Journal of Environmental Biology
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November 2009, 30(6) 977-981 (2009)
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Dynamics of nitrogen in subtropical wetland and its uptake and storage by *Pistia stratiotes*

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(Received: June 12, 2008; Revised received: November 15, 2008; Accepted: November 21, 2008)

Abstract: *The paper describes the dynamics of nitrogen in different components (water, soil and plants) of Kabar wetland situated in Begusarai district of Bihar. Contents of nitrogen in the natural components were determined and were compared with the rate of uptake and accumulation under the experimental conditions. Physico-chemical characteristics of natural water and of test basins were quite similar. The trend of seasonal variation of* NO_3^- *-N in water and total N in soil and P. stratiotes tissue was almost similar but content of nitrogen differed significantly in the different components. The accumulation of nitrogen in the tissues of P. stratiotes was 5 to 15 fold higher than the concentration of nitrogen in the water and 2 to 3 fold higher than the nitrogen content measured in the soil. Maximum accumulation of nitrogen in P. stratiotes was 15.25 mg* g^{-1} *when the concentration of* NO_3^- *-N in water was 0.86 mg* l^{-1}*. Under experimental conditions six different nitrogen concentrations were supplied and determined the uptake and accumulation of nitrogen in P. stratiotes. Maximum uptake and accumulation was 82.87 g* m^{-2} *at the end of 60 days after starting the experiment but still the rate of accumulation was in rising trend. In another part of experiment no nitrogen was left in the basins of low concentrations (0.5 and 5 mg N* l^{-1}*) at the end of 60 days of experiment but at higher concentrations (50 and 65 mg N* l^{-1}*) significant amount of N was left in the test basin. The biomass enhancement was parallel with nitrogen supply till 15 mg N* l^{-1}*. This was opposite to the relationship between the nitrogen accumulation in the tissues and nitrogen supply in the experimental basins. Though, potassium was added as an essential growth nutrient but its accumulation was 95 g* m^{-2} *at 5 mg* l^{-1}*.*

Key words: *Nitrogen dynamics, Wetland, Uptake, Storage, P. stratiotes*
PDF of full length paper is available online

Introduction

The application of higher aquatic plants for nutrient removal from the waste water of various origin is one of the emerging aspects of environmental biotechnology that promises to clean up the environment effectively (up to 90% of inorganic nitrogen) (Reddy and DeBusk, 1985; Liikanen *et al*., 2004). The use of natural and constructed wetlands is a cost effective alternative for the wastewater treatment from primary to tertiary level. It is well-practiced technology in many subtropical countries (Hammer and Knight, 1994). Free water surface (FWS) and subsurface (SS) constructed wetlands planted with macrophytes are very effective in N removal *i.e.* up to 70 to 80% (Knight *et al*., 1993; Healy and Cowley, 2002). In constructed wetland biological and physical environment interact to provide a mechanical and biogeochemical filter capable of removing nitrogen from the wastewater. Nitrogen removal in constructed wetlands is accomplished primarily by physical settlement, denitrification and plant and microbial uptake. Plant uptake does not represent permanent removal unless plants are routinely harvested. Among floating leaved macrophytes, the free-floating species constitute a widely distributed group especially in subtropical region that flourish in mesotrophic and eutrophic lakes (Sculthorpe, 1967). In these systems nutrient availability in the water is sufficient to satisfy the nutrient requirements of the plants. The test organism *P. stratiotes* is a monotypic genus of family Araceae, which has a rosette like plant body with submerged fibrous root system and surface floating foliages. This aquatic angiosperm extract nutrients dissolved in water via root and subsequently translocate them to the upper leafy shoots. However, massive root system is the important factor in nitrogen uptake by *P. stratiotes.*

The science of understanding of wetland detoxification mechanisms is in its infancy where surplus and unutilized nutrients producing undesirable changes on aquatics and biota are not well studied. Some studies have been done on the trace element removal efficiency of wetland species (Sato and Kondo, 1981; Reddy *et al.,* 1989; Shardendu and Ambasht, 1991; Shardendu *et al*., 2003; Azaizeh *et al*., 2006) but comprehensive studies on *P. stratiotes* complementing natural field study with laboratory determinations are a few only. This study is very significant in Indian context where even major cities are not provided with sewage treatment system due to its high constructive and operative cost, the small, natural and constructed wetland can be utilized as alternative technology to sewage treatment systems.

