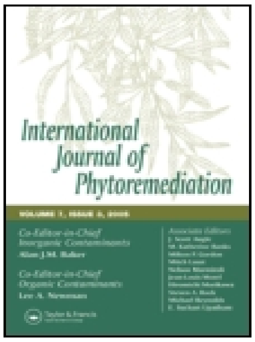

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Phytoremediation potential of *Lemna minor* L. for heavy metals

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ABSTRACT

Phytoremediation potential of *L. minor* for cadmium (Cd), copper (Cu), lead (Pb), and nickel (Ni) from two different types of effluent in raw form was evaluated in a glass house experiment using hydroponic studies for a period of 31 days. Heavy metals concentration in water and plant sample was analyzed at 3, 10, 17, 24, and 31 day. Removal efficiency, metal uptake and bio-concentration factor were also calculated. Effluents were initially analyzed for physical, chemical and microbiological parameters and results indicated that municipal effluent (ME) was highly contaminated in terms of nutrient and organic load than sewage mixed industrial effluent (SMIE). Results confirmed the accumulation of heavy metals within plant and subsequent decrease in the effluents. Removal efficiency was greater than 80% for all metals and maximum removal was observed for nickel (99%) from SMIE. Accumulation and uptake of lead in dry biomass was significantly higher than other metals. Bio-concentration factors were less than 1000 and maximum BCFs were found for copper (558) and lead (523.1) indicated that plant is a moderate accumulator of both metals. Overall, *L. minor* showed better performance from SMIE and was more effective in extracting lead than other metals.

KEYWORDS
municipal and industrial effluents; duckweed; heavy metals; removal efficiency; phytoremediation

Introduction

Increasing urbanization, industrialization and over population are considered as leading causes of environmental degradation and pollution. Discharge of both industrial and municipal effluents to surface water bodies (ponds, rivers, streams etc.) leading to environmental degradation which is a matter of great concern (Sood *et al.* 2012), especially for developing countries like Pakistan. There is no proper system for disposal of domestic and industrial effluent which has adverse effects on human, environment and agricultural sector (Amahmid *et al.* 2002). Most of the health problems related to waterborne diseases due to exposure of bacteria (Farid *et al.* 2014). Presence of coli forms bacteria shows danger of fecal pollution and consequent hazard of contracting diseases through pathogenic organisms (Vijaya 2008).

Among various environmental contaminants, heavy metals are of particular concern because of their potential toxic effect, non-biodegradable nature and ability to bio-accumulate in aquatic ecosystems (Censi *et al.* 2006). The heavy metals such as lead, copper, nickel, chromium, zinc and cadmium are common pollutants discharged from Industrial Estate Islamabad, Pakistan. Copper is a heavy metal which is reported to have an increasing role in metabolic processes of plant cells and is regarded as an essential micronutrient for the growth and development of plant (Maksymiec 1997). When copper is found in excess concentration in water, it has been shown to be one of the most toxic heavy metals to human and animals (Kara and Zeytunluoglu 2007). Cadmium is not an essential element and can be strongly phyto-toxic to aquatic organisms (Kuzovkina *et al.* 2004). Lead and chromium are regarded as toxic contaminants, even in very low concentrations (Flegal *et al.* 2001). Given sufficient exposure, lead can exert severe and chronic health effects (Juberg *et al.* 1997).

Conventional remediation techniques such as adsorption, ion exchange columns, electrochemical removal, filtration etc., used for removal of heavy metal contamination are not economical and may also adversely effects on aquatic ecosystems (Rai 2008). Phytoremediation technique for treatment of heavy metals contaminated industrial wastewater is simple, cost effective and self-sustaining alternative of the traditional treatment methods (Chandra and Yadav 2011). Phytotechnologies involve using plants for metal removal has gained an increasing development during last two decades (Miretzky *et al.* 2004) after the discovery of hyper accumulating plants that can accumulate, translocate, and concentrate high amount of heavy metals in their above-ground tissues (Rahman and Hasegawa 2011). Smits and Pilon (2002) stated that an ideal plant for phytoremediation is one having high biomass production, capacity of pollutant tolerance as well as accumulation and its degradation which depend on the type of pollutant and choice of phytoremediation technology.

A number of aquatic plant species have been known for the remediation of heavy metals such as Cd, Cu, Pb, Cr, Hg and Ni, etc. Among floating aquatic plants *Lemna minor*, *Lemna*

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gibba, *Lemna aequinoctialis*, and *Spirodela polyrhiza* (Charles *et al.* 2006; Axtell *et al.* 2003; Zhang *et al.* 2011), *Azolla filiculoides*, *Azolla caroliniana*, and *Azolla pinnata* (Pandey 2012; Sood *et al.* 2012), *Eichhornia crassipes* and *Pistia stratiotes* (Aurangzeb *et al.* 2014) are the best candidates which have been studied for investigation of their metal uptake ability and potential in phytoremediation technology.

Duckweed is a small, free floating aquatic plant belonging to the Lemnaceae family. Duckweed species are used in water quality studies for monitoring heavy metals (Radic *et al.* 2010) and are considered as better alternative and have been recommended for wastewater treatment because they are more tolerant to cold than water hyacinth, more easily harvested than algae, and capable of rapid growth (Sharma and Gaur 1995).

