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ASSESSMENT OF FOUR DIFFERENT MEDIA FOR THE MASS CULTURE OF *CERIODAPHNIA RETICULATA* (JURINE) AS A LIVE FISH FEED

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Abstract

Experiments on the mass culture of *Ceriodaphnia reticulata* (Jurine) were carried out in aquarium water for 54 days with different media like cowdung (1.5g/L), pulse bran water (50g/L), poultry manure (0.45g/L) and snail faeces (faeces of six apple snails). All the media were fertilized by 50-100% of the initial amount of feed in every 7 days. About 100 individuals of *C. reticulata* were inoculated as starter in 50 litres of water (2 individuals/ml).The temperature of the media ranged from 24-30^{o}C during study period. p^{H} of the culture media varied i.e., 9.1 ± 0.40 in cowdung; 8.72 ± 0.73 in pulse bran water, 8.82 ± 0.72 in poultry manure and 7.5 ± 0.55 in snail faeces. The highest average population of *C. reticulata* was observed in cowdung (8.56 ± 4.11individuals/ml), moderate in poultry manure (4.21 ± 2.97 individuals/ml) and snail faeces (2.52 ± 3.01individuals/ml). The lowest growth of *C. reticulata* was recorded in pulse water (0.37 ± 0.69 individuals/ml). The culture media with cowdung as well as poultry manure and snail faeces were found to be useful for artificial mass production of *C. reticulata*.

Key words: Assessment, Food media, Mass culture, Live fish feed, *Ceriodaphnia reticulata*

Introduction

Zooplanktons are important food item for the young and some adults of many freshwater fishes which represent a major component of the human diet (Kenneth 1990). Among freshwater zooplankton, rotifers, cladocerans and copepods are dominant groups throughout the year (Hutchinson 1967). Successful hatchery production of the fish fry and crustaceans for aquaculture depends on the availability of zooplankton of appropriate size of larval feeding. Freshly hatched *Artemia nauplii* has been popular larval feed used by the scientists and aquaculturists for a long time. But the high cost of *Artemia* cysts has led to the aquaculturists to search for alternative suitable zooplankton which could be easily reared in large scale. The rotifer *Brachionus plicatilis*, the cladoceran, *Moina* sp, the harpacticoid copepods such as *Tigriopus* spp and *Tispe* spp., nematodes, *Panagrellus* spp. and the ciliate *Fabrea salina* all of which have high reproductive rate, short generation time and the ability to live and grow in crowded culture conditions that are found to be useful as live feed for larval rearing of cultivable species of fish and crustaceans (Muthu 1982).

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Zooplankton play an important food item of omnivorous and carnivorous fishes (Alam *et al.*1987). Cladocerans often known as 'water fleas' because of their shape and "hop-sink" type of locomotion are the major group of zooplankton available in freshwater ponds. Larger fry and even adults of some fish species often selectively prey on the crustaceans (Ludwig 1999). *Ceriodaphnia* is a small cladoceran genus that has higher protein content than *Daphnia* and is an excellent feed for fish fry with minute mouths. The males range from 0.4-0.8mm in length, whereas the size of females is 0.4mm to 1.4 mm in length that varied depending upon various species (Balcer *et al*. 1984).

In the study, *Ceriodaphnia reticulata*, an important cladoceran as fish food had been selected for mass culture in aquaria with different types of media. Development of a suitable culture media for commercial production of *Ceriodaphnia* sp. will be an inexpensive alternative approach to live feeds needed for fish rearing.

Materials and Methods

The experiments were conducted over a period of 54 days in the Zoology Section of BCSIR Laboratories, Dhaka. *Ceriodaphnia reticulata* was collected from local water bodies of Dhaka city and this species was identified according to Brooks 1959. The culture media maintained in 12 aquarium tanks of 75 cm x 36 cm x 36 cm size with aeration for 24 hrs. Each tank was washed, left to dry and then filled with 30 litres of tap water. The tap water was kept for two days for seasoning. On the 3rd day, the tanks were fertilized by four different types of food media with three replicates for each treatment. The media were cowdung (44.88g dried manure + 30 litres of water) (Rottman 1992), pulse bran water with *Chlorella* (1.5g Urea+0.3g TSP + 3g salt + 600 ml pulse bran water + 100 ml *Chlorella* + 30 litres of water), poultry manure (13.45g dried manure + 30 litres of water) and snail faeces (six apple snails feed on 2-3 cabbage leaf daily). Additional feed, approximately 50-100% of the initial amount were added to 5 days later. On the third day of the experiment, about 100 individuals of *C. reticulata* were introduced to the culture medium of each tank as starter. Following initiation of different growth experiments, the number of living individuals of each tank was counted daily. These processes continued until population study in each replication that started to decline. The population of *C. reticulata* was recorded by using the Sedgewick-Rafter counter cell which is 50 mm long, 20 mm wide and 1 mm deep. Zooplankton number (no./ml) was calculated according to the formula outlined by Boyd and Lichktoppler (1979):

$$\text{Number of zooplankton/ml} = \frac{T \times 1000}{A \times N \times \text{Vol. of concentrate in ml/Vol. of sample}}$$

Where, T= total number of zooplankton counted
A=area of grid in mm^2
D=Number of grids counted
1000 = area of counting chambers in mm^2

Water temperature (^oC) of the culture media were recorded by using a mercury thermometer and pH was detected with the help of a portable pH meter (model-HI 98103) before sampling started at 10.00 a.m. once in every 3 days. The statistical analysis of different physico-chemical parameters were carried out by using one-way ANOVA and any difference at 5% level of significance by using the statistical package of SPSS-16(SYSTA, USA) to express the results.

Results and Discussion

Media, pH level and water temperature, affect the growth and reproduction of *Ceriodaphnia*. The growth of *Ceriodaphnia reticulata* varied from medium to medium used in this experiment (Fig. 1). Among the media, the highest average number of individuals were recorded in cowdung medium of 8.56 ± 4.11 no./ml whereas *C. reticulata* reared on pulse bran water medium exhibited the lowest density of 0.37 ± 0.69 no./ml. The other cultured media like poultry manure and snail faeces showed moderate population growth of 4.21 ± 2.97 and 2.52 ± 3.01 no./ml respectively on an average (Table 1).

Table 1. Average pH, temperature, survival and number of individuals of four different culture media.

Feeds	pH(Mean ± SD)	Temperature (^oC)	Days of survival	Number Individuals (Mean ± SD)
Cowdung	*9.1 ± 0.40^a	26.28 ± 1.88^c	29.08 ± 15.29^a	8.56 ± 4.11^a
Poultry manure	8.82 ± 0.72^b	27.52 ± 1.42^b	25.21 ± 16.94^b	4.21 ± 2.97^b
Pulse bran water	8.72 ± 0.73^b	26.42 ± 1.67^{bc}	26.22 ± 15.46^{ab}	0.37 ± 0.69^d
Snail faeces	7.5 ± 0.55^c	30.41 ± 0.88^a	27.43 ± 16.94^{ab}	2.52 ± 3.01^c

*means containing the same letters do not differ significantly at 5% level of significance.

Effects of days of survival: The parameter on the days of survival was studied to make a comparison of the effects of cultured food media in cultivation of *C. reticulata*. At the end of 54 days of experiment, average days of survival were highest in cowdung media (29.08 ± 15.29) and lowest in poultry manure media (25.21 ± 16.94).

There was a significant ($p < 0.01$) relationship between the number of individuals and days of survival ($r = 0.441$) and that the number of organisms increased with time in cowdung medium. The effects of days of survival on the growth of individual cultured in four types of media were not the same (Fig. 2). In cowdung, the population of *C. reticulata* showed more or less average growth rate over the period with two peaks abundances on the 15th and the 40th day. There was no figure for complete decline of this organism in cowdung medium. In poultry manure, the population started to grow from first day to fourth day and became steady till 10th day. It grew upto 6 individual/ml in

similar fashion till 23rd day and then suddenly increased to 10 individual/ml by 29th day which continued till 36th day and fell to 2 indi./ml by 40th day. In pulse bran water, the population of *C. reticulata* started to grow from 8th day with 1 indi./ml and became stable till 14th day and then sudden increase to 2 individual/ml by 15th to 20th day. After that population declined abruptly to 0 indi./ml. The number of individuals in snail faeces started to grow from 8th day and gradually increase till 32th day upto 3 indi./ml and then sudden increase to 14 indi./ml by 35th day and then fell to 4 indi./ml by 39th day. After that growth of *C. reticulata* continued with slight fluctuation from 1-3 indi./ml (Fig. 2).

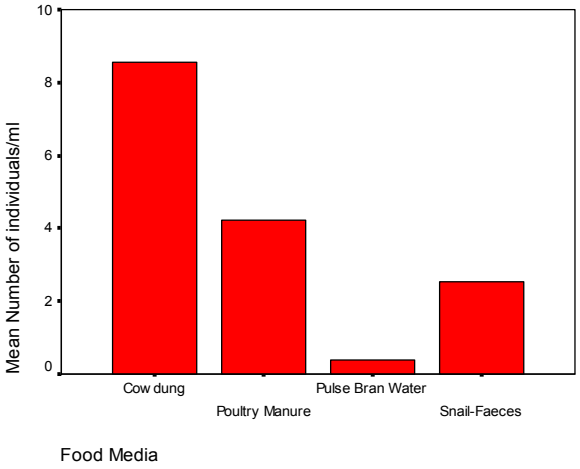

Fig.1. Average number of individuals produced in four types of feed media.

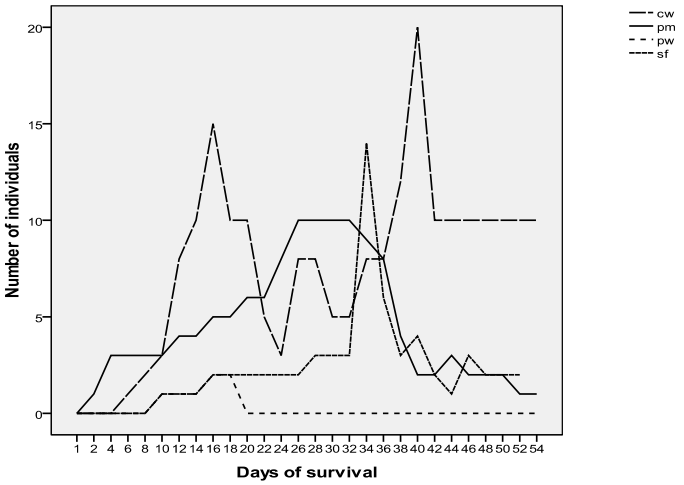

Fig. 2. Effects of days of survival on number of individuals in four types of feed media.

*cw = cowdung, pm = poultry manure, pw = pulse water, sf = snail feaces.

Effects of pH: pH of the culture media showed profound effects on the growth of individuals which ranged from 9.1 ± 0.40 (snail faeces) to 7.5 ± 0.55 (cowdung) on an average (Table 1). It is evident that pH of the poultry manure indicated a significant positive relationship (0.757) with the number of individuals up to a certain range (7.4-10). Result showed that there were no significant differences ($p > 0.05$) between the number of individuals and pH of the three culture media (Table 2). Fig. 3 showed the overall effects of pH comparatively in four cultured media under experiment. In cowdung, number of individuals increased with increase of pH up to a point between 8.4-9.1 and then population decreased sharply with short growth phase. In poultry manure, there was some extension period of population growth with few lag phases but showed optimum abundances within pH 9.2-9.8. The population of culture organism showed irregular pattern of growth in pulse bran water medium with few remarkable fluctuations at pH 8.2 to 9.4. Number of individuals showed a downward trend with the increase of pH values despite of two peak curves with certain pH level such as at pH 7.2 and 7.7 in snail faeces.

Table 2. Correlation of number of individuals with pH, temperature and days of survival in different culture media.

Food levels	p^H	Temperature	Days of survival
Cowdung	-0.248	0.408*	0.441*
Poultry manure	0.757**	0.286	0.020
Pulsebran water	0.024	-0.042	-0.378
Snail feaces	-0.356	-0.388	0.383

** significant at the 0.01 level, * significant at the 0.05 level

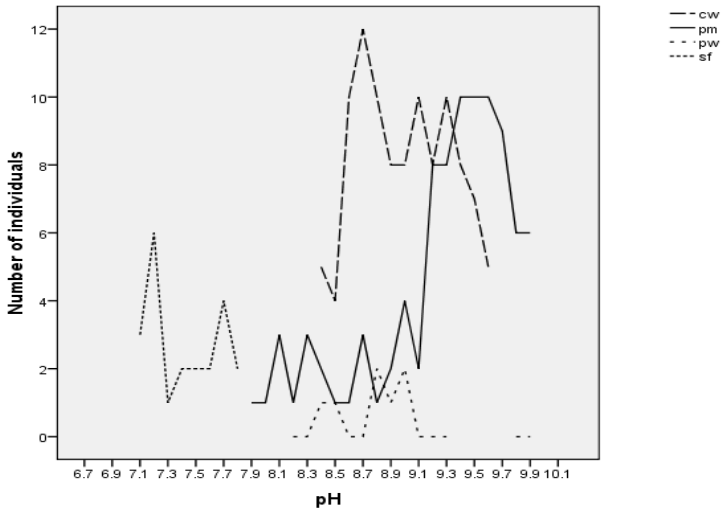

Fig. 3. Effect of pH on number of individuals in four types of feed media.
*cw = cowdung, pm = poultry manure, pw = pulse water, sf = snail feaces.

Effects of Temperature: Experimental results showed that the highest mean temperature was in snail faeces (30.41 ± 0.88^{o}C) whereas lowest in cowdung medium (26.28 ± 1.88^{o}C). The correlation between mean temperature (0.408) and population density of cowdung medium shows positive relationship (Table 2).

Fig. 4 showed the comparative effects of temperature on number of individuals cultured in four types of feed media with a great variation. In cowdung, number of individuals increased with the increase of temperature following few fluctuations. The figure reached at peak sharply at 23^{o}C, 25^{o}C and 26^{o}C temperature and dramatic declined at 23-24^{o}Cand 25.5^{o}C. After a short lag phase, growth of individuals continued its upward trend till 29^{o}C. In poultry manure, the number of individuals increased steadily from 25 to 28^{o}C and then declined abruptly at 28.5^{o}C. Then population increased sharply with little fluctuation within 29 to 30 ^{o}C. The number of individuals in pulse bran water medium did not increase beyond the inoculation density (2 ind./ml) and showed temperature range between 26^{o}C to 29.5^{o}C with a small peak at 27 ^{o}C. The temperature recorded in snail faeces culture medium was confined between 29 to 31^{o}C with a single peak at 30 ^{o}C (Fig. 4).

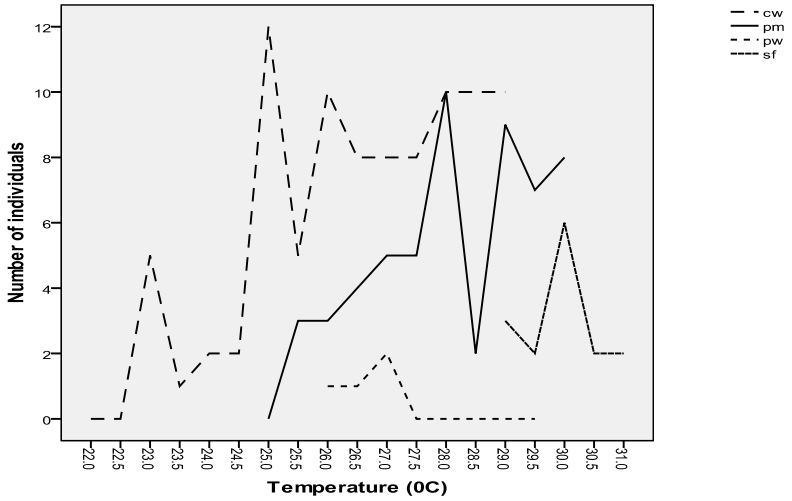

Fig.4. Effect of temperature on number of individuals in four types of feed media.
*cw = cowdung, pm = poultry manure, pw = pulse water, sf = snail feaces

Table 3 depicts the simple regression analysis with pH, water temperature and days of survival on the number of *C. reticulata* in different culture media. In cow dung media, the contribution of pH is inversely proportional to the production of number of individuals whereas temperature and days of survival are more contributing parameters for the production of *C. reticulata* than that of pH parameter. In poultry manure, pH and temperature are more important for the number of individuals than days of survival which is inversely proportional to the production of days of survival. Similar type of result was found in pulse bran water medium. In snail faeces, pH and temperature are more

inversely proportional to the production of number of individuals and days of survival are less prominent parameter for detecting the number of *C. reticulata.*

Table 3. Regression of pH, temperature and days of survival on number of individuals.

Food levels	Regression line	R^2	Adjusted R^2
Cow dung	No. of individuals = 23.531 - 3.948 pH + 0.602 Temperature + 6.72 Days of survival	0.309	0.210
Poultry manure	No. of individuals = - 46.21 + 3.49 pH + 0.810 Temperature - 0.106 Days of survival	0.736	0.704
Pulse bran water	No. of individuals = - 3.39 + 0.294 pH + 7.04 Temperature - 2.69 Days of survival	0.25	0.152
Snail faeces	No. of individuals = 31.908 - 1.05 pH -0.74 Temperature + 3.11 Days of survival	0.219	0.081

Comparison of the number of the culture organisms in four different media revealed that growth rate of *C. reticualta* was considerably good in cowdung medium, average in poultry manure and then snail faeces and pulse bran water respectively (Fig. 1). The highest growth rate (20 individuals/ml) was achieved in the cowdung medium might be the cause for rich nutrient component available than those of others. Muthupriya and Altaf (2009) have observed 3593 ± 258 to 9333 ± 203 individuals/litre of *C. cornuta* population in chicken manure medium which was more or less similar to the findings in present study for poultry manure (1-10 individuals/ml). In this medium, the peak population density was observed on the 24^{th} to the 34^{th} day which is in contradiction to the findings of Altaf and Mehraj-ud-Din (2010) who detected peak on the 17^{th} day. Malhotra and Langer (1993) studied on the four cladoceran species of importance as fish food organisms, viz. *Daphnia similes* (Claus), *Simocephalus vetulus* (Schodler). *Moina macrocopa* (Straus) and *Ceriodaphnia cornuta* (Sars) which were maintained in the laboratory on nutrient sources including manure, rice bran and *Chlorella.* The organisms responded better with rice bran and *Chlorella.* The findings of the present study indicate that *C. reticulata* cultured in pulse bran water medium with *Chlorella* inoculums had exhibited the lowest growth rate with an average (0.37 ± 0.69 indi./ml) that differed from the previous findings of Malhotra and Langer (1993).

Studies revealed that nutrient and temperature have significant effects on the life cycles of the planktonic species (Ebert *et al.* 1993, Gillooly 2000 and Savage *et al.* 2004) which in turn affected the population growth of zooplanktons. This was evident in *C. reticualta* cultured in cowdung medium up to a certain range (25-29^{o}C) of temperature. Normally under optimal range of culture conditions, the population growth rates of cladocerans are directly related to the food density and different temperature gradients (Nandini and Sarma 2003, Sarma *et al*. 2005, Xi *et al*. 2005). *C. reticulata* had optimal growth rates at 25^{o}C in cowdung medium, 28^{o}C in poultry manure, 27^{o}C in pulse bran water media and 30^{o}C in snail faeces medium.

Temperature is one of the major determinants of the feeding rate of *Ceriodaphnia*. Gopen (1976) showed that *Ceriodaphnia* feed at higher rates when the water temperature increased and it occurred upto a certain level. According to Gopen (1976), the optimum temperature for *C. reticulata* ranged from 20-22^{o}C. He also opined that reproduction and growth rates of *C. reticulata* decreased at temperature above 22^{o}C due to the increase in energy requirements released from increases in the respiration rates and slightly different results obtained from previous findings which support the results of Tauson (1930). He studied *Daphnia pulex* and observed the temperatures between 15 and 25^{o}C were favourable for egg production, but above and below these temperatures; there was a considerable decline in the number of egg production. Similar effects at higher temperature have also been recorded in *Ceriodaphnia* sp. (Green 1978) and *Moina macrocopa* (Malhothra and Langer 1990). Hall (1964) stated that temperature tolerance may be utilized to predict the frequency of molting, reproduction, and duration of the egg development. Bellosillo (1937) reported that temperature ranging from 26–31°C to be favourable for laboratory and outdoor cultures of zooplankter, *Moina macrocopa*.

It is apparent that temperature alone may not account for variations in plankton densities as other parameters such as high pH, alkalinity, carbon dioxide but nutrients are also responsible for the organic mass production (Pulle and Khan 2003). Hydrogen ion concentrations have great impact on the survival, growth and reproduction rates of cladocera (Walton *et al*. 1982 and Moustafa 2007). The results of the present study exhibited that pH recorded in the poultry manure medium have a significant effects on the growth of *C. reticulata* (Table 2).

C. reticualta as a live food is appeared to be the best food we can possibly feed to different adult fish, fish fry and fingerlings because they are natural and healthy. Live food gives healthier fry, more successful spawns, and better colouration than any prepared food available in the market. Hence, adequate research is needed before stepping into a large scale production of *C. reticulata* using different doses of applied food media as fertilizer and to obtain optimum growth rate and continuous production of live fish feed.

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PHYTOCHEMICAL SCREENING OF SOME ANTIDYSENTERIC MEDICINAL PLANTS OF BANGLADESH

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Abstract

In this report, 40 antidysenteric medicinal plant species representing 24 families were considered for qualitative assessment of their secondary metabolites like alkaloids, flavonoids, glycosides, sterols and tannins. Alkaloids were present in all plant species, though in different degrees and the relative effectiveness of Dragendorffs' reagent was better than others. Distribution of flavonoids, glycosides, sterols and tannins was sporadic in different plant species except *A. cepa, A. marmelos, I. coccinea, M. indica, S. dulcis* and *Z. officinale*, where all these metabolites were present. Abundance and mode of distribution of secondary metabolites in different test plants and their organs were discussed.

Key words: Antidysenteric medicinal plants, Therapeutic principles, Secondary metabolites

Introduction

The use of medicinal plants has been a central component of health care in many cultures for centuries. The first recorded culturally significant plant residues of about 60000 years old were found in Iraq in 1960 at Neanderthal human burial site (Solecki and Shanidar 1975). About 30000 to 70000 plants are currently used by 80% of the rural people across the world for primary health care and WHO upholds quality, recommends and encourages the use of herbal drugs because of their easy availability, efficacy and, specially cost effectiveness compared to modern allopathic drugs (WHO 2002). More than 1000 plant species of Bangladesh are considered to have medicinal properties and about 455-747 have been described with their therapeutic uses for different diseases including dysentery (Mia 1990, Ghani 2003 and Yousuf *et al*. 2009).

Herbs which help in curing dysentery are antidysenteric and antidysenteric plants contain some active chemical agents, usually secondary metabolites, which function as therapeutic principles against dysentery. Dysentery is an inflammatory disorder of the lower intestinal tract, usually caused by microbial infection and resulting pain or fever or bloody diarrhoea. Dysenteric disease has long been recognized as a leading cause of morbidity and mortality among children (1-4 years) and aged people (≥60 years) in Bangladesh (Mitra *et al.* 1990).

Medicinal plants produce a diverse assortment of secondary metabolites of therapeutic importance (Croteau *et al*. 2000 and Terryn *et al.* 2006) and plants with antidysenteric and antidiarrhoeal properties were found to contain alkaloids, flavonoids, saponins,

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sterols, tannins and reducing sugars as well as show antibacterial and antiprotozoan activity (Longanga *et al.* 2000). However, phytochemical characteristics of many of the antidysenteric medicinal plants of Bangladesh are unknown. This paper deals with the phytochemical screening of antidysenteric medicinal plants available in the hilly area of Chittagong University campus and around.

Materials and Methods

During this investigation, 40 antidysenteric medicinal plant species, enlisted in different published literature (Ghani 2003 and Yousuf *et al*. 2009) were collected from the hilly area of Chittagong University campus and around covering different life forms such as herbs, shrubs and trees. Secondary metabolites like alkaloids, flavonoids, glycosides, sterols and tannins were analyzed qualitatively in the whole plant and plant parts within 6 h of collection. For alkaloids, a modified method (Amarasingham *et al.* 1965 and Apline and Cannon 1971) was followed. Dragendorff (D), Wagner (W), Mayer (M), Hager (H) and Tannic acid (T) were prepared following standard method (Cromwell 1955) and were used for alkaloid detection in 2% HCl extract of the plant. Ethanol extract was used for the detection of flavonoids (Wall *et al.* 1954 and Farnsworth 1964). Sterols (Bhattacharjee and Das 1969), tannins (Wall *et al*. 1954) and glycosides (Eyjolfesson 1970) were assessed following the reported methods. The absence, presence and abundance of different secondary metabolites in test samples were indicated by –, + and multiple of + signs, respectively. Each test was replicated thrice. The results are presented in Tables 1 and 2.

Results and Discussion

Qualitative analysis of alkaloids, flavonoids, glycosides, sterols and tannins of 40 antidysenteric medicinal plant species and/or their organs, e.g., root-rhizome, stem, bark, leaf, flower and fruit were carried out. From Table 1, it is apparent that alkaloids were present in all plant species and the extracts from various sources showed different responses to Dragendorff (D), Wagner (W), Mayer (M), Hager (H) and Tannic acid (T) reagents for alkaloids. Out of 225 tests by different reagents for alkaloids, 207 tests were positive indicating the presence of alkaloids in range of slight to abundant (1+ to 4+) and the number of positive response to reagents D, W, M, H and T were 45, 44, 39, 38 and 41, respectively. Negative response (-) in 18 tests indicated the inefficiency of the reagent used in the test. On the basis of alkaloid detecting efficacies, the relative effectiveness of the reagents may be graded as: D>W>T>M>H. While evaluating 102 plant species of 47 families, Pasha (1977) reported positive response for alkaloids in 48 plant species only. On the other hand, positive response for alkaloids in 32 out of 42 plant species was reported by Tariq *et al*. (1987). In the present work, the degrees of responses (1+ to 4+) of 40 plant species and their parts to different alkaloid detecting reagents were different but a large number of tests were appeared to be strong positive (3+ to 4+). Kapoor *et al*.

Table 1. Qualitative analysis of alkaloids in 40 antidysenteric medicinal plants.

Plant species	Family	Plant part used	Present+ / absent – abundant n+				
			Reagents used				
			D	W	M	H	T
Allium cepa	Liliaceae	bulb	+	+	+	+	+
Allium sativum	Liliaceae	bulb	3+	3+	2+	2+	2+
Alstonia scholaris	Apocynaceae	bark	2+	3+	3+	2+	3+
Andrographis paniculata	Acanthaceae	leaf	3+	2+	2+	2+	4+
Aegle marmelos	Rutaceae	fruit	3+	2+	+	-	+
Ageratum conyzoides	Asteraceae	leaf	2+	-	-	-	-
Anacardium occidentale	Anacardiaceae	bark	+	+	+	+	+
Barringtonia acutangula	Barringtoniaceae	leaf	4+	4+	2+	2+	2+
Calotrotropis gigantea	Asclepiadaceae	root	+	3+	+	+	+
Calotropis procera	Asclepiadaceae	root	+	3+	+	+	+
Catharanthus roseus	Apocynaceae	leaf	2+	3+	-	-	2+
Cassia fistula	Caesalpiniaceae	stem bark	2+	+	-	-	-
Centella asiatica	Apiaceae	whole plant	2+	+	+	+	4+
Cicer arietinum	Fabaceae	seed	3+	3+	3+	3+	3+
Cleome viscosa	Capparidaceae	leaf	3+	+	3+	2+	4+
Cuminum cyminum	Apiaceae	seed	3+	+	2+	2+	2+
Cocos nucifera	Arecaceae	flower	+	+	+	+	+
		kernel	+	+	+	+	+
Curcuma longa	Zingiberaceae	rhizome	2+	2+	+	+	+
Daucas carota	Apiaceae	rhizome	2+	+	+	+	2+
Dalbergia sissoo	Fabaceae	leaf	+	+	2+	2+	+
Eupatorium odoratum	Asteraceae	leaf	3+	2+	2+	2+	3+
Euphorbia hirta	Euphorbiaceae	leaf	4+	+	+	2+	2+
		stem	3+	+	-	+	+
		flower	4+	+	+	2+	2+
Ficus hispida	Moraceae	leaf	2+	2+	2+	3+	+
Holarhena antidysenterica	Apocynaceae	leaf	3+	+	2+	2+	+
		bark	4+	4+	3+	3+	+
Hibiscus rosa-sinensis	Malvaceae	flower	2+	+	2+	+	2+
Ixora coccinea	Rubiaceae	flower	2+	2+	+	2+	2+
Jatropa gossypifolia	Euphorbiaceae	leaf	+	+	+	+	+

(Contd.)

Kalanchoe pinnata	Crassulaceae	leaf	2+	+	+	+	2+
Melastoma malabathricum	Melasomaceae	leaf	3+	+	-	-	-
Mimosa pudica	Mimosaceae	root	+	+	+	+	+
Mikania cordata	Asteraceae	leaf	3+	2+	2+	2+	3+
Morinda citrifolia	Rubiaceae	fruit	2+	+	+	+	4+
Murraya koenigii	Rutaceae	leaf	4+	2+	2+	2+	3+
Mangifera indica	Anacardiaceae	bark	4+	3+	3+	3+	4+
Ocimum sp	Lamiaceae	leaf	+	+	+	+	+
Rauvolfia serpentina	Apocynaceae	leaf	3+	3+	2+	3+	3+
Scoparia dulcis	Scrophulariaceae	leaf	4+	3+	3+	3+	4+
Solanum nigrum	Solanaceae	leaf	+	+	+	+	+
		fruit	+	+	-	-	-
Tridax procumbens	Asteraceae	leaf	3+	3+	3+	3+	3+
Zinziber officinale	Zingiberaceae	rhizome	2+	+	+	+	2+

(1969) noted weak positive response for alkaloids while others (Pasha1977, Affandi *et al.* 2004) observed strong positive reactions (3+ to 4+) for alkaloids in a few plant species. The relative abundance of alkaloids found in the present work was higher in leaf, bark and rhizome than other organs of the test plants. With some minor exceptions leaf, stem and root of different medicinal plants were found to contain a broad spectrum of secondary metabolites including alkaloids, flavonoids, saponins etc. (Viji and Murugesan 2010, Pascaline *et al.* 2011). It appears that the distribution of alkaloids is uneven and sporadic within and among different antidysenteric medicinal plants of the present work. This finding supports the previous report (Chhetri *et al*. 2008) of a phytochemical screening for alkaloids and other bioactive chemicals.

Table 2. Qualitative analysis of flavonoids, glycosides, sterols and tannins in 40 antidysenteric medicinal plants.

Plant species	Family	Plant part used	Secondary metabolites: + present /-absent			
			Flavonoids	Glycosides	Sterols	Tannins
Allium cepa	Liliaceae	bulb	+	+	+	+
Allium sativum	Liliaceae	bulb	+	+	-	+
Alstonia scholaris	Apocynaceae	bark	-	+	+	-
Andrographis paniculata	Acanthaceae	leaf	+	+	+	-
Aegle marmelos	Rutaceae	fruit	+	+	+	+
Ageratum conyzoides	Asteraceae	leaf	+	-	+	-

(Contd.)

Anacardium occidentale	Anacardiaceae	bark	-	-	+	-
Barringtonia acutangula	Barringtoniaceae	leaf	+	-	+	+
Calotrotropis gigantea	Asclepiadaceae	leaf	-	+	+	-
Calotropis procera	Asclepiadaceae	leaf	-	+	+	-
Catharanthus roseus	Apocynaceae	leaf	-	+	+	-
Cassia fistula	Caesalpiniaceae	stem bark	+	+	-	-
Centella asiatica	Apiaceae	whole plant	-	+	+	+
Cicer arietinum	Fabaceae	seed	+	+	-	-
Cleome viscosa	Capparidaceae	leaf	-	-	+	-
Cuminum cyminum	Apiaceae	seed	+	+	-	-
Cocos nucifera	Arecaceae	kernel	+	-	+	-
Curcuma longa	Zingiberaceae	rhizo me	+	+	-	+
Daucas carota	Apiaceae	rhizo me	-	-	-	+
Dalbergia sissoo	Fabaceae	leaf	+	+	-	+
Eupatorium odoratum	Asteraceae	leaf	+	-	-	-
Euphorbia hirta	Euphorbiaceae	leaf	+	+	-	+
Ficus hispida	Moraceae	leaf	-	+	-	+
Holarhena antidysenterica	Apocynaceae	leaf	+	-	+	+
Hibiscus rosa-sinensis	Malvaceae	flower	+	-	-	+
Ixora coccinea	Rubiaceae	flower	+	+	+	+
Jatropa gossypifolia	Euphorbiaceae	leaf	+	-	-	+
Kalanchoe pinnata	Crassulaceae	leaf	+	-	-	-

(Contd.)

