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Phytoaccumulation of Lead from Wastewater by *Azolla pinnata* and *Lemna gibba* for Comparative Assessment

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ABSTRACT

In this study, two aquatic macrophytes namely, *Azolla pinnata* and *Lemna gibba* 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 investigate their bioindicative value by evaluating their ability to accumulate different concentrations of Pb^{+2} (0, 10, 20, 30, 40 and 50 ppm) in the form of $Pb(No_3)_2$ for 25 days experimentation period under greenhouse conditions. The results indicated that, *A. pinnata* exhibited more tolerance for Pb^{+2} than *L. gibba* and recorded the highest values of fresh, dry weights, the lowest values of doubling time and showed the highest capacity of accumulation during all the tested incubation periods in comparison to that recorded with *L. gibba*. Accordingly, *A. pinnata* is a good accumulator and is a potential candidate for the removal of Pb^{+2} from wastewater.

Key words: *Azolla pinnata*, *Lemna gibba*, Phytoaccumulation, Wastewater, Lead

Introduction

The environmental pollution is one of the most severe problems nowadays. Among various water pollutants, heavy metals are of major concern because of their persistent and bioaccumulative nature (Chang *et al*., 2009). Heavy metal contamination in aquatic and soil environments is a serious environmental problem, which threatens aquatic ecosystems, agriculture, water resources and human health (Overesch *et al*., 2007). Heavy metals are metallic chemical elements with a high atomic weight and density much greater (at least five times) than water (Anjuli *et al*., 2012). When the heavy metals absorbed by the human body, some kinds of them can react with human physiology odd molecular substances such as protein and enzyme (Dejun *et al*., 2014). A high concentration of lead can be found in industrial wastewater, in domestic detergents and other laundry products and in cigarettes (Celebi and Kendir, 2002). The major source of environmental lead is metal smelting (Caussy *et al*., 2003), but agriculture, industry and urban activities are also important sources of Pb pollution (Marchiol *et al*., 2004).

Lead is one of the very toxic heavy metals that affect the entire food chain and disrupt the health system of human beings, animals and phytoplanktons (Divya *et al*., 2012). In human, it is absorbed directly into the blood stream and stored in soft tissues, bones and teeth (95% in bones and teeth) (David *et al.,* 2003). Chronic intoxication can lead to encephalopathy mainly in children (Jordao *et al*., 2002). Because heavy metal pollution affects the quality of drinking water supply and wastewater discharge, great efforts have been made in the last two decades to reduce pollution sources and remedy polluted water resources (Akpor and Muchie, 2010).

The term ‘‘phytoremediation’’ is a combination of two words: Greek phyto (meaning plant) and Latin remedium (meaning to correct or remove an evil) (Hazrat *et al*., 2013). Phytoremediation has also been called green remediation, botano-remediation, agro-remediation and vegetative remediation (Erakhrumen, 2007). Phytoremediation can be prepared from the naturally abundant plants which are very economical (Rai, 2011). 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 (Alaa and Elsayed, 2015).

The application of *Azolla* and *Lemna* is a very common practice in phytoremediation, because they have very good potential for hyperaccumulation of different pollutants, minerals and heavy metals, restoring polluted aquatic resources (Hossein *et al*., 2014). *Azolla* spp. is heterosporous free-floating freshwater ferns that

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live symbiotically with *Anabaena azollae*, a nitrogen-fixing blue-green algae (Punita and Soma, 2015). Extensive work has been done using *Azolla* in the removal of heavy metals from aquatic environments (Rai and Tripathi, 2009; Rai, 2010). *Azolla* is a better macrophyte for aquatic phytoremediation because of its short doubling time (2-3 d), easy harvest, nitrogen fixation ability and tolerance to and accumulation of a wide range of heavy metals (Sood *et al*., 2012).

According to Stêpniewska *et al*. (2005), *Azolla* can bioaccumulate heavy metals and also remove organic substances from wastewater. It has been reported that *Azolla* has a high capacity to accumulate toxic elements such as mercury, cadmium, chromium, copper, nickel and zinc (Rai, 2008) and can be used to remove contaminants from wastewater (Arora and Saxena, 2005; Rakhshaee *et al*., 2006). This fern can also remove nutrients (Forni *et al*., 2001) and organic substances like sulphonamides (Forni *et al*., 2002). The bioaccumulation potential of *Azolla* spp. for various heavy metals has been compared with other aquatic macrophytes by many workers (Rai and Tripathi, 2009; Rai, 2010).

Lemnaceae (duckweeds) are small aquatic herbs that are widely dispersed below or on the surface of water (Iram *et al*., 2012). Previous studies have demonstrated that, several species of duckweeds can be used to remove toxic heavy metals and organic compounds from wastewater (Öbek and Sasmaz, 2011). 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). Megateli *et al*. (2009) reported that *L. gibba* can accumulate large amounts of metals and has great potential for phytoremediation.

Therefore, the aim of this investigation is to evaluate the role of *A. pinnata* and *L. gibba* in accumulation of heavy metals such as lead existing in wastewater. *A. pinnata* and *L. gibba* were compared for their growth, fresh, dry weights, doubling time and Pb^{+2}accumulation.

Materials and Methods

Propagation of A. pinnata and L. gibba

A. pinnata and *L. gibba* used in this study were kindly provided by Agric. Microbial Dept., Soils, Water and Environ. Res. Inst. (SWERI), Agric. Res. Center (ARC), Giza, Egypt. *A. pinnata* was cultured on modified Yoshida medium (Yoshida *et al*., 1976) and *L. gibba* was cultured on Hoagland medium (Hoagland and Arnon, 1950). *A. pinnata* and *L. gibba* (10 g) were grown separately in plastic pots (32 cm in diameter and 15 cm depth) containing 1kg clay soil saturated with 3 liters of tap water. These pots were kept in a greenhouse till *A. pinnata* and *L. gibba* covered the entire water surface according to El- Shahat (1988). Then they were collected and washed gently in running deionized water for several times by using 0.2 meshes screen and then the plants were air dried for 30 min.

Composition of modified Yoshida medium (Yoshida et al., 1976)

Modified Yoshida medium contained of 40.00 mg L^{-1} $NaH_2PO_4.H_2O$, 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} H_3BO_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.

Composition of Hoagland medium (Hoagland and Arnon, 1950)

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} H_3BO_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.

