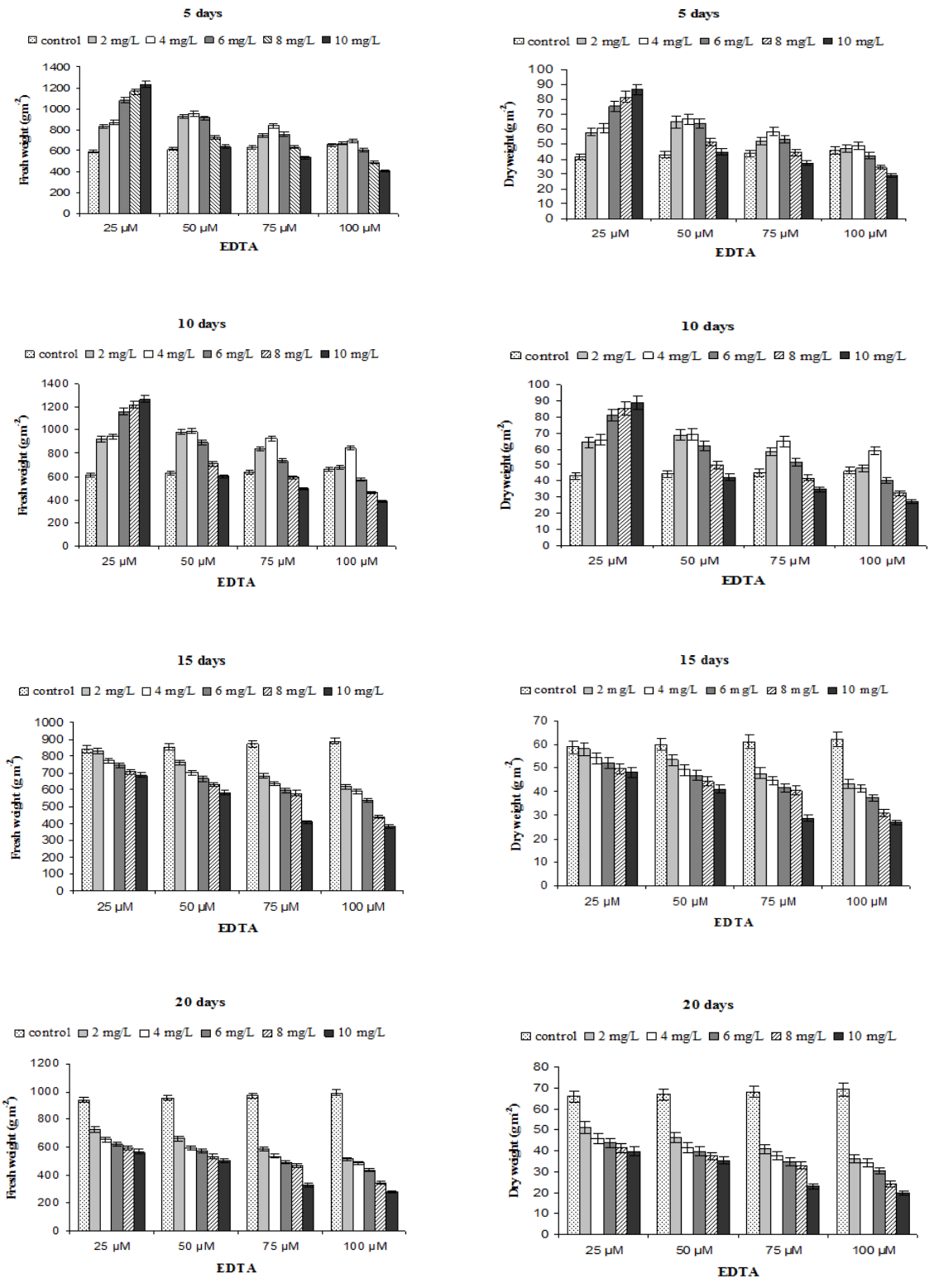

Fig. (3): Effect of different concentrations of Cr and EDTA on fresh and dry weights of *L. minor* at pH 5 after different treatment periods.

Table (2): Effect of EDTA on accumulation of Cr in *L. minor* at pH 5 after different treatment periods.

