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Remediation of nitrite contamination in ground and surface waters using aquatic macrophytes

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Abstract

The study was carried out to determine the seasonal variation of nitrite levels in drinking and surface waters of urban, peri-urban and rural areas of Lucknow, during 2007-2008, and to evaluate the nitrite removal and accumulation potential of certain native aquatic macrophytes. Most of the drinking and surface water samples were collected from urbanized region of the city. All drinking water samples detected, showed higher nitrite level in winter, when compared with that in summer and rainy seasons. However, in drinking water samples nitrite level was below the permissible limit *i.e.* 3.29 mg l^{-1} NO_2. The surface water showed more than 3 fold higher levels of nitrite over the permissible level *i.e.* 0.06 mg l^{-1}, and the level was higher during rainy season than in summer and winter seasons. Eight macrophytes *viz. Peltandra virginica, Utricularia vulgaris, Eichhornia crassipes, Trapa natans, Mimulus glabratus, Marsilea quadrifolia, Pistia stratiotes* and *Polygonum persicaria* were studied for phytoremediation potential of nitrite from the water under simulated laboratory conditions. The gradual diminution in the level of nitrite in the water and simultaneously it's increase in the plant tissues was recorded at 5^{th}, 10^{th} and 15^{th} d after plant culture. All the plants selected, removed nitrite from water but *Polygonum persicaria, Mimulus glabratus, Trapa natans* and *Pistia stratiotes* were found more efficient and removed nitrite upto 60.91, 58.09, 60.97 and 72.28%, respectively. Observations revealed that *Pistia stratiotes* can be used for the effective removal of nitrite from the contaminated water.

Key words

Drinking water, Nitrite level, *Pistia stratiotes*, Surface water, Aquatic macrophytes

Introduction

Nitrite is a natural component of the nitrogen cycle in ecosystems, and its presence in the environment is a potential problem due to its well documented toxicity to animals (Sinha and Nag, 2010). It has been reported in various water sources (Bingbing *et al.,* 2009). It is therefore, necessary to remove it from water in order to reduce it's harm to the animal and human consumers of the water which cannot assimilate nitrite like bacteria and plants (Alonso and Camargo, 2009). Nitrite can damage aquatic life, causing hypoxia, where oxygen concentration declines below 2 mg l^{-1} (Taylor *et al.,* 2005), and is recognized as a toxic compound that can induce a number of physiological disturbances, when its concentration in the organism is high (Jensen, 2007). Nitrite is however, also a natural constituent in the body, and recent research has suggested that nitrite has important biological functions at its natural low endogenous concentrations and thus, nitrite may participate in blood flow regulation (hypoxic vasodilation), may act as a signaling molecule and regulate gene expression (Gladwin *et al*., 2006; Fago and Jensen, 2007). Nitrite physiology therefore, seems to be a balance between toxic disruptions of functions at high concentrations and beneficial effects at low concentrations (Taylor *et al*., 2006).

Most of ammonium in surface waters is oxidized to nitrite by *Nitrosomonas* bacteria. Being an intermediate compound nitrite is very unstable, it's excessive amount deplete dissolved oxygen in water column as the oxidation of nitrite to nitrate consumes oxygen (Lam *et al*., 2007). The major metabolites of nitrites are nitric oxide and nitrosamine. The nitrosamine is highly carcinogenic and associated with a high risk of stomach, liver and esophagus cancer (Kim-Shapiro *et al*., 2006). Nitrite has been shown to cause methaemoglobinaemia in animals. Humans appear to be more sensitive to nitrite-induced methaemoglobin formation. The fraction of nitrate reduced to nitrite in adults is at least 5%, and knowing that nitrate reduction in infants is significantly greater than in adults (due to the presence of gastrointestinal bacteria), the conversion rate is estimated to be at least 10% in infants (U.S. EPA, 1990). Anyway, nitrite when present at high concentration in blood can react with Fe (II) of the haemoglobin, forming methaemoglobin [NO_2^- + oxyHb (Fe_2^+) $\rightarrow$ metHb (Fe_3^+) + NO_3^-] which has no oxygen-carrying ability. This condition is called methaemoglobinaemia (Dejam *et al*., 2005).