Experimental procedure

This experiment was carried out in the greenhouse of Soils, Water and Environ. Res. Inst. (SWERI), Agric. Res. Center (ARC), Giza, Egypt during July 2014. Cultivation of *A. pinnata* and *L. gibba* was carried out in plastic pots separately (10.0 cm diameter and 7.0 cm in depth). Pots were filled with 1000 ml of medium (Yoshida medium for *A. pinnata* and Hoagland medium for *L. gibba*) and supplemented with different concentrations of Pb^{+2}. Wastewater samples were prepared by dissolving their corresponding analytical grade salts of $Pb(No_3)_2$ in deionized water at nominal concentrations of 0, 10, 20, 30, 40 and 50 ppm. The pots were inoculated with 1 g fresh of *A. pinnata* and *L. gibba* separately, which was used as a standard inoculum in all experiments (El- Berashi, 2008). Every concentration of Pb^{+2} was represented by 3 replicates which carried out for this treatment. The inoculated pots were incubated at 35°C ± 2, 14 hr light and 10 hr dark for 25 days under greenhouse conditions. Samples of the treatments were taken after zero time, 5, 10, 15, 20 and 25 days of incubation.

Control treatment (plants without metal) which contained only a nutrient medium, was used to compare it with the effects Pb^{+2} concentrations on fresh, dry weight (El-Shahat, 1997), doubling time of *A. pinnata* and *L. gibba* growth and the accumulation of Pb^{+2} by these plants were determined on dry weight basis by using Inductively Coupled Plasma Spectrometry (ICP) (Model Ultima 2 JY Plasma- Jobin Yvon) according to

Chapman and Pratt (1961). *Azolla* and *Lemna* cultures were kept at a constant volume throughout the experimental periods by frequent changing of culture medium every 5 days to compensate water loss by evaporation when it is necessary (El- Berashi, 2008).

The measured parameters

Fresh and Dry Weight

Samples of *A. pinnata* and *L. gibba* fronds were harvested, washed by deionized water and placed under shade between two thick layers of blotting tissue papers for approximately 1 hr. before determining fresh weight. The dry weight of *A. pinnata* and *L. gibba* was determined by drying fronds in an oven at 70°C to constant weight. Fresh and dry weights of *A. pinnata* and *L. gibba* were expressed as g/m^2 (El- Berashi, 2008).

Doubling time calculation

Growth rate of *A. pinnata* and *L. gibba* in terms of doubling time (D.T.) was calculated by using the following equation according to Aziz and Watanabe (1983):

Doubling time = t/r, whereas:

t = the duration of *Azolla* and *Lemna* growth,
r = [log (wt/wo) / 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.

Determination of Pb^{+2} accumulation by A. pinnata and L. gibba

Before digestion to analyze heavy metals, harvested plants were washed with deionized water, air dried, dried at 70°C until constant weight and weighted for the dry weight. The digestion method was applied involving sulfuric acid and perchloric acid as wet digestion procedure according to Chapman and Pratt (1961). 0.1 g dry weight of *A. pinnata* or *L. gibba* was used for digestion for each sample. Concentrations of Pb^{+2} were determined by using ICP (Chapman and Pratt, 1961). Read of the instrument (mg L^{-1}) multiplied by an inverted extraction ratio (total volume for sample (cm) / sample weight (g)) = mg kg^{-1}.

Statistical analysis

The data were presented by mean ± standard deviation (n=3). Statistical analysis was carried out as a randomized complete design (Snedecor and Cochran, 1980) using LSD test to compare means of treatments in investigation. Statistical significance was defined as $p < 0.05$.

Results

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

Fresh and dry weights gradually increased with increasing the incubation period from zero time up to 25 days. Fresh and dry weights of *A. pinnata* also gradually increased with increasing the concentrations of Pb^{+2} from 10 to 40 ppm and then decreased at 50 ppm after 20 days of incubation. After 25 days of incubation, fresh and dry weights gradually decreased with increasing the concentrations of Pb^{+2} from 10 up to 50 ppm as illustrated in (Table and Fig. (1)). Maximum growth density was observed for *A. pinnata* at 40 ppm (823.95 ± 30.07 and 61.80 ± 2.26 g/m^2) for fresh and dry weight, respectively after 20 days of incubation. These parameters were compared with the control (733.33 ± 24.61 and 55.00 ± 1.85 g/m^2) for fresh and dry weight, respectively. The values of fresh and dry weights were significantly different at 40 ppm compared to the control after 20 days of incubation.

Doubling time of *A. pinnata* growth generally decreased with increasing the concentrations of Pb^{+2} from 10 to 40 ppm and then increased at 50 ppm after 20 days of incubation (Table and Fig. (1)). After 25 days of incubation, the doubling time gradually increased with increasing the concentrations of Pb^{+2} from 10 up to 50 ppm. The lowest value of doubling time was clearly demonstrated at 40 ppm (7.46 ± 0.15 days) and this value decreased less than that of the control (7.94 ± 0.16 days) after 20 days of incubation.

Fresh and dry weights of *L. gibba* gradually decreased with increasing the concentrations of Pb^{+2} from 10 up to 50 ppm during all the tested incubation periods from zero time up to 25 days as illustrated in (Table and Fig. (2)). The highest values of *L. gibba* were observed at 10 ppm (745.43 ± 27.11 and 52.18 ± 1.90 g/m^2) for fresh and dry weights, respectively after 25 days of incubation. These parameters were compared with the control (796.71 ± 34.86 and 55.77 ± 2.45 g/m^2) for fresh and dry weights, respectively. There was non significant difference between fresh, dry weights at all concentrations and control during all the tested incubation periods from zero time up to 25 days. Doubling time of *L. gibba* growth generally increased with

increasing the concentrations of Pb^{+2} from 10 to 50 ppm during all the tested incubation periods up to 25 days (Table and Fig. (2)). The lowest doubling time value was recorded at 10 ppm (9.84 ± 0.20 days) and this value increased more than that of the control (9.47 ± 0.24 days) after 25 days of incubation.

Table 1: Effect of different concentrations of Lead (Pb^{+2}) on fresh, dry weight (g/m^2) and doubling time (days) of *A. pinnata* (Data expressed as mean ± SD).

