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Accumulation of Iron, Zinc and Lead by Azolla pinnata and Lemna minor and activity in contaminated water

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Abstract

In this study, two aquatic macrophytes namely, Azolla pinnata and Lemna minor are floating plants were obtained from Agric. Microbial Dept., Soils, Water and Environment Research Institute (SWERI), Agric. Res. Center (ARC), Giza, Egypt and used to some heavy metal such as Iron, Zinc and lead This study reported the ability of two aquatic plants (A. pinnata and L. minor) to remove Iron, Zinc and lead from aqueous solutions FeSO4.7H2O, ZnSo4.7H2O and C4H6O4Pb.3H2O of four different initial concentrations (0–100 ppm) for 20 days under greenhouse conditions. The results indicated that A. pinnata gave higher growth density than that recorded for L. minor during all the tested incubation periods from zero time up to 20 days. Results obtained in this study showed a maximum removal of Fe, Zn (88.18, 84.63 %) by L. minor at 100ppm initial metal concentration however the maximum removal by A. pinnata at the same concentration was (86.97, 81.14%) after 20 day of incubation. These A. pinnata appeared to be more efficient than L. minor for removing Pb .On the other hand A. pinnata was better than L. minor in biomass for each of the elements used in the experiment during the incubation period.

Keywords: Azolla pinnata, Lemna minor, Phytoaccumulation, Heavy metals, Metal accumulation

1. Introduction

Water pollution is one of the major problems for most countries. Pollutants may enter water bodies as leachates or through the improper disposal of industrial wastes which may include pesticides, heavy metals, textile wastes, inorganic anions and radioactive compounds [1].

Water contaminations, along with limited availability of water, have put a severe burden on the environment. Around 40% population of the world is facing the problem of water scarcity due to climate change, rapid urbanization, food requirement and unchecked consumption of natural resources [2, 3].

The word “heavy metals” mean an element having high density greater than 4–5 g/cm3 and toxic to human being even at very low concentration [4]. Examples of heavy metals are the element present in platinum group, copper, iron, lead, arsenic, mercury, silver, chromium, zinc, and cadmium [5, 6, 7]. According to [8], about 0.84 million people die every year by diarrhea due to the intake of unsafe drinking water.

The most important heavy metals from the point of view of water pollution are Zn, As, Cu, Pb, Cd, Hg, Ni and Cr as some of these metals (e.g. Cu, Fe, Mn, Ni, and Zn) are required as nutrients in trace amount for life processes in plants and microorganisms but become toxic at higher concentrations [9].

Lead is not an essential element to the human body, and excessive Pb intake can have adverse impacts on the nervous, skeletal, enzymatic, endocrine, immune, and circulatory systems [10].

Fe for example prevent anemia while Zn is a core

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factor for over 100 enzymes reaction. Because they may be needed in small quantity, metals such as mercury, lead and cadmium has no known vital or beneficial effect on organisms and accumulation over time in the body of mammals can cause serious health effect [11, 12]. The mining activities for metals, such as Pb or Zn, are a well-known environmental worry due to the potential release and spread of heavy metals during extraction, transportation, metal smelting, and the activities of metallurgical industries. [13]

Phytoremediation can provide a long lasting, cost effective, long lasting and aesthetic solution to the remediation of this wastewater, since macrophytes such as Lemna minor, Azolla pinnata, Pistia stratiotes, Eicchornia crassipes and Salvinia molesta which are easily accessible have been proof to have phytoremediation potentials by researchers such as [14,15,16] etc. Aquatic macrophytes are known as good indicators of heavy metal contamination in aquatic ecosystems and they act as biological filters by accumulating heavy metals from the surrounding environments [17].

Plants should have the following characteristics in order to make the phytoremediation an eco-sustainable technology: native and quick growth rate, high biomass yield, the uptake of a large amount of heavy metals, the ability to transport metals in aboveground parts of plant, and a mechanism to tolerate metal toxicity [18, 19]. Other factors like pH, solar radiation, nutrient availability and salinity greatly influence the phytoremediation potential and growth of the plant [20, 21].

Removal of different heavy metals along with other contaminants through the application of aquatic plants is the most proficient and profitable method [18, 22]. Constructed wetlands along with aquatic plants were extensively applied throughout the world for the treatment of wastewater [23, 24]. The selection of aquatic plant species for the accumulation of heavy metal is a very important matter to enhance the phytoremediation [25, 26].

Over the years, aquatic plants have gained an overwhelming reputation because of their capacity to clean up contaminated sites throughout the world [23, 27]. Aquatic plants always develop an extensive system of roots which helps them and makes them the best option for the accumulation of contaminants in their roots and shoots [28, 29]. Aquatic plants Pistia stratiotes, Azolla pinnata, and Salvinia, molesta were found very competent for the elimination of Fe, Cu and Mn at 25% concentration of the textile effluents [30]. A hairy root system of aquatic plants plays a vital part in the remediation of pollutants from wastewater in phytoremediation [31].

Azolla is an aquatic fern or small leafed floating plant, seen in quiet and slow-moving water bodies and is present in countries like Africa, Asia, and some parts of Australia. It produces maximum biomass in a relatively shorter period of time [32] and is of great applications in both developing as well as developed countries [33, 34]. Some advantages for Azolla is it can grow rapidly and double its biomass in every three days. It produces more than 4 to 5 times the protein compared with hybrid Napier and Lucern [35] and proved to a potent aquatic water fern for the bio filtration of various toxic metals [36].

It has high biomass productivity coupled Also, it can remove sulfa drugs (Forni et al., 2001) and metals like Sr, Cu, Cd, Zn, Cr, Ni, Pb, Fe, Au, Pt and even radioactive elements as U [37, 38, 39, 40].

Among aquatic plants, Lemna. minor is one of the best candidates that has been investigated for its metal uptake abilities and potential for phytoremediation [41]. Adult Lemna. minor fronds generate daughter fronds from two side pouches, which make up a colony composed of a mother and several (typically 3–4) of spring [42] Lemna minor is known for its simple structure, compactness, rapid generation, asexual reproduction, and facile culturing [43, 44].