Nitrite is produced inside the body as an oxidative metabolite of the physiological messenger molecule (NO). The NO in blood can react with plasma oxygen and form nitrite (Dejam *et al*., 2005). Other sources of nitrite include intake *via* the diet and reduction of nitrate (present in diet or drinking water) to nitrite by bacteria in the oral cavity (Lundberg and Weitzberg, 2005). It is well documented that blood gas transport is disturbed by increased methaemoglobin level and decreased total haemoglobin during nitrite exposure and thus metabolism may also be influenced by inhibition of mitochondrial respiration (Shiva *et al*., 2007). The oxygen-carrying properties of haemoglobin depend on oxygenbinding to ferrous iron at each of the four heme groups. Onceiron is in the ferric (Fe^{3+}) state, as in methaemoglobin, itis unable to combine reversibly with oxygen and transport itin the body (Gladwin *et al*., 2006). Clinically, methaemoglobin concentrations >10-20% result in obvious cyanosis, with headaches, weakness, and asphyxiation becoming apparent at levels of 35% or greater (Duranski *et al*., 2005).

Availability of N in water plays an important role for the growth and establishment of aquatic plants. The role of aquatic plants in phytoremediation technology is well established (Skinner *et al*., 2007). Aquatic plants in particular exhibit exorbitant potential to remove various contaminants including heavy metals, organic compounds, radionuclide from the aquatic environment (Xu *et al*., 2009). Macrophytes based wastewater treatment systems are relatively inexpensive, easy to maintain, provide effective and reliable wastewater treatment and resulting vegetative biomass provide economic returns when harvested (Polomski *et al*., 2008).

The projected population of Lucknow urban agglomeration is 71.66 lakh which is covered by an area of 369.01 km^2 (web: www.ldalucknow.com). The present study was aimed to find out the extent of nitrite pollution in ground and surface waters of Lucknow, during summer, rainy and winter seasons in the repeated year (2007-2008). An unconventional access was taken to evaluate the ability of eight different aquatic plant species to thrive and recover the nitrite rich water under uniform controlled environmental conditions.

Materials and Methods

The drinking water samples of hand pumps were collected from twenty two sites of urban, peri-urban and rural vicinities of Lucknow. Surface water samples were also collected simultaneously from eleven different surface water bodies *i.e.* ponds and river, randomly at uniform depth of 5 cm. Six samples were collected from each site. The nitrite level was determined during summer, rainy and winter seasons of the year 2007 and 2008.

Nitrite removal rate was measured by eight aquatic plant *viz*. *Peltandra virginica, Utricularia vulgaris, Eichhornia crassipes, Trapa natans, Mimulus glabratus, Marsilea quadrifolia, Pistia stratiotes* and *Polygonum persicaria* which were obtained from surface water bodies. The experiment was conducted during the months of May and June, 2009 (min/max temp- 24.6/42.4°C) in a prefabricated net house to stimulate removal of nitrite. The day length during the experimental period was approximately 14 hr and the min./max mean of humidity recorded was 45.4 to 82.7%. Macrophytes were placed in the plastic pots having capacity of 3 l (4 replicates per treatment), in the net house, and pots without plants served as control. Initially, 0.25 mg l^{-1} NO_2 concentration was maintained by employing requisite amount of $NaNO_2$ salt in the treatments and control pots. Average value of electrical conductivity (EC) and pH measured during treatment tenure were 1.4 mg l^{-1} and 8.99, respectively. In each treatment, macrophytes with identical biomass *i.e.* 100 g were used and their culture was monitored for 15 d. At every 5^{th} d water samples were collected in the sampling tubes from the treatments and control pots between 8:30 to 9:30 a.m., prior to sampling deionized water was added to each pot to replenish the water lost by evaporation and to maintain the initial level. Simultaneously, fresh leaves from the treatment plants were also collected and homogenized in the distill water in a ratio 1:6 w/v (1 g fresh leaf per 6.0 ml). The homogenates were filtered and the filtrates were centrifuged at 6,000 rpm for 15 min. The supernatants were used to measure the content of nitrite.