Treatment periods (day)	Cr concentration (mg L^{-1})	Cr accumulation (µg g^{-1} dry wt)				*F-value*	*P-value*
		EDTA (25 µM)	EDTA (50 µM)	EDTA (75 µM)	EDTA (100 µM)		
5	2	1708.39^{a}±117.63	1623.36^{b}±119.86	1472.08^{c}±109.29	1223.07^{d}±99.05	109.14	0.000
	4	3485.14^{a}±132.71	3333.24^{b}±128.83	3187.05^{a}±129.29	3024.31^{c}±109.00	73.03	0.000
	6	5422.99^{a}±158.17	3256.40^{b}±121.11	2794.93^{c}±110.84	1814.29^{d}±100.55	298.98	0.000
	8	7420.47^{a}±204.80	3117.91^{b}±119.47	2687.92^{c}±120.45	1633.75^{d}±95.55	693.98	0.000
	10	9285.60^{a}±232.39	3006.76^{b}±122.77	2441.77^{c}±92.00	1406.14^{d}±83.95	130.50	0.000
10	2	1770.19^{a}±126.54	1682.47^{b}±117.67	1528.02^{c}±116.30	1326.03^{d}±93.20	102.38	0.000
	4	3721.01^{a}±153.42	3282.94^{b}±120.41	2986.64^{c}±103.72	2333.75^{d}±105.15	155.91	0.000
	6	5674.16^{a}±174.15	1561.91^{b}±119.64	1488.29^{c}±112.65	1288.57^{d}±87.23	455.33	0.000
	8	7653.48^{a}±210.92	1403.85^{b}±121.23	1334.31^{c}±91.18	1161.32^{d}±88.26	917.87	0.000
	10	9926^{a}±235.84	1223.81^{b}±107.82	1151.17^{c}±82.52	1090.25^{d}±79.71	169.60	0.000
15	2	1689.84^{a}±113.66	1559.91^{b}±112.68	1354.20^{c}±114.45	1239.93^{d}±100.85	83.44	0.000
	4	1596.49^{a}±114.89	1476.69^{b}±110.49	1311.24^{c}±99.15	1205.61^{d}±94.44	71.08	0.000
	6	1443.72^{a}±100.72	1358.26^{b}±99.20	1291.52^{c}±96.23	1114.33^{d}±93.34	104.91	0.000
	8	1326.55^{a}±100.42	1299.74^{b}±91.85	1245.48^{c}±86.81	1038.12^{d}±86.04	122.10	0.000
	10	1283.09^{a}±97.32	1199.35^{b}±87.69	1123.43^{c}±73.85	974.42^{d}±68.31	184.14	0.000
20	2	1340.55^{a}±82.73	1091.28^{b}±79.03	1064.17^{c}±69.33	1012^{d}±78.11	123.91	0.000
	4	1263.30^{a}±76.94	1065.91^{b}±77.17	1039.05^{c}±65.70	992.76^{d}±63.97	108.68	0.000
	6	1213.57^{a}±81.13	1017^{b}±68.93	984.97^{c}±59.13	966.24^{d}±57.34	217.23	0.000
	8	1149.46^{a}±63.73	991.46^{b}±71.25	931.18^{c}±58.84	857.43^{d}±54.23	139.50	0.000
	10	1062.82^{a}±58.73	954.12^{b}±61.43	872.63^{c}±49.64	837.14^{d}±39.88	82.38	0.000

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

3.6. Effect of EDTA on bioconcentration factor (BCF) for Cr at pH 5

The results of bioconcentration factor (BCF) for Cr with different concentrations of EDTA at pH 5 are shown in Fig. 4. The highest value of BCF for Cr was (992.6±24.56) at 10 mg L^{-1} with 25 µM of EDTA after 10 days of treatment.