In this paper we have -

(i) determined the physical characteristics like temperature, transparency and pH of natural and test basin water,
(ii) determined the mean monthly concentrations of nitrogen in water, soil and in *P. stratiotes* tissues,

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(iii) measured the uptake and storage of total nitrogen by *P.stratiotes* at weekly intervals for 60 days under different nitrogen loads (0.5, 5, 15, 25, 50 and 65 mg N l^{-1}),

(iv) determined the effect of different nitrogen concentrations on biomass of *P. stratiotes at* weekly intervals,

(v) measured the potassium accumulation in the tissues of *P. stratiotes* at weekly intervals for 60 days and

(vi) determined the amount of NO_3^--N left in the culture medium to correlate the remediation efficiency of monospecific culture of *P. stratiotes*.

Materials and Methods

Kabar wetland, a perennial fresh water system, situated between 86° 05´ E to 86° 09´ E longitude and 25° 30´ N to 25° 32´ latitude is located in Begusarai district of Bihar. *P. stratiotes* were brought and grown in the cemented culture tank. The area of the main culture tank having surface area of 7.29 m^2 was divided into four units measuring 1m^2 each by a cemented wall, but all four units were connected to each other at the bottom for the movement of water. The water level of the tank was maintained regularly with tap water and plants are exposed to solar radiations. The phytoremediation experiment was performed in another smaller cemented basin. Surface area of the each basin was 0.375 m^2 (0.75 m length, 0.5 m widths and 0.25 m depth). Each experimental basin was filled with 30 liters of tap water and kept independent of each other. The source of nitrogen salt was ammonium nitrate (NH_4^--NO_3). Six concentrations of nitrogen (0.5, 5, 15, 25, 50 and 65 mg N l^{-1}) were supplied that corresponds to 6, 57, 171, 286, 571 and 743 mg N m^{-2} day^{-1}. A fixed amount of phosphorus (3 mg l^{-1}) and potassium (25 mg l^{-1}) were added which is essential for the growth of the plant. Micronutrients were added at the rate equivalent to modified Hoagland solution (Reddy and DeBusk, 1984). The nutrient medium was replaced with freshly prepared nutrient solution at weekly intervals. Temperature and pH of culture water for each basin was recorded daily at 11 pm during the experiment period.

The initial fresh weight of *P. stratiotes* was kept at 160 g m^{-2} for first four basins (0.5, 5, 15 and 25 mg N l^{-1}) and for the last two basins (50 and 65 mg N l^{-1}) the fresh weight of *P. stratiotes* was 200 g m^{-2}. At the end of each week two individuals of the *P. stratiotes* was taken out randomly and their weight was measured. The fresh plants (roots + shoots) were kept in the oven at 80 °C for 48 hr and were oven dried. The oven-dried materials were mixed, grounded and sieved. The powders were utilized for the determination of nitrogen and potassium content.

Temperature of water of Kabar wetland was determined Monthly (during July 2001 to July 2002) by Celsius thermometer. Transparency was measured by secchi disc (Welch, 1952). pH of water was determined by systronics digital pH meter. NO_3^--N in water samples was determined by Phenyldisulphonic acid method. Total nitrogen in sediment was estimated by macro Kjeldahl method (Misra, 1968) whereas plant total nitrogen was estimated by Kjeldahl digestion method (Jackson, 1967). Potassium content of plant samples was estimated using Systronic type 121-flame photometer.

Statistical analysis including ANOVA and regression analysis has been done as prescribed in Snedecor and Cochran (1987).

