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NITROGEN AND PHOSPHOROUS REMOVAL FROM LEACHATE BY DUCKWEED (*Lemna minor*)

Two separate experiments were conducted during the months of June and September, 2014 to investigate the nutrient (nitrogen and phosphorous) removal from leachate by growing duckweed, *Lemna minor* in various leachate dilutions under natural climatic conditions of Islamabad, Pakistan. The highest uptake of nitrogen and phosphorous by duckweed was 95% and 90%, respectively, whereas the highest growth rate of duckweed was 6.4 $g\cdot m^{-2}\cdot day^{-1}$ during both experiments. The highest rates of nitrogen and phosphorous removal from leachate media were 380 and 200 $mg\cdot m^{-2}\cdot day^{-1}$, respectively, during both experiments. Nutrient uptake by duckweed and its growth rate was rapid at more diluted leachate whereas the nutrient removal rates from leachate media were higher in more concentrated leachate. The duckweed growth and its nutrient uptake ability under natural climatic conditions were directly affected by seasonal climatic variations. Relatively higher temperature and more intense solar radiation were more favorable for the duckweed growth and its nutrient uptake ability. Both parameters can be improved by pre-acclimation of duckweed with leachate which prevents the lag phase of the duckweed growth.

1. INTRODUCTION

Sustainable management of solid waste is one of the emerging challenges faced by developing countries like Pakistan. As an existing practice, open dumping of solid waste in low lying areas is very common in Pakistan [1]. Besides other direct and indirect environmental hazards, production of large amount of leachate is one of the major problems associated with solid waste dumping.

Leachate is the concentrated wastewater. At open dump sites, it is produced by percolation of rainwater through solid waste layers [2]. Physical and bio-chemical activities in solid waste transfer variety of pollutants from waste into percolating rain water [3].

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Depending on nature of solid waste, climatic conditions (temperature, precipitation), management practices and age of dump site, leachate may contain variety of inorganic and organic pollutants [4]. Major components of general landfill leachate are: dissolved organic matter characterized as chemical oxygen demand (COD), total organic carbon, volatile fatty acids and refractory compounds; inorganic macronutrients such as calcium (Ca^{2+}), sodium (Na^{+}), potassium (K^{+}), and ammonium (NH_4^{+}) ions, sulfates (SO_4^{2-}) and hydrogen carbonates (HCO_3^{-}); heavy metal ions such as copper (Cu^{2+}), lead (Pb^{2+}), nickel (Ni^{2+}) and zinc (Zn^{2+}) and xenobiotic organic compounds [3]. Leachate poses a serious threat to surface as well as ground water quality [5]. Leachate hazards for human health, flora, fauna, and ecosystems have also been frequently investigated and documented [6]. Presently, it has become major concern worldwide to impose more stringent environmental requirements related to leachate treatment and surface and ground water quality [7].

Use of aquatic plants such as duckweed, water hyacinth, water lettuce in wastewater treatment has received greater attraction during the recent years [8–10]. Production of aquatic plants on wastewater has two fold benefits: treatment of wastewater and, as an alternate technology, converting wastewater nutrients into potentially useful forms [11].

Duckweed is a small floating macrophyte belonging to family *Lemnaceae* of monocotyledon plants. *Lemnaceae* consists of 4 genera (*Lemna*, *Spirodela*, *Wolffia* and *Wolffiella*) and 28 species [12, 13]. Duckweed has efficient ability of nutrient uptake and shows high growth rate when grown under nutrient rich wastewaters [14]. In eight week time, a duckweed based swine lagoon treatment system can remove up to 83.7% of total nitrogen (TN), and 89.4% of total phosphorus (TP) from the media when harvested twice a week [15]. High level of nutrients tolerance in duckweed is of particular importance for treatment of landfill leachate which usually has high concentrations of nitrogen and phosphorous. For example, the high concentrations of ammonia-N (3032 $mg\cdot dm^{-3}$), nitrate-N (22 $mg\cdot dm^{-3}$), and nitrite-N (120 $mg\cdot dm^{-3}$), and 3000 $mg\cdot dm^{-3}$ of phosphates have been reported in leachate samples from Hong Kong [16].