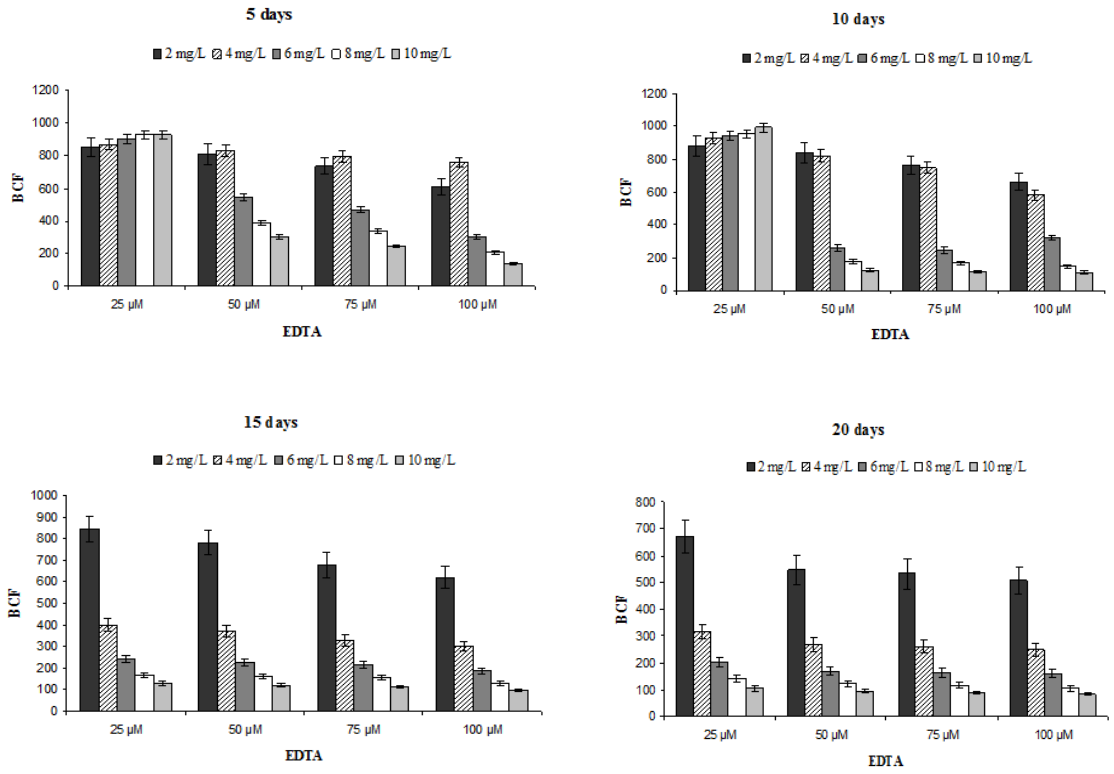

Fig. (4): Effect of EDTA on bioconcentration factor ((BCF) for Cr after different treatment periods.

3.7. Removal efficiency of chromium by *L. minor*

Effect of pH 5 and 25 µM of EDTA on removal efficiency of different concentrations of Cr by *L. minor* after different treatment periods is shown in Fig. 5. The highest removal efficiency of Cr by *L. minor* was 99.26 % at 10 mg L^{-1} after 10 days of treatment.

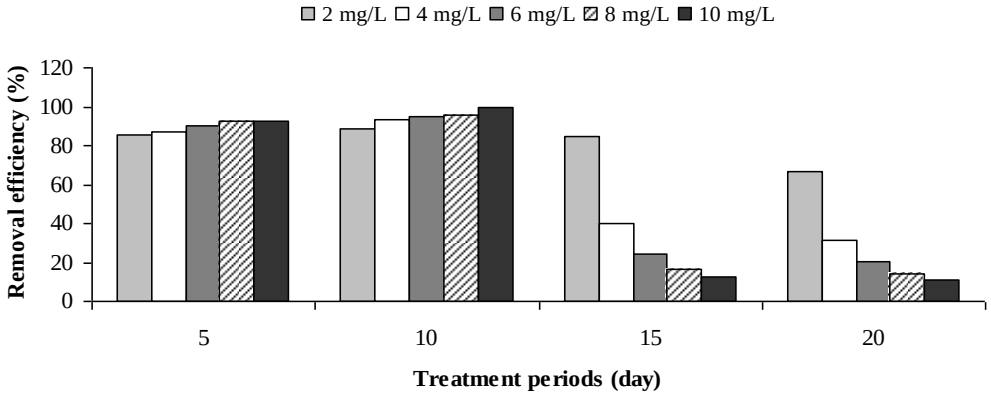

Fig. (5): Effect of pH 5 and 25 µM of EDTA on removal efficiency of Cr by *L. minor* after different treatment periods