Nitrite content in the water and in the plants was estimated by the method as described by Stevens and Oaks (1973) using 1% sulphanilamide (w/v in 1N HCl) and 0.01% N-1-napthyl ethylene diamine dihydrochloride (w/v in deionized water), with the help of Varian, carry 100 Bio, UV-visible spectrophotometer. The recorded data was subjected to one-way analysis of variance (ANOVA) difference between individual means was tested using least significance difference (LSD) tests at 0.05 significance level.

Results and Discussion

In the summer 2007-2008, the mean level of nitrite in drinking water was found in the range of 0.06 to 0.28 mg l^{-1}. In rainy season it ranged from 0.05 to 0.28 mg l^{-1}, while during winter it varied from 0.09 to 0.31 mg l^{-1}. The highest mean value of nitrite content (0.31 mg l^{-1}) was estimated in a urban region (Mall Avenue) during winter, 2008 and lowest (0.06 mg l^{-1}) during summer, 2007 in peri-urban region (PGI) (Table 1). Nitrite level in this study was found below the acceptable drinking water standard *i.e.* 3.29 mg l^{-1} NO_2^- (U.S.

Table - 1: Seasonal variation of the nitrite (NO_2^-) level in drinking water samples collected from the hand pumps situated in the urban, peri-urban and rural regions of Lucknow, during 2007-08

Sites	mg nitrite l^{-1} water					
	Summer		Rainy season		Winter	
	2007	2008	2007	2008	2007	2008
Urban regions						
Hazratganj	0.25 ± 0.024	0.28 ± 0.02	0.24 ± 0.02	0.27 ± 0.02	0.27 ± 0.02	0.29 ± 0.03
Kesharbagh	0.16 ± 0.01	0.20 ± 0.02	0.15 ± 0.01	0.20 ± 0.009	0.19 ± 0.02	0.23 ± 0.02$^{\#}$
Charbagh	0.19 ± 0.01	0.25 ± 0.02	0.19 ± 0.02	0.24 ± 0.02	0.23 ± 0.025*	0.27 ± 0.03
Zoo	0.25 ± 0.026	0.26 ± 0.02	0.25 ± 0.02	0.25 ± 0.026	0.26 ± 0.027	0.28 ± 0.03
Mall avenue	0.26 ± 0.02	0.27 ± 0.03	0.24 ± 0.03	0.28 ± 0.03	0.28 ± 0.03	0.31 ± 0.04$^{\#}$
Ruchi khand	0.26 ± 0.03	0.28 ± 0.03	0.25 ± 0.02	0.27 ± 0.01	0.27 ± 0.04	0.30 ± 0.03
Rajni khand	0.09 ± 0.01	0.15 ± 0.01	0.08 ± 0.007	0.14 ± 0.009	0.12 ± 0.01*	0.18 ± 0.02$^{\#}$
Gomti nagar	0.09 ± 0.005	0.12 ± 0.01	0.07 ± 0.005	0.13 ± 0.02	0.12 ± 0.008*	0.15 ± 0.01$^{\#}$
Nishatganj	0.07 ± 0.008	0.13 ± 0.007	0.07 ± 0.005	0.12 ± 0.01	0.11 ± 0.009*	0.15 ± 0.015
Triveni nagar	0.08 ± 0.01	0.11 ± 0.01	0.07 ± 0.008	0.10 ± 0.009	0.09 ± 0.02	0.13 ± 0.02
Aliganj	0.08 ± 0.005	0.12 ± 0.02	0.08 ± 0.004	0.13 ± 0.02	0.10 ± 0.005	0.16 ± 0.01$^{\#}$
Peri-urban regions						
Telibagh	0.13 ± 0.01	0.15 ± 0.01	0.12 ± 0.01	0.14 ± 0.02	0.14 ± 0.01	0.17 ± 0.01
Bhadrukh	0.20 ± 0.02	0.21 ± 0.02	0.18 ± 0.02	0.19 ± 0.01	0.22 ± 0.03	0.24 ± 0.02$^{\#}$
Qila	0.10 ± 0.009	0.14 ± 0.01	0.09 ± 0.007	0.14 ± 0.009	0.13 ± 0.01*	0.17 ± 0.02$^{\#}$
PGI	0.06 ± 0.006	0.10 ± 0.008	0.05 ± 0.002	0.11 ± 0.007	0.09 ± 0.007*	0.13 ± 0.009$^{\#}$
Saleh nagar	0.09 ± 0.01	0.13 ± 0.01	0.09 ± 0.01	0.12 ± 0.01	0.12 ± 0.02*	0.14 ± 0.01
B.B.A.University	0.08 ± 0.007	0.10 ± 0.008	0.07 ± 0.008	0.11 ± 0.01	0.11 ± 0.01*	0.11 ± 0.006
Rural regions						
Beli kala	0.11 ± 0.01	0.12 ± 0.009	0.11 ± 0.009	0.10 ± 0.008	0.13 ± 0.01	0.15 ± 0.017$^{\#}$
Bijnour	0.08 ± 0.005	0.11 ± 0.01	0.07 ± 0.006	0.10 ± 0.01	0.10 ± 0.008	0.13 ± 0.009
Chiraiyabagh	0.07 ± 0.006	0.10 ± 0.009	0.08 ± 0.006	0.11 ± 0.009	0.09 ± 0.007	0.13 ± 0.01$^{\#}$
Sabha khera	0.08 ± 0.007	0.13 ± 0.02	0.07 ± 0.007	0.13 ± 0.01	0.12 ± 0.01*	0.15 ± 0.017
Aurangabad	0.08 ± 0.008	0.12 ± 0.01	0.08 ± 0.009	0.11 ± 0.007	0.11 ± 0.009*	0.14 ± 0.01

