Chapter 7. Comparative study on remediation of nitrate, nitrite, ammonium and phosphate from water using *Pistia stratiotes* and *Hydrilla verticillata*

7.1. Introduction

Agriculture, fishing, industries and municipal drainage have become an increasing concern for point and non-point pollution sources of nitrogen and phosphorous in many countries, especially developing countries (Wu, 2011; Souza *et al.,* 2013). Excessive release of nitrogen and phosphorus may enter freshwater ecosystems such as lakes, rivers, leads to undesirable eutrophication of surface waters and different diseases among animals and humans due to consumption of water contaminated with nitrate, nitrite, ammonium, which is the most widespread problem to water environment quality around the world (Rawat *et al*., 2012; Rezania *et al*., 2015). Nitrite has been shown to cause methaemoglobinaemia in animals. Humans appear to be more sensitive to nitrite-induced methaemoglobin formation. Nitrogenous ions especially NO^-_2 can combine with organic pollutants to produce cancer causing nitrosyls which is a big health hazard (Kim Shapiro et al. 2006; Duplay et al. 2013).

The removal of reactive nitrogenous species (Nitrate, Nitrite and Ammonium) from drinking water is a challenging problem which demands high cost technologies (Abou-Elela, 2013). On the other hand phosphorus also needs to be removed from waste water entering the fresh water sources. Plants are considered to play an important role in nutrient removal from constructed wetlands (Vymazal and Kropfelova, 2011). Macrophytes are considered to be the main biological component of constructed wetlands that directly take up nitrogen and phosphorous, which are key nutrients in the life cycles of wetland plants for the growth and reproduction (Xu et al., 2009). Research indicates that different macrophytes have different nutrient preferences. Although most wetland plants prefer absorbing NH4–N in anoxic wetland substrates some wetland plants prefer absorbing NO3–N (Zhang et al., 2009). Mostly the macrophytes have been employed to remediate the wastewater. The study was undertaken to remediate nitrate, nitrite, ammonium and phosphate from drinking water under simulated conditions.

7.2. Material and Methods

7.2.1. Collection of plants and experimental design

The experimental plants *Pistia stratiotes* and *Hydrilla Verticellata* were collected from the Gomti River and local ponds of Lucknow city. The plants were washed with running tap water to remove any attached particles. They were acclimatized for 15 days in Hoagland solution (Hoagland and Arnon, 1950) in constructed cemented ponds. After 15 days of acclimatization, fully grown and healthy plants were transferred to the treatment tubs. Cemented tubs filled with 20 liters of simulated water were employed to evaluate the phytoremediation potential of *Pistia stratiotes* and *Hydrilla verticillata* for the removal of inorganic pollutants. 100 grams of each plant was put into 4 tubs for their monocultures, covering 80% of surface area of water in the tub. A control treatment without any plant was also setup. Simulated water was having the following concentration of Nitrate: 40.00 mg/l; Nitrite: 10.00 mg/l; Ammonium: 10.00 mg/l and Phosphate: 5 mg/l.

