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The Effects of two Aquatic Floating Macrophytes (*Lemna* and *Azolla*) as Biofilters of Nitrogen and Phosphate in Fish Ponds

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Abstract

Effects of different fish–edible floating aquatic macrophytes on water quality in aquaculture ponds through biofiltration of organic pollutants were studied for a period of four months from July to November 2001. *Lemna* sp. and *Azolla* sp. were used in treatment 1 and treatment 2, respectively. And treatment 3 remained as control. The stocking density of Rohu (*Labeo rohita*), Catla (*Catla catla*), Mrigal (*Cirrhina mrigala*), Thai sharpunti (*Puntius gonionotus*) and feeding regimes (rice bran and mustard oil) and fertilization rate were the same in all treatments. The values of dissolved oxygen, temperature, nitrate-nitrogen, phosphate-phosphorus, chlorophyll-a, alkalinity and plankton abundance varied among the treatments. It was observed that the lowest concentration of PO_4-P (0.01 mg/L) and chlorophyll-a (26.99 µg/L) were found in treatment 1 and treatment 2 followed by treatment 3. This was probably due to the utilization of nutrients by the experimental aquatic macrophytes. Lowest concentration of NO_3-N was found in treatment 2 during July, but the fortnightly average values of NO_3-N were mostly found lower in treatment 1 followed by treatment 2 and treatment 3. The phytoplankton was composed of Euglenophyceae, Cyanophyceae, Bacillariophyceae and Chlorophyceae. Throughout the experimental period the dominant genus were *Euglena*, *Anabeana* and *Microcystis*. These macrophytes also appeared as a nutrient filter for absorption of nitrogen and phosphorus and removed the excessive amount of nutrients from the water body in treatments 1 and 2 and the aquatic environment remained in sustainable conditions.

Key words: Duckweed, *Lemna* sp., *Azolla* sp., Water quality, nutrient, fish pond.

Introduction

A very serious problem in aquaculture arising from modern technology is pollution. One of the widespread types of pollution is the addition of large quantities of inorganic nutrients, particularly nitrogen and phosphorus to freshwater lakes and ponds. Now days with gradual increase of aquaculture, especially intensive and semi-intensive aquaculture, high doses of fertilizers, feed etc. are used in the confined waters of ponds and lakes year after year to increase the fish production. Regular fertilization in fishponds accumulates nutrients in pond mud. The nutrients budget in intensive and semi-intensive fish culture ponds reveals that large quantity of these elements is not utilized by fish and often accumulated in pond (Boyd, 1990).

As a result, most of the fish ponds become hypernutrified and the fish farmers are experiencing many new unexpected problems such as environmental degradation with noxious algal blooms, ammonia toxicity, different fish diseases etc. that ultimately hampered or reduced fish production. Toxic and noxious algal blooms have a serious negative effect causing severe economic losses to aquaculture/fisheries and having major environmental and human health impacts (Anderson, 1989; Smayda, 1992)

Nutrients in feed are not directly consumed by culture species but also leached into the culture system. Unconsumed particles were decomposed by heterotrophic activity, affecting all levels of nutrient availability and organism growth in such system (Moore, 1986). These nutrients, which are otherwise wasted and created problems, can be used to grow aquatic macrophytes, which will help to maintain desirable water quality for fish culture; and on the other hand, they will also serve as fish food.

Aquatic macrophytes play a significant role in maintaining water quality. Their presence may enhance water quality due to their ability to absorb excessive loads of nutrients. There has been a great deal of interest in the use of floating aquatic macrophytes to reduce the concentration of noxious phytoplanktons in the effluent from stabilization ponds and to remove nitrogen and phosphorous from the water (Steward, 1970). Aquatic floating macrophytes take up inorganic nutrients mainly by the roots, although uptake through the leaves may also be significant. Members of free floating duckweeds (Lemnaceae), namely *Lemna minor*, *L. gibba*, *Wolffia arrhiza*, and *Azolla pinnata* have shown potential usefulness in the treatment of eutrophicated water system (Sutton and Ornes, 1975).

In recent years, the use of floating macrophytes as a dietary supplement in polyculture of Indian and Chinese carps in freshwater ponds has been increased in Bangladesh.

Therefore, the aim of this study is to investigate the effects of different aquatic floating macrophytes in maintaining water quality in fishponds through biofiltration of organic pollutants.