Melastoma malabathricum	Melasomaceae	flower	-	-	-	-
Mimosa pudica	Mimosaceae	root	-	-	-	+
Mikania cordata	Asteraceae	leaf	+	-	+	-
Morinda citrifolia	Rubiaceae	leaf	-	+	+	+
Murraya koenigii	Rutaceae	leaf	-	+	+	-
Mangifera indica	Anacardiaceae	bark	+	+	+	+
Ocimum sp	Lamiaceae	leaf	-	+	+	-
Rauvolfia serpentina	Apocynaceae	leaf	-	-	+	-
Scoparia dulcis	Scrophulariaceae	leaf	+	+	+	+
Solanum nigrum	Solanaceae	leaf	-	+	+	-
Tridax procumbens	Asteraceae	leaf	+	-	+	-
Zinziber officinale	Zingiberaceae	rhizome	+	+	+	+

Results presented in Table 2 for 4 other metabolites (e.g., flavonoids, glycosides, sterols and tannins) show that all except the flower of *M. malabathricum* gave positive response to tests for one or more metabolites. Out of the total 160 tests, about 92 tests were positive. Among the lot, 6 plant species or plant parts (e.g., *A. cepa, A. marmelos, I. coccinea, M. indica, S. dulcis* and *Z. officinale*) gave positive response for all 4 metabolites (e.g., flavonoids, glycosides, sterols and tannins) while 9, 17 and 7 plant species or plant parts gave positive responses for 3, 2 and 1 metabolite, respectively. For each of flavonoids and glycosides, 24 plant species gave positive response whereas for sterols and tannins positive responses were in 25 and 19 species, respectively. It appears that the distribution of flavonoids, glycosides and sterols in the test plants and their parts was comparatively wider than that of tannin but all showed sporadic and uneven distribution in different plant species and their parts. Tariq *et al*. (1987) in his work with the members of Asteraceae, noted positive responses for flavonoids, sterols, tannins and saponins in 21, 22, 20 and 4 plant species, respectively. In the present work, 6 plant species, e.g., *A. cepa, A. marmelos, I. coccinea, M. indica, S. dulcis* and *Z. officinale* contained all 5 secondary metabolites but in the rest of the plants their distribution is uneven. The presence of alkaloids, flavonoids, glycosides and steroids in *Citrullus* seeds has been reported (Ambi1 *et al.* 2007). Ayoola *et al*. (2008) reported on the presence of flavonoids, terpenoids, saponins, tannins and reducing sugars in *Carica papaya,*

Magnifera indica, Psidium guajava, and *Vernonia amygdalina* has been found. Cardiac glycosides and alkaloids were absent in *M. indica* while alkaloids and phenolic compounds, anthraquinones, were absent in *P. guajava* and *V. amygdalina*, respectively. Sivasankari *et al. (*2010) while examining the major metabolites like carbohydrates, tannins, saponins, flavonoids, alkaloids, betacyanins, quinones, terepenoids, phenols, glycosides and cardiac glycosides in *Caesalpinia pulcherrima* (a domesticated shrub) and *Caesalpinia bonduc* (a wild shrub) leaf extracts reported their uneven distribution in the plant species and the wild plants contributed high values for the secondary metabolites than the domesticated. A wide range of secondary metabolites were reported to be present in different antidysenteric, antidiarrhoeal and other medicinal plants (Longanga *et al.* 2000, Satyanarayana and Eswaraiah 2010 and Narayanasamy and Ragavan 2012). Therefore, the secondary metabolites identified in different medicinal plants of the present work may be considered as active therapeutic agents against the dysenteric disease.

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ANGIOSPERM FLORA OF MANIKGONJ SADAR UPAZILA, BANGLADESH

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Abstract

Angioperm flora of Manikgonj Sadar Upazila has been partially inventoried. A total of 207 species under 72 families and 174 genera has been recorded. Among those, the division Magnoliopsida represents 147 species belonging to 56 families and 127 genera, and the division Liliopsida represents 60 species belonging to 16 families and 47 genera.

Key words*:* Angiopermic flora; Manikgonj Sadar Upazila; Bangladesh

Introduction

The Sadar upazila of Manikgonj district occupies an area of 214.81 sq. km. including 12.97 sq. km. of river. The upazila lies between 23º42′ to 23º55′ N latitudes and 89º58′ to 90º07′ E longitudes. It is bounded by Saturia upazila on the North, Nawabganj (Dhaka) and Harirampur upazilas on the South, Singair and Dhamrai upazilas on the East, and Harirampur and Ghior upazilas on the West. Main rivers of the upazila are the Dhaleshwari, the Ichamati, the Kaliganga and the Gazikhali (Ramzan 2003).

The Sadar upazila is mainly dominated by the extension of shallow upland soil. The soil of the area is mainly formed by the Brahmaputra river representing silty and sandy alluvial soil of moderate class. (Rizvi 1969). The area enjoys a tropical climate characterized by high precipitation from May to October and relatively dry period from November to April. The mean annual rainfall of the area is about 1671mm. Temperature of the area ranges from 19.5 to 33.9ºC. The maximum temperature oberved in May and minimum temperature observed in January. (Bangladesh Meteorological Department, personal communication).

A number of floristic works has so far been done in greater Dhaka district including Ismail and Mia (1973), Alam (1995), Hossain *et al.* (1995), Rahman and Hassan (1995), Rashid *et al.* (1995) and Alam *et al.* (2006). But no studies are found in Manikganj Sadar upazila. Moreover, the area supports a large number of angiopermic species including herbs, shrubs, trees, climbers, epiphytes, parasites and hydrophytes. Like other parts of the country, the floristic elements of this area are in risk because of various anthropogenic activities including irrigation, modern agriculture, population settlements, firewood collection, industrilization and also habitat degradation. In order to make a documentation of the angiospermic vegetation of the area an attempt has made to prepare a preliminary flora of the angiopermic plant species available at Manikganj Sadar upazila.

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Materials and Methods

This work is mainly based on the fresh plant materials collected by the author and the herbarium specimens collected from the same area and stored at different herbaria of the country. A standard survey method was developed to collect maximum number of plant species from all representative habitats found in the area. Four visits were made at three months interval during the year 2006 to collect specimens with either flowering and/or fruiting condition for recording seasonal variations.Those specimens were identified by matching with correctly identified herbarium specimens of Bangldesh National Herbarium and Dhaka University Salar Khan Herbarium (DUSH). In some cases, standard taxonomic literature [viz. Hooker (1872 - 1897), Prain (1903) and Uddin and Hassan (2004)] were also consulted to identify critical specimens. Voucher herbarium sheets were prepared for all species by using traditional herbarium technique and stored at DUSH."

The families have been arranged according to Cronquist (1981). The genera under each family and the species under each genus are arranged in an alphabetic order. For each species, nomenclature has been brought up-to-date; impotant synonyms, local name (wherever available) and a short annotation are also provided.

Results and Discussion

In the present survey, a total of 207 angiopermic species under 174 genera and 72 families were recorded from the Sadar upazila of Manikganj district. Magnoliopsida is represented by 56 families, 127 genera and 147 species, while Liliopsida is represented by 16 families, 47 genera and 60 species. Habit-wise itemization of plant species shows that 58% of the total species are represented by herbs, 21% by trees, 11% by shrubs, 10% by other groups (climbers, epiphytes and parasites).

Magnoliopsida (Dicots)

1. Annonaceae

Polyalthia longifolia (Sonn.) Thw., Enum. Pl. Zeyl.: 398 (1864). *Uvaria longifolia* Sonn. *Local name: Debdaru*. A tall tree. *Representative specimens:* Putail, 03.03.06, Kanika 42 (DUSH); Jaigir, 30.12.06, Kanika 241(DUSH).

P. suberosa (Roxb.) Thw., Enum. Pl. Zeyl.: 398 (1864). *Uvaria suberosa* Roxb. A shrub or small tree. *Representative specimen:* Putail, 15.06.06, Kanika 122 (DUSH).

Uvaria hamiltonii Hook. f. & Thoms., Fl. Ind. 1: 96 (1820). A large climber. *Representative specimen***:** Nabagram, 04.03.06, Kanika 85 (DUSH).

2. Lauraceae

Litsea monopetala (Roxb.) Pers., Syn. Pl. 2: 4 (1807). *Tetranthera monopetala* Roxb. *Local names: Menda*, *Chapaitta*. A medium-sized tree. *Representative specimen:* Manikgonj Sadar, 10.09.06, Kanika 154 (DUSH).

3. Piperaceae

Peperomia pellucida (L.) Kunth. Nov. Gen. Sp. 1: 64 (1815). *Piper pellucidum* L. *Local names: Luchipata, Peperomia*. A small annual herb. *Representative specimen:* Putail, 11.09.06, Kanika 175 (DUSH).

Piper longum L., Sp. Pl. 1: 9 (1753). *Local name: Pepul*. A perennial climbing herb. *Representative specimen:* Putail, 03.03.06, Kanika 83 (DUSH).

4. Aristolochiaceae

Aristolochia tagala Cham., Linnaea 7: 207 (1832). *Local name: Ishwarmul*. A glabrous climber. *Representative specimen:* Jaigir, 16.06.06, Kanika 133 (DUSH).

5. Nymphaeaceae

Nymphaea nouchali Burm. f., Fl. Ind.: 120 (1768). *Local names: Shapla, Kamal.* A perennial aquatic herb. *Representative specimens:* Betila, 12.09.06, Kanika 194 (DUSH); Jaigir, 30.12.06, Kanika 242 (DUSH).

N. rubra Roxb. *ex* Andr., Bot. Rep. 8: 104, t. 503 (1808). *Local names: Lal-Shapla, Ogul phul.* A perennial aquatic herb. *Representative specimen:* Manikgonj Sadar, 02.03.06, Kanika 17 (DUSH).

6. Ranunculaceae

Ranunculus sceleratus L., Sp. Pl.: 551 (1753). An erect, annual herb. *Representative specimen:* Manikgonj Sadar, 02.03.06, Kanika 35 (DUSH).

7. Menispermaceae

Stephania japonica (Thunb.) Miers, Ann. Mag. Nat. Hist. Ser. 3, 18: 14 (1866). *Menispermum japonicum* Thunb. *Local names: Aknadi, Maknadi, Nimukha.* A slender climber. *Representative specimen:* Betila, 15.06.06, Kanika 127 (DUSH).

Tinospora sinensis (Lour.) Merr., Sunyatsenia 1: 193 (1934). *Campylus sinensis* Lour. *Local name: Gulonchoe*. A climbing shrub. *Representative specimen:* Manikgonj Sadar, 02.03.06, Kanika 01(DUSH).

8. Papaveraceae

Argemone mexicana L., Sp. Pl. 1: 508 (1753). *Local name: Sialkanta*. An annual herb. *Representative specimen:* Manikgonj Sadar, 02.03.06, Kanika 36 (DUSH).

9. Ulmaceae

Trema orientalis (L.) Bl., Mus. Bot. Lugd.-Bat. 2: 62 (1856). *Celtis orientalis* L. *Local names: Jilan, Chikan, Nars.* An ever green, small tree. *Representative specimen:* Manikgonj Sadar, 02.03.06, Kanika 37 (DUSH).

10. Moraceae

Artocarpus lacucha Buch.-Ham., Mem. Wern. Soc. 5: 333 (1826). *Local name: Dephul.* A large deciduous tree. *Representative specimen:* Garpara, 28.12.06, Kanika 222 (DUSH).

Ficus benghalensis L., Sp. Pl. 1: 1059 (1753). A large tree. *Representative specimen***:** Nabagram, 04.03.06, Kanika 86 (DUSH).

F. heterophylla L. f., Suppl. Pl.: 442 (1781). A scandent shrub. *Representative specimen:* Putail, 03.03.06, Kanika 43 (DUSH).

F. hispida L. f., Suppl. Pl.: 442 (1781). *Local name: Kakdumur*. A low tree. *Representative specimen:* Putail, 11.09.06, Kanika 176 (DUSH).

F. religiosa L., Sp. Pl.: 1059 (1753). *Local names: Aswatha, Pakur.* A large tree. *Representative specimen:* Garpara, 17.06.06, Kanika 144 (DUSH).

11. Urticaceae

Laportea interrupta (L.) Chew, Gard. Bull. Singapore. 21: 200 (1965). *Urtica interrupta* L. *Local name: Lal Bichuti.* A shrub. *Representative specimen:* Putail, 15.06.06, Kanika 123 (DUSH).

Pilea microphylla (L.) Liebm., Vidensk. Selsk. Skr. 5, Ser: 2: 302 (1851). *Parietaria microphylla* L. An annual, prostrate herb. *Representative specimen:* Manikgonj Sadar, 10.09.06, Kanika 155 (DUSH).

Pouzolzia zeylanica (L.) Benn., Pl. Jav. Rar.: 67 (1833). *Parietaria zeylanica* L. An erect or trailing herb. *Representative specimen:* Betila, 15.06.06, Kanika 128 (DUSH).

12. Nyctaginaceae

Boerhaavia diffusa L., Sp. Pl. 1: 3 (1753). *Local name: Punarnava*. A perennial procumbent herb. *Representative specimen:* Putail, 03.03.06, Kanika 82 (DUSH).

13. Chenopodiaceae

Chenopodium album L., Sp. PI. 1: 219 (1753). Local name: Batua Shak. An erect, annual herb. *Representative specimen***:** Nabagram, 27.12.06, Kanika 206 (DUSH).

C. ambrosioides L., Sp. Pl. 1: 219 (1753). An erect, annual herb. *Representative specimen:* Jaigir, 16.06.06, Kanika 134 (DUSH).

14. Amaranthaceae

Achyranthes aspera L., Sp. Pl. 1: 204 (1753). *Local name: Apang*. A perennial herb. *Representative specimen:* Garpara, 28.12.06, Kanika 223 (DUSH).

Alternanthera philoxeroides (Mart.) Griseb., Symb. Argent. in Abh. Ges. Wiss. Gott. 24: 36 (1879). *Bucholzia philoxeroides* Mart. *Local name: Helencha Sak*. A perennial or annual herb. *Representative specimen***:** Nabagram, 04.03.06, Kanika 102 (DUSH).

A. sessilis (L.) A. Dc., Cat. Pl. Horti Monsp.:77 (1813). *Gomphrena sessilis* L. *Local names: Chanchi, Haicha, Sachisak.* An annual or perennial, prostrate herb. *Representative specimen:* Putail, 15.06.06, Kanika 124 (DUSH).

A. spinosus L., Sp. Pl. 1: 991 (1753). *Local name: Kantanotey, Kantadenga, Katamiris*. An annual, erect, spinescent herb. *Representative specimen:* Betila, 12.09.06, Kanika 195 (DUSH).

A. viridis L., Sp. Pl. ed. 2: 1405 (1753). *Local names: Marissag, Notey, Notey shak*. An annual, erect herb. *Representative specimen:* Manikgonj Sadar, 02.03.06, Kanika 02 (DUSH).

15. Portulacaceae

Portulaca oleracea L., Sp. Pl. 1: 445 (1753). *Local names: Bara Lanya, Bara Nunia*. A prostrate annual herb. *Representative specimen:* Putail, 03.03.06, Kanika 44 (DUSH).

16. Polygonaceae

Persicaria hydropiper (L.) Spach., Hist. Nat. Veg. 10: 536 (1841). *Polygonum hydropiper* L. *Local name: Pakurmul*. An annual herb. *Representative specimens:* Manikgonj Sadar, 10.09.06, Kanika 156 (DUSH); Garpara, 28.12.06, Kanika 224 (DUSH).

Polygonum effusum Meissn. in DC., Prodr. 14: 93 (1857). *Polygonum plebeium* R.Br. var *effusum* Hook. f. A much-branched, prostrate annual herb. *Representative specimens:* Jaigir, 30.12.06, Kanika 261(DUSH).

Rumex maritimus L., Sp. Pl. 1: 335 (1753). *Local name: Bon-palong.* An annual erect herb. *Representative specimen:* Betila, 15.06.06, Kanika 129 (DUSH).

17. Dilleniaceae

Dillenia indica L., Sp. Pl.: 535 (1753). *Local name: Chalta.* A medium sized tree. *Representative specimen:* Jaigir, 30.12.06, Kanika 259 (DUSH).

18. Tiliaceae

Grewia nervosa (Lour) G. Panigrahi, Taxon 34 (2): 702 (1985). *Microcos paniculata* L. *Local names: Asar, Assar, Patka, Dattoi.* A shrub or small tree. *Representative specimen:* Manikgonj, 02.03.06, Kanika 18 (DUSH).

19. Sterculiaceae

Abroma augusta (L.) L. f., Suppl. Pl.: 341 (1781). *Theobroma augusta* L. *Local name: Ulatkambal*. A shrub. *Representative specimen:* Putail, 11.09.06, Kanika 178 (DUSH).

20. Bombacaceae

Bombax ceiba L., Sp. Pl.: 511 (1753). *Local name*: *Shimul*. A large tree with buttress base. *Representative specimen:* Putail, 03.03.06, Kanika 81 (DUSH).

21. Malvaceae

Abelmoschus moschatus Medic., Malv.: 46 (1787). A tuberous, prickly herb. *Representative specimen***:** Nabagram, 27.12.06, Kanika 207 (DUSH).

Abutilon indicum (L.) Sweet., Hort. Brit. ed. 1: 54 (1826). *Sida indica* L. An annual or perennial herb. *Representative specimen:* Putail, 03.03.06, Kanika 84 (DUSH).

Sida cordata (Burm. f.) van Boriss., Blumea 14 (1): 182 (1966). *Melochia cordata* Burm. f. *Local names*: *Junka, Sunate.* An annual, prostrate or ascending herb. *Representative specimen***:** Nabagram, 04.03.06, Kanika 87 (DUSH).

22. Lecythidaceae

Barringtonia acutangula (L.) Gaertn., Fruct. 2: 97, t. 101 (1791). *Eugenia acutangula* L. A medium-sized tree. *Representative specimen:* Jaigir, 30.12.06, Kanika 265 (DUSH).

Careya arborea Roxb., Pl. Corom. 3: 14, t. 218 (1811). *Local names*: *Kumba, Gade*. A low tree. *Representative specimen:* Manikgonj Sadar, 10.09.06, Kanika 160 (DUSH).

23. Cucurbitaceae

Coccinia grandis (L.) Voigt, Hort. Suburb. Calcut.: 59 (1845). *Bryonia grandis* L. *Local name*: *Telakucha*. A climber. *Representative specimen***:** Nabagram, 04.03.06, Kanika 103 (DUSH).

Mukia maderaspatana (L.) M. Roem., Fam. Nat. Syn. Monogr. 2: 47 (1846). *Local name: Bilari.* A perennial, climbing herb. *Representative specimens:* Putail, 03.03.06, Kanika 45 (DUSH); Garpara, 28.12.06, Kanika 226 (DUSH).

24. Capparaceae

Cleome viscosa L., Sp. Pl. 2: 672 (1753). An erect, annual herb. *Representative specimen:* Betila, 12.09.06, Kanika 196 (DUSH).

Crataeva magna (Lour.) DC., Prodr. 1: 243 (1824). *Capparis magna* Lour. *Local name: Barun.* Deciduous tree. *Representative specimen:* Putail, 03.03.06, Kanika 80 (DUSH).

25. Brassicaceae

Rorippa indica (L.,) Hiern, Cat. Afr. Pl. Welw.1: 26 (1896). *Sisymbrium indicum* L. An annual herb. *Representative specimen:* Jaigir, 16.06.06, Kanika 135 (DUSH).

26. Sapotaceae

Mimusops elengi L., Sp. Pl.: 349 (1753). *Local name: Bakul.* A large evergreen tree. *Representative specimen:* Putail, 15.06.06, Kanika 125 (DUSH).

27. Ebenaceae

Diospyros montana Roxb., Pl. Corom. 1: 37 (1795). *Local name: Gab.* A small tree. *Representative specimen:* Putail, 03.03.06, Kanika 46 (DUSH).

28. Mimosaceae

Acacia nilotica (L.) Delile subsp. **indica** (Benth.) Brenan in Kew Bull. 12: 84 (1957). *Mimosa nilotica* L. *Local name: Babla.* A tree. *Representative specimen***:**

Nabagram, 04.03.06, Kanika 101 (DUSH).

Albizzia procera Benth. in Hook., London J. Bot. 3: 89 (1844). *Local names*: *Koroi, Jat Koroi, Sada Koroi, Sil Koroi.* A medium-sized tree. *Representative specimen:* Garpara, 17.06.06, Kanika 146 (DUSH).

Mimosa pudica L., Sp. Pl.: 518 (1753). *Local names*: *Lojjabati, Lajak*. A prickly, woody herb**.** *Representative specimens***:** Nabagram, 04.03.06, Kanika 87 (DUSH); Garpara, 28.12.06, Kanika 228 (DUSH).

Pithecellobium angulatum Benth. in Hook., London J. Bot. 3: 208 (1844). A tree. *Representative specimen:* Manikgonj Sadar, 02.03.06, Kanika 38 (DUSH).

29. Caesalpiniaceae

Cassia fistula L., Sp. Pl.: 377 (1753). *Local name*: *Badarlathi*. A small tree. *Representative specimen*: Nabagram, 27.12.06, Kanika 208 (DUSH).

Senna sophera (L.) Roxb., Fl. Ind. 2: 347 (1832). *Cassia sophera* L. *Local names*: *Chotokalkesunda*, *Jhingi*. A shrub or undershrub. *Representative specimen:* Manikgonj Sadar, 02.03.06, Kanika 34 (DUSH).

S. tora (L.) Roxb., Fl. Ind. 2: 340 (1832). *Cassia tora* L. A perennial, erect herb or undershrub. *Representative specimen:* Jaigir, 30.12.06, Kanika 259 (DUSH).

30. Fabaceae

Butea monosperma (Lamk.) Taub. in Engl. & Prantl, Nat. Pflanz. 3(3): 366 (1894). *Erythrina monosperma* Lamk. *Local name: Palash.* A deciduous tree. *Representative specimen:* Putail, 03.03.06, Kanika 79 (DUSH).

Cajanus cajan (L.) Druce, Rep. Bot. Exch. Cl. Brit. Isles, 1916: 611 (1917). *Cytisus cajan* L. *Local name*: *Orhor*. A shrub. *Representative specimen***:** Nabagram, 04.03.06, Kanika 100 (DUSH).

Crotalaria pallida Aiton, Hort. Kew. 2: 20 (1789). *Local name*: *Bara jhunjhuni*. An annual herb. *Representative specimen:* Jaigir, 30.12.06, Kanika 266 (DUSH).

Dalbergia sissoo Roxb., Fl. Ind. 3: 223 (1832). *Local name*: *Sisso*. A tree. *Representative specimens***:** Nabagram, 04.03.06, Kanika 104 (DUSH); Garpara, 28.12.06, Kanika 229 (DUSH).

Desmodium hetrophyllum (Willd.) DC., Prodr. 2: 334 (1825). *Hedysarum heterophyllum* Willd. A small herb. *Representative specimen:* Garpara, 28.12.06, Kanika 230 (DUSH).

D. triflorum (L.) DC., Prodr. 2:334 (1825). *Hedysarum triflorum* L. A trailing herb. *Representative specimen:* Putail, 03.03.06, Kanika 47 (DUSH).

D. triquetrum (L.) DC., Prodr. 2: 326 (1825). *Hedysarum triquetrum* L. A shrub. *Representative specimen:* Jaigir, 30.12.06, Kanika 255 (DUSH).

Erythrina ovalifolia Roxb., Fl. Ind. 3: 251 (1832). *Local name*: *Mandar*. A small deciduous tree. *Representative specimen:* Jaigir, 30.12.06, Kanika 254 (DUSH).

E. variegata L., Diss. Herb. Amb. Amoen. Acad. 4: 122 (1754). A small to medium sized tree. *Representative specimen:* Betila, 15.06.06, Kanika 130 (DUSH).

Sesbania grandiflora (L.) Poir. in Lamk., Encycl. Met. 7: 127 (1806). *Aeschynomene grandiflora* L. A tree. *Representative specimen***:** Nabagram, 04.03.06, Kanika 88 (DUSH).

31. Lythraceae

Lagerstroemia speciosa (L.) Pers., Syn. 2: 72 (1807). *Munchausia speciosa* L. *Local name*: *Jarul*. A large, deciduous tree. *Representative specimen:* Garpara, 17.06.06, Kanika 147 (DUSH).

32. Myrtaceae

Syzygium fruticosum (Roxb.) DC., Prodr. 3: 260 (1828). *Eugenia fruticosa* Roxb. *Local names*: *Banjam, Khudijam*. A small tree. *Representative specimen***:** Nabagram, 04.03.06, Kanika 99 (DUSH).

33. Onagraceae

Ludwigia adscendens (L.) Hara, J. Jap. Bot. 28: 291 (1953). *Jussiaea abyssinica* L. *Local name*: *Keshardam*. A creeping aquatic herb. *Representative specimen:* Manikgonj Sadar, 10.09.06, Kanika 161 (DUSH).

L. hyssopifolia (G. Don) Exell., Fernand. Garica de Orta 5: 471 (1957). *Jussiaea hyssopifolia* G. Don. A herb. *Representative specimen:* Jaigir, 16.06.06, Kanika 136 (DUSH).

34. Combretaceae

Terminalia arjuna (Roxb. *ex* DC.) Wight. & Arn., Prodr.: 314 (1834). *Pentaptera arjuna* Roxb. *ex* DC. *Local name*: *Arjun*. A medium-sized tree. *Representative specimen***:** Nabagram, 27.12.06, Kanika 209 (DUSH).

T. citrina (Gaertn.**)** Roxb. *ex* Fleming, Asiat. Res. 11: 183 (1810). *Myrobalanus citrina* Gaertn. *Local name: Horitaki.* A tall tree. *Representative specimen:* Jaigir, 30.12.06, Kanika 243 (DUSH).

35. Loranthaceae

Dendrophthoe falcata (L. f.) Etting., Denkschr. Akad. Wisscnch. Wicn. Math.- Natur. Cl. 32: 51 (1872). *Loranthus falcatus* L. f. *Local names: Bancha, Phorolla.* A parasite shrub. *Representative specimen:* Manikgonj, 02.03.06, Kanika16 (DUSH).

36. Euphorbiaceae

Croton bonplandianus Bill., Adansonia 4: 339 (1864). *Local name*: *Moricha*. An annual herb. *Representative specimen:* Jaigir, 30.12.06, Kanika 257 (DUSH).

Euphorbia hirta L., Sp. Pl.: 454 (1753). *Local names*: *Ghaopata, Dudhia*. An annual, robust herb. *Representative specimen:* Putail, 11.09.06, Kanika 180 (DUSH).

Jatropha curcas L., Sp. Pl. 2: 1006 (1753). A shrub or small tree. *Representative specimen:* Manikgonj Sadar, 02.03.06, Kanika 19 (DUSH).

J. gossypifolia L., Sp. Pl. 2: 1006 (1753). *Local names: Lalbherenda, Laljeol.* A small shrub. *Representative specimen***:** Nabagram, 04.03.06, Kanika 105 (DUSH).

Macaranga denticulata (Blume) Muell.-Arg. in DC., Prodr. 15 (2): 1000 (1886). *Mappa denticulata* Blume. *Local name*: *Bura*. A small, ever green tree. *Representative specimen:* Garpara, 17.06.06, Kanika 145 (DUSH).

Phyllanthus reticulatus Poir. in Lamk., Encycl. Meth. B. 5: 298 (1804). *Local names*: *Panseuli, Panku*. A large, scandent shrub. *Representative specimen:* Manikgonj Sadar, 02.03.06, Kanika 39 (DUSH).

Ricinus communis L., Sp. Pl. 2, 1007 (1753). *Local name*: *Redhi*. An ever green shrub. *Representative specimen:* Putail, 03.03.06, Kanika 49 (DUSH).

Trewia nudiflora L., Sp. Pl.: 2, 1193 (1753). *Local names: Bhatam, Lattu, Latim. Pitali.* A medium-sized deciduous tree. *Representative specimen***:** Nabagram, 04.03.06, Kanika 89 (DUSH).

37. **Vitaceae**

Cayratia trifolia (L.) Domin, Biblioth. Bot. 89: 370 (1927). *Vitis trifolia* L. *Local name*: *Amal-lata*. A herbaceous climber. *Representative specimen***:** Nabagram, 27.12.06, Kanika 210 (DUSH).

Cissus quadrangularis L., Syst. Nat. ed. 12 (2): 124 (1767). *Local names*: *Harjora lata*, *Kumor lata*. A herbaceous climber. *Representative specimen:* Jaigir, 30.12.06, Kanika 267 (DUSH).

38. Anacardiaceae

Lannea coromandelica (Houtt.) Merr., J. Arnold Arbor. 19: 353 (1938). *Dialium coromandelicum* Houtt. *Local names*: *Jiga, Jeol, Jika, Bhadi*. A medium-sized, deciduous tree. *Representative specimen:* Putail, 03.03.06, Kanika 78 (DUSH).

39. Meliaceae

Aphanamixis polystachya (Wall.) Parker, Ind. For. 57: 486 (1931). *Aglaia polystachya* Wall. *Local names*: *Royna, Roonna, Pitraj.* A tree. *Representative specimen:* Jaigir, 30.12.06, Kanika 256 (DUSH).

Azadirachta indica A. Juss., Mem. Mus. Hist. Nat. Paris 19: 221, t. 13 (1830). *Local name: Neem.* An evergreen tree. *Representative specimen:* Putail, 11.09.06, Kanika 181 (DUSH).

Melia azedarach L., Sp. Pl.: 384 (1753). *Local names*: *Mohanim, Bokain, Poma, Ghora neem*. A medium-sized cultivated tree. *Representative specimen:* Manikgonj, 02.03.06, Kanika 33 (DUSH).

40. Rutaceae

Aegle marmelos (L.) Corr., Trans. Linn. Soc. 5: 223 (1800). *Crataeva marmelos* L. *Local name*: *Bel*. A small, deciduous tree. *Representative specimen***:** Nabagram, 04.03.06, Kanika 106 (DUSH). Cultivated.

Glycosmis pentaphylla (Retz.) A. DC., Prodr. 1: 538 (1824). *Limonia pentaphylla* Retz. *Local names*: *Datmajan, Matkila, Ranggach, Matmati*. A shrub or small tree. *Representative specimen***:** Nabagram, 27.12.06, Kanika 211 (DUSH).

Limonia acidissima L., Sp. Pl. ed. 1: 554 (1763). *Limonia elephantum* (Correa) Panigrah. *Local name: Kathbel*. A medium-sized, semi-deciduous, spiny tree. *Representative specimen:* Putail, 03.03.06, Kanika 50 (DUSH).

41. Oxalidaceae

Averrhoa carambola L., Sp. Pl.1: 428 (1753). *Local name*: *Kamranga*. A bushy tree. *Representative specimen***:** Nabagram, 04.03.06, Kanika 90 (DUSH).

Oxalis corniculata L., Sp. Pl. 1: 435 (1753). *Local names*: *Amrul, Amboli, Chukatripati.* An annual herb. *Representative specimen:* Putail, 15.06.06, Kanika 126 (DUSH).

42. Apiaceae

Centella asiatica (L.) Urban in Mart., Fl. Bras. 11: 287 (1879). *Hydrocotyle asiatica* L. *Local names*: *Thankuni, Thulkuri, Brahmabuti*. A perennial herb. *Representative specimen:* Jaigir, 16.06.06, Kanika 137 (DUSH).

43. Apocynaceae

Alstonia scholaris (L.) R. Br., Mem. Wern. Nat. Hist. Soc. 1: 75 (1811). *Echites scholaris* L. *Local names*: *Chhatim, Chatwan*. A medium-sized tree. *Representative specimen:* Jaigir, 30.12.06, Kanika 250 (DUSH).

Ichnocarpus frutescens (L.) R. Br. in Mem. Wern. Soc. 1: 62 (1809). *Apocynum frutescens* L. *Local names*: *Dudhilata, Kalilata, Loi, Paralilata, Shamalata.* A climbing shrub. *Representative specimen:* Manikgonj Sadar, 02.03.06, Kanika 39 (DUSH).

Rauwolfia serpentina (L.) Benth. *ex* Kurz, For. Fl. Brit. Burma 2: 171 (1877). *Ophioxylon serpentinum* L. *Local name*: *Sarpaganda*. A woody herb. *Representative specimen:* Garpara, 17.06.06, Kanika (DUSH).

44. Asclepiadaceae

Calotropis gigantea (L.) W.T. Aiton, Hort. Kew. ed. 2, 2: 78 (1811). *Aselepias gigantea* L. A large shrub. *Representative specimen:* Putail, 03.03.06, Kanika 77 (DUSH).

C. procera (Ait.) R. Br. in Ait. f., Hort. Kew. ed. 2, 2:78 (1811). *Asclepias procera* Ait. *Local name: Akanda*. A large shrub. *Representative specimen:* Garpara, 17.06.06, Kanika 148 (DUSH).

45. Solanaceae

Datura metel L., Sp. Pl.: 179 (1753). *Local name*: *Dutra*. A pernnial herb. *Representative specimen:* Jaigir, 16.06.06, Kanika 137 (DUSH).