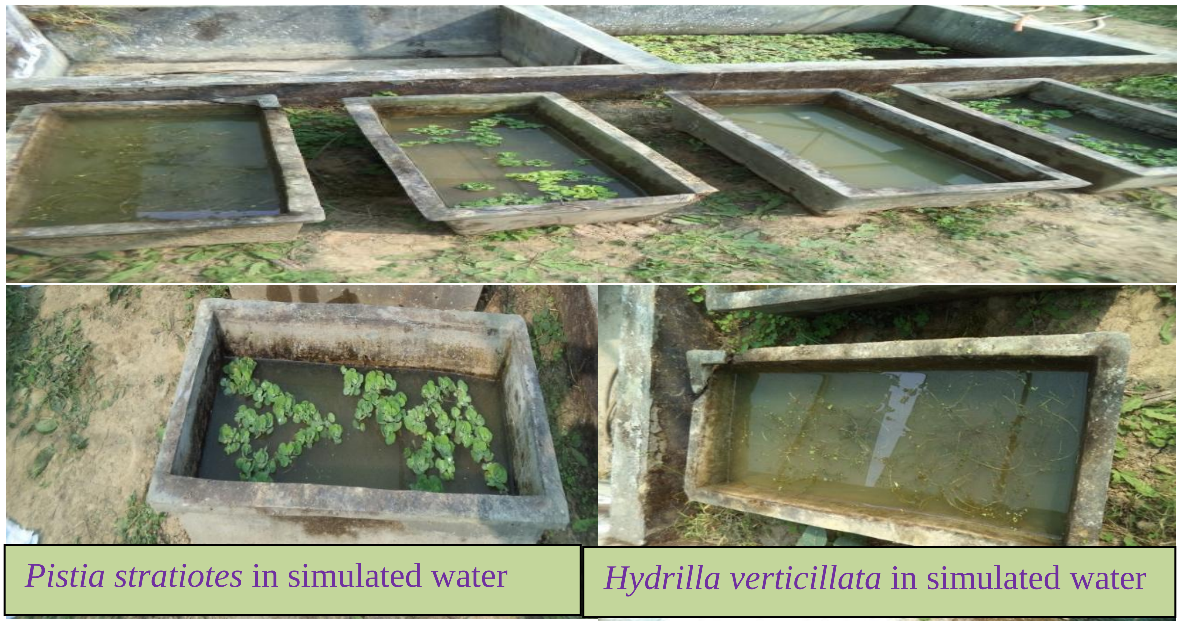

Plate7.1. Showing the experiment conducted in the net house of DES, to evaluate the efficiency of selected plants for removal of nitrate, nitrite, ammonium and phosphate.

7.2.2. Water sampling and analysis

Water samples from each treatment were collected on 5th, 10th, 15th, 20th and 25th day from the start of the experiment from each treatment including the control. On each sampling day, randomly plants were selected from each treatment and rinsed with deionized water and divided into root and shoot components for further analysis. The changes in the temperature, pH, conductivity of water and the removal of nitrate, nitrite, ammonium and phosphate from the simulated water were observed during the study period. The details of the protocols are given in chapter 3.

7.2.3. Plant sampling and biomass analysis

Plant samples from each treatment were collected on 5th, 10th, 15th, 20th and 25th day from the start of the experiment. On each sampling day, randomly plants were selected from each treatment and rinsed with deionized water and divided into root and shoot components for biomass analysis. The biomass of plants was expressed on dry weight (DW) basis. The dry weight of the samples was recorded after drying it in hot air oven at 60º C for 48 hr. until the concordant values were obtained.

7.3. Results

7.3.1. Changes in the physicochemical characteristics of nutrient simulated water due to treatment with *Pistia stratiotes* and *Hydrilla verticillata*

In the present study the changes in the temperature, pH and electrical conductivity (EC), nitrate, nitrite ammonium and phosphate of nutrient simulated water were observed due to treatment with *Pistia stratiotes* and *Hydrilla verticillata* are presented in Tables 6.1 and 6.2. The marked variations were observed in temperature, pH and electrical conductivity of water treated with *Pistia stratiotes* and *Hydrilla verticillata.* The temperature in water treated with *P. stratiotes* decreased with time reaching minimum of 23.33 º C from a maximum of 24.66º C at the beginning of experiment. In case of water treated with *H. verticillata,* temperature increased from 24.6 º C to 25.43 º C at the end of the experiment. The pH of water treated in both case decreased with time and reached 7.21 and 7.19 for water treated with *P. stratiotes* and *H. verticillata,* respectively. A sharp decrease in EC (µs/cm) for both treatments was observed. EC decreased from 2.06 (µs/cm) to 0.17 (µs/cm) in water treated with *P. stratiotes* and decreased from 2.02 (µs/cm) to 0.32 (µs/cm) in water treated with *H. verticillata.*

Table 7.1. Changes in Temperature, pH and Electrical Conductivity (EC) of water due to *Pistia stratiotes* and *Hydrilla verticillata* (n=6±SD)