Various studies have highlighted the heavy metal removal potential of duckweed species except from Pakistan where little research is conducted on phytoremediation capacity of floating aquatic plants especially *Lemna* species which is commonly found in natural wastewater ponds of Islamabad. Moreover, most of the previous studies were performed in laboratory experiments with metal solutions by using different spiked metal concentration in water. The present study was conducted to determine the heavy metal removal performance of *L. minor* from two different types of effluent in raw form which were also loaded with nutrients, organic contaminants and microbes in addition to heavy metal contents.

Materials and methods

Sampling of effluent and laboratory analysis

Effluent samples were collected from two different sources. The first sample (sewage mixed industrial effluent) was collected from a natural drain, located at Industrial Estate Islamabad (IEI) which receives wastewater from industrial unit (steel melting furnaces, re-rolling mills, flour mills, oil and ghee, marble cutting and polishing, pharmaceuticals, soap, auto body shops, and recycling of lead storage batteries etc.) as well as residential areas. The second sample (municipal effluent) was collected from the inlet of Shahzad town, Islamabad which receives sewage wastewater directly from the colony houses. Two separate samples were collected from both sites; first for initial composition of effluents and second for phytoremediation experiment (collected in plastic cans). The effluent samples for initial analysis and experiment were used in raw form as collected from the sampling site. Sample for initial composition of heavy metals was filtered immediately and preserved with nitric acid to prevent the microbial activity and analyzed for Cd, Cr, Cu, Pb, and Ni using graphite furnace Atomic Absorption Spectrometer (Perkin Elmer AAnalyst-700). A separate sample for analysis of coli form bacteria was collected in 100 ml autoclave bottle and transported to Pakistan Council of Research in Water Resources (PCRWR) Islamabad for analysis of total and fecal coli form by Most Probable Number (MPN) method (APHA 2005). The analysis of pH, EC, turbidity, TDS, hardness, chloride, bicarbonate, and calcium were performed of original sample without filtration. Parameters including pH, electrical conductivity (EC) and TDS (multifunction meter-WA-2015) were determined in the field using portable instruments. Turbidity was measured in nephelometric turbidity units (NTUs) using microprocessor (turbidity meter−Hi 93703−11Hanna). Alkalinity (acid−base titrometry), chloride (silver nitrate titrometry), total hardness and calcium (EDTA titrimetric method), phosphate (ammonium molybdate method), sodium and potassium (flame photometric), nitrate (ion chromatography), and COD (open reflux method) were determined in the laboratory according to standard methods prescribed by American Public Health Association (APHA 2005).

Experimental set up

Phytoremediation experiment was carried out at National Agricultural Research Center Islamabad. Fresh plants of *L. minor* were obtained from a pond maintained by National Institute of Bioremediation, National Agriculture Research Centre, Islamabad, Pakistan. Two sets of experimental containers were arranged, one set in triplicate was filled with 25 L of SMIE and second set with ME and 200 (g) fresh weight of duckweed was added to each container/tubs. Experimental tubs were placed in glass house for a period of 31 days and both water and plant samples were collected from each tub at 3, 10, 17, 24, and 31 day (total of 6 observations with pre-treatment data) for analysis of heavy metal.

Heavy metal estimation in water and plant samples

Plant samples were washed three times with distilled water, oven-dried at 70°C till constant weight, milled and sieved to <1 mm. Plant material (0.25 g) was digested with 10 ml of double acid (HNO_3-HClO_4 in the ratio of 2:1 respectively) on hot plate (Type 2200 Hot Plate) by slowly raising the temperature. The digested sample was diluted to 50 ml with de-ionized water and filtered through what man no. 42 filter paper. Periodically collected water samples were filtered immediately after collection by using 0.45 μm membrane filter through vacuum filtration apparatus and preserve/acidify with HNO_3. Determination of heavy metal (Cd, Cr Cu, Pb, and Ni) contents in plant and water samples were carried out by graphite furnace Atomic Absorption Spectrometer (Perkin Elmer, A Analyst 700).

Calculation methods

Amount of heavy metal in plant was calculated using dilution factor.

Metal ($\mu g\ g^{-1}$) in plant = metal reading of digested sample ($mg\ L^{-1}$) × dilution factor

Where,

$$\text{Dilution factor} = \frac{\text{Total volume of sample (ml)}}{\text{weight of plant material (g)}}$$

The percentage efficiency was calculated from initial and remaining concentration of metal according to Tanhan *et al.* (2007):

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

Where C_0 and C_1 are initial and remaining concentrations respectively in the medium ($mg\ L^{-1}$).

The metal uptake in whole plant was calculated using dry weight:

Metal uptake (mg/tub) = Metal concentration in plant (μg g^{-1}) × plant's whole dry weight (g)

The bio-concentration factor was calculated as follows (Zayed *et al.* 1998):

$$\mathrm{BCF} = \frac{\text{metal concentration in plant } (\mu g\, g^{-1})}{\text{metal concentration in medium } (mg\, L^{-1})}$$

Statistical analysis

The experiment was set up in replicates and all the data was mean of triplicate (n = 3). Heavy metal concentration in plant and effluent samples at different exposure time (days) as well as calculated values for removal efficiency, uptake in whole plant and bio-concentration factor were subjected to two-way Analysis of Variance (ANOVA) using complete randomized design (CRD) and significance between means were tested by Least Significant Difference (LSD) test on statistix 8.1 package.

