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Duckweed *Lemna minor* (Liliopsida, Lemnaceae) as a natural biofilter in brackish and fresh closed recirculating systems

[1,2]Mutaz A. Al-Qutob, [2]Tharwat S. Nashashibi

[1] Department of Earth and Environmental Studies, Faculty of Science and Technology, Al Quds University, East Jerusalem, Palestinian Authority; [2] Aquatic and Aquaculture Research Laboratory, Al-Quds University, East Jerusalem, Palestinian Authority.
Corresponding author: M. A. Al-Qutob, qutob@planet.edu

Abstract. This study attempted to assess the potential use of common duckweed *Lemna minor* as natural biofilter in brackish closed recirculating systems of 4 g/L salinity and to evaluate the effect of salinities in the ranges of 1-7 g L^{-1} on the duckweed's ammonium NH_4^{+1} uptake. Furthermore the possibility of nitrification as a second mechanism of nitrogen removal in closed recirculating systems was investigated. Three closed recirculating systems each having 20 mixed *Oreochromis niloticus* were used. Duckweed *L. minor* and fresh water were added in system 1, fresh water only in system 2, and duckweed with brackish water in system 3. Ammonium NH_4^{+1} and nitrate NO_3^{-1} were tested periodically in each system during a 1.5 month period. Another three experiments were run in parallel using aquariums incubated with 60.00 g fresh weight *L. minor* at salinities in the ranges of 1-7 g L^{-1} for two weeks. Aquariums were exposed directly to sunlight in experiment 1 and 3, and were placed in the dark in experiment 2. The nitrogen source in experiment 1 and 2 was 100 mg L^{-1} of NH_4Cl, and 280 mg L^{-1} of NH_4Cl in experiment 3. NH_4^{+1} and NO_3^{-1} levels were monitored as above. *L. minor* managed to reduce the average NH_4^{+1} levels to 0.5 mg L^{-1}, 0.43 mg L^{-1} below the standard recommended levels in both brackish and fresh water systems, respectively. Ammonium NH_4^{+1} uptake was coupled with nitrate NO_3^{-1} uptake under favorable conditions of sunlight. Salinity in the ranges from 1-7 g L^{-1} enhanced ammonium NH_4^{+1} uptake ($r = 0.8819$, $p = 0.023$) without affecting nitrate NO_3^{-1} uptake or any observed mortality of the duckweed. Nitrification was a second mechanism of nitrogen removal in a closed recirculating system, but it was affected by salinity and light. Average ammonium NH_4^{+1} of 51.8 mg L^{-1} was toxic to *L. minor* and death was observed within a week in experiment 3. The duckweed *L. minor* was an efficient natural biofilter in both brackish and fresh water closed recirculating systems.
Key Words: duckweed, *Lemna minor*, natural biofilter, ammonium NH_4^{+1} uptake, nitrification, brackish water.

Resumen. Este estudio intenta evaluar el potencial uso de la lenteja común de agua *Lemna minor* como biofiltro natural en sistema de recirculación cerrado con agua salubre de salinidad 4 g L^{-1} y evaluar el efecto de 1-7 g L^{-1} salinidades sobre absorción de amonio NH_4^{+1} por *L. minor*. Por otra parte, la posibilidad de nitrificación como un segundo mecanismo de eliminación de nitrógeno en sistema de recirculación cerrado fue investigada. Tres sistemas de recirculación cerrados cada uno de ellos con 20 mixtos *Oreochromis niloticus* se utilizaron con lenteja *L. minor* y agua dulce en el sistema 1, agua dulce sólo en sistema 2 y lenteja con agua salubre de salinidad 4 g L^{-1} en sistema 3. El amonio NH_4^{+1} y nitratos NO_3^{-1} fueron probados periódicamente en cada sistema durante 1.5 meses. Otros tres experimentos fueron realizados en paralelo con acuarios incubados con 60.00 g peso fresco *L. minor* a salinidades en las gamas de 1-7 g L^{-1} durante dos semanas. Acuarios fueron expuestos directamente a la luz del sol, en el experimento 1 y 3 y se colocaron en la sombra en el experimento 2. La fuente de nitrógeno en el experimento 1 y 2 fue de 100 mg L^{-1} de NH_4Cl, y 280 mg L^{-1} de NH_4Cl en el experimento 3. Los niveles de amonio NH_4^{+1} y nitratos NO_3^{-1} fueron probados como se ha descrito anteriormente. *L. minor* logró reducir el promedio NH_4^{+1} niveles a 0.5 mg L^{-1}, 0.43 mg L^{-1} por debajo del nivel recomendado tanto en la sistema de agua salubre y sistema de agua dulce respectivamente. El amonio NH_4^{+1} asimilación era junto con los nitratos NO_3^{-1} absorbidos en condiciones favorables de la luz solar. La salinidad en las gamas de 1-7 g L^{-1} ha mejorado la absorción de amonio NH_4^{+1} ($r= 0.8819$, $p = 0.023$), sin que ello afecte al absorción de nitrato NO_3^{-1} o cualquier mortalidad observada de la lenteja de agua. La nitrificación fue un segundo mecanismo de eliminación de nitrógeno en un sistema de recirculación cerrada, pero se vio afectada por la salinidad y la luz. Promedio de amonio NH_4^{+1} de 51.8 mg L^{-1} fue tóxico para *L. minor* y la muerte fue observada dentro de una semana en el experimento 3.
Palabras Clave: lenteja de agua, *Lemna minor*, biofiltro natural, absorción de amonio NH_4^{+1}, nitrificación, agua salubre.

Introduction. Plants are not normally used as a primary biofilter in aquaculture systems. They do however provide a very good sink for the nitrates produced by a well functioning biofiltration system. Common duckweed *Lemna minor* (Linnaeus, 1753) is a small floating aquatic plant belonging to the botanical family Lemnaceae. It is green and have a small size (1-3 mm) with short but dense roots (1-3 cm) (Les et al 2002). Fronds can grow in colonies that, in particular growing conditions, form a dense and uniform surface mat in full sunlight as well as dense shade (Cheng et al 2002). Their high productivity and efficient nitrogen removal make them suitable for wastewater treatment (Zimmo et al 2005) and as biofilters in fish ponds (Ferdoushi et al 2008). They tolerate different pH conditions ranging between 4.5–7.5 with optimum water temperature for growth between 17ºC and 35ºC (Iqbal 1999) which corresponds well to tilapia growth conditions. Duckweeds are salinity tolerant, adapt with time to high salinity, remove salinity, and have a potential for desalination in agricultural detention ponds (Omar & Balla 2010). According to Haller et al (1974), growth of *L. minor* gradually decreases at levels above 6.66 ‰ diluted sea water. Buckley et al (1996) showed that the *L. minor* growth decreases when NaCl increases with concentrations: control, 2.59, 4.32, 7.20 and 12 g/l. Nitrogen removal is affected by different conditions of dissolved oxygen (DO) and pH in duckweed-based waste stabilization ponds as reported by Zimmo et al (2003).

