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TREATMENT OF WASTEWATER BY *LEMNA MINOR*

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Abstract

The aim of the present study was to study the performance of bio-treatment ponds after one year of functioning at National Agricultural Research Center, Islamabad, Pakistan. The physical parameters (colour, pH, EC, TDS, turbidity) and chemical parameters (Zn, Cu, Cd, Ni, Mn, Fe and Pb) are within the limits which are not sub-lethal for fish rearing. *Lemna* accumulates higher concentration of heavy metals as compared to wastewater and best for phytoremediation purpose. The treated wastewater is currently used for rearing of fish and irrigation of crops and plants. The plants around the bio-treatment ponds are healthy, green and showing enough production. The present investigation indicates that in future it would be possible to construct bio-treatment ponds in polluted areas of Pakistan.

Introduction

Human impact on environment can be scaled by the measurements of heavy metals in soil, plants and animals because metal pollution adversely affects the density and diversity of biotic communities including humans (Mountouris e*t al.,* 2002). Water is probably the most important resource and humans can survive without food for several weeks, but without water one would die in less than a week. On a slightly less dramatic note, millions of liters of water are needed every day worldwide for washing, irrigating crops, and cooling industrial processes, not to mention leisure industries such as swimming pools and water-sport centers. Domestic wastewater contains plant nutrients and has traditionally been used for crop irrigation and fertilization in many countries. The reuse of treated wastewater in aquaculture/agriculture practices is encouraged to minimize demand on freshwater resources. A major concern for reuse of wastewater is the bioaccumulation of hazardous wastes especially heavy metals and pesticides in food chain (Teisseire & Guy, 2000).

The Lemnaceae family consists of four genera (*Lemna, Spirodela, Wolffia* & *Wolffiella*) and 37 species have been identified so far. Compared to most other plants, duckweed (*Lemna minor*) has low fiber content (about 5%), since it does not require structural tissue to support leaves and stems. Of these, applications of duckweed in wastewater treatment was found to be very effective in the removal of nutrients, soluble salts, organic matter, heavy metals and in eliminating suspended solids, algal abundance and total and fecal coliform densities (Nieder *et al*., 2004). Duckweed is a floating aquatic macrophyte, which can be found world-wide on the surface of nutrient rich fresh and brackish waters (Zimmo *et al.,* 2005). Duckweed acts as a purifier of domestic wastewater in shallow mini-ponds (20 & 30 cm deep). The treated wastewater can be used for irrigation purpose (Oron, 1994) and wastewater ammonia was converted into a protein rich biomass, which could be used for animal feed or as soil fertilizer. Zayed (1998) found that under experimental conditions, duckweed proved to be a good accumulator of Cd, Se and Cu, a moderate accumulator of Cr, and a poor accumulator of Ni and Pb. The toxicity effect of each trace element on plant growth was in the order: Cu > Se > Pb > Cd > Ni > Cr. He also concluded that duckweed showed promise for the removal of Cd, Se and Cu from contaminated wastewater since it accumulated high concentrations of these elements. Further, the growth rates and harvest potential make duckweed a good species for phytoremediation.

The aim of the present investigation was to evaluate the effectiveness of *Lemna* to remove heavy metals from the wastewater of bio-treatment ponds. For the first time in Pakistan bio-treatment ponds were constructed to understand the reuse of such treated water for agriculture and aquaculture purpose. In Pakistan phytoremediation techniques are on initial scale and detail investigations are necessary for further research. Environmental assessment studies on the reuse of wastewater for agriculture and aquaculture are being conducted by the National Agricultural Research Center (NARC), Islamabad, Pakistan.

Materials and Methods

Study area and samples collection: Samples of wastewater and *Lemna minor* (duckweed) were collected from the bio-treatment ponds (Fig.1). The bio-remediation work was commenced at National Agricultural Research Center (NARC), Islamabad in October 2008. The assignment was to reclaim the sewage water of main NARC office buildings and hostels through bio-remediation for irrigation purposes. The used water treatment garden project was started in October 2008 and finalized in February 2009. The total area of bio-treatment pond is 0.3 acre and total storage capacity is 264870 (0.265 million gallons) for irrigation (Table 1). All ponds are aerobic while Pond 6 including wetland and Pond 7 is fish rearing pond. The water and plant samples were collected in replicates.

Table 1. Bio-treatment ponds specification.