Results and discussion

Initial composition of effluents

The results of initial composition (physical, chemical and microbiological) of effluents are given in Table 1. Chemical composition of both types of effluent was slightly alkaline in nature having pH greater than 7.5. The EC value of the sewage mixed industrial effluent (SMIE) was 882 μS cm^{-1} and of municipal effluent (ME) was 1133 μS cm^{-1}. Turbidity of the SMIE and ME was 5.3 NTU and 13.66 NTU respectively while the value of TDS was 585 mg L^{-1} in SMIE and 755 mg L^{-1} in the ME. The concentration of total hardness, Cl, HCO_3, Ca, Na and K was 400 mg L^{-1}, 160.1 mg L^{-1}, 250 mg L^{-1}, 105.1 mg L^{-1}, 58 mg L^{-1}, and 24 mg L^{-1} respectively in the SMIE whereas, the concentration was 550 mg L^{-1}, 125.1 mg L^{-1}, 520 mg L^{-1}, 130.3 mg L^{-1}, 79 mg L^{-1}, and 28 mg L^{-1} respectively as found from the ME. High load of all the major chemical parameters except chloride was observed in municipal effluent compared to the SMIE.

Nutrient composition of the ME was also greater than SMIE which was 2.3 mg L^{-1} for nitrate and 5.5 mg L^{-1} for phosphate in SMIE whereas, 10 mg L^{-1} for nitrate and 10.75 mg L^{-1} for phosphate was found in the municipal effluent. The concentration of COD was 33 mg L^{-1} in SMIE and 200 mg L^{-1} in the ME. Results showed that concentration of COD was moderate to high in the municipal effluent compared to sewage mixed industrial effluent and was also above the permissible limit set by PNEQS (1999). High value of COD in municipal effluents confirm heavy load of organic contaminants. The number of total coli form and fecal coli form bacteria was greater than 1600 in both types of effluent. Results showed that municipal effluent was more contaminated in terms of nutrients and organic load than SMIE. Chapman (1996) reported that municipal wastewater consists of sewage effluents, urban drainage and other collected wastewater which usually contain high levels of fecal materials and organic matter such as BOD, COD, chloride, ammonia, and nitrogen compounds.

Effluents collected from two different sources were also analyzed for initial heavy metal concentration before treatment (Table 1). Cadmium concentration in the sewage mixed industrial effluent (SMIE) was 0.038 mg L^{-1}, copper concentration was 0.062 mg L^{-1}, lead concentration was 0.608 mg L^{-1} and nickel concentration was 0.059 mg L^{-1}. In the municipal effluent (ME), the concentration of cadmium, copper, lead and nickel was 0.054 mg L^{-1}, 0.032 mg L^{-1}, 0.419 mg L^{-1}, and 0.051 mg L^{-1} respectively, whereas chromium was not detected in both types of effluent. The concentration of all heavy metals except the cadmium was high in the sewage mixed industrial effluent than municipal effluent and the concentration of lead in the SMIE was above the permissible limit (0.5 mg L^{-1}) described by PNEQS (1999).

Table 1. Initial composition (Physical, chemical and microbiological) of effluents.

Parameters	Units	Sewage mixed industrial effluent	Municipal effluent	Permissible limits*
pH		7.9	7.61	6–10
EC	μS/cm	882	1133	–
Turbidity	NTU	5.3	13.66	–
TDS	mg L^{-1}	585	755	3500
Hardness	mg L^{-1}	400	550	–
Cl	mg L^{-1}	160.1	125.1	1000
HCO_3	mg L^{-1}	250	520	–
Ca	mg L^{-1}	105.1	130.3	–
Na	mg L^{-1}	58	79	–
K	mg L^{-1}	24	28	–
Nitrate	mg L^{-1}	2.3	10	–
Phosphate	mg L^{-1}	5.5	10.75	–
COD	mg L^{-1}	33	200	150
Total coli form	MPN/100 ml	≥1600	≥1600	–
Fecal coli form	MPN/100 ml	≥1600	≥1600	–
Cd	mg L^{-1}	0.038	0.054	0.1
Cu	mg L^{-1}	0.062	0.032	1
Cr	mg L^{-1}	BDL	BDL	1
Pb	mg L^{-1}	0.608	0.419	0.5
Ni	mg L^{-1}	0.0591	0.051	1

BDL: Below Detection Limit. *PNEQS (1999).