In aquaculture we are interested in ammonia (NH_3) since it is toxic to fish. Studies on the effect of salinity on nitrogen removal showed that removal of nitrate NO_3 decreased significantly with the increase of salinity between temperature ranges of 25 and 35ºC (Omar & Balla 2010). Nitrogen, in particular, occurs at very high levels in recirculating systems since fish excrete waste nitrogen directly into the water through their gills in the form of ammonia. El-Shafai et al (2004) reported negative effects on the growth performance of juvenile Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) between 0.07 and 0.14 mg L^{-1} unionized ammonia NH_3 (UIA-N), no mortality up to 0.434 mg L^{-1} over 75 days and recommended UIA-N<0.1 ppm. Another study reported the 48 hr-LC50 for tilapia fingerlings to be 7.1 mg L^{-1} unionized ammonia NH_3 (El-Sherif & El Feky 2008). In aqueous solutions, an equilibrium exists between unionized ammonia (NH_3) and ammonium ion (NH_4^{+1}). Raising the pH and temperature shift the equilibrium toward the toxic unionized ammonia (NH_3) (Environment Canada 1999).

This study is an attempt to assess the potential use of common duckweed *L. minor* as biofilter in closed recirculating systems containing adult Nile tilapia *O. niloticus* in brackish water from Jericho aquifers with salinity of 4 g L^{-1}, and to evaluate the effect of salinities in the ranges of 1-7 g L^{-1} on the duckweed's ammonium NH_4^{+1} uptake. In addition, nitrification coupled to nitrogen uptake contributes to the removal of nitrogen in duckweed covered system during wastewater treatment. The possibility of nitrification as a mechanism of nitrogen removal in closed recirculating systems was investigated.

Material and Method. The study was carried out at the Aquaculture Research Laboratory, in AL-Quds University, Jerusalem from 26 February 2012 till 10 April 2012. To assess the potential use of duckweed *L. minor* as natural biofilter in closed brackish and fresh recirculating system, three closed recirculating systems each having 20 mixed *O. niloticus* were used. Duckweed *L. minor* and fresh water were added in system 1, fresh water only in system 2, and duckweed with brackish water in system 3. Each system consisted of three aquariums each with a capacity of 45 L in a vertical position (Figures 1.1 and 1.2). The first aquarium was stocked with 60.00 g fresh weight of duckweed *L. minor* delivered from wet lands waste water treatment plants in the West Bank. Into each of the two bottom aquariums 10 mixed *O. niloticus* were stocked. Brackish water of 4 g L^{-1} salinity was collected from aquifers in Jericho area (Palestinian Territories). Water flowed down into the two bottom aquariums by the effect of gravity and was recirculated up to the first aquarium through a pump. Water temperature was maintained at ~28^{o}C with a constant photoperiod of 12 h light and 12 h dark. Oxygen saturation was maintained above 80% by an aquarium air pump. Ten percent of each system's water was changed weekly. Commercial feed was used as diet. Feed composition included 91.8% dry matter (OM), 43.5% crude protein, 10.9% crude lipid,13.0% crude ash and 18.7Kj GROSS energy. Samples for ammonium NH_4^{+1} and

nitrate NO_3^{-1} testing were collected periodically from each system during a 1.5 month period at the following intervals: 1, 3, 15, 7, 12, 2 and 4 days, respectively (Figures 2.1, 2.2). Ammonium NH_4^{+1} and nitrate NO_3^{-1} determination was according to methods described by Searle (1984) and Eaton et al (1998), respectively.

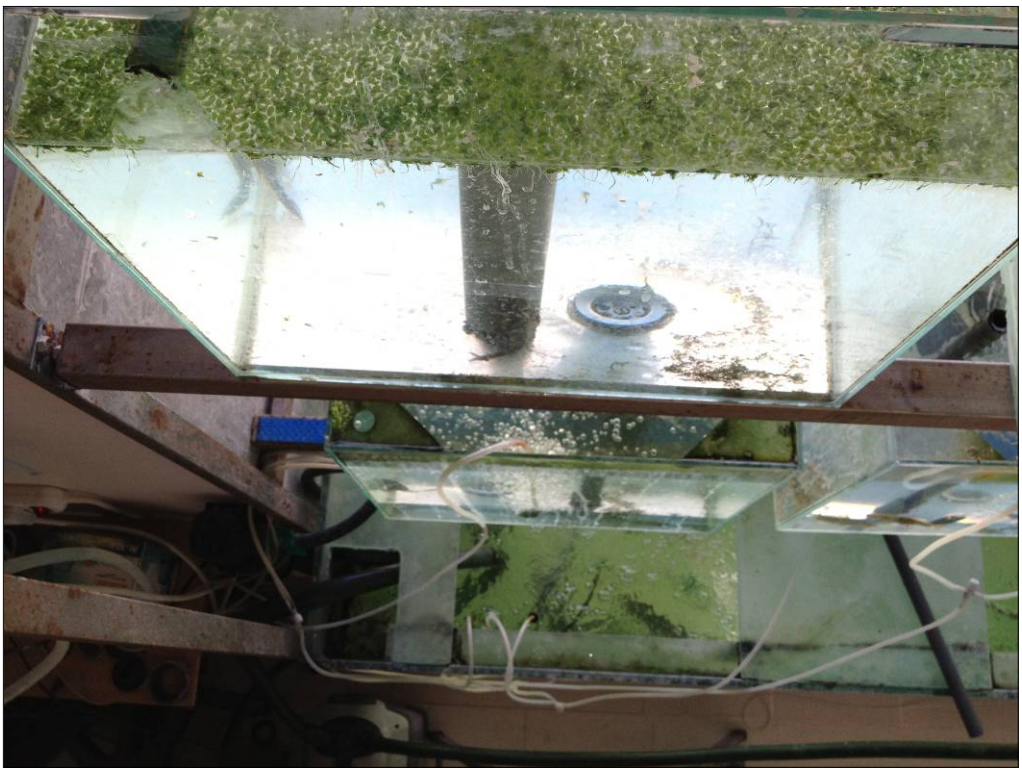


Figure 1.1. System 1 (duckweed *L. minor* & fresh water): duckweed stocked in the first aquarium & Nile tilapia *O. niloticus* stocked in the two bottom aquariums.

