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Phytoremediation of nutrients from water by aquatic floating duckweed (*Lemna minor*) in rearing of African cichlid (*Labidochromis lividus*) fingerlings

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ABSTRACT

Water treatment, reuse, and reducing the nutrients loading to the aquatic environments are key ways to achieve sustainable aquaculture. The usage of aquatic plants is an effective and environment-friendly method for water treatment. This study was conducted to investigate the nutrient removal efficiency of aquatic plant *Lemna minor* by static test and flow test using a water recirculation system for rearing of African cichlid (*Labidochromis lividus*) fingerlings during 7 and 30 days, respectively. The growth performance of fish and water quality parameters were compared between the *L. minor* and control groups in triplicate. The results of static test showed that *L. minor* removed the total nitrogen ammonia (TAN) and total phosphorus (TP) by 43.7% and 52.38% after 48 h and 7 days, respectively. The results of flow test revealed that the survival rate (%) and growth performance including final weight, final length, weight gain, specific growth rate (SGR%), body weight increase (BWI%) and daily growth index (DGI) of fish cultured in a water recirculation system containing *L. minor* as a biofilter were significantly higher than the control ($P<0.05$). The utilization of *L. minor* decreased the concentrations of TAN, TP, electrical conductivity (EC) and total suspended solids (TSS) by 41%, 37.80%, 2.60% and 81.11% compared to the control after 30 days of cultivation period. The nitrate (NO_3^-) concentration increased to the maximum level on day 20 and then it decreased significantly on day 30 in the *L. minor* treatment ($P<0.05$). These findings indicated that the usage of aquatic plant, *L. minor* could be considered as an effective biological method for water treatment in aquaculture.

1. Introduction

Nowadays, aquaculture industry is growing rapidly worldwide (Mehana et al., 2015). One of the serious problems in aquaculture is the discharge of inorganic nutrients, particularly nitrogen and phosphorus into surface waters (Ferdoushi et al., 2008). The discharge of nutrient rich effluents into water bodies lead to eutrophication and algal blooms, which ultimately depletes oxygen and reduces the water quality (Selvarani et al., 2015). Furthermore, the concentrations of nitrogen compounds and phosphorus are regularly increased in intensive and semi-intensive aquaculture systems, which adversely effect on water quality and subsequently fish health and production.

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Ammonia is the major nitrogenous waste product excreted by aquatic animals (Alabaster and Lloyd, 1980), which exists in equilibrium as both molecular ammonia (NH_3) and ammonium ion ($NH_4{}^+$) in aquatic environments (Rezagama et al., 2017). The two forms of ammonia can be converted into one another depends on the pH and temperature. The molecular ammonia is predominantly formed at higher pH and temperature and is more toxic than ionic form because of its higher permeability to biological membranes (Randall and Tsui, 2002; Rezagama et al., 2017). Ammonia induce acute toxicity to vertebrates through effects on the central nervous system, which lead to an increase in extracellular glutamate level of brain, excessive activation of NMDA receptors and eventually neuronal cell death (Randall and Tsui, 2002). Chronic ammonia concentrations also decrease the growth and disease resistance of aquatic animals (Lemarie et al., 2004).

Various methods including biological nitrification, ion exchange and air-stripping have been administrated to remove ammonia from water and wastewater (Widiastuti et al., 2011; Sandip and Kalyanraman, 2017). Phytoremediation is an energy-efficient and less expensive alternative method that acts based on biological processes, in which plants are used to remove nutrients and pollutants from medium (Fang et al., 2007; Lee, 2013; Zhang et al., 2014). Some plants are preferential to absorb ammonium ion ($NH_4{}^+$) as nitrogen source, so that phytoremediation can be used as one effective way for $NH_4{}^+$ removal (Olguin et al., 2007). Xu and Shen (2011) showed that $NH_4{}^+$ as a preferred nitrogen source can be absorbed by duckweeds.

Duckweed is a small and simple floating aquatic plant that belongs to lemnaceae family (Wang et al., 2014). This aquatic plant is distributed all over the world and has ability to grow rapidly and absorb mineral nutrients (Priya et al., 2012; Zhang et al., 2014). In the recent years, the duckweed has been used in treatment of municipal wastewater (Dalu and Ndamba, 2003; El-Kheir et al., 2007) and as biofilter in aquaculture (Velichkova and Sirakov, 2013). Selvarani et al. (2015) reported that duckweed (*Lemna minor*) achieved the maximum removal efficiency of NH_3, $NO^-{}_3$ and $PO_4{}^-$ in different municipal wastewaters at the rate of 96%, 98% and 96%, respectively. The results of another study showed that ammonia and phosphate removal efficiency of duckweed from stabilized domestic wastewater was 30.8% and 28.7%, respectively (Matos et al., 2014). In recent years, several studies have evaluated the nutrient removal efficiency of different aquatic plants from municipal and industrial wastewaters (El-Shafai et al., 2007; Xu and Shen, 2011; Samimi and Shahriari Moghadam, 2018). To the best of our knowledge, there are no enough reports on the administration of aquatic plants as biological filter to remove the ammonia and phosphorous from water in aquaculture.