Period (days) / Concentrations (ppm)	F.wt. (g/m^2)						D.wt. (g/m^2)						D.t. (days)					
	Zero-time	5	10	15	20	25	Zero-time	5	10	15	20	25	Zero-time	5	10	15	20	25
Control	128.21	333.33 ± 11.05	393.20 ± 9.26	543.60 ± 17.34	733.33 ± 24.61	1135.07 ± 31.76	9.62	25.00 ± 0.83	29.49 ± 0.69	40.77 ± 1.30	55.00 ± 1.85	85.13 ± 2.38	0.00	3.62 ± 0.12	6.17 ± 0.12	7.21 ± 0.14	7.94 ± 0.16	7.94 ± 0.10
10	128.21	340.18 ±10.55	406.80 ± 13.46	557.24 ± 18.78	770.93 ± 25.87	947.02 ± 29.12	9.62	25.51 ± 0.80	30.51 ± 1.01	41.79 ± 1.41	57.82 ± 1.94	71.03 ± 2.26	0.00	3.55 ± 0.12	5.99 ± 0.16	7.08 ± 0.15	7.72 ± 0.15	8.65 ± 0.14
20	128.21	352.18 ± 9.24	435.91 ± 17.29	632.49 ± 22.37	810.27 ± 23.14	933.34 ± 27.35	9.62	26.41 ± 0.70	32.69 ± 1.30	47.44 ± 1.68	60.77 ± 1.74	70.00 ± 2.05	0.00	3.42 ± 0.10	5.65 ± 0.20	6.52 ± 0.16	7.52 ± 0.13	8.74 ± 0.14
30	128.21	364.09 ± 13.26	458.13 ± 12.88	694.00 ± 21.05	818.80 ± 26.32	899.16 ± 26.74	9.62	27.31 ± 1.00	34.36 ± 0.96	52.05 ± 1.65	61.41 ± 1.97	67.44 ± 2.01	0.00	3.31 ± 0.12	5.43 ± 0.11	6.15 ± 0.12	7.46 ± 0.13	8.90 ± 0.13
40	128.21	379.51 ± 15.19	487.20 ± 20.20	705.96 ± 24.43	823.95 ± 30.07	858.13 ± 26.30	9.62	28.46 ± 1.14	36.54 ± 1.52	52.95 ± 1.83	61.80 ± 2.26	64.36 ± 1.97	0.00	3.18 ± 0.11	5.18 ± 0.22	6.10 ± 0.12	7.46 ± 0.15	9.12 ± 0.15
50	128.21	348.76 ± 10.82	418.80 ± 9.63	599.96 ± 18.93	668.36 ± 22.44	752.09 ± 24.15	9.62	26.16 ± 0.81	31.41 ± 0.73	45.00 ± 1.42	50.13 ± 1.76	56.41 ± 1.81	0.00	3.47 ± 0.09	5.85 ± 0.12	6.73 ± 0.14	8.40 ± 0.18	9.80 ± 0.17
LSD at 0.05	-	21.080	29.317	36.692	45.419	49.229	-	1.580	1.910	2.773	3.428	3.780	-	0.190	0.280	0.436	0.624	0.649

F.wt: Fresh weight; D.wt: Dry weight; D.t: Doubling time; L.S.D: Least Significant Differences

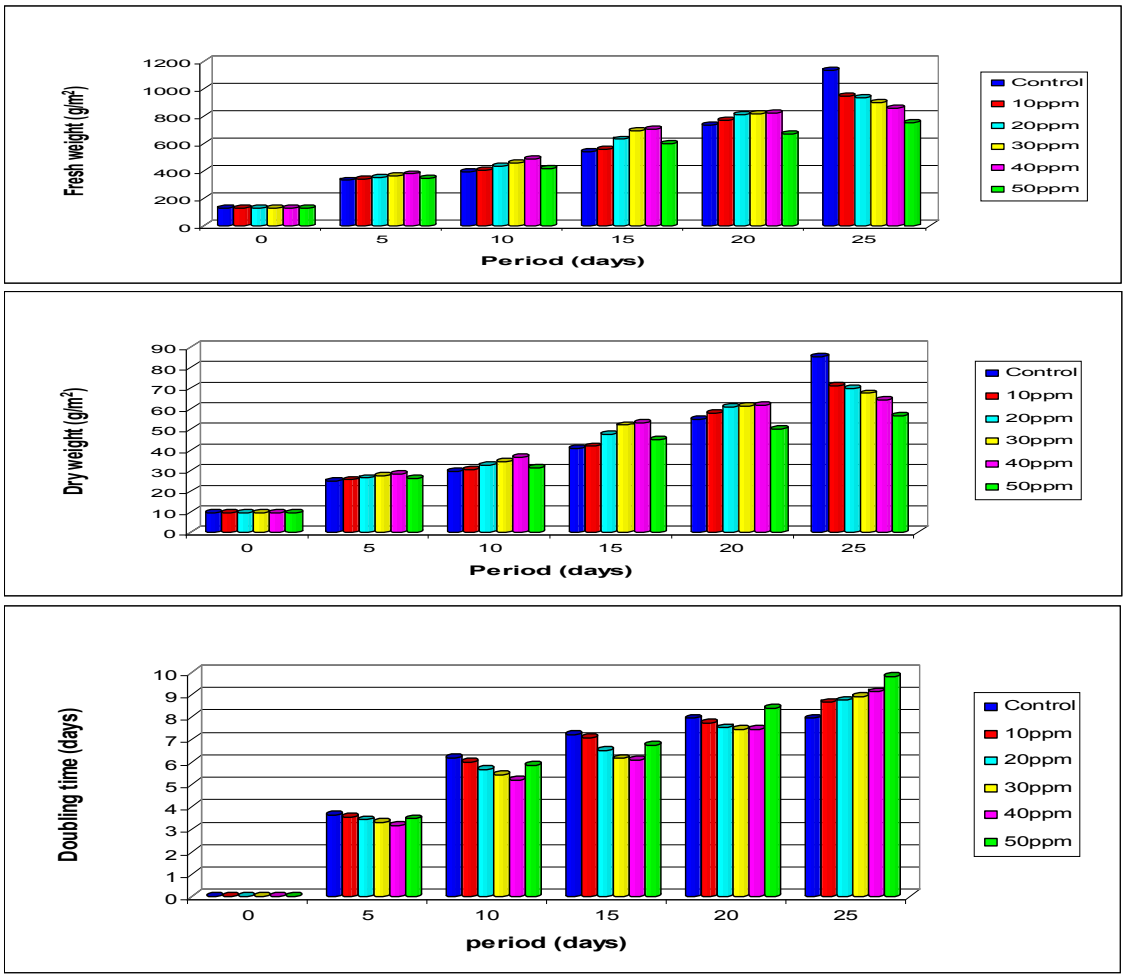

Fig. 1: Effect of different concentrations of Lead (Pb^{+2}) on fresh, dry weight (g/m^2) and doubling time (days) of *A. pinnata.*

Table 2: Effect of different concentrations of Lead (Pb^{+2}) on fresh, dry weight (g/m^2) and doubling time (days) of *L. gibba* (Data expressed as mean ± SD).

