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ORIGINAL ARTICLE

Ex-situ Management of Ammonium Contamination in Water using Eight Aquatic Plants

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ABSTRACT
The study was conducted to determine the ammonium (NH_4^+) level in drinking and surface waters of Lucknow and to assess the NH_4^+ accumulation potential of 8 aquatic plants. Ammonium level in drinking water was higher than permissible limit (PL) i.e. 0.5 mg L^{-1}. A few urban sites showed 6-8 folds high NH_4^+ over the PL. Ammonium in the peri-urban and rural site was comparatively lower, though it was still 2-4 folds higher than PL. On the other hand, all surface water sites, except one residential site (Aashiana), showed NH_4^+ level less than PL (5 mg L^{-1}). Eight aquatic plants viz. Peltandra virginica, Utricularia vulgaris, Eichhornia crassipes, Trapa natans, Mimulus glabratus, Marsilea quadrifolia, Pistia stratiotes and Polygonum persicaria were investigated and cultured in 10 L plastic pots for 15 days (d) under simulated net house conditions. Reduction in NH_4^+ level in water and its simultaneous increase in plant tissues was recorded at 5th, 10th and 15th d. Pistia stratiotes was found more efficient and removed NH_4^+ upto 70%. Highest accumulation of NH_4^+ i.e. 46% was noticed in the tissues of Pistia stratiotes.
Key words: *Aquatic plants, Drinking water, Ammonium, Phytoremediation, Pistia stratiotes, Surface water.*

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INTRODUCTION

Ammonium (NH_4^+) is an important nitrogenous pollutant in soil and aquatic systems, and has been found frequently in the water sources [1]. It is the major end product of protein catabolism and major excretory product which remains in the form of unionized (NH_3) and ionized (NH_4^+) ammonia. Ammonia is a critical water quality parameter and toxic to aquatic life, but the NH_4^+ is harmless except in extremely high concentrations. At higher concentration NH_4^+ can damage aquatic life, by declining the oxygen level below 2 mg L^{-1}, as it oxidizes to nitrite (NO_2^-) and nitrate (NO_3^-) [2], and can induce a number of physiological disorders, when its level in the organism is high [3]. Most of NH_4^+ in surface waters is oxidized to NO_2^- by *Nitrosomonas* bacteria. The presence of NO_2^- in the environment is a potential threat due to its well documented toxicity to animals [4]. The major metabolites of NO_2^- are nitric oxide and nitrosamine. Latter one is highly carcinogenic and associated with a high risk of stomach, liver and esophagus cancer [5].
Ammonium removal is accomplished through biological nitrification in which it is converted into NO_2^- and NO_3^- by nitrifying bacteria called nitrifers. On the other hand, anammox (Anaerobic Ammonium Oxidation) bacteria co-function with autotrophic nitroso-bacteria *via* the following route $NH_4^+ + 1.32\ NO_2^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 2.03H_2O$. This pathway of NH_4^+ removal is known as 'Completely Autotrophic Nitrogen removal Over Nitrite' (CANON) pathway [6].
Phytoremediation is a rapidly expanding area of environment science that holds great promise for cleaning up the polluted and contaminated environment [7]. There have been a number of reports using the aquatic plants in phytoremediation technology [8]. Because of its low cost and lack of technical problems, phytoremediation has globally become very popular. This technique is currently being studied for potential use in waste water treatment [9]. The high productivity and nutrient removal capability of aquatic plants have created substantial interest for waste water treatment [10, 11].
The present study was intended to find out the extent of NH_4^+ pollution in ground and surface waters of Lucknow during various seasons in the repeated year, 2007-2008 and an unconventional access was taken to evaluate the ability of eight aquatic plants.

MATERIALS AND METHODS

Water Sample: The drinking water samples of hand pumps were collected from 22 sites of urban, peri-urban and rural vicinities of Lucknow during 2007-2008. Simultaneously, surface water samples were also collected from 11 different surface water bodies i.e. ponds, lake and river, randomly at uniform depth of 5 cm. Six samples from each site were collected and analyzed for NH_4^+ concentration on the same day to avoid the effect of storage and preservatives.

Plant Culture: Aquatic plants viz. *Peltandra virginica, Utricularia vulgaris, Eichhornia crassipes, Trapa natans, Mimulus glabratus, Marsilea quadrifolia, Pistia stratiotes* and *Polygonum persicaria* were procured from the different surface water bodies, during the month May and June, 2009 (min./max. temp- 24.6/42.4°C). Plants having biomass i.e. 100 g were placed in 3 L plastic pots (4 replicates per treatment), in the net house for 15 days (d). Initial NH_4^+ concentration i.e. 10 mg L^{-1} in water was maintained by employing requisite amount of ammonium chloride (NH_4Cl) salt. At every 5^{th} d water/plant samples were collected from the treatment pots. Prior to sampling deionized water was added to the pots to replenish the water lost by evaporation and to regain the initial level.