3.8. Effect of the treatment on Nile tilapia growth

3.8.1. Estimation of growth parameters

The results of growth parameters of fish that exposed to the treated water for 30 days are shown in Table (3). At the end of exposure period, the average final weight and length were (43.8±2.42 gm and 32.9±1.74 cm) respectively. Meanwhile; a specific growth rate (SGR) of fish was 1.2±0.01 %/day after 30 days of exposure the same as control group. Overall, the exposed fish and control group had no mortality rate during the exposure period.

3.8.2. Analysis of chromium accumulation in fish muscles

Accumulation of Cr in fish muscles was measured at the end of the exposure period and compared with control group (Table 3). Fish that exposed to the treated water had no chromium in their muscles.

Table (3): Effect of the treated water by *L. minor* on Nile tilapia growth.

Exposure period (day)	Fish	Average initial weight (gm)	Average final weight (gm)	Average initial length (cm)	Average final length (cm)	Specific growth rate (SGR) (%/day)	Accumulation of Cr in fish muscles (µg g^{-1} dry wt)
30	Control	30.4±1.60	43.6±2.30	21.4±1.0	32.7±1.71	1.2±0.01	ND
	Exposed	30.6±1.63	43.8±2.42	21.8±1.0	32.9±1.74	1.2±0.01	ND

* Data are presented as mean of three samples ± S.D, ND refers to non determined.

DISCUSSION

In recent years much attention has been given on wastewater treatment with the help of aquaculture (growth of aquatic plants having economic values) and recycling of treated water. The green plants degrade, assimilate, metabolize, or detoxify inorganic and organic pollutants from the environment or render them harmless. *L. minor* has been commonly used as a test organism in ecotoxicological and environmental studies (Khellaf and Zerdaoui, 2010) due to its high sensitivity to various chemicals, small size, rapid vegetative reproduction and easy handling in laboratory conditions. Chandra and Kulshreshtha (2004) reported duckweeds to have greater tolerance to Cr relative to other aquatic plants. Compared to most other aquatic plants, *L. minor* is less sensitive to low temperatures, very high nutrient levels, pH fluctuations, pests and diseases (Irfana *et al*., 2017). Goswami and Majumdar (2015) reported a significant reduction in specific growth rate of *L. minor* with increase in Cr(VI) concentration in ambient solution. According to Thayaparan *et al*. (2015), at the end of the experiment period (after 7 days) Cr(VI) caused a distinct limitation of *Lemna*'s growth compared to the control. In this study, we investigated the accumulation of Cr in *L. mionr* under the effect of pH and EDTA variation and assessed the treatment impact on Nile tilapia growth. The parameters that monitored during this study were fresh and dry weights of *L. minor*, accumulation of Cr and BCF; as well as accumulation of Cr in Nile tilapia muscles. Both chromium concentration and pH directly affect the growth of *L. minor* and the data in this study emphasized that the toxic effects of Cr on *L. minor* is dependent on pH. The selection of pH range was done on the basis of the survival potential of duckweed.

Our results indicated that fresh and dry weights were positively affected by pH 5> pH 6> pH 7> pH 8> pH 9 during all the treatment periods. A similar observation has been reported in different studies that found duckweed growth declines with increase in alkaline pH (Kumar *et al*., 2015). According to Sekomo *et al*. (2012), the pH range of 4.5-7.5 was reported to be best suited range for the growth of duckweed species. Some characteristics e.g. *L. minor* can grow well in pH from 6 to 9 while the lowest value of pH it can tolerate in between pH 5-6 make it a suitable plant for phytoremediation (Chaudhary and Sharma, 2014). The chemical nature of the metals ion; strength and pH tends to be a master variable in accumulation process (Nwabunike, 2016). In acidic conditions, hydrogen ions occupy many of the negatively charged surfaces and little space is left to bind heavy metals, hence more heavy metals remain in the soluble phase. The present study appeared that the highest value of Cr accumulation was recorded with pH 5; as well as accumulation of Cr in *L. minor* increased with increasing Cr concentration in nutrient solution followed by other pH (6, 7, 8 and 9) during all the treatment periods. These results agreed with that reported by Rashmi and Surindra (2015), the uptake yield of Pb was the maximum in 5 mg/L set-up with 5 pH followed by other set-ups. The uptake yield in set-ups was directly related to the metal loads and pH in culture medium. The effects of EDTA and other chelators on the uptake and toxicity of metals in aquatic biota have been investigated by numerous researchers (Borgrnann *et al*., 1991). Several studies suggest that the toxicity of different metals can also be mitigated by EDTA binding (Postma *et al*., 2000).