Period (days) / Concentrations (ppm)	F.wt. (g/m^2)						D.wt. (g/m^2)						D.t. (days)					
	Zero-time	5	10	15	20	25	Zero-time	5	10	15	20	25	Zero-time	5	10	15	20	25
Control	128.21	307.66 ± 13.77	386.43 ± 27.10	525.57 ± 26.27	598.86 ± 33.63	796.71 ± 34.86	9.62	21.54 ± 0.97	27.05 ± 1.90	36.79 ± 1.84	41.92 ± 2.36	55.77 ± 2.45	0.00	3.97 ± 0.21	6.29 ± 0.39	7.35 ± 0.27	9.01 ± 0.33	9.47 ± 0.24
10	128.21	261.91 ± 11.06	316.85 ± 15.73	428.62 ± 20.18	540.29 ± 23.94	745.43 ± 27.11	9.62	18.33 ± 0.77	22.18 ± 1.10	30.00 ± 1.41	37.82 ± 1.63	52.18 ± 1.90	0.00	4.85 ± 0.28	7.63 ± 0.41	8.62 ± 0.32	9.62 ± 0.30	9.84 ± 0.20
20	128.21	232.62 ± 10.87	294.90 ± 16.94	390.09 ± 22.04	516.43 ± 23.43	727.71 ± 34.81	9.62	16.28 ± 0.76	20.64 ± 1.19	27.31 ± 1.55	36.15 ± 1.64	50.94 ± 2.44	0.00	5.81 ± 0.45	8.33 ± 0.61	9.32 ± 0.47	9.95 ± 0.33	9.96 ± 0.26
30	128.21	214.33 ± 9.87	289.38 ± 14.65	369.95 ± 15.86	470.71 ± 20.30	538.43 ± 23.93	9.62	15.00 ± 0.69	20.26 ± 1.03	25.90 ± 1.11	32.95 ± 1.42	37.69 ± 1.68	0.00	6.76 ± 0.65	8.55 ± 0.55	9.80 ± 0.39	10.64 ± 0.38	12.08 ± 0.35
40	128.21	204.71 ± 8.24	263.67 ± 12.54	349.81 ± 17.59	412.05 ± 19.38	511.00 ± 23.28	9.62	14.33 ± 0.57	18.46 ± 0.88	24.49 ± 1.23	28.84 ± 1.36	35.77 ± 1.63	0.00	7.35 ± 0.61	9.62 ± 0.65	10.34 ± 0.54	11.90 ± 0.46	12.50 ± 0.42
50	128.21	198.57 ± 7.94	243.57 ± 13.28	338.81 ± 15.12	391.95 ± 16.38	481.71 ± 20.80	9.62	13.90 ± 0.56	17.05 ± 0.93	23.72 ± 1.06	27.44 ± 1.15	33.72 ± 1.46	0.00	7.94 ± 0.69	10.75 ± 0.93	10.71 ± 0.46	12.42 ± 0.46	13.09 ± 0.41
LSD at 0.05	-	18.634	30.960	33.151	41.775	48.590	-	1.304	2.166	2.476	2.846	3.494	-	0.913	1.095	0.746	0.676	0.577

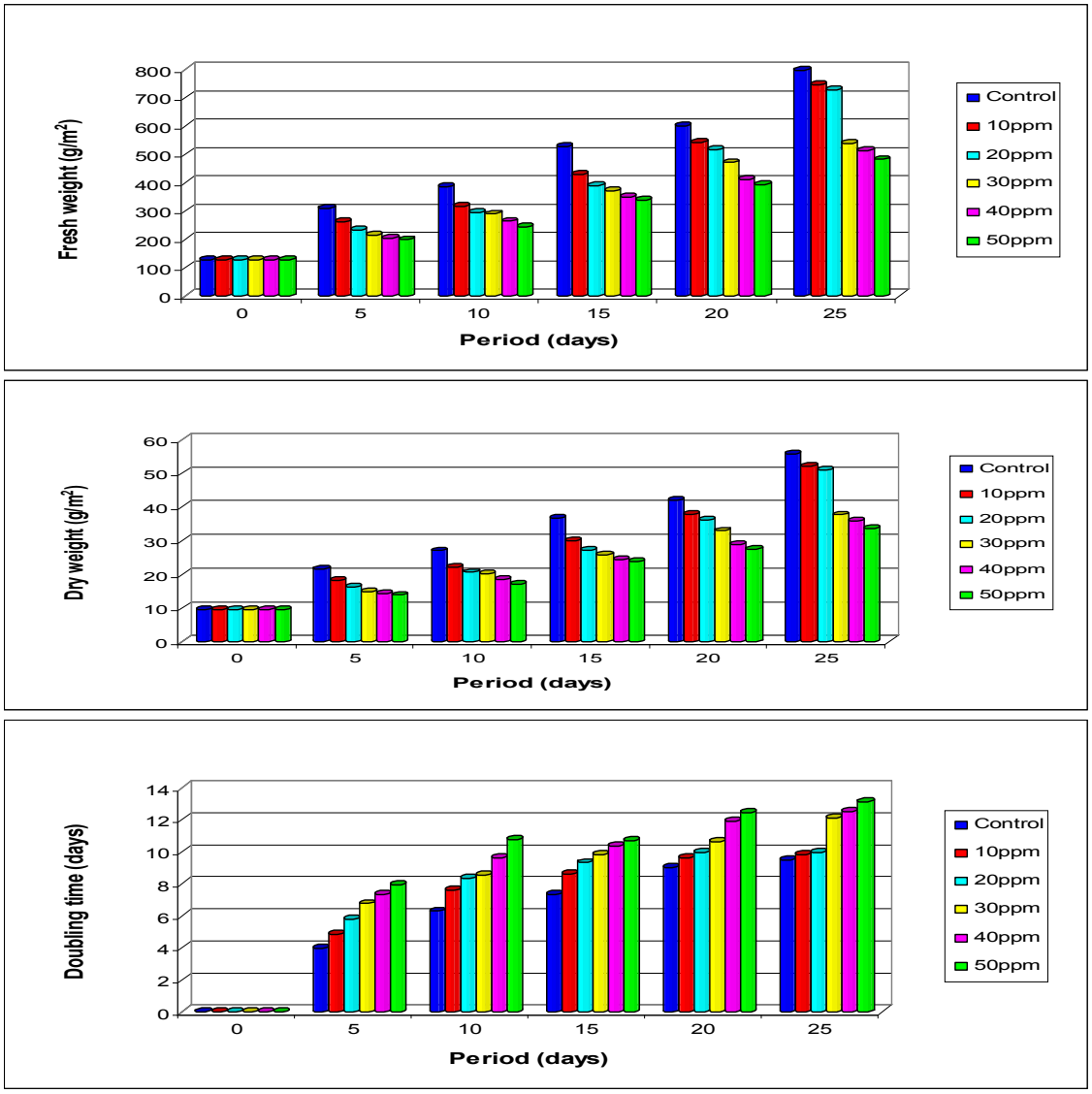

Fig. 2: Effect of different concentrations of Lead (Pb^{+2}) on fresh, dry weight (g/m^2) and doubling time (days) of *L. gibba*.