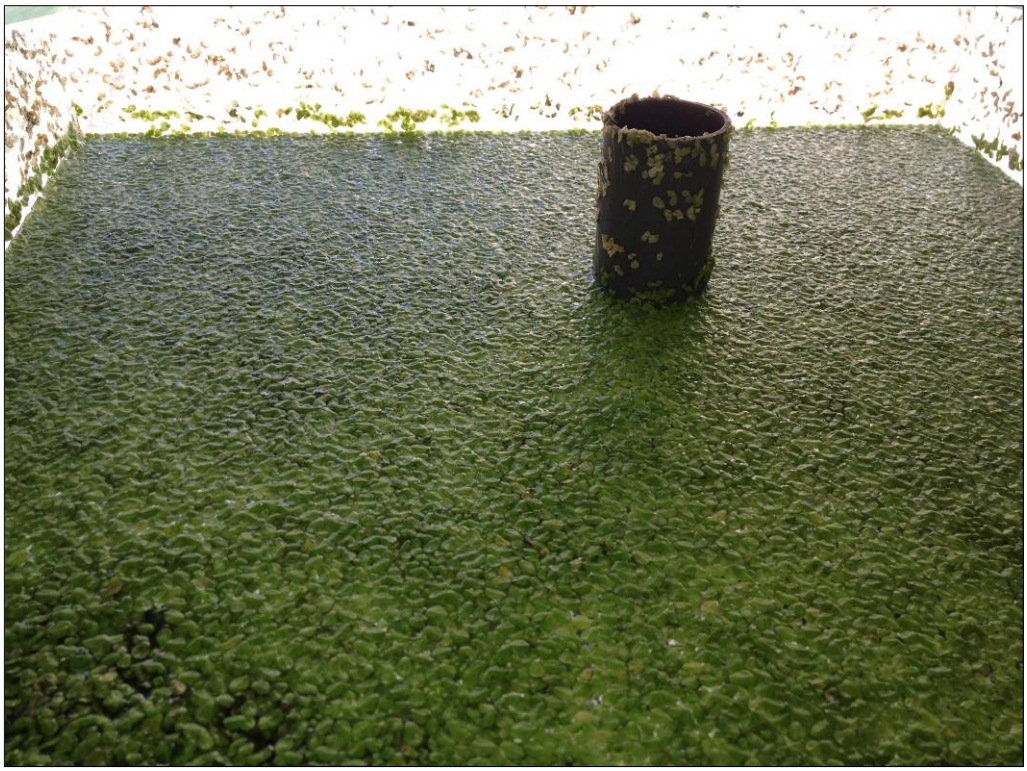


Figure 1.2. Duckweed *L. minor* in the first aquarium at the end of the experiment in system 3.

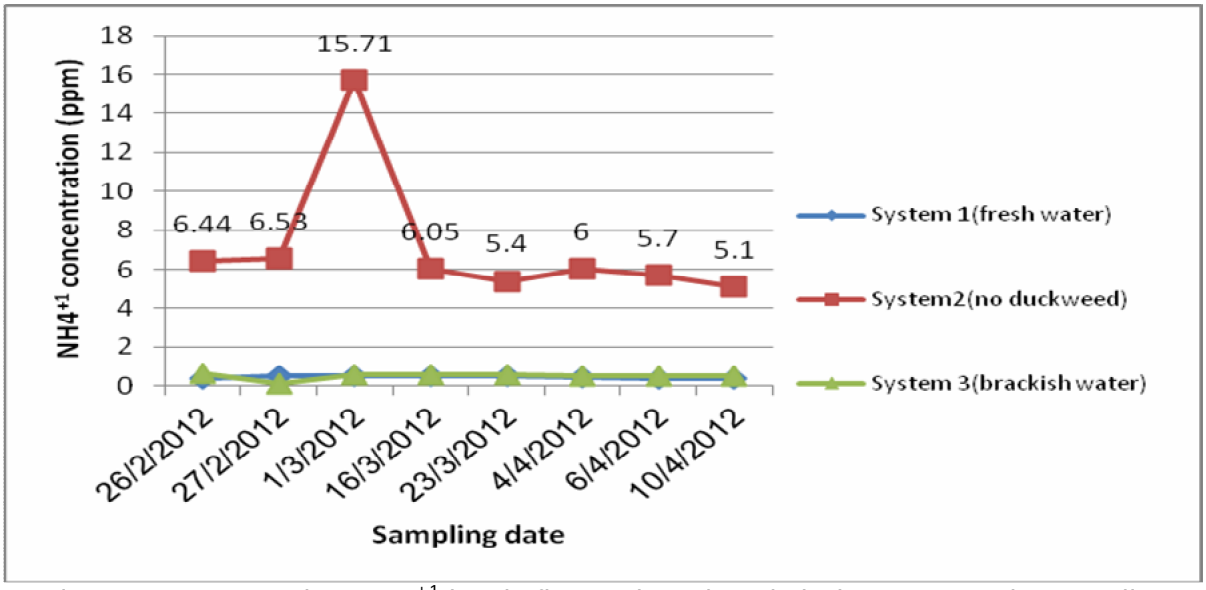


Figure 2.1. Ammonium NH_4^{+1} levels fluctuations (ppm) during 1.5 months sampling period in system 1, system 2, and system 3.

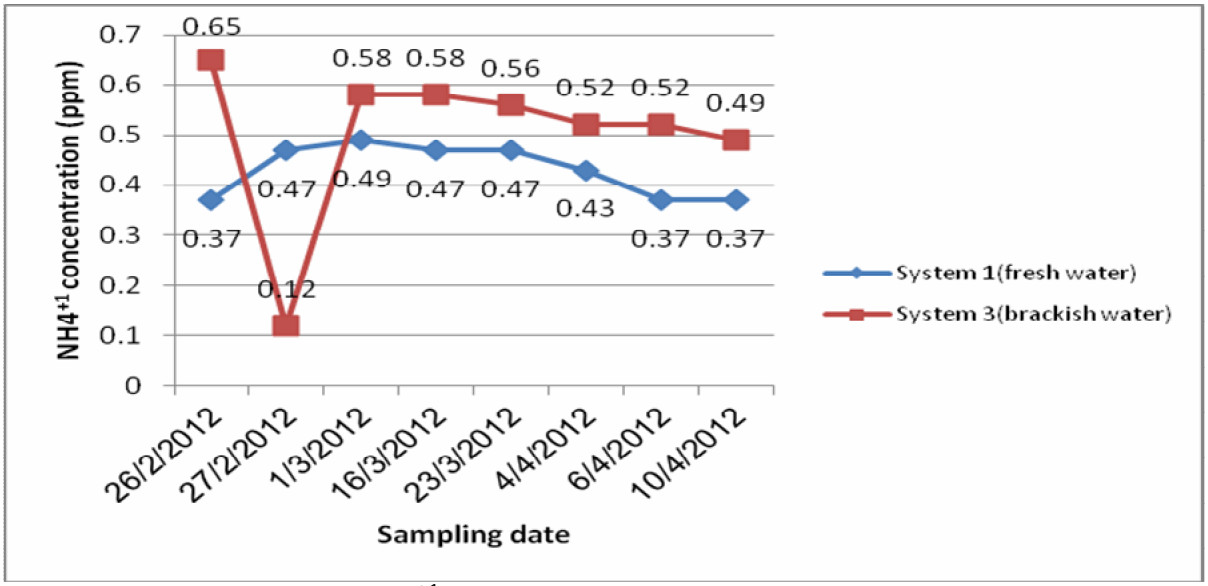


Figure 2.2. Ammonium NH_4^{+1} levels fluctuations (ppm) during 1.5 months sampling period in system 1 compared to system 3.