Heavy metal removal from effluents

The removal of heavy metals from both types of effluent by *L. minor* at different exposure time (days) was observed in the study. The results of Cd, Cu, Pb, and Ni removal are shown in Figures 1–4. In general, a decrease in the concentration of metals was observed from effluents and maximum removal was found at 31 day of experiment with significant differences ($p < 0.01$). Reduction in concentration of different metals was different at different exposure times. Initial cadmium concentration of sewage mixed industrial effluent (0.038 mg L^{-1}) and municipal effluent (0.054 mg L^{-1}) was reduced to 0.002 mg L^{-1} and 0.003 mg L^{-1} respectively at day 31 (Figure 1). Initial copper concentration of SMIE was 0.062 mg L^{-1} in which a significant decrease ($p < 0.01$) was observed in first three days interval after which non-significant changes were found till day 24. At the end of experiment, the remaining concentration of Cu was 0.0033 mg L^{-1}. A similar response of removal of copper from municipal effluent was observed by treatment with *L. minor* and initial concentration (0.032 mg L^{-1}) was reduced to 0.0024 mg L^{-1} with significant differences at termination of

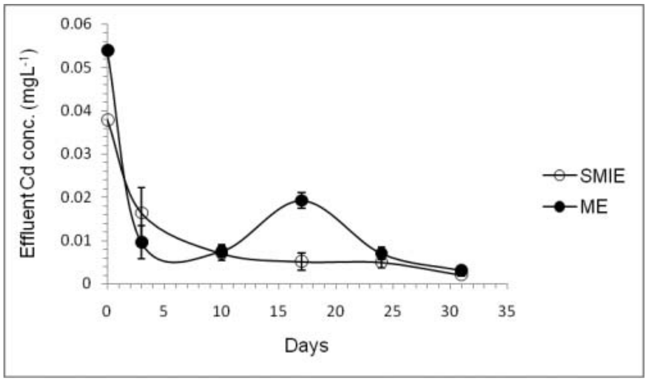

Figure 1. Cadmium concentration (mg L^{-1}) in effluents treated with *L. minor* at different exposure time (days).

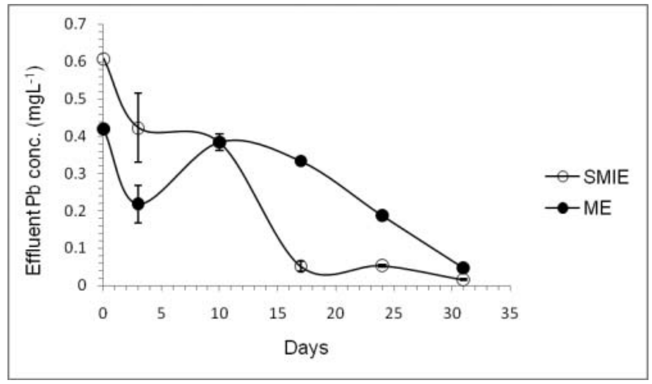

Figure 3. Lead concentration (mg L^{-1}) in effluents treated with *L. minor* at different exposure time (days).

experiment (Figure 2). Role of duckweed spp., *Spirodela polyrrhiza* in removal of heavy metals such as Cd and Cu in 15 days laboratory experiment from a metal solution of three different concentrations (1.0, 2.0, and 5.0 mg L^{-1}) was also investigated by Mishra and Tripathi (2008).

Compared to cadmium and copper, a slow reduction in the concentration of Pb and Ni was observed till tenth day of experiment from SMIE. But a highly significant decrease in concentration was observed at seventeenth day which further decrease at the end of experiment. Results showed that growth of *L. minor* in the sewage mixed industrial effluent reduced the lead concentration from 0.608 mg L^{-1} (initial concentration) to 0.015 mg L^{-1} (remaining concentration) with statistically highly significant differences in 31 day period (Figure 3). Singh *et al.* (2012) stated that *L. minor* has potential for removing lead from industrial wastewater.

Like lead, the nickel concentration of sewage mixed industrial effluent was also decrease over time and it was reduced to negligible amount (0.000) at the end of experiment from initial concentration of 0.059 mg L^{-1} with significant differences ($p < 0.01$) (Figure 4). Lead and nickel removal followed a similar pattern from municipal effluent. The removal rate was slow till twenty fourth day of experiment after which highly significant reduction in concentration was observed on day 31. Initial lead concentration was 0.419 mg L^{-1} which was reduced to remaining concentration of 0.046 mg L^{-1} while initial nickel concentration was 0.051 mg L^{-1} which was reduced to 0.008 mg L^{-1} at termination of experiment (Figures 3–4). Overall, plant demonstrated the ability to remove all metals and significantly higher ($p < 0.01$) removal rate for Cd, Cu, Pb, and Ni from both types of effluent was observed. Mant *et al.* (2007) reported that higher removal rate of metals by free floating macrophytes (*P. stratiotes* and *S. polyrrhiza*) is due to their efficient growth and high biomass accumulation in nutrient and metals contaminated environment.

The results of the present study demonstrated that removal efficiency of duckweed for four metals was greater than 80% from both types of effluent (Figure 5). During the experiment, 94% cadmium and 92–94% copper removal was found from both types of effluents in 31 day period. For Ni and Pb, the results revealed high percentage removal efficiency (99% and 97.4% for Ni and Pb respectively) from sewage mixed industrial effluent. The initial lead concentration in the effluent was 0.608 mg L^{-1} and nickel concentration was 0.059 mg L^{-1}. Axtell *et al.* (2003) examined the ability of *L. minor* to remove soluble lead and nickel under various laboratory conditions. Initial lead concentrations were 0.0, 5.0, and 10.0 mg L^{-1}, and nickel concentrations were 0.0, 2.5, and 5.0 mg L^{-1} in the experiment. Overall, *L. minor* removed an average of 76% of the lead and 82% of the nickel from solution.