Recently, the phytoremediation of landfill leachate by aquatic plants has received growing attention [12]. Duckweed due to its high rates of growth and nutrient uptake is one of the promising aquatic plants used for leachate treatment. *L. minor* can uptake significant amount of inorganic nitrogen through roots and fronds as well [18]. Ammonium ions are the preferred forms of nitrogen uptake by duckweed [19]. Majority of total Kjeldahl nitrogen (TKN) in leachate is ammonium which is an advantage for the duckweed growth. A range of 0.2–13 000 $mg\cdot dm^{-3}$ of N has been reported in leachate samples collected from various parts of the world [6]. Duckweed has ability to grow under broad range of temperature making it an advantageous macrophyte to grow round the year in the areas where other tropical aquatic plants such as water hyacinth are unable to perform in summer [9, 11]. Duckweed can survive below freezing temperatures for many days [11]. Duckweed has high protein contents (10–40% of protein on dry mass basis). Some species of duckweed have ability to produce protein six to ten times faster than soybeans planted at an equivalent surface area [10]. A positive correlation has been reported between TN of wastewater medium and protein contents of duckweed grown on it [10]. High protein content of duckweed is an indication of its higher capacity to assimilate nitrogen. The protein content of 35% was reported in duckweed with fastest nitrogen removal rates (4.4 $g \cdot m^{-2} \cdot$day of TKN) from swine wastes [23]. Protein content of duckweed is very useful for its end use as high protein feed for ducks, cattle, poultry and fish [24–26].

Lemna minor species of duckweed has enormous potential of nutrients removal from wastewaters of high strength such as swine lagoon wastewater [11, 13]. Under favorable climatic conditions and nutrient balance in growth media, *Lemna minor* grows well and doubles its biomass within two days [25]. The growth rate of *L. minor* close to 29 $g \cdot m^{-2} \cdot day^{-1}$ as well as TKN and TP uptake of 90% and 88.6%, respectively have been reported when grown on high strength swine lagoon under natural conditions in Raleigh, North Carolina [11].

This study has been conducted to investigate the nutrient removal dynamics from leachate by *L. minor* under the natural conditions of temperature and light intensity in Islamabad, Pakistan. The duckweed growth in relation to various nutrient concentrations in leachate and nutrient uptake rates are determined by growing duckweed on various initial concentrations of nutrients in leachate (various leachate dilutions). This study will be useful to initiate the duckweed based leachate treatment systems by providing an understanding of nutrient removal dynamics from such leachate treatment systems under natural climatic conditions.

2. MATERIALS AND METHODS

Leachate. Leachate was prepared by the processing of decomposed solid waste collected from various residential, commercial and industrial areas of Islamabad and Rawalpindi, Pakistan. 100–120 kg well decomposed solid waste was collected from each residential, commercial and industrial dump sites. Waste was collected from pre-determined lowest points at each dump site at soil depths of 0.5–1.5 m [27]. Solid waste samples were collected during both dry and wet season of the year. The samples were mixed in a large plastic water storage tank having the internal diameter of 1.5 m, 1.8 m hogh. A sieve (pore size 1 mm) was fixed at an internal height of 10 cm of the plastic container. Thorough shaking was applied to well mix the waste and obtain a homogenized sample. The homogenized waste was soaked with leaching solution (distilled water) and maintained for 30 days after which the leachate was collected from the bottom outlet in the container. Remaining solid waste was again mixed thoroughly and soaked with distilled water. Afterwards, the leachate was collected three times at an interval of ten days each, during both summer and fall. Each time the solid waste samples were thoroughly mixed and shaken. The leachate collected from various runs was mixed to form single homogenized sample to be used for this research.