Effect of salinity on ammonium NH_4^{+1} uptake and nitrification as a mechanism of nitrogen removal.

Experiment number (1): five aquariums, each with a capacity of 45 L, were exposed directly to sunlight and used for the incubations. Tap water from Al Quds University was used to start incubations and each aquarium was filled with 40 L. Different salinity ranges were achieved by dissolving sea salt (Coral reef red sea salt, Red Sea Fish Pharm Ltd) in each aquarium in order to obtain the following salinities: 2 g L^{-1}, 4 g L^{-1}, 6 g L^{-1}, 7 g L^{-1}. One aquarium served as a control and was incubated with tap water only. Initial and final salinities were measured using the manual refractometer (Model 2483, ATAGO). The nitrogen source was obtained by adding 4 g of NH_4Cl to each aquarium obtaining a final concentration of 100 mg L^{-1} of NH_4Cl. Each aquarium was stocked with 60.00 g fresh weight duckweed *L. minor* delivered from wet lands waste water treatment plants in West Bank. Each incubation was monitored for two weeks. Samples for ammonium NH_4^{+1} and nitrate NO_3^{-1} testing were collected from each aquarium and were determined according to methods described by Searle (1984) and Eaton et al (1998), respectively. Duckweed

biomass was weighted at the beginning and the end of the experiment. Duckweed growth was evaluated on the basis of the relative growth rate RGR (Caicedo et al 2000) as given by:

$$\ln(N_t) = \ln(N_0) + RGR * t$$

where N_t = dry weight, at time t and N_0 = dry weight at time 0.

Experiment number (2): six aquariums each with a capacity of 45 L placed in the dark were used for the incubations. One aquarium used as a control was incubated with tap water and duckweed. Another was incubated with tap water and no duckweed. The rest of the aquariums had the following salinities: 2 g L^{-1}, 4 g L^{-1}, 6 g L^{-1}, 7 g L^{-1} as in experiment (1). The experiment followed the same steps as in experiment number (1).

Experiment number (3): this experiment followed the same steps as in experiment number (1) except that higher nitrogen content was used by adding 11.2 g of NH_4Cl to each aquarium obtaining final concentration of 280 mg L^{-1} of NH_4Cl.

Statistical analysis. Data was analyzed using paired t-test. Statistical significance was assessed using a probability level of $p = 0.05$. Significant negative or positive correlation coefficient between salinities and ammonium removal was determined.

Results and Discussion. Duckweeds prefer ammonia nitrogen (NH4-N) as a source of nitrogen and will remove ammonia preferentially, even in the presence of relatively high nitrate concentrations. Common duckweed *L. minor* reduced ammonium NH_4^{+1} levels significantly in both system 1 and system 3 compared to system 2 with no duckweed ($p = 0.001$, $p < 0.05$) (Figures 2.1 and 2.2). Ammonium NH_4^{+1} levels ranged from 0.37-0.49 ppm, 5.1-15.71 ppm, 0.12-0.65 ppm, in system 1, system 2, and system 3, respectively (Figure 2.1).

Tilapia *Oreochromis* sp. excrete 25.5 mg Kg^{-1} h^{-1} total ammonia nitrogen (TAN) in response to 45% protein in diet. TAN production is affected by temperature, salinity, alkalinity and dissolved oxygen (Meyer & Pena 2001). The mean daily ammonia excretion rate of 200 g fed red tilapia (*Oreochromis* sp.) is 5.83±0.19 mg kg $fish^{-1}$ h^{-1} (Rafiei et al 2006). In our experiments, 20 tilapia fish were fed 40% protein in diet with controlled temperature and dissolved oxygen in each system excreting 453.33 mg Kg^{-1} h^{-1} TAN. Recommended TAN for tilapia culture is < 1 ppm (Lucas & Southgate 2003). TAN include both the unionized NH_3(UIA-N) and the ionized NH_4^{+1} forms. At pH 7 and temperature 25°C the percentage of unionized NH_3 in the NH_3/ NH_4^{+1} equilibrium is only 0.56% of the total ammonia (Lucas & Southgate 2003). In general average ammonium NH_4^{+1} and unionized NH_3 concentrations (0.5, 0.0028; 0.43, 0.0024, respectively), decreased in systems that used duckweeds as biofilters below the standard recommended levels (UIA-N < 0.1 ppm, TAN < 1 ppm) in both brackish and fresh water compared to control (system 2) (Figures 2.1, 2.2). Mortality was not observed in systems 1 and 3, but 30% mortality occurred in system 2. According to El-Sherif & El Feky (2008) tilapia fingerlings can tolerate up to 7.1 mg L^{-1} unionized ammonia NH_3.

Nitrogen movement through the system proceeded as the following: ammonia concentrations spiked as the bacteria was grown to consume it and produced nitrites. Once the ammonia consuming bacteria were of sufficient quantity, the ammonia concentration dropped to zero and the nitrite levels spiked. Bacteria were grown to consume the nitrite and produced nitrate. The cycle was completed when ammonia and nitrite levels were zero and nitrate levels were climbing. As previously mentioned duckweeds remove nitrogen from the medium for growth and photosynthesis. The duckweed *Lemna gibba* can uptake 80% of the ammonia in less than 48 h (Porath & Pollock 1982). Nitrification coupled to nitrogen uptake contributes to removal of nitrogen in duckweed covered systems during wastewater treatment (Zimmo et al 2003). Nitrification, in the presence of *L. minor* mat could represent a second mechanism for ammonia removal in aquariums discussed later. Average ammonium NH_4^{+1} concentration (0.5) in system 3 (brackish water) was raised compared to system 1 (fresh water), but was not statistically significant ($p > 0.05$) (Figure 2.2). This could be contributed to lower

nitrification rates and an increased rate of ammonia production by the stressor saline environment (Wood 2001).

In reference to Figure 2.3, there were statistically significant differences in nitrate NO_3^{-1} levels between system 1, system 2 without duckweed ($p = 0.0001$, $p < 0.05$), and system 3. Although there is uptake preference for ammonia over nitrate by duckweed, these results indicated that there was also uptake of nitrate NO_3^{-1} in both brackish and fresh water systems, or that ammonia removal was largely dependent on duckweed uptake and only a small proportion was diverted toward nitrification pathway.