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

Table - 2: Seasonal variation of the nitrite (NO_2^-) level in surface water samples collected from ponds and river in urban, peri-urban and rural regions of Lucknow, during 2007-08

Sites	mg nitrite l^{-1} water					
	Summer		Rainy season		Winter	
	2007	2008	2007	2008	2007	2008
Urban regions						
Gomti river	0.14 ± 0.009	0.16 ± 0.01	0.18 ± 0.014*	0.19 ± 0.02	0.15 ± 0.017	0.18 ± 0.01
Aashiana pond	0.21 ± 0.01	0.22 ± 0.02	0.26 ± 0.03*	0.27 ± 0.03$^{\#}$	0.23 ± 0.02	0.24 ± 0.02
Aliganj pond	0.12 ± 0.007	0.13 ± 0.009	0.14 ± 0.014	0.18 ± 0.015$^{\#}$	0.13 ± 0.009	0.15 ± 0.01
Fazullaganj pond	0.14 ± 0.013	0.15 ± 0.01	0.17 ± 0.008	0.20 ± 0.015$^{\#}$	0.16 ± 0.02	0.16 ± 0.01
Bharat nagar pond	0.16 ± 0.012	0.16 ± 0.01	0.18 ± 0.014	0.19 ± 0.01	0.17 ± 0.01	0.17 ± 0.011
Peri-urban regions						
Qila pond	0.16 ± 0.014	0.18 ± 0.009	0.20 ± 0.02*	0.22 ± 0.018$^{\#}$	0.18 ± 0.01	0.19 ± 0.01
B.B.A.University lake	0.11 ± 0.01	0.14 ± 0.007	0.16 ± 0.01*	0.17 ± 0.01	0.13 ± 0.01	0.14 ± 0.009
Telibagh pond	0.13 ± 0.008	0.16 ± 0.01	0.18 ± 0.007*	0.19 ± 0.015	0.16 ± 0.01	0.17 ± 0.008
Sharda naher	0.23 ± 0.022	0.25 ± 0.02	0.26 ± 0.017	0.27 ± 0.021	0.24 ± 0.02	0.24 ± 0.018
Rural regions						
Devi khera pond	0.20 ± 0.014	0.23 ± 0.02	0.24 ± 0.018*	0.26 ± 0.025	0.22 ± 0.009	0.24 ± 0.013
Aurangabad pond	0.17 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.23 ± 0.02$^{\#}$	0.19 ± 0.01	0.21 ± 0.016