Parameter	Days	*Pistia stratiotes*	*Hydrilla verticillata*	Control
Temperature (°C)	Day5	24.66 ± 0.05	24.6±0.05	24.64± 0.01
	Day10	23.66±0.01	25.06±0.15	24.43± 0.01
	Day15	22.33±0.05	25.16±0.05	24.33± 0.02
	Day20	23.1±0.03	25.23±0.52	23.21± 0.03
	Day25	23.33±0.01	25.43±0.02	23.1± 0.01
pH	Day 5	7.56±0.05	7.57±0.01	7.55± 0.02
	Day10	6.8±0.01	7.63±0.03	7.49± 0.03
	Day15	6.67±0.02	7.31±0.09	7.32± 0.02
	Day20	7.13±0.02	7.46±0.01	7.22± 0.01
	Day25	7.21±0.01	7.19±0.02	7.17± 0.05
EC(µs/cm)	Day5	2.06±0.01	2.02±0.01	2.02± 0.05
	Day10	0.66±0.015	1.06±0.03	2.00± 0.05
	Day15	0.32±0.03	0.71±0.026	1.96± 0.05
	Day20	0.26±0.015	0.41±0.05	1.87± 0.05
	Day25	0.17±0.03	0.32±0.01	1.65± 0.05

An overall decrease in the four inorganic pollutants i.e., nitrate, nitrite ammonium and phosphate was observed with water treated with *P. stratiotes* and *H. verticillata.* Nitrate content in water decreased from (40 mg/l) to (3.7 mg/l) and (7.83 mg/l) treated with *P. stratiotes* and *H. verticillata*, respectively at the 25th day of the experiment. Nitrite content in water treated with *P. stratiotes* decreased from (10mg/l) to (3.28 mg/l) and (2.23 mg/l) in case of treatment with *H. verticillata.* For ammonium the final content in water at the end of the experiment was (1.98 mg/l) in case of *P. stratiotes* and (1.23 mg/l) for *H. verticillata.* The phosphate content in water reached a final concentration of (0.69 mg/l) from (5 mg/l) treated with *P. stratiotes* and (0.44 mg/l) in *H. verticillata* treated water. From the control treatment the changes in concentration of selected pollutants was not significant.

Table 7.2. Changes in nitrate, nitrite , ammonium and phosphate during water treatment with *Pistia stratiotes* and *Hydrilla verticillata* (n=6±SD)

Parameter	Days	*Pistia stratiotes*	*Hydrilla verticillata*	Control
Nitrate (mg/l)	Day 5	31.01±0.14	35.79±0.30	39.45±0.02
	Day10	23.96±0.16	28.66±0.40	39.1±0.01
	Day15	13.41±0.19	17.52±0.4	38.65±0.02
	Day20	7.08±0.15	14.88±0.12	37.93±0.03
	Day 25	3.7±0.18	7.83±0.38	37.65±0.02
Nitrite (mg/l)	Day 5	8.52±0.05	10.7±0.3	9.87±0.01
	Day10	7.29±0.14	8.6±0.4	9.88±0.03
	Day15	6.33±0.38	7.52±0.4	9.72±0.02
	Day20	4.59±0.43	4.8±0.1	9.7±0.01
	Day 25	3.28±0.34	2.23±0.3	9.67±0.03
Ammonium(mg/l)	Day 5	8.35±0.8	8.62±0.5	9.87±0.02
	Day10	7.14±0.3	7.54±0.4	9.66±0.03
	Day15	6.72±0.05	4.35±0.5	9.56±0.03
	Day20	2.45±0.07	3.35±0.2	9.45±0.02
	Day 25	1.98±0.2	1.23±0.3	9.23±0.05
Phosphate(mg/l)	Day 5	4.41±0.8	4.19±0.2	4.91±0.01
	Day10	3.63±1.8	2.82±0.6	4.9±0.02
	Day15	2.18±0.2	1.45±1.1	4.88±0.03
	Day20	1.27±0.2	1.04±0.6	4.86±0.03
	Day 25	0.69±0.5	0.44±0.4	4.87±0.01

7.3.2. Percentage removal of nitrate, nitrite, ammonium and phosphate from water treated with *Pistia stratiotes* and *Hydrilla verticillata*

The percent removal of studied inorganic pollutants by *Pistia stratiotes* and *Hydrilla verticillata* has been presented in table 6.3. *P. stratiotes* was more efficient for the removal of nitrate and phosphate. 90.83% of nitrate and 91.07% of phosphate was removed from water treated with *P. stratiotes*. However, nitrite and ammonium was maximum removed by submerged macrophyte *H. verticillata.* The removal percentage for nitrite and ammonium by *Hydrilla verticillata* was 77.48% and 81.81%, respectively. The removal of pollutants from the control treatment was not significant.