Previous results showed that, *A. pinnata* gave higher growth density than that recorded for *L. gibba* during all the tested incubation periods from zero time up to 25 days. After 20 days of incubation, *A. pinnata*

gave higher value of fresh and dry weight at 40 ppm (823.95 ± 30.07 and 61.80 ± 2.26 g/m^2), respectively in comparison to that of *L. gibba* (412.05 ± 19.38 and 28.84 ± 1.36 g/m^2) as illustrated in Tables (1, 2). Concering the doubling time of *A. pinnata* and *L. gibba*, the longevity of period required for doubling the fresh weight of *A. pinnata* and *L. gibba*, gradually increased by the time with considerable differences between them. The doubling time was recorded at 40 ppm (7.46 ± 0.15 and 11.90 ± 0.46 days) for *A. pinnata* and *L. gibba*, respectively after 20 days of incubation.

Effect of different concentrations of Lead (Pb^{+2}) on accumulation of this metal (g/m^2) by *A. pinnata* and *L. gibba*.

Results showed that, Pb^{+2}accumulation by *A. pinnata* was increased with increasing the concentrations of Pb^{+2} from 10 up to 40 ppm and then decreased at 50 ppm after 20 days of incubation as illustrated in (Table and Fig. (3)). After 25 days of incubation, accumulation of Pb^{+2} decreased with increasing the concentrations of Pb^{+2} from 10 up to 50 ppm. Hence, the greatest accumulation of Pb^{+2} was observed in *Azolla* fronds grown with 40 ppm (854.54 ± 26.51 g/m^2) after 20 days of incubation. The values of Pb^{+2}accumulation by *A. pinnata* were highly significantly different compared to the control at all concentrations during all the tested incubation periods from zero time up to 25 days.

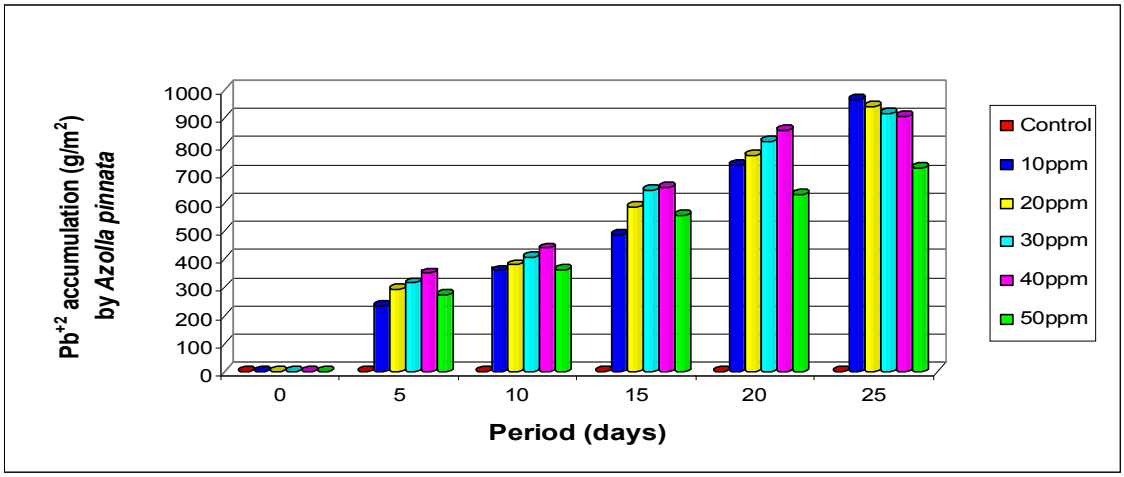

Fig. 3: Effect of different concentrations of Lead (Pb^{+2}) on accumulation of this metal (g/m^2) by *A. pinnata*.

Table 3: Effect of different concentrations of Lead (Pb^{+2}) on accumulation of this metal (g/m^2) by *A. pinnata* (Data expressed as mean ± SD).

Concentrations (ppm) \ Period (days)	Pb^{+2} accumulation (g/m^2)					
	Zero-time	5	10	15	20	25
Control	0.00	0.00	0.00	0.00	0.00	0.00
10	0.00	233.16 ± 6.89	359.34 ± 13.00	483.41 ± 15.90	732.78 ± 23.71	963.15 ± 28.63
20	0.00	291.16 ± 7.62	378.53 ± 15.38	584.58 ± 17.96	767.37 ± 25.18	939.75 ± 27.21
30	0.00	314.33 ± 7.61	405.61 ± 15.23	643.21 ± 21.36	816.37 ± 22.19	914.32 ± 25.34
40	0.00	349.52 ± 9.22	437.89 ± 16.54	652.87 ± 22.05	854.54 ± 26.51	903.15 ± 26.97
50	0.00	273.61 ± 6.31	362.70 ± 11.12	553.28 ± 15.11	627.88 ± 22.26	720.50 ± 23.88
LSD at 0.05	-	12.328	23.362	30.349	39.025	42.966

Results showed that, Pb^{+2}accumulation by *L. gibba* was generally decreased from 10 up to 50 ppm during all the tested incubation periods from zero time up to 25 days as illustrated in (Table and Fig. (4)). However, the highest value of Pb^{+2}accumulation was recorded at 10 ppm (286.04 ± 10.79 g/m^2) after 25 days of incubation. The values of Pb^{+2}accumulation by *L. gibba* were highly significantly different compared to the control at all concentrations during all the tested incubation periods from zero time up to 25 days. Previous results showed that, *A. pinnata* gave the highest results of Pb^{+2} accumulation than that recorded for *L. gibba* during all the tested incubation periods from zero time up to 25 days. After 20 days of incubation, Pb^{+2}accumulation by *A. pinnata* was recorded at 40 ppm (854.54 ± 26.51 g/m^2) in comparison to that of *L. gibba* (175.20 ± 6.84 g/m^2) as illustrated in Tables (3, 4).