Duckweed can eliminate a vast variety of different heavy metals, inorganic and organic contaminants, pesticides, nutrients arise from agricultural runoff, sewage, industrial and domestic wastewater [45, 46, 47].

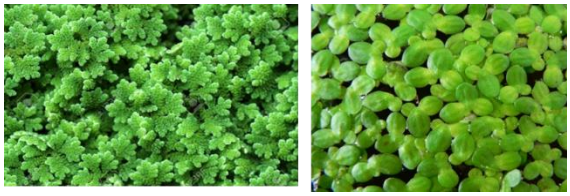
In this study aim of this investigation is to evaluate the role of A. pinnata and L. minor in absorption of heavy metals such as Fe, Zn, and Pb and their effects on growth, fresh, dry weights, doubling time and Fe, Zn, and Pb accumulation. Comparing of A. pinnata and L. minor in resisting heavy metals such as Fe, Zn, and Pb.

2. Materials and Methods

2.1. Azolla pinnata and Lemna minor Strains

Azolla pinnata and *Lemna minor* used in the present study was illustrated in picture 1. The *Azolla pinnata*

and *Lemna minor* kindly provided by Microbial Res. Depart., Soils, Water and Enviro. Res. Institute Agric. Res. Center (ARC) Giza, Egypt.



(a) (b)

Fig. 1. (a) *Azolla pinnata*, (b) *Lemna minor*

2.2. Standard Inoculation

The collected *Azolla pinnata* and *Lemna minor* surface were sterilized with a concentrations 0.1% solution of mercury chloride for 30 Sec. according to [48] washed by distilled water for several times and then air dried on tissue papers for 30 minutes.

2.3. Media Used

2.3.1. Yoshida medium

This medium [49]. was prepared using the following chemical Composition in ppm: Modified Yoshida medium contained of 40.00 mg L^{-1} $NaH2PO^4.H^2O$, 40.00 mg L^{-1} K_2SO_4, 40.00 mg L^{-1} $CaCl_2$, 40.00 mg L^{-1} $MgSO_4.7H_2O$, 0.50 mg L^{-1} $MnCl_2.2H_2O$, 0.20 mg L^{-1} $H3BO_3$, 0.01 mg L^{-1} $ZnSO_4.7H_2O$, 0.01 mg L^{-1} $CuSO_4.5H_2O$, 2.00 mg L^{-1} Iron (II) ethylene diamine tetra acetic acid (Fe-EDTA) and pH was adjusted to 5.5.

2.3.2. oagland solution

This medium [50] was prepared using the following chemical Composition in ppm: Hoagland medium contained of 136.00 mg L^{-1} KH_2PO_4, 246.40 mg L^{-1} $MgSO_4.7H_2O$, 555.00 mg L^{-1} $CaCl_2$, 372.80 mg L^{-1} KCl, 2.86 mg L^{-1} $H3BO_3$, 1.55 mg L^{-1} $MnSO_4.H_2O$, 0.22 mg L^{-1} $ZnSO_4.7H_2O$, 0.08 mg L^{-1} $CuSO_4.5H_2O$, 0.02 mg L^{-1} $Na_2MoO_4.2H_2O$, 30.00 mg L^{-1} $FeSO_4.7H_2O$ and pH was adjusted to 7.

3. Experimental layout

The experiment was carried out in the greenhouse of Soils, Water and Environ. Res. Inst. (SWERI), Agric. Res. Center (ARC), Giza, Egypt during September and October 2019. Cultivation of *A. pinnata* and *L. minor* was carried out in plastic pots separately (32.0 cm diameter and 13.0 cm in depth). Pots were filled with 3000 ml of medium (Yoshida medium for *A. pinnata* and Hoagland medium for *L.minor*) and supplemented with different concentrations of Fe^{+2}, Zn^{+2} and Pb^{+2}.

Wastewater samples were prepared by dissolving their corresponding analytical grade salts of $FeSO_4.7H_2O$, $ZnSo_4.7H_2O$ and $C_4H_6O_4Pb.3H_2O$ in deionized water at nominal concentrations of control, 25, 50, and 100 ppm. The pots were inoculated with 10 g fresh of *A. pinnata* and *L. minor* separately, which was used as a standard inoculum in all experiments (El-Berashi, 2008). Every concentration of Fe^{+2}, Zn^{+2}, and Pb^{+2} were represented by 3 replicates which carried out for this treatment. The inoculated pots were incubated at 35°C ± 2, 14 h light and 10 h dark for 20 days under greenhouse conditions. Samples of the treatments were taken after zero time, 5, 10, and 20 days of incubation.

Control treatment (plants without metal) which contained only a nutrient medium, was used to compare it with the effects Fe^{+2}, Zn^{+2} and Pb^{+2}. concentrations on fresh, dry weight [51], doubling time of *A. pinnata* and *L. minor* growth and the accumulation of Fe^{+2}, Zn^{+2} and $Pb^{+2}$by these plants were determined on dry weight basis by using the atomic absorption spectrophotometer (analytikjena, nov AA 350, Germany).

4. Vegetative growth parameters of Azolla pinnata and Lemna minor

4.1. Fresh Weight

A. pinnata and *L. minor* fronds were harvested washed with deionized water and placed under shade between two thick layers of blotting tissue papers for approximately 1-2 h before determining fresh weight. Fresh weight of *A. pinnata* and *L. minor* fronds were measured and expressed as g m^{-2}

4.2. Dry Weight

The dry weight of *A. pinnata* and *L. minor* were determined by drying fronds at 70 0C to constant weight. Dry weight of *A. pinnata* and *L. minor* were

expressed as g m^{-2}.

4.3. Doubling Time

Doubling time was calculated as growth rate of *A. pinnata* and *L. minor* was calculated by using the following Eq (1). according to [52]:

$$\text{Doubling time (D.T)} = \frac{t}{r} \quad (1)$$

Where:

t = the duration of Azolla and *Lemna* growth,

$r = \text{Log}(\frac{wt}{wo \times 0.301})$

wt = weight of *Azolla* and *Lemna* at time t,

wo = weight of *Azolla* and *Lemna* at zero time *i.e*. weight of inoculum.