Table 7.3. Percent removal of nitrate, nitrite, ammonium and phosphate in water during application of *Pistia stratiotes* and *Hydrilla verticillata*

	Parameter	Day1 (mg/l)	Day 25 (mg/l)	% Remediation
P. stratiotes	NO_3^-	40.01±0.14	3.790±0.18	90.83
	NO_2^-	10.02 ± 0.05	3.28 ± 0.34	70.06
	NH_4^+	10.05± 0.8	1.98±0.2	80.88
	PO_4^-	5.01±0.8	0.69±0.5	93.05
H. verticillata	NO_3^-	40.01 ±0.14	7.82±0.053	81.08
	NO_2^-	10.02 ±0.03	2.23± 0.3	77.48
	NH_4^+	10.02 ±0.5	1.23 ±0.3	81.81
	PO_4^-	5.01 ±0.2	0.46± 0.4	91.07
Control	NO_3^-	40.01 ±0.14	37.65±0.02	5.54
	NO_2^-	10.02 ±0.03	9.67±0.03	3.21
	NH_4^+	10.02 ±0.5	9.23±0.02	7.68
	PO_4^-	5.01 ±0.2	4.87±0.01	2.04

7.3.3. Changes and correlation of biomass accumulation with nutrient removal by *Pistia stratiotes* and *Hydrilla verticillata*.

In the present study, the changes in the biomass accumulation expressed as dry weight (DW/plant) of *Pistia stratiotes* and *Hydrilla verticillata* are given in table 6.4. The correlation of biomass accumulation with nutrient removal by the plants has been presented in figures 6.1 (A-H). The biomass of both plants increased with the removal and concurrent accumulation of inorganic nutrients from the water. Increase in root and shoot biomass of *Pistia stratiotes* and *Hydrilla verticillata* was observed during the treatment phase. *P. stratiotes* accumulated greater biomass both in root and shoot parts than *H. verticillata* at the end of the experiment.

Table 7.4. Changes in biomass of *Pistia stratiotes* and *Hydrilla verticillata* during the treatment phase (n=6±SD)

Parameter	Days	*P. stratiotes*		*H. verticillata*	
		Shoot	Root	Shoot	Root
Biomass (mg/plant DW)	Day5	0.47±0.04	0.223±0.04	0.75±0.03	0.19±0.04
	Day10	0.9±0.06	0.342±0.04	1.17±0.01	0.27±0.06
	Day15	2.1±0.06	0.481±0.05	2.71±0.04	0.57±0.04
	Day20	6.85±0.04	0.831±0.01	3.15±0.02	1.45±0.02
	Day25	9.55±0.04	3.24±0.06	5.57±0.03	2.27±0.02