Globally, ornamental fish production is growing rapidly as a profitable activity in aquaculture industry (Jaleel et al., 2015). The increased demand for aquarium fish can be fueled using and semi-intensive and intensive systems. A consequence of high-density aquaculture is the increased nitrogenous wastes excretion especially ammonia which adversely affects the growth, health and survival of fish. Ammonia toxicity depends on several factors such as the life stage of fish. Several studies have reported more sensitivity of fish species to ammonia in the growing stages (Mallett and Eddy, 1995; Karasu Benli and Koksal, 2005; Gomułka et al., 2014). Ambient ammonia concentration is a key factor for intensive culture of fish larvae and juvenile. The administration of suitable method for treatment and reuse of water is also vital for cost-effective fish production. Therefore, this study was conducted to evaluate the efficiency of duckweed (*L. minor*) for removal of total nitrogen of ammonia (TAN) and total phosphorous from recirculation water system used to culture African cichlid (*Labidochromis lividus*) fingerlings. The survival rate (%) and growth performance of fish were evaluated at the end of experimental period. Moreover, the fluctuations of total suspended solids (TSS), electrical conductivity (EC) and pH during cultivation period were determined.

2. Materials and methods

We conducted two separate experiments to evaluate the efficiency of a species of duckweed for removal of the total ammonia nitrogen (TAN) and total phosphorus from water. The duckweeds were collected from a pond in a greenhouse in Mashhad, Khorasan Rasavi province, Iran. They were transferred to Aquatic Lab. in Ferdowsi University of Mashhad, Iran and stocked in a 200-L tank to acclimatize to laboratory conditions for two weeks. Taxonomy experts identified the plant as *Lemna minor* using the identification keys.

2.1. Static test

For this experiment, we designed two treatments consisted of the *L. minor* and control in triplicate. Six-glass aquariums (45 × 30 × 40 cm) were filled with 15 L of Tap water whose properties are shown in Table 1. The concentrations of the total ammonia nitrogen (TAN) and total phosphorus (TP) were reached to 5 mg L^{-1} and 3 mg L^{-1}, respectively by adding the analytical salts (Merck)[NH_4Cl and K_2HPO_4] into water of each glass aquarium. These concentrations were chosen based on the literature review on the TAN and TP concentrations in effluents of fish farms (Mustapha and Akinshola, 2016; Coldebella et al., 2018). The duckweeds (*L. minor*) were inoculated in each aquarium with a surface area of 0.13 m^2 at density of 150 g wet weight. The aquarium without duckweeds was considered as control treatment. The water temperature was kept at 25 °C using an aquarium heater. The auxiliary light for growth of duckweeds was provided using white fluorescent light in a 16-h light/8-h dark cycle. The concentration of TAN was measured after 0, 6, 12, 24 and 48 h using ultraviolet visible spectrophotometer (DR 5000^{TM} model, HACH Co., USA) at the wavelength of 425 nm. The TP concentration also detected on days 0, 3, 5 and 7 using UV/visible spectrophotometer (WPA model, Biochrom Co., USA). The experiment was done in triplicate. Removal efficiency of TAN or TP in each time was calculated using following

Table 1
The physicochemical properties of water used in static test of duckweed (*L. minor*).

Parameters	Unit	Concentration
Total ammonia nitrogen (TAN)	mg L^{-1}	0
Nitrate (NO_3^--N)	mg L^{-1}	2.47 ± 0.16
Total phosphorus	mg L^{-1}	0.17 ± 0.02
pH	–	7.60 ± 0.2
Electrical conductivity (EC)	μS cm^{-1}	938 ± 33 ± 12.58
Dissolved oxygen	mg L^{-1}	6.21 ± 0.10

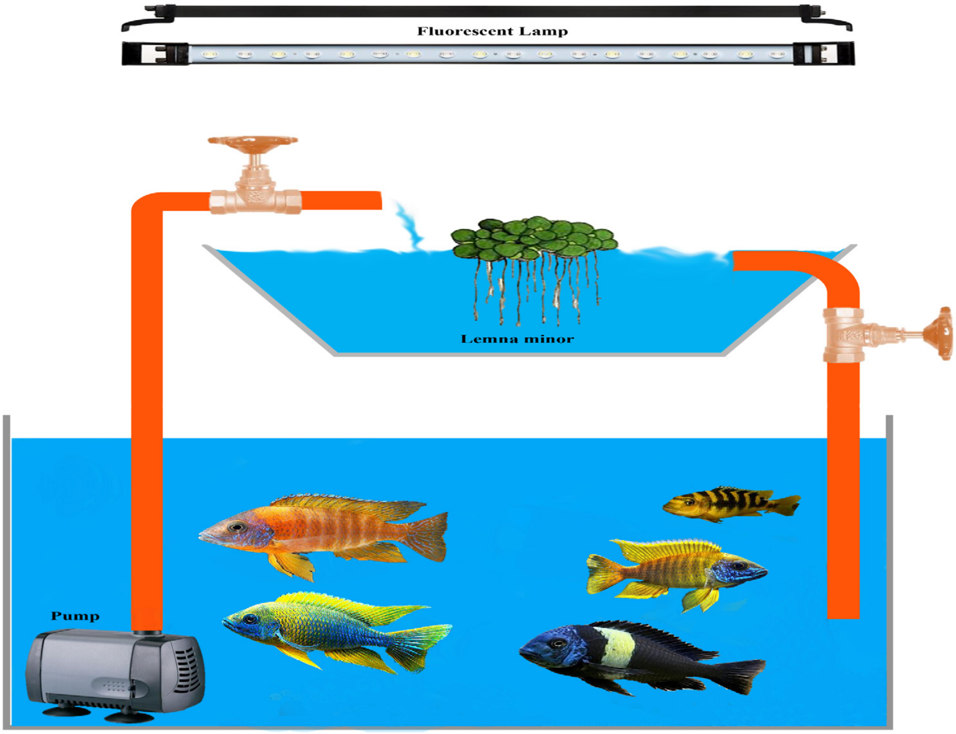

Fig. 1. Schematic figure of a water recirculation system for the flow test of duckweed (*L. minor*).