Nicotiana plumbaginifolia Viv., Elench. Pl. Hort. Dinegro: 26. t. 5 (1802). *Local name*: *Ban-tamak.* An erect, annual herb. *Representative specimen***:** Nabagram, 27.12.06, Kanika 212 (DUSH).

Physalis minima L., Sp. Pl.: 183 (1753). *Local names*: *Byakur, Gurkamai.* An annual herb. *Representative specimen:* Jaigir, 30.12.06, Kanika 268 (DUSH).

Solanum nigrum L., Sp. Pl.: 186 (1753). *Local name*: *Tit begun*. An annual shrub. *Representative specimen:* Putail, 03.03.06, Kanika 51 (DUSH).

S. torvum Sw., Nov. Gen. Sp. Pl.: 47 (1788). *Local name*: *Bot begun*. A small shrub. *Representative specimen:* Betila, 12.09.06, Kanika 198 (DUSH).

46. Convolvulaceaess

Ipomoea aquatica Forssk., Fl. Aeg. Arab.: 44 (1775). *Local name*: *Kalmilata*. An aquatic herb. *Representative specimen***:** Nabagram, 04.03.06, Kanika 108 (DUSH).

I. fistulosa Mart. *ex* Choisy in DC., Prodr. 9: 349 (1845). *Local names*: *Dholkalmi, Durakalma*. A shrub. *Representative specimen:* Jaigir, 30.12.06, Kanika 269 (DUSH).

Merremia hederacea (Burm. f.) Hallier f., Bot. Jahrb. 18: 118 (1994). *Evolvulus hederaceus* Burm. f. A twinner. *Representative specimen:* Garpara, 17.06.06, Kanika 149 (DUSH).

M. hirta (L.) Merr., Philip. J. Sc. Bot. 7: 244 (1912). *Convolvulus hirtus* L. A slender twiner. *Representative specimen:* Putail, 03.03.06, Kanika 76 (DUSH).

47. Cuscutaceae

Cuscuta reflexa Roxb., Pl. Corom. 2: 3, t. 104 (1798). *Local names*: *Algusi, Jarbuti, Swarnalata*. A fleshy parasite. *Representative specimen***:** Nabagram, 27.12.06, Kanika 211 (DUSH).

48. Boraginaceae

Heliotropium indicum L., Sp. Pl.: 139 (1753). *Local name*: *Hatisur*. An annual herb. *Representative specimen:* Manikgonj Sadar, 10.09.06, Kanika 165 (DUSH).

49. Verbenaceae

Clerodendrum indicum (L.) Kuntze, Rev. Gen. Pl. 2: 586 (1891). *Siphonanthus indica* L. *Local names: Bamunhati, Banchat.* An undershrub. *Representative specimen:* Manikgonj Sadar, 02.03.06, Kanika 31 (DUSH).

C. viscosum Vent., Jard. Malm. 1: t. 25 (1803). *Local names*: *Bhant, Ghetu, Ghetuphul.* A perennial, woody herb. *Representative specimen:* Putail, 11.09.06, Kanika 191 (DUSH).

Duranta repens L., Sp. P1. 1: 637 (1753). *Local names: Duranta, Kantamehedi.* A shrub or small tree. *Representative specimens***:** Nabagram, 04.03.06, Kanika 109 (DUSH); Garpara, 28.12.06, Kanika 231 (DUSH).

Lantana camara L., Sp. Pl.: 627 (1753). A shrub. *Representative specimens:* Putail, 11.09.06, Kanika 183 (DUSH); Garpara, 28.12.06, Kanika 232 (DUSH).

Lippia javanica (Burm. f.) Spreng., Syst. 2: 752 (1825). An undershrub. *Representative specimen:* Jaigir, 30.12.06, Kanika 270 (DUSH).

.**Vitex pubescens** Vahl, Symb. Bot. 3: 85 (1794). A large tree. *Representative specimen:* Manikgonj Sadar, 02.03.06, Kanika 40 (DUSH).

50. Lamiaceae

Anisomeles indica (L.) O. Kuntze, Rev. Gen.: 512 (1891). *Nepeta indica* L. *Local name: Gobura.* A bushy undershrub. *Representative specimen:* Putail, 03.03.06, Kanika 52 (DUSH).

Dysophylla crassicaulis Benth. in Wall., Pl. As. Rar. 1: 30 (1830). An annual herb. *Representative specimen:* Garpara, 17.06.06, Kanika (DUSH).

Leonurus sibiricus L., Sp. Pl.: 584 (1735). An erect sherb. *Representative specimen***:** Nabagram, 04.03.06, Kanika (DUSH).

Leucas aspera Spreng., Syst. 2: 743 (1825). *Local name*: *Durung pata*. An annual herb. *Representative specimen:* Jaigir, 30.12.06, Kanika 251 (DUSH).

Ocimum tenuiflorum L., Sp. Pl.: 597 (1753). *Ocimum sanctum* L. *Local name*: *Kalo tulshi*. A much branched, soft hairy, perennial herb. *Representative specimen:* Betila, 12.09.06, Kanika 199 (DUSH).

51. Scrophulariaceae

L. crustacea (L.) F. Muell., Census Austral. Pl. 1: 97 (1882). *Capraria crustacea* L. A small annual herb. *Representative specimen:* Putail, 15.06.06, Kanika 127 (DUSH).

Scoparia dulcis L., Sp. Pl.: 166 (1753). *Local names*: *Bandhuni, Bandhoney, Phurphuri*. A herb. *Representative specimen:* Manikgonj Sadar, 02.03.06, Kanika 30 (DUSH).

52. Acanthaceae

Andrographis paniculata (Burm.f.) Nees in Wall., P1. Asiat. Rar. 3: 116 (1832). *Justicia paniculata* Burm. f. *Local name: Kalmegh.* An erect annual herb. *Representative specimen:* Putail, 03.03.06, Kanika 63 (DUSH).

Justicia adhatoda L., Sp. Pl.: 15 (1753). *Adhatoda vasica* Nees. *Local name*: *Basak*. A shrub. *Representative specimens***:** Nabagram, 04.03.06, Kanika 91 (DUSH); Jaigir, 30.12.06, Kanika 245 (DUSH).

J. gendarussa Burm. f., Fl. Ind.: 10 (1768). *Local names: Jagatmadan, Jagmadan*. An undershrub. *Representative specimen:* Jaigir, 30.12.06, Kanika 248 (DUSH).

Rungia pectinata (L.) Nees in DC., Prodr. 11: 470 (1847). *Justicia pectinata* L. *Local name: Pindi.* A prostrate or suberect herb. *Representative specimen:* Manikgonj Sadar, 02.03.06, Kanika 15 (DUSH).

53. Bignoniaceae

Fernandoa adenophyllla (Wall. *ex.* G. Don.) van Steenis, Blumea 23: 135 (1976). *Bignonia adenophylla* Wall. *ex.* G. Don. *Local name: Banpata.* A tree. *Representative specimen***:** Nabagram, 04.03.06, Kanika 110 (DUSH).

54. Campanulaceae

Lobelia radicans Thunb., Trans. Linn. Soc. 2: 330 (1794). A prostrate herb. *Representative specimen:* Putail, 03.03.06, Kanika 53 (DUSH).

55. Rubiaceae

Dentella repens (L.) J. R. & G. Forst., Char. Gen. P1. Ins. Mar. Austr.: 26, t, 13 (1776). *Oldenlandia repens* L. *Local name: Bhuipat.* An annual prostrate herb**.** *Representative specimen:* Manikgonj Sadar, 02.03.06, Kanika 20 (DUSH).

Ixora acuminata Roxb., Fl. Ind. 1: 393 (1820). An undershrub. *Representative specimen***:** Nabagram, 27.12.06, Kanika 212 (DUSH).

Neolamarckia cadamba (Roxb.) Bosser, Bull. Mus. Hist. Nat. Paris, Ser. 6, Sec. B, Adans. 3: 247 (1984). *Anthocephalus cadamba* (Roxb.) miq.; *Anthocephalus chinensis* (Lamk.) A. Rich. *ex* walk. *Local name: Kadam. A* large tree spreading sub whorled branches. *Representative specimen:* Putail, 03.03.06, Kanika 64 (DUSH).

Paederia foetida L., Mant. Pl. 1: 52 (1767). *Local name*: *Gandha badhuli*. A slender climber. *Representative specimen***:** Nabagram, 04.03.06, Kanika 111 (DUSH).

56. Asteraceae

Ageratum conyzoides L., Sp. Pl.: 839 (1753). *Local names*: *Dulkuri, Hialmuti*. An annual herb. *Representative specimen:* Manikgonj Sadar, 10.09.06, Kanika 166 (DUSH).

Blumea lacera (Burm. f.) DC. in Wight, Contrib. Bot. Ind.: 14 (1834). *Conyza lacera* Burm. f. *Local names*: *Barakukshima, Barakosing, Barasaksang, Kukursunga, Kuksung*. An erect, annual herb. *Representative specimen***:** Nabagram, 04.03.06, Kanika 92 (DUSH).

Chromolaena odorata (L.) King & Robinson, Phytologia 20: 204 (1970). *Eupatorium odoratum* L. *Local names: Germanlata, Barashialmuti.* An erect herb. *Representative specimen:* Jaigir, 30.12.06, Kanika 252 (DUSH).

Eclipta alba (L.) Hassk., Pl. Jav. Rar.: 528 (1848). *Local name: Kesoraj.* An annual herb. *Representative specimen:* Manikgonj Sadar, 02.03.06, Kanika 04 (DUSH).

Enhydra fluctuans Lour., Fl. Cochinch.: 511 (1790). *Local name*: *Helencha*. An annual aquatic herb. *Representative specimen:* Putail, 03.03.06, Kanika 54 (DUSH).

Gnaphalium luteo-album L., Sp. Pl.: 851 (1753). *Local name: Bara kamra*. An erect, annual herb. *Representative specimen:* Jaigir, 30.12.06, Kanika 247(DUSH).

Grangea maderaspatana (L.) Poir., Enc. Suppl. 2: 825 (1811). *Artemisia maderaspatana* L. *Local name: Nemuti.* An annual herb. *Representative specimen:* Manikgonj, 02.03.06, Kanika 14 (DUSH).

Mikania cordata (Burm. f.) B.L. Robinson, Contrib. Gray Herb. 104: 65 (1934). *Eupatorium cordatum* Burm. f. *Local names*: *Assamlata, Tarulata*. A perennial herb. *Representative specimen:* Putail, 03.03.06, Kanika 69 (DUSH).

Spilanthes calva DC. in Wight, Contrib. Bot. Ind.: 19 (1834) *Spilanthes acmella* auct. *non* L. *Local name*: *Marhatitiga*. An annual herb. *Representative specimen:* Putail, 03.03.06, Kanika 70 (DUSH).

Synedrella nodiflora (L.,) Gaertn., Fruct. 2: 456. t. 171 (1791). An erect annual herb. *Representative specimen:* Jaigir, 16.06.06, Kanika 140 (DUSH).

Tridax procumbens L., Sp. Pl.: 900 (1753). *Local name: Tridara*. An annual or perennial, procumbent herb. *Representative specimen:* Betila, 12.09.06, Kanika 200 (DUSH).

Vernonia cinerea (L.) Less., Linnaea 4(1): 291 (1829). *Conyza cinerea* L. *Local name: Shial lata.* An erect herb. *Representative specimen:* Putail, 03.03.06, Kanika 55 (DUSH).

Wedelia chinensis (Osbeck) Merr., Philipp. J. Sci. Bot. 12: 111(1917). *Solidago clinensis* Osbeck. *Local name: Kesharaja.* A procumbent or decumbent herb. *Representative specimen:* Putail, 03.03.06, Kanika 65 (DUSH).

Xanthium strumarium L., Sp. Pl. 2: 987 (1753). *Local name: Gaghra.* An erect annual herb. *Representative specimen:* Manikgonj Sadar, 02.03.06, Kanika 21 (DUSH).

Liliopsida (Monocots)

57. Alismataceae

Sagittaria guayanensis H.B. & K. subsp. **lappula** (D. Don) Bogin in Mem. N.Y. Bot.,Gard. 9: 192 (1955). *Sagittaria guayanensis sensu* Hook. An annual, scapigerous herb. *Representative specimen***:** Nabagram, 27.12.06, Kanika 213 (DUSH).

S. sagittifolia L., Sp. P1. 2: 993 (1753). *Local names: Muyamuya, Chhotokut.* A scapigerous aquatic herb. *Representative specimen:* Putail, 11.09.06, Kanika 185 (DUSH).

58. Hydrocharitaceae

Blyxa octrandra (Roxb.) Planch. *ex.* Thw. Enum. Pl. Zeyl.: 332 (1864). A stemless, submerged herb. *Representative specimen***:** Nabagram, 04.03.06, Kanika 97 (DUSH).

Hydrilla verticillata (L. f.) Royle, 3. Bot. Himal. t. 376. (1839). *Serpicula verticillata* L. f. An auquatic herb. *Representative specimen:* Putail, 03.03.06, Kanika 68 (DUSH).

Ottelia alismoides (L.) Pers., Syn. P1. 1: 400 (1805). *Stratiotes alismoides* L. A submerged herb. *Representative specimens:* Manikgonj Sadar, 02.03.06, Kanika 05 (DUSH); Garpara, 28.12.06, Kanika 234 (DUSH).

59. Aponogetonaceae

Aponogeton appendiculatus van Bruggen in Blumea 16: 265 (1968). *Local name: Gheehu.* A perennial stoloniferous herb. *Representative specimen:* Manikgonj Sadar, 02.03.06, Kanika 29 (DUSH).

A. natans (L.) Engl. & Krause, Pflanzenreich, Aponogetonaceae: 11 (1906). *Saururus natans* L. A herb with stoloniferous rootstock. *Representative specimen***:** Nabagram, 27.12.06, Kanika 214 (DUSH).

60. Arecaceae

Calamus viminalis Willd, Sp. Pl. 2 (1): 203 (1799). A thicket forming climbers. *Representative specimen:* Putail, 11.09.06, Kanika 189 (DUSH).

Caryota urens L., Sp. Pl.: 1189 (1753). An erect, solitary palm. *Representative specimens:* Putail, 03.03.06, Kanika 66 (DUSH); Garpara, 28.12.06, Kanika 235 (DUSH).

61. Araceae

Alocasia macrorrhizos (L) G. Don in Sweet, Hort. Brit. ed. 3: 631(1839). *Arum macrorrhizon* L. *Local name: Man kachhu.* A stout herb. *Representative specimen:* Manikgonj Sadar, 02.03.06, Kanika 06 (DUSH).

Amorphophallus bulbifer (Roxb.) Blume in Rumphia 1: 148 (1837). *Arum bulbiferum*. Roxb. A tuberous herb. *Representative specimen:* Manikgonj Sadar, 10.09.06, Kanika 167 (DUSH).

Colocasia esculenta (L.) Schott in Schott & Endl., Melet. Bot.: 18 (1832). *Arum esculenta* L. A perennial herb. *Representative specimen:* Putail, 03.03.06, Kanika 56 (DUSH).

Lasia spinosa (L.) Thw., Enum. P1. Zeyl.: 336 (1864). *Dracontium spinosum* L. *Local name: Kanta kanchhu.* A prickly, aquatic herb. *Representative specimen***:** Nabagram, 04.03.06, Kanika 93 (DUSH).

Pistia stratiotes L., Sp. Pl. 963 (1753). A free floating, monoecious herb. *Representative specimen:* Manikgonj Sadar, 02.03.06, Kanika 23 (DUSH).

T. trilobatum (L.) Schott., Wien. Zeitschr. 3: 72 (1829). *Arum trilobatum* L. A tuberous herb. *Representative specimen:* Putail, 03.03.06, Kanika 71 (DUSH).

62. Lemnaceae

Lemna perpusilla Torrey, Fl. N. York. 2: 245 (1843). A free floating, aquatic herb. *Representative specimen:* Putail, 03.03.06, Kanika 57 (DUSH).

63. Commelinaceae

Commelina benghalensis L., Sp. Pl.: 41 (1753). *Local nams: Kanchira, Dholpata*. A slender herb. *Representative specimen***:** Nabagram, 04.03.06, Kanika 112 (DUSH).

64. Cyperaceae

Cyperus compressus L., Sp. Pl. ed. 1: 46 (1753). A tufted annual herb. Representative specimen: Manikgonj, 02.03.06, Kanika 08 (DUSH).

C. diffusus Vahl, Enum. Pl. 2: 321 (1806). A perennial herb. *Representative specimens*: Putail, 27-12-06; Manikgonj Sadar, 10.09.06, Kanika 168 (DUSH).

C. distans L. f., Suppl. Pl.: 103 (1781). *Local name*: *Panimalancho*. A perennial herb. *Representative specimen:* Jaigir, 30.12.06, Kanika 271 (DUSH).

C. iria L., Sp. Pl. ed. 1: 45 (1753). *Local name: Barachancha.* An annual herb. *Representative specimen***:** Nabagram, 27.12.06, Kanika 219 (DUSH).

C. michelianus (L.) Link., Hort. Bot. Berol. Descr. 1: 303 (1827). *Scirpus michelianus* L. An annual herb. *Representative specimen:* Putail, 03.03.06, Kanika 58 (DUSH).

C. rotundus L., Sp. Pl.: 45 (1753). *Local name*: *Mutha ghas*. A perennial herb. *Representative specimen:* Putail, 03.03.06, Kanika 72 (DUSH).

Diplacrum caricinum R. Br., Prodr. Fl. Nov. Holl.: 241 (1810). An annual herb. *Representative specimen:* Betila, 12.09.06, Kanika 203 (DUSH).

Fimbristylis acuminata Vahl, Enum. Pl. 2: 285 (1806). An annual herb. *Representative specimen:* Manikgonj, 02.03.06, Kanika 09 (DUSH).

F. cymosa R. Br., Prodr. Fl. Nov. Holl.: 228 (1810). A rhizomatous, perennial herb. *Representative specimen***:** Nabagram, 04.03.06, Kanika 113 (DUSH).

65. Poaceae

Axonopus compressus (Sw.) P. Beauv., Ess. Agrost. 12: 154 (1812). *Milium compressum* Sw. A perennial, tufted grass. *Representative specimen***:** Nabagram, 04.03.06, Kanika 94 (DUSH).

Bambusa balcooa Roxb., Fl. Ind. 2: 196 (1832). *Local names: Barakbans, Balukbans, Valku, Balkua.* A tall, densely caespitose bamboo. *Representative specimen:* Betila, 15.06.06, Kanika 132 (DUSH).

Chrysopogon aciculatus (Retz.) Trin., Fund. Agrost.: 188 (1820). *Andropogon aciculatus* Retz. A glabrous grass. *Representative specimen***:** Nabagram, 04.03.06, Kanika 96 (DUSH).

Cynodon dactylon (L.) Pers., Syn. Pl. ed. 1: 85 (1805). *Panicum dactylon* L. *Local name*: *Durba*. A creeping grass. *Representative specimen***:** Nabagram, 04.03.06, Kanika 120 (DUSH).

Cyrtococcum accrescens (Trin.) Stapf in Hook., Ic. Pl.: sub t. 3096 (1922). *Panicum accrescens* Trin. An annual, scrambling grass. *Representative specimen:* Putail, 03.03.06, Kanika 73 (DUSH).

Dactyloctenium aegyptium (L.) P. Beauv., Ess. Agrost. Expl. Pl.: 15 (1812). *Cynosurus aegyptius* L. A stoloniferous, annual or short-lived perennial grass. *Representative specimen:* Putail, 11.09.06, Kanika 186 (DUSH).

Echinochloa crus-galli (L.) P. Beauv., Ess. Agrost. 53: 161 (1812). *Panicum crus-galli* L. *Local name*: *Bara shyama ghas*. An annual or perennial grass. *Representative specimen:* Manikgonj Sadar, 02.03.06, Kanika 12 (DUSH).

Echinochloa stagnina (Retz.) P. Beauv., Ess. Agrost.: 161 (1812). *Panicum stagninum* Retz. An aquatic, perennial grass. *Representative specimen***:** Nabagram, 04.03.06, Kanika 114 (DUSH).

Eleusine indica (L.) Gaertn., de Fruct. 1: 8 (1789). *Cynusurus indicus* L. *Local name*: *Malanga kuri*. An annual, tufted herb. *Representative specimen:* Manikgonj Sadar, 02.03.06, Kanika 24 (DUSH).

Hemarthria protensa Steud., Syn. Pl. Glam.: 359 (1854). *Local names: Chalia, Chailla.* An erect to decumbent grass. *Representative specimen:* Putail, 03.03.06, Kanika 75 (DUSH).

Hygroryza aristata (Retz.) Nees in Wight & Arn., Edinburgh. New Philos. J. 15: 380 (1833). *Pharus aristatus* Retz. A floating grass. *Representative specimen:* Garpara, 28.12.06, Kanika 236 (DUSH).

Imperata cylindrica (L.) Reaeschel, Nom. Bot. ed. 3: 10 (1797). *Lagurus cylindricus* L. *Local names*: *Chan, Chau, Sarkanb, Son*, *Ulukhar*. A perennial, rhizomatous grass. *Representative specimen:* Manikgonj Sadar, 02.03.06, Kanika 26 (DUSH).

Oplismenus compositus (L.) P. Beauv., Ess. Agrost. 54: 168 (1812). *Panicum compositum* L. A perennial grass. *Representative specimen***:** Nabagram, 04.03.06, Kanika 115 (DUSH).

Panicum montanum Roxb., Fl. Ind. 1: 315 (1820). A perennial, tufted grass. *Representative specimen:* Putail, 03.03.06, Kanika 59 (DUSH).

P. repens L., Sp. Pl. ed. 2: 87 (1753). A perennial, rhizomatous grass. *Representative specimen:* Jaigir, 30.12.06, Kanika 253 (DUSH).

Paspalum scrobiculatum L., Mant. 1: 29 (1767). *Local name*: *Goicha*. An annual grass. *Representative specimen:* Putail, 03.03.06, Kanika 61 (DUSH).

Saccharum spontaneum L., Mant. Pl. 2: 183 (1771). *Local name*: *Kash*. A perennial, tall grass. *Representative specimen:* Jaigir, 30.12.06, Kanika 246 (DUSH).

Setaria palmifolia (Koen.) Stapf, J. Linn. Soc. Bot. 42: 186 (1914). *Panicum palmaefolium* Koen. *Local name*: *Urodhan*. A perennial grass. *Representative specimen:* Putail, 03.03.06, Kanika 74 (DUSH).

Themeda quadrivalvis (L.) O. Kuntze, Rev. Gen. Pl. 2: 793 (1891). *Andropogon quadrivalvis* L. An annual herb. *Representative specimen:* Manikgonj Sadar, 02.03.06, Kanika 11 (DUSH).

Vetiveria zizanioides (L.) Nash in Small, Fl. Southeast U.S.: 67 (1903). *Phalaris zizanoides* L. *Local names: Bena, Ghandhabena, Gandamul, Benamul.* A rhizomatous, aromatic, perennial grass. *Representative specimen:* Manikgonj Sadar, 02.03.06, Kanika 26 (DUSH).

66. Zingiberaceae

Alpinia nigra (Gaertn.) Burtt., Notes Roy. Bot. Gard. Edinb. 35: 213 (1977). *Zingiber nigrum* Gaertn. *Local name*: *Tara*. A rhizomatous perennial herb. *Representative specimen:* Manikgonj Sadar, 02.03.06, Kanika 13 (DUSH).

Curcuma zedoaria (Christm.) Rosc. in Trans. Linn. Soc. Lond. 8: 354 (1807). *Amomum zedoaria* Christm. *Local name: Shoti.* A rhizomatous herb. *Representative specimen***:** Nabagram, 27.12.06, Kanika 217(DUSH).

67. Costaceae

Costus speciosus (Koen.) Smith, Trans. Linn. Soc. London 1: 249 (1791). *Banksea speciosa* Koen. *Local names*: *Gardong*, *Jongliphul*. A rhizomatous herb. *Representative specimen:* Betila, 12.09.06, Kanika 204 (DUSH).

68. Pontederiaceae

Eichhornia crassipes (Mart.) Solms in A. DC., Mon. Phan. 4: 527 (1883). *Pontederia crassipes* Mart. *Local name*: *Kachuripana.* A free-floating herb. *Representative specimen***:** Nabagram, 04.03.06, Kanika 95 (DUSH).

Monochoria hastata (L.) Solms. in A. DC., Mon. Phan. 4: 523 (1883). *Pontederia hastata* L.. An aquatic, emergent herb. *Representative specimen:* Putail, 03.03.06, Kanika 60 (DUSH).

69. Liliaceae

Asparagus acerosus Roxb., Fl. Ind. 2: 150 (1832). *Local name*: *Shatamuli*. A perennial, subscandent undershrub. *Representative specimen:* Betila, 15.06.06, Kanika 133 (DUSH).

Curculigo orchioides Gaertn., de Fruct. 1: 63, t. 13 (1788). *Local names*: *Tali, Talamuli, Talura, Langtipata*. A rhizomatous, perennial herb. *Representative specimen:* Manikgonj Sadar, 10.09.06, Kanika 170 (DUSH).

Molineria recurvata (Dryand.) Herbert, Amaryl.: 84 (1834). *Curculigo recurvata* Dryand. *Local name*: *Satipata*. A herb with tuberous rootstocks. *Representative specimen***:** Nabagram, 04.03.06, Kanika 116 (DUSH).

70. Smilacaceae

Smilax zeylanica L., Sp. Pl. 2: 1029 (1753). *Local name*: *Kumarilata*. A prickly climber. *Representative specimens:* Garpara, 28.12.06, Kanika 237 (DUSH); Jaigir, 30.12.06, Kanika 220 (DUSH).

71. Dioscoreaceae

Dioscorea bellophylla (Prain) J.O. Voigt *ex* Haines, For. Fl. Choto Nagpur: 530 (1910). *Dioscorea nummularia* var. *belophyla* Prain. *Local name*: *Shora Alu*. A perennial climber. *Representative specimen***:** Nabagram, 04.03.06, Kanika 118 (DUSH).

D. bulbifera L., Sp. Pl. 2: 1033 (1733). *Local name: Bon Alu.* A large climber. *Representative specimen:* Manikgonj Sadar, 10.09.06, Kanika 171 (DUSH).

D. hispidia Dennst., Hort. Ind. Malabar.: 33 (1818). *Dioscorea daemona* Roxb. A twining climber. *Representative specimen***:** Nabagram, 27.12.06, Kanika 215 (DUSH).

D. pentaphylla L., Sp. Pl. 2: 1032 (1753). *Local name*: *Jhum Alu*. A climber. *Representative specimen:* Manikgonj Sadar, 02.03.06, Kanika 27 (DUSH).

72. Orchidaceae

Vanda tessellata (Roxb.) Hook. f. *ex* G. Don in Loud., Hort. Brit.: 372 (1830). *Epidendrum tessellatum* Roxb. An epiphytic herb. *Representative specimen:* Putail, 03.03.06, Kanika 67 (DUSH).

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GROWTH OF CYANOBACTERIA IN SALINE SOIL AMENDED WITH NP FERTILIZERS

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Abstract

Assessment of cyanobacterial population in saline soil amended with three rates of each of N (0, 50, 100 kg ha^{-1}) and P (0, 25, 50 kg ha^{-1}) fertilizers in a factorial combination showed a significant variation during growth of rice. Quantitatively the population of cyanobacteria ranged from 26.90 to 70.83 × 10^4 g^{-1}, 32.07 to 82.03 × 10^4 g^{-1} and 31.03 to 74.47 × 10^4 g^{-1} soil at 30, 60 and 90 days of transplantation of rice seedlings respectively. The highest and lowest values were encountered in $N_{50}P_{50}$ and $N_{100}P_0$ treatments respectively irrespective of the sampling intervals. Addition of P accentuated the proliferation of cyanobacteria while that of N inhibited their abundance significantly with increasing level of the applied fertilizers. Joint contribution of P and N stimulated significantly better growth of cyanobacteria.

Key words: Cyanobacteria, Nitrogen, Phosphorus, Saline soil

Introduction

The significant contribution of cyanobacteria (blue-green algae) in the flooded rice soils to atmospheric nitrogen fixation has now been well recognized (Roger and Kulasooriya 1980, Kaushik 2002, Rinaudo *et al*. 1971 and Araragi *et al*. 1978). Among the nitrogen fixing agents in the rice field ecosystem, the immence significance of cyanobacteria as an alternative source of N_2-fixation deserves more attention, recently, due to continuous increase of the cost of synthetic N-fertilizer. The process of nitrogen fixation is virtually a complex biochemical process and mainly depends on environmental conditions. Abundance of cyanobacteria in soil and their potential capacity practically determine the extent of N to be fixed.

Intensive cultivation of rice causes the tremendous depletion of nutrients like NPK Zn in the soil. To over come this problem, the use of these chemical fertilizers become unavoidable for rice farming areas. The significant positive role of P-fertilization on growth of the indigenous cyanobacteria in rice field ecosystem has already been established by Roger and Kulasooria (1980), Khushik (2002) and Begum *et al*. (2008).

Reports are available on the performance of cyanobacteria in the normal rice field (Rinaudo *et al*. 1971 and Araragi *el al*. 1978), in contrast, very little information about their role in salt affected rice field is available (Kaushik 2002). A field experiment was, therefore, designed to evaluate of the role of N and P on the growth of cyanobacteria in a saline induced rice field situated in the belt of southern part of Bangladesh.

Materials and Methods

A field experiment was conducted in a moderately saline (Ec 7.3 dsm^{-1}) rice field of Khulna district during boro season. N (urea) (0, 50, 100 kg N ha^{-1}) and P (TSP) (0, 25, 50 kg P ha^{-1}) in a factorial combination together with a basal dose of K (MP) (50 kg K ha^{-1}) were applied. The field was ploughed mechanically, watered and leveled. The land was, finally, divided into three blocks. Each block was again subdivided into nine sub-plots. The unit plot size was 4m × 2m. Nine treatments, in triplicate, were allocated following a randomized block design. N and P were applied, accordingly, into two equal splits during final land preparation and at maximum tillering stage of rice.

Thirty days old seedlings of BR dhan-28 variety of rice collected from farmer seed bed were transplanted at the rate of three seedlings per hill. The hill-to-hill distance was 15 cm. The line to line spacing was 20 cm. Weeds were removed manually whenever required. Irrigation was given frequently to maintain the water level (1.25 cm above the soil surface). Soil samples were collected at 30, 60 and 90 days after transplanting for quantitative enumeration of cyanobacteria following standard method.

Results and Discussion

Impact of N and P on the abundance of cyanobacteria was found to be modified appreciably (Table 1). Incorporation of P-fertilizer caused a significant increase in the number of cyanobacteria with the increase in the rate of the fertilizer at 30, 60, and 90 days of transplantation.

Table 1. Abundance of indigenous cyanobacteria (× 10^4 g^{-1} soil) in rice field amended with NP fertilizers.

Treatments (kg ha^{-1})	Days of transplantation		
	30	60	90
N_0P_0	28.00 f	37.87 cd	35.00 cd
N_0P_{25}	42.00 d	46.70 c	37.77 c
N_0P_{50}	64.37 b	65.73 b	57.10 b
$N_{50}P_0$	25.64 f	38.00 cd	26.43 e
$N_{50}P_{25}$	54.00 c	62.28 b	62.11 b
$N_{50}P_{50}$	70.83 a	82.03 a	74.47 a
$N_{100}P_0$	26.90 f	30.07 d	31.03 de
$N_{100}P_{25}$	34.40 e	45.73 c	36.00 cd
$N_{100}P_{50}$	35.40 e	44.27 c	41.77 c

Level of significance, P = 0.05. In a column, figures having similar letter(s) do not differ significantly whereas figures with dissimilar letter(s) differ significantly as per DMRT.

At 30 days of transplantation of rice seedlings, the maximum number of cyanobacterial population (70.83×10^4 g^{-1} soil) was recorded in the plot receiving 50 kg P and 50 kg N ha^{-1} together. Similarly, the second highest number (64.37×10^4 g^{-1} soil) was in the sub-plot with 50 kg P ha^{-1} only. In contrast, the minimal number of population (26.90×10^4 g^{-1} soil) was in the treatment where 100 kg N ha^{-1} was applied alone. Very identical number of the population i.e. 34.40×10^4 and 35.40×10^4 g^{-1} soil was recorded in the treatments with 100 kg N along with 25 kg P and 100 kg N together with 50 kg P ha^{-1}, respectively. Cyanobacterial population decreased with the increase of applied N though not significantly.

However, at 60 days of transplantation, the maximal number of cyanobateria (82.03×10^4 g^{-1} soil) was in the plot with N and P at the rate of 50 kg ha^{-1}. Incorporation of 25 kg P ha^{-1} showed 46.70×10^4 g^{-1} soil of the cyanobacteria. The number, however, increased significantly to 65.73×10^4 g^{-1} soil when the amount of P increased from 25 to 50 kg ha^{-1}. Number of cyanobacterial population decreased insignificantly from 37.87×10^4 to 30.07×10^4 g^{-1} soil due to increase of nitrogen from 0 to 100 kg ha^{-1}.