4.4. Determination Heavy metal removal from contaminated water

The heavy metals are expressed as percentage of metal removal as given below in Eq. (2);

$$\text{Metalremoval} = \frac{C_0 - C_e}{C_0} * 100 \quad (2)$$

Where Co and Ce are the initial and final metal concentration in solution (mg/L) respectively

4.5. Determination of mineral Heavy Metals concentrations for (Iron, Zinc and Lead) in plant and water

A 0.2 ground powder of plant (*A. pinnata* and *L. minor)* oven dried 70^0C and 5ml of sulphuric acid were placed in 100 ml digestion flask. The samples were digested on for electric heater for 10 min. then 1.00 ml of perchoric acid was added. The digestion was completed until dense white fumes appeared and finally the solution became clear. The samples were left to cool diluted with distilled water and quantitatively transferred in to 50ml volumetric flask. The volume was made up to a known volume with distilled water according to the method of [53] For Iron**,** Zinc and Lead in plant and water the solution obtained was measured by using the atomic absorption spectrophotometer (analytikjena, nov AA 350, Germany). Iron**,** Zinc and Lead determination by atomic absorption

4.6. Statistical analysis

A randomize complete block design with three factors [Plant (A), Concentrations (C) and Days (D)] was used for analysis all data was randomized complete block design with three replications for each parameter. The treatment means were compared by least significant differences (L.S.D.) test as given by[54]. The second test was performed to determine relationships between the treatments with correlation coefficients (R^2). All analyses were done by using the MSTAT program (MSTAT is written in the C programming language and runs on DOS compatible machines) [55]

5. Result and DISCUSSION

5.1. Heavy metal removal

The ability of *A. pinnata* and *L. minor* to remove iron from contaminated water after 20 days of incubation was shown in Fig.2. The values of residual Fe varied according to the initial concentration of iron. Results indicated that the best values of residual Fe. Results indicated that the best values of residual Fe achieved by *L. minor* after 20 days of incubation (4.51, 9.80 and 11.82 ppm) with removal efficiency 81.96%, 80.4% and 88.18%, respectively. While the values achieved by *A. pinnata* were (5.66, 6.56 and 13.03 ppm) with removal efficiency 77.6%, 86.88% and 86.97%, respectively by the different initial concentrations 25, 50 and 100 ppm, respectively after 20 days of incubation that when *A. pinnata* and *L. minor* were reached to the saturation level. [56] who reported that *L. minor* was able to remove greater amount of metals when there was high metal concentration was *added* in the solution The present study demonstrates that zinc removal efficiency of *A. pinnata* and *L. minor* contaminated water after 20 days more than 70 to 80% was shown in Fig.3

Results indicated that the best values of residual Fe achieved by *L.* minor after 20 days of incubation (6.72, 10.71 and 15.37 ppm) with removal efficiency 73.12%, 78.58% and 84.68%, respectively. While the values achieved by *A. pinnata* were (6.77, 9.03 and 17.86 ppm) with removal efficiency 72.92%, 81.94% and 82.14%, respectively by the different initial concentrations 25, 50 and 100 ppm, respectively after 20 days of incubation that when *A. pinnata* and *L. minor* were reached to the saturation level. In a previous study, *L. minor* was reported to accumulate higher amount of zinc as compared to *L. gibba* [57].

Zinc is an essential trace element which plays an important role in the growth and development of plants. Zinc is a most commonly found element in several enzyme The results of lead indicated a lower percentage of removal compared to iron and zinc *A. pinnata* and *L. minor* contaminated water after 20 days shown in Fig.4

Results indicated that the best values of residual Fe achieved by *L. minor* after 20 days of incubation (5.91, 11.79 and 28.42 ppm) with removal efficiency 76.36%, 76.42% and 71.58%, respectively. While the values achieved by *A. pinnata* were (9.99, 11.69 and 20.85ppm) with removal efficiency 60.04%, 76.62% and 79.15%, respectively by the different initial concentrations 25, 50 and 100 ppm, respectively after 20 days of incubation that when *A. pinnata* and *L. minor* were reached to the saturation level. *A. pinnata* possesses a remarkable capacity to hyperaccumulate heavy metals from polluted water bodies [58] Ex situ research carried out by [59],[60] , [61], [62], [63], [64], [65], [66] have shown the uptake and retention capacities of *A. pinnata* species to different heavy metal ions. These findings suggest at the potential and the applicability of *A.pinnata* species to phytoremediate heavy metal polluted water reservoir.

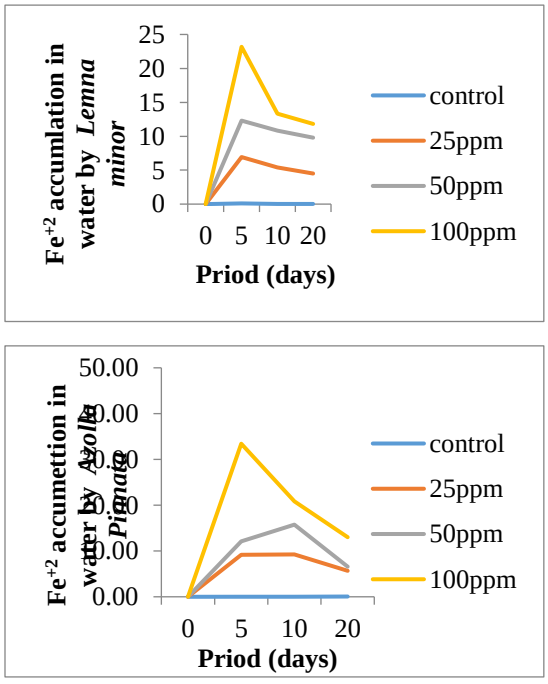


Fig. 2: Effect of different concentrations of of Iron (Fe^{+2}) on accumulation of this metal (ppm) by *L. minor and A. pinnata*.