Duckweed. Duckweed (*L. minor*) used in this research was collected from a wastewater pond in Islamabad, Pakistan. Prior to grow on leachate, duckweed was repeatedly washed with excess water to remove bacteria, algae and other unwanted compounds [28]. During initial experiments, duckweed was grown at various leachate dilutions in order to reach the optimal range of dilutions duckweed can tolerate. Before starting the experiments, duckweed was adopted to new environment for fifteen days by growing on experimental leachate under the same natural conditions.

Duckweed was grown in 300 cm^3 transparent plastic containers. 250 cm^3 of initial volume of leachate was used in each container at the leachate depth of 9.5 cm. Surface area of each container was 25.8 cm^2. Duckweed containers were placed within a porous iron rack having three compartments. The rack with duckweed containers was placed in an open environment at the Institute of Environmental Sciences and Engineering (IESE), National University of Sciences and Technology, Islamabad under natural climatic conditions. Seasonal effect on growth of duckweed and its nutrient removal efficiency were determined during the study. Seasonal data related to ambient air temperature and day lengths during both experiments was retrieved from the website of Pakistan Metrological Department, whereas the solar radiation data during the experimental period was obtained from the web site of LEO Corporation, Pakistan. Nutrient removal and growth of duckweed was tested at various initial concentrations of nutrients.

Experimental setup. Two batch experiments were conducted during the months of June (summer experiment) and September (fall experiment), 2014, to determine the duckweed growth and nutrient (N and P) removal rates from leachate. Five dilutions of leachate with leachate/water ratios of 50/50, 40/60 , 30/70, 20/80 and 10/90) were prepared with tap water and the duckweed was grown at each leachate dilution. About 30 mg of initial fresh mass of pre-acclimated duckweed was used during each test. Each batch test consisted of 44 containers containing a dilution of leachate corresponding to triplicate samples of 11 time points. Out of these, 33 containers contained duckweed cultures whereas 11 control containers were without duckweed. The leachate and duckweed were mixed briefly for five min every day. During each experiment, three duckweed containers and a control one were removed for destructive sampling after every 2 days to monitor the duckweed growth and nutrient levels. Each experiment lasted for 22 days. Average ambient air temperature was 39.1 °C, solar radiation 4.5–5.0 $kWh\cdot m^{-2}\cdot day^{-1}$, and day lengths during summer experiment 14.20 h. During fall it was 24–30 °C, 4–4.5 $kWh\cdot m^{-2}\cdot day^{-1}$ and 12.20 h, respectively.

Laboratory analysis. Samples of the leachate were analyzed for TKN, ammonium nitrogen (NH_4^+-N), total phosphorous (TP), *o*-phosphate-phosphorous (*o*-PO_4^{3-}-P), chemical oxygen demand (COD) and pH. Dry biomass of duckweed was analyzed for TKN and

TP contents. All chemical analyses were conducted in environmental analytical laboratories of the Institute of Environmental Sciences and Engineering (IESE), National University of Sciences and Technology, Islamabad. All chemical analyses were performed using the standard methods of American Public Health Association (APHA, 1998) [25] and US-EPA Methods (EPA, 1983) [26].

3. RESULTS AND DISCUSSION

To investigate the nutrients dynamics of duckweed based leachate treatment system, two batch experiments were performed under the natural climatic conditions of Islamabad, Pakistan during the summer and fall of 2014. Five dilutions of leachate (50%, 40%, 30%, 20% and 10%) were used as medium for the duckweed growth. Initial concentrations of average N and P and other characteristics of each leachate dilution during each season were determined (Table 1). In initial leachate medium, P:N ratio has relatively higher ranges in summer experiments (0.75–0.86) than that in the fall experiments (0.77–0.82).