Pond	Length (ft)	Width (ft)	Depth (ft)	Storage capacity	
				(ft3)	gal
P1	30	20	6	3600	27000
P2	48	18	6	5184	38880
P3	70	16	6	6720	50400
P4	42	16	6	4032	30240
P5	44	20	6	5280	39600
P6	46	24	6	3600	24840
P7	46	25	6	6900	51750
Total				**35316**	**264870.00**

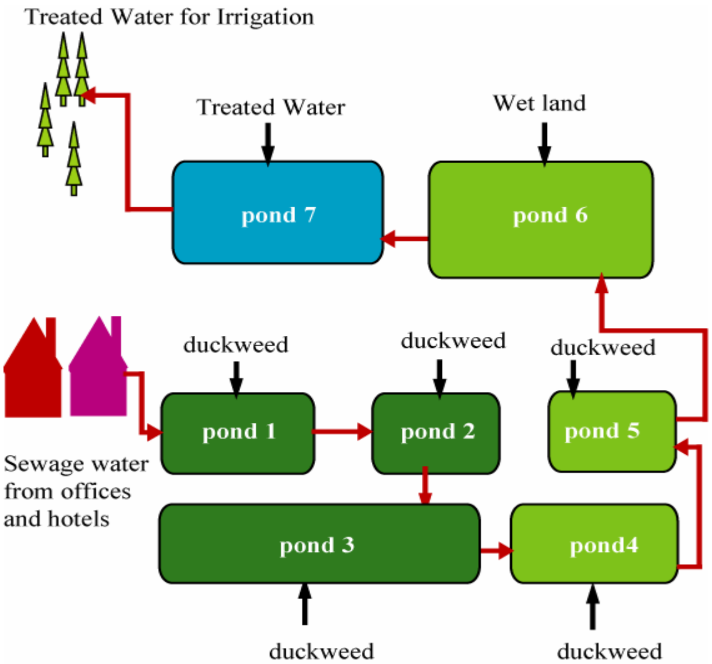

Fig. 1. Schematic diagram of bio-treatment ponds.

Plant sampling and heavy metal analysis: *Lemna minor* plants were collected to study the pattern of heavy metals and comparison of phytoremediation process among seven bio-treatment ponds. From each pond, samples were collected in replicates. Plant samples were put in clean plastic bags and labeled carefully by permanent marker. All the collected plant samples were placed in newspapers for the absorption of excessive water and put in oven at 70ºC for 48 hours. The dried plant samples were grinded with pestle and mortar so that their size was 2mm after grinding. Grinded plant samples (1g) were added in 10mL of double acid HNO_3:$HClO_4$ (2:1 v/v) in conical flask (300mL) and placed on hot plate in fume hood at 200ºC. After 24 hours plant samples were digested and filtered, and volume rose to 100mL. All prepared samples were analyzed for heavy metals (Zn, Pb, Cu, Ni, Cd, Cr, Mn, Fe) by flame atomic absorption spectrophotometer Model No Perkin Elmer Analyst 700 (Ryan *et al.,* 2001).

Wastewater sampling and physio-chemical analysis: One and half-liter of water samples were collected from all bio-treatment ponds. For sample collection the bottles were washed with hot water followed by distilled water. During collection bottles were filled, rinsed with the sample water 2-3 times, tightly capped and properly labeled. Physical parameters of collected water samples were studied immediately, which were collected in replicates from all the 7 bio-treatment ponds. In physio-chemical analysis different physical parameters were studied. Colour was determined by direct comparison with standards and presented in somewhat arbitrary terms of colour scale, which was observed by naked eye. It was done during the sampling of water on the spot (Peavy *et al.,* 1985). Qualitative tests that employ the human sense of taste and smell were used for odour purpose (Peavy *et al.,* 1985).

Temperature was measured by using a mercury thermometer of 0°C to 50°C range and with 0.2°C least count. The temperature of water samples was measured on the spot (Trivedi & Gurdeep, 1992). The pH of water samples was determined in laboratory with pH meter (Inolab pH 720). The conductivity of water samples was determined in laboratory with the help of conductivity meter. First of all the instruments were washed with distilled water and rinsed with the water sample. Bulb was also washed with distilled water before putting in each water sample. The same procedure was repeated for all water samples. For the measurement of total dissolved solids (TDS) clean china dishes were put into oven at 103 to 105°C for dryness, which were then cooled and weigh. Filtered water samples (20mL) were put in china dish and placed in oven at 103 to 105°C for evaporation, later on cooled in desiccators and weighed. The increase in weight of china dish gave the weight of dissolved solids (Trivedi & Gurdeep 1992; Greenberg *et al*., 2005). The results are shown in mg/liter using the following formula:

$$TDS = \frac{\text{Final weight of china dish-initial weight of china dish}}{\text{mL of water sample used}} \times 1000$$

Water samples were collected in triplicates and nitric acid (HNO_3) was added in water samples after it in situ pH measurement. All the collected samples of water (100mL) were filtered with the filtration assembly using the filter paper nitrocellulose membrane diameter of 0.45 µm. For the analysis of water and plant samples atomic absorption was powered on and warmed up for 30 minutes. After the heating of hollow cathode lamp, the air acetylene flame was ignited and instrument was calibrated or standardized with different working standards. By atomic absorption spectrophotometer heavy metals (Zn, Pb, Ni, Mn and Fe) of each water and plant sample were noted (Perkin Elmer Analyst 700).