Values are mean of six replicates ± S.D, significance at $p \leq 0.05$. * = Significant values of 2007, $^{\#}$ = Significant values of 2008

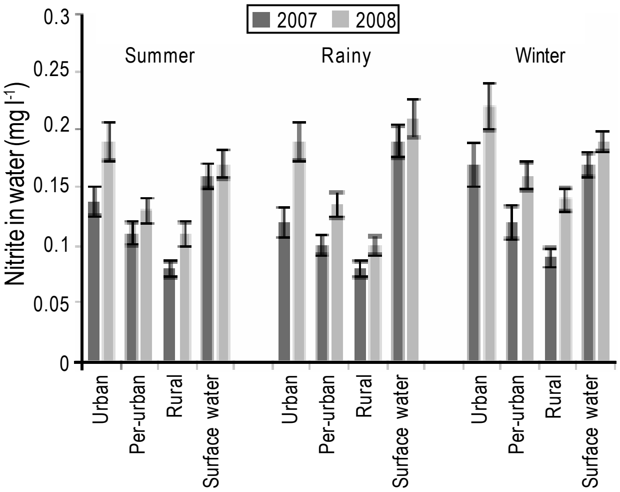

Fig. 1: Seasonal average of nitrite content in drinking water of urban, peri-urban and rural regions and in surface waters, during 2007-08