equation, where R is removal efficiency (%), C_A is concentration in control group (mg L^{-1}) and C_B is concentration in *L. minor* group (mg L^{-1}).

$$R\,(\%) = [C_A - C_B/C_A] \times 100$$

2.2. Flow test

This experiment was performed using a water recirculation system as experimental unit (Fig. 1). The components of each unit consisted of a glass aquarium (45 × 30 × 40 cm), a plastic container (50 × 30 × 20 cm), water pump (RS-4000 model, China), pipes and a controllable valve. The glass aquarium equipped with aeration and heating system was filled with 30 L of Tap water. The plastic container also w filled with 20 L of water and inoculated with 150 g wet wt. of duckweed (*L. minor*). The water of aquarium was supplied to the top of the plastic container by a pump at a flow rate of 3.5 L min^{-1}. The effluent water from the plastic container was returned into the aquarium. The water level inside the plastic container was controlled with a valve at the level of 15 cm. The system without duckweed was considered as the control. This experiment was done in three replicates.

One hundred and twenty healthy African cichlid (*Labidochromis lividus*) fingerlings were purchased from a local ornamental fish farm and transferred to the Aquatic Lab. (Ferdowsi University of Mashhad, Iran). The fish were stocked into a 300-L tank to acclimatize to laboratory conditions and fed on commercial pellets (Salvea 9015F, BioMar®, France) at rate of 3.5% of body weight for two weeks. The proximate composition of the supplied feed was as follows: crude protein 42%, crude lipid 28%, carbohydrates (NFE) 20.9% and ash 6.1%. Then, 17 fish (average weight of 2.07 ± 0.041 g; average length of 5.65 ± 0.098 cm) were randomly stocked into glass aquarium of each water recirculation system that aerated for 48 h. After startup of system, the fish were fed three times daily (8:00, 12:00, and 16:00) at rate of 3.5% of body weight during 30 days of experimental period. The duckweeds were kept under 16-h light/8-h dark regime. Water temperature was also tried to adjust to 25 °C using an aquarium heater. Water of each system was replaced with freshwater at rate of 20% every 10 days. Water quality parameters including temperature, dissolved oxygen, pH and electrical conductivity (EC) were measured daily using the portable multi-meter model AZ-8603. The water temperature and dissolved oxygen were recorded as 24.74 ± 1.06 °C and 6.9 ± 0.18 mg L^{-1}, respectively. Water samples were collected from each system on days 0, 10, 20 and 30 to measure the concentrations of total ammonia-nitrogen (TAN), nitrate-nitrogen (NO_3^--N), total phosphorus (TP) and total suspended solids (TSS). The nitrate concentration was measured using ultraviolet visible spectrophotometer (DR 5000^{TM} model, HACH CO., USA) at the wavelength of 500 nm. The TAN and TP concentrations were measured as mentioned in 2.1 section. At the end of the experimental period, the all fish of each aquarium were individually anesthetized using 500 mg L^{-1} clove powder and their weight and length were measured. The growth performance and survival rate of the fish were calculated using following equations:

$$\text{Weight gain (g)} = (W_f - W_i)$$
$$\text{Specific growth rate (SGR; \% body weight day}^{-1}) = [\mathrm{Ln}W_f - \mathrm{Ln}W_i/\mathrm{Time}] \times 100$$
$$\text{Condition factor \% (CF)} = [W_f(g)/L_F\ (cm)^3] \times 100$$
$$\text{Daily growth index (DGI)} = [(W_f - W_i)/\mathrm{Time}]$$
$$\text{Body weight increase \% (BWI)} = [(W_f - W_i)/W_i] \times 100$$
$$\text{Survival rate (\%)} = (N_f/N_i) \times 100$$

where W_i, W_f, L_F, N_i and N_f and are initial weight, final weight, final length, initial number of fish and final number of fish, respectively.

2.3. Statistical analysis

The data were presented as mean ± SD . Statistical analysis was assessed using IBM SPSS software (version 19.0). The normality of data was evaluated using Kolmogorov–Smirnov test. One-way repeated measures analysis of variance (ANOVA with repeated measures) was used to assess the significant differences between the means. The significant differences between two independent and paired samples were determined using Independent-Sample T test and Paired-Sample T-test, respectively. A probability level of $P < 0.05$ was applied for all analyses.

3. Results

3.1. Static test

The variations in total ammonia nitrogen (TAN) concentration of water during 48-h of static test is shown in Fig. 2. The TAN concentration in *L. minor* treatment showed no significant difference compared to the control treatment after 0, 6 and 12 h ($P > 0.05$), but this value decreased significantly compared to the control at times of 24 h and 48 h ($P < 0.05$). In the control treatment, TAN concentration did not change significantly during 48 h ($P > 0.05$). The TAN level in *L. minor* treatment decreased significantly after 12 h until the 48 h ($P < 0.05$).

The comparison of the total phosphorus (TP) concentration in two treatments of static test revealed the reduction of TP concentration in *L. minor* treatment compared to the control on days 5 and 7 of the experiment ($P < 0.05$). The TP concentration decreased significantly during the 7-days of the experiment in the control treatment ($P < 0.05$). The statistical analysis indicated that the TP concentration decreased significantly from 3th day to 7th day in *L. minor* treatment ($P < 0.05$) (Fig. 3).

3.2. Flow test

3.2.1. Growth performance and survival rate of fish

The growth performance and survival rate of African cichlid (*L. lividus*) cultured in a recirculation water system (flow test) for 30 days are shown in Table 2. The final weight, final length, weight gain, specific growth rate (SGR%), daily growth index (DGI) and body weight increase (BWI%) of fish cultured in system containing *L. minor* were significantly higher than the control ($P < 0.05$). In treatment of *L. minor*, the survival rate of fish was significantly higher than the control ($P < 0.05$).