Results and Discussion

Results of temperature, transparency and pH of surface water of Kabar wetland has been presented in Fig. 1. Data of physical characteristics of water indicated that they were influenced by atmospheric changes. Water temperature was higher in the summer season and was lower in the winter season. Contrary to

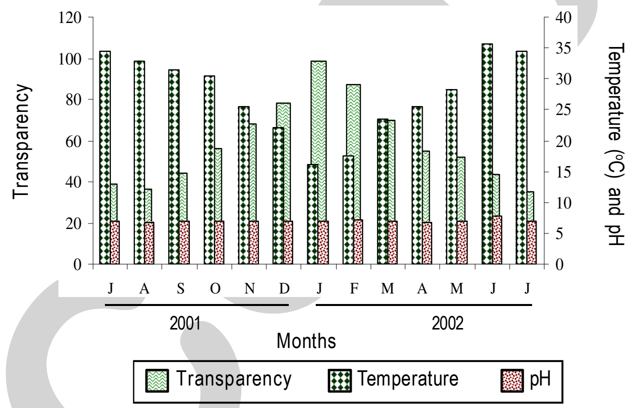

Fig. 1: Monthly variation in temperature, transparency and pH in surface water of Kabar wetland during the period of July 2001 to July 2002

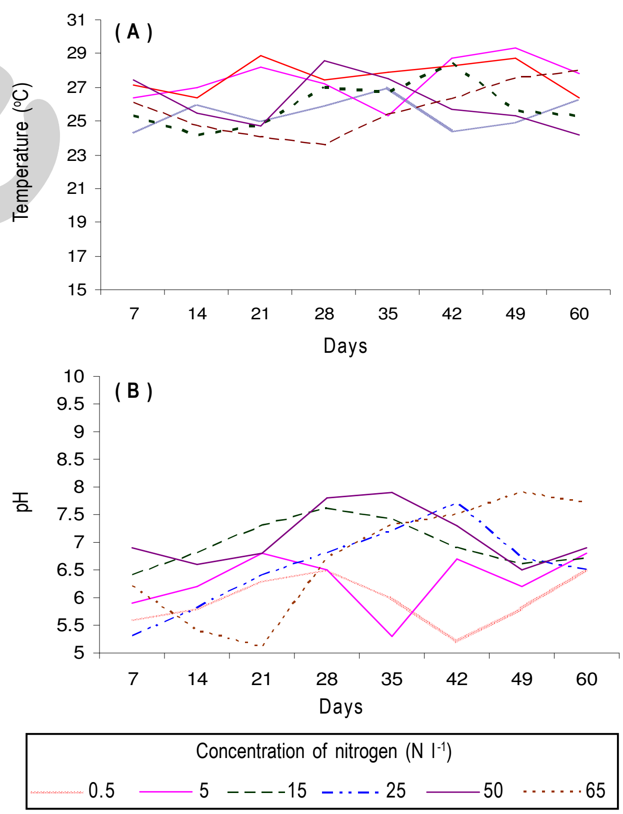

Fig. 2: Weekly variations in temperature (A) and pH (B) of water in the six test basins during 60 days of experimental period

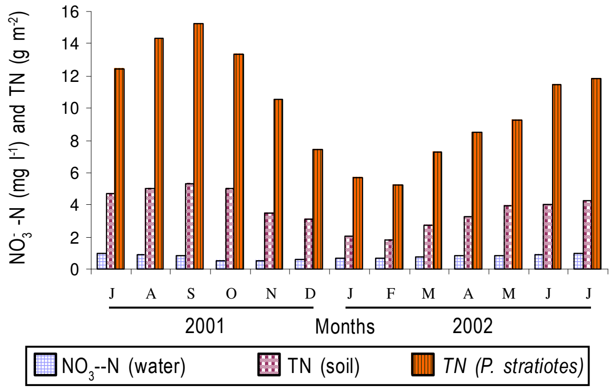

Fig. 3: Distribution of NO$_3^-$-N in water, total nitrogen (TN) in soil and *P. stratiotes*

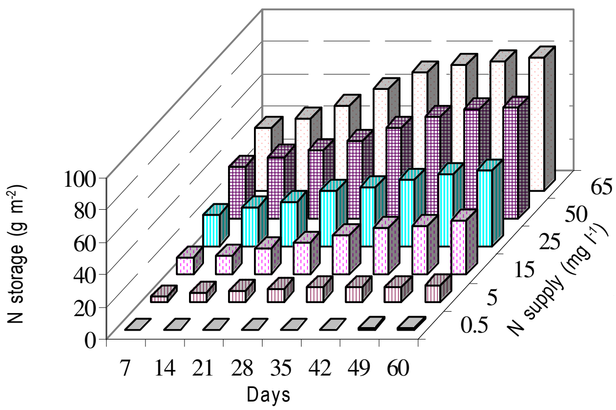

Fig. 4: Effect of N loading on N storage in the tissue of *P. stratiotes* during 60 days of experimental period