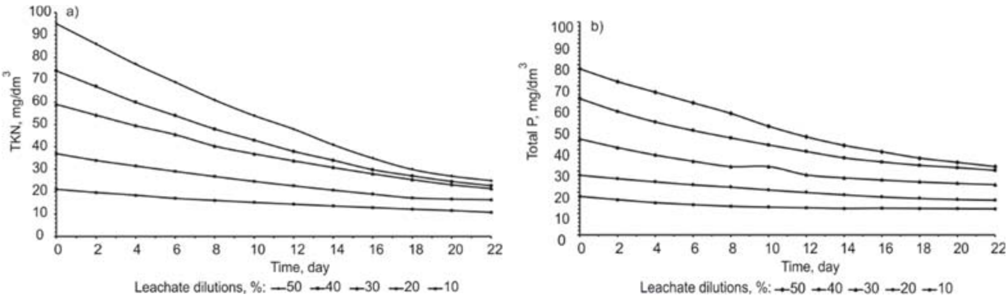
Table 1

Initial characteristics of leachate dilutions used as medium for growth of *L. minor* in natural climatic conditions of Islamabad, Pakistan in 2014

Leachate dilutions[a] [%]	Nutrient concentration[b] $[mg\cdot dm^{-3}]$				COD[b] $[mg\cdot dm^{-3}]$	pH[b]
	TKN	NH_4^+-N	TP	*o*-PO_4^{3-}-P		
Summer experiment						
50	95±1.63	55±0.82	78±0.41	18±1.47	2760±2.83	7.45±0.02
40	74±0.82	40±1.63	64±3.08	14±1.22	2248±2.16	7.36±0.01
30	59±1.63	35±1.08	45±2.68	10±1.08	1732±1.41	7.29±0
20	37±1.78	20±1.41	28±1.41	8±0.71	1088±2.16	7.18±0.07
10	21±1.08	12±0.41	18±0.71	5±0.41	540±2.83	7.14±0
Fall experiment						
50	102±2.16	58±2.16	82±1.63	32±1.41	2922±7.48	7.89±0.02
40	82±0	46±1.41	66±3.74	26±1.41	2308±5.72	8.00±0.08
30	61±1.41	32±0	47±0.82	22±2.94	1695±5.10	7.94±0.01
20	40±0.82	17±2.16	33±2.83	12±1.41	1216±8.16	7.82±0.01
10	23±0	10±1.41	19±0.82	9±0.82	522±3.74	7.76±0.02

[a]Initial concentrations of nutrients and COD for all leachate dilutions may not be exactly same as intended because of the deviations caused during the dilution operation.

[b]Each value is the average of those obtained from three replicate experimental containers.



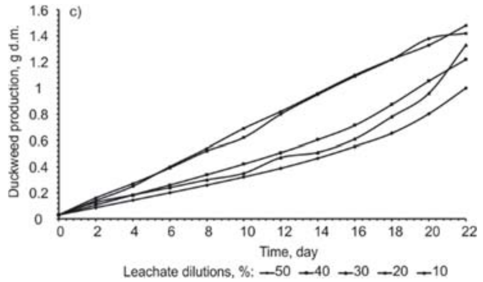
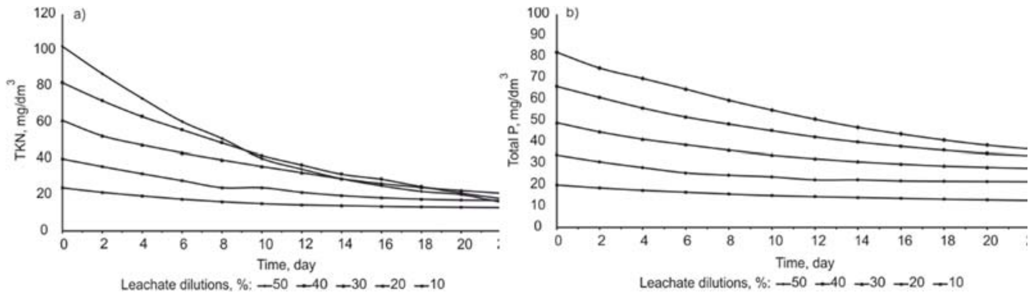