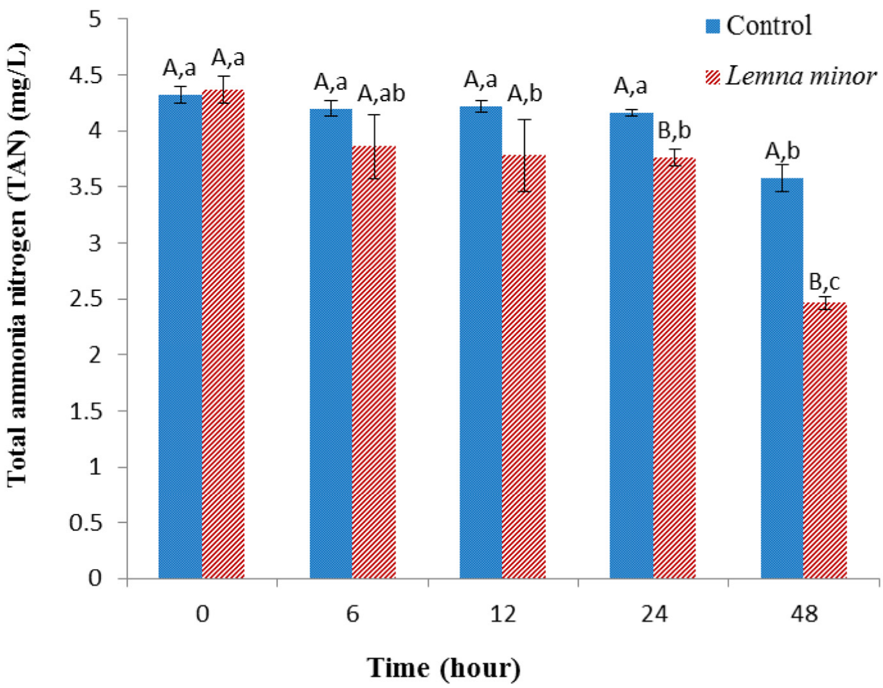

Fig. 2. Total ammonia nitrogen (TAN) concentration of water in different treatments of static test at various time intervals. Bars with different capital letters in each time are significantly different (mean ± SD, Independent-Sample T test, $P < 0.05$). Bars with different lowercase letters in each treatment are significantly different (mean ± SD, ANOVA with Repeated Measures, $P < 0.05$).

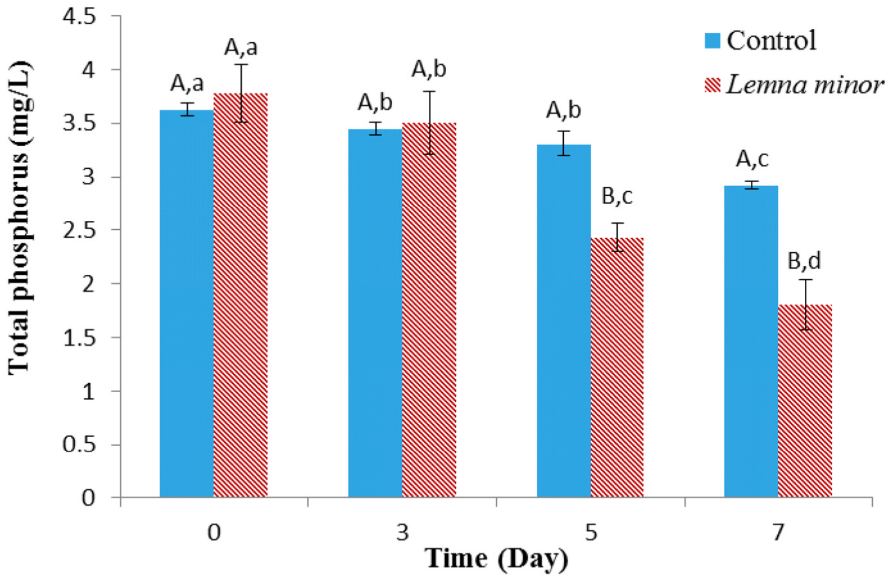

Fig. 3. Total phosphorus concentration of water in different treatments of static test at various time intervals. Bars with different capital letters in each time are significantly different (mean ± SD, Independent-Sample T test, $P < 0.05$). Bars with different lowercase letters in each treatment are significantly different (mean ± SD, ANOVA with Repeated Measures, $P < 0.05$).

3.2.2. Water quality assessment

The efficiency of *L. minor* used in a water recirculation system for removal of the total ammonia nitrogen (TAN), nitrate (NO_3^-) and total phosphorus (TP) during 30 days of flow test is shown in Fig. 4. The TAN concentration was 0 mg L^{-1} at the first day of the experiment in the both treatments. On the days 20 and 30, the TAN concentrations were significantly lower in *L. minor* than the control ($P < 0.05$). During 30 days, the concentration of TAN did not change significantly in the

Table 2
Growth performance, feed utilization parameters and survival rate of African cichlid (*L. lividus*) fingerlings cultured in different treatments of flow test for 30 days (Mean ± SD, n = 3).