Overall, the results of the second experiment found that fresh and dry weights with pH 5 and 25 μM of EDTA have significantly increased after 10 days of treatment. Chelators are an essential component of a complete nutrient medium and should be included when measuring metal toxicity in aquatic plants, since *L. trisulca* grown without EDTA had low multiplication rates and appeared stunted and chlorotic (David and Jennifer, 1992). *L. minor* had the capacity to accumulate large quantities of Cr in solutions containing different concentrations of EDTA (25, 50, 75 and 100 μM). On the other hand, Kwan and Smith (1991), found that increasing the EDTA concentration from 6.8 to 50 pM completely inhibited the Cd uptake in *L. minor*. Bioconcentration factor (BCF) is an indicator of the metal accumulation ability of plants in respect of metal concentration in the medium and allows for a comparison of the results (Mountouris *et al*., 2002). In the current study, BCF value for Cr by *L. minor* was high at 10 mg L^{-1} with pH 5 and 25 μM of EDTA after 10 days of treatment as the best combinations for the optimum removal. On the basis of obtained results, *L. minor* can be considered as a hyperaccumulator for Cr under given conditions. In general, when the metal concentration in the feed solution increases, the amount of metal accumulating in plant increases, whereas, the BCF value decreases (Lu *et al*., 2004).

The highest removal efficiency of Cr by *L. minor* was 99.26 % at 10 mg L^{-1} with pH 5 and 25 μM of EDTA after 10 days of treatment. Chromium amount decreased by 99.26 % in nutrient solution and subsequently this element exhibited an increasing concentration in the plant fronds. The results of Cr removal are in accordance with a study which conducted by Abdallah (2012) found that *L. gibba* is a potential candidate in removing Cr and Pb about 95% and 84%, respectively after 12 days of incubation. Also, Leela *et al*. (2012) stated that the maximum removal by *L. mionr* was found to be 99.99% for Pb at pH 5-6 and 99.3% for Ni at pH 6 after 28 days of exposure. Their study found

that Pb removal was lowest (95.94%) at pH 10 after 7 days of exposure. In the same way the lowest Ni removal was achieved at pH 10 after 7 days (73.78%). The results of the present study indicated that the toxic effects of chromium on *L. minor* is dependent on pH and EDTA. Thus, pH and EDTA operated together on the removal of Cr. According to Campbell and Stokes (1985), the presence of chelators and pH influence the metal toxicities to aquatic organisms. The assessment of the treatment by *L. minor* under the effect of pH and EDTA using Nile tilapia as a test organism is very important. According to Gado and Midany (2003), fishes have been recognized as a good accumulator of organic and inorganic pollutants. Therefore, many international monitoring programs have been established in order to assess the quality of fish for human consumption and to monitor the health of the aquatic ecosystem (Meche *et al*., 2010).