Similarly at 90 days of transplantation, the lowest number of cyanobacterial population (26.43×10^4 g^{-1} soil) was in the treatment provided with 50 kg N ha^{-1} alone. Supply of 25 kg P ha^{-1} showed 37.77×10^4 g^{-1} soil of cyanobacterial population and the number increased significantly to 57.10×10^4 g^{-1} soil due to increase of P from 25 to 50 kg ha^{-1}. The population was estimated to be 62.11×10^4 g^{-1} soil ranking the second highest in the treatments receiving 25 kg P and 50 kg N ha^{-1} applied in combination. However, the number increased significantly to 74.47×10^4 g^{-1} soil when 50 kg N with 50 kg P applied together ranking the highest position among the treatments imposed in the experiment. The number of cyanobacterial population decreased significantly to 41.77×10^4 g^{-1} soil when 100 kg N and 50 kg P ha^{-1} was applied together. Addition of P (0, 25, 50 kg P ha^{-1}) resulted a significant increase in the number of cyanobacteria with the increase in the level of P over the control irrespective of the duration of sampling.

Retardation in the number of cyanobacteria was assessed to be the highest in the plot supplied with N (50 and 100 kg N ha^{-1}). However, this depressive situation was significantly improved due to supplementation of P at intermediate level of N (50 kg N ha^{-1}). The stimulatory and positive interaction of P with N promoted the growth of cyanobacteria significantly at all stages of sampling. However, the stimulative effect of P was found to be reduced at the highest level of N when applied in combination. Moreover, the efficacy of P became leveled off in the presence of 100 kg N ha^{-1} resulting a nonsignificant variation in the number of cyanobacteria in the treated plot. The number of cyanobacteria was found to increase with the growth span up to 60 days and thereafter their abundance decreased at 90 days of transplantation of the rice crop.

Application of N in the rice field significantly retarded the cyanobacteria enumerated in the location under investigation at 30, 60 and 90 days of transplantation of rice seedlings.

This suggests that enrichment of the soil with fertilizer-N inhibited the growth of cyanobacteria. The reason might be attributed to the fact that N_2- fixing cyanobacteria are profoundly favoured by a lack or competitiveness of the other algae and can proliferate profusely in soil poor in nitrogen content particularly in rice field. This possibly explains the fact that nitrogen fixing cyanobacteria are retarded or at least affected to some extent in the presence of nitrogen in soil. Similarly the selective action and inhibitory effect of added N-fertilizers on N_2- fixing blue-green algae in rice fields of Ivory Coast was reported by other workers (Renaudo 1974).

Supply of P generally encouraged the growth of cyanobacteria resulting a significant flush in their number irrespective of the duration of sampling intervals. These findings are in agreement with the reports of other investigators (Than Thun 1969 and Srinivasan 1978). The positive relationship of growth of blue-green algae with the available P content of soil has also been demonstrated earlier (Araragi *et al*. 1978, Okuda and Yamaguchi 1952, Ishizawa *et al*. 1975 and Yamaguchi 1975).

Results revealed that the inhibitory impact of fertilizer N on the abundance of cyanobacteria can be overcome significantly due to incorporation of P in the with N-fertilized plot. However, this positive interaction of P with N was found to be only statistically significant at intermediate dose of N i.e. 50 kg N ha^{-1}. The role of P became leveled off resulting an insignificant impact on the growth of cyanobacteria at the highest dose of N (100 kg N ha^{-1}). Nevertheless, the interaction of P with intermediate dose of N ($N_{50}P_{25}$, $N_{50}P_{50}$) was significantly better to promote the growth of cyanobacteria than the individual impact of intermediate rate of N i.e. 50 kg N ha^{-1} irrespective of sampling intervals.

The outcome of the investigation suggests that modern methods of biotechnology may be improved through cyanobacterial biofertilization, provided the relationship of cyanobacteria with soil is well understood through coordinated research in the laboratory and field.

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LIMNOLOGICAL STATUS OF TRIMOHINI BEEL OF RAJSHAHI, BANGLADESH

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Abstract

A total of 38 zooplankton genera and 26 physico-chemical variables were recorded in Trimohini *Beel*. This *beel* marked as a medium level of polluted wetland based on the values of the redox characteristics i.e. pH, DO, BOD, COD, Eh and rH_2, chlorides, nitrites, ammonium, phosphate values etc. and on the presence of some zooplankton as indicator of pollution. A large number of inland fresh water non-culturable fishes and other aquatic biota of the Trimohini *Beel* may be eliminated in future due to mixing of continuous chemicals from agriculture fields. It is necessary to conserve the ecosystem of Trimohini *Beel* for the fresh water non-culturable fishes and other aquatic biota.

Key words*:* Wetland, Agro-chemicals, Zooplankton, Fish disease, Catchment area, Eutrophic nature

Introduction

The wetland ecosystem of Bangladesh is composed of more than 700 rivers, streams, numerous haors, baors, beels, seasonal and perennial floodplain etc. (BBS 1997). During the recent years captive or ponds fishery and beel fishery have become popular in the country. The total number ponds in the country are 12888222 covering an area of 150000 ha. A total of 2832792 ha seasonal floodplain and 1031563 ha is permanent riverine and estuary water body (Nuruzzaman 1990, SPARRSO 1984 and FIB 1986). Through proper culture based fishery and efficient management, an increased fish production of about 16 million m.t./year is possible in inland waters in Bangladesh (Islam 1992).

The agro-chemicals from terrestrial runoff of agricultural fields enter into surface water. This has resulted in the total elimination of a large number of inland fresh water non-cultureable fishes and other biota. Ammonia is produced in surface water by decomposition of organic matters and hydrolysis of urea. Acidification and pollution of the surface water are created by PO_4, SO_4, chlorides etc. along with insecticides and herbicides, and this acidic polluted water is responsible for fish epizootic ulcerative syndrome (Conway and Pretty 1991, Swarup *et al*.1992, Bhatt *et al.* 1999, Bandela *et al*.1999, Cudchodkar and D'souza 1996 and Islam 2004). Limnological knowledge and their proper applications for better fish yield and management of the ecosystems are prerequisites to sustainable fish sectoral development and maintenance of the eutrophic nature of the wetland ecosystem (Wetzel 1983). Fish health and management of other

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biodiversity are practical applications of limnology and aquacultural environment (Khan and Chowdhury 1974 and Toetz 1971). It is most important to study the interrelationship between the physico-chemical aspects and between phyto-zooplankton and the effect of agricultural effluent on the biota. Bhatt *et al*. (1999), Das *et al*. (2002), Mishra and Trivedy (1993), Moyle (1946), Rai (1974) studied the limnology and biology of rivers, lakes and wetlands in India. Islam and Khatun (1966), Islam and Nahar (1967), Islam and Shaha (1975) studied the limnology of the polluted waters in Bangladesh. In the recent years environmental scientists of the country are interested to study the limnological status of surface water bodies to assess the water quality and biodiversity for conservation planning of the wetlands. A natural perennial wetland known as Trimohini *Beel* is situated under Mohonpur *upazila* of Rajshahi, Bangladesh. Intensive cultivation is being practiced in the catchment area of this wetland and organic, inorganic manures, insecticides, herbicides and fungicides are used at heavy doses, as a result water quality changed (Islam 2004). The available scientific documents in Bangladesh reveal that Tromohini *Beel* has not been studied up to date. So it is necessary to know the limnological conditions of this wetland for sustainable conservation of aquatic ecosystem. Thus the present investigation has been carried out to find out the physico-chemical and zooplankton conditions of Trimohini *Beel* of Rajshahi for evaluating the pollution level of water of this wetland.

Materials and Methods

Trimohini wetland is near Rajshahi, Bangladesh. The investigated wetland lies between 24º35' to 24º40'N latitudes and between 88º35' to 88º40'E longitudes (Anon 1997). This perennial wetland is 24 km^2 (8 km×3 km) and covers an area of 50 km^2 (10 km×5 km) during monsoon. The catchments area is 150 km^2 (20 km×7.5 km), where intensive cultivation is being practiced by using organic, inorganic manures, insecticides, herbicides and fungicides. Water and plankton samples were collected from January 2010 to December 2011 in four different spots of the wetlands. Fortnightly samplings were done in this wetland and in each sampling date physico-chemical and biological samples were collected three times (8 A.M, 12 Noon and 4 P.M.). An average of these data for each spot was made and average depth of each spot was also determined. Water samples were collected from a depth 10-15 cm below the surface using a 250 ml. BOD bottle. Water colour was detected by following method as stated by Welch (1948). Temperature was noted by a digital thermometer (Model China Empex-range 10-110˚C). Transparency was determined by a Secchi disc. A digital pH meter (Model pH epi HANNA instruments CEEN 50081-1) and portable conductivity meter (Model OSK CM-1K) were used for the measurement of pH and conductivity respectively. DO content of water was measured by DO meter (Model- JENWAY-9015). Free CO_2, CO_3, HCO_3, alkalinity, total hardness, chloride, BOD_5, COD, mobile NH_3 and NH_4-N were determined by following APHA (1989), FAO (1984) and (Welch 1948). Total phosphate, Oxidation-reduction Potential

(Eh) and Oxidation reduction index (rH_2) were measured by following Gautam (1990). Primary productivity was measured by Gaarder and Graan (1927).

Plankton were collected by using a plankton net No. 20 silk bolting cloth of mesh size 76µm. Identification of plankton was done immediately after collection. Plankton abundance was measured by using a Sedgewick-Rafter counting chamber (Welch 1948) and expressed in unit/l whether it is an individual or part thereof.

Results and Discussion

In this study 26 physico-chemical variables were measured and 38 genera of zooplankton were recorded. Average data of 4 spots are presented in Tables 1 and 2 respectively.

During the period of study water colour was found always transparent except monsoon (June to August) in the four study spots. Air temperature was almost similar in all study spots and ranged from 19-33.5º C (yearly mean value 29.00±5.88º C). Water temperature varied from 20-32.5º C (yearly mean value 28.29±4.96º C). Average depth of water was 60-320 cm (yearly mean value 158.8± 94.6 cm). Transparency was 20-110 cm (yearly mean value 72.08± 29.88 cm). TSS varied from 250-380 mg/l (yearly mean value 273.3±49.92 mg/l) in all spots. Electric conductivity ranged from 84.6-110.5 µmho/cm (yearly mean value 93.65±10.15 µmho/cm) in all spots. pH varied from 7.2-7.5 (yearly mean value 7.35±0.10). Free CO_2 ranged from 13-23 mg/l (yearly mean value 17.50±3.23 mg/l). CO_3 alkalinity was nil in all spots and HCO_3 alkalinity varied from 45-72 mg/l (yearly mean value 61.20±11.75 mg/l). Ca-hardness and Mg-hardness ranged from 75.0-85.0 mg/l (yearly mean value 80.28±3.53 mg/l) and 40.0-52.4 mg/l (yearly mean value 48.12±3.96 mg/l) respectively in all spots. Total hardness varied from 120-137 mg/l (yearly mean value 128.40±7.03 mg/l). Chloride ranged from 60-100 mg/l (yearly mean value 78.79±12.62 mg/l). DO values varied from 5.3-5.7 mg/l (yearly mean value 5.57±0.16 mg/l) and percentage of sat. of oxygen ranged from 64.5-78.2 (yearly mean value 71.66±5.14) in all spots. BOD and COD values varied from 5.9-6.4 mg/l (yearly mean value 6.21±0.16 mg/l) and 13.70-14.64 mg/l (yearly mean value 14.28±0.30 mg/l) respectively. Nitrite-nitrogen and NH_4-N ranged from 0.15-0.59 mg/l (yearly mean value 0.39±0.16 mg/l) and 0.15-0.36 mg/l (yearly mean value 0.28±0.08 mg/l) respectively. PO_4 values varied from 0.20-0.45 mg/l (yearly mean value 0.33±0.07 mg/l). Eh and rH_2 values were from 0.28-0.32 mv (yearly mean value 0.30±0.01 mv) and 24.48-25.40 (yearly mean value 25.02±0.31) respectively. Gross primary productivity (GPP) and net primary productivity (NPP) ranged from 0.016-0.020 mgC/h/l (yearly mean value 0.018±0.001 mgC/h/l) and 0.008-0.011 mgC/h/l (yearly mean value 0.009±0.0012 mgC/h/l) respectively in all spots.

Table1. Average Physico-chemical conditions of all study spots of Trimohini wetland, Mahonpur of Rajshahi.

Parameters	Jan.	Feb.	Mar.	April.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Mean±SD
Air Temp. ºC	19	20	32.5	33	33	32.5	32.5	33	33.5	32	27	20	29.00±5.88
Water Temp.ºC	20	21	30	32	32	31	31	32	32.5	30	28	20	28.29±4.96
Av. Depth (cm)	110	100	85	75	60	85	300	320	270	230	150	120	158.8±94.6
S.D. Depth (cm)	110	100	85	75	60	50	20	20	70	80	95	100	72.08±29.88
T.S.S (mg/l)	250	250	250	255	250	260	380	380	255	250	250	250	273.3±49.92
E. Con (µmoh/cm)	110.5	108.5	90.6	86.6	86.6	88.6	88.6	86.6	84.6	86.6	95.5	110.5	93.65±10.15
pH	7.2	7.3	7.3	7.4	7.5	7.4	7.4	7.4	7.5	7.3	7.3	7.2	7.35±0.10
Free CO_2 (mg/l)	20	20	15	15	13	13	20	23	15	18	18	20	17.50±3.23
CO_3 (mg/l)	0	0	0	0	0	0	0	0	0	0	0	0	0
HCO_3 (mg/l)	72	72	70	50	45	48	48	49	68.4	70	70	72	61.20±11.75
Ca hard (mg/l)	76	78	81	82	83	83	80	81	84.4	85	75	75	80.28±3.53
Mg hard (mg/l)	44	47	49	50	52	52	40	49	52.4	52	45	45	48.12±3.96
Total hard (mg/l)	120	125	130	132	135	135	120	130	136.8	137	120	120	128.40±7.03
Chloride (mg/l)	75	77	65	63	60	88	95	100	87.5	85	75	75	78.79±12.62
DO (mg/l)	5.7	5.6	5.5	5.4	5.4	5.8	5.7	5.7	5.3	5.4	5.6	5.7	5.57±0.16
% of Sat. of O_2	64.5	64.5	73.04	73.8	73.8	78.2	76.8	77.90	72.90	67.70	72.30	64.50	71.66±5.14
BOD_5 (mg/l)	6.2	6.3	6.2	6.1	6.0	5.9	6.3	6.3	6.4	6.1	6.4	6.3	6.21±0.16
COD (mg/l)	14.26	14.45	14.26	14.07	13.88	13.70	14.45	14.45	14.64	14.07	14.64	14.45	14.28±0.30
Nitrite-nitrogen (mg/l)	0.30	0.30	0.25	0.20	0.15	0.58	0.59	0.58	0.56	0.50	0.40	0.30	0.39±0.16
NH_4-N (mg/l)	0.35	0.30	0.15	0.15	0.20	0.25	0.25	0.30	0.35	0.36	0.36	0.36	0.28±0.08
PO_4 (mg/l)	0.30	0.30	0.25	0.25	0.20	0.35	0.40	0.45	0.40	0.35	0.35	0.30	0.33±0.07
Eh (mv)	0.32	0.32	0.30	0.29	0.28	0.30	0.30	0.30	0.28	0.30	0.31	0.32	0.30±0.01
rH_2	25.4	25.35	25.09	24.48	24.63	25.04	25.05	25.05	24.58	25.05	25.15	25.40	25.02±0.31
GPP (mgC/h/l)	0.018	0.018	0.019	0.020	0.020	0.016	0.017	0.017	0.020	0.020	0.018	0.018	0.018±0.001
NPP (mgC/h/l)	0.009	0.009	0.010	0.011	0.011	0.008	0.008	0.008	0.010	0.011	0.009	0.009	0.009±0.001

In total 38 zooplankton genera were recorded from four spots during the period of study of which 12 genera belonged to Copepoda (31.58%), 12 to Cladocera (31.58%) and 14 to

Rotifer (36.84%) (Table 2). The average zooplankton abundance of four spots varied from 15015-26000 units/l with mean abundance (21240 units/l).

The abundance of Copepoda, Cladocera and Rotifera varied from 6740-11170 units/l (mean abundance 9138 units/l; 43.02%), 3730-6565 units/l (mean abundance 5418 units/l; 25.51%) and 4545-8265 units/l (mean abundance 6684 units/l; 31.47%) respectively.

The *Copepoda* were *Allodiaptomus* sp., *Cyclops* sp., *Diaptomus* sp., *Eucyclops* sp., *Heliodiaptomus* sp., *Paradiatomus* sp., *Phyllodiaptomus* sp., *Rhinediaptomus* sp., *Macrocyclops* sp., *Mesocyclops* sp.,*Orthocyclops* sp. and *Paracyclops* sp.. *Diaptomus* sp. (22.28%) and *Cyclops* sp. (20.46%) were found to occur in higher abundance followed by *Eucyclops* sp. (12.22%), *Mesocyclops* sp. (9.83%) and others.

The *Cladocerans* were represented by *Alona* sp., *Alonella* sp., *Bosmina* sp., *Bosminopsis* sp. *Ceriodaphnia* sp., *Daphnia* sp., *Diaphanosoma* sp., *Macrothrix* sp., *Moina* sp., *Polyphemus* sp., *Sida* sp. and *Simocephalus* sp.. The *Cladoceran* were dominated by *Diaphanosoma* sp.(10.50%), *Simocephalus* sp. (10.27%), *Daphnia* sp. (9.89%) and others.

The Rotifers were represented by *Brachionus* sp., *Dorystoma* sp., *Filinia* sp., *Gastropus* sp., *Harringia* sp., *Hexarthra* sp., *Keratella* sp., *Monostyla* sp., *Notholca* sp., *Philodina* sp., *Platyias* sp., *Polyarthra* sp., *Scaridium* sp. and *Trichocerca* sp.. The Rotiferans were dominated by *Trichocerca* sp.(11.20%), *Keratella* sp. (10.86%), *Brachionus* sp. (9.91%) and others.

The Rotifers are tolerant to varying degree of physico-chemical and biological conditions. Islam *et al*. (2005), Islam *et al*. (2001) and Arora (1966) observed that the rotifers occur in eutrophic waters in high abundance. Islam *et al*. (2005), Islam *et al*. (2001), Arora (1966) and Bergins (1949) designated a large number of rotifers genera including *Barchionus* sp., *Keratella* sp., *Filinia* sp. *Gastropus* sp., *Hexarthra* sp., *Polyarthra* sp., *Trichocerca* sp. and others as indicators of eutrophic or polluted waters and the findings of the present study appears to be in conformity with their works.

Air and water temperature was always found with low values. Indiscriminately uses of agro-chemicals like urea, TSP, chloride in catchment area of the wetland caused the water of the wetland acidic (Islam 2004). In unpolluted rivers the values of chloride are usually low (between 2 to 10 mg/l). The desirable level of chloride in water should be below 200 mg/l for human consumption (Koshy and Nayar 1999). In the studied wetland the chloride content showed higher values. The higher amounts of nitrite nitrogen (NO_2-N) and ammonium nitrogen (NH_4-N) indicated the higher loads of organic matters and excessive use of inorganic fertilizers which was washed into natural water by the rain and flood water (Islam 2004 and Swarup *et al*. 1992). Low pH, Eh, DO and high rH_2, BOD, COD are the indication of presence of both organic and inorganic load in study water coupled with NH_4-N, NO_2-N and PO_4 values and the whole physical-chemical variable

together formed a complex of nutrient status of biological importance imparting an eutrophic nature to study water (Morris and Stumm 1967, Morrissette and Mavinic 1978, Lakshminarayana 1965 and Jayangaudar 1964).

Table 2. Monthly average of abundance (units/l) of zooplankton of Trimohini wetland.

Zooplankton	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Yearly Mean	%
Copepoda														
Allodiaptomus 2sps.	275	270	265	275	280	290	285	250	195	175	200	230	249	2.73
Cyclops 3sps.	1800	2000	1890	2050	2100	2150	2100	2000	1650	1500	1590	1600	1869	20.46
Diaptomus 2sps.	1900	2050	2200	2250	2300	2400	2350	1800	1775	1650	1750	2000	2035	22.28
Eucyclops 2sps.	1000	1120	1200	1250	1300	1400	1300	1200	1100	800	825	900	1116	12.2
Heliodiaptomus sp.	450	475	500	550	575	600	575	500	450	350	375	400	483	5.29
Paradiaptomus sp.	295	300	320	350	375	400	350	300	250	195	200	260	300	3.28
Phyllodiaptomus sp.	300	250	350	370	380	400	385	300	270	200	230	270	309	3.38
Rhinediaptomus sp.	290	320	340	350	380	430	350	325	275	230	240	250	315	3.45
Macrocyclops sp.	900	940	960	980	1000	1050	950	900	600	500	540	870	849	9.29
Mesocyclps sp.	100	1020	1050	1090	1120	1150	1000	950	900	700	750	950	898	9.83
Orthocyclops sp.	290	300	310	350	350	320	300	250	195	160	200	250	273	2.99
Paracyclops sp.	400	470	500	550	570	580	490	400	350	280	295	400	440	4.82
Total	8000	9515	9885	10415	10730	11170	10435	9175	8010	6740	7195	8380	9138	100
Cladocera														
Alona 2sps.	360	355	370	380	390	340	300	290	270	210	290	350	325	6.01
Alonella 2sps.	390	385	395	430	450	470	450	430	340	290	330	400	397	7.32
Bosmina 2sps.	410	420	450	470	490	500	495	445	395	300	390	440	434	8.01
Bosminopsis 2sps.	390	380	400	430	450	470	490	400	350	320	350	345	398	7.34
Ceriodaphnia sp.	400	430	450	470	490	520	490	410	370	330	370	400	428	7.89
Daphnia 2sps.	500	540	580	600	620	670	620	600	400	340	460	490	535	9.87
Diaphanosoma 2sps.	550	590	620	640	670	690	640	610	420	370	495	530	569	10.50
Macrothrix 2sps.	540	570	600	620	640	680	610	500	400	350	410	450	531	9.80
Moina 3sps.	380	390	420	450	480	500	440	400	360	300	340	345	400	7.39

(Contd.)

Zooplankton	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Yearly Mean	%
Polyphemus 2sps.	500	490	520	540	580	600	550	520	440	320	480	500	503	9.29
Sida 2sps.	300	330	350	380	400	430	400	350	300	250	295	320	342	6.31
Simocephalus 2sps.	520	540	580	600	640	695	640	600	500	350	495	515	556	10.27
Total	5240	5420	5735	6010	6300	6565	6125	5555	4545	3730	4705	5085	5418	100
Rotifera														
Brachionus 3sps.	640	650	690	720	750	800	760	700	600	420	590	630	663	9.91
Dorystoma 2sps.	400	420	450	490	520	580	500	470	400	370	400	430	453	6.77
Filinia 2sps.	390	400	430	460	500	580	500	450	400	300	370	400	432	6.46
Gastropus 2sps.	460	480	500	530	560	600	550	500	450	390	450	475	495	7.41
Harringia 2sps.	450	425	440	460	495	620	490	400	370	300	430	440	443	6.63
Hexarthra 2sps.	330	360	390	400	430	470	400	320	295	240	320	330	357	5.34
Keratella 3sps.	700	730	760	790	820	850	790	720	650	440	740	720	726	10.86
Monostyla sp.	250	270	290	310	330	370	370	300	250	230	225	260	288	4.31
Notholca 2sps.	220	240	270	300	360	400	330	300	200	195	210	220	270	4.05
Philodina 2sps.	320	350	370	390	420	450	400	370	295	250	300	340	355	5.30
Platyias 3sps.	450	590	600	630	670	695	650	550	450	350	420	450	542	8.11
Polyarthra 2sps.	345	320	360	390	450	480	400	390	290	240	500	530	391	5.85
Scaridium 2sps.	500	530	580	600	640	680	520	420	390	320	520	550	521	7.79
Trichocerca 3sps.	750	725	750	800	840	690	920	840	750	500	700	720	749	11.20
Total	6205	6490	6880	7270	7785	8265	7580	6730	5790	4545	6175	6495	6684	100
Grand Total	19445	21425	22500	23695	24815	26000	24140	21460	18345	15015	18075	19960	21240	

The GPP and NPP values, higher amounts of CO_2 and absence of CO_3 throughout the study period indicate the low rate of photosynthesis and high rate of respiration by the aquatic biota in Trimihini *Beel*. Similar findings were recorded by Islam (2004), Bhatt *et al.* (1999) and Das *et al.* (2002) in their studies. Islam (2004) also observed that low air and water temperature, low pH etc. are the causes of epizootic ulcerative syndrome of fin fishes in natural wetlands. Higher amounts of nitrite nitrogen (NO_2-N) and ammonium nitrogen (NH_4-N) were recorded in this wetland, which are the causes of death of more than hundred species of fish (Cudchodkar and D'souza 1996, Islam *et al.* 2001 and Islam 2004). Islam (2004) mentioned that toxic chemicals including fungicides, insecticides,

herbicides, urea, chlorides, sulphates and phosphates were used at heavy doses in agricultural crops fields around the studied wetland; as a result residual effluent of used chemicals or as direct solution of chemicals entered into the water of wetland.

According to Gautam (1990), Chowdhury *et al*. (1996), Islam and Nahar (1967), Lakshiminarayana (1965), Jayangauder (1964), Zafar (1964), George (1968), Moyle (1946), Montgomery *et al.* (1964), Mortimer (1956), Islam *et al.* (1998), Zaman *et al.* (1993), Mishra *et al.* (1992) and Ameen *et al.* (1986) the studied wetland marked as a medium level polluted wetland on the basis of values of the redox characteristics i.e. pH, DO, BOD, COD, Eh, and rH_2 chlorides, nitrites, ammonium, phosphate values etc. and on the presence of some zooplankton as pollution indicator. The findings of the present study indicate that a large number of inland fresh water non-culturable fishes and other aquatic biota of the Trimohini *Beel* may be eliminated in future due to mixing of continuous chemicals from agriculture fields. Toxic chemicals have a deleterious effect on the wetlands ecosystem as a whole (Cudchodkar and D'souza 1996, Chowdhury *et al.* 1996 and Gautam 1990). So, it is necessary to conserve the ecosystem of Trimohini *Beel* for the fresh water non-culturable fishes and other aquatic biota. A sustainable management plan as well as area demarcation is necessary for the agriculture to protect the further degradation of water quality and biodiversity of the study area. Findings of this study will be helpful for further study and sustainable management plan of the fresh water wetlands.

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ELECTROSYNTHESIS OF Cu / ZnO NANOCOMPOSITE ELECTRODE ON ITO ELECTRODE AND ITS APPLICATION IN OXIDATION OF ASCORBIC ACID AND GLUCOSE

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Abstract

In the present study Cu nanoparticles (NPs), ZnO nanorods have been deposited on indium tin oxide (ITO) electrode and thereby Cu-NPs /ZnO / ITO composite film electrode has been prepared. The prepared nanostructures have been characterized by using scanning electron microscope (SEM). The shape of the electrochemically deposited ZnO follow rod like structure on ITO electrode, Cu–NPs follow irregular spherical shape when electrodeposited on ITO electrode. Oxidation of ascorbic acid on bare ITO electrode is favorable than that of ZnO modified ITO electrode but Cu-NPs/ ZnO composite electrode had high sensitivity and stability and showed higher catalytic current for glucose oxidation.

Key words: Nanoparticles (NPs), Nanocomposite electrode, Indium tin oxide (ITO), Ascorbic acid, Glucose

Introduction

Electrochemical sensors and biosensors for the electroanalysis of biologically active compounds have attracted a great deal of interest due to their analytical performances (Xu *et al.* 2006). Electrochemical sensors provide low detection limits, a wide linear response range, and good stability and reproducibility, among other advantages (Zhang *et al.* 1997 and Chen *et al*. 2008). However, bare electrodes are more likely than chemically modified electrodes to suffer from interferences or surface fouling by products arising from follow-up reactions, associated to the main electrochemical process. The modification of electrode surfaces with inorganic or organic coatings often avoids these drawbacks and represents a rapid and versatile resource for the preparation of stable and selective new electrochemical sensors (Evans *et al.* 2004 and Wang 2008).

Electrochemical methods have potential application in the detection of D (+)-glucose in blood samples (Wilson and Turner 1992) and also in bio-fuel cells (Park *el al.* 2006). Glucose oxidase (GOD) is one of the most extensively studied enzyme and has been successfully employed for preparing glucose biosensors. The development of glucose biosensors utilizing GOD is an active research area (Chen *et al.* 2002, Evans *et al.* 2004 and Wang 2008). A majority of glucose sensors, especially those used in in-vivo application are based on the electrochemical oxidation of hydrogen peroxide which is formed in the course of the enzyme-catalyzed oxidation of glucose by dissolved oxygen.

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Recently, excellent review articles have been published and discussed about the principles of electrochemical glucose biosensors and key challenges in their further development and use. It is shown that nano-ZnO film provides better environment and enhanced electron transfer between ChOx and electrode (Lupo *et al.* 2010).

Over the past decades, a number of studies have been conducted to alleviate the drawbacks of enzymatic glucose sensors. The most common and serious problem is insufficient stability originated from the nature of the enzymes which is hardly overcome. Although glucose oxidase (GOD) is quite stable compared with other enzymes, the glucose sensors based on GOD are always exposed to the possible thermal and chemical deformation during fabrication, storage or use. Further, GOD quickly loses its activity below pH 2 and above pH 8 and temperature above 40 °C can cause fatal damages (Wilson and Turner 1992). Ionic detergents also deactivate GOD as well. To overcome the above obstacles, non-enzymatic glucose sensors have been developed and kept coming closer to practical applications. Park and coworkers discussed about the merits and mechanism of glucose oxidation on non-enzymatic sensors (Park *et al.* 2006). Nanostructured particles have attracted extensive scientific and industrial interest due to their unique electronic, optical and catalytic properties. Nanoparticles can display four unique advantages over microelectrodes when used for electro analysis. These are: enhancement of mass transport, catalysis, high effective surface area and control over electrode microenvironment (Lupo *et al.* 2010). Electrochemical deposition of copper nanoparticles (Cu-NPs) and their applications in electro-catalysis is an active research area (Miao *et al.* 2008). Though, the Cu-NPs were prepared in several matrixes by several researchers, no effort has been made to prepare Cu-NPs embedded in functional matrix material like zinc oxide (ZnO). In our laboratory zinc oxide (ZnO) nano particles have been prepared by using simple combustion methods and by electrochemical methods onto ITO electrode in order to use as nano-electrodes. In the present study, ZnO NPs were deposited on the ITO electrodes by electrochemical method. Copper NPs were deposited onto ZnO/ITO electrode to obtain Cu-NPs / ZnO /ITO composite film electrode using electrochemical technique. The characterization of these electrodes have been performed by using SEM and possible applications of these electrodes as sensors have been examined by using electrochemical methods.

Materials and Methods

This research work was carried out at the physical chemistry research laboratory, Dhaka University, during the period of 2010.

Chemicals: Potassium chloride, KCl (BDH. UK), zinc nitrate, $Zn(NO_3)_2.6H_2O$ (MERCK, India), zinc acetate, $Zn(CH_3COO)_2.2H_2O$ (MERCK, Germany), copper sulfate, $CuSO_4.5H_2O$ (MERCK, Germany), absolute ethyl alcohol, (AR, BDH), ethylene glycol (AR, BDH), sodium hydroxide, NaOH (AR, BDH), N_2, 99.99% pure (BOC, Bangladesh), sulfuric acid, H_2SO_4(AR, BDH), potassium per chlorate, $KClO_4$ (AR, BDH), Glucose,

$C_6H_{12}O_6$ (AR, BDH), cetyltrimethyl ammonium bromide (CTAB) (BDH, UK), ascorbic acid, ($C_6H_8O_6$), (Merck, Germany) and potassium ferricyanide, $K_3Fe(CN)_6$ (Merck, Germany) were used without further purification.

Instruments: A Computerized Electrochemistry system, Model HQ 2040 was used in the present study. A pyrex glass volumetric cell (10 mL) with three electrode configurations, ITO as working electrode (area 3.2cm^2), platinum wire as the counter electrode and Ag/AgCl (satd. KCl) as the reference electrode was used in this study.

SEM (Hitachi, S-3400, Japan) was used in this experiment**.** In this SEM accelerating voltage was fixed ranging from 0.3 kV to 30.0 kV. Resolution was set in 3.0 nm at 30.0 kV accelerating voltage in high vacuum mode for Secondary Electron (SE) image. Back Scattered Electron (BSE) image resolution was set in 4.0 nm at 30.0 kV accelerating voltage in low vacuum mode. For all these images magnification was set at a range 5 times to 300000 times. From this SEM image size and different shapes of nanoparticles were characterized.

Supporting electrolyte solution:

(a) 100 mL of 0.1M KCl solution was prepared in deionized water.

(b) 100 mL of 0.1M NaOH solution was prepared in deionized water.

(c) $CuSO_4.5H_2O$ solution: 10 mL of 0.1M Cu^{2+} stock solution was prepared in 0.1M KCl.