Parameter	Treatment	
	Control	*Lemna minor*
Initial weight (g)	2.04 ± 0.010[a]	2.10 ± 0.30[a]
Initial length (cm)	5.59 ± 0.079[a]	5.72 ± 0.068[a]
Final weight (g)	3.41 ± 0.01[a]	4.42 ± 0.41[b]
Final length (cm)	6.79 ± 0.10[a]	7.31 ± 0.18[b]
Weight gain (g)	1.37 ± 0.15[a]	2.31 ± 0.43[b]
SGR (%BW day^{-1})	1.71 ± 0.14[a]	2.46 ± 0.34[b]
Daily growth index (DGI)	0.045 ± 0.005[a]	0.077 ± 0.014[b]
Body weight increase (BWI%)	67.45 ± 7.27[a]	109.96 ± 21.55[b]
CF (%)	1.08 ± 0.004[a]	1.12 ± 0.02[a]
Survival rate (%)	76.46 ± 5.88[a]	96.07 ± 3.40[b]

The values with different letters in the same row are significantly different (Independent-Sample T test, $P < 0.05$).

control treatment ($P > 0.05$), whereas this value decreased significantly in *L. minor* treatment ($P < 0.05$) (Fig. 4a). In control treatment, the nitrate concentration increased during the first 10 days of experiment and it did not alter significantly until 30th day ($P > 0.05$). In *L. minor* treatment, the nitrate concentration increased to the highest level on day 20, and then it decreased significantly on day 30 ($P < 0.05$). The nitrate concentration was significantly higher in *L. minor* treatment compared to the control on day 20 ($P < 0.05$) (Fig. 4b). The results showed that the total phosphorous (TP) concentration was lower in *L. minor* treatment compared to the control on days 20 and 30 ($P < 0.05$). The TP concentration increased significantly during 30 days of the flow test in the both treatments ($P < 0.05$) (Fig. 4c).

The trend of pH changes during 30 days of the flow test in two different treatments is shown in Fig. 5. The pH value altered from 7.70 ± 0.015 to 7.29 ± 0.14 and from 7.72 ± 0.01 to 7.22 ± 0.12 in the control and *L. minor* treatments, respectively. There were no significant differences between pH values of the both treatments at different time intervals ($P > 0.05$).

The comparison of electrical conductivity (EC) value between two treatments of the flow test revealed the reduction of this value in *L. minor* treatment compared to the control on days 20 and 30 ($P < 0.05$). In the control treatment, the EC concentration increased significantly from the first day of experiment to 30th day ($P < 0.05$). In the *L. minor* treatment, the lowest EC concentration was observed on day 20 but this value increased significantly at the end of experiment ($P < 0.05$) (Fig. 6).

Fig. 7 shows the fluctuations of total suspended solid (TSS) in two treatments of the flow test at different time intervals. There were no significant differences between the TSS concentrations of two treatments on days 0, 10 and 20 ($P > 0.05$). The TSS value in *L. minor* treatment was significantly lower than the control treatment on day 30 ($P < 0.05$). In the control treatment, the TSS concentration increased from the first day of experiment to day 10 ($P < 0.05$), but it did not change significantly until 30th day ($P > 0.05$). The TSS concentration increased during the first 10 days, but it decreased significantly until the 30th day in the *L. minor* treatment ($P < 0.05$).

4. Discussion

Phytoremediation, the use of plants for treatment of contaminated water systems, is an emerging technology that promises efficient, cost-effective and environment-friendly cleanup of wastewater (Schnoor et al., 1995; Mahujchariyawong and Ikeda, 2001). Duckweeds as small free-floating aquatic plants are able to absorb the nutrients from wastewater (Goopy and Murray, 2003; Nafea, 2016). In the present study, the potential efficiency of duckweed (*L. minor*) to remove total ammonia nitrogen (TAN), nitrate (NO_3^-) and total dissolved phosphorous (TP) from water used in cultivation of African cichlid (*L. lividus*) fingerlings was investigated under static test and flow test.

In fish, ammonia as a byproduct of protein metabolism is excreted through gill epithelium, and occurs in two forms of un-ionized ammonia (NH_3) and ionized ammonia (NH_4^+) in natural waters (Francis-Floyd et al., 2009). Increased ammonia concentration in the water bodies either damage ammonia excretion or cause a net absorption of ammonia from the water that lead to an elevation in body ammonia levels. The end results are a decline in survival, growth inhibition, variety of physiological dysfunctions and death (Tomasso, 1994; Randall and Tsui, 2002). Ammonia tolerance varies between 0.068–2.00 mg NH_3L^{-1} and between 0.09–3.35 mg NH_3L^{-1} in freshwater and marine fish, respectively (Eddy, 2005). Therefore adjusting the ammonia concentration of water bodies is essential for successful production of different fish species. Duckweed preferentially absorbs ammonium more than other sources of nitrogen from water medium (Porath and Pollock, 1982). The results of the static test revealed that duckweeds were able to absorb NH_4^+ from water, so that the TAN concentration of water decreased by 13.95% and 43.7% after 24 h and 48 h of treatment with *L. minor*, respectively. Selvarani et al. (2015) reported that *L. minor* achieved the removal efficiency of 96% NH_3–N in 25% dilution of municipal wastewater after a week. The maximum ammonia removal percentage (96.449%) from wastewater of a petrochemical