Materials and Methods

The experiment was carried out for a period of four months from July to November 2001 in 9 similar sized experimental ponds (each of 80 m^2) in the field laboratory of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. All the ponds were free from water hyacinth and other undesirable species. Ponds were completely well exposed to sunlight and without inlet and outlet. Three treatments, each with triplicates were used in the study. Treatment 1 was designed with Rohu (*Labeo rohita*), Catla (*Catla catla*), Mrigal (*Cirrhina mrigala*), Thai sharpunti (*Puntius gonionotus*) and aquatic macrophyte *Lemna* sp., Treatment 2 with same fish species and aquatic macrophyte *Azolla* sp. and Treatment 3 was designed with similar fish species and without any aquatic macrophyte. The stocking densities were similar in all treatments and densities were of the above three native carps: *Labeo rohita*, *Cirrhina mrigala*, *Catla catla* and one exotic carp, Thai sharpunti (*Puntius gonionotus*) were 2,000, 2,000, 2,000 and 5,000/ha respectively.

The ponds were prepared by draining and application of lime at the rate of 550 lb/ha. Five days after liming, ponds were filled with groundwater and fertilized with organic manure i.e. cow dung and poultry droppings at the rate of 4,400 and 1,100 lb/ha. Inorganic fertilizers i.e. urea and TSP were applied at the rate of 55 lb/ha for each pond after three days. Then the fish were stocked in all ponds after five days of fertilization. Sufficient *Lemna* and *Azolla* stocked in other ponds separately, adjacent to the experimental ponds and periodically supplied at the rate of 4% of body weight of Thai sharpunti (*Puntius gonionotus*) to the experimental ponds and made available for 24 hours, per day. Ponds were fertilized weekly with urea and TSP at a rate of 55 lb/ha. Commonly available ingredients such as rice bran and mustard oil cake were also used as supplementary feed daily at the rate of 4% of body weight of total fish in all the treatments.

Water quality parameters of the experimental ponds were recorded throughout the study period. Water samples were collected between $9.^{00}$ to $10.^{00}$ am fortnightly and water temperature, dissolved oxygen (DO), pH, nitrate-nitrogen (NO_3-N), phosphate-phosphorus (PO_4-P), chlorophyll-a and total alkalinity were analyzed. Temperature and DO were measured by a digital oxygen meter (YSI Model 58, USA). Transparency and pH of water were measured by a Secchi disc and a pH meter (CORNING pH meter 445), respectively. NO3-N was determined by a HACH kit (DR/2001), a direct reading spectrophotometer using pillow Nitravar-5 nitrate reagent and PO_4–P was determined by a HACH kit (DR/2001) using pillow phosver-3. Chlorophyll-a was measured spectrophotometrically after acetone extraction and total alkalinity was determined by titrimetric method (Stirling, 1985).

Qualitative and quantitative study on plankton were from each of the experimental ponds. 14 L of water samples were collected from different areas and depths of each pond and pass through a fine mesh sized (25 µ) plankton net and then the collected plankton samples were preserved in 5% buffered formalin in 250 ml plastic bottle for subsequent studies. Then, 1 ml of sub-samples were examined using Sedgewick-Rafter counting cell under a compound binocular microscope. The plankton cell density was calculated following Stirling (1985). Identification of plankton was performed by APHA (1992) and Bellinger (1992).

The observed data were analyzed following Analysis of Variance (ANOVA) in randomized block design. The significant differences among the treatment means were determined using Duncan's Multiple Range test (Gomez and Gomez, 1984). Statistical significance was assessed using a probability level of $P=0.05$.

Results

Mean values and range of each physico-chemical properties of water such as temperature, pH, transparency, dissolved oxygen (DO), alkalinity, nitrate-nitrogen (NO_3-N), phosphate-phosphorous (PO_4-P) and chlorophyll-a concentration were shown in Table 1. During the experimental period, the values of water temperature were more or less similar in all the treatments and fluctuated from 23.6°C to 35.5°C. pH values of experimental ponds were found to be slightly alkaline and mean values of pH were 7.53±0.34, 7.61±0.39 and 8.09±0.46 in treatments 1, 2 and 3, respectively (Table 1) which were conducive for fish culture. The values of water transparency showed a wide fluctuation, varied from 14 to 73.5 cm and ranged from 19 to 60.59, 17 to 73.5 and 14 to 56.5 cm in treatments 1, 2 and 3, respectively. During the study period mean values of dissolved oxygen were found higher in treatment 3 (5.27±1.86 mg/L) followed by treatment 2 (4.84±1.63 mg/L) and treatment 1 (4.80±1.93 mg/L). On some occasion, DO concentrations fell below 3 mg/l in all treatments and significant difference was not observed between the treatments ($P>0.05$) (Table 1). The highest concentration of alkalinity was (367 mg/L) found in treatment 3 and the lowest was (98 mg/L) found in treatment 1. Throughout the study period nitrate nitrogen (NO_3-N) and phosphate phosphorus (PO_4-P) were also found higher (3.20 and 3.5 mg/L) in treatment 3. The concentration of nutrients gradually increased with the culture period. Chlorophyll-a concentration varied significantly during the study period (P<0.05), was found the highest in treatment 3 (332.70±198.90 mg/L) followed by treatment 2 (175.84±158.41 mg/L) and treatment 1 (125.33±97.07 mg/L) (Table 1).