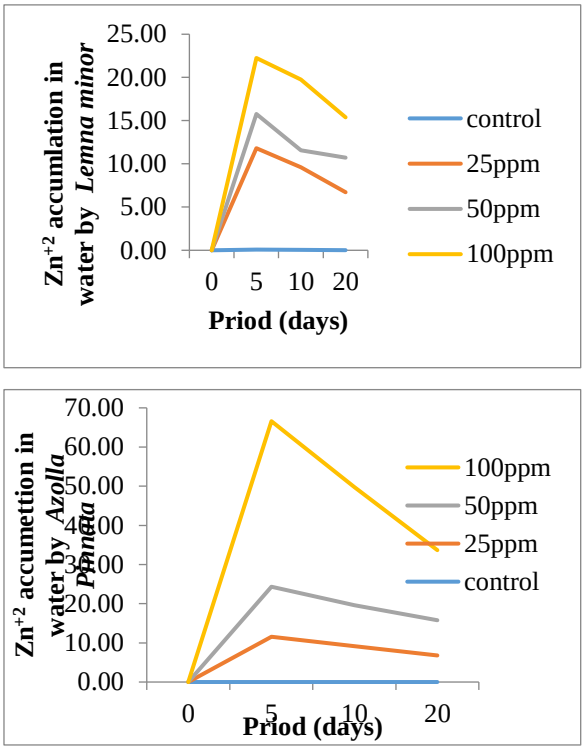


Fig. 3: Effect of different concentrations of Zinc (Zn+2) on accumulation of this metal (ppm) by L. minor and A. pinnata

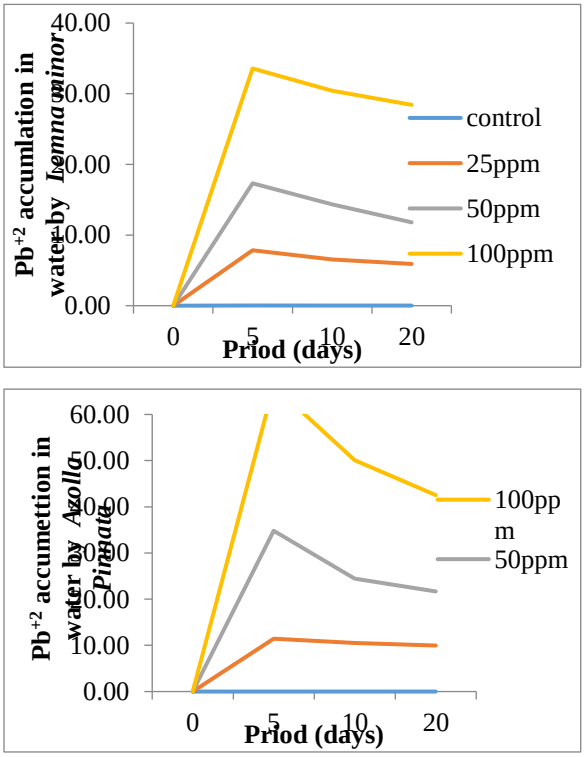


Fig. 4: Effect of different concentrations of Lead (Pb+2) on accumulation of this metal (ppm) by L. minor and A. pinnata

5.2. Growth parameters

5.2.1. Biomass

There was variation in biomass values during incubation period in *L. minor* and *A. pinnata* depending upon the combination of the levels of the parameters was shown in Fig. 5. In case of *A. pinnata*, the Highest value of fresh and dry wieght was recorded in control treatment after 20 days of incubation period it was (659.8 g / m^2 and 26.4 g /m^2). The lowest value was recorded (100 ppm) after 5 days; it was (316.7 g / m^2 and 12.7 g /m^2). It was shown that the treatment (Iron). Case of *L. minor*, the highest value of fresh and dry wieght was recorded in (100ppm) treatment after 20 days of incubation period it was (312.69 g / m^2 and 15.44 g /m^2). The lowest value was recorded in the control treatment after 5 days; it was (88.31 g / m^2 and 2.48g/m^2) of Iron respectively.

Doubling time of *A. pinnata* and *L.minor* growth generally increased with increasing the concentrations of Fe^{+2} from 25 to 100 ppm during all the tested incubation periods up to 20 days (Fig. 4). The lowest doubling time value was recorded at 25 ppm (17.39 and 24.37 days) and this value increased more than that of the control (16.05 and 23.93 days) after 20 days of incubation. Removal efficiency of *A. pinnata* showed the plant has different absorption potential for each metal with higher affinity for iron and lead and lower affinity for cadmium and zinc. *A. pinnata* has the potential to be used for absorption of iron and lead at high concentration of 25% produced water concentration.

The use of *A. pinnata* as a phytoremediation agent has also been reported by [67].

As for zinc, the highest value of fresh and dry wieght was of *A. pinnata* was recorded in(50 ppm) treatment after 20 days it was (1000g/m^2 and 40g/m^2). The lowest value was recorded (25 ppm) after 5 days; it was (419.6 g / m^2 and 16.8 g /m^2). Case of *L. minor*, the highest value of fresh and dry wieght was recorded in (100ppm) treatment after 20 days of incubation period it was (315.07 g / m^2 and 12.37 g /m^2). The lowest value was recorded in the control treatment after 5 days; it was (81.06 g / m^2 and 2.48g/m^2) of Zinc respectively.

Doubling time in Zinc of *A. pinnata* and *L. minor* generally decreased at treatment (control and 100ppm) respectively and then gradually increased from (25ppm) to (100ppm) up to 20 days of incubation. After 20 days of incubation, the doubling time gradually increased from (control and 100 ppm) to (100ppm) as illustrated in Fig. 6. The lowest value of the doubing time was obtained at treatment (control and 100ppm) (2.64 and 10.64 days) [68] The uptake ability and the Bio concentration factor of Azolla sps. for lead and Zinc increased with the increase of concentration in the growth medium. Azolla can absorb maximmum at only 4%. But its uptake capacity significantly increased with the increase of exposure time.