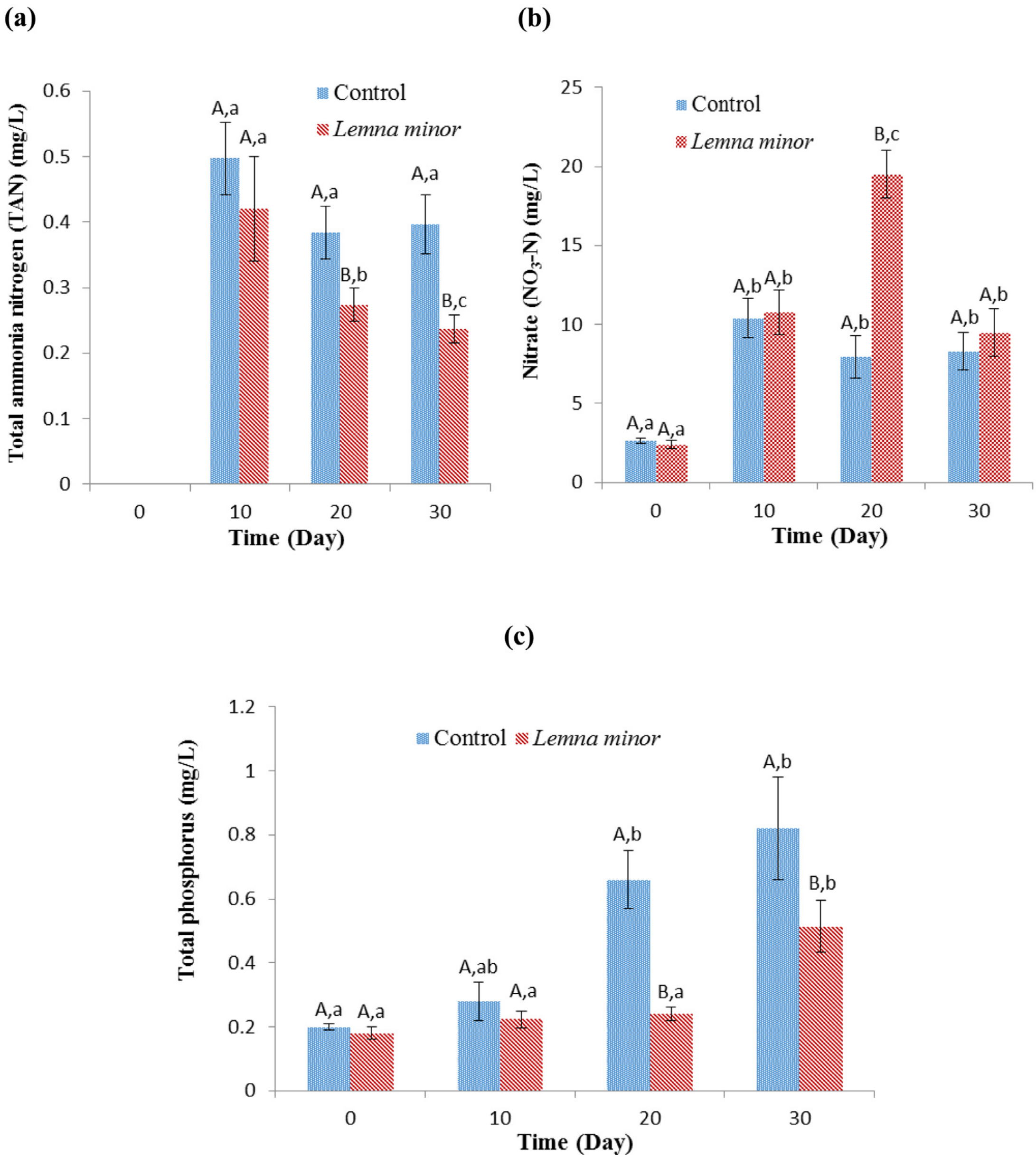

Fig. 4. Total ammonia nitrogen (TAN) (a), nitrate (b) and total phosphorus (c) concentrations of water in different treatments of flow test at various time intervals. Bars with different capital letters in each time are significantly different (mean ± SD, Independent-Sample T test, $P < 0.05$). Bars with different lowercase letters in each treatment are significantly different (mean ± SD, ANOVA with Repeated Measures, $P < 0.05$).

company by *L. gibba* was also determined at ammonia concentration of 5 ppm and duckweeds residence time of 11 days in wastewater (Samimi and Shahriari Moghadam, 2018). Matos et al. (2014) showed that the ammonia removal efficiency of duckweed from domestic wastewater was 30.8%. The different removal efficiency rate may be due to difference in duckweed species, residence time and ammonia concentration and pH of wastewater.

The effluent from fish farms contains different concentrations of pollutants such as phosphorous, which plays important role in causing eutrophication of natural waters (Nordvarg, 2001; Gyllenhammar and Hakanson, 2005). The removal of phosphorous from wastewater using aquatic macrophytes has been discussed in the literatures (Srivastava et al., 2009; Gao et al., 2009; Yu et al., 2019). The results of static test showed that the total phosphorous (TP) concentration decreased significantly from 3.63 mg L^{-1} to 2.92 mg L^{-1} and from 3.78 mg L^{-1} to 1.80 mg L^{-1} in control and *L. minor* treatments after a week, respectively. The TP concentration in *L. minor* treatment was significantly lower than the control treatment on days 5 and 7. The reduction of TP concentration in the control treatment was probably due to its deposition during the experiment period. The maximum TP removal efficiency by *L. minor* was 52.38% after a week. Nassar et al. (2015)