There were 32 genera of phytoplankton belonging to Euglenophyceae, Cyanophyceae, Bacillariophyceae and Chlorophyceae. During the study period, the mean values of total phytoplankton cell densities were found the highest in treatment 3 (263.40±178.33 x10^3 cells/L) (Table 2).

Phytoplankton abundance of all classes varied significantly among the treatments except the Bacillariophyceae. Cyanophyceae ranked first position in respect of abundance and second position in order of species composition (10 genera). The most dominant identified bloom forming genuses of Cyanophytes were *Anabaena* sp., and *Microcystis* sp., Euglenophyceae comprised of only 2 genera: *Euglena* sp. and *Phacus* sp. Euglenophyceae was the most abundant in August and the least abundant in July. Cell density of Chlorophyceae was found to be the highest (142.5x10^3 cells/L) in treatment 3 in August and the lowest (0.37x10^3 cells/L) in treatment 1 in July (P<0.05). Bacillariophyceae comprised of 7 genera and the most dominant identified genus was *Nitschia* sp. The changes of nitrate-nitrogen and phosphate-phosphorus with the growth of Euglenophyceae and Cyanophyceae were also presented in Figure 1 to 3.

Discussion

In the present study, water temperature was found to vary from 23.6ºC to 33.6°C. The change of water temperature might be due to the change of weather condition from summer to winter season and no significant differences in temperature and

Table 1. Mean values (±SD) and ranges of each water quality parameters in different treatments throughout the period of study

Parameters	Treatment 1	Treatment 2	Treatment 3	F-Value	Level of Significance
Temperature (^oC)	29.60±2.97 (23.60-35.50)	29.30±2.63 (23.80-33.40)	29.57±2.59 (23.90-33.60)	0.39	NS
Transparency (cm)	34.69^a ±11.93 (19.00-60.59)	36.32^a ±19.16 (17.00-73.5)	26.27^b ±13.47 (14.00-56.50)	14.58	**
Dissolved oxygen (mg/L)	4.80±1.93 (2.10-9.60)	4.84±1.63 (2.50-8.40)	5.27±1.86 (2.38-6.90)	1.08	NS
pH	7.53^b ±.034 (6.89-8.50)	7.61^b ±0.39 (7.12-8.68)	8.09^a ±0.46 (7.20-9.01)	97.46	**
Alkalinity (mg/L)	133.87^b ±18.95 (98.00-172.00)	144.93^b ±25.09 (106.00-196.00)	224.41^a ±47.36 (145.00-367.00)	39.16	**
NO_3-N (mg/L)	1.29^b ±0.39 (0.90-2.30)	1.33^b±0.40 (0.60-2.40)	1.87^a±0.63 (1.00-3.20)	88.58	**
PO_4-P (mg/L)	1.04^b ±0.75 (0.01-2.29)	1.04^b ±0.76 (0.01-2.17)	1.64^a±1.02 (0.02-3.50)	6.64	**
Chlorophyll-a (µg/L)	125.33^b ±97.07 (26.99-345.21)	175.84^b ±158.41 (26.99-593.25)	332.70^a ±198.90 (27.50-690.36)	35.63	**

NS indicates, non significant at 5% level
** Indicates significant difference at 5% level
Figure in the same row having same superscript (a or b) are not significant different

Table 2. Mean values (±SD) and ranges of cell density (x 10^3 cells/L) of different phytoplankton classes in four treatments throughout the period of study