Fig. 1. Removal of a) total Kjeldahl N (TKN), b) total P from various dilutions of leachate by growing *Lemna.minor* and, c) duckweed production under natural climatic conditions of Islamabad, Pakistan in summer (June), 2014



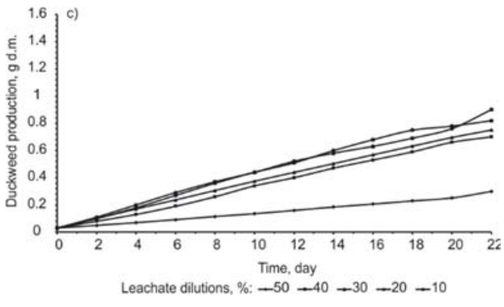


Fig. 2. Removal of a) total Kjeldahl N (TKN), b) total P from various dilutions of leachate by growing *Lemna.minor* and, c) duckweed production under natural climatic conditions of Islamabad, Pakistan in fall (September), 2014

pH in test containers remained stable within the range of 7.1–7.5 during summer experiments and 7.7–8.0 during the fall experiments. This indicates a strong buffering capacity of leachate (slightly greater during the fall experiments) which is very important for the growth of duckweed. It tends to rapidly decrease the pH of growth media without buffering (from approximately 7 to approximately 5 within 24 h) [22]. COD reduction from 46% to 79% and from 44% to 67% was achieved during fall and summer experiments, respectively, which is the indication of high microbial activity in the test containers during the experiments. The highest level of COD reduction was achieved during two first days after of each experiment. It is because of the pre-acclimation of duckweed in leachate media which resulted in high rates of pollution removal. Close to linear rates of N and P removal and duckweed growth were observed during both experiments (Figs. 1, 2). At the end of experiments, considerable amounts of nutrients were still present in more concentrated leachate.

Lag phase of the duckweed growth was not observed in both experiments (Figs. 1c, 2c). It was because, prior to start the experiments, duckweed was acclimated to the nutritional environment of leachate under natural climatic conditions. Acclimation also helps the duckweed to adjust under drastic changes within a system [27]. The highest ducked growth rate (6.4 $g\cdot m^{-2}\cdot day^{-1}$) was achieved at 10% leachate dilution in summer experiment, whereas the lowest rate of the duckweed growth (1.2 $g\cdot m^{-2}\cdot day^{-1}$) was observed at 50% leachate dilution during the fall experiment (Table 2).

Table 2

Rates of nutrient (N and P) removal and duckweed (*L. minor*) growth for various dilutions of leachate under the natural climatic conditions of Islamabad, Pakistan in 2014

Dilutions of leachate [%]	Nutrient removal rate[a] [$mg\cdot m^{-2}\cdot day^{-1}$]				Duckweed growth rate[a] [$g\cdot m^{-2}\cdot day^{-1}$]
	TKN	NH_4^+-N	TP	*o*-PO_4^{3-}-P	
Summer experiment					
50	310	200	200	60	4.3
40	230	130	150	50	5.2
30	160	110	90	30	5.7
20	90	50	50	20	6.1
10	40	30	30	10	6.4
Fall experiment					
50	380	230	200	120	1.2
40	280	170	140	90	3.0
30	180	110	90	70	3.2
20	100	60	60	30	3.5
10	50	30	30	20	3.8

[a]Each value is the average of the results obtained from three replicate experimental containers.

Overall rates of nutrient removal were higher in a more concentrated leachate, whereas the nutrient uptake by duckweed was higher in a more diluted one (Tables 2, 3). More concentrated growth media may be more favorable for microbial growth than for the duckweed growth [11]. Some nitrogen was probably lost through algal and microbial assimilation [28]. Ammonia volatilization was negligible in our experiments due to average pH of the media which was lower than 8 throughout the experiments. Large portion of N and P was not taken up by duckweed particularly in 50% leachate dilution during fall experiment which agrees with the duckweed growth (Table 3, Fig. 2).