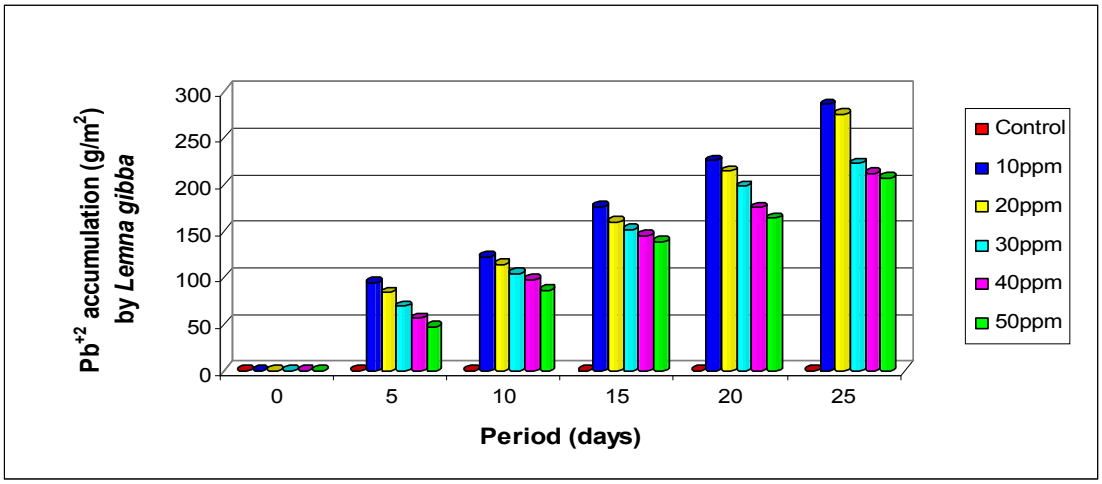

Fig. 4: Effect of different concentrations of Lead (Pb^{+2}) on accumulation of this metal (g/m^2) by *L. gibba*.

Table 4: Effect of different concentrations of Lead (Pb^{+2}) on accumulation of this metal (g/m^2) by *L. gibba* (Data expressed as mean ± SD).

Period (days) / Concentrations (ppm)	Pb^{+2} accumulation (g/m^2)					
	Zero-time	5	10	15	20	25
Control	0.00	0.00	0.00	0.00	0.00	0.00
10	0.00	94.87 ± 4.65	122.43 ± 6.82	176.50 ± 6.04	225.78 ± 8.18	286.04 ± 10.79
20	0.00	83.96 ± 3.30	114.62 ± 5.81	159.57 ± 5.28	214.46 ± 8.11	275.20 ± 9.45
30	0.00	69.08 ± 2.97	103.81 ± 5.67	152.08 ± 6.23	198.16 ± 7.28	222.87 ± 8.17
40	0.00	56.38 ± 2.91	97.33 ± 4.48	144.90 ± 5.11	175.20 ± 6.84	211.93 ± 7.48
50	0.00	47.25 ± 2.33	86.50 ± 3.32	138.12 ± 5.98	163.77 ± 5.89	206.91 ± 7.08
LSD at 0.05	-	5.402	8.699	9.332	11.871	14.133

Discussion

Recently, there has been growing international interest in the use of metal-accumulating plants for the removal of heavy metals from contaminated aqueous streams, in the biological purification of wastewater and in biomonitoring of pollution (Lafabrie *et al*., 2013). Plants that can accumulate and tolerate high levels of heavy metals are good candidates for phytoremediation (Ruley *et al*., 2006). Gupta and Sinha (2007) reported that heavy metal uptake and accumulation in the different plant organs depend on the concentration of the available metals in the surrounding environment, solubility sequence and plant species it self.

In this study, *A. pinnata* and *L. gibba* were grown in Yoshida and Hoagland media, respectively (Yoshida *et al*., 1976; Hoagland and Arnon, 1950). These media were the most suitable environments where they had the essential nutrients needed for *Azolla* and *Lemna* growth. Fresh and dry weights of *A. pinnata* gradually increased with increasing the incubation period from zero time up to 25 days. The recorded results are in the same line with those of El- Araby *et al*. (1999); they found that *A. pinnata* recorded its maximum growth with increasing the incubation period up to 25 days. The values of fresh and dry weights were significantly different at 40 ppm compared to the control after 20 days of incubation.

According to Nuzhat *et al*. (2015), *A. pinnata* doubles its biomass in less than two days in laboratory conditions and 5-10 days in normal field conditions. Moreover, Watanabe *et al*. (1977) reported that doubling time in *A. pinnata* is 3 days, also Tung and Shen (1985) recorded a doubling time of 2.8 days for *A. pinnata*, while Kannaiyan (1993) 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 (El-Berashi, 2008).

A. pinnata do not show any visible toxicity symptoms up to 50 mg/l Pb treatment when was grown in different concentrations of $Pb(NO_3)_2$, this result was similarly with that recorded by Monica *et al*. (2014). On

the other hand, Stêpniewska *et al*. (2005) also reported that, growth of *Azolla* sp. in water solution enriched with $(Pb)SO_4$ was inhibited by about 30-37%. In this respect, Arora *et al*. (2004) reported that, *A. pinnata* have been shown to absorb Cr, Pb, Cd, Zn and other heavy metals and showed tolerance when present in low concentrations. The values of Pb^{+2}accumulation by *A. pinnata* were highly significantly different compared to the control at all concentrations during all the tested incubation periods from zero time up to 25 days.

In a microcosm investigation, *L. minor* removed 76% of Pb and 82% of Ni and no synergistic/antagonistic was noted in the multimetallic conditions (Axtell *et al*., 2003). According to Iqbal (1999), biomass of duckweeds gets doubled in 2-3 days under ideal conditions of nutrient availability, sunlight, pH (6.5-7.5), temperature (20-30°C) and can be cultured, harvested and sun dried without much cost, labor and expertise. After 25 days of incubation, there was non significant difference between the values of fresh, dry weight and doubling time at 10 ppm compared to the control. The values of Pb^{+2}accumulation by *L. gibba* were highly significantly different compared to the control at all concentrations during all the tested incubation periods from zero time up to 25 days.