Parameters	Treatment 1	Treatment 2	Treatment 3	F-Value	Level of Significance
Euglenophyceae	34.05^b ±21.93 (4.20-87.60)	55.57^b ±44.18 (2.00-156.00)	93.48^a ±73.28 (3.00-318.00)	26.57	**
Cyanophyceae	44.90^b ±33.37 (3.67-135.89)	51.26^b ±31.08 (4.56-101.66)	95.94^a ±70.45 (2.88-282.00)	6.56	**
Bacillariophyceae	18.63±26.19 (0.8-117.93)	15.97±19.12 (0.51-75.20)	20.69±16.58 (3.84-70.91)	0.47	NS
Chlorophyceae	32.14^b ±28.32 (0.37-99.66)	36.17^b ±29.73 (1.34-102.00)	52.56^a ±43.12 (3.07-142.50)	7.95	**
Total Phytoplankton	130.62^b ±80.25 (15.04-301.92)	154.86^b ±101.44 (11.66-342.13)	263^a ±178.33 (17.76-774.00)	10.22	**

NS indicates, non significant at 5% level
** Indicates significant difference at 5% level
Figure in the same row having same superscript (a or b) are not significant different

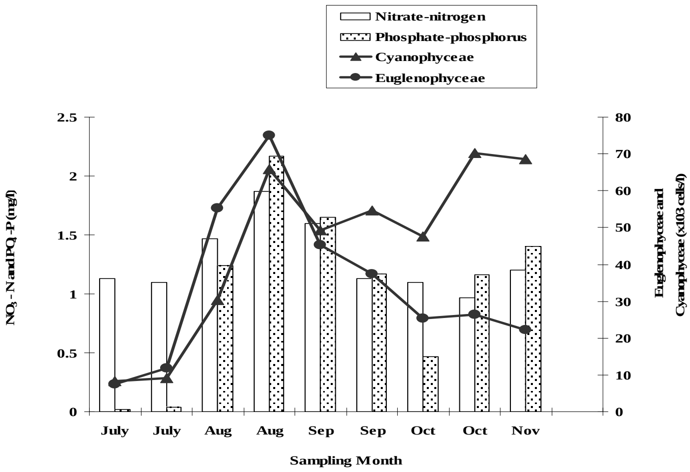

Figure 1. Effect of nitrate-nitrogen and phosphate-phosphorus (mg/L) on the growth of Cyanopyceae and Euglenophyceae ($x10^3$ cell/L) in Treatment 1.

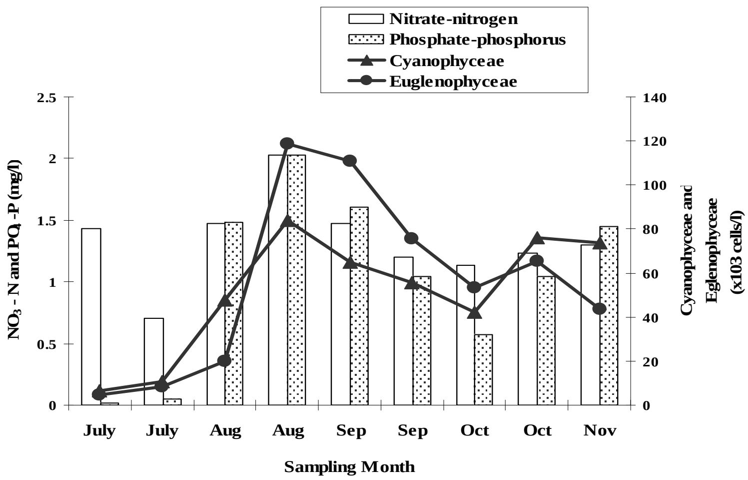

Figure 2. Effect of nitrate-nitrogen and phosphate-phosphorus (mg/L) on the growth of Cyanopyceae and Euglenophyceae ($x10^3$ cell/L) in Treatment 2.

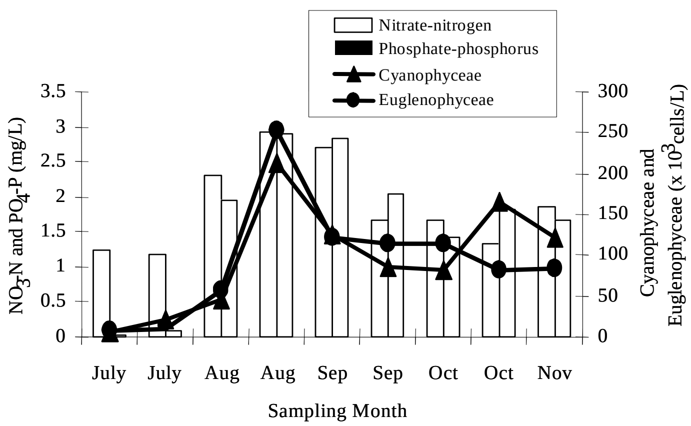

Figure 3. Effect of nitrate-nitrogen and phosphate-phosphorus (mg/L) on the growth of Cyanopyceae and Euglenophyceae ($x10^3$ cell/L) in Treatment 3.