Results and Discussion

The results of efficiency of *Lemna* in scavenging contaminants indicate that the presence of this macrophyte was an important element for contaminant removal in wastewater. Hydrophytes can supply required oxygen by oxygen leakage from the roots into the rhizosphere to accelerate aerobic degradation of organic compounds in wetlands. This assumption was confirmed in the present study, since the accumulation of heavy metals were higher in plants than water. Phytoremediation can be classified as phytoextraction, phytodegradation, phytostabilization, phytostimulation, phytovolatilization and rhziofiltration (Susarla *et al*., 2002). Rhziofiltration, also referred to as phytofiltration, is based on hydroponically grown plants that have shown to be most efficient in removing heavy metals from water (Raskin *et al*., 1994). Phytoextraction was considered to have taken less part relatively in metal removal but it should have been promoted.

Physical analysis of wastewater: In physio-chemical analysis different parameters (colour, odour, pH, EC, total dissolved solids, temperature and turbidity) were studied (Table 2). During sample collection colour of the wastewater samples was turbid or slightly yellowish. The level of colour in the wastewater may be due to the presence of total dissolved solids in the groundwater. Wastewater of six ponds was odourless while pond 7th water gave fishy smell because this pond is fish rearing. The pH of all ponds was alkaline, 7.5 pH was found to be the most ideal for the successful establishment of a duckweed system and optimum pond performance (Dalu & Ndamba, 2002). Hicks (1932) found that duckweed grew well at pH 6-7.5 with outer limits of 4 and 8. Korner *et al*., (2003) reported that sewage temperature is one of the crucial design parameters of duckweed ponds. In the present experiment temperature ranged between 21.7°C and 23°C (Table 2), which was within temperature tolerance limit for duckweed growth as mentioned by Culley *et al*., (1981) who found that the upper temperature tolerance limit for duckweed growth was around 34°C. On the other hand the plants showed a slight decrease in growth below 10°C. It was also proved that duckweed survived in outdoor wastewater treatment tanks at below freezing temperatures and resumed growth when the temperature rose above freezing (Classen *et al*., 2000). Duckweed cold tolerance allows it to be used for year–round wastewater treatment in areas where tropical macrophytes, such as water hyacinths, can only grow in summer (Cheng *et al.*, 2002).

Table 2. Mean concentration of pH, temperature, turbidity, EC and TDS of wastewater of seven bio-treatment ponds.

Ponds	pH	Temp. °C	Turbidity us/cm	EC dS/m	TDS mg/L
1	7.1	21.7	5.5	601	421
2	7.4	22.0	4.4	578	404
3	6.8	22.0	6.5	518	363
4	7.7	22.5	5.3	495	347
5	7.2	22.5	4.4	459	321
6	7.1	23.0	5.5	416	291
7	7.1	22.8	3.8	437	306
Mean	**7.20**	**22.36**	**5.08**	**501**	**350**

Turbidity was observed to increase if the color of the water changes from white to light-yellowish, reddish or grayish and from there to greenish or brown **(**Omezuruike1 *et al*., 2008). Electric conductivity (EC) values in the present study were high (Table 2) and usually high EC values indicate the presence of high contents of dissolved salts in water (Abdullah & Mustafa, 1999). The EC values are a good measure of the relative difference in water quality between different aquifers (Anon., 1990)**.** The recommended permissible limit for EC is 300µS cm^{-1}(Jafari *et al.*, 2008). Total dissolved solids showed variation in all biotreatment ponds. Total dissolved solids **(**TDS) in the present study ranged from 291.43-420.7. Whereas ISI prescribed the desirable limit of TDS is 500 mg/L, the maximum permissible level is 2000mg/L. High TDS in ground water may be due to ground water pollution when waste waters from both residential and dyeing units are discharged into pits, ponds and lagoons (Shyamala *et al*., 2008).