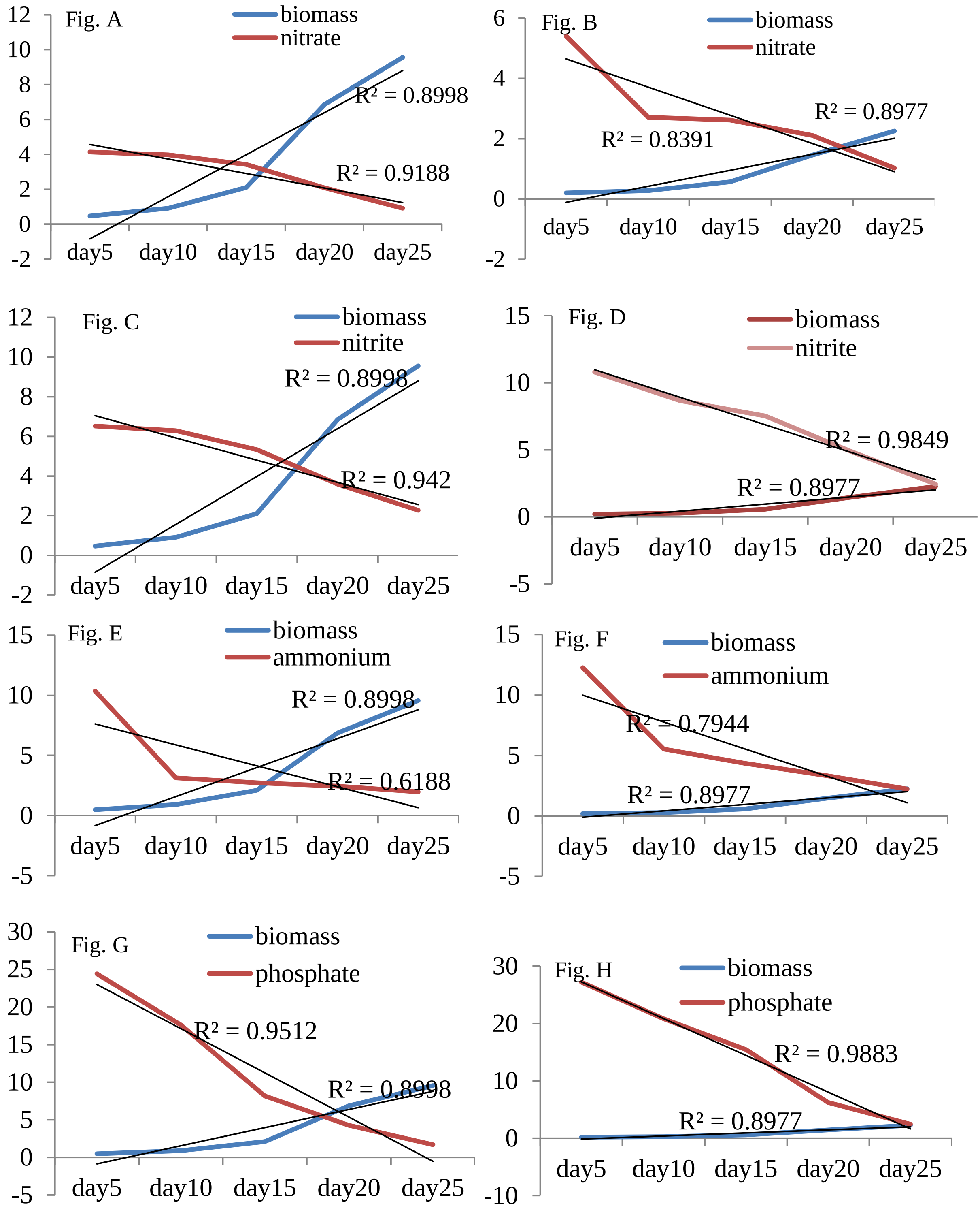

Figure7.1. (A-H). Fig. A and B: Correlations of biomass accumulation with nitrate removal by *P. stratiotes* and *H. verticillata*, respectively. **Fig. C and D:** Correlation of biomass accumulation with nitrite removal by *P. stratiotes* and *H. verticillata*, respectively. **Fig. E and F:** Correlation of biomass accumulation with ammonium removal by *P. stratiotes* and *H. verticillata*, respectively. **Fig. G and H:** Correlation of biomass accumulation with Phosphate removal by *P. stratiotes* and *H. verticillata*, respectively.

Nitrate removal was observed to be positively correlated with plant biomass accumulation for *P. stratiotes* and *H. verticillata*. The value of R^2 was found to be (0.9188) for *P. stratiotes*, while as for *H. verticillata* it was (0.8391).Also nitrite removal was positively correlated with plant biomass accumulation. The value of R^2 was found to be (0.942) for *P. stratiotes*, while as for *H. verticillata* it was (0.9849).Ammonium removal was observed to be positively correlated with plant biomass accumulation for *P. stratiotes* and *H. verticillata*. The value of R^2 for *P. stratiotes* was found to be (0.6188) for *P. stratiotes*, while as for *H. verticillata* it was (0.7944). Phosphate removal was found to be positively correlated with plant biomass accumulation for *P. stratiotes* and *H. verticillata*. The value of R^2 for *P. stratiotes* was found to be (0.9512) for *P. stratiotes*, while as for *H. verticillata* it was (0.9883). Therefore, the removal of pollutants and their accumulation within the plants was significantly correlated with the increase in the biomass of both *P. stratiotes* and *H. verticillata.*