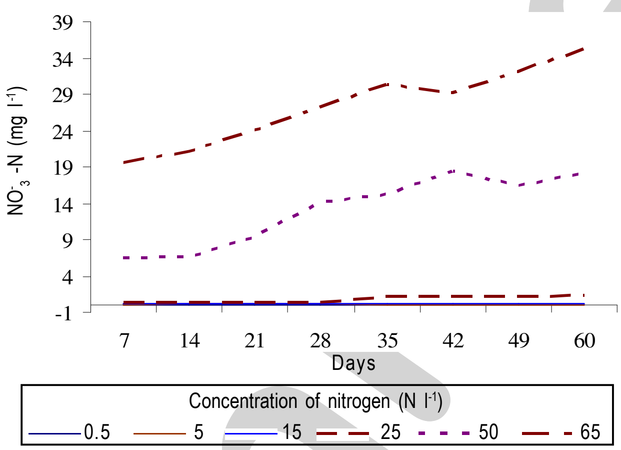

Fig. 5: Left over NO$_3^-$-N in the six test basins at the weekly intervals during 60 days of experimental period

seasonal variation in temperature, transparency was higher in winter season in comparison to summer and rainy months. Variations in temperature, pH and transparency in water of Kabar wetland were significant ($p<0.01$) (Table 1). A similar study has been done by Raja *et al.* (2008). In winter months suspended particles settle down due to less anthropogenic activities and allowed light to penetrate whereas rains brought neighbouring suspended particles to raise the turbidity. Kabar wetland water

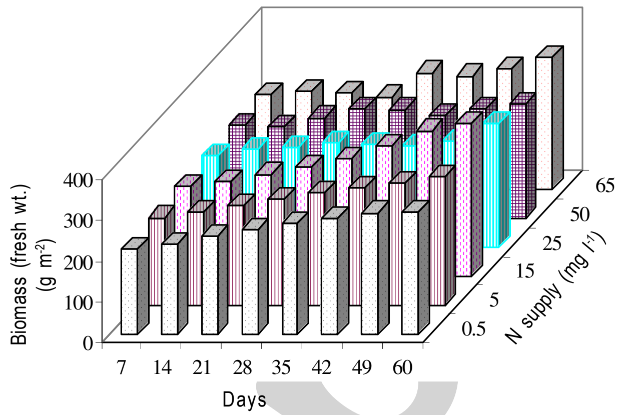

Fig. 6: Effect of N loading on biomass (fresh wt.) of *P. stratiotes* during 60 days of experimental period

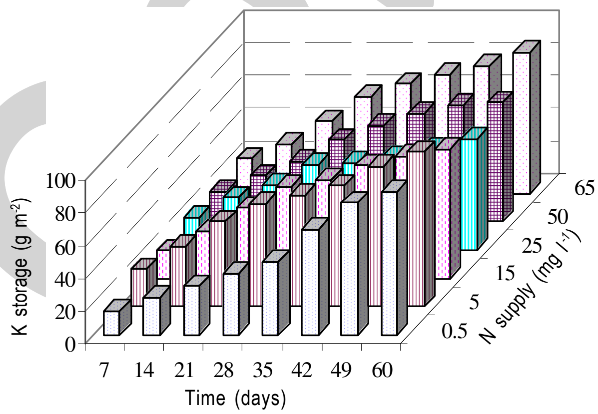

Fig. 7: Effect of N loading on potassium storage in the tissue of *P. stratiotes* during 60 days of experimental period.