Analysis of variance showed significant differences ($p < 0.01$) in the percentage efficiency of plant for Ni and Pb from SMIE and ME. The removal efficiency of plant for Ni and Pb was 84% and 89% respectively from municipal effluent. The following pattern for different metal removal (%) was observed from SMIE and ME:

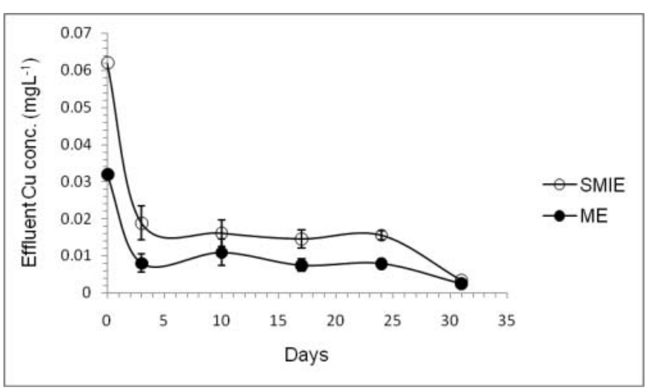

Figure 2. Copper concentration (mg L^{-1}) in effluents treated with *L. minor* at different exposure time (days).

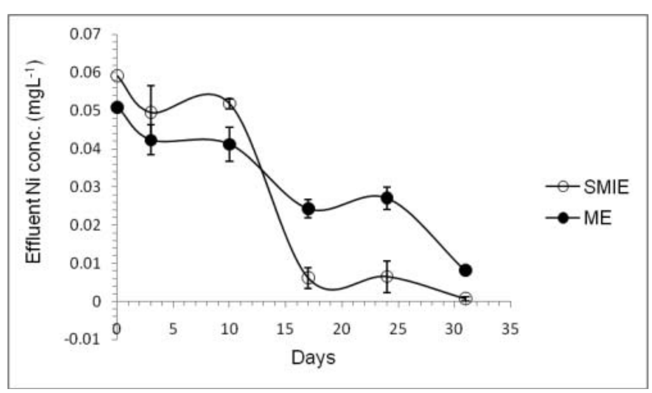

Figure 4. Nickel concentration (mg L^{-1}) in effluents treated with *L. minor* at different exposure time (days).

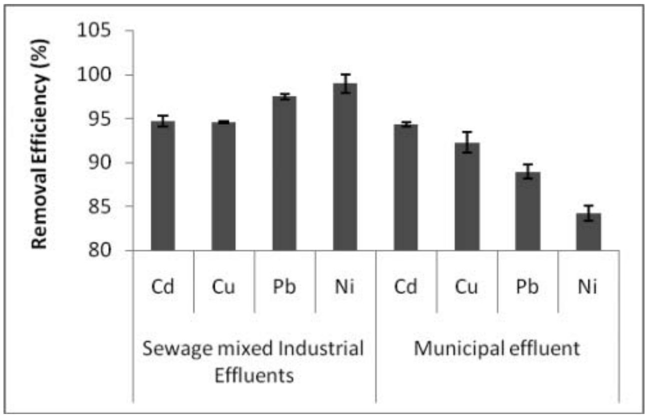

Figure 5. Percentage efficiency of *L. minor* for different metals from both types of effluent.

Sewage mixed industrial effluent: Ni (99%) > Pb (97.4%) > Cd (94.7%) > Cu (94.5%)

Municipal effluent: Cd (94.3%) > Cu (92.2%) > Pb (89%) > Ni (84.2%)

Heavy metal percentage efficiency of floating aquatic macrophyte under the present study was high (Figure 5) and this is in agreement with the findings of Loveson *et al.* (2013) and Aurangzeb *et al.* (2014). Yilmaz and Akbulut (2011) used floating macrophytes (*L. minor* and *L. gibba)* in wastewater treatment for removal of heavy metals under various laboratory tests. The removal efficiency for Cu, Ni, and Pb from the wastewater were more than 60% for *L. minor* (58%, 68%, and 62% for Cu, Ni, and Pb respectively).

Accumulation of heavy metals and uptake in dry biomass

Aquatic plants are well known to accumulate metals from contaminated water and substrates (Rai *et al.* 1995). Denny (1980) reported that heavy metals were taken up by plants by absorption and translocation, and released by excretion. He further stated that the main route of heavy metal uptake in wetland plants such as surface floating was through the roots. Heavy metal accumulation capacity of plant in present study for different metal is shown in Figures 6–9. Accumulation (concentration) of cadmium from both types of effluent at different exposure time was ranged between 5.8 and 24 μg g^{-1} and

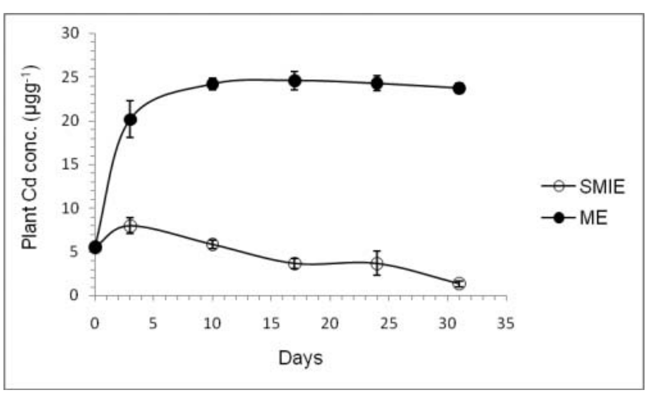

Figure 6. Cadmium concentration (μg g^{-1}) in *L. minor* at different exposure time (days) from effluents.