dissolved oxygen were observed among the three treatments. Ondok *et al*. (1984) said that diel oxygen changes as large as 8 ppm occur in the waters of dense submerged macrophytes stands. Macrophytes oxygenate the water more effectively. In this experiment, dissolved oxygen was found higher in treatment 1 during September and lower concentration was also found in July due to rainfall and cloudy weather. But the fortnightly mean values were always found higher in treatment 3.

Banforth (1958) said that transparency indicates the presence and absence of food particles and productivity of a water body, which is influenced by the suspended materials, silt and microorganisms. Water bodies with medium and dense macrophytes cover are characterized by a low concentration of suspended sediments, hence high water transparency. Where aquatic macrophytes disappear, such as under the impact of eutrophication, water transparency is reduced. It was observed that the mean values of transparency were found higher in treatment 1 and 2 (33.69 and 36.32 cm respectively) due to the application of *Lemna* sp. and *Azolla* sp. than the treatment 3 (26.27 cm)

Mitzner (1978) reported a significant increase in alkalinity following a 91% reduction of aquatic macrophytes by grass carp in a lake in Iowa. Crutchfield *et al*. (1992) also found that elimination of macrophytes in a cooling reservoir in North Carolina using *Tilapia zillii* also resulted in an increase of alkalinity concentration. In the present study, the concentration of alkalinity was found higher in treatment 3 followed by the treatment 1 and treatment 2.

Landolt and Kandeler (1987) reported that *Lemna* sp. requires high phosphorus concentration to grow in water. Perniel *et al*. (1998) also found that *Lemna minor* monoculture consistently removed the largest amount of ammonia and phosphorus from storm water in 8 weeks. During the present experiment, aquatic macrophytes, *Lemna* sp. and *Azolla* sp. used inorganic fertilizers for growth. On the other hand, the absence of aquatic macrophytes leads to accumulate large amount of nutrients in treatment 3. Those excessive amounts of nutrients sometimes cause phytoplankton blooms especially the blooms, of blue green algae such as Microcystis and Anabeana.

Leslie *et al*. (1983) detected a significant increase in nitrate-nitrogen concentration in two Florida lakes following macrophytes removed by grass carp. In aquariums, the macrophyte *Anacharis canadensis* was eliminated by the grazing activity of rudd (Hansson *et al*., 1987). This grazing activity resulted in an increase in phosphorus concentration in water. Rakocy and Allison (1981) also stated that in the experimental use of macrophytes for removal of nutrients originating from fish production (*Oreochormis aureus*) tanks, water hyacinth was the fastest growing plant in the wastewater treatment tank and removed the greatest amount of nitrogen. These statements are supportive to the findings of the present study.

There is a positive relationship between chlorophyll-a concentration and total phytoplankton abundance. In the present study, the highest concentration of chlorophyll–a and phytoplankton cell densities were found in treatment 3 followed by treatments 2 and 1. The dominant phytoplankton classes were Euglenophyceae and Cyanophyceae in the study. The identified dominant genuses were *Euglena* sp., *Anabaena* sp. and *Microcystis* sp. The aquatic macrophytes applied in ponds of treatments 1 and 2 acted as a biofilter and established a partial recycling system. So the nutrient concentrations remained in a suitable range in ponds of treatments 1 and 2. But in case of treatment 3 due to the absence of aquatic macrophytes, the highest concentration of nutrients were found which lead to the heavy phytoplankton bloom of Euglenophyceae and Cyanophyceae. So, chlorophyll-a concentration was also found the highest in ponds of treatment 3. When ANOVA was performed in case of chlorophyll-a concentration and plankton cell density, significant difference was observed among the three treatments. Comparatively better performance was observed in treatment 1 (using *Lemna* sp.) than treatment 2 (using *Azolla* sp.)

The results showed that the introduction of fish-edible floating aquatic macrophytes in ponds of treatments 1 and 2 enhances the water quality in a sustainable way, especially reducing the nutrient loading to the aquatic environment for sustainable aquaculture.

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