was slightly acidic to neutral. The range of temperature and pH of test basin (0.375 m^2) water (Fig. 2 a,b) was similar to wetland water and had also the similar seasonal effects. Monthly variation of NO$_3^-$-N concentration in surface water, total nitrogen content in sediment and tissues of *P. stratiotes* has been presented in Fig. 3. There is similarity in the seasonal changes in nitrogen forms of components (water, soil, *P. stratiotes*) of Kabar wetland ecosystem, whereas variation in contents was measured. NO$_3^-$-N content in water was higher in rainy months followed by summer and lowest was noted in winter months (Fig. 3). Nitrogen accumulation in the tissues of *P. stratiotes* was 5 to 15 fold higher than in water and 2 to 3 fold higher than the N accumulation in soil (Fig. 3). Variation in forms of 'N' in three components (water, soil and *P. stratiotes*) between the months were significant ($p<0.01$) (Table 1). A similar study has been performed by Saravanakumar *et al.* (2008) and Saksena *et al.* (2008). Soil is the reservoir of nitrogen and when nitrogen is higher in the reservoir, these are released by microbial activity in water resulted to higher uptake and accumulation. In most natural ecosystems, there is competition between submerged and floating species for space and nutrients. *P. stratiotes* is cosmopolitan species and grows round the year in nutrient enriched water. Kabar wetland has big agricultural practices in its catchments and in rainy season nutrients are

Table - 1: ANOVA for temperature, pH, transparency and NO_3^- -N in water and total nitrogen (TN) in soil and *P. stratiotes* between months for Kabar wetland

Nutrient	Source of variation	d.f.*	F	p<
Temperature in water	Months	12	14.3	0.01
	Error	52		
pH in water	Months	12	1.9	0.01
	Error	52		
Transparency in water	Months	12	13.8	0.01
	Error	26		
NO_3^- -N in water	Months	12	13.4	0.01
	Error	52		
TN in soil	Months	12	17.5	0.01
	Error	26		
TN in *P. stratiotes*	Months	12	392	0.01
	Error	26		

*With df 12 and 52 (Temperature, pH, NO_3^- -N) and 12 and 26 for Transparency, TN in soil and *P. stratiotes*

Table - 2: Multiple regression (R^2) equations showing the relationship between nitrogen uptake (NU) by the *P. stratiotes* population with plant biomass (PB) and nitrogen left (NL) in the culture tank. The squared multiple correlation coefficient (R) and F statistics are also shown

N supply (mg l^{-1})	Regression equation	R^2	F	Significance
0.5	NU= 8.873-0.029 PB+46.0 NL	0.032*	0.34	p<0.05
5.0	NU= - 0.490+0.027 PB+10.4 NL	0.850	59.32	p<0.001
15	NU= - 4.627 +0.079 PB- 6.0 NL	0.950	201.04	p<0.001
25	NU= - 13.267+ 0.128 PB- 12.0 NL	0.925	129.97	p<0.001
50	NU= - 1.921+0.086 PB+ 1.7 NL	0.924	128.15	p<0.001
65	NU= - 20.216+ 0.005 PB + 3.0 NL	0.952	210.36	p<0.001

R^2 values are significant p< 0.001 except*< 0.05 with d.f. 7

carried with runoff, which promotes growth and accumulation of nitrogen in the tissues.

Availability of nitrogen in water and sediment plays an important role in establishment and growth of plants. Denitrification promotes nitrogen loss from sediment to the water layers and then to atmosphere through biochemical processes. Mobilization of nitrogen from sediment also occurs through submerged plants of the wetlands (Barko and Smart, 1981). Nitrogen in the *P.stratiotes* ranged from 5.22 to15.25 mg g^{-1}. Plants need nitrogen in early growth phase when the plant tissues are metabolically active. In *P. stratiotes* maximum nitrogen accumulation was in the rainy season. This is because the rainy season was growing phase of *P. stratiotes* and water nitrogen was higher in the rainy season.

Figure 4 shows the effect of different nitrogen loads on its uptake and storage by *P. stratiotes* tissues at different time intervals. The uptake is concentration and time dependent. There were six concentrations of nitrogen loads. The result of uptake and storage shows distinct trends. At initial two loads (0.5 and 5.0 mg N l^{-1}) there was a regular increase in the uptake of nitrogen. Total accumulation of nitrogen was 1.54 g N m^{-2} at 0.5 mg N l^{-1} concentration and 10.21 g m^{-2} at 5.0 mg N l^{-1} concentration after 60 days. At middle level nitrogen supply (15 and 25 mg N l^{-1}) there was further increase in accumulation of nitrogen in the tissues that later increased to maximum (82.8 g m^{-2}) accumulation at the highest nitrogen load (65 mg N l^{-1}).