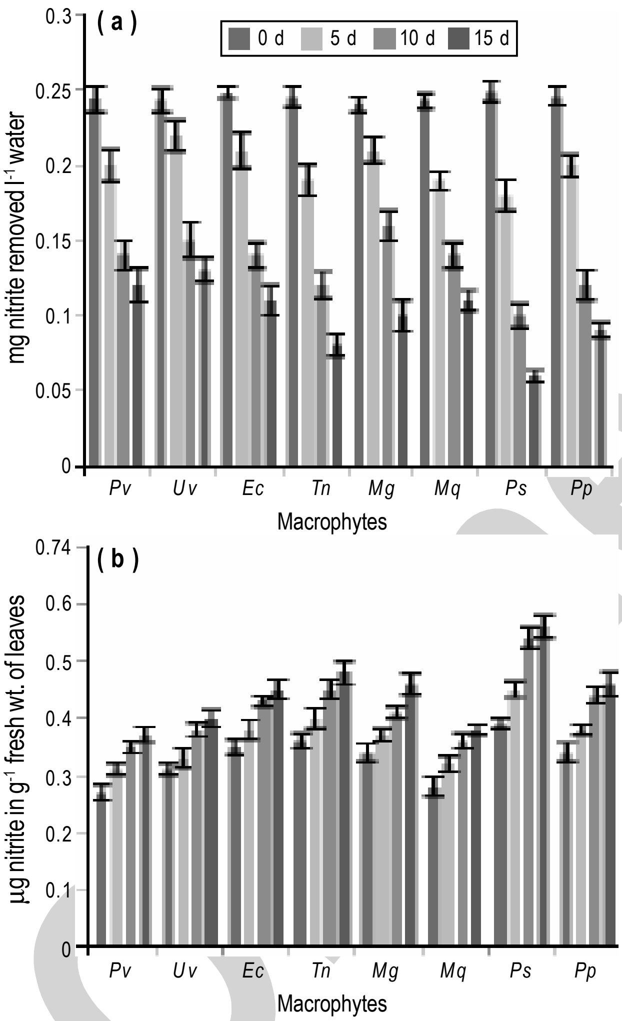

Fig. 2: (a) Nitrite removed from water at 5th, 10th and 15th d (b) Nitrite accumulated in the plants at 5th, 10th and 15th d. (Pv: *Peltandra virginica*, Uv: *Utricularia vulgaris*, Ec: *Eichhornia crassipes*, Tn: *Trapa natans*, Mg: *Mimulus glabratus*, Mq: *Marsilea quadrifolia*, Ps: *Pistia stratiotes* and Pp: *Polygonum persicaria*)

EPA, 1997). However, nitrite content was found higher in winter season as compared to summer and rainy seasons; it may be due to the continuous leaching and percolation of nitrogenous species after rainy season, to the ground water table (Singh and Singh, 2008).

But in case of surface water samples, significant high nitrite content was detected, that was more than 3 folds higher, over maximum acceptable concentration *i.e.* 0.06 mg l^{-1} (CCME, 2006). The mean of nitrite level during summer, rainy and winter season (2007-2008) varied from 0.11 to 0.23, 0.14 to 0.27 and 0.13 to 0.24 mg l^{-1}, respectively (Table 2). Data revealed that maximum elevation in nitrite content was during rainy season in surface water bodies. This may be due to the receiving of agricultural, human and animal waste in excess by the surface water bodies during the period, that cause exceedance of surface water quality standard (Miller *et al.,* 2007). In drinking water average nitrite level (0.22 mg l^{-1}) in urban region during course of winter, 2008 was found to be higher as compared to peri-urban and rural region and lowest (0.08 mg l^{-1}) in rural region in summer, 2007 (Fig. 1). During rainy season (2008), surface water bodies showed enhanced nitrite level (average value= 0.21 mg l^{-1}) that was much more above the recommended limit (Fig. 1).

The highest mean percent of nitrite removed at 15th d by selected aquatic macrophytes ranged from 45.26 to 72.28% (Fig. 2a). Initial concentration of nitrite employed to the pots containing aquatic plants was 0.25 mg l^{-1}, and at the end of experimental period it's concentration (mean ± S.D.) was found in the range of 0.06 to 0.13 (Fig. 2a). Concentration of nitrite in the plants tissues before plantation was recorded between 0.27 to 0.39 µg g^{-1}. Finally, total nitrite accumulated in the plants at 15th d was found in the range of 0.37 to 0.56 µg g^{-1} at 15th d (Fig. 2b). The maximum accumulation of nitrite (0.56 µg g^{-1}) was measured in the tissues of *Pistia stratiotes* at 15th d.

Concentrations of nitrite in water treated by eight species of aquatic macrophytes were significantly lowered when compared with initial value during the treatment tenure. Nitrite recovery rates of the eight aquatic plants were evaluated by comparing the amount of nitrite supplied and accumulated in whole plant tissues. Nitrite content of whole plant tissues for all eight species increased linearly, at 5th, 10th, and 15th d, with simultaneous decrease in nitrite level of the water (Fig. 3). Nitrite uptake was dependent on it's concentration in the medium. The nitrite recovery rate of all the selected aquatic plants was almost similar except *Pistia stratiotes*. However, the highest nitrite removal and accumulation was attained by *Pistia stratiotes* as compared to other aquatic plants used in the experiment. *Pistia stratiotes* had nitrite uptake efficiencies similar to *Thalia geniculata* f. *rheumoides* and *Rhyncospora colorata* (Polomski *et al.,* 2008).