Chemical analysis of wastewater and *Lemna minor*: It was observed that the concentration of Zn was highest in the water of 1st pond, Pb was highest in 5th pond, Ni was highest in the 6th pond, Mn was highest in 7th pond and Fe was highest in the 1st and 7th ponds (Table 3). It was found that Zn was lowest in the 6th and 7th ponds, Pb and Ni were lowest in 1st pond, Mn was lowest in the 2nd pond and Fe was lowest in the 3rd pond. Concentration of all the heavy metals in the biotreatment ponds was lower than the permissible limits. No metals was found in the fish Pond (7th pond) in abnormally high concentration. For example, the average concentrations of Zn and Ni were close to 5th pond, whereas the concentrations of Mn, and Fe were higher than the other ponds and this may be attributed to the turbidity of this pond, and the movement of fishes also causes continuous fluctuation of the waters and consequently release of these metals from the bottom sediments (Abdelmoneim *et al.*, 1997).

Table 3. Mean concentration of heavy metals (µg/L) in the wastewater of seven biotreatment ponds.

Biotreatment ponds water	Zn	Pb	Ni	Mn	Fe
1	0.09^{+}	0.03^{-}	0.06^{-}	0.21	0.15^{+}
2	0.08	0.06	0.08	0.17^{-}	0.10
3	0.06	0.06	0.09	0.22	0.07^{-}
4	0.06	0.05	0.10	0.25	0.09
5	0.06	0.08^{+}	0.10	0.28	0.09
6	0.05^{-}	0.04	0.13^{+}	0.46	0.09
7	0.05^{-}	0.00	0.12	0.47^{+}	0.15^{+}
Mean	**0.06**	**0.04**	**0.10**	**0.29**	**0.11**

Table 4 shows that the concentration of Zn was highest in the *Lemna minor* plants present in the 2nd pond, Pb was highest in fifth pond, Ni was highest in the 6th pond, Mn was highest in 4th pond and Fe was highest in the 6th pond. The concentrations of Mn and Fe were lowest in the 1st pond while the concentration of Ni was lowest in the 1st, 3rd and 4th bio-treatment ponds. The Pb was lowest in 2nd pond and Zn was lowest in the 6th pond.

Table 4. Mean concentration of heavy metals (µg/L) in *Lemna minor* from seven biotreatment ponds.

Biotreatment ponds water	Zn	Pb	Ni	Mn	Fe
1	147	13	1^-	592^-	1173^-
2	213^+	8^-	5	1000	2746
3	60	9	1^-	962	1270
4	99	9	1^-	1873^+	1330
5	84	23^+	8	676	2563
6	35^-	12	15^+	1225	3039^+
Mean	**106**	**12**	**5**	**1055**	**2020**

The concentrations of heavy metals were higher in *Lemna* as compared to water and the result indicates that *Lemna* plants are good hyper accumulators. Aquatic plants take up metals from the environment and contribute to the accumulation of metals in the trophic chains (Miretzky *et al.,* 2004). The final metal concentration in aquatic plants is usually significantly larger than in the surrounding water. Similar study was reported by Junshum *et al*.,(2007) that the levels of heavy metals in water showed a reducing trend from the settleable solid and oxidation pond to the Mae Moh reservoir. This trend may have been due to precipitation of suspended matter and by the aquatic plant root systems (*Eichhornia* sp., *Ipomoea* sp., *Typha* sp. & *Canna* sp.) in the wastewater treatment system, which can remove heavy metals from water (Hastuti, 1998; Izaguirre *et al.*, 2001; Razo *et al.,* 2004; Saygideger *et al*., 2004). In general, phytoextraction and phytovolazation are considered to be the main options for the removal of heavy metals and other elemental compounds (Meagher, 2000).

Of the tested metals, the numbers of metals are accumulated at certain extents with plant species and responsible for lowering the hardness of textile effluent with *B. juncea*. The heavy-metal pollutants along with other nutrients get bound to the root surfaces, as in the case with *B. juncea*, which can concentrate Cd, Ni, Pb, and Sr into root tissues at higher levels (Salt *et al*., 1995), *B. juncea* is already reported for the phytoextraction of metals. This is important because this plant species reduces the metal content as well dye concentration from the textile effluent, which is very useful to reduce the toxicity and total dissolved solids (TDS). Phytoremediation has recently become a subject of intense public and scientific interest and a topic of many recent researches (Raskin *et al.*, 1994; Cunningham *et al.*, 1995; Salt *et al.,* 1995; Cunningham & Ow, 1996; Ike, *et al.*, 2007; Kumar & Jaiswal, 2007; Muneer *et al.,* 2007; Sun *et al.*, 2007). Phytoremediation of heavy metals is a cost-effective green technology; there are more advantages, when it comes to the use of native and naturally growing plants. This is the first report on the ability of *Lemna* in wastewater phytoremediation.

Conclusion

This study will help to make better choice of wastewater plant system for other cities of Pakistan because this technology is not expensive and easy to adopt. The ponds are suitable as fish farms and we can use this water for crops and farming.

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