(d) $K_3Fe(CN)_6$ solution: 10 mL of 0.1M $K_3Fe(CN)_6$ solution was prepared in 0.1M KCl.

(e) Glucose solution: 10 mL of glucose solution was prepared in 0.1M NaOH.

(f) Ascorbic acid solution: 10 mL of 0.1M Ascorbic acid stock solution was prepared in 0.1M KCl.

Electrochemical Method for the preparation of ZnO NPs:The conventional three electrode cell in which ZnO NPs were prepared was maintained at 40^{0}C in a water bath. The working electrode was a commercial ITO glass (30×66.66×6 mm, R(s) < 10 ohm). An aqueous solution of 0.1 M Zn $(NO_3)_2$ mixed with 0.1 M KCl was used and deposition was carried out for an hour. ZnO nanoparticles were electrodeposited on ITO electrode at -1.1 V vs. Ag/AgCl reference electrode and a Pt electrode was used as counter electrode. After deposition the resulting nanodeposits were thoroughly rinsed with water and dried under a nitrogen atmosphere.

Results and Discussion

Preparation of copper nanoparticles/zinc oxide composite modified electrode**:** ITO substrates were cleaned by using detergent, diluted hydrochloric acid and then finally rinsed with distilled water. Transparent ZnO films have been cathodically deposited onto conductive ITO glasses from a simple aqueous zinc nitrate electrolyte bath kept at 335 K. Electrodeposition of ZnO film was carried out potentiostatically (−0.7 V for 20 min)

using a potentio/ galvanostat without stirring. ZnO-modified ITO was rinsed with distilled water to remove unbounded materials from the electrode surface and then dried by nitrogen gas. Cu-NPs were deposited onto a ZnO film coated ITO from the solutions of 0.01 M $CuSO_4$+0.1 M $KClO_4$ under fixed applied potential of −0.9 V (vs. Ag/AgCl) for 3 min . For comparative studies, Cu-NPs were deposited onto an unmodified ITO as described above. Finally, the Cu-NPs/ZnO composite modified electrode was thoroughly washed with doubly distilled water to remove unbounded materials from the electrode surface and then dried in air for 30 min before use.

SEM images of ZnO nanoparticles:It is not possible to determine the exact particle size from SEM images which can be done through TEM analysis. The SEM images show the approximate concept about the formation of nanoparticles.

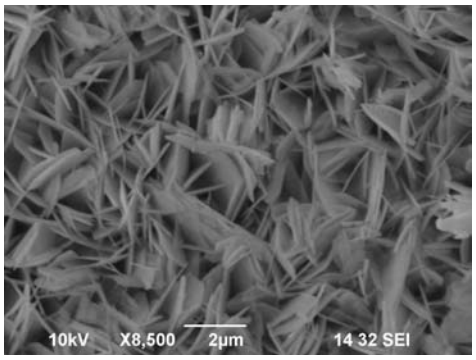

Fig. 1 (a) SEM image of ZnO NPs on ITO.

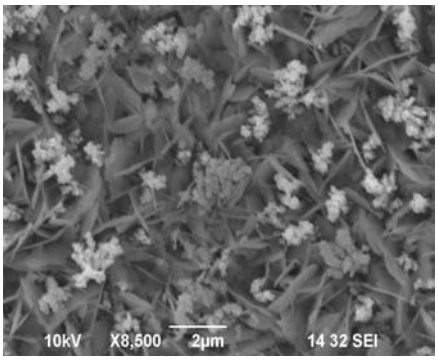

Fig. 1 (b) SEM image of Cu/ZnO NPs on ITO.

Fig. 1 shows the SEM images of (a) ZnO deposited on ITO electrode and (b) Cu particles formed on ZnO/ ITO electrode. It is evident from above figures that ZnO NPs prepared by electrochemical methods on ITO electrode exhibits a rod like structures. ZnO NPs modified ITO electrode can be used as nanoelectrode. Cu NPs when deposited electrochemically on these modified electrode follow irregular flower like structures as shown in Fig. 1 (b). ZnO / ITO electrode and Cu NPs / ZnO / ITO composite film electrodes have been used in the present study as sensors.

Electrochemical behaviour of $K_3Fe(CN)_6$ on ITO electrode for different scan rates: Fig.2 shows the CV of 0.40 mM $K_3Fe(CN)_6$ solution at different scan rates on ITO electrode. The potential was stepped from +0.650 V to a vertex potential of -0.150 V and finally the potential was reversed back to +0.650 V. The cathodic peak was observed at +0.158V. The corresponding anodic peak was observed at +0.230 V. Both cathodic and anodic peak current increases with increasing scan rate and potential remain almost constant.

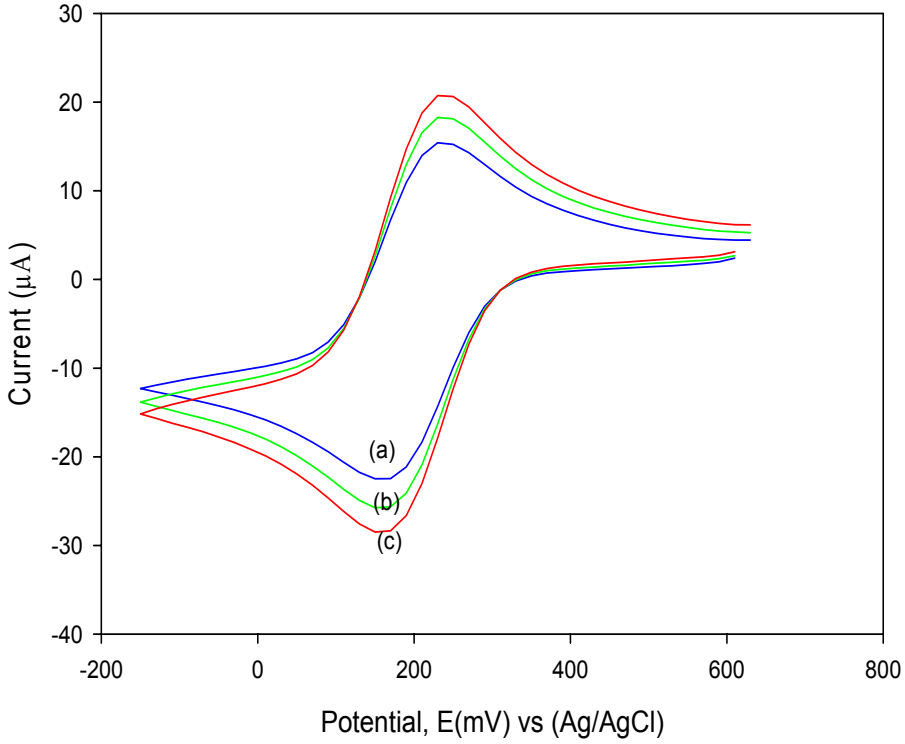

Fig. 2. CV of 0.40 mM $K_3Fe(CN)_6$ solution at (a) 15 mVs^{-1} (b) 20 mVs^{-1} (c) 25 mVs^{-1} S.R.

Electrochemical behaviour of $K_3Fe(CN)_6$ on ZnO Modified ITO electrode for different scan rates : Fig. 3. Shows the CV of 0.40 mM $K_3Fe(CN)_6$ solution at different scan rates on modified ITO electrode. The potential was stepped from +0.650 V to a vertex potential of -0.150 V and finally the potential was reversed back to +0.650 V. The cathodic peak was observed at +0.162 V. The corresponding anodic peak was observed at +0.245 V. Both cathodic and anodic peak current increases with increasing scan rate and potential remain almost constant.

Comparison of CV of $K_3[Fe(CN)_6]$ solution between ITO electrode and ZnO modified ITO electrode : Here the CV of $K_3[Fe(CN)_6]$ solutions (Fig. 4.) show that both the cathodic and anodic peak potential are almost at the same position for ITO electrode and modified ITO electrode.

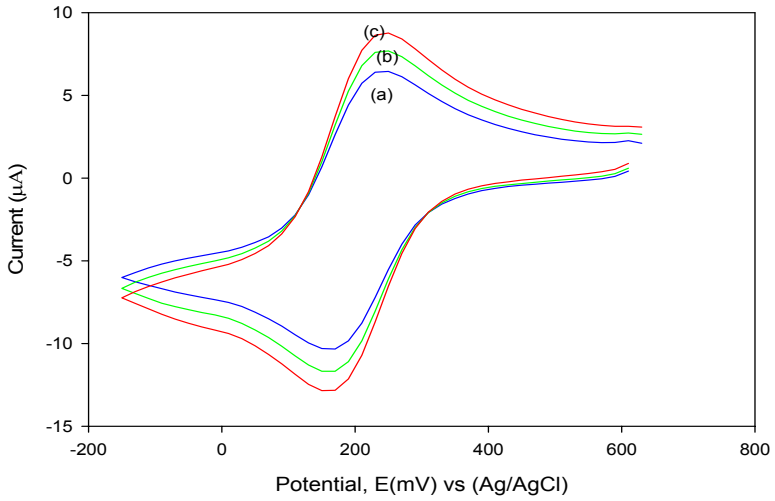

Fig.3. CV of 0.40 mM $K_3Fe(CN)_6$ solution at (a) 15 mVs^{-1} (b) 20 mVs^{-1} (c) 25 mVs^{-1} S.R.

But the current decreases significantly in the case of ZnO modified ITO electrode. The diffusion coefficient values were found as follows:

(a) The Diffusion co-efficient at ITO electrode =13.03×10^{-8} cm^2/s

(b) The Diffusion co-efficient at ZnO modified ITO electrode =2.22×10^{-8} cm^2/s.

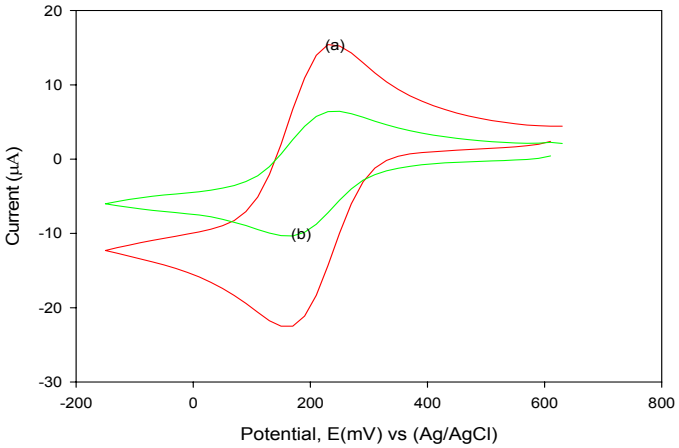

Fig.4. CV of 0.40 mM $K_3[Fe(CN)_6]$ solution at (a) ITO electrode (b) ZnO modified ITO electrode at scan rate 15 mVs^{-1}.

Oxidation of glucose on ITO, ZnO / ITO and Cu NPs/ ZnO / ITO composite electrode: From Fig. 5. it is evident that glucose exhibits an oxidation peak at around +0.8 V on ITO electrode (curve c) the peak current decreases when the CV is run on ZnO modified ITO electrode. However, the glucose oxidation enhances remarkably when the CV is carried out onto Cu NP/ ZnO/ ITO composite electrode. The peak current for glucose oxidation increases almost ten times indicating that Cu NPs play a strong catalytic role when deposited onto ZnO particles. CVs of the Cu NPs/ZnO composite modified electrode recorded in 0.1M NaOH in the presence (curve a) and absence (curve b) of 5 mM glucose. It exhibited a single oxidation peak around 0.4 to 0.8 V vs Ag/AgCl. Fig.5 (curve c and d) shows CVs obtained for glucose at a bare ITO electrode (curve c) and a ZnO modified electrode (curve d).

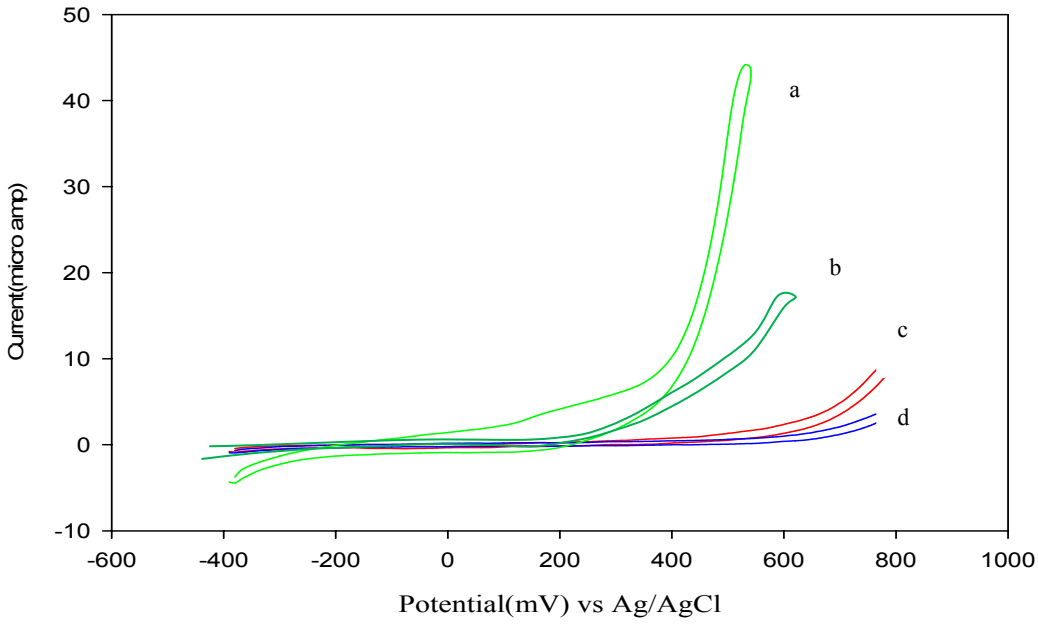

Fig. 5. The CVs of 5 mM glucose in 0.1 M NaOH solution at Cu-NPs/ZnO / ITO composite electrode (a) CV without glucose (curve b) The CVs of 5 mM glucose in 0.1 M NaOH solution at bare ITO (curve c) and at ZnO/ ITO (curve d) at a scan rate of 20 mV s^{-1}.

In this study ZnO nanostructures and Cu-NPs have been deposited onto ITO electrode by using electrochemical method. It was found that Cu-NPs / ZnO composite modified electrode exhibits higher electrocatalytic activity towards glucose oxidation. The obtained results revealed that determination of glucose can be easily performed using Cu-NPs/ZnO composite film electrode and the modified electrode enhanced the eletrocatalytic activity remarkably towards glucose with high stability in its solution. The Cu-NPS, ZnO nanostructures and composite electrodes were characterized by SEM and CV. This method may open an avenue for electrochemical sensing of glucose, ascorbic acid, other

biomolecules and organic pollutants present in the environment. Further study for the development of non enzymatic electrochemical sensors is in progress.

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ANTIAGING, ANTIOXIDANT FLAVONOIDS; SYNTHESIS, ANTIMICROBIAL SCREENING AS WELL AS 3D QSAR CoMFA MODELS FOR THE PREDICTION OF BIOLOGICAL ACTIVITY

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Abstract

Flavonoids, polyphenolic heteronuclear compounds which are naturally occurring antioxidants are widely used as antiaging substances. Synthesis of new naturally occuring organic compounds with basic skeleton of chalcones, flavones and oxygenated flavones and their antimicrobial activity were reported by this research group for long. Presently comparative molecular field analysis (CoMFA) implemented in Sybyl 7.3 was conducted on a series of substituted flavones. CoMFA is an effective computer implemented 3D QSAR technique deriving a correlation between set of the biologically active molecules and their 3D shape, electrostatic and hydrogen bonding characteristics employing both interactive graphics and statistical techniques. Evaluation of 38 compounds were served to establish the models with grid spacing (2.0 Å). CoMFA produced best predictive model for compound 1C (2 – Phenyl – 1,4 – benzopyrone) and compound 2C (5 – Fluoro – 3′– hydroxy flavone) among all. Model for compound 2C [$r^2_{conv\ (no\text{-}validation)}$ = 0.956, SEE = 0.211, F value = 111.054) is better than that of compound 1C [$r^2_{conv\ (no\text{-}validation)}$ = 0.955, SEE = 0.212, F value = 110.261) but comparing superimposed model 1C being suggested as the best predictive model. 3D contour maps were generated to correlate the biological activities with the chemical structures of the examined compounds and for further design.

Key words: Antioxidant, 3D QSAR, CoMFA, Flavones, 3D contour map

Introduction

Flavonoids, the most common naturally occurring antioxidants are found ubiquitously in plants as pigments for flower coloration, in fruits, vegetables and beverages. Chemically flavonoids are polyphenolic, heteronucler compounds which are the characteristics of antioxidants. Antioxidants are compounds that protect cells against the damaging effects through the formation of phenoxy radical which combine with reactive oxygen species, such as superoxide, peroxyl radicals, hydroxyl radicals, and terminate the unwanted free radical chain reaction in cells. The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health specially its antiaging effect (Shirley 2001, Tapas *et al*. 2008 and Lee *et al*. 2011). A library of new naturally occuring organic compounds with basic skeleton of chalcones, flavones, and oxygenated flavones have been reported and their antimicrobial screening being carried out by this research

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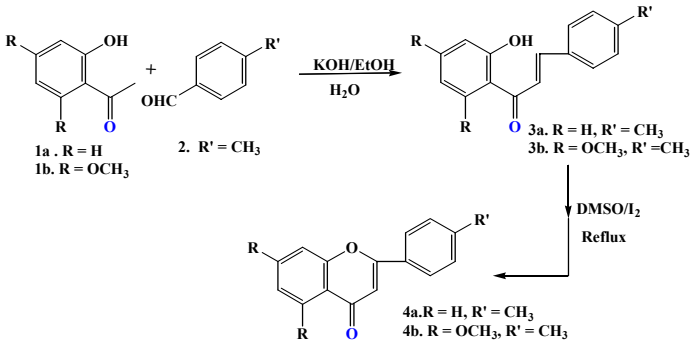
group since last thirty years (Islam *et al*. 1980, Islam *et al*. 1981, Alam *et al*. 2004, Morshed *et al*. 2005 and Mostahar *et al*. 2006 and 2007). A general synthetic scheme being suggested (scheme 1) where by varying substituents several flavones can be obtained (eg, 4′– Methyl flavone, **4a**, and 5,7- Dimethoxy – 4′– methyl flavones, **4b**) with satisfactory percent yield. Antimicrobial screening usually being carried out against Gram positive bacteria (eg, G^+, *Bacillus megateriam*), Gram negative bacteria (eg, G^-, *Escheria coli*) by qualitative technique (Disk diffusion) and quantitative technique (Minimum Inhibitory Concentration). Most of the cases significnt biological activity was found and the variation in substituents can enhance the biological and medicinal activity and should be studied more to explore a single therapeutic tool for the treatment of cancer, cardiovascular, inflammatory diseases (Alam *et al*. 2004, Morshed *et al*. 2005 and Mostahar *et al*. 2006 and 2007).



Scheme 1. Substituted acetophenon (**1a**, R=H, **1b**, R=OCH_3), substituted benzaldehyde (**2**, R′=CH_3), produce the precursor of flavones, substituted chlacones (**3a**, R=H, **3b**, R=OCH_3) followed by the cyclisation to flavones (**4a**, R=H, **4b**, R=OCH_3).

In addition, Comparative molecular field analysis (CoMFA) implemented in Sybyl 7.3 were conducted on a series of substituted flavones (Fig. 1). CoMFA is an effective computer implemented 3D QSAR technique deriving a correlation between the set of biologically active molecules and their 3D shape, electrostatic and hydrogen bonding characteristics employing both interactive graphics and statistical techniques. Classical QSAR correlates biological activities of drugs with physicochemical properties or indicator variables which encode certain structural features (Patrick 2001, Young 2001, Samee *et al*. 2004 and Putambaker *et al*. 2006). CoMFA is a powerful 3D QSAR method which has already shown its practical value in many cases. Most of the applications are in the field of ligand-protein interactions, describing affinity, inhibition constant, and also to correlate steric and electronic parameter (Patrick 2001). The molecules, which showed high activity results such as K_i, IC_{50} values are required. Charges should be added to the

molecules so that electrostatic energy can be determined (Young 2001). A good alignment is the single most important part of doing a CoMFA analysis. The common substructures should have the same conformation in all molecules, and other parts should be superimposed as much as possible by adjusting internal torsional angles. Evaluation of 38 compounds (Fig. 1 and Table 1) was served to establish the models with grid spacing (2.0 Å) is to find the best predictive model.

Materials and Methods

CoMFA Precedure (Patrick 2001, Young 2001, Samee *et al*. 2004 and Putambaker *et al*. 2006): CoMFA describes 3D structure- activity relationship in a quantitative manner. For th**is** purpose, a set of 38 derivative compounds of flavones (Fig. 1 and Table 1) have been constructed. Each of the structure is provided with charges (Minimization Process, scheme 2). Each of the molecules is saved and Molecular database (MDB) is created.

Scheme 2. Least energised structure determining process.

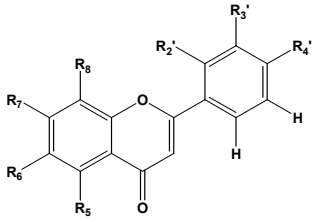


Fig. 1. Substitued flavones for CoMFA Analysis (R values are presented in Table 1).

Database Alignment **(**Young 2001, Samee *et al*. 2004 and Putambaker *et al*. 2006): As one of the most important preconditions, all molecules have to interact with the same kind of receptor in the same manner so each conformation is taken in turn, and the molecular fields (steric and electrostatic) around it are measured at the lattice points of a regular Cartesian 3D grid; the lattice spacing is typically 2 Å. The "measured" interaction is between the molecule and a probe atom (an sp^3-hybridised carbon with +1 charge). In the next step, a certain subgroup of molecules are selected which constitutes a training set to derive the CoMFA model. The residual molecules are considered to be a test set which independently proves the validity of the derived model. A pharmacophore hypothesis is derived to orient the superposition of all individual molecules and to afford a rational and consistent alignment. The best superimposed alignment structure for compound 1C (2 – Phenyl – 1,4 – benzopyrone) and compound 2C (5 – Fluoro – 3′– hydroxy flavone) have been shown in Figs. 2 and 3 respectively.

Table 1. Substituted flavone Compounds in this study and their BZ (Benzodiazopine) site binding affinities [-logK_i, K_i in nM] (Huang *et al*. 2001).

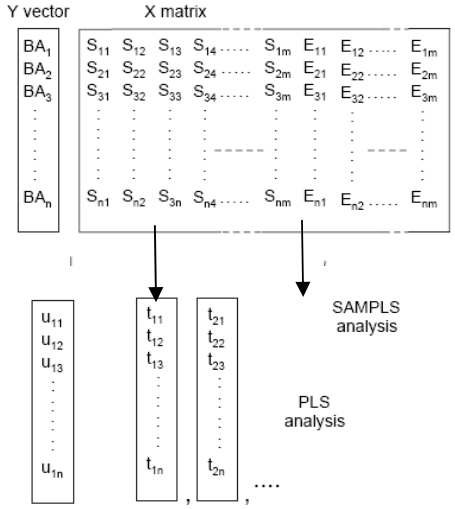
Compound	R_5	R_6	R_7	R_8	R_2'	R_3'	R_4'	–logK_i
1C	-H	-H	-H	-H	-H	-H	-H	6.00
2 C	-H	-F	-H	-H	-H	-OH	-H	5.60
3 C	-H	-Cl	-H	-H	-H	-OH	-H	6.07
4 C	-H	-Br	-H	-H	-H	-OH	-H	6.22
5 C	-H	-F	-H	-H	-H	$-NO_2$	-H	6.74
6 C	-H	-Cl	-H	-H	-H	$-NO_2$	-H	8.10
7 C	-H	-Cl	-H	-H	-H	-H	$-OCH_3$	5.90
8 C	-H	-Br	-H	-H	-H	-H	$-OCH_3$	5.68
9 C	-H	-Br	-H	-H	$-NO_2$	-H	-H	6.68
10 C	-H	$-NO_2$	-H	-H	-H	-H	-Br	7.60
11 C	-H	-Cl	-H	-H	-F	-H	-H	6.38
12 C	-H	-Br	-H	-H	-F	-H	-H	6.42
13 C	-H	-H	-H	-H	-H	-F	-H	5.45
14 C	-H	-F	-H	-H	-H	-F	-H	6.04
15 C	-H	-Cl	-H	-H	-H	-F	-H	6.93
16 C	-H	-Br	-H	-H	-H	-F	-H	7.38
17 C	-H	-H	-H	-H	-H	-H	-F	5.44
18 C	-H	-F	-H	-H	-H	-H	-F	5.60
19 C	-H	-Cl	-H	-H	-H	-H	-F	6.74
20 C	-H	-Br	-H	-H	-H	-H	-F	6.94
21 C	-H	-H	-H	-H	-H	-Cl	-H	6.21
22 C	-H	-F	-H	-H	-H	-Cl	-H	6.70
23 C	-H	-Cl	-H	-H	-H	-Cl	-H	7.64
24 C	-H	-Br	-H	-H	-H	-Cl	-H	7.77
25 C	-H	-H	-H	-H	-H	-Br	-H	6.38
26 C	-H	-F	-H	-H	-H	-Br	-H	6.63
27 C	-H	-Cl	-H	-H	-H	-Br	-H	7.64
28 C	-H	-Br	-H	-H	-H	-Br	-H	7.72
29 C	-H	-Br	-H	-H	-H	-H	-H	7.15
30 C	-H	-Br	-H	-H	-H	-H	$-NO_2$	6.70
31 C	-H	$-NO_2$	-H	-H	-H	$-NO_2$	-H	7.92
32 C	-H	-Br	-H	-H	-H	$-NO_2$	-H	9.00
33 C	-OH	-Br	-OH	-Br	-H	-H	-H	6.15
34 C	-OH	-H	-OH	-H	-H	-H	-H	5.52
35 C	-OH	-H	-OH	-H	-H	-H	-OH	5.52
36 C	-OH	-H	-OH	-H	-Cl	-H	-H	5.10
37 C	-OH	-H	-OH	-H	-F	-H	-H	5.10
38 C	-OH	$-OCH_3$	-OH	-H	-H	-H	-OH	6.00

Statistical analysis: It includes statistical averages of all possible interactions of the probe molecule and others. These sort of processes can be modelled on a molecular level by obtaining many results and then using a statistical distribution of those results. Partial Least Square (PLS) analysis is the most appropriate method for this purpose. The value of the resulting QSAR can be determined through the cross validated r^2 (referred to as q^2) reported by the PLS (Table 3). If acceptable, The CoMFA rederived in final in non cross validated form (referred to as r^2 in Table 3).

Equations: 3D properties of a molecule are considered as a whole, size, shape, electronic properties etc. Biological activities correlate the physicochemical properties (hydrophobicity) in following way:

$$\log (1/C) = \log P \text{ (partition coefficient)} + k_2\sigma\text{(electronic effect)}$$
$$+ k_3 Es \text{ (steric effect)} + k_4 \qquad (1) \text{ [simple relation]}$$

It can be represented by the following Matrix



It can be written as

$$BA_i = a_1S_{i1} + a_2S_{i2} + a_3S_{i3} + \ldots\ldots + a_mS_{im} +$$
$$+ b_1E_{i1} + b_2E_{i2} + b_3E_{i3} + \ldots\ldots + b_mE_{im} \qquad [2]$$

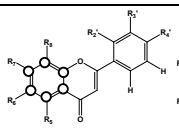
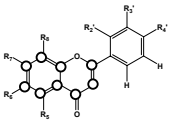
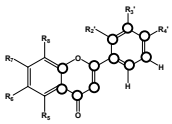
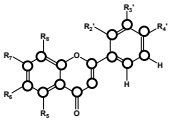
[PLS analysis derives vectors from the Y block; BA_i = logarithm of realative affinities or other biological activities and the X block; S_{ij} = steric field variable of molecule i in the grid point j, E_{ij} = electrostatic field variable of molecule i in the grid point j]

Results and Discussion

The best CoMFA predictive model is suggested by varying common substructures (in Table 2 for compound 1C) for all 38 compounds are presented in Table 3. For better PLS result the search of proper common substructure during molecular alignment is a vital part. Based on regression and statiscal error from Table 2, 1C (ii) being selected as common substructure for all the molecules during PLS analysis. As 3D properties of a molecule are considered as a whole, size, shape, electronic properties so 1C (2 – Phenyl –

1, 4 – benzopyrone) and compound 2C (5 – Fluoro – 3′– hydroxy flavone) presented in Table 3 showed better results among all. Model for compound 2C [$r^2_{\text{conv (no-validation)}}$ = 0.956, SEE = 0.211, F value = 111.054) is better than that of compound 1C [$r^2_{\text{conv (no-validation)}}$ = 0.955, SEE = 0.212, F value = 110.261). But if we compare the superimposed Figures (Fig. 2 for 1C and Fig. 2 for 2C) the best superimposed model is 1C (Fig. 2). So the best predictive model being suggested as compound 1C with common substructure 1C(ii).

Table 2. For better PLS results the search of common substructures observed during molecular alignment of compound 1C.

Selected substructures				
	1C(i)	1C(ii)	1C(iii)	1C(iv)
No. of Components[a]				
Set	6	6	6	6
Obtained	6	3	3	3
Cross validation				
q^2	0.710	0.743	0.722	0.738
No validation				
R^2	0.933	0.955	0.947	0.953
SEE[b]	0.261	0.213	0.232	0.217
F value	71.512	110.261	91.835	105.608

[a]Set component no. 6-13 and column filtering 0.5-2 kcal/mol giving similar results, [b] Standard Error of Estimation.

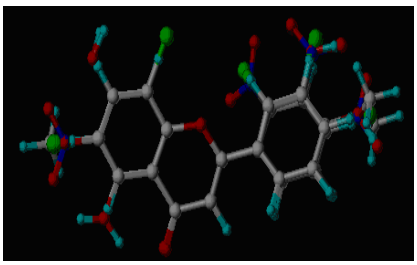


Fig. 2. Alignment picture for compound 1C.

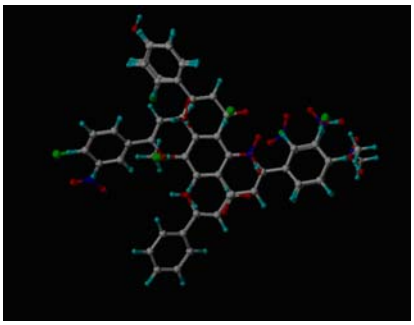


Fig. 3. Alignment picture for compound 2C.

Table 3. PLS Analysis results for aligning 38 substituted flavones by Sybyl 7.3.

Compounds	Min. energy Ges-Huck model(kcal/mol)	No. of compo nents	Cross validation q2	No Validation R^2	SEE	F value
01C	4.461	3	0.743	0.955	0.213	110.261
02C	1.537	6	0.815	0.956	0.212	111.054
03 C	2.408	6	0.811	0.954	0.216	106.710
04 C	2.523	6	0.787	0.940	0.247	80.991
05 C	3.990	6	0.693	0.917	0.291	56.746
06 C	4.863	4	0.778	0.945	0.236	88.448
07 C	4.618	6	0.666	0.949	0.228	95.654
08 C	4.724	6	0.531	0.874	0.357	35.844
09 C	5.386	4	0.771	0.956	0.212	111.738
10 C	5.373	5	0.804	0.937	0.253	76.317
11 C	2.069	4	0.804	0.961	0.199	127.206
12 C	2.172	6	0.777	0.937	0.252	77.270
13 C	2.818	6	0.711	0.931	0.265	69.386
14 C	0.589	6	0.701	0.931	0.264	69.814
15 C	1.457	6	0.712	0.930	0.267	68.471
16 C	1.575	6	0.712	0.930	0.267	68.377
17 C	3.699	6	0.703	0.930	0.266	69.054
18 C	1.462	6	0.703	0.930	0.265	69.155
19 C	2.334	6	0.702	0.930	0.266	69.051
20 C	2.453	6	0.702	0.930	0.266	69.009
21 C	4.461	6	0.786	0.934	0.258	73.235
22 C	1.537	6	0.777	0.945	0.239	86.127
23 C	2.408	6	0.778	0.942	0.243	83.359
24 C	2.523	6	0.769	0.941	0.245	81.937
25 C	3.990	6	0.774	0.942	0.243	83.843
26 C	4.863	4	0.720	0.953	0.218	104.762
27 C	4.618	4	0.720	0.953	0.218	104.770
28 C	4.724	4	0.720	0.953	0.218	104.757
29 C	5.386	4	0.720	0.953	0.218	104.782
30 C	5.373	6	0.740	0.900	0.319	46.436
31 C	2.069	6	0.691	0.895	0.327	43.895
32 C	2.172	6	0.762	0.944	0.238	87.011
33 C	2.818	6	0.684	0.916	0.291	56.465
34 C	0.589	6	0.724	0.936	0.255	75.500
35 C	1.457	6	0.732	0.942	0.243	83.222
36 C	1.575	4	0.726	0.949	0.227	96.768
37 C	3.699	4	0.736	0.946	0.235	89.996
38 C	9.722	6	0.456	0.888	0.337	41.030

The result of this analysis is presented as a set of contour maps. These contour maps show favourable and unfavourable steric regions around the molecules as well as favourable

and unfavorable regions for electropositive and electronegative substituents in certain positions. Predictions for the compounds not included in the analysis can be made by calculating the fields of this molecules and by inserting the grid values into the PLS model. 3D contour maps were generated to correlate the biological activities with the chemical structures of the examined compounds and for further design. Steric contour map for 1C (Fig. 4) is shown by (a) and (b) polyhedra. Polyhedra (a) indicate region where more steric bulk will enhance the activity and arround polyhedra (b) less steric bulk will enhance the activity. The variation in substituents can enhance the biological and medicinal activity and should be studied more to explore the moleclar drug design.