The highest value of fresh and dry wieght was of *A. pinnata* was recorded incontrol treatment after 20 days it was (659.8g/m^2 and 26.4g/m^2). The lowest value was recorded (100 ppm) after 5 days; it was (275.1 g / m^2 and 11.00 g /m^2). Case of *L. minor*, the highest value of fresh and dry wieght was recorded in (100ppm) treatment after 20 days of incubation period it was (279.85 g / m^2 and 14.46 g /m^2).

The lowest value was recorded in the (25ppm) treatment after 5 days; it was (80.85 g/$m^2$and 2.37g/m^2) of Lead respectively. Doubling time of *A. pinnata* and *L.minor* growth generally increased with increasing the concentrations of Pb^{+2} from 25 to 100 ppm during all the tested incubation periods up to 20 days Fig. 7. The lowest doubling time value was recorded at 25 ppm (17.39 and 24.37 days) and this value increased more than that of the control (16.05 and 23.93 days) after 20 days of incubation.

According to [69], *A. pinnata* doubles its biomass in less than two days in laboratory conditions and 5-10 days in normal field conditions. Moreover, [70] reported that doubling time in *A. pinnata* is 3 days, also [71] recorded a doubling time of 2.8 days for *A. pinnata*, while [72] reported higher biomass production by Azolla hybrids. The dense growth, consumption of nutrients and the production of some substances due to metabolic processes which may have a toxic effect on Azolla growth might be the main reasons of increasing doubling time of Azolla species under investigation [73] *A. pinnata* do not show any visible toxicity symptoms up to 50 ppm Pb treatment when was grown in different concentrations of C4H6O4Pb.3H2O, this result was similarly with that recorded by [74].

However, the highest value of Pb^{+2}accumulation by *L. minor* was recorded at 25 ppm after 20 days of incubation period. These results are in agreement with those of [75] According to [69] has revealed the role

of free floating macrophyte (*A. pinnata*) in phytoremediation technology has an excellent performance in removing the metals and was able to remove huge amount of heavy metals in 10 days of the experimentation period

Analysis of variance (ANOVA) was performed on the factors affecting on bioremediation as described in Table 1. There was highly significant difference between treatments (Residual Iron, Zinc and lead, Fresh, dry weight, Doubling time and removal efficiency (RE)) while there wasn't any significant difference between biomass and each other under the studied factors (Plant, concentrations and days) at 0.05 level.

5.3. Mean performance

Table 2 showed the mean performance of the three factors (Plant, concentrations and days) for studied treatments. The two aquatic plant were significantly different from one another at the 0.05 level. The results indicated that *L. minor* was better in removing Fe, Zn and Pb from water however *A. pinnata* showed better quality in pigments (Fresh, Dry weight and Doubling time). The different concentrations were significantly different from one another at the 0.05 level.