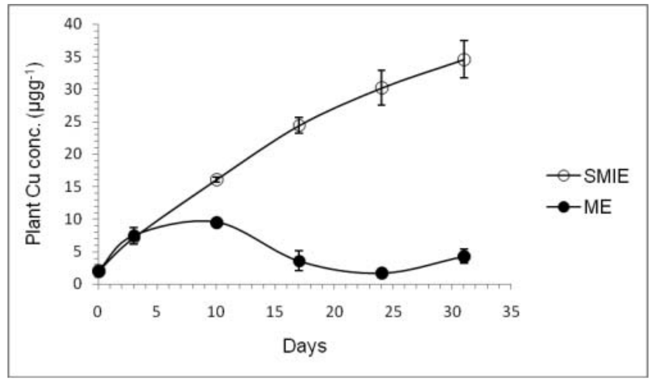

Figure 7. Copper concentration (μg g^{-1}) in *L. minor* at different exposure time (days) from effluents.

maximum concentration was observed from plant grown in municipal effluent. A significant increase in the initial concentration of Cd was found on day 3 from municipal effluent. After that minor changes were revealed till the end of experiment. Whereas, plant accumulates maximum concentration of cadmium on day 3 from SMIE, after which a decrease in the concentration was found with increasing time (Figure 6). Mishra and Tripathi (2008) reported that Cd^{2+} ions have become a problem for biological system because cadmium is a non-essential and toxic element but plants taken up the element by root system and transport it to shoot which adversely affects their growth and metabolism.

Results revealed increasing trend of accumulation of Cu in plant with increasing exposure time from SMIE. Initial copper concentration (2.0 μg g^{-1}) was increased to 7.13 μg g^{-1}, 16.06 μg g^{-1}, 24.4 μg g^{-1}, 30.2 μg g^{-1}, and 34.6 μg g^{-1} at 3, 10, 17, 24, and 31 days of treatment respectively. The pattern of Cu concentration in plant at various exposure times in ascending order was: day 31 > day 24 > day 17 > day 10 > day 3 > day 0. From the municipal effluent, plant accumulated Cu at the initiation of experiment till day 10, afterward no further increase was observed. The Cu concentration on days 3 and 10 was 7.46 μg g^{-1}, and 9.53 μg g^{-1} respectively, whereas, it was reduced to 4.26 μg g^{-1} at the experiment termination (Figure 7).

Compare to the other metals, *L. minor* accumulated high concentration of lead from both types of effluent. Lead has the highest initial concentration (mg L^{-1}) in the effluents among four metals studied. Initial concentration of lead in the plant was 5.8 μg g^{-1}and maximum concentration was 318 μg g^{-1} from SMIE. Lead concentration was increased over time till day 24 with highly significant differences. The concentration in plant was increased to 81.3 μg g^{-1},158 μg g^{-1}, 237.8 μg g^{-1}, and 318 μg g^{-1} on days 3, 10, 17, and 24, respectively. However slight reduction in the concentration was observed at experiment termination. Lead accumulation by plant from municipal effluent (ME) was very slow at initial stages of experiment which was 15.6 μg g^{-1} and 16.13 μg g^{-1} on days 3 and 17, respectively. The concentration of lead in the plant was increased to 53.46 μg g^{-1} and 103.6 μg g^{-1} on days 24 and 31 respectively. Analysis of variance showed significant differences ($p < 0.01$) between initial and final concentration (Figure 8). Our result conforms to the earlier report that *L. minor* could

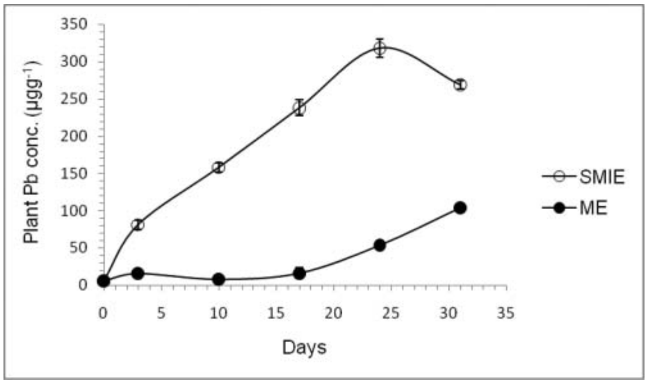

Figure 8. Lead concentration (μg g^{-1}) in *L. minor* at different exposure time (days) from effluents.

accumulate high concentration of lead and concentration increase with passage of time (Singh *et al.* 2012). In a study by Leblebici and Aksoy (2011), *L. minor* was exposed to different Pb concentrations (0, 5, 10, 25, and 50 mg l^{-1}) under laboratory conditions for a period of 1, 3, 5, and 7 days. Results showed that *L. minor* accumulated 561 mg g^{-1} dry weight (dw) Pb on day 7 at 50 mg L^{-1} concentration.