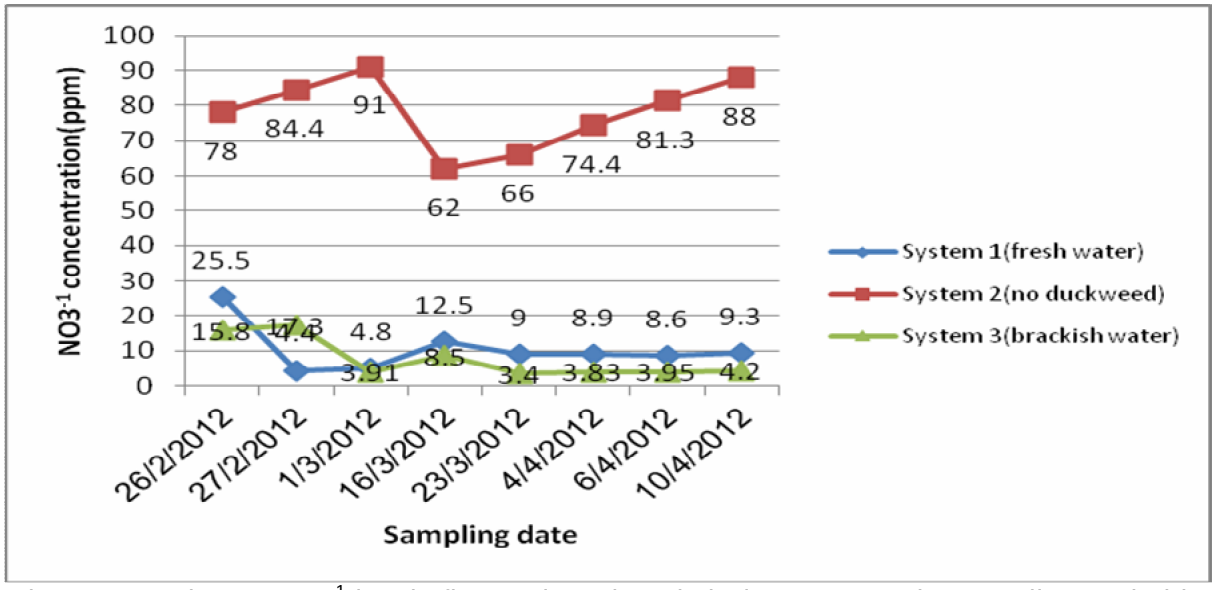


Figure 2.3. Nitrate $NO3^{-1}$ levels fluctuations (ppm) during 1.5 months sampling period in system 1, system 2, and system 3.

Nitrifying bacteria in small nitrifying biofilters and those attached to *L. minor* mat contributed to nitrate production in systems 1 and 3. *Lemna* sp. were reported to lower nitrate NO^3-N levels in fish pond (Ferdoushi et al 2008). Nitrate is relatively non-toxic and is reported to reach as high as 400-500 mg L^{-1} NO_3-N in recirculating systems (van Rijn et al 2006). Nitrate NO_3^{-1} ranges (62-91) in system 2 (no duckweed) were not extremely elevated during the 1.5 months experimental period due to weekly water replacement. In addition, nitrate accumulation in the system could have increased acidity which have decreased efficiency of filter leading to lower nitrification rates and higher ammonia levels was observed (Figure 2.3) (van Rijn et al 2006). Average nitrate NO_3^{-1} concentration (10.3) in system 1 (fresh water), was higher than system 3 (7.6) (brackish water), but was not statistically significant ($p > 0.05$) (Figure 2.3). Possible explanations include the decrease in the rate of nitrification by the biological filter in brackish water compared to fresh water until the filter nitrifying bacteria's adaptation to the new environment took place (Grommen et al 2005). In addition, greater percentage of ammonia removal by uptake or the enhancement of both ammonia and nitrate uptake in the saline environment (Omar & Balla 2010) could have affected the results. It is worth mentioning that nitrification over the duckweed mat will be not affected since brackish water in system 3 was collected from aquifers and saline environments harbor diverse bacterial groups, which exhibit modified physiological and structural characteristics under prevailing saline conditions (Zahran 1997).

Effect of salinity on ammonium NH_4^{+1} uptake. A significant positive correlation ($p = 0.023$, $p < 0.05$) was found between salinity and ammonium NH_4^{+1} removal in experiment 1 (exposed to sunlight) (Table 1, Figure 3a) but not in experiment 2 (in the dark) (Table 2, Figure 3b).

Table1

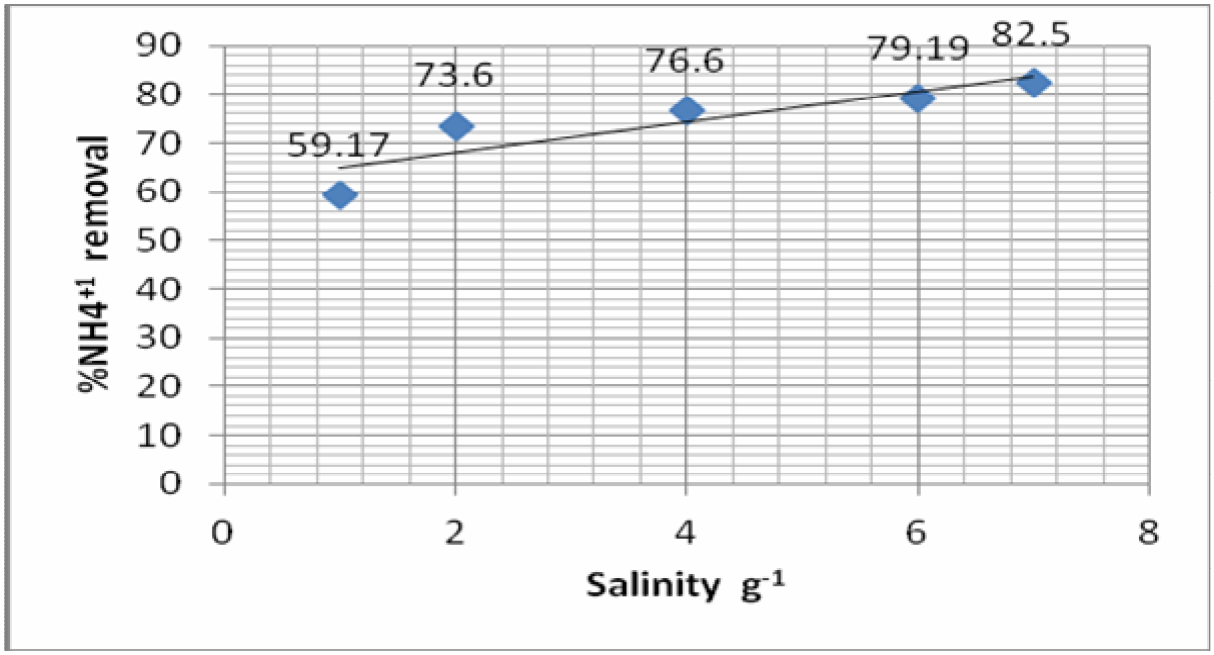
Ammonium NH_4^{+1} levels (ppm) during 2 weeks sampling period, salinity changes (initial, final) and relative growth rate (RGR) of duckweed *L. minor* during experiment 1 (exposed to sunlight)