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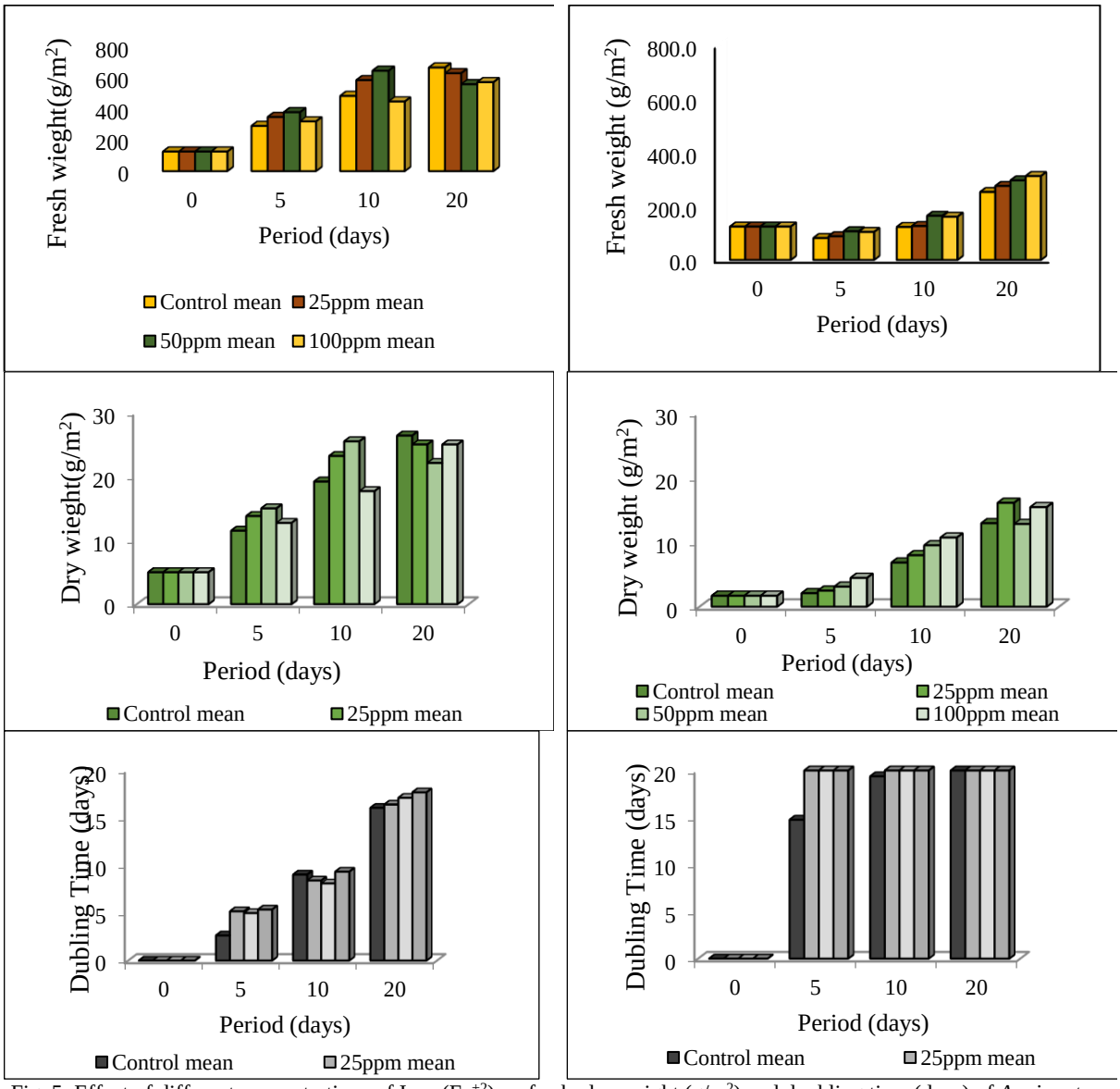
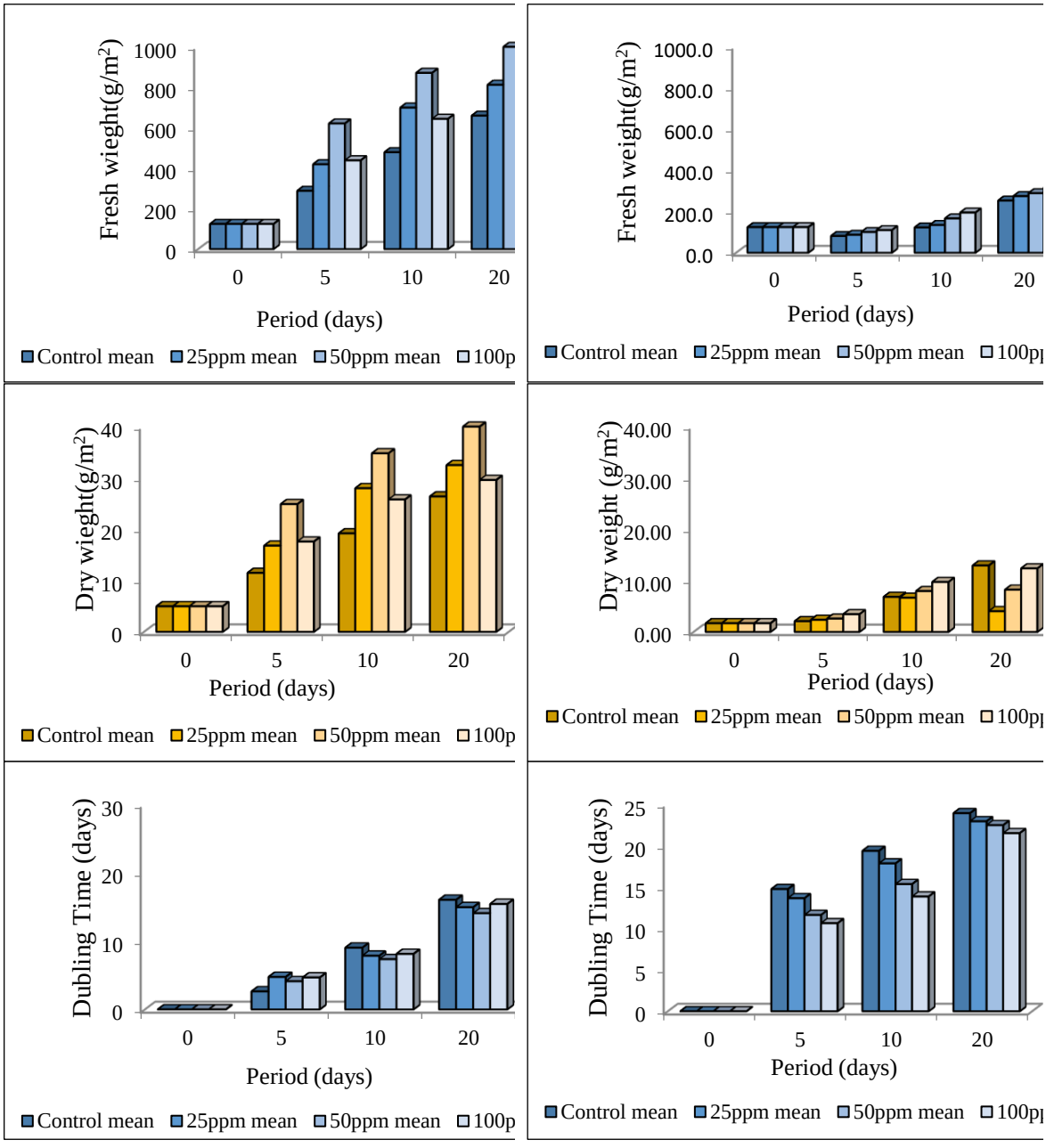


Fig. 5: Effect of different concentrations of Iron (Fe^{+2}) on fresh, dry weight (g/m^2) and doubling time (days) of *A. pinnata*. and *L. minor*.

The results indicated that *L. minor* was better in removing Fe, Zn and Pb from water and also was better in OD's value however *A. pinnata* showed better quality in pigments (fresh, Dry weight and D.T.). The different concentrations were significantly different from one another at the 0.05 level.

The results indicated that the better result for Res. Fe, Zn by different concentrations and after control treatment (C0) was the second concentration (C25), while Pb control treatment (C0) was the second concentration (C100), was there was significant difference between (C0, C25, C50 and C100) in Absorb. Plant for every heavy metal