During the study, it was found that the nickel concentration in plant at different days was nearly same from both types of effluent. The initial concentration of nickel in both effluent samples was also close to each other. The initial concentration of metal in the effluent appears to have effect on the accumulation capacity of plant. Maximum concentration of Ni in the plant (19.6 μg g^{-1}) was found on day 10 of experiment from sewage mixed industrial effluent while maximum concentration from municipal effluent was observed on day 17 which was 17.13 μg g^{-1}. Overall, minor changes (decrease/increase) were observed in Ni concentration after 10th day which was statistically non-significant. The accumulation pattern of maximum metal concentration (μg g^{-1}) in *L. minor* was in order of: Pb (318) > Cu (34.6) > Ni (19.6) > Cd (8.0) from sewage mixed industrial effluent and Pb (103.6) > Cd (24.6) > Ni (17.1) > Cu (9.53) from municipal effluent.

Dry biomass of *L. minor* is an excellent bio sorbent for a few metal and metalloids (Axtell *et al.* 2003). Ekvall and Greger (2003) stated that a plant with relatively high biomass may

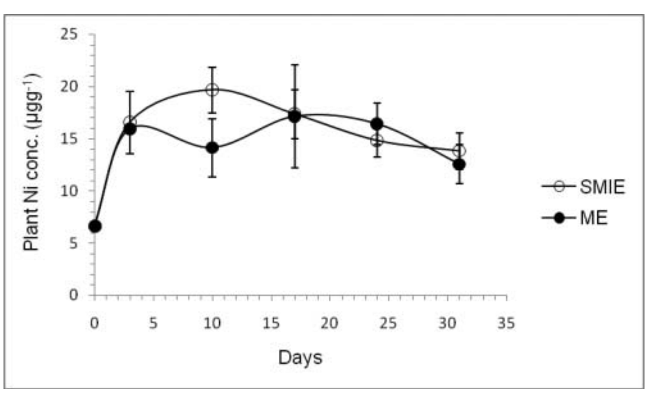

Figure 9. Nickel concentration (μg g^{-1}) in *L. minor* at different exposure time (days) from effluents.

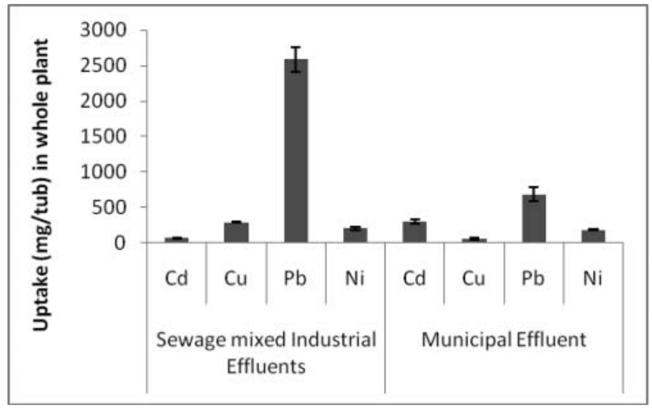

Figure 10. Metal uptake in whole plant (mg tub^{-1}) from both types of effluent.

have a greater metal uptake capacity due to lower metal concentration in its tissues and a growth rate that exceeds its uptake rate. Figure 10 shows uptake of different heavy metals in whole dry biomass of *L. minor* from two effluents. The results obtained from the present study indicated that uptake in plant was high when the metal concentration (mg L^{-1}) in the effluent was comparatively greater. The initial concentration of metal in the effluent appears to have an effect on the total uptake by plant. Our results conforms the findings of Axtell *et al.* (2003) who reported that *L. minor* was able to remove greater amount of metals when there was high metal concentration was added in the solution. Analysis of variance showed significant differences ($p < 0.01$) in uptake of all metals except nickel between SMIE and ME. Results revealed high uptake for lead (2587 mg tub^{-1}) from sewage mixed industrial effluent. Uptake of other metals by *L. minor* was ranged between 55.7 mg tub^{-1} and 686 mg tub^{-1}. The pattern of heavy metal uptake from two different effluents was in order of:

$$\text{Sewage mixed industrial effluent}: \text{Pb}(2587) > \text{Cu}(290.5) > \text{Ni}(201.7) > \text{Cd}(65.6)$$

$$\text{Municipal effluent}: \text{Pb}(686) > \text{Cd}(297.3) > \text{Ni}(185) > \text{Cu}(55.7)$$

Among four metals, maximum uptake was observed for lead from both types of effluents while uptake values for nickel were close (with non-significant differences) from sewage mixed industrial effluent and municipal effluent.

Bio concentration factor for cadmium, copper, lead, and nickel

The bioconcentration factor is the ability of the plant to accumulate heavy metal with respect to the metal concentration in the surrounding medium (Zayed *et al.* 1998). BCF values at different exposure time (days) calculated from initial concentration of metal in the effluent are given in Table 2. Maximum bio concentration factor for different metals were ranged between 210.5 and 558. Maximum BCF value for cadmium was 210.5 on day 3 from SMIE and 455.5 on day 17 from municipal effluent. Bio-concentration factor for copper was increased with

Table 2. Bio concentration factor of *L. minor* for heavy metals at different exposure time (days) from both types of effluent.