NH_4^{+1} levels (ppm)	*Salinity (g L^{-1})*				
Sampling date	*Tap water(1 g L^{-1})*	*2*	*4*	*6*	*7*
24/3/2012	33.36	31.55	34.36	34.6	34.55
27/3/2012	31.03	29.91	29.63	27.5	27.33
3/4/2012	22.42	16.01	16.2	14.8	13.75
7/4/2012	13.62	8.32	8.04	7.2	6.02
The initial weight of *L. minor (g)*	60.05	60.05	60.05	60.05	60.05
The final weight of *L. minor (g)*	101.34	98.30	97.78	93.8	90.2
RGR (d^{-1})	0.037	0.033	0.032	0.030	0.027
The final salinity (g L^{-1})	2	3.5	6	7	8.5

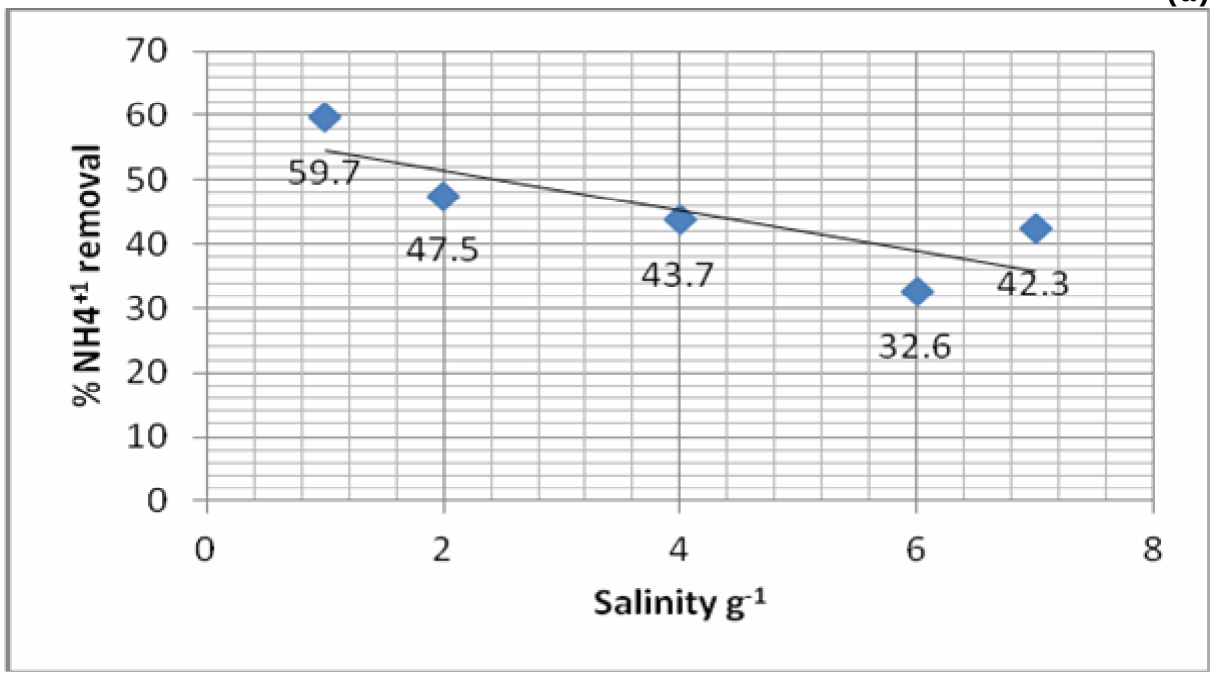
Table2

Ammonium $NH4^{+1}$ levels (ppm) during 2 weeks sampling period, salinity changes (initial, final) and relative growth rate (RGR) of duckweed *L. minor* during experiment 2 (in the dark)

NH_4^{+1} levels (ppm)	*Salinity (g L^{-1})*					
Sampling date	*No duckweed (1 g L^{-1})*	*Tap water (1 g L^{-1})*	*2*	*4*	*6*	*7*
24/3/2012		27.8	26.3	27.4	27.6	26.2
27/3/2012	28	27.8	25.7	27.2	27.2	24.8
3/4/2012	28	27.9	24.1	25.3	25.9	22.8
7/4/2012	27.9	11.2	13.8	15.4	18.6	15.1
The initial weight of *L. minor (g)*	22	60.05	60.05	60.05	60.05	60.05
The final weight of *L. minor (g)*		66.82	66.50	65.45	66.05	72.20
RGR (d^{-1})		0.007	0.007	0.006	0.007	0.012
The final salinity (g L^{-1})	2	2	3	5	8	9.5



(a)



(b)

Figure 3. Influence of salinity on ammonium NH_4^{+1} removal in experiment 1 exposed to sunlight (r = 0.8819, p = 0.023) (a); ammonium NH_4^{+1} removal in experiment 2 in the dark (r = 0.9941, p = 0.0002) (b)

Duckweeds absorb nutrients through the whole plant, not through a central root system, directly assimilating organic molecules needed for growth requirements. *L. minor* is well documented to accumulate salts in their tissues and to be used in water desalination (Omar & Balla 2010). In plants NO_3^{-1} and NH_4^{+1} influx across the plasma membrane increases as external ion concentration increases (Glass et al 2002). Sea salt was used to conduct our experiments, which is composed of several elements, in addition to NaCl that, constitute only 80% of its relative concentration (Atkinson & Bingman 1998). These factors could have enhanced NH_4^{+1} uptake with increasing salinity (Figure 3a). On the other hand, there was little NH_4^{+1} uptake and lower RGR% in experiment 2 (in the dark) (Figure 3b, Table 2). NH_4^{+1} uptake was found to display diurnal patterns in several plants with amplitude of the diurnal pattern highest on high irradiance days (Macduff et al 1997,

Glass et al 2002). Light is needed for the photosynthesis and altering the amount of light received by plants will alter its photosynthetic rate (Bohning & Burnside 1956). Low light intensity affects the rate of growth of *L. minor*. In continuous darkness growth rate is very low and significant increase in multiplication rate occurs at light intensities below 0.1 uWcm^{-2} (Rombach 1976). Higher RGR% were achieved in experiment 1 (exposed to sunlight) (Table1). Although RGR% decreased with increasing salinity, *L. minor* tolerated shifts in salinities by evaporation (Tables 1 and 2) without any observed physical changes or mortality in both experiments 1 and 2. This was in accordance with Haller et al (1974), who reported gradually decreased growth of *L. minor* at levels above 6.66 ‰ diluted sea water and did not contraindicate previous studies of Buckley et al (1996) that reported EC50 of 4.88 to 5.50 g L^{-1} or Omar & Balla (2010) that reported low EC50 of 1.6 g L^{-1}. As mentioned above we used sea salt in our experiments not NaCl that contains other elements reported to be essential for duckweed survival (Leng 1999). Furthermore, *L. minor* survived well in brackish water system of 4 g L^{-1} salinity collected from Jericho aquifers (Figure 2.2). These brackish waters also contain various elements like Ca, Mg, K, SO4, Br that may be required for better duckweed growth (Marie & Vengosh 2001).