Table 3

Mass balance of total N and P removal and nutrient uptake by duckweed (*Lemna minor*) from diluted leachate in two batch experiments under the natural climatic conditions of Islamabad, Pakistan in 2014

Leachate dilutions [%]	Total nutrients removed from media[a]				Nutrient uptake by duckweed[a]			
	[mg]		[%]		mg		[%]	
	N	P	N	P	N	P	N	P
Summer experiment								
50	20	10	16	49	9.4	4.6	47	46
40	10	8	46	50	5.6	4.8	56	60
30	9	5	39	55	5.9	3.3	66	66
20	5	3	46	58	4.0	2.5	80	83
10	2	2	62	56	1.9	1.8	95	90
Fall experiment								
50	20	10	22	52	2.4	1.4	12	14
40	20	8	03	52	10.2	3.5	51	44
30	10	5	35	58	6.7	2.6	67	52
20	6	3	40	64	4.6	2.1	76	70
10	3	2	48	58	2.7	1.6	90	80

[a]Each value is the average of those obtained from three replicate experimental container.

Overall rate of NH_4^+-N removal in the fall experiment was higher than in summer which is similar to the TKN removal pattern (Figs. 3a, 4a). Initial concentrations of *o*-PO_4^{3-}-P and its removal rates were significantly higher in the fall experiments than in summer and same was the pattern of its removal i.e. higher removal rate at higher leachate concentration (Figs. 3b and 4b). It suggests that removal rate of *o*-PO_4^{3-}-P is more dependent on its initial concentrations than on climatic conditions which were relatively less favorable for the duckweed growth during the fall experiment. Some of the *o*-PO_4^{3-}-P removal may also occur by microbial assimilation and precipitation with minerals in leachate media [28].

Duckweed production rate was smaller in the fall experiment (Table 2). This is likely due to the combination of climatic factors during this period which were less favorable for the duckweed growth such as light intensity and air temperature were comparatively lower during fall experiment and day light hours in Islamabad were also shorter in September than in June. Short term temperature fluctuations in experimental containers were observed during both seasons which are probably due to small volume of leachate (250 cm^3) in each container. Such kind of temperature fluctuations would not be expected in large ponds or lagoons for leachate treatment.

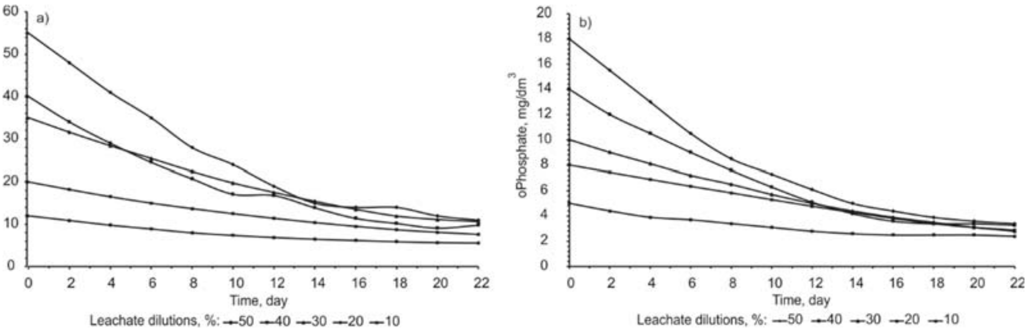


Fig. 3. Removal of a) ammonium-N, b) o-PO_4^{3-}-P from various leachate dilutions by growing *Lemna minor* under natural climatic conditions of Islamabad, Pakistan in summer (June), 2014