		Exposure time (Days)				
Sample	Metal	3	10	17	24	31
Sewage mixed industrial effluent	Cd	210.5 ± 39.7 a	154.3 ± 21.2 ab	96.49 ± 26 bc	96.49 ± 63.6 bc	35.08 ± 15.1 c
	Cu	115 ± 27.4 e	259.1 ± 12.2 cd	393.5 ± 32.7 bc	487 ± 75.1 ab	558 ± 80.6 a
	Pb	133.7 ± 17.4 e	260 ± 18 d	391.2 ± 30.1 bc	523.1 ± 34.2 a	442.1 ± 17.1 ab
	Ni	279.7 ± 87.1 a	332.7 ± 64 a	293.2 ± 69.7 a	250.4 ± 47 a	233.5 ± 52 a
Municipal effluent	Cd	372.8 ± 67.2 a	448.1 ± 22.5 a	455.5 ± 34 a	449.3 ± 28 a	439.5 ± 18.2 a
	Cu	233.3 ± 68.5 ab	298 ± 32 a	111.4 ± 83.4 ab	53.12 ± 14.3 b	133.3 ± 59 ab
	Pb	37.39 ± 24.8 c	18.93 ± 2.45 c	38.5 ± 29.8 c	127.6 ± 22 b	247.2 ± 5.38 a
	Ni	312.4 ± 8.16 a	277.1 ± 94.7 a	336 ± 168 a	321.5 ± 67 a	245.7 ± 63.7 a

Means ± standard deviation (n = 3) of BCFs values; means with different letters are significantly different ($p < 0.01$) according to LSD test.

increasing exposure time with significant differences ($p < 0.01$) and maximum value (558) was found on day 31 in the plant grown on sewage mixed industrial effluent (SMIE). Whereas from the municipal effluent, maximum BCF value for copper was observed on day 10. BCF value for Pb was increased over time and maximum value was found on days 24 and 31 from SMIE and ME respectively. Bio-concentration factor values for Ni by *L. minor* were nearly same from both types of effluent. Maximum BCF value was 332.7 (on day 10) and 336 (on day 17) from SMIE and ME respectively, with non-significant differences. For copper and lead, maximum BCF values were observed from the plant grown on SMIE while for Cd from municipal effluent. Overall, the pattern of maximum BCF values for four metals was in order of:

$$Cu(558) > Pb(523.1) > Cd(455.5) > Ni(336)$$

According to the results demonstrated from the present study, BCF values for all metals were less than 1000 and maximum BCFs were found for copper (558) and lead (523.1) considering the plant a moderate accumulator of both metal. In the previous studies, BCF value for Cu in *Lemna* spp., was also under 1000 and found as 850 by *L. minor* (Zayed *et al.* 1998) and 650 by *L. polyrrhiza* (Jain *et al.* 1990), whereas the findings of Kwan and Smith (1991) reported very high BCF value for copper by *L. minor* which was 12600. In another study, *Spirodella polyrrhiza* (member of family Lemnaceace) proves to be an excellent accumulator of both Cd and Cu having BCF values 36500 and 5750 for Cd and Cu respectively (Rai *et al.* 1995).

According to literature survey, some floating aquatic plants have been shown to exhibit higher accumulation of metals with higher bio-concentration factor. Sela *et al.* (1989) reported very high BCF value for Cd (24,000) in the roots of floating plant, water fern (*Azolla filiculoides*). In comparison, some plants proved to be poor accumulator of metals having low BCF values. Miller *et al.* (1983) reported that the BCF value for Cd in soft-water macrophyte was only 2.7. Another study with different habitats of aquatic plants showed that the level of BCF for cadmium in floating hydrophytes were 4 to 5 times greater than those in water and sequence of BCF for Cu, Cd, Pb, and Ni in different habitats of plants was in order of: free floating > submerged > emergent plant in water (Ndeda and Manohar 2014).

Conclusions

L. minor showed better performance from SMIE than ME (which was loaded with high level of nutrient and organic contaminants especially COD). Effluent with high organic load and nutrients concentration may recommend primary treatment to reduce the organic contaminants before treatment by duckweed species. During the study, initial concentration of metal in effluent appear to have an effect on the accumulation capacity of plant and results revealed that accumulation and uptake of different heavy metals by plant was significantly high when the initial metal concentration in the effluent was comparatively greater. On the other hand, metal removal percentage was high when the initial metal concentration was low as it was based on initial metal concentration. Among four metals, accumulation and uptake of lead in dry biomass of *L. minor* was significantly high. Excellent metal efficiency was shown by plant and percentage removal was greater than 80% for all metal. Maximum bio-concentration factors values for different metals was less than 1,000 and varies between 210.5 and 558. Maximum BCF were shown for copper and lead which were 558 and 523.1 respectively. During the experiment, plant demonstrated the ability to remove metals from raw sewage mixed industrial effluent as well as municipal effluent. Moreover, high percentage efficiency and metal accumulation and uptake capacity give evidence of its phytoremediation efficacy.

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