Sample Analysis: Ammonium in the water and plants samples was assayed by the method as described by Herbert *et al.*[12], using Nessler's reagent (K_2HgI_4) with the help of UV-1601, UV-visible spectrophotometer (Shimadzu) and Varian, carry 100 Bio, UV-visible spectrophotometer, by preparing standard curve.

Statistical Analysis: The data was analyzed using one way ANOVA (Analysis of Variance). The difference between treatments was considered significant at $p \le 0.05$.

RESULTS AND DISCUSSION

Drinking Water: In the summer 2007-2008, the level of NH_4^+ in drinking water was found in the range of 0.18 to 4.08 mg L^{-1}. In rainy season it ranged from 0.1 to 3.75 mg L^{-1}, while during winter it varied from 1.12 to 4.51 mg L^{-1}. The highest value of NH_4^+ (4.51 mg L^{-1}) that was beyond 8 folds over the Permissible Limit (PL) i.e. 0.5 mg L^{-1} [13] was estimated in an urban site (Hazratganj, a business centre of the city) during winter-2008 and lowest (0.10 mg L^{-1}) during rainy season-2007 in a peri-urban site (Saleh nagar, a developing area). Later on during 2008, latter one (Saleh nagar) showed more than 2 fold higher NH_4^+ level than the PL, which was in accordance with the urbanization resulting in the increase of sewage water content (table 1). Ammonium in peri-urban and rural regions was comparatively lower than that in urban region, though it was still 2-4 folds higher than the PL at most of the sites (table 1). The annual variation was not very significant and seasonal variation was yet marginal which was statistically significant only at few sites. Ammonium level in drinking water was found above the PL however, its level was often found higher in winter season as compared to summer and rainy season that may be due to the continuous leaching and percolation after rainy season to the ground water table [14].

Surface Water: In surface water samples, low NH_4^+ level was detected, that was below the PL i.e. 5 mg L^{-1} [15]. At only one urban site (Aashiana, a residential area), NH_4^+ level was estimated between 5.40 to 6.65 mg L^{-1} during the repeated year of 2007-2008, that was beyond but close to the PL. Ammonium level during summer, rainy and winter season (2007-2008) varied from 1.10 to 5.66, 1.71 to 6.52 and 1.43 to 6.02 mg L^{-1} respectively (table 2). Data revealed that maximum elevation in NH_4^+ level was during rainy season in surface water bodies that may be due to the receiving of surplus quantity of agricultural, human and animal waste during the period [11, 16]. In most cases, the level of NH_4^+ was higher in 2008 as compared to that in 2007, which indicated yearly increase in NH_4^+ level in the water (table 2). The values were almost consistent in two different years and in the three different seasons, and the minor variations observed were significant only in few cases.

Table 1 Seasonal variation of ammonium (NH_4^+) level in drinking water samples of hand pumps in urban, peri-urban and rural regions of Lucknow, during 2007-08

	mg NH_4^+ L^{-1} water					
	Summer		Rainy season		Winter	
Sites	**2007**	**2008**	**2007**	**2008**	**2007**	**2008**
Urban regions						
Hazratganj	3.71 ± 0.40	4.08 ± 0.28	3.54 ± 0.24	3.75 ± 0.27	3.93 ± 0.27	4.51 ± 0.29
Kesharbagh	2.47 ± 0.20	3.15 ± 0.22	2.28 ± 0.15	2.64 ± 0.20	2.98 ± 0.19	4.09 ± 0.27#
Charbagh	2.60 ± 0.11	2.80 ± 0.15	2.34 ± 0.13	2.90 ± 0.24	2.82 ± 0.23	3.33 ± 0.23
Zoo	2.45 ± 0.14	2.75 ± 0.19	2.56 ± 0.20	2.59 ± 0.25	2.58 ± 0.26	2.98 ± 0.28
Mall avenue	2.50 ± 0.25	3.25 ± 0.23	1.97 ± 0.24	2.78 ± 0.28	3.45 ± 0.28*	4.22 ± 0.31#
Ruchi khand	2.30 ± 0.50	2.98 ± 0.38	1.65 ± 0.25	2.51 ± 0.27	2.23 ± 0.27	3.17 ± 0.30