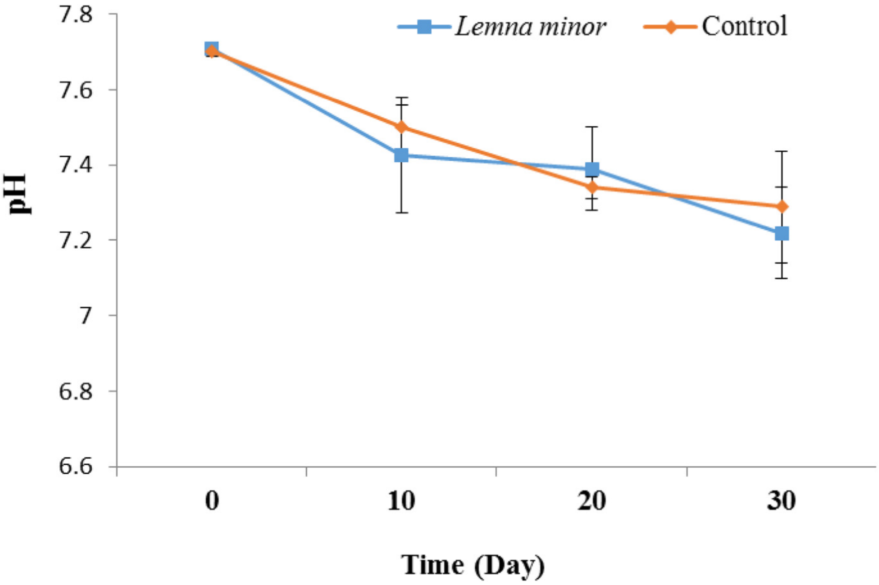

Fig. 5. Fluctuations trend of pH during 30 days of flow test in different treatments (mean ± SD).

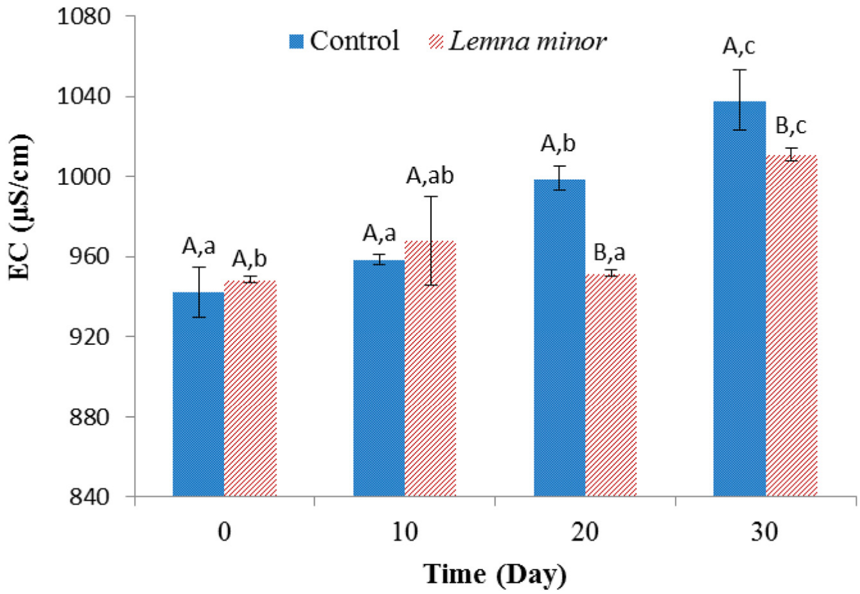

Fig. 6. Electrical conductivity (EC) of water in different treatments of flow test at various time intervals. Bars with different capital letters in each time are significantly different (mean ± SD, Independent-Sample T test, $P < 0.05$). Bars with different lowercase letters in each treatment are significantly different (mean ± SD, ANOVA with Repeated Measures, $P < 0.05$).

reported that phosphorus removal efficiency from agriculture wastewater by duckweed at 10 days hydraulic detention times (HDT) was 68.3%. The phosphorus removal rate from 50% diluted swine lagoon liquid by growing *L. minor* under field condition was 45.7% after six weeks (Cheng et al., 2002).

The results of flow test revealed the enhancement of the survival rate and the growth performance including final length, weight gain, SGR%, DGI and BWI% of African cichlid fingerlings cultured in a water recirculation system contained *L. minor* compared to the control after 30 days. The survival rates of fish were recorded as 96.07 ± 3.40% and 76.46 ± 5.88% in the *L. minor* and control groups, respectively. Similarly, the usage of two macrophytic plants *L. minor* and *Wolffia arrhiza* in recirculation aquaculture system (RAS) improved the growth performance of common carp (*C. carpio*) fingerlings but they had no significant effect on the survival rate of fish (Velichkova and Sirakov, 2013). In fact, this issue could be attributed to the improvement of water quality treated with *L. minor*. Indeed, water quality parameters are key factors

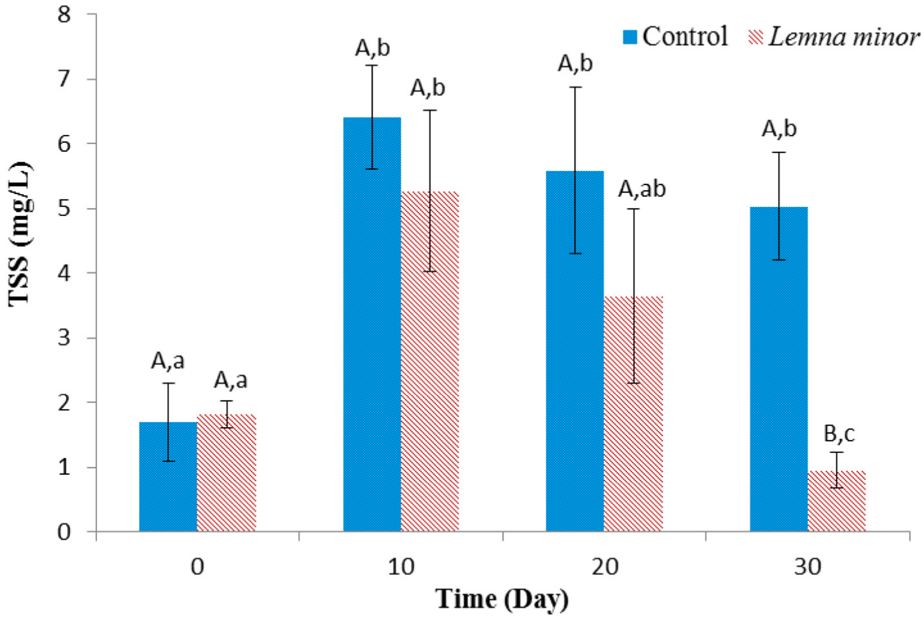

Fig. 7. Total suspended solids (TSS) of water in different treatments of flow test at various time intervals. Bars with different capital letters in each time are significantly different (mean ± SD, Independent-Sample T test, $P < 0.05$). Bars with different lowercase letters in each treatment are significantly different (mean ± SD, ANOVA with Repeated Measures, $P < 0.05$).