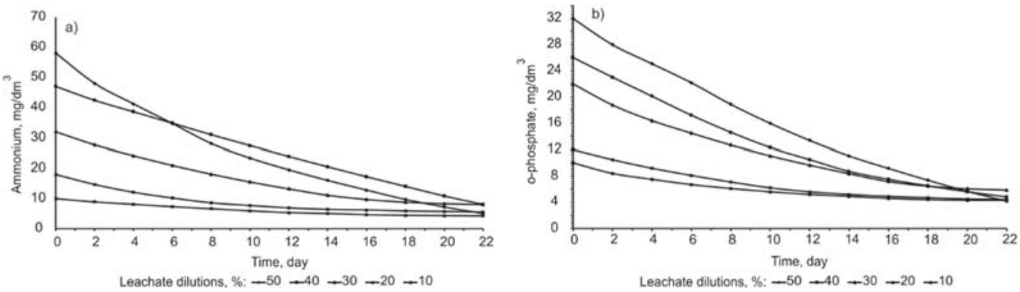


Fig. 4. Removal of a) ammonium-N, and b) o-PO_4^{3-}-P from various leachate dilutions by growing *Lemna minor* under natural climatic conditions of Islamabad, Pakistan in fall (September), 2014

Lower duckweed production at concentrated leachate media may be due to many factors such as presence of microorganisms, insects and undefined chemicals in field containers with more concentrated leachate which caused the duckweed to take up relatively smaller percentage of nutrients, overall removed from the media (Table 3). At higher leachate concentration, the inhibitory action caused by the ammonia (NH_3) and ammonium ions (NH_4^+) may also contribute to lower duckweed growth. It has been reported that the ammonia concentration of more than 50 $mg \cdot dm^{-3}$ in domestic wastewater is inhibitory to the duckweed growth [8]. Effect of ammonia to weaker duckweed growth seems less significant in our experiments because of the narrow fluctuations in pH of growth leachate media during both experiments (summer 7.1–7.5 and fall 7.7–8.0) as duckweed growth inhibition by ammonia is more significant at pH values above 8 [8].

Reasons for the weaker duckweed growth at more concentrated leachate need to be further investigated. Effects of high content of phosphorous and nitrogen (in more concentrated leachate) on the duckweed growth also need to be determined in future. Data reported in this paper indicate that to maintain a rapid growth and nutrient uptake by duckweed, leachate should be diluted to below 60 mg $TKN \cdot dm^{-3}$ and 45mg $TP \cdot dm^{-3}$.

Data presented in this paper is useful to initiate duckweed based leachate treatment system. Diluted leachate should be used in such duckweed systems in order to initiate high growth and nutrient removal rates. Fresh leachate generally has high values of nutrients and other pollutants. Therefore to avoid nutrient shock loading, leachate may be treated by mixing with less concentrated wastewater. Recycling of duckweed treated leachate and mixing it into influent leachate may also reduce the nutrient loading of influent leachate [11].

Lemna minor has potential to remove N and P from landfill leachate therefore it is important to establish duckweed for in-field leachate treatment. In Pakistan, small duckweed based ponds may be established adjacent to solid waste dump sites and local strains of *Lemna minor* may be used as potential aquatic plant to treat diluted leachate under natural climatic conditions.

4. CONCLUSIONS

- *Lemna minor* maintained a healthy growth at leachate efficiently removing the nutrients (N and P) from the media. The highest duckweed growth rate of 6.4 $g \cdot m^{-2} \cdot day^{-1}$ was achieved at least concentrated leachate during summer and fall experiments, whereas the nitrogen and phosphorous uptake by duckweed (95% and 90%, respectively) was also achieved at 10% leachate dilution during summer experiment.
- Highest rates of nitrogen and phosphorous removal (380 $mg \cdot m^{-2} \cdot day^{-1}$ and 200 $mg \cdot m^{-2} \cdot day^{-1}$, respectively) were achieved at most concentrated leachate during summer and fall experiments.
- Lag phase of the duckweed growth can be prevented by pre-acclimation of duckweed with leachate which also improves the nutrient removal efficiency of duckweed.
- More concentrated leachate is less favorable for the duckweed growth and its nutrient uptake efficiency.
- Relatively ability of duckweed to remove nutrients from leachate media and its growth rates were affected by the climatic conditions during both experiments. Comparatively higher temperature and solar radiation during summer was more favorable for the duckweed growth and its nutrient uptake ability.

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