Rajni khand	1.50 ± 0.20	2.36 ± 0.15	1.13 ± 0.08	1.78 ± 0.14	2.24 ± 0.12*	2.64 ± 0.18
Gomti nagar	1.30 ± 0.20	2.05 ± 0.12	1.08 ± 0.07	1.53 ± 0.13	1.87 ± 0.12	2.14 ± 0.15
Nishatganj	1.40 ± 0.17	1.98 ± 0.13	1.17 ± 0.07	1.71 ± 0.12	2.09 ± 0.11*	2.08 ± 0.15
Triveni nagar	1.30 ± 0.16	2.10 ± 0.11	1.16 ± 0.07	1.58 ± 0.10	1.93 ± 0.09	2.07 ± 0.13
Aliganj	1.40 ± 0.26	2.22 ± 0.14	1.23 ± 0.08	1.67± 0.13	1.72 ± 0.10	2.34 ± 0.16
Peri-urban regions						
Telibagh	1.60 ± 0.20	1.93 ± 0.15	1.49 ± 0.12	1.89 ± 0.14	1.98 ± 0.14	2.43 ± 0.17
Bhadrukh	1.50 ± 0.10	2.13 ± 0.21	1.54 ± 0.18	1.98 ± 0.19	2.05 ± 0.22	3.04 ± 0.24#
Qila	1.30 ± 0.06	2.24 ± 0.14	1.16 ± 0.09	1.35 ± 0.14	2.18 ± 0.13*	2.57 ± 0.17
PGI	1.40 ± 0.20	1.88 ± 0.10	1.26 ± 0.05	1.77 ± 0.11	2.01 ± 0.09	2.31± 0.13
Saleh nagar	0.18± 0.07	1.06 ± 0.13	0.10 ± 0.09	0.87 ± 0.08	1.12 ± 0.12*	1.36 ± 0.11
BBAUniversity	1.30 ± 0.14	1.48 ± 0.10	1.16 ± 0.07	1.29 ± 0.11	1.56 ± 0.11	1.67 ± 0.11
Rural regions						
Beli kala	1.80 ± 0.30	1.98 ± 0.12	1.71 ± 0.11	1.73 ± 0.10	2.12 ± 0.13	2.23 ± 0.15
Bijnour	1.30 ± 0.07	2.18 ± 0.11	1.33 ± 0.07	2.22 ± 0.10	2.33 ± 0.10*	2.53 ± 0.13
Chiraiyabagh	1.30 ± 0.08	1.95 ± 0.10	1.21 ± 0.08	1.82 ± 0.11	1.87 ± 0.09	1.99 ± 0.13
Sabha khera	1.20 ± 0.25	1.67± 0.13	1.31 ± 0.07	1.53 ± 0.13	1.75 ± 0.12	2.13 ± 0.15
Aurangabad	1.60 ± 0.17	1.72 ± 0.12	1.36 ± 0.08	1.75± 0.11	1.55 ± 0.11	1.98 ± 0.14

Values are mean of 6 replicates ± S.D., significance at $p \leq 0.05$.

Table 2 Seasonal variation of ammonium (NH_4^+) level in surface water samples of ponds, lake and river in urban, peri-urban and rural regions of Lucknow, during 2007-08

Sites	mg NH_4^+ L^{-1} water					
	Summer		Rainy season		Winter	
	2007	2008	2007	2008	2007	2008
Urban regions						
Gomti river	2.60 ± 0.10	2.99 ± 0.16	3.48 ± 0.18	3.91 ± 0.19	2.91 ± 0.15	3.22 ± 0.18
Aashiana pond	5.40 ± 0.30	5.66 ± 0.22	6.65 ± 0.26*	6.52 ± 0.27	5.55 ± 0.23	6.02 ± 0.24
Aliganj pond	2.20 ± 1.0	2.76 ± 0.13	3.18 ± 0.14	3.85 ± 0.18#	2.71 ± 0.13	3.56 ± 0.15
Fazullaganj pond	2.01 ± 0.30	2.31 ± 0.15	2.91 ± 0.17	3.26 ± 0.20	2.36 ± 0.16	3.12 ± 0.16
Bharat nagar pond	2.20 ± 0.40	3.01 ± 0.16	4.09 ± 0.18*	3.97 ± 0.19	2.91 ± 0.17	3.45 ± 0.17
Peri-urban regions						
Qila pond	2.60 ± 0.70	2.81 ± 0.18	3.15 ± 0.20	3.80 ± 0.22#	2.86 ± 0.18	3.65 ± 0.19
B.B.A.University lake	1.80 ± 0.50	2.26 ± 0.14	2.50 ± 0.16	2.83 ± 0.17	2.17 ± 0.13	3.44 ± 0.14
Telibagh pond	2.0 ± 0.20	2.27 ± 0.16	2.45 ± 0.18	2.91 ± 0.19	2.24 ± 0.16	2.42 ± 0.17
Sharda naher	1.10 ± 0.08	1.39 ± 0.25	1.71± 0.26	1.95 ± 0.27	1.43 ± 0.24	1.57 ± 0.24
Rural regions						
Devi khera pond	2.60 ± 0.70	3.11 ± 0.23	3.35 ± 0.24	3.77 ± 0.26	3.08 ± 0.22	3.43 ± 0.24
Aurangabad pond	2.50 ± 0.80	2.95 ± 0.19	3.18 ± 0.20	3.34± 0.23	2.97 ± 0.19	3.17 ± 0.21