However, the highest value of Pb^{+2}accumulation by *L. gibba* was recorded at 10 ppm after 25 days of incubation period. These results are in agreement with those of Divya *et al*. (2012) they found that, accumulation of Pb decreased when *L. minor* was treated with higher concentrations of Pb (30, 40 and 50 mg/L). In another study with bioremoval of lead from water using duckweed, North Dakota, USA, exposed to a single dose of lead (from $Pb(NO_3)_2$) at a concentration of 5.0 mg/l for a time period of 21 days. Rahmani and Sternberg (1999) investigated that viable biomass of *L. minor* removed 85-90% of the lead, viable duckweed previously exposed to lead removed 70-80% of the lead, non-viable biomass (control group) removed 60-75% of the lead and there was no removal in the no-biomass or control group. Therefore, they concluded that the viable biomass is effective in removing lead present at sub-lethal levels.

Aquatic macrophytes can accumulate significant quantity of heavy metals in their tissues ($10\text{-}10^6$) times greater concentration than in the water (Snežana *et al*., 2005). Albers and Camardese (1993) found that the concentrations of metals in aquatic plants can be more than 100,000 times greater than in the associated water. The previous results indicated that, *A. pinnata* gave higher growth density than that recorded for *L. gibba* during all the tested incubation periods from zero time up to 25 days. The results also indicated that, *A. pinnata* gave the highest results of Pb^{+2} accumulation than that recorded for *L. gibba* during all the tested incubation periods from zero time up to 25 days. According to Nuzhat *et al*. (2015) 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.

Conclusion

Present study highlights the fact that *A. pinnata* bioaccumulates high concentrations of Pb^{+2} as compared to *L. gibba*. Thus, *A. pinnata* is a good accumulator for Pb^{+2} and is a potential candidate for the removal of Pb^{+2} from wastewater, therefore *A. pinnata* can play an important role in the bioremediation of aquatic ecosystems and wastewater treatment which are under heavy stress of anthropogenic pressure.

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References

Akpor, O. B. and M. Muchie, 2010. Remediation of heavy metals in drinking water and wastewater treatment systems: Processes and applications. International Journal of the Physical Sciences, 5(12): 1807-1817.

Alaa, M. Y. and M. A. N. Elsayed, 2015. Heavy metals and nutritional composition of some naturally growing aquatic macrophytes of Northern Egyptian Lakes. Journal of Biodiversity and Environmental Sciences (JBES), 6(3): 16-23.

Albers, P. H. and M. B. Camardese, 1993. Effects of acidification on metal accumulation by aquatic plants and invertebrates. 1. Constructed wetlands. Environmental Toxicology and Chemistry, 12: 959.

Anjuli, S., L. U. Perm, P. Radha and S. A. Amrik, 2012. Phytoremediation potential of aquatic macrophyte, *Azolla*. A.M.B.I.O., 41:122-137.

Arora, A. and S. Saxena, 2005. Cultivation of *Azolla microphylla* biomass on secondary-treated Delhi municipal effluents. Biomass Bioenerg., 29: 60-64.

Arora, A., A. Sood and P. K. Singh, 2004. Hyperaccumulation of cadmium and nickel by *Azolla* Species. Indian J. Plant Physi., 9: 302-304.

Axtell, N. R., S. P. K. Sternberg and K. Claussen, 2003. Lead and nickel removal using *Microspora* and *Lemna minor*. *Biores.* Technol., 89: 41-48.

Aziz, T. and I. Watanabe, 1983. Influence of nutrients on the growth and mineral composition of *Azolla pinnata*. Bangladesh J. Bo., 12 (2): 166-170.

Caussy, D., M. Gochfeld, E. Gurzau, C. Neagu and H. Ruedel, 2003. Lessons from case studies of metals: investigation exposure, bioavailability and risk. *Ecotoxicol. Environ. Safe*., 56: 45-51.

Celebi, S. and S. Kendir, 2002. Toxicity assessment of a dye industry treatment sludge. Waste Manage. Res., 20: 541-545.

Chang, J. S., I. H. Yoon and K. W. Kim, 2009. Heavy metal and arsenic accumulating fern species as potential ecological indicators in As-contaminated abandoned mines. Ecological Indicators, 9: 1275-1279.

Chapman, H. D. and R. E. Pratt, 1961. Methods of analysis for soil, plants and water. Dept. of Soil, Plant Nutrition, Univ. of California, U.S.A.

David, T. W., M. M. Than and S. Tun, 2003. Lead removal from industrial waters by water hyacinth. A.U. J. T., 6(4): 187-192.

Dejun, K., T. Hong, X. Danyu and K. Pengzhen, 2014. Adsorption abilities by heavy metals and inorganic particles and activated sludge in domestic wastewater treatment plant. Journal of Chemical and Pharmaceutical Research, 6(6): 2918-2926.

Divya, S., G. Richa and T. Archana, 2012. Potential of duckweed (*Lemna minor*) for removal of lead from wastewater by phytoremediation. Journal of Pharmacy Research, 5(3): 1578-1582.

El- Araby, M.M., R. M. El- Shahat, and N. Y. El- Berashi, 1999. Comparison between *Azolla pinnata* and *Azolla filiculoides* application as green manure. In: Development of the desert Environment in Egypt. VI th National Conference on Environmental studies and research II. Institute of Environmental Studies and Research. Ain shams Univ., Cairo, Egypt, 445-460.

El- Berashi, N. M. Y., 2008. Impacts of *Azolla* in increasing rice production and reducing environmental pollution. Ph.D. Ain Shams Univ., 110-157.

El- Shahat, R. M., 1988. Prospects of *Azolla* as biofertilizer in Egypt condition. M.Sc.Thesis In Agricultural Microbiology, Department of Agric. Microbiology, Faculty of Agriculture, Ain Shams University, Cairo, Egypt, 53-72.

El- Shahat, R. M., 1997. Prospects of *Azolla* as biofertilizer in Egypt. Ph.D, Thesis In Agricultural Microbiology, Department of Agric. Microbiology, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Erakhrumen, A. A., 2007. Phytoremediation: an environmentally sound technology for pollution prevention, control and remediation in developing countries. Educational Research and Review, 2(7): 151-156.

Forni, C., A. Casconeb, M. Fioric and L. Migliorea, 2002. Sulphadimethoxine and *Azolla filiculoides* Lam.: a model for drug remediation. Water Res., 36(13): 3398-3403.

Forni, C., J. Chen, L. Tancioni and M. G. Caiola, 2001. Evaluation of the fern *Azolla* for growth, nitrogen and phosphorus removal from wastewater. Water Res., 35(6): 1592-1598.

Gupta, A. K. and S. Sinha, 2007. Phytoremediation capacity of the plants growing on tannery sludge dumping site. Bioreso. Technol., 98: 1788-1794.