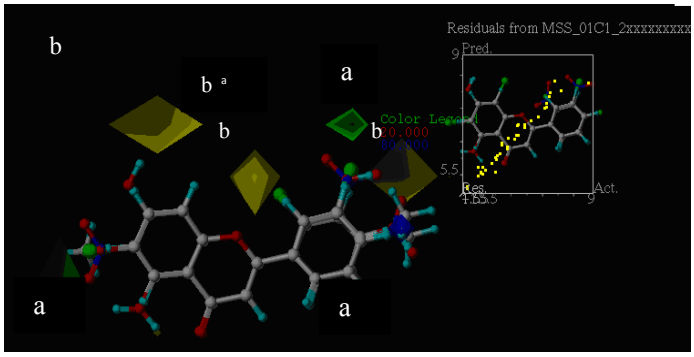


Fig. 4: CoMFA contour maps for compound 1C.

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PERFORMANCES OF 2 PHENOXYETHANOL AND QUINALDINE WITH OXYGEN IN THE LIVE TRUCK TRANSPORTATION OF ROHU FINGERLINGS

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Abstract

The effectiveness of 2 phenoxyethanol (2 PE), quinaldine (2-methyl quinoline), benzocaine (ethyl 4 aminobenzoate) and tertiary amyl alcohol (TAA) was tested to determine their optimal dosages for rapid induction and recovery in the first experiment, while the second experiment evaluated the effects of low dose quinaldine (175 µl/L) and 2 PE (250 µl/L) with oxygen for 1, 3, 6 and 9 h on the mortality and water qualities at 400 g/L rohu *Labeo rohita* fingerlings in a truck transport simulation. Optimum dosages of 2 PE (250 µl/L), quinaldine (175 µl/L), benzocaine (40 mg/L) and TAA (1.5 ml/L) were found to have rapid (within 6 min) immobilization and recovery. Very low level of immediate and delayed mortality (<1%) was found across all three transport methods. While 2 PE had dissolved oxygen (DO) concentration above 13 mg/L, quinaldine had lower level. Results suggest that rohu fingerlings at 400 g/L can be transported for 9 hours with oxygen with or without sedatives.

Key words: 2 Phenoxyethanol, Quinaldine, Oxygen, Transportation, Rohu fingerlings

Introduction

Rohu is a high valued cultured fish in Bangladesh contributing 24.84% to the country's total pond production (DoF 2008). This fish fetches a good market price and consumer demand. Typically, small batches of rohu fingerlings (25 kg) are packed into plastic drum (200 L), given hand agitation carried on truck-beds for transportation to cover distances as much as 500 km. Handling stress, however, can result in large-scale mortalities of fish transported over long distances; especially if high densities of fish are maintained during transit (Smit 1980). Hasan and Bart (2006) have estimated the annual loss (6200 mt) of carp fingerlings resulting from mortality due to transport stress which is nearly 13.62 million US$ in Bangladesh.

Modern aquaculture practices frequently expose fish to a variety of acute stresses that have the potential to negatively affect fish performance for survival (Barton 2000). The

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effects of stress can be manifested at several levels of biological organizations from sub-cellular to individual, population and community levels by altering the plasma hormones, physiological activities such as iono-osmotic function, metabolism and growth rates (Adams 1990, Pickering 1992 and 1993, McCormick *et al*. 1998 and Weil *et al*. 2001). Higher survival, better health and growth can be achieved by minimizing stress, maintaining good water quality, proper loading density and less stressful handling and transportation. One method commonly used to mitigate or minimize the effect of stress on fish is the use of sedatives (McFarland 1959 and Berka 1986).

Anesthesia has been introduced as an aid to prevent stress in fish for long time (Coyle *et al*. 2004). It also reduces excitement and hyperactivity-related trauma that can occur during routine handling and thus directly reduces mortality. The decrease in movement minimizes skin damage, associated osmoregulatory disturbances (Kumlu and Yanar 1999) and reduces metabolism, resulting in decreased oxygen demand and the production of less waste (i.e., CO_2 and ammonia) during transportation (Guo *et al*. 1995, Pirhonen *et al*. 2003 and Cooke *et al*. 2004). Use of sedatives generally depends on their efficacy, availability, cost-effectiveness and safety.

Among different sedatives, quinaldine was found to be more effective in alkaline water, efficient at low dose and less toxic with a shorter recovery period (Bell 1964, Sills and Allen 1973, Lambert 1982 and Coyle *et al*. 2004). Quinaldine (25 mg/L) induced light sedation in tilapia (*Sarotherodon melanotheron*) fingerlings (3-13 cm) (Sado 1985). Quinaldine was also found to induce quick lethargy and recovery in sea bream (*Sparus sarba*) fingerlings (3-3.5 g) compared to other sedatives (e.g., quinate, MS-222, benzocaine and 2 phenoxyethanol (Hseu *et al*. 1998). Munday and Wilson (1997) reported quinaldine as the most effective anesthetic to induce total loss of equilibrium compared to benzocaine, MS 222, 2 phenoxyethanol and clove oil. Durve (1975) achieved 97% survival of mullet *Mugil cephalus*, *Liza tade* fry/fingerlings using quinaldine during 24 h transport while Lambert (1982) recommended a lower dose (1-4 mg/L) to minimize handling stress in yearling Atlantic mackerel (*Scomber scrombrus*). However, in fish 95% clearance of quinaldine was found within 24 h of withdrawal (Hunn and Allen 1974). Hasan and Bart (2007a) have demonstrated that low dose quinaldine with mechanical aeration can be used for high density (400 g/L) long distance (6 h) safe transport (no mortality) of silver carp (*Hypophthalmichthys molitrix*) and rohu fingerlings.

Two phenoxyethanol is an opaque, colorless, a moderately water-soluble clear or straw-colored oily and aromatic liquid. It is relatively inexpensive and remains active in the diluted state for 3 days (Hseu *et al*. 1998). Coyle *et al*. (2004) suggested that MS 222, benzocaine, quinaldine, 2 phenoxyethanol, metomidate, clove oil, CO_2, hypothermia and electro-anesthesia as useful in the transportation of fish. The efficacy of 2 phenoxyethanol varies with the size of the fish and with the temperature of the water.

Benzocaine and quinaldine have been found to be used successfully for transportation of rohu and silver carp fingerlings with mechanical aeration at a loading density of 400 g/L (Hasan and Bart 2007a). But 2 phenoxyethanol has never been reported in the transport of rohu fingerling with oxygen. In a moving truck bed, lack of power prevents from using mechanical aerator to diffuse oxygen. However, use of oxygen instead of mechanical aerator would provide the most valuable life support for the fingerlings without requirement of any energy during long distance transport.

This study, therefore, was designed to determine the efficacy of 2 phenoxyethanol, quinaldine, benzocaine and tertiary amyl alcohol and effects of quinaldine and 2 PE with oxygen on the mortality and water quality variables in rohu fingerlings.

Materials and Methods

Rohu fingerlings (9.37 ± 0.09 cm; 9.66 ± 0.22 g; mean ± SE) were collected from the "Ma Fatema Fish Hatchery", Chachra, Jessore, Bangladesh, some 6-7 hours bus ride from the capital city Dhaka for getting reliable supply of the fingerlings throughout the entire experimental period. The experiments were carried out in the hatchery between May and June 2011. For simulation purposes, the fingerlings were held in flow-through-tank system with vertical spray. Feeding was suspended 12 hours before loading the fingerlings in to transport drums.

Efficacy of 2 PE, quinaldine, benzocaine and TAA was tested in a series of preliminary trials to determine the appropriate dosages which works best to properly tranquilize rohu fingerlings in 2-4 min with no or lowest mortality depending on the availability in the local market and associated cost. Several aluminum vessels (10 L) were filled with 1 L well water and mixed with one sedative of several dosages to observe the mobility of the fingerlings. Initial DO content and temperature of the water were also monitored. Afterwards 15 rohu fingerlings were released in each vessel. The changes in the condition of the fingerlings in different vessels were observed over 1, 3, 6 and 9 hour duration. The time requirements in terms of immobilization and recovery of the rohu fingerlings exposed to different sedatives were determined.

All sedatives were purchased from Sigma-Aldrich. To determine the optimal dosages, 2 PE at the rate of 0.20, 0.25 and 0.30 ml/L; quinaldine (4%) 50, 175, 200, 225 and 250 µl/L; benzocaine 30, 40 and 50 mg/L and TAA (2–methyl 2-butanol) 1.5, 2.0, 2.5, 3.0 and 4.0 ml/L were applied. Mobility of fish fingerlings during application of different dosages of the above mentioned sedatives was observed. From the efficacy test quinaldine (175 µl/L) and 2 PE (250 µl/L) were selected to use in the transport simulation.

A 3 × 4 factorial design was used in triplicates by truck. The experimental variables were three transport methods and four (1, 3, 6 and 9 h) transport durations. The indicator

variables were immediate and delayed mortality. Water quality variables such as dissolved oxygen (DO) concentration and temperature were also measured.

Plastic drums of 200 L filled with 100 L subsurface well water was used as the experimental system. For simulation purpose, each drum was loaded with 40 kg fingerlings at 400 g/L loading density. Before loading, sedatives were mixed well with water and oxygen was injected. Oxygen cylinder containing 9.8 m^3 pure (99.9%) oxygen at 2200 PSI was used as the source of oxygen and released at 8 PSI. Dead fingerlings were removed and counted to determine the immediate mortality rate at 1, 3 6 and 9 h after transport. Water quality variables were also measured at the time of fingerling sampling for mortality. After collection, samples were held in frozen condition at -20^0 C. For determination of delayed mortality after simulation experiment, dead fingerlings were removed from stocking hapa and counted 24 hours after the experiment began. Dissolved oxygen concentration and temperature of transport water were measured by using a portable DO meter (HACH sension^{TM} 6, USA) after 1, 3, 6 and 9 h of transport simulation. The results were subjected to statistical evaluation. All percent data were transformed into square root before statistical analysis. Treatments were compared by ANOVA followed by Tukey's HSD post hoc for multiple comparisons. Data were analyzed by using SPSS software version 10.0 with the level of significance at $p<0.05$.

Results and Discussion

Efficacy tests: Optimum doses of four sedatives for complete immobilization and recovery were found to vary (Table 1). Apparently better result was found when TAA was applied. Because of unavailability and high cost, TAA was not used in the simulation experiment.

In efficacy test, low dose (1.5 ml/L) of tertiary amyl alcohol gave better result for immobilization and recovery of fingerlings. Quinaldine (175 µl/L), 2 phenoxyethanol (250 µl/L) and benzocaine (40 mg/L) were also found to be very effective at low dose. All four anesthetics at different optimal doses were found to impart light sedation in rohu fingerlings, above which significant loss of equilibrium and mortality resulted. The effective concentration of an ideal anesthetic was defined as the minimum concentration that produced anesthesia within 4 min and allowed recovery within 5 min. This study has clearly demonstrated that compressed oxygen and low-dose of quinaldine and 2 PE are effective in the transportation of rohu fingerlings for a period of 9 h. Hasan and Bart (2007b) have found overall low level (4-12%) immediate and high level (27-49%) delayed mortality in rohu fingerlings when transported for 9 hours at 200, 300 and 400 g/L loading densities without any sedative and oxygen, but with only hand splashing of water. However, much higher levels of immediate (38 and 83-92%) and delayed mortality (90%) have been observed in carps, large mouth bass (*Micropterus salmoides*), freshwater drum (*Aplodinotus grunneiens*) and striped bass (*Morone saxatilis*) (Johnson

and Metcalf 1982, Carmichael *et al*. 1984, Mazik *et al*. 1991, Lewis *et al*. 1996). From this study, it was found that longer period transport of rohu fingerlings caused a significant decrease in DO concentration especially in the control group. Although temperature had an increasing trend in all treatments, it was less variable. Low level of mortality (<1%) in every transport method suggests that rohu fingerlings at a loading density of 400 g/L can be transported successfully for 9 h with high rate of survival aided with low dose quinaldine, 2 PE and pure oxygen.

Table 1. Anesthetics and their doses with levels of immobilization.

Anesthetics	Dose	State of the findings
2 Phenoxyethanol	0.20 ml/L	Some fishes found immobilized and some with little mobility within 5 minutes
	0.25 ml/L	All fishes found completely immobilized within 5 minutes
	0.30 ml/L	All fishes found upside down within 5 minutes
Quinaldine (4%)	50 µl/L	No lethargy found within 2 minutes
	175 µl/L	All fishes found completely immobilized within 2 minutes
	200 µl/L	Some fishes found immobilized and some upside down within 2 minutes
	225 µl/L	Most fishes found upside down within 2 minutes
	250 µl/L	All fishes found upside down within 2 minutes
Benzocaine	30 mg/L	Some fishes found immobilized and some with little mobility within 2 minutes
	40 mg/L	All fishes found completely immobilized within 2 minutes
	50 mg/L	All fishes found upside down within 2 minutes
Tertiary amyl alcohol (2-methyl 2-butanol)	1.5 ml/L	All fishes found completely immobilized within 2.5 minutes
	2.0 ml/L	Half of the total fishes found upside down within 2.5 minutes
	2.5 ml/L	More than half of the total fishes found upside down within 2.5 minutes
	3.0 ml/L	Most fishes found upside down within 2.5 minutes
	4.0 ml/L	All fishes found upside down within 2.5 minutes
Control	0	All fishes found were highly mobile

Considering the cost for transportation of rohu fingerlings at a loading density of 400 g/L, TAA was found to be most expensive followed by 2 PE and quinaldine, however, benzocaine was found to be the cheapest (Table 2). Since TAA was unavailable and most expensive, and benzocaine does not remain effective after 3 h of transport (Hasan and Bart 2007a), these were not used in the transport simulation.

Table 2. Optimum dose, time of induction and recovery period and cost of sedatives in rohu fingerling (Bangladeshi Taka = BDT; 1 US$ = 82 BDT).

Sedatives	Dose	Time required to be immobilized (min)	Recovery period (min)	Cost BDT/kg
2 Phenoxyethanol	250 µl/L	4.0 to 5.0	5.0 to 6.0	9.75
Quinaldine (4%)	175 µl/L	1.5 to 2.0	2.0 to 3.0	5.25
Benzocaine	40 mg/L	2.0 to 2.5	2.0 to 3.0	2.60
TAA	1.5 ml/L	1.5 to 2.5	1.5 to 2.0	97.50

Effects of Transport Methods on the Immediate and Delayed Mortality Rate, Dissolved Oxygen Concentrations and Temperature: In the control group, 6 and 9 h samples had nearly 1 and 3% mortality, respectively, while 3 h had very low level of mortality (<1 %). In quinaldine treatment group, only 9 h sample resulted in very little immediate mortality (< 1%), while other three sampling durations did not have any mortality. No immediate mortality was observed up to 6 h sampling duration and very low level (<1 %) was found at 9 h in the treatment group of 2 PE (Table 3).

Table 3. Immediate and delayed mortality rates (%) of rohu *Labeo rohita* fingerlings sampled from three transport simulation methods.

Duration (h)	Immediate mortality rate (%) of rohu fingerlings sampled from three transport methods at 1, 3, 6 and 9 h of transport		
	Control (O_2 only)	2 phenoxyethanol	Quinaldine
1	0.00±0.00	0.00±0.00	0.00±0.00
3	0.13±0.03	0.00±0.00	0.00±0.00
6	1.14±0.25	0.00±0.00	0.00±0.00
9	2.62±0.03[a]	0.15±.002[b]	0.08±0.01
Delayed mortality			
1	0.00±0.00	0.00±0.00	0.00±0.00
3	0.00±0.00	0.00±0.00	0.00±0.00
6	0.00±0.00	0.00±0.00	0.00±0.00
9	0.08±0.07	0.29±0.02	0.46±0.04

Control received only pure oxygen. Within row means (± SE) with different letters are significantly different ($p<0.05$).

Mortality rates were used as the main indicators in this study which plays a central role in the management of fish seed distribution. In the control group in which no sedative was used, the observed no or little immediate mortality (<1%) up to 3 h duration indicates less stressful phase and good environmental condition. Nearly 2 and 3% deaths occurred in the control group that might be associated with dropped DO level at 6 and 9 h transport duration. While in the quinaldine and 2 PE treatment groups, the mortality levels observed were zero throughout the first three transport durations (1, 3 and 6 h) with a little immediate and delayed mortality at 9 h duration treatment. Such survivals could be explained by the effective sedation by 2 PE and quinaldine which induced rapid immobilization of the fingerlings which in turn resulted in reduction in metabolism, excretion, physical stress and thus prevented deterioration of water quality. As no sedative was used in the control group the water quality particularly the DO level

dropped below 1 mg/L which could be the underline cause of the observed little mortality. Hasan and Bart (2007a) reported (0-2 %) mortality of rohu and silver carp fingerlings using quinaldine and benzocaine. Almost similar survival rate (97%) was reported by Durve (1975) in mullet fry/fingerlings transport using quinaldine.

Similar to immediate mortality, levels of delayed mortality found in all three transport methods were little and similar (Table 3). No delayed mortality was observed in the first three treatment durations while very little (<1 %) was detected after 9 h of transport in all three transport methods. Levels of delayed mortality in all four transport durations were similar to that of immediate mortality.

The observed low level of delayed mortality could be associated with physical injury of fish, loss of scale, improper handling during weighing and releasing of fingerlings into the drums for the treatment. High survival in all treatments could have been a consequence of continuous use of pure oxygen. Hasan and Bart (2007a) found no delayed mortality by using low dose quinaldine and benzocaine when transported for 6 h at a loading density of 400 g/L in rohu and silver carp fingerlings aided with mechanical aeration.

Successive fall in dissolved oxygen concentration was observed during four sampling durations in all three transport methods. In the control group while comparing, a significant fall in DO concentration was observed after 6 and 9 h during experiment but 1 and 3 h had, respectively, nearly 14 and 7 mg/L (Table 4). While 1 h sample resulted in nearly 18 mg/L, 9 h sample had only about 4 mg/L of dissolved oxygen in the quinaldine treatment group. Although a continuous fall in the DO concentration across all four transport durations was detected in the 2 PE treatment group, it did not went below 13 mg/L.

Table 4. Dissolved oxygen concentration (mg/L) and temperature (ºC) sampled from three transport methods at 1, 3, 6 and 9 h.

Duration (h)	Dissolved oxygen concentration (mg/L) sampled from three transport methods at 1, 3, 6 and 9 h		
	Control (O_2 only)	2 phenoxyethanol	Quinaldine
Pre-loading	28.87±0.00[a]	28.87±0.00[a]	28.87±0.00[a]
1	13.96±1.46[b]	25.35±0.14[a]	17.01±1.01[b]
3	6.49±0.62[c]	18.89±1.24[b]	11.52±0.43[c]
6	0.98±0.06[d]	17.61±1.24[b]	5.85±0.15[d]
9	0.93±0.03[d]	13.36±0.95[c]	3.36±0.52[d]
Temperature			
Pre-loading	27.30±0.00	27.30±0.00	27.30±0.00
1	27.60±0.06	27.53±0.03	27.73±0.03
3	28.25±0.28	28.12±0.04	28.57±0.18
6	29.14±0.28	28.77±0.03	29.23±0.09
9	29.44±0.39	28.87±0.03	29.57±0.07

Within column means (± SE) with different letters are significantly different ($p<0.05$).

In this study, DO concentrations dropped progressively with a negligible increase in temperature throughout the experimental durations in all three treatments. Maintaining

high concentration of DO in 2 PE treatment group throughout the entire 9 hours study period indicates it's suitability in persisting sedation over long period. Sedation reduces oxygen consumption by reducing hyperactivity. DO concentration, however, in the quinaldine and control groups had declining trend which could be due to high consumption of oxygen resulted from increased hyperactivity of the fingerlings over duration. Reduction of the effectiveness of the sedatives with increased duration may be responsible for enhancing mobility and high consumption of oxygen by the fingerlings. While comparing, similar levels of water temperature were observed in all three treatment groups (Table 4).

We found that rohu fingerlings at 400 g/L can be safely transported for 9 hours with no or little immediate and delayed mortality by using oxygen with or without sedatives. Use of 2 PE and quinaldine maintains high DO concentration. .

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COGNITIVE EMOTION REGULATION IN CHILDREN AS RELATED TO THEIR PARENTING STYLE, FAMILY TYPE AND GENDER

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Abstract

This study aimed to investigate whether cognitive emotion regulation in children varies with parenting style, family type and gender. Toward this end, cognitive emotion regulation and perceived parenting style of 206 school children were measured. Standard regression analyses of data revealed that the models were significant and explained 17.3% of the variance in *adaptive* emotion regulation (Adjusted R^2=0.173; F=9.579, p<.001), and 7.1% of the variance in *less adaptive* emotion regulation (Adjusted R^2=.071, F=4.135, p=.001). Results showed that children's cognitive emotion regulation is functionally associated with parenting style, but not with family type and their gender. Amongst the three types of parenting, authoritative parenting was the strongest predictor of overall *adaptive* emotion regulation while authoritarian parenting was the strongest predictor of overall *less adaptive* emotion regulation. Permissive parenting has impact on neither *adaptive* nor *less adaptive* emotion regulation. The findings have implications for parents, caregivers, child psychologists and other professionals working with children/ adolescents.

Key words*:* Cognitive emotion regulation, Adaptive, Less adaptive, Parenting style, Family type

Introduction

In everyday life we often experience strong emotions that need to be managed in order to function well in the family, office or workplace, and community or society etc. Managing or regulating emotions mean understanding and filtering emotional experience, using healthy strategies to control uncomfortable emotions and engaging in appropriate behavior (e.g. attending classes, going to work, engaging in friendships or social relationships) when distressed. Emotion regulation has both cognitive and behavioral aspects. We are interested here in the cognitive aspect of emotion regulation of adolescents. Cognitive emotion regulation is defined as the conscious, cognitive way of managing the intake of emotionally arousing information (Thompson 1994). The cognitive strategies that people generally use to regulate their emotions in different settings can be divided into two broad categories: *adaptive* strategies and *less adaptive* strategies.

Researchers have shown that the neurological changes improve the regulation of emotion over the course of adolescence. As adolescents grow they also learn how to regulate emotions which has both positive and negative impacts on their relationships with

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family, neighbors and friends. However, we still do not know about the role of parents or family in its development. Thus we are interested to see what kind of strategies children use (Adaptive or Less Adaptive?) in what kind of family and parenting situations to regulate their emotions. The child's interactions with the family members in general and with the parents in particular can play a crucial role in its cognitive and socio-emotional development (McLanahan and Bumpass 1988). This role can be much more important in a collectivistic society such as in Bangladesh where there are mainly two types of family: nuclear family and extended family.

Although children at adolescence give more importance to their peer group than parents, but parents and families have strong influence over them. Berndt (1979), and Young and Ferguson (1979), for example, found that although both sexes are highly peer-oriented, males and females at different times in adolescence are influenced by their parents. Parenting styles have been described as the collection of parents' behaviors which create an atmosphere of parent-child interactions across situations (Mize and Pettit 1997). *Authoritative* parents offer a balance between high nurturance and high control, they do not reward dependency (Baumrind and Black 1967), but instead set a standard of responsibility and self-control. Here, expectations are clear, rules are firm and rational, and discipline is administered in a consistent manner (Baumrind 1978). Thus *authoritarian* parenting is characterized by high control with low levels of parental warmth, involvement, support, or emotional commitment (Baumrind and Black 1967). *Permissive/indulgent* parents allow their children to do whatever they wish and they are characterized by high levels of warmth and low levels of control over children. In this style of parenting children are often left to regulate their own activities, behavior, and emotions at a young age.

The period of adolescence forms an important stage in the development of cognitive coping skills as this is the period in which the more advanced cognitive abilities are being mastered. Studies have shown that children's emotion regulation can be dependent on the style of parenting. Children of *authoritarian* parents on the other hand have been shown to be dissatisfied, apprehensive, fearful, socially inhibited, aggressive, and experience difficulty in regulating emotions (Baumrind 1967, Baumrind and Black 1967, Hart *et al.* 1998, Hart *et al.* 2003, Nix *et al.* 1999). *Permissive* parenting has been linked to bossy, dependant, impulsive behavior in children, with low levels of self-control and achievement; these children do not learn persistence and emotional control (Baumrind 1967). One limitation of these studies is that they are biased to the scenario of individualistic societies. A second limitation is that they mostly focused on the behavioral part of emotion regulation. The cognitive part of emotion regulation has been studied in relation to parenting style and child's gender insufficiently and not at all in relation to family type. Therefore, the present study investigated whether parenting style can determine that a child would employ *adaptive* or *less adaptive* strategies in

regulating emotions and whether the type of family (nuclear/extended) and child's gender have any impacts on such regulation.

Materials and Methods

Participants: 206 children (40% boys, 60% girls) aged 12-15 from four randomly chosen secondary schools of Dhaka City participated in the study. Of the participants, 48 were from grade VII, 44 from grade VIII, 44 from grade IX and 70 from grade X. The grades were chosen at random, one from each selected school. 71% of the participants came from nuclear families and 29% from extended families.

Measures: Two psychometric measures were used in the study. These were the Cognitive Emotion Regulation Questionnaire (CERQ) and the Parental Authority Questionnaire (PAQ). The CERQ is a 36-item questionnaire originally developed by Garnefski *et al.* (2002). It is a five-point Likert type scale ranging from 1 (almost never) to 5 (almost always). The scale has nine sub-scales, each consisting of four items, each item referring to what someone thinks after the experience of threatening or stressful life events. The sub-scales are grouped broadly into *Adaptive* and *Less Adaptive* emotion regulation strategies. The *adaptive* strategies are acceptance, positive refocusing, refocus on planning, positive reappraisal, and putting into perspective. The *less adaptive* strategies include self-blame, rumination, catastrophizing and blaming others. The sub-scales have good internal consistencies ranging from 0.68 to 0.83 and test-retest reliabilities ranging from 0.48 to 0.65 and the CERQ has good factorial validity, discriminant validity and construct validity (Garnefski *et al.* 2002). In this study, the scale was translated into Bangla and adapted within the socio-cultural context of Bangladesh by administering on a sample of 100 secondary school students. The split-half reliability of the Bangla version as calculated by the Spearman-Brown formula is 0.78. As reported by the judges the Bangla version has good content and face validity.

The PAQ is a 30-item measure originally developed by Buri (1991). It is a five-point Likert type scale ranging from 1 (disagree) to 5 (agree). The scale has three subscales, namely *authoritative parenting, authoritarian parenting* and *permissive parenting*. Each sub-scale contains 10 items. Cronbach α coefficients for the sub-scales are 0.61, 0.79 and 0.72 respectively. The full scale has Cronbach α coefficients of 0.74 to 0.87 and test-retest reliabilities of 0.77 to 0.92 (Buri 1991). The construct validity of the original scale was tested by correlating parenting style with self-esteem. As in the above, the scale was translated into Bangla and adapted within the socio-cultural context of Bangladesh. The Bangla version has good content and face validity as reported by the judges. The split-half reliability of the full scale calculated by the Spearman-Brown formula is 0.72.

Procedures: Standard data collection procedures were followed in the study. One of the authors of this paper personally met each head of the selected schools, briefed about the general purpose of the study and got permission to collect relevant data from the

students. At the beginning of survey administration, good rapport was established with the students. The surveys were distributed to them individually asking to read the instructions printed on questionnaires, record the socio-demographic information (e.g., age, gender, class, family type, socio-economic status) and respond to the items. Necessary clarifications were made whenever they faced any problems to understand the items. Thus the CERQ and the PAQ were administered to them at a single sitting. They responded to the CERQ followed by the PAQ. Thus data collection was completed in four selected schools.

Data Analysis: Participants' responses were scored according to the scoring systems of the PAQ and CERQ respectively. Each participant received three types of scores on the PAQ: permissive score, authoritative score and authoritarian score, and two scores on the CERQ: adaptive score and less adaptive score. As the present study was correlational in its design, data were analyzed in multiple regressions using 'Enter' method on SPSS with overall adaptive and overall less adaptive emotion regulations as criterion/dependent variables and permissive, authoritative, authoritarian parenting, family type (levels: nuclear and extended) and child's gender (levels: male and female) as predictor/independent variables. Major assumptions of the multiple regression analysis (linearity, normality, homoscedasticity and multi-collinearity) were met in the present data.

Results and Discussion

The results of this study are illustrated below showing how children's adaptive cognitive emotion regulation and less adaptive cognitive emotion regulation vary with parenting style, but not with family type and child's gender.

Adaptive Cognitive Emotion Regulation: A significant regression model was emerged explaining 17.3% of the variance in *adaptive* cognitive emotion regulation (Adjusted R^2=0.173; F=9.579, p<.001). Table1 indicates that *adaptive* cognitive emotion regulation has a functional relationship with parenting style. As revealed by the standardized β, *authoritative* parenting style (β=.376, p<.001) was the strongest predictor of *adaptive* cognitive emotion regulation when the variance explained by all other variables in the model was controlled. A second significant predictor of this type of emotion regulation was the *authoritarian* parenting style (β=.197, p<.005). Thus a change of 1 standard deviation in *authoritative* parenting resulted in a change of .376 standard deviations in *adaptive* cognitive emotion regulation, whereas a change of 1 standard deviation in *authoritarian* parenting resulted in a change of .197 standard deviations in *adaptive* cognitive emotion regulation. Part correlation coefficients for the *authoritative* and *authoritarian* parenting styles were .334 and .190 respectively (not shown in table). When computed their unique contributions (squared of part correlation multiplied by 100), *authoritative* parenting style excelled (11.16%) over *authoritarian* parenting style (3.61%). Furthermore, when

Table 1. Regression of adaptive cognitive emotion regulation on permissive parenting, authoritative parenting, authoritarian parenting, family type and child's gender.

Predictor variables	Unstandardized coefficients		Standardized coefficients		
	B	*SE*	*β*	*t*	*p*
(Constant)	36.68	6.475		5.66	.0001
Permissive parenting	.121	.187	.047	.646	.519
Authoritative parenting	. 641	.122	.376	5.256	.0001
Authoritarian parenting	. 317	.106	.197	2.995	.003
[1]Family type (nuclear)	2.451	1.793	.088	1.367	.173
[2]Child's gender (male)	- 2.29	1.725	-.087	-1.32	.187

Adjusted R^2=0.173 (F =9.579, p<.001)

Note: [1]Family type (N) was used here and subsequently as a dummy variable coded as '1' or '0'. '1' stands for membership of a nuclear family and '0' stands for non-membership of a nuclear family. So, when '1' changes to '0' the variable switches to Family Type (Extended). The same logic applies for [2]Child's gender (M) variable.

data for each *adaptive* strategy were analyzed separately, results were highly consistent with the results for overall *adaptive* cognitive emotion regulation as above. That is, *authoritative* parenting was the strongest and/or only predictor of the child's scores in positive refocusing (β=.341, p<.001), refocus on planning (β=.286, p<.001), positive reappraisal (β=.310, p<.001) and putting into perspective (β=.256, p=.001) strategies. Although authoritarian parenting was identified as a significant predictor of positive refocusing (β=.138, p<.05) and putting into perspective (β=.183, p=.01) strategies it was weaker than authoritative parenting. However, none of the variables explained child's score in acceptance strategy.

The above findings suggest that authoritative parenting works best for adaptive emotion regulation (i.e., rational and positive thoughts, happy and pleasant thoughts) in children. The present findings are consistent with the past findings. For example, past studies found that children reared by authoritative parents show higher levels of social competence (Baumrind 1978), a greater ability to regulate emotions, high social skills (Isley et al. 1996) and self-regulation (Baumrind 1967). They also excel in areas of independence, creativity, persistence, academic competence, leadership skills, and social perspective taking (Baumrind 1967, 1991, 1993). Why is *authoritative* parenting conducive for *adaptive* cognitive emotion regulation in children? As discussed earlier in this paper, *authoritative* parents set reasonable demands on and have high expectations for their children while being warm and responsive. As parents give them chance to explore the event, they can analyze and handle the situation more efficiently, and approach forward to reach the goals. They can develop thoughts to give a positive meaning even to the negative and stressful events. Baumrind (1978) explained that

authoritative parents openly discuss the problems or actions that may arise in relation to the child and exhibit firm control when necessary.