Relationship between nitrogen uptake in *P. stratiotes* tissue with nitrogen concentration left in culture tank and plant biomass at six supplied concentrations were examined by multiple regression equation analysis (Table 2). Nitrogen supply rate has positive effect on the increase in plant biomass except at concentration of 65 mg N l^{-1} (p<0.001). This is because in early growth phase, more nitrogen is needed. There was no addition in biomass even at higher supply of nitrogen. Plant nitrogen uptake was strongly related to concentration of nitrogen in culture tank, this is evidenced by the amount of nitrogen left in the culture tank. At the initial concentration, all nitrogen is taken up and only at higher concentration some amount of nitrogen is left in the culture medium (Table 2).

NO_3^- -N left in the experimental basins has been presented at the weekly intervals (Fig. 5). The result clearly shows the complete utilization of NO_3^- -N at two lower concentrations. There was negligible concentration of NO_3^- -N left in the two basins of 15 and 25 mg N l^{-1}. At higher range of nitrogen supply the significant amount of NO_3^- -N was left in the culture tank as there was no further uptake and utilization of nitrate nitrogen by the *P. stratiotes* population.

NO_3^- -N uptake by *P.stratiotes* was dependent on the nitrogen in the medium and the time period allowed to grow the plant in the medium. The maximum accumulation of nitrogen (82.8 g m^{-2}) was measured at 65 mg N l^{-1} supply at the end of 60 days of experiment. This result is supported by the result of nutrient left in the medium as uptake and storage rate was almost parallel to the nutrient left in the medium for at least two lower concentrations but not at two higher concentrations. This result is comparable to Reddy *et al*. (1989) in which he has shown that 80 g m^{-2} of nitrogen was accumulated on the supply rate of 50 mg N l^{-1}. In a study by Cedergreen and Madsen (2002) *Lemna* plant showed high tissue nitrogen (1.95 ± 0.12 m mol N g^{-1} dry wt) and population growth under high N supply.

The biomass enhancement was not parallel to N supply rates (Fig. 6) unlike nitrogen storage in the tissues. The biomass was maximum (507 g m^{-2}) at 15 mg N l^{-1} supply. After that there was no further increase in biomass even at higher supply of nitrogen to the culture medium. This indicates that a supply of nitrogen supported enhancement of biomass till maturity level of the species while further increase of nitrogen supply enhanced the nitrogen storage in plant tissues. This means nitrogen is not the only requirement for plant growth and biomass yield.

In water hyacinth, which has similar habit to water lettuce there was an increase in biomass up to 84 mg N l^{-1} (Sato and Kondo, 1981). The present experiment showed maximum biomass of *P. stratiotes* at 15 mg N l^{-1} whereas Sato and Kondo (1981)

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observed maximum fresh weight biomass of water hyacinth at 28 mg N l^{-1}. This might be due to smaller habitat of *P. stratiotes* in comparison to water hyacinth, which require more nutrients to reach maximum biomass. In high nutrient experimental basins high concentrations of N may have damaging effects on the plant population.

Barko (1982) concluded that even for rooted submerged species like *Hydrilla verticillata*, there was limited mobilization of potassium from the soil and even interstitial water is not available to plants in aquatic systems so only source of potassium to free floating species like *P. stratiotes* is open water. We supplied six different doses of potassium to all culture tank of nitrogen as an essential element and measured the storage of potassium in plant tissue after its utilization for growth (Fig. 7). All concentrations had shown remarkable potassium storage but maximum accumulation was 95 g m^{-2} at 5.0 mg N l^{-1} at 60 days of growth.

By the analysis of data presented in this paper, we can conclude that *P. stratiotes* can be utilized in natural or constructed wetland as a hyperaccumulator of 'N' that can phytoremediate the nitrogen up to 82%.

Acknowledgments

We are thankful to University Grants Commission, Kolkatta (Minor Project No. PSB 046106-07) for financial support.

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