Hazrat, A., K. Ezzat and A. S. Muhammad, 2013. Phytoremediation of heavy metals-Concepts and applications. Chemosphere, 91: 869-881.

Hoagland, P. R. and D. I. Arnon, 1950. The water culture method for growing plants without soil. California, College of Agriculture, Agricultural Experiment Station, 347: 1-32.

Hossein, A., B. Pejman, D. Kondiram and P. Gorakh, 2014. Wastewater remediation by using *Azolla* and *Lemna* for selective removal of mineral nutrients. International Journal of Biosciences, 4(3): 66-73.

Iqbal, S., 1999. Duckweed aquaculture: potentials, possibilities and limitations for combined wastewater treatment and animal feed production in developing countries. SUNDEC Report no. 6/99. Switzerland.

Iram, S., I. Ahmad, Y. Riaz and A. Zahra, 2012. Treatment of wastewater by *Lemna minor*. Pak. J. Bot., 44(2): 553-557.

Jordao, C., M. Pereira and J. Pereira, 2002. Metal contamination of river waters and sediments from effluents of kaolin processing in Brazil. Water, Air and Soil Pollution, 140(1): 119-138.

Kannaiyan, S., 1993. *Azolla- Anabaena* symbiosis-a potential biofertilizer for rice. Biodigest., 4: 21-31.

Lafabrie, C., K. M. Major, C. S. Major and J. Cebrián, 2013. Trace metal contamination of the aquatic plant *Hydrilla verticillata* and associated sediment in a coastal Alabama creek (Gulf of Mexico - USA). Marine Pollution Bulletin, 68: 147-151.

Marchiol, L., S. Assolari, P. Sacco and G. Zerbi, 2004. Phytoextraction of heavy metals by canola (*Brassica napus*) and radish (*Raphanus sativus*) grown on multicontaminated soil. Environ. Pollut., 132: 21-27.

Megateli, S., S. Semsari and M. Couderchet, 2009. Toxicity and removal of heavy metals (cadmium, copper and zinc) by *Lemna gibba*. *Ecotox.* Environ. Safe., 72: 1774-1780.

Monica, M., P. Chinmay and B. S. Kunja, 2014. Decontamination of lead from aquatic environment by exploitation of floating macrophyte *Azolla microphylla* Kauf., 8(12): 17-23.

Nuzhat, S., K. P. Ashok, N. K. Azra and M. Basharat, 2015. Heavy metal accumulation by *Azolla pinnata* of Dal Lake Ecosystem, India, 1(1): 8-12.

Öbek, E. and A. Sasmaz, 2011. Bioaccumulation of aluminum by *Lemna gibba* L. from secondary treated municipal wastewater effluents. Bulletin of Environmental Contamination and Toxicology, 86: 217-220.

Overesch, M., J. Rinklebe, G. Broll and H. U. Neue, 2007. Metals and arsenic in soils and corresponding vegetation at Central Elbe river floodplains (Germany). Environmental Pollution, 145(3): 800-812.

Punita, S. P. and K. M. Soma, 2015. Capacity of *Azolla pinnata var. imbricata* to absorb heavy metals and fluorides from the wastewater of oil and petroleum refining industry at Vadodara. *International Peer Reviewed Referred Journal. Vol. II, Issue I, 37-43.*

Rahmani, G. N. H. and S. P. K. Sternberg, 1999. Bioremoval of lead from water using *Lemna minor*. Biores. Technol., 70: 225-230.

Rai, P. K., 2008. Heavy-metal pollution in aquatic ecosystems and its phytoremediation using wetland plants: An eco-sustainable approach. Int. J. Phytoremediation, 10(2): 133-160.

Rai, P. K., 2010. Heavy metal pollution in lentic ecosystem of subtropical industrial region and its phytoremediation. International Journal of Phytoremediation, 12: 226-242.

Rai, P. K., 2011. An eco-sustainable green approach for heavy metals management: two case studies of developing industrial region. Environmental Monitoring and Assessment, 1-28.

Rai, P. K. and B. D. Tripathi, 2009. Comparative assessment of *Azolla pinnata* and *Vallisneria spiralis* in Hg removal from G.B. Pant Sagar of Singrauli Industrial region, India. Environmental Monitoring and Assessment, 148: 75-84.

Rakhshaee, R., M. Khosravi and M. T. Ganji, 2006. Kinetic modeling and thermodynamic study to remove Pb(II), Cd(II), Ni(II) and Zn(II) from aqueous solution using dead and living *Azolla filiculoides*. J. Hazard. Mater., 134: 120-129.

Ruley, A. T., N. C. Sharma, S. V. Sahi, S. R. Singh and K. S. Sajwan, 2006. Effects of lead and chelators on growth, photosynthetic activity and Pb uptake in *Sesbania drummondii* grown in soil. Environmental pollution, 144: 11-18.

Snedecor, G. W and W. G. Cochran, 1980. Statistical Methods 7th ed lowa State Univ. press lowa, USA.

Snežana, P., P. Dragana and T. Marina, 2005. Comparative analysis of heavy metal content in aquatic macrophytes in the reservoirs Gruža, Bubanj and Memorial park. Kragujevac J. Sci., 27: 147-156.

Sood, A., L. Perm, U. R. Prasanna and A. S. Ahluwalia, 2012. Pytoremediation potential of aquatic macrophyte, *Azolla*. A Journal of the Human Environment, 41: 122-137.

Stêpniewska, Z., R. P. Bennicelli, T. I. Balakhnina, K. Szajnocha, A. Banach and A. Woliñska, 2005. Potential of *Azolla caroliniana* for the removal of Pb and Cd from wastewaters. Int. Agrophysics, 19: 251-255.

Tung, H. F. and R. C. Shen, 1985. Studies of the *Azolla pinnata- Anabaena azollae* symbiosis: Concurrent growth of *Azolla* with rice. Aquatic Bot., 22: 145-152.

Uysal, Y. and F. Taner, 2009. Effect of pH, temperature and lead concentration on the bioremoval of lead from water using *L. minor*. Int. J. Phytoremed., 11: 591-608.

Watanabe, I., C. R. Espinas, N. S. Berja and B. V. Alimagno, 1977. Utilization of the *Azolla-Anabaena* complex as a nitrogen fertilizer for rice. Intl. Rice. Res. Inst. Res. Pap. Ser., 11: 1-15.

Yoshida, S., D. A. Forno, J. H. Corck and K. A. Gomez, 1976. Laboratory manual for physiology studies of rice. The Int. Rice. Res. Int. (IRRI), Los Banos, Laguna, Manila, Philippines, 61-65.