Values are mean of 6 replicates ± S.D., significance at $p \leq 0.05$. *significant values of 2007, #significant values of 2008

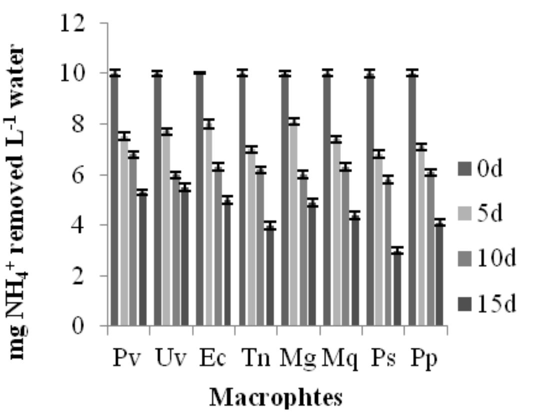

Fig. 1(a) Ammonium removed from water at 5th, 10th and 15th d.

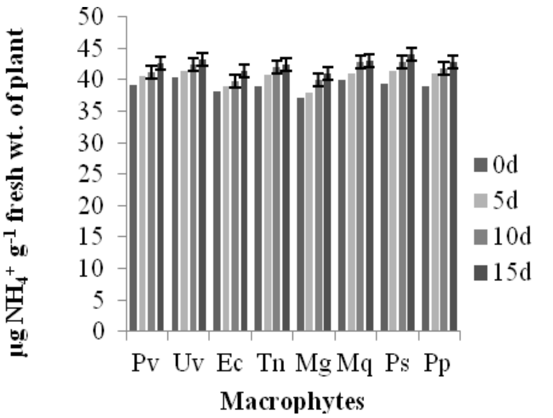

Fig. 1(b) Ammonium accumulated in the plants at 5th, 10th and 15th d

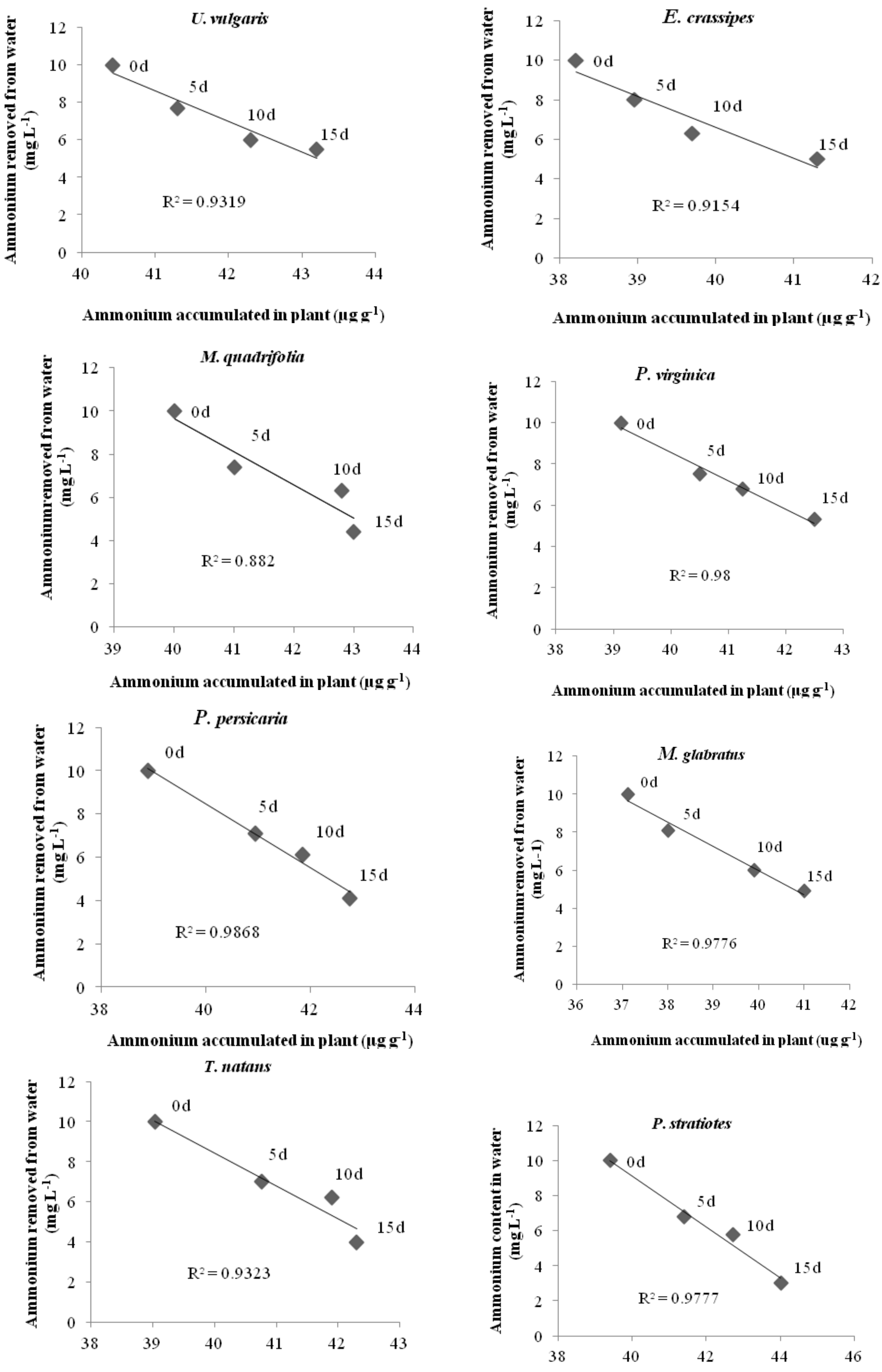

Fig. 2 Linear correlation between ammonium removed from water and accumulated in the plants at 5th, 10th and 15thd.

Ammonium Remediation: Ammonium removal from water and accumulation in the plants water was measured by 8 native aquatic plant species. Initial concentration of NH_4^+ employed to the pots containing aquatic plants was 10 mg L^{-1}, and at the end of experimental period it's level was found in between 3 to 5.5 mg L^{-1} in the treated water (fig. 1a). The percent of NH_4^+ removed from the water at 15th d by the selected aquatic plants ranged from 45%-70% (fig. 1a). Content of NH_4^+ in the plants tissues before plantation was in between 37.12-40.42 µg g^{-1}. At 15th d, NH_4^+ estimated in the different plants was found in between 41-44.01 µg g^{-1} (fig. 1b).

The highest content of NH_4^+ i.e. 4.6 µg g^{-1} equivalents to 46% was accumulated in the tissues of *Pistia stratiotes* whereas least i.e. 3.88 µg g^{-1} or 38.8% NH_4^+ was accumulated in the tissues of *Mimulus glabratus* at 15th d (fig. 1b). On the basis of above findings it is observed that the amount of NH_4^+ reduced from the water was 6.2-24% more than that accumulated in the different aquatic plants. This difference may be due to the oxidation of ammonium into NO_2^-/NO_3^- by nitrifying/denitrifying aerobic/anaerobic microorganisms [6]. Results indicated that NH_4^+ content increased linearly at 5th, 10th, and 15th d in the plants tissues with simultaneous decrease in its level in water (fig. 2).

All the selected aquatic plants accumulated NH_4^+ from the water. However, NH_4^+ content was found greater in *Pistia stratiotes* which is similar to other studies [17]. *Pistia stratiotes* is a cosmopolitan species and grows round the year in nutrient enriched water. This aquatic plant may also be recommended for the NH_4^+ phytoremediation from surface water because of lower gain of mass, thus reducing problems from exceeded biomass [17, 18].

CONCLUSIONS

The result of this study indicated that ground water NH_4^+ level was more than 8 folds over the PL that may pose threat to humans. On the other hand all the surface water bodies exhibited NH_4^+ level which was below the PL, except one site Aashiana of urban region. By the analysis of data presented in this paper, we can conclude that *Pistia stratiotes* can be utilized as a hyper-accumulator of NH_4^+ and can phytoremediate it upto 70%.

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