Ammonium NH_4^{+1} removal by nitrification. Nitrification is a two step biological process in which ammonia/ammonium is first oxidized to nitrite by ammonia-oxidizing bacteria, then further to nitrate by nitrite oxidizing bacteria. Nitrification, coupled to nitrogen uptake contribute to removal of nitrogen in duckweed covered system during wastewater treatment (Zimmo et al 2003).

In reference to Figures 4 and 5, nitrification, in the presence of *L. minor* mat could represent a second mechanism for ammonia removal in aquariums. Percentages of nitrate increase were significantly lower than percentages of ammonium removal in experiment 1 (exposed to sunlight), but not in experiment 2 (in the dark). In addition higher percentages of nitrate increase in experiment 2 (in the dark) compared to experiment 1. These observations indicated that nitrification over the duckweed mat was favored in the dark and reduced in the sunlight which on the other hand accelerated the process of ammonium uptake. This is obvious since nitrifying bacteria are photosensitive, to UV/sun light especially before they colonize surfaces (Guerrero & Jones 1997; Alleman 1987).

On the other hand, in experiment 3 (average NH_4^{+1} 51.8 mg L^{-1}), percentages of nitrate increase were significantly higher than percentages of ammonium removal (Figure 6). Ammonium uptake by duckweed was blocked and removal was only by nitrification. Duckweeds are subjected to direct toxicity from pH variations, and both high levels of ionized and unionized ammonia (Korner et al 2001). Clement & Merlin (1995) reported 56% inhibition of dry weight increase in *L. minor* at total ammonia concentrations of 152 mg L^{-1} N, calculated unionized ammonia of 5.7 mg L^{-1} NH_3 with pH adjusted at 8 and no growth at UIA-N>10.5 mg L^{-1}. As mentioned earlier, high total ammonia causes a shift in pH causing both direct toxicity and higher fraction of UIA-N forms. This could explain the extra nitrification from dead debris of *L. minor* (Figure 6). Death of *L. minor* was observed after the first week in experiment 3 (average NH_4^{+1} 51.8 mg L^{-1}).

In reference to Table 3 and Figure 5, the effect of salinity on nitrification, the percentage of nitrate NO_3^{-1} increase was the highest in tap water, and decreased as the salinity increased in experiment 2 (in the dark) (Table 3, Figure 4). Nitrate NO_3^{-1} levels doubled after two weeks but remained constant in the first five days (Table 3). Usually, nitrifying bacteria have a very slow reproductive rate and adaptation to different salinities may involve a lag time of 1-3 days before exponential growth begins. Moreover, there is loss of diversity of nitrifying bacteria related to increasing salinity with selection for a less diverse ammonia-oxidizing population (Moussa et al 2006; Grommen et al 2005). This did not hold true during experiment 1 (exposed to sunlight) since the percentage of nitrate NO_3^{-1} increase was significantly lower than ammonium NH_4^{+1} removal at different salinities (Table 4, Figure 4). This could be contributed to nitrate NO_3^{-1} uptake by *L. minor* coupled to ammonium NH_4^{+1} uptake in experiment 1 (exposed to sunlight). Although Omar & Balla (2010) reported inhibition of nitrate NO_3^{-1} uptake at salinity above

0.5 g L^{-1} NaCl, it was mentioned earlier that sea salt containing the necessary required elements for duckweed growth was used in our experiments (Leng 1999).

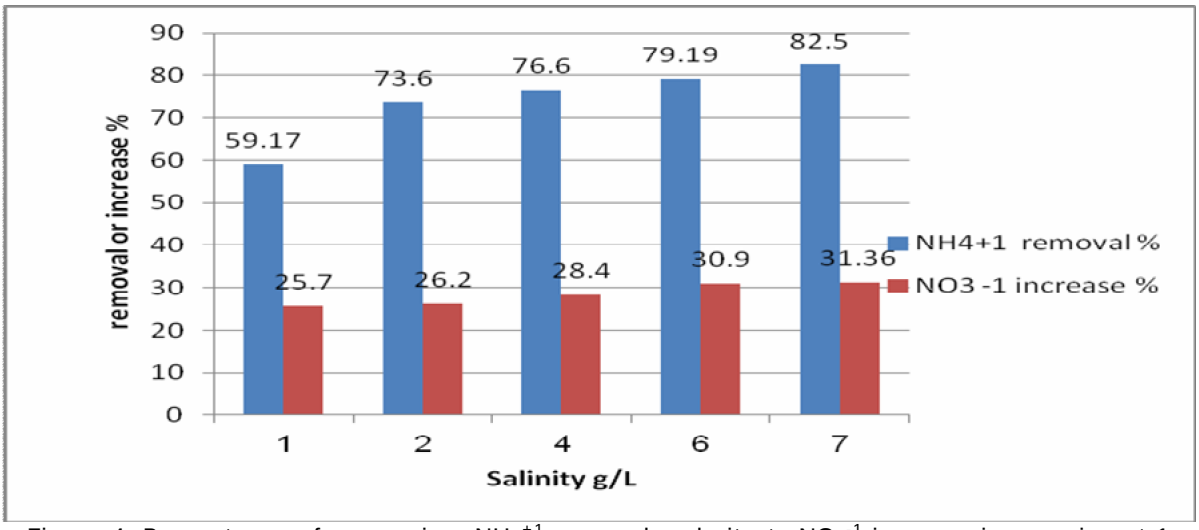


Figure 4. Percentages of ammonium NH_4^{+1} removal and nitrate NO_3^{-1} increase in experiment 1 (exposed to sunlight).