Nitrite concentration was found greater in *Pistia stratiotes* than other aquatic plants which was similar to other studies (Irfan

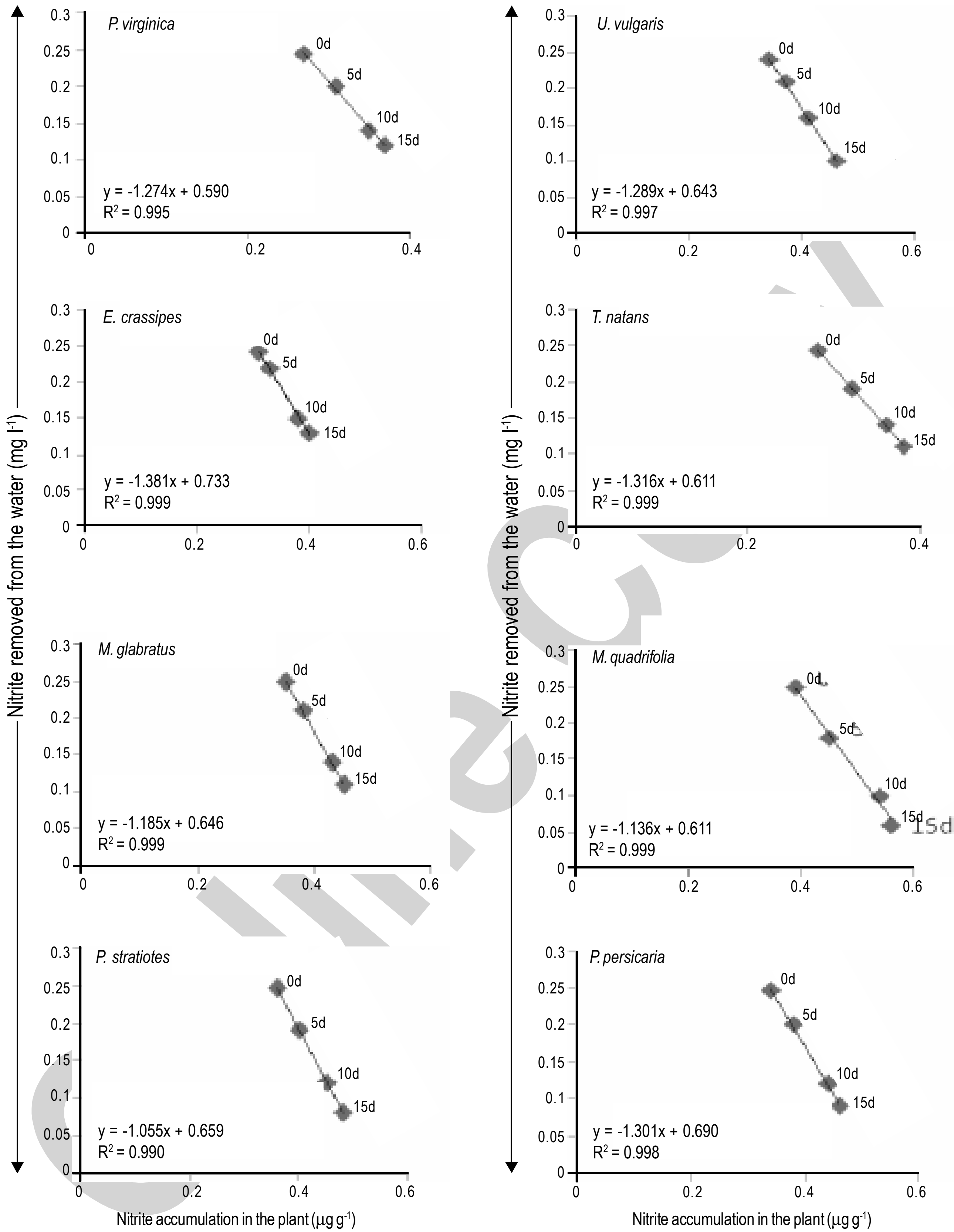

Fig. 3: Linear correlation between nitrite removed from the water and accumulated in the plants at 5th, 10th and 15th d

and Shardhendu, 2009). Analysis of data presented in this paper, revealed that *Pistia stratiotes* can be utilized as a hyperaccumulator of nitrite that can phytoremediate it upto 72.28%.

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