7.4. Discussion

The current study was undertaken to evaluate the comparative potential of two different aquatic macrophytes *Pistia stratiotes* and *Hydrilla verticillata* for the removal of inorganic pollutants from the simulated water under NET house conditions of Department. The changes in temperature, pH, EC, nitrate, nitrite, ammonium and phosphate were observed during the 25days study period. The removal efficiency for pollutants and biomass accumulation by the plants were evaluated. The fluctuations in temperature were evident during the study period in both treatments. This may be due to minimal temperature stratification within the system (Tripathi *et al*; 2013).

The pH in water determines the extent of acidity or basicity in water. Our study pertaining to the changes in pH reveals that pH of simulated water after treatment with selected plants was reduced. Reduction in pH may be due to the plant uptake of significant amount of soluble nutrients by rhizosphere or by acidifying the rhizosphere, via excreting H^+ in exchange for cation and ending organic acids and carbon dioxide (Gikas and Tsihrintzis, 2012). The changes in EC reveal that EC of water was significantly reduced during the treatment. This is expected due the removal of dissolved nitrogen and phosphorus salts (Souza *et al*, 2013).

The inorganic nutrients in water were nitrate, nitrite, ammonium and phosphate degrades the quality of water and depletes the dissolved oxygen present in water. 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, 2011). 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 its harm to the animal and human consumers of the water which cannot assimilate nitrite like bacteria and plants (Alonso and Camargo, 2009). Our study pertaining to the changes in the inorganic nutrients reveals that there is a significant decrease in the concentration of inorganic pollutants from the treatment system. Similar results pertaining to the current study were reported by Rawat *et al*., (2012). The potential rate of nutrient uptake by plant is limited by its net productivity (growth rate) and the concentration of nutrients in the plant tissues (Vymazal, 2007). Decrease in the concentration of nitrate could be due to the increased plant uptake rather than microbial denitrification. Kadlec *et al.*, (2009) and Bindu *et al.*, (2008) reported the removal of nitrate might be due to uptake by the plant roots. Denitrification is believed to be the major pathway for ammonia removal in the constructed wetland (Rai et al., 2013).

The mechanism of the phosphorus removal could be reported to occur by sorption, complexation, precipitation and assimilation into microbial and plant biomass. The better removal of phosphorus in the planted system in the presented study could be attributed to plant uptake and microbial assimilation. The main role of wetland plants with respect to removal of phosphate are direct uptake and provision of suitable conditions for microorganisms that use phosphorus as a nutrient (Mbuligwe; 2004). Nutrient removals by plants accounted for 15–80% N and 24–80%P (Greenway and Woolley, 2001). Different species, nutrient loading rates and climates accounted for different nutrient uptake by plants in various studies (Vymazal, 2007).

Growth characteristics like biomass were also observed during the study period. The changes in growth parameters reveal that the biomass of both the plants increased continuously throughout the experiment. Increase in biomass could be due the uptake of pollutant and all other nutrient in water by the plant. Nutrients removal coupled with biomass accumulation and plant productivity varied widely with the species. This variation resulted partly from relative differences in climate, intrinsic species and possible ecotype as well as growth characteristics (Brisson and Chazarenc, 2009).

7.5. Conclusions

Removal of inorganic pollutants from water can help reduce eutrophication of water bodies and also improve the quality of water thereby preventing human and animal life from different health aliments. The studied plants were able to remove nitrate, nitrite, ammonium and inorganic phosphate from simulated water. Both plants showed growth and development by increasing their biomass from day 5 until day 25. Moreover, plants were able to survive in high concentration of nutrients and effectively removed all the four nutrients/pollutants from water. Analysis of data revealed that *Pistia stratiotes* can be utilized as a hyper-accumulator of nitrate and phosphate, while as *Hydrilla verticellata* could serve the purpose of stabilizing nitrite and ammonium in contaminated waters.