Fish, in comparison with invertebrates, are more sensitive to many toxicants and are a convenient test subject for indication of ecosystem health (Zaki *et al*., 2014). Also, Fishes are considered to be most significant biomonitors in aquatic systems for the estimation of metal pollution level (Authman, 2008). However, fish are relatively situated at the top of the aquatic food chain; therefore, they normally can accumulate heavy metals from food, water and sediments (Zhao *et al*., 2012). Toxic effects of Cr in fish include: hematological, histological and morphological alterations, inhibition/reduction of growth, production of reactive oxygen species (ROS) and impaired immune function (Vera *et al*., 2011). According to Sfakianakis *et al*. (2015), poor treatment of the effluents can lead to the presence of Cr (VI) in the surrounding water bodies, where it is commonly found at potentially harmful levels to fish. Dupuy *et al*. (2014) reported that the fish health status in some polluted systems (estimated by the condition factor) indicated that the fish have a lower condition. Very low-levels of pollution may have no apparent impact on the fish itself, which would show no obvious signs of illness, but it may decrease the fecundity of fish populations, leading to a longterm decline and eventual extinction of this important natural resource (Ebrahimi and Taherianfard, 2011). In the third experiment, the fish that exposed to the treated water were healthy; as well as fish muscles had no Cr accumulation and this result due to the efficiency of *L. minor* in removal of Cr from polluted water under the effect of pH and EDTA. However, in polluted aquatic habitats the concentration of metals in fish muscles may exceed the permissible limits for human consumption and imply severe health threats (Elnabris *et al*., 2013).

CONCLUSION

Our results concluded that *L. minor* is a better candidate for treating high concentrations of chromium in polluted water under the effect of pH and EDTA. Fish that exposed to the treated water by *L. minor* were healthy and safe. Therefore, this method can be applied on the large scale for wastewater treatment. Finally, we recommend that the treatment of all kinds of wastewater, sewage and agricultural wastes must be conducted before discharge into the aquatic systems. Also, enforcement of all articles of laws and legislations regarding the protection of aquatic environment must be taken into considerations.

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ARABIC SUMMARY

تراكم عنصر الكروم فى عدس الماء تحت تأثير إختلاف رقم الحموضة والإيديتا وتقييم تأثير المعالجة على سمك البلطى النيلي

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تلوث الماء بعنصر الكروم هو تهديد رئيسى للبيئة وصحة الإنسان. لذا هدف الدراسة الحالية هو تقييم كفاءة نبات عدس الماء كمادة حيوية طبيعية فى تراكم الكروم من الماء الملوث تحت تأثير أرقام الحموضة والإيديتا المختلفة، بالإضافة إلى تقييم تأثير هذه المعالجة على نمو سمك البلطى. نبات عدس الماء عُرّضَ لمجموعة من التركيزات المختلفة من الكروم (٠، ٢، ٤، ٦، ٨، ١٠ ملليجرام/ لتر) مع رقم حموضة مختلف (٥، ٦، ٧، ٨، ٩) لفترة (٥، ١٠، ١٥، ٢٠ يوم). أعلى تراكم للكروم سجل (٢٢٢,٤٧±٩٥٦٣,٨١ ميكروجرام/جرام وزن جاف) للتركيز ١٠ ملليجرام/لتر مع رقم الحموضة ٥ بعد ١٠ أيام من المعالجة، بينما مع تركيزات الإيديتا المختلفة (٢٥، ٥٠، ٧٥، ١٠٠ ميكرومول) أعلى تراكم للكروم سجل (٢٣٥,٨٤±٩٩٢٦ ميكروجرام/جرام وزن جاف) للتركيز ١٠ ملليجرام/لتر مع رقم الحموضة ٥ وتركيز ٢٥ ميكرومول من الإيديتا بعد ١٠ أيام من المعالجة. أعلى كفاءة إزالة للكروم بإستخدام عدس الماء كانت ٩٩,٢٦% للتركيز ١٠ ملليجرام/ لتر مع رقم الحموضة ٥ وتركيز ٢٥ ميكرومول من الإيديتا بعد ١٠ أيام من المعالجة. هذه الدراسة أشارت إلى أن رقم الحموضة والإيديتا أثرا على تراكم الكروم في عدس الماء. تأثير الماء المعالج على نمو سمك البلطى أشار إلى أن عضلات السمك لم يكن لديها كروم متراكم والماء المعالج أصبح مقبول وآمن. فى هذه الأثناء، معدل النمو كان (٠,٠١±١,٢ %/ يوم) في نهاية فترة التعرض التى دامت ٣٠ يوم تماما مثل المجموعة التى لم تعرض. لذا، يمكن أن نستنتج بأن سمك البلطى لعب دورا مهما لمراقبة الكروم فى الماء المعالج بإستخدام عدس الماء تحت تأثير كلا من رقم الحموضة والإيديتا.