for the growth and heath of fish in a water recirculation system. Timmons et al. (2002) reported that deterioration of water quality in recirculation aquaculture system (RAS) resulted in reduced growth and increased stress in the fish. In the current study, the use of *L. minor* led to the reduction of the TAN by 28.94% and 41% compared to the control on days 20 and 30. Velichkova and Sirakov (2013) found that ammonium concentration decreased by 19.6% in the RAS with a biofilter consisting of *L. minor* and *W. arrhiza* compared to the control. Our results showed that the nitrate concentration was higher in *L. minor* treatment than the control on day 20, probably the results of the growth of the bacteria involved in the nitrification process, which can lead to accumulation of nitrate in the water. Velichkova and Sirakov (2013) also attributed the higher accumulation of nitrate in the RAS contained *L. minor* and *W. arrhiza* to nitrification process. In fact, the presence of duckweeds in water medium can provide additional surface for the attachment of nitrifies (Zimmo et al., 2004). The accumulation of nitrate may be also due to the preferential absorption of TAN by duckweeds as a nitrogen source. It was found that the free-floating macrophyte plant *Landoltia punctate* preferred to absorb $NH_4{}^+$ more than $NO_3{}^-$ when both nitrogen sources were available (Fang et al., 2007). Hasan and Chakrabarti (2009) also reported that duckweed plants uptake available ammonium before beginning to absorb nitrate. According to the results, the nitrate concentration reached the maximum level of 19.5 mg L^{-1} on day 20, but it decreased to 9.4 mg L^{-1} at the end of cultivation period. The removal of $NO_3{}^-$ may be attributed to the microbial denitrifying activity in the rhizosphere of *L. minor*. Lu et al. (2013) found that root of two common aquatic duckweed species; *Spirodela polyrrhiza* and *L. minor* secrete bioactive compounds that stimulate the nitrogen-removal efficiency of the denitrifying bacterium *Pseudomonas fluorescens*.

In the present study, the total phosphorous (TP) concentration of water increased gradually in the two treatments of the flow test with culture period. The TP concentration increased to 0.82 mg L^{-1} after feeding African cichlid fingerlings for 30 days in the control treatment. The usage of *L. minor* as biofilter resulted in the reduction of TP by 37.80% compared to the control after 30 days of rearing period. These findings were in line with Velichkova and Sirakov (2013) who stated that *L. minor* and *W. arrhiza* as biofilter decreased the TP by 20% in a RAS for the cultivation of common carp (*C. carpio*) fingerlings compared to the control. Ferdoushi et al. (2008) also reported that phosphate phosphorus (PO_4-P) decreased by 36.5% from water of fish pond filtrated by *L. minor* for four months.

The pH values of water were slightly alkaline during cultivation period in the both treatments. The pH value decreased from 7.72 ± 0.01 to 7.22 ± 0.12 in the *L. minor* treatment. Mal et al. (2015) found that pH of effluent from fish farm decreased by 15% after passage through the aquatic macrophyte *Eichhornia crassipes* as a biofilter. The pH of water was weakly alkaline in a RAS containing *L. minor* and *W. arrhiza* (Velichkova and Sirakov, 2013). Our results showed the elevation of electrical conductivity (EC) of water during 30 days of flow test in the both treatments. Treatment of water with *L. minor* led to the decrease in EC values by 4.73% and 2.60% compared to the control on days 20 and 30, respectively. Velichkova and Sirakov (2013) also reported that the EC value of water treated with *L. minor* and *W. arrhiza* decreased by 10.37% after 40 days. The reduction of EC may be attributed the uptake of macronutrients (calcium, magnesium, potassium, sodium, chloride, sulfate, carbonate) by aquatic plants from water medium (Mal et al., 2015). In the current study, the maximum total suspended solids (TSS) removal of 81.11% was observed in *L. minor* treatment on day 30. The turbidity of 25% diluted municipal wastewater decreased by 93% after treatment with *L. minor* for one month (Selvarani et al., 2015). It was suggested that all the plant matter decrease the turbidity by absorption of colloidal materials (Center et al., 2002). The concentration of suspended sediments can be reduced in water bodies covered with dense macrophytes (Ferdoushi et al., 2008).

5. Conclusion

In the present study, we evaluated the potential efficiency of duckweed (*L. minor*) for removal of total nitrogen ammonia (TAN) and total phosphorus (TP) from water under the static and flow tests. The results of static test revealed that *L. minor* was able to remove effectively the TAN and TP from water medium. The administration of *L. minor* as a biofilter in a water recirculation system for cultivation of African cichlid (*L. lividus*) fingerlings improved the survival rate and growth performance of fish by enhancement of water quality. The treatment of water with *L. minor* decreased significantly the concentrations of TAN, nitrate, TP, electrical conductivity (EC) and total suspended solids (TSS) during cultivation period. These findings indicated that the duckweed (*L. minor*) could be considered as an effective and environment-friendly biofilter in aquaculture industry.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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