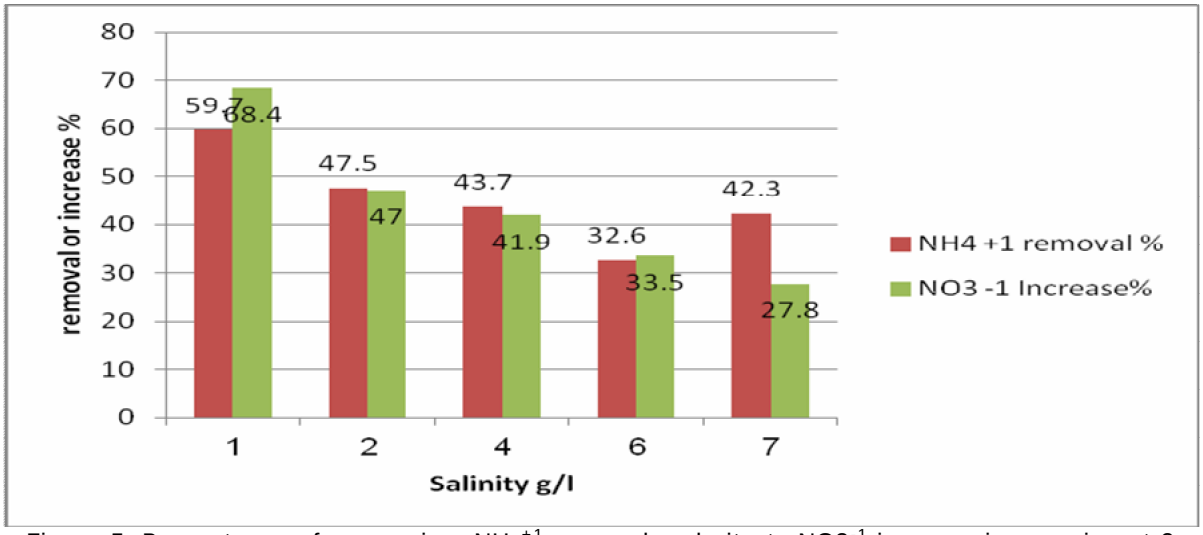


Figure 5. Percentages of ammonium NH_4^{+1} removal and nitrate $NO3^{-1}$ increase in experiment 2 (in the dark).

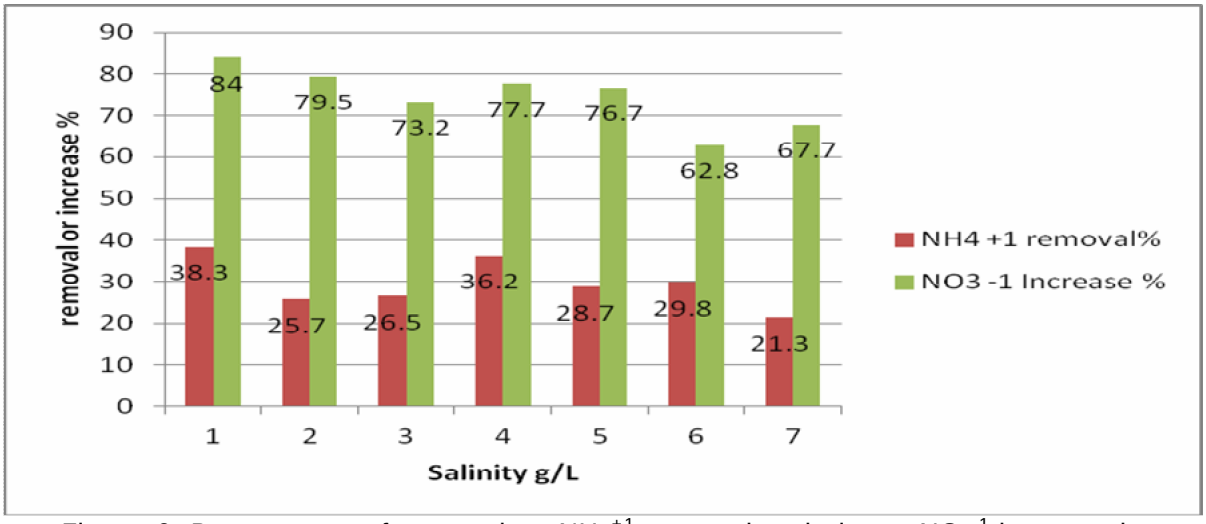


Figure 6. Percentages of ammonium NH_4^{+1} removal and nitrate NO_3^{-1} increase in experiment 3 (average NH_4^{+1} 51.8 mg L^{-1}).

Table3

Nitrate NO_3^{-1} levels (ppm) during 2 weeks sampling period in experiment 2 (in the dark)

NO_3^{-1} levels (ppm)	*Salinity (g L^{-1})*					
Sampling date	*No duckweed (1 g L^{-1})*	*Tap water (1 g L^{-1})*	*2*	*4*	*6*	*7*
24/3/2012	16.3	14.9	16.0	16.9	18.3	19.4
27/3/2012	16.2	16.5	16.0	16.9	18.6	18.8
3/4/2012	17.1	17.0	18.3	18	17.04	17.8
7/4/2012	18.1	36.5	30.2	29.1	27.4	26.9

Table 4

Nitrate NO_3^{-1} levels (ppm) during 2 weeks sampling period in experiment 1 (exposed to sunlight)

NO_3^{-1} levels (ppm)	*Salinity (g L^{-1})*				
Sampling date	*Tap water (1 g L^{-1})*	*2*	*4*	*6*	*7*
24/3/2012	14.78	15.41	15.39	15.39	15.80
27/3/2012	17.12	17.12	16.89	17.20	17.94
3/4/2012	18.94	19.87	18.88	19.30	19.83
7/4/2012	19.9	20.90	21.50	22.30	23.02

Conclusions. The duckweed *L. minor* was an efficient natural biofilter in both brackish and freshwater closed recirculating systems. *L. minor* reduced ammonia levels below the standard recommended levels (UIA-N<0.1 ppm, TAN<1 ppm). Ammonium NH_4^{+1} uptake was coupled with nitrate NO_3^{-1} uptake under favorable conditions of sunlight. This could indicate that *L. minor* could also be used as nitrate filters in aquariums eliminating the need of daily water exchange. Salinity in the ranges from 1 to 7 g L^{-1} enhanced ammonium NH_4^{+1} uptake, without affecting nitrate NO_3^{-1} uptake or any observed mortality of the duckweed. This does not contraindicates a previous study that reported inhibition of nitrate NO_3^{-1} uptake at salinity above 0.5 g L^{-1} NaCl, since sea salt containing other necessary required elements for duckweed growth beside NaCl was used in our experiments. Nitrification is a second mechanism of nitrogen removal in closed recirculating systems, which is affected by factors that influence the nitrifying bacteria population growth (eg. salinity, light). Average ammonium NH_4^{+1} of 51.8 mg L^{-1} was toxic to *L. minor* and death was observed within a week. In recirculating systems there is no fear of ammonium raising to such toxic levels since pH is under control and there is a continuous production of ammonia by fish and removal by duckweed.

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Authors:
Mutaz A. Al-Qutob, Faculty of Science and Technology, Department of Earth and Environmental studies, Al-Quds University, P. O. Box 19164, Jerusalem – Israel, e-mail: qutob@planet.edu
Tharwat S. Nashashibi, Aquatic and Aquaculture Laboratory, Al-Quds University, P. O. Box 19164, Jerusalem – Israel, e-mail: tharwatnash33@hotmail.com

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