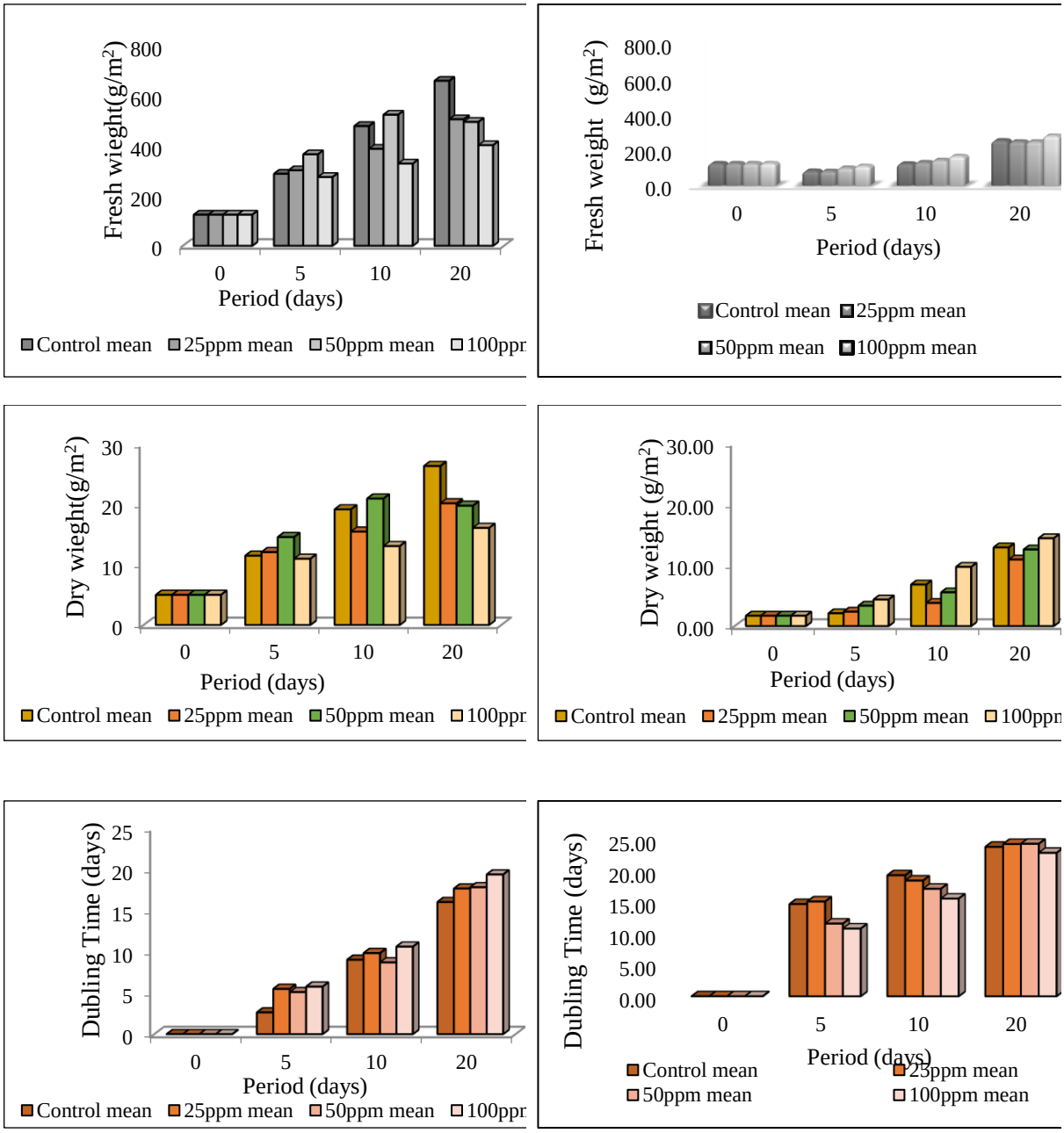


Fig. 7: Effect of different concentrations of Lead (Pb^{+2}) on fresh, dry weight (g/m^2) and doubling time (days) of *A. pinnata.* and *L. minor.*

In F .W and D.W a treatment for Fe, there wasn't any significant difference between (C25, C50) and in treatment for Zn and Pb that was there was significant difference between (C0, C25, C50 and C100) the better result, while the better result in D.T was achieved by control treatment (C100)I n each of the heavy metal there wasn't any significant difference between (C25, C50) and in treatment for Fe and Pb as for zn wasn't any significant difference between (C0, C25). Statistical Analysis results at the 0.05 level gave the best value of Res. Fe, Zn and Pb after 20 days of incubation and there were significantly different between different days (D0, D5, D10, D20), while the best results of Absorb. Plant Fe, Zn and Pb were achieved by the days (D5, D10) and there was significantly different between them, also there wasn't any significant difference between (D0, D4, D8). Pigments (fresh, dry weight and dubling time) also had significantly different from one another at the 0.05 level, the best results were obtained by the D20.

5.4. *Statistical analysis*

5.4.1. Analysis of variance

Table 1 Mean Square values of studied treatments from ANOVA table

Source of variation	D.F.	Mean Square			Source of variation	D.F.	Mean Square	
		F.W(g/m^2)	D.W(g/m^2)	D.T(D)			Res. in water Fe (ppm)	Absor.. in plant Fe (ppm)
Replications	2	860.614^{ns}	1.502^{ns}	0.004^{ns}	Replications	2	0.001^{ns}	0.002^{ns}
Plant (A)	1	2597.94**	10.689**	15.9243**	Plant (A)	1	55.770**	102.911**
Concentrations (C)	3	14406.298**	22.850**	2.717**	Concentrations (C)	3	776.750**	10029.275**
AC	3	84904.62**	571.874**	1135.67**	AC	6	429.06**	11830.146**
Days (D)	3	533397.804**	852.675**	612.215**	Days (D)	2	170.942**	471.654**
AD	9	438.19**	2.358**	2.697**	AD	6	22.540**	25.769**
CD	9	9481,140**	15.259**	1.579**	CD	6	74.566**	93.295**
Error	30	585.598	0.922	0.020	Error	22	0.001	0.01
Source of variation	D.F.	Mean Square			Source of variation	D.F.	Mean Square	
		F.W(g/m^2)	D.W(g/m^2)	D.T(D)			Res. in water Zn (ppm)	Absor.. in plant Zn (ppm)
Replications	2	1154.712^{ns}	1.828**	0.001^{ns}	Replications	2	0.001^{ns}	0.003^{ns}
Plant (A)	1	3787.81**	20.904**	11.41**	Plant (A)	1	54.842**	137.481**
Concentrations (C)	3	145260.361**	232.478**	1.040**	Concentrations (C)	3	1440.353**	8382.172**
AC	3	82483.73**	172.287**	1234.65**	AC	6	568.804**	9614.911**
Days (D)	3	1064532.49**	1701.660**	499.862**	Days (D)	2	203.515**	193.580**
AD	9	639.23**	12.4843**	2.791**	AD	6	7.719**	17.455**
CD	9	17703.659**	28.361**	1.799**	CD	6	90.383**	82.229**
Error	30	1036.150	1.667	0.003	Error	22	0.002	0.001
Source of variation	D.F.	Mean Square			Source of variation	D.F.	Mean Square	
		F.W(g/m^2)	D.W(g/m^2)	D.T(D)			Res. in water Pb (ppm)	Absor.. in plant Pb (ppm)
Replications	2	882.218^{ns}	1.474^{ns}	0.016^{ns}	Replications	2	0.212^{ns}	0.024^{ns}
Plant (A)	1	1524.24**	16.707**	21.65**	Plant (A)	1	30.267**	150.419**
Concentrations (C)	3	27938.092**	44.211**	8.454**	Concentrations (C)	3	1059.502**	8803.514**
AC	3	61785.48**	291.309**	1106.55**	AC	6	1578.974**	9028.48**
Days (D)	3	546148.96**	554.256**	687.582**	Days (D)	2	109.574**	208.502
AD	9	261.212**	3.82**	3.53**	AD	6	5.310**	33.264**
CD	9	11417.926**	18.154**	1.847**	CD	6	30.225**	37.869**
Error	30	589.263	1.037	0.20	Error	22	0.113	0.065