Less Adaptive Cognitive Emotion Regulation: The regeression model was also significant here which explained 7.1% of the variance in *less adaptive* cognition emotion regulation (Adjusted R^2=.071, F=4.135, p=.001). Table 2 indicates that the *less adaptive* cognitive emotion regulation has a functional relationship with parenting style. As revealed by the standardized β, *authoritarian* parenting style (β=.294, p<.001) was the strongest and only significant predictor of *less adaptive* cognitive emotion regulation when the variance explained by all other variables in the model was controlled. Thus a change of 1 standard deviation in *authoritarian* parenting resulted in a change of .294 standard deviations in *less adaptive* cognitive emotion regulation. Part correlation coefficient for this predictor was .283 (not shown in table) indicating that *authoritarian* parenting alone contributes 8.01% (squared of part correlation multiplied by 100) of the

Table 2. Regression of less adaptive cognitive emotion regulation on permissive parenting, authoritative parenting, authoritarian parenting, family type and child's gender.

Predictor variables	Unstandardized coefficients		Standardized coefficients		
	B	*SE*	*β*	*t*	*p*
(Constant)	34.597	5.394		6.414	.0001
Permissive parenting	.166	.156	.083	1.064	.289
Authoritative parenting	-.028	.102	-.021	-.273	.785
Authoritarian parenting	.371	.088	.294	4.210	.0001
[1]Family type (nuclear)	-.053	1.493	-.002	-.035	.972
[2]Child's gender (male)	-.950	1.437	-.046	-.661	.509

Adjusted R^2=.071 (F=4.135, p<.001)

variance in *less adaptive* cognitive emotion regulation. Furthermore, when data for each *less adaptive* strategy were analyzed separately, results were consistent with the results for the overall *less adaptive* cognitive emotion regulation as above. That is, *authoritarian* parenting was the strongest and only predictor of child's scores in self-blame (β= .235, p<.001), rumination or focus on thought (β=.262, p<.001) and catastrophizing (β=.214, p<.005) strategies. However, none of the variables explained child's score on blaming others.

The above findings suggest that authoritarian parenting leads to a less adaptive emotion regulation in children. The positive association of *authoritarian* parenting with *less adaptive* cognitive emotion regulation indicates that children of *authoritarian* parents always emphasize their thoughts of negative aspects of the situation. Consistent with these findings past studies have shown that *authoritarian* parenting is positively associated with the child's negative outcomes and negatively with the positive outcomes such as self-esteem (Buri *et al.* 1987). *Authoritarian* parents are obedience- and status-

oriented, and expect their orders to be obeyed without explanation (Baumrind 1991). They are demanding and unresponsive to the emotional needs of the child, as well as being controlling, detached and unsupportive (Baumrind 1967). Thus offering one-way style of parenting authoritarian parents might block the development of emotion regulation in adaptive manner, increasing the likelihood that the child will be less adaptive in interaction with the surroundings.

The study further revealed that permissive/indulgent parenting has a neutral role in developing cognitive emotion regulation making children neither adaptive nor less adaptive (Tables 1 and 2). As permissive parents exhibit high levels of warmth and low levels of control, children of these parents become neither adaptive nor less adaptive in emotional setting. Research has shown that children of permissive parents get inconsistent and confusing guidelines or no outlines of the boundaries in their environment (Baumrind 1967). Under such parenting, little is required of children, especially in the areas of maturity and responsibility (Baumrind 1991). Also, permissive parents often surrender to the demands of their child. According to Baumrind (1968), children of permissive parents are often left to regulate their own activities, behavior, and emotions at a young age.

One important aspect of the findings is that children's cognitive emotion regulation is not associated with the type of family they are raised in (Tables 1 and 2). Family is the first and foremost important psychosocial setting for every child. According to Karim *et al.* (2004), to know the future of a society one should look into the ways the children are raised in, but not into the family structure. As the difference in family structure did not produce any difference in children's cognitive emotion regulation in this study, we give more importance on quality parenting over family structure, suggesting that all parents should be trained on good parenting rather than family structure. However, there are studies demonstrating that family type can facilitate or limit the ways in which parents are able to positively influence the outcomes of their children (Amato 2001, Amato and Keith 1991, Sigle-Rushton and McLanahan 2002). But, in those studies the concept of family type was different from what we mean by family type in the present study. As demonstrated by Amato (2001), for example, children coming from divorced families have more difficulties in school, more behavior problems, more negative self-concept and more trouble getting along with their parents. Children who live with a single mother family fare poorly across a wide range of adolescent and adult outcomes, including educational attainment, economic security and physical and psychological well-being (Sigle-Rushton and McLanahan 2002). Thus whether family type is important for the child's emotional or other psychosocial development depends on how it is defined. A family type just defined by the number of people living together is not important at all.

Another interesting demonstration is the gender equality in children's cognitive emotion regulation. This is inconsistent with the findings in other cultures (Zlomke and Hahn 2010, Martin and Dahlen 2005, Garfenski 2004). American women scored higher on

rumination, catastrophizing, positive refocusing, refocusing on planning and positive reappraisal whereas American men scored higher on blaming others (Martin and Dahlen 2005). Likewise, Dutch women reported to use rumination, catastrophizing and positive refocusing more often than Dutch men (Garfenski 2004). Nowadays, parents in Bangladesh are conscious enough to deal with the male and female children alike, thus promoting no difference in cognitive emotion regulation between the two sexes.

In summary, the study demonstrated that the style of parenting determines the cognitive strategies children will employ to regulate their emotions. Of the three types of parenting, *authoritative* parenting was the best for children's *adaptive* cognitive emotion regulation, and authoritarian for *less adaptive* cognitive emotion regulation. *Permissive* parenting has no impact on children's cognitive emotion regulation. Type of family or child's gender has also nothing to do with such functioning. It can, therefore, be concluded that good parenting style as characterized by parental warmth, acceptance, and readiness for childhood needs and proper control is crucial for adaptive emotion regulation at adolescent period and to handle the problems skillfully.

Limitations and Implications: The present study offers some inconsistent results. For example, authoritarian parenting contributes significantly in both the adaptive and less adaptive cognitive emotion regulations. This was unexpected and cannot be explained by the present data. The study has also some inherent limitations such as it cannot explain a large proportion of the variance in cognitive emotion regulation. Further research on a large scale sample from different parts of Bangladesh will possibly exclude the inconsistency.

Despite the above limitations, the present findings will have important implications for research and practice. The findings will give rise to new researches in family matters, parental practices and adolescents' outcomes. For practice, the study provides important information about the good parenting need in adolescence. Adolescence is a very sensitive age, when guidance and proper press of their emotion and emotion regulation must go together with affection, support, and freedom. The findings will be helpful for parents, caregivers, child psychologists, and other professionals working with children or adolescents for guiding them to become resources of the country.

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ACCUMULATION AND HISTOPATHOLOGICAL EFFECTS OF ARSENIC IN TISSUES OF SHINGI FISH (STINGING CATFISH) *HETEROPNEUSTES FOSSILIS* (BLOCH, 1794)

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Abstract

A 60-day experiment was conducted to compare the accumulation and toxicological effects of arsenic in muscle, intestine and liver of shingi fish, *H. fossilis* (Bloch) after exposure to two concentrations (7.0 and 20.0 ppm) of arsenic trioxide. The highest/maximum level of accumulation of arsenic was observed in the liver whereas the lowest level of arsenic was found to accumulate in the muscle tissues at the end of exposure period. It is apparent from the study that the damage of the liver of test fish due to 15 days exposed period was less compared to the damage caused by 60 days exposure periods. The intensity of histological alterations was observed to increase gradually with the arsenic concentration and the exposure time.

Key words: Arsenic, *Heteropneustes fossilis*, Accumulation, Tissues, Histopathology

Introduction

Study of toxicology pertaining to aquatic animals has become important in water pollution studies. Heavy metal contaminants in aquatic ecosystems pose a serious environmental hazard because of their persistence and toxicity. Among the heavy metal pollutants, arsenic (As) receives a special attention due to its potential health hazard to aquatic fauna and human life in particular. The recent research has suggested that As acts as an endocrine disruptor at extremely low concentrations (Stoica *et al.* 2000). The presence of As in industrial wastes and its high toxicity along with considerable bioaccumulation in freshwater fishes make it a toxicant that should be given due consideration in aquatic toxicology. The term bioaccumulation refers to the wastes which have been reconcentrated in organisms often having undergone initial dilution in environment producing toxic effects in fishes (Dallinger *et al.* 1987). Availability of heavy metals in the aquatic ecosystem and its impact on the flora and fauna had been reported by many investigators (Nayak 1999 and Shrinivas and Balaparameswara 1999).

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The accumulation of heavy metals in different tissues of fish may cause various physiological defects and mortality (Torres *et al.* 1987). Heavy metals accumulated in the tissues of aquatic animals may become toxic when accumulation reaches a substantially high level (Kalay and Canli 2000). The pattern of accumulation of metals in animals differs from metal to metal and organ to organ during their functional status. Most of the investigations pertaining to heavy metals contaminants in aquatic systems are dealt either with toxicity or with accumulation (Rushforth *et al.* 1981 and Khadiga *et al.* 2002). Heavy metals have been shown to be concentrated in the liver of various fishes (Sorensen 1991 and Rao *et al.* 1998). The highest concentrations of As was recorded in the liver, while the lowest one was in the muscle. Mormede and Davies (2001) suggested that the liver was the target organ, showing the detoxification and accumulation role of the liver. Muscle is generally considered to have a weak accumulating potential (Erdoğrul and Erbilir 2007, Uysal *et al.* 2009 and Bervoets and Blust 2003). Histological changes associated with heavy metals in fish have been studied by many authors (Thophon *et al.* 2003, Mohamed and Gad 2005, Athikesavan *et al.* 2006, Giari *et al.* 2007, Jiraungkoorskul *et al.* 2007 and Van Dyk *et al.* 2007). Hence, the present study was aimed to investigate the accumulation of As in *Heteropneustes fossilis* (Bloch) and the associated histopathological changes in three organs (muscle, liver and intestine) at laboratory condition.

Materials and Methods

Special care was taken to make sure that the fish were approximately of similar size and weight. Fishes with almost similar length (8.15±0.51 cm) and weight (6.25±0.75 g) were collected from local market and were acclimatized under laboratory conditions (29.0±1 ^{0}C). Fishes were transported to the laboratory in large buckets with proper covering and frequent agitation. On arrival at the laboratory, these were immediately released into three big tanks containing tap water and then maintained there for about 6-7 days in a static condition. Fishes were fed on artificial feed twice daily. Any debris or unwanted particles were removed from the tank after feeding. The water medium was changed at 24 hours interval to remove the metabolic-pollutants. Air compressor with air stones was used for oxygenation of water. The water quality parameters of the acclimation tank were studied at times. However, after acclimation, only healthy fishes were used for experiment. Arsenic trioxide (As_2O_3) was collected from the BDH laboratory (England) in original package form. By mixing with tap water two different concentrations of As_2O_3 was used as stock solution. The fishes were exposed to two concentrations for a period of 1, 15 and 60 days in glass aquaria containing 10-20 L water. Tap water stored in the tank for two months confirming the settlement of iron, were used for the experiment. The water was aerated for one day before starting the experiment. Stone aerators connected to a compressed air supply were used to maintain an adequate level of dissolved oxygen in each aquarium. The liver, intestine and muscle tissues of control and treated fishes were isolated and dried in an oven at 105 ^{0}C for 24 hours. The known amounts of dried tissues

were digested with nitric acid and perchloric acid. After the accomplishment of complete digestion, the digested samples were made-up to 25 ml with metal free double distilled water and arsenic measurements were made using atomic absorption spectrophotometer (Begum *et al.* 2005). Values were expressed as µg/g, dry weight.

At the end of the exposure period, muscle, intestine and liver were collected from the *H. fossilis* and preserved in small plastic vials with 10% buffered neutral formalin (Begum *et al.* 1996). The number at section of samples was prepared using a microtome, stained and studied under a photomicroscope (Olympus, CH4O, Japan). Photomicrographs were taken after the examination of histological condition of each of the tissue slides.

Results and Discussion

The physico-chemical properties (temperature, dissolved oxygen, pH, carbon dioxide, alkalinity, total hardness and ammonia concentration) of the tap water were monitored during the acclimation period and trial with fishes exposed to As_2O_3 (Table 1). It is evident from the data that (Table 1), the water quality parameters did not fluctuate greatly among the different treatment aquariums as well as between different experimental trials. Moreover, the water quality was always within the normal ranges.

Table 1. Physico-chemical parameters of aquarium water with different treatments of arsenic (As).

Concentration of arsenic (ppm)	Temperature (^{0}C)	Dissolved O_2 (mg/l)	P^H	CO_2 (mg/l)	Alkalinity (mg/l)	Hardness (as $CaCO_3$) mg/l	Ammonia mg/l
BDL (control)	29 (27.5-30.0)	6.2 (6.1-6.3)	7.1 (7.1-7.3)	19.1 (18.2-20.0)	129.8 (125.1-134.5)	226 (224-229)	0.27 (0.24-0.30)
7		5.7 (5.4-6.1)	7.32 (7.5-7.6)	20.9 (19.8-22.0)	130 (129-131)	225 (220-230)	0.18 (0.10-0.26)
20		5.3 (5.2-5.4)	7.26 (7.2-7.31)	20.2 (18.0-22.4)	129 (128-130)	213 (212-214)	0.15 (0.089-0.21)

BDL- Below detection level

The levels of arsenic (As) accumulation in the liver, intestine and muscle tissues of *H. fossilis,* during exposed to control, 7 and 20 ppm of concentration for 1, 15 and 60 days are presented in Table 2. In the present investigation, the highest level of As accumulation (10.01 ± 0.55) and (16.26 ± 0.34) was found in the liver and lowest level (3.24 ± 0.25) and (6.55 ± 0.10) in muscle to 15 and 60 days of exposure periods, respectively. Similar pattern of accumulation of As in the liver tissues of *Mugil cephalus* has been reported by Maher *et al.* (1999) and found significantly higher than in any other tissues. Pazhanisamy *et al.* (2007) investigated the accumulation of As in *Labeo rohita*

after exposed in two sub lethal concentration of As trioxide. They found that the maximum level of accumulation of As was in the liver whereas, the lowest level in the muscle tissue at the end of 28 days of exposure. Similarly, the distribution and accumulation pattern of heavy metals in the liver of various teleosts fishes have been studied by Noel-Lambot *et al.* (1978) and Thiruvalluvan *et al.* (1997). In the present study, the rate of accumulation was found to increase gradually with the As concentration and the exposure time. The findings are identical with the report of Karuppasamy (1999) while, described the bioaccumulation as dose and time dependent in phenyl mercuric acetate exposed fish *Channa punctatus*.

Table 2. Accumulation of arsenic (µg/g, dry weight) in tissues of *H. fossilis* exposed to different concentration and exposure periods (days).

Tissues	Concentration	Exposure period in days		
		1	15	60
Muscle	0.28 (Control)	0.53 ± 0.17	1.38 ± 0.12	2.16 ± 0.11
	7.0	1.99 ± 0.90	3.24 ± 0.25	6.55 ± 0.10
	20.0			
Intestine	2.43 (Control)	2.10 ± 0.17	3.81 ± 0.29	8.50 ± 0.21
	7.0	3.47 ± 0.14	5.67 ± 0.27	11.10 ± 0.23
	20.0			
Liver	3.39 (Control)	5.38 ± 0.33	6.62 ± 0.46	7.49 ± 1.11
	7.0	8.09 ± 0.67	10.01 ± 0.55	16.26 ± 0.34
	20.0			

Then, with the rate of accumulation of As and days of exposure, the histopathological study also showed drastic changes in muscle, intestine and liver tissues of the exposed fish. These findings suggested that the observed changes were undoubtedly as a result of various toxicological impact of As exposure. Even, these changes were directly related with the concentration of 7 and 20 ppm of As along the 1, 15 and 60 days of exposure (Plate 1a). However, at 7 ppm exposed to As, the muscle tissue exhibited dystrophic changes with marked thickening and separation of muscle bundles after 15 days of exposure (Plate 1b), but, such a change after 60 days of exposure, the vacuolar degeneration in muscle bundles with aggregations of inflammatory cells between them and focal areas of necrosis were observed (Plate 1c). Again, at 20 ppm of As concentration, the muscle tissues exhibited dystrophic changes with marked thickening and vacuolar degeneration of muscle bundles along with severe intramuscular edema after 15 days of exposure (Plate 1d) while, atrophy and edema of muscle bundles as well as splitting of muscle fibers were seen in 60 days of As exposure of fish (Plate 1e). It is true that like gills, muscle tissue also come into close contact with pollutants dissolved in water. Therefore, the muscles of *H. fossilis* showed degeneration in muscle bundles accompanied with focal areas of necrosis as well as atrophy and vacuolar degeneration. Similar findings had been observed in Nilotica fish by Mohamed (2008).

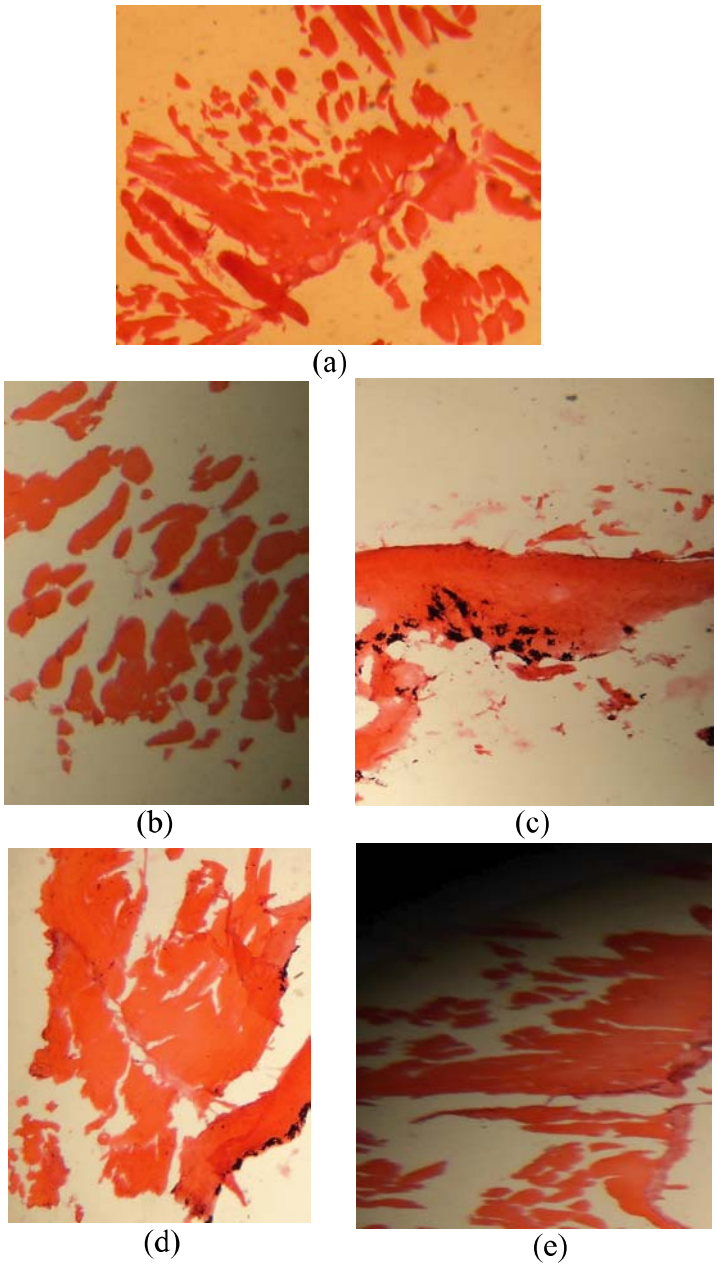


Plate 1. (a) Muscles of fish showing the normal, (b) separation in muscle bundles, (c) focal area of necrosis, (d) intramuscular edema and (e) splitting of muscle fibers and atrophy of muscle bundles.

In the case of intestine (Plate 2a), the histopathological changes so obtained in 7 ppm of As concentration and 15 days of exposed included partial intactness of serosa but, more or less organized mucosa and disorganized villi (Plate 2b). The same organ, even at same concentration after 60 days of exposure exhibited partially damage of muscles, but disorganized, slightly swollen and shorten of villi (Plate 2c). This in 20 ppm concentration of As and after 15 days of exposure showed damaged serosa disorganized and consequent fussion of mucosa, degeneration and edema between the intestinal submucosa and lamina propria (Plate 2d). Further, these damages were characterized by the increases in number of goblet (mucosal) cells, width of the lamina propria and degeneration of villi after 60 days of As exposure (Plate 2e). The findings suggest that however, uptake of As and other metals occur mainly through gills but may also occur *via* intestinal epithelium. Therefore, the histopathological alterations so far observed in the intestine tissues of studied fish may be a result of uptake of toxic As. The present results are in agreement with those observed by many investigators about the effects of metals on fish intestine (Giari *et al.* 2007 and Hanna *et al.* 2005).

Observations on the fish liver (Plate 3a) revealed that in 7 ppm of As concentration, and 15 days of exposure primary degeneration occurred in the hepatocytes (Plate 3b), which at the end of 60 days showed further changes like focal areas of necrosis, haemorrhage and haemolysis between the hepatocytes (Plate 3c). On the other hand, fish exposed to As concentration of 20 ppm, the hepatocytes became more irregular and lose their polygonal shape, areas of hepatocytes with eosinophilic cytoplasm (Plate 3d). Moreover, haemosiderin was seen around central veins and hepatoportal blood vessels. In some cases, dilation and intravascular haemolysis in hepatoportal blood vessels were noticed at 60 days of exposure (Plate 3e). Sorensen (1991) stated that the liver is a critical target organ for As toxicity in fish due to the role it plays in metabolism and detoxification. Such a statement is supported by the results of our present study, as livers of shingi fish exposed to different concentrations of As showed significant changes in architectural and structural arrangements, as well as areas of inflammation and focal necrosis. Similar alterations have been observed in fish liver being exposed to As in both the laboratory and field conditions (Gilderhus 1966 and Joshi and Sahu 2007).

Thus, comparing the entire findings it is clear that the highest/maximum level of As accumulation obtained in the liver and intestine, whereas, the lowest in the muscle tissues at the end of exposure periods. At the same time, it indicates about the intensity of histological alterations are also dose (As concentration) and time dependent. In conjugation of these two facts, the As trioxide affects severely on the tissues of internal organs of *H. fossilis* leading to life threat and poor reproductive performance. Moreover, the present study suggests further detailed investigation on the possible As pollution sources as well as the rate of accumulation in different fish tissues and possible histological changes.

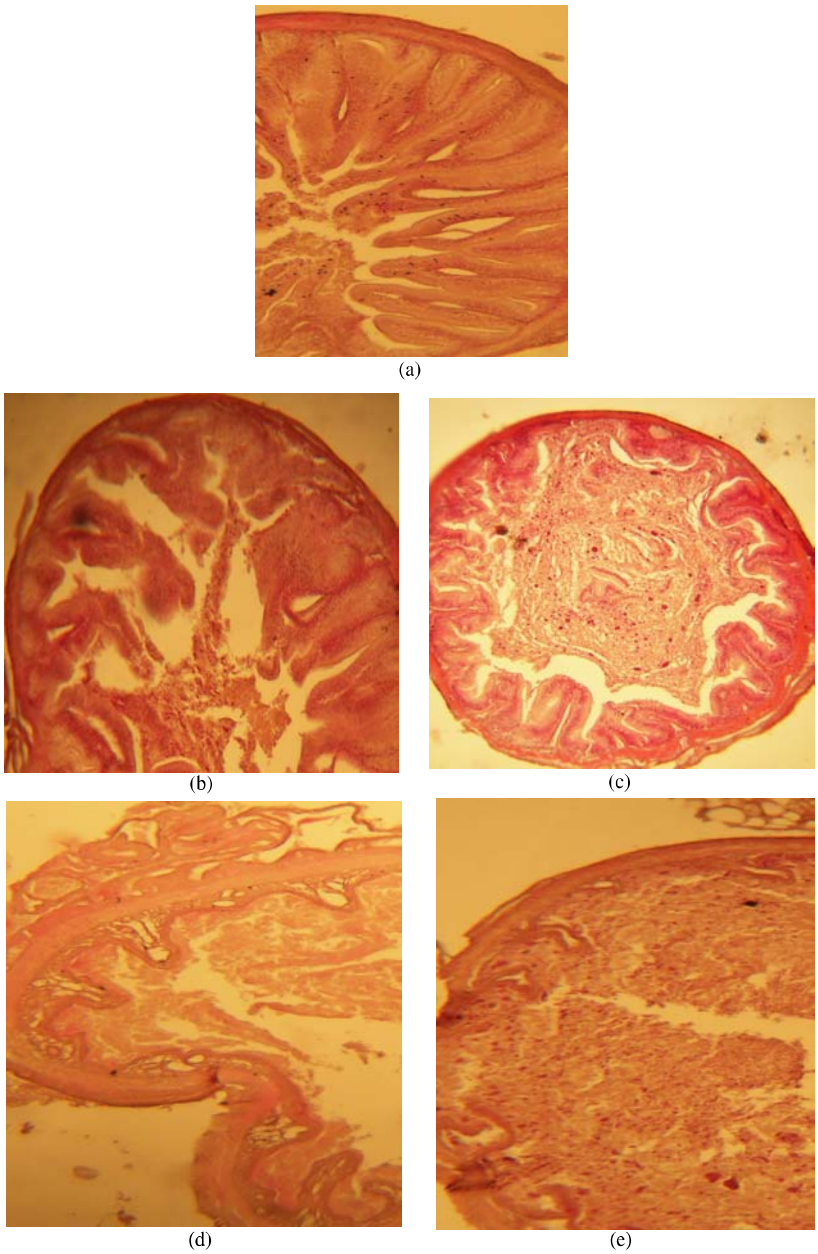
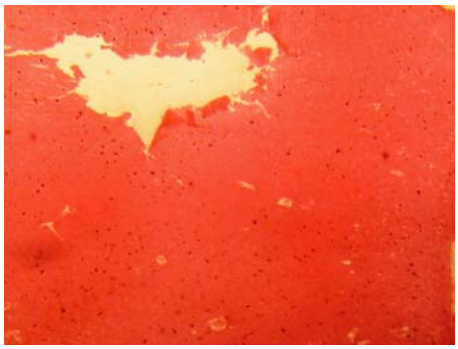
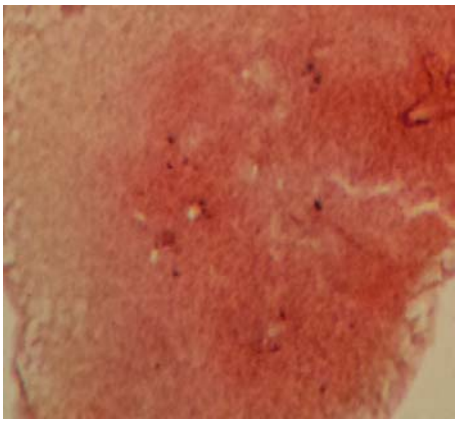


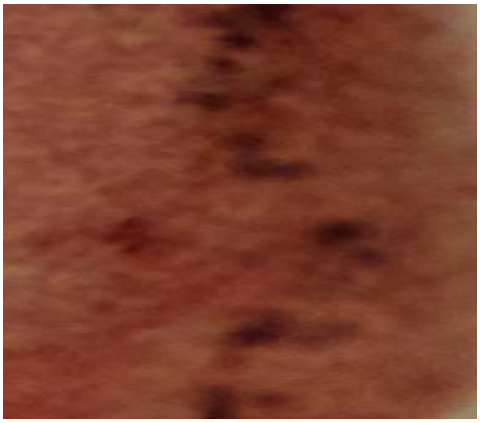
Plate 2. (a) Intestine of fish showing the normal, (b) serosa showed partial intacness and disorganized villi, (c) severe damage in muscularis and villi slightly swollen and shortened, (d) degeneration and edema between the intestinal submucosa and lamina propria and (e) increase in the width of lamina propria and villi degenerated.



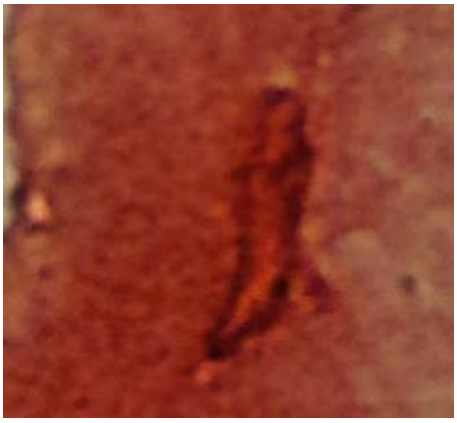
(a)



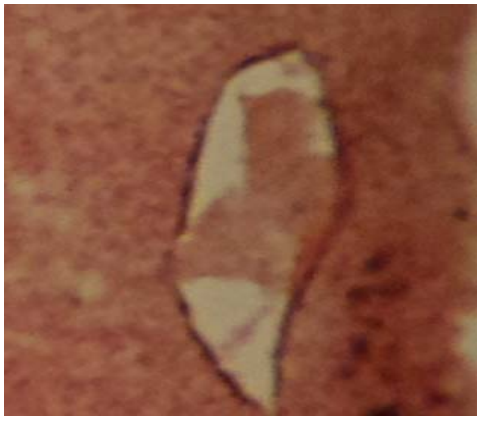
(b)



(c)



(d)



(e)

Plate 3. (a) Liver of fish showing the normal, (b) vacuolar degeneration, (c) focal areas of necrosis, (d) haemorrhage and haemolysis between the hepatocytes, intravascular haemolysis in blood vessels and (e) intravascular haemolysis in hepatoportal blood vessels.

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COPPER, CADMIUM, CHROMIUM AND LEAD BIOACCUMULATION IN STINGING CATFISH, *HETEROPNEUSTES FOSSILIS* (BLOCH) AND FRESHWATER MUSSEL, *LAMELLIDENS CORRIANUS* LIA AND TO COMPARE THEIR CONCENTRATION IN SEDIMENTS AND WATER OF TURAG RIVER

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Abstract

The present study was carried out to determine the level of bioaccumulation of some heavy metals namely Copper (Cu), Cadmium (Cd), Chromium (Cr) and Lead (Pb) in freshwater fish Stinging catfish (*Heteropneustes fossilis* Bloch, 1794) and freshwater Mussel (*Lamellidens corrianus* Lia, 1834) collected from Turag river during the months of October to December 2010. The accumulation levels were then compared with the concentration levels of sediments and water of the same river. In *H. fossilis* the average bioaccumulations were Cu 13.27 ± 2.47 mg/kg ; Cd 0.215 ± 0.208 mg/kg ; Cr 1.46 ± 0.431 mg/kg and Pb 0 mg/kg in dry weight while Cu 31.90 ± 6.202 mg/kg ; Cd 0.182 ± 0.025 mg/kg ; Cr 0.0367 ± 0.039 mg/kg and Pb 3.865 ± 1.041 mg/kg in dry weight of *L. corrianus*. Average concentration of metals in sediments of Turag river were Cu 54.95 ± 9.218 mg/kg ; Cd 0.05 ± 0.011 mg/kg ; Cr 5.575 ± 0.608 mg/kg and Pb 34.89 ± 5.554 mg/kg in dry weight and in water these levels were Cu 0.0253 ± 0.024 ppm ; Cd 0.0012 ± 0.001 ppm ; Cr 0.2335 ± 0.044 ppm and Pb 0.1169 ± 0.041 ppm. The bioaccumulation level of heavy metals in Turag river were higher than the FAO approved standard level.

Key words*:* Bioaccumulation, Heavy metal, *Heteropneustes fossilis, Lamellidens corrianus*, Turag river

Introduction

Heavy metals are the metallic elements of high atomic weight which would mainly include the transition metals, some metalloids, lanthanides, and actinides. Many different definitions have been proposed on the basis of density, atomic number, atomic weight as well as their chemical properties or toxicity. Moreover, the term heavy metal has been called a "misinterpretation" in an IUPAC technical report due to its contradictory definitions and lack of a "coherent scientific basis". Thus, an alternative term toxic metal has been proposed, but no consensus of exact definition exists either (Davies *et al*. 2006). These are natural components of the Earth's crust and cannot be degraded or destroyed. A small extent of these may enter our body via food, drinking water and inhalation of contaminated air. As a result, gradual concentration of higher level leads to poisoning (Bendicho and Lavilla 2003). The thing is more acute in aquatic ecosystems,

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where the industrial and municipal wastes as well as the soil deposited forms are constantly being washed out by the normal and acid rain waters into the nearby waterbodies and therein bioaccumulate in the body of all kinds of aquatic organisms. In small amounts, trace or heavy metals are normal constituents of aquatic organisms but at higher concentrations, they exert ranges of toxic effects that are metabolic, physiologic, behavioral and ecological in nature (Kebede and Wondimu 2004). The sources are then transmitted those and even some essential trace elements in human body through food chain can cause damage to health or even death at increasing concentrations. The form in which an element is ingested also plays a major role in its restorability or toxicity.