(ns) No significant difference between the treatments. ** Highly significant difference between the treatments (P ≤0.05).

6. Conclusion

Contaminated water with toxic heavy metals is a serious environmental problem which may be solved with bioremediation. In the present study, two cyanobacteria sp. (*A. pinnata* and *L. minor*) were tested to remove Iron, Zinc and lead at four concentrations (0, 25, 50 and 100 mg/ L).

The main conclusions of this research are:

- It was proved that aquatic ecosystems and effective method to treat contaminated water.
- *L. minor* was found to be more effective than *A. pinnata* for bioremoval of Iron, Zinc and lead from contaminated water.
- Based on these results, biomass of *L. minor* and *A. pinnata* can be used as an efficient low cost biomass for the removal of Iron, Zinc and lead from wastewater
- It's recommended to increasing the experiment after 20 days of incubation period to get the highest efficiency of Iron, Zinc and lead removal by the two aquatic plants sp.

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Table 2 Mean performance of three factors under study (Plant, concentrations and days) for studied treatments.

Treatments	F.W(g/m^2)	D.W(g/m^2)	D.T(D)	Treatments	Res. in water Fe (ppm)	Abso. in plant Fe (ppm)
Plant (A)				Plant (A)		
A1	312.7a	15.82a	315.08a	A1	23.18a	88.96a
A2	296.6b	12.44b	289.b	A2	13.33b	87.20b
F. Test	**	**	**	F. Test	**	**
Concentrations (C)				Concentrations (C)		
C0	387.8b	15.52b	6.932c	C0	0.05d	145.5d
C25	418.0a	16.72a	7.496b	C25	5.62c	155.3c
C50	422.9a	16.91a	7.550b	C50	10.97b	170.2b
C100	347.6c	13.91c	8.096a	C100	16.11a	186.2a
LSD	20.18	0.8006	0.117	LSD	0.285	0.0083
Days (D)				Days (D)		
D0	124.4d	4.980d	0.00d			
D5	331.1c	13.25c	4.544c	D5	10.63a	24.89c
D10	535.1b	21.39b	8.725b	D10	7.40b	32.17b
D20	585.8a	23.44a	16.80a	D20	6.53c	37.37a
LSD	20.19	0.8006	0.117	LSD	0.246	0.026
Treatments	F.W(g/m^2)	D.W(g/m^2)	D.T(D)	Treatments	Res. in water Zn(ppm)	Abso. in plant Zn(ppm)
Plant (A)				Plant (A)		
A1	247.1b	12.37b	22.95b	A1	19.77b	82.13a
A2	164.8a	12.94a	23.93a	A2	22.35a	74.74b
F. Test	**	**	**	F. Test	**	**
Conc. (C)				Conc. (C)		
C0	387.8d	15.52c	6.93b	C0	0.037d	0.29d
C25	514.1b	20.56b	6.89b	C25	8.021c	15.57c
C50	654.0a	26.18a	6.392c	C50	11.48b	33.73b
C100	487.2c	19.49b	7.06a	C100	22.43a	75.92a
LSD	26.84	1.076	0.045	LSD	0.043	0.075
Days (D)				Days (D)		
D0	124.4d	4.98d	0.00d			
D5	442.1c	17.69c	4.050c	D5	13.69a	28.10c
D10	673.78b	26.95b	8.097b	D10	11.47b	31.17b
D20	803.3a	32.12a	15.14a	D20	6.32c	43.86a
LSD	26.85	1.076	0.045	LSD	0.037	0.065
Treatments	F.W(g/m^2)	D.W(g/m^2)	D.T(D)	Treatments	Res. in water Pb (ppm)	Abso. in plant Pb (ppm)
Plant (A)				Plant (A)		
A1	279.9a	14.46a	22.94b	A1	30.40b	78.40b
A2	253.4b	12.94b	24.41a	A2	33.57a	79.75a
F. Test	**	**	**	F. Test	**	**
Concentrations (C)				Concentrations (C)		
C0	387.8a	15.52a	6.93d	C0	0.00d	0.025d
C25	330.2b	13.20b	8.25b	C25	10.65c	14.81c
C50	377.5a	15.02a	7.91c	C50	16.34b	40.35b
C100	282.7c	11.30c	8.94a	C100	5.95a	69.54a
LSD	20.24	0.849	0.117	LSD	0.328	0.249
Days (D)				Days (D)		
D0	124.4d	4.98d	0.00d			
D5	307.8c	12.22c	4.75c	D5	16.55a	27.08c
D10	430.0b	17.19b	9.56b	D10	12.52b	31.35b
D20	516.0a	20.64a	17.74a	D20	10.63c	35.11a
LSD	20.24	0.849	0.117	LSD	0.284	0.215

Similarity between one or more letters indicates no statistically significant differences ($P \leq 0.05$)

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