Besides, heavy metal pollution may also arise from many other sources, but most common sources are the purification of metals, e.g., the smelting of copper and the preparation of nuclear fuels. Electroplating is the primary source of chromium and cadmium. Through precipitation of their compounds or by ion exchange into soils and mud, heavy metal pollutants can localize and lay dormant. Unlike organic pollutants, heavy metals do not decay and thus pose a different kind of challenge for remediation.

Some of these elements (cobalt, copper, chromium, manganese, nickel) are necessary for human in minute amounts while others are carcinogenic or toxic. Manganese, mercury, lead and arsenic are harmful to the central nervous system. Mercury, lead, cadmium and copper are carcinogenic to the kidneys and liver. Similarly, nickel, cadmium, copper and chromium are harmful to the skin, bones, or teeth (Bhattacharya *et al.* 2007).

Copper is an essential substance to human life, but in high doses it can cause anemia, liver and kidney damage, and stomach and intestinal irritation. Similarly, long-term exposure of cadmium is associated with renal disfunction. High exposure can lead to obstructive lung disease and has been linked to lung cancer, although data concerning the latter are difficult to interpret due to compounding factors. Cadmium may also produce bone defects (osteomalacia, osteoporosis) in humans and animals. Low-level of chromium exposure can irritate the skin and cause ulceration. Long-term exposure can cause kidney and liver damage, and damage too the circulatory and nerve tissues. Chromium often accumulates in aquatic life, adding to the danger of eating fish that may have been exposed to high levels of chromium. In humans exposure to lead can result in a wide range of biological effects depending on the level and duration of exposure. Various effects occur over a broad range of doses, with the developing foetus and infant being more sensitive than the adult. High levels of exposure may result in toxic biochemical effects in humans which in turn causes problem in the synthesis of haemoglobin, effects on the kidneys, gastrointestinal tract, joints and reproductive system, and acute or chronic damage to the nervous system. (Lenntech 2010).

Contaminations of aquatic ecosystems with trace elements received great attention since the events of Hg and Cd poisoning through fish and shellfish in Minamata, Japan. In recent years, there has been an increasing interest in the utilization of fishes as bio-

indicators of the integrity of aquatic environmental systems. Turag and Buriganga rivers encircled the major part of Dhaka city and thereby highly polluted with municipal and industrial wastes. Local people use its water for many of their daily activities and fishes for consumption. Considering all these things, the main objectives of this research were to determine the contamination levels of heavy metals in sediments and water as well bioaccumulation in aquatic organisms like fish and mollusks.

Materials and Methods

Freshwater Stinging catfish or Shing, *Heteropneustes fossilis* (Bloch, 1794) and freshwater mussel, *Lamellidens corrianus* Lia, 1834 as well as sediment and water samples were collected from Turag river at Diabari to Chatbari region ($23^0$47′59.45″N-$23^0$49′44.29″ N and $90^0$20′35.10″E- $90^0$20′32.82″ E) during the months of October to December, 2010. Samples were prepared step by step according to recommended procedures by AOAC (AOAC International 2002). Sediment, water, fishes and mussels were separately digested by nitric acid (HNO_3) and perchloric acid ($HClO_{4)}$ in a hot plate apperatus. The present work was completed by Flame Atomic Absorption Spectrophotometry (FAAS, model: Perkin Elmar Analyst 800) in the Institute of Nuclear Science and Technology (INST) of Atomic Energy Research Establishment, Savar, Dhaka. Before each metal determinations the FAAS instrument was calibrated with four standard solutions to measure the error. Each of the four metal calibrations showed the linear calibration curve which indicates the negligible error. The readings for each target sample solutions were taken thrice and obtained data was calculated statistically by Winlab 32^{TM} and then by SPSS (version 16.0). The levels of bioaccumulations in *H. fossilis* and *L. corrianus* were determined without separating organs. Sediments and water were also examined to compare bioaccumulation between living organisms and their habitats.

Results and Discussion

Sediments of Turag river showed a higher level of contamination. Mussels showed a higher Cu bioaccumulations as 25.57- 43.53 mg/kg (average 31.9 ± 6.21 mg kg) than that of fish but lesser than that of sediments. In the body of *H. fossilis* it was 10.21- 17.15 mg/kg (average: 13.27 ± 2.47 mg/kg), while in sediments and water it was 43.15 - 73.56 mg/kg (average: 54.95 ± 9.21 mg/kg) and 0 - 0.073ppm (average: 0.0253 ± 0.023 ppm) respectively, which in all cases was higher than the FAO approved standard level. (FAO 2010) (Table 1 and Fig. 1).

Table 1. Concentrations of copper, cadmium, lead and chromium in fish, mollusk, sediment and water of Turag river.

Samples	N	Copper (Mean±SD)	Cadmium (Mean±SD)	Lead (Mean±SD)	Chromium (Mean±SD)
H. fossilis	15	13.2700±2.470	0.2148±0.208	BDL[1]	1.4636±0.431
L. corrianus	15	31.9011±6.212	0.1826±0.025	3.8656±1.041	0.0367±0.039
Sediments	15	54.9511±9.218	0.0507±0.011	34.8922±5.554	5.5749±0.608
FAO standards		30.00	2.00	0.10	7.00
Water	15	0.0253±0.024	0.0012±0.001	0.1169±0.041	0.2335±0.044
[2]MAC in Bangladesh		1.00	0.001	0.05	0.05

[1]BDL= Below the Detection Level; [2]MAC= Maximum Allowable Concentrations

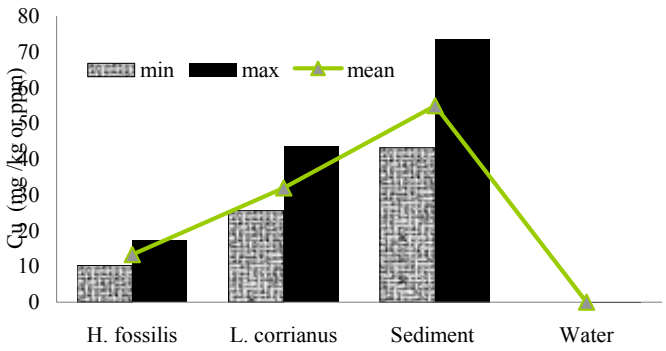

Fig. 1. Mean, minimum and maximum concentration of copper in *H. fossilis, L. corrianus*, sediment and water.

Similarly, the cadmium (Cd) concentrations in *H. fossilis, L. corrianus* and in sediments were 0.052 - 0.46 mg/kg (average: 0.2143 ± 0.20 mg/kg), 0.1235 - 0.220 mg/kg (average: 0.1826 ± 0.025 mg/kg), and 0.0325 - 0.722 mg/kg (average: 0.0507 ± 0.011 mg/kg) respectively (Table 1). This contamination level of Cd in water was 0.00012 - 0.00233 ppm (average: 0.0012 ± 0.001 ppm). (Table 1 and Fig. 2).

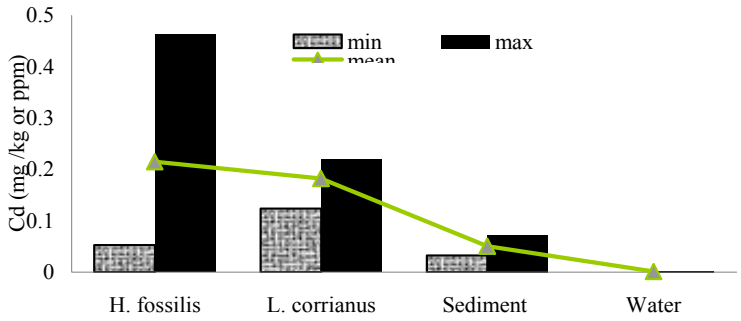

Fig. 2. Mean, minimum and maximum concentration of cadmium in *H. fossilis, L. corrianus*, sediment and water.

The lead concentrations were found as 0 mg/kg (below detection limit), 2.54 - 6.26 mg/kg (average: 3.86 ± 0.01 mg/kg), and 25.89 - 42.33 mg/kg (average: 34.89 ± 5.55 mg/kg) and 0.0856 - 0.210 ppm (average: 0.117 ± 0.04 ppm) in *H. fossilis, L. corrianus*, sediments and water respectively during the same study period. (Table 1 and Fig. 3).

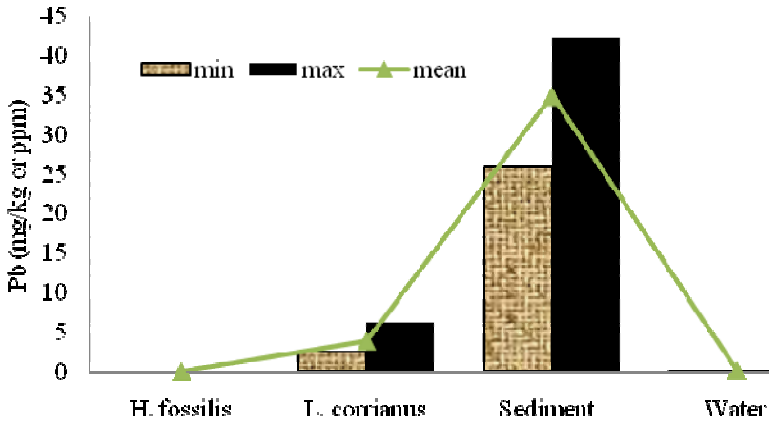

Fig. 3. Mean, minimum and maximum concentration of lead in *H. fossilis, L. corrianus*, sediment and water.

Again, the bioaccumulation of Cr in the body of *H. fossilis, L. corrianus* as well as in the sediments and water of Turag river was 0.95 - 2.1 mg/kg (average: 1.46 ± 0.431 mg/kg), 0 - 0.99 mg/kg (average: 0.03667 ± 0.03 mg/kg), and 4.50 - 6.33 mg/kg (average: 5.57 ±

0.607 mg/kg) and 0.165 - 0.291 ppm (average: 0.233 ± 0.04 ppm) respectively. (Table 1 and Fig. 4).

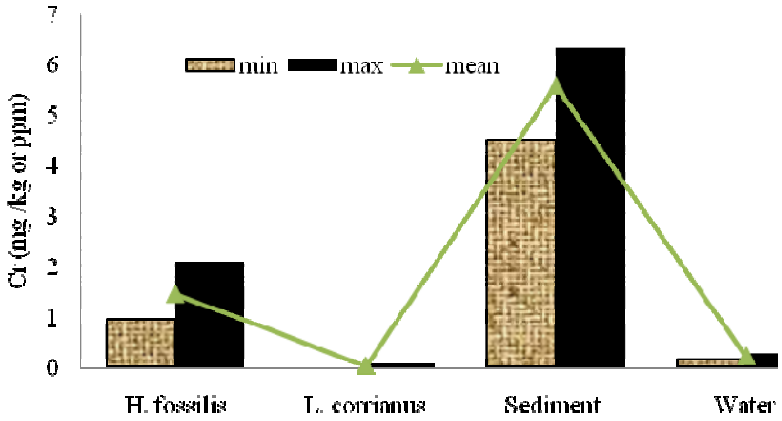

Fig. 4. Mean, minimum and maximum concentration of chromium in *H. fossilis, L. corrianus*, sediment and water.

Sharma and Fulekar (2009) found the concentration pattern as Cu 43.0 mg/kg ; Cd 4.05 mg/kg and Pb 2.43 mg/kg and their order of toxicity was Cu > Cd > Pb in similar kind of study on some commercial fishes of the Gulf of Cambay. In the present study, the level of heavy metals concentration in fish body were obtained as Cu 13.27 mg/kg ; Cr 1.46 mg/kg ; Cd 0.21 mg/kg and Pb 0.0 mg/kg (Table 1) and their order of toxicity was Cu > Cr > Cd > Pb. Ezemonye *et al.* (2006) observed bioaccumulation of heavy metals in freshwater Snail (*Pila ovata*) from the Ikpoda river of Southern Nigeria by using AAS and obtained the mean concentration of Cu 4.56 mg/kg. Whereas, in this study, the average bioaccumulation levels of different selected metals were found in mussel as Cu 31.9 mg/kg ; Cr 0.04 mg/kg ; Cd 0.18 mg/kg and Pb 3.8 mg/kg and in *H. fossilis* as Cu 13.27 mg/kg ; Cr 1.46 mg/kg ; Cd 0.21 mg/kg and Pb 0.0 mg/kg. From this data, it is evident that the bioaccumulation rate of Cu and Pb in mussel were greater than in fish, but Cr and Cd accumulations were higher in fish (Table 1).

Ahmed *et al.* (2012) investigated the level of heavy metals in Ayre fish (*Sperata aor*) in Dhaleshwari river of Bangladesh and found the bioaccumulation levels in fish were Cu 31.50 mg/kg ; Pb 18.776 mg/kg ; Cr 1.458 mg/kg and Cd 0.4873 mg/kg of dry weight and in sediments these levels were Cu 37.45 mg/kg ; Cr 27.3933 mg/kg ; Pb 15.7967 mg/kg ; Cd 2.0830 mg/kg and in water the same were Cu 0.00 ppm ; Pb 0.2014 ppm ; Cd 0.0012 ppm ; Cr 0.1302 ppm. Thus their suggested order of accumulations in fish Cu > Pb > Cr > Cd, sediments Cu > Pb > Cr > Cd and water Cu < Cd < Cr < Pb. Haque *et*

al.(2003) determined the bioaccumulation of heavy metals in *M. vittatus,* collected from Buriganga river and reported Cu 3.595 - 5.139 mg/kg ; Pb 1.031 - 3.3578 mg/kg ; Cr 2.04 - 11.79 mg/kg and Cd 0.109 - 1.102 mg/kg. These in the present study, bioaccumulations in *H. fossilis* were Cu 13.27 mg/kg and Cr 1.46 mg/kg. In the sediments of Turag river, the toxicity order was Cu > Pb > Cr > Cd. Against that in water samples, the levels of Pb and Cr were higher but, Cd and Cu were lower. In numerical values, these were Cu 0.0253 ppm ; Cr 0.2335 ppm ; Pb 0.1169 ppm and Cd 0.0012 ppm which in comparison to maximum allowable concentration levels of Bangladesh (Cu 1.0 ppm ; Cr 0.05 ppm and Pb 0.05ppm) were more or less higher and even exceeded the MAC (GoB 1997) (Table 1).

From this study, it is apparent that the contamination levels of heavy metals in sediments and water exceeded the standard level and consequently accumulated into living organisms like fish and mollusks and even exceeded the safe levels. Thus, the Government should take immediate action to mitigate contamination of toxic heavy metals as well as other pollutants by regulating the industrial and municipal effluents discharges directly into Turag and other rivers all over Bangladesh. At the same time, the local people should be aware about harmful effect of such pollutants.

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OCCURRENCE OF INTESTINAL PARASITES AMONG THE TEACHERS, STUDENTS AND STAFFS OF DHAKA UNIVERSITY

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Abstract

The present study was conducted to find out the incidence of the intestinal parasites and their prevalence among the teachers, students and staffs of University of Dhaka, Bangladesh. The study revealed that the prevalence of intestinal protozoa and helminth parasites are common among the outdoor patients of University of Dhaka. A total of 350 stool samples was examined in the Pathology department of Medical Center of University of Dhaka from June 2009 to May 2010, four species of intestinal parasites were identified of which two species were protozoa *(Entamoeba histolytica* and *Giardia intestinalis)* and two species were nematodes *(Ascaris lumbricoides* and *Trichuris trichiura).* The overall prevalence of infestation was 23.14% where *Entamoeba histolytica, Giardia intestinalis, Ascaris lumbricoides* and *Trichuris trichiura* were found as 4.86%, 3.71%, 11.14% and 3.43% respectively. Highest prevalence was recorded in *Ascaris lumbricoides* (11.14%) and the seasonal pattern showed that highest (30%) prevalence occurred in rainy season and lowest (17.19%) in winter season. The prevalence of intestinal parasites was higher in female (30.56%) than in male (22.29%).

Key words: Intestinal parasites, Teachers, Students, Staffs, Dhaka University

Introduction

Intestinal parasitic diseases are considered as a worldwide problem including Bangladesh. They have a detrimental effect on the health of millions of people every year. The prevalence of intestinal parasite in Bangladesh is quite high where infestation with protozoa and helminth parasites such as *Giardia intestinalis, Entamoeba histolytica, Ascaris lumbricoides* and *Trichuris trichiura* are major public health concern both in rural and urban areas (Saha and Chowdhury 1961, Muazzem and Ali 1968, Muttalib 1975, Muttalib *et al.* 1976, Islam *et.al*. 1975, Chowdhury 1978, Stoll *et al*. (1982), Khanum *et al*. (1999 and 2001). The first five year plan of Bangladesh (1973-1978) reported that 64% of the children of the country suffered from intestinal parasitic infection. Khanum *et al*. (1999 and 2005) worked on infestation of three intestinal worms in 600 children of different socio-economic status residing in three selected rural areas. They reported single and multiple infections by a number of parasites including *A. lumbricoides, T. trichiura* and hookworms. The prevalence was higher among the children who used pond water for drinking and washing utensils, open field

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for defecation, used clay after defecation and lived in mud and straw houses.

The World Health Organization (WHO) estimated that there were around 1000 million cases of ascariasis due to *Ascaris lumbricoides,* and 500 million cases of *Trichuris trichiura* infection worldwide (WHO 1990 and Bundy *et al.* 1992). On the other hand, WHO (1990) estimated that worldwide there were 1447 million and 1048 million cases of *A. lumbricoides* and *T. trichiura* infections, respectively. Children are more exposed to these soil–transmitted worms which are also associated with stunted growth (Kuntz 1960, Ahmed 1986, Ahmed 1989, Robert 1990, Rawsan 1993, Uddin and Khanum 2008 and Khanum *et al*. 2010) and impaired cognitive functions. The high prevalence rate of the parasites was correlated with poverty, poor environmental hygiene and impoverished health services (WHO 1990 and Uddin and Khanum 2006).

Dhaka, being the capital city of Bangladesh has more than 7 million people. A large part of this population resides in the slum areas with very poor living condition, where there is practically no provision for safe water supply and safe excreta disposal system. A number of studies has so far been conducted in slum areas and other parts of Dhaka city where the socio-economic condition is miserable. Low socio-economic condition, poor hygienic habits, lack of sanitary latrines and lack of health education have been found to be related to wider prevalence of different parasitic infections (Nuruzzaman and Huda 1976, Khanum *et al*. 1999 and Uddin and Khanum 2006). Children, the most vulnerable among all the members of the low-income group families are suffering from various diseases and malnutrition. The aim of the present study was to identify the prevalence of four important parasitic infections affecting staffs, students and teachers working in University of Dhaka and to understand their seasonal prevalence rates.

Materials and Methods

A total of 350 stool samples was collected, processed and prepared for both macroscopic and microscopic examinations to detect the parasitic infestation of the teachers, students and staffs who came as out-door patient in Dhaka University Medical Center. Fresh stool samples were collected and examined as quickly as possible. In case of delayed examination, formalin (10% solution) was used as preservative. The samples were primarily examined microscopically by direct smear technique and also by formal- ether concentration method (Cheesbrough 1987). A formatted questionnaire was used to record the observations and results of the examinations of the present study. At first the selected persons were explained about the nature of the study through direct interview with each. The demographic, socioeconomic and sanitation of the household were observed and all relevant information were recorded in predefined questionnaire. The patients were then provided with clean containers for stool collection and all collected stools were preserved with 10% formalin for further examination.

In microscopic examination, saline preparation, iodine preparation and floating technique were followed (Cheesbrough 1987). The presence of ova / cysts of protozoan parasites and eggs of helminth parasites by microscopy was indicative for the presence of adult/mature stages of parasites in the patients.

During this study, a predefined questionnaire were used to collect information concerning the lifestyle, socio-economic status, knowledge level of the patients and personal hygienic practices followed by the individuals who were included in the study.

Results and Discussion

Out of 350, only 81 (23.14%) were found as positive cases for helminth and protozoan infection among the Teacher, Students and Staffs at Dhaka University Medical Center during the study period. The comperative analysis indicates that protozoan infection was higher than helminth infection in studied population (Fig. 1).

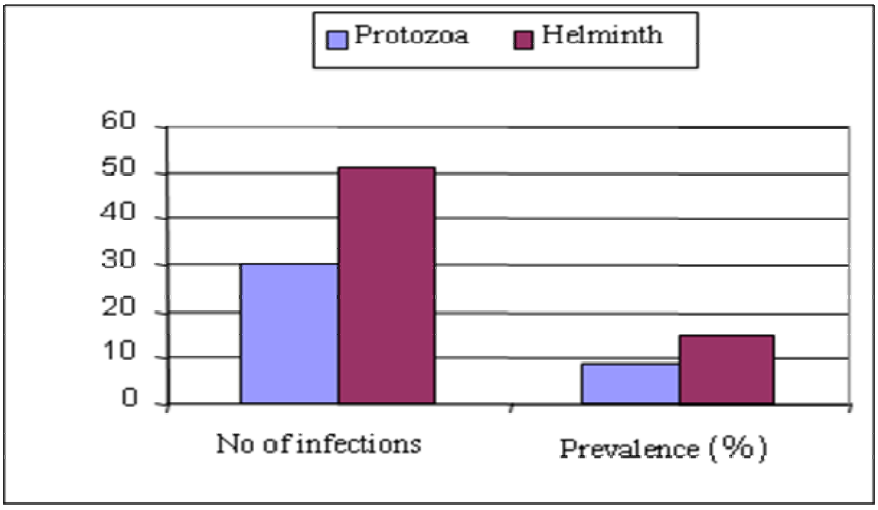

Fig 1. The prevalence of intestinal protozoan and helminth parasites.

Out of 350 only 17 were positive for *Entamoeba histolytica* and prevalence of infection was 4.86%, 13 were positive for *Giardia intestinalis* and prevalence of infection was 3.714 %. The highest rate of infection (11.142%) was recorded with *Ascaris lumbricoides* where 39 cases were found to be positive. The lowest prevalence rate was recorded in *Trichuris trichiura* with only 3.43% positive cases (Table 1).

Table 1. Overall prevalence of four different types of intestinal parasitic infection recorded during study period.

Different parasites	Total no. of sample examined	No. of infected sample	Prevalence (%) (n=350)
*Entamoeba histolytic*a	350	17	4.86
Giardia intestinalis	350	13	3.174
Ascaris lumbricoides	350	39	11.14
Trichiuris trichiuria	350	12	3.43

The seasonal prevalence of different gastrointestinal parasites were investigated during the study period. Table 2 shows the prevalence rates of different parasites over three seasons namely winter, summer and rainy season. The sample size in different seasons were different and the difference in prevalence was recorded. Graphical representation of the same data ispresented in Fig. 2. The highest prevalence of *E. histolytica* was 7.33% and 5.33% of *G. intestinalis* in rainy season. The prevalence of *A. lumbricoides* was highest (15.33%) in rainy season while that of *T. trichiura* was (4.69%) in winter (Table 2 and Fig. 3).

Table 2. Prevalence of different parasitic infection in three seasons.

Parasites	Winter (n=128)			Summer (n=72)			Rainy (n=150)		
	Total sample exam.	No .of infected sample	Prev. (%)	Total sample exam.	No. of infected sample	Prev. (%)	Total sample exam.	No. of infected sample	Prev. (%)
E. histolytica	128	4	3.13	72	2	2.78	150	11	7.33
G. intestinalis	128	2	1.56	72	3	4.17	150	8	5.33
A. lumbricoides	128	10	7.81	72	6	8.33	150	23	15.33
T. trichiura	128	6	4.69	72	3	4.17	150	3	2

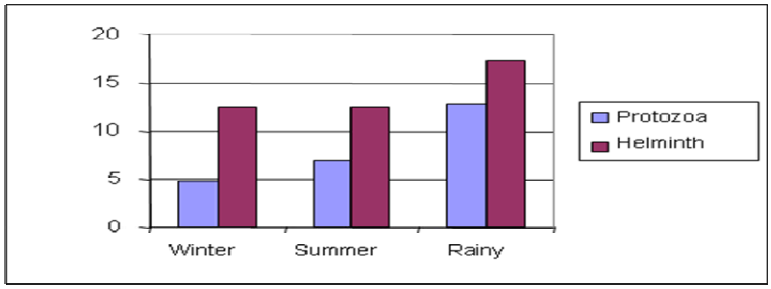

Fig 2. Seasonal prevalence of different parasitic groups among the patients.

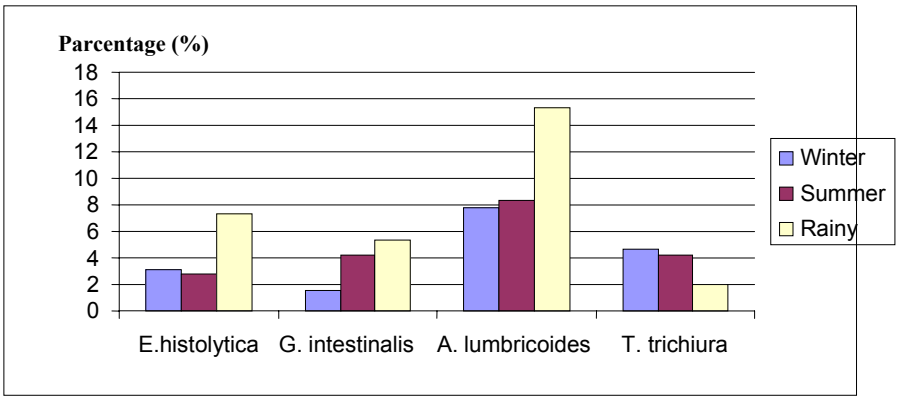

Fig 3. The prevalence of different parasitic infections in three seasons among the patients.

The monthly variation in prevalence of intestinal parasites was also analyzed during this study. The prevalence of infection was highest (66.67%) in staffs group during October, 2009 and absence of positive cases was observed in teacher group in a number of months. However, the students group had the highest (33.33%) prevalence rate recorded in September, 2009 and lowest (7.14%) in March, 2010 of the following year (Table 3).

Table 3. Monthly variations in prevalence of the total gastrointestinal parasites among the patients.

	Teacher			Student			Staff		
Months	Total sample	No. of infected	Prev.	Total sample	No. of infected	Prev.	Total sample	No. of infected	Prev.
Jun ’09	0	0	0	16	3	18.75	4	2	50
July ’09	3	1	33.33	23	6	26.08	19	7	36.84
Aug ’09	1	0	0	14	4	28.37	12	4	33.33
Sep ’09	2	0	0	27	9	33.33	5	1	20
Oct ’09	1	0	0	17	4	23.53	6	4	66.67
Nov ’09	2	1	50	12	2	16.67	15	3	20
Dec ‘09	1	0	0	27	5	18.52	21	4	19.04
Jan ‘10	0	0	0	21	3	14.29	4	0	0
Feb ‘10	1	0	0	18	2	11.11	6	2	33.33
Mar ‘10	0	0	0	14	1	7.14	5	2	40
Apr ‘10	1	0	0	26	4	15.38	4	1	25
May ‘10	1	0	0	10	3	30	11	3	27.27

Helminthic infection specially soil transmitted nematodes are major health problem in this country. Many factors like, poor hygienic habits, poor standard of living, lack of health education, ignorance, poverty, poor socio-economic conditions are some of the many reasons behind high prevalence of parasitic infections in Bangladesh. Previous investigators have shown that intestinal parasite is a major health problem in our country and present data also support this statement.

During the present study, helminth infection was recorded to be higher compared to protozoan infection. This condition usually contributed by different level of environmental conditions which facilitate the transmission of the infective stages of the parasites. The seasonal prevalence was also informative to predict any possible rise of infection in any specific time of the year. This information will be useful for the medical facilities to be prepared with the necessary medications or planning the control of programs. Dhaka University is the largest University of the country and has high number of employees. Some of them live in good accommodations while many minimum-paid employees live in places near slums and they might not have access to clean drinking water or food. Therefore they are more prone to suffer from different intestinal parasites. This could be an important observation from the present study as it was found that staffs were having high infection rate over the years. The students' accommodation is also not very clean as suggested by the significant infection in different times of the year. The teachers are usually economically more stringer group out of the three groups in the study. They have comparatively better accommodation and therefore have less infection rate. One other issue could be that as the teachers have financial capacity, many of them may not be visiting the Dhaka University Medical Centre, rather visiting any good and modern hospitals located in Dhaka. Therefore these cases might not be available for analysis during this study.

The gastrointestinal parasitic infestation is a common public health problem in Bangladesh. Though clinically very small number of parasitic infestations are manifested, but this should be considered as the tip of iceberg. The prevalence of helminthiasis and protozoan infestivity is rampant in Bangladesh, which is leading to a cumulative economical loss of the country. The present study was carried out to find out the incidence of parasitic infections among the selected patients visiting the Dhaka University Medical centre. However this study is concentrated on only four parasites while one would expect many other protozoans and helminthes prevalent in the same community.

Further epidemiological study is essential to understand the distribution of different other parasites throughout the country and mass campaign is necessary to develop awareness among different communities to combat this infection which has considerable economic significance.

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HIGH TEMPERATURE TREATMENT ON THE EGGS OF THE MOSQUITO, *CULEX QUINQUEFASCIATUS* SAY AND ITS EFFECTS ON THE SUBSEQUENT STAGES DEVELOPED THEREFROM

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Abstract

The eggs of *Culex quinquefasciatus* Say (Diptera: Culicidae) were exposed to 40^{o}C for different exposure periods (viz. half an hour, one, two and four hours) and control (room temperature, 28±6^{o}C); the percentage of egg hatching ranged from 74.14 to 96.33 ($F=215.593$, $P<0.05$), larval mortalities were from 24.52 to 0.00% ($F=73.287$, $P<0.05$), pupal mortalities ranged from 10.2 to16.71% ($F=34.056$, $P<0.05$), mean larval periods ranged from 127.9 to 155.3 hours ($F=124.002$, $P<0.05$), mean pupal periods ranged from 30.5 to 36.1 hours ($F=10.531$, $P<0.05$), lengths of 2nd instar ranged from 3.82 to 4.67 mm ($F=16.50$, $P<0.05$), lengths of 3rd instar ranged from 6.195 to 7.195 mm ($F=7.558$, $P<0.05$), lengths of 4th instar ranged from 7.395 to 8.025 mm ($F=3.961$, $P<0.05$), mean diameter of the head capsule of 1st instar larvae was 0.316 to 0.384 mm ($F=8.308$, $P<0.05$), that of 2nd instar larvae was 0.395 to 0.468 mm ($F=4.953$, $P<0.05$), that of 3rd instar larvae was 0.652 to 0.71 mm ($F=2.629$, $P>0.05$), that of 4th instar larvae was 0.806 to 0.91 mm ($F= 13.871$, $P<0.05$), length of the cephalothorax of pupae ranged from 1.862 to 2.062 mm ($F=0.662$, $P>0.05$), body length of male adults ranged from 3.41 to 3.58 mm ($F=0.59$, $P>0.05$), and that of female ranged from 3.75 to 4.09 mm ($F=1.98$, $P>0.05$), mean egg- rafts laid per female ranged from 1.4 to 2.0 and mean numbers of eggs per raft were 230 to 260.

Keywords: High temperature, *Culex quinquefasciatus*, Mosquito, Immature stages

Introduction

Mosquitoes are important group of insects and one of the serious pests from the public health point of view throughout the world. *Cx. quinquefasciatus* breeds primarily in stagnant water with different degrees of organic contaminations. In Dhaka city *Cx. quinquefasciatus* constitutes the major population of mosquitoes throughout the year (Ameen and Moizuddin 1973). The open and semi open manholes, polluted water, logged drains, ditches, marshy areas, shabby water containers, etc. are potent breeding grounds of this species of mosquito (Hamid 1979). The peak population of this species in the city is observed during the dry weather from November to December (Hamid 1979 and Begum *et al*. 1996). In recent years the population of this species increased significantly in Dhaka city. The species comprised nearly 60% of total mosquito population of the city in 1984 (Ameen *et al*. 1984), while Ahmed (1986) reported that 84% of the mosquitoes of Dhaka city belonged to this species.

Temperature has a profound influence upon insects in various ways. The extremes of temperature hamper their activities; control the rate of metabolism and consequently those of growth, reproduction and general behaviour. According to Wigglesworth (1965) temperature and humidity are the most important factors in the environment that influence the physiology of insect. Chapman (1969) noted that enzyme functions are efficient only within a limited range of temperature and for this reason, environmental temperature is of great importance in the lives of all insects. The higher temperature has a harmful influence on the development of insect (Davidson 1944). The optimum temperature for the insect species is generally taken to be 28^{o}C; any increase above this appears to be increasingly unfavourable, and over 40^{o}C it is quickly fatal and insects die from the effects of heat (Mellanby 1932). The effects of temperature on various stages of mosquito were studied by Dakshinamurty and Sharma (1951), Nayar (1968), Ameen and Huda (1976), Ameen and Bhuiya (1979) and Rahman (2006).

Global warming may affect the future pattern of many arthropod-borne diseases, yet the relationship between temperature and development has been poorly described for many key vectors (Bayoh and Lindsay 2003). With this view in mind, the effects of high temperature at 40^{o}C on the eggs of *Cx. quinquefasciatus* exposed for different time periods and the subsequent stages developed there from after egg hatching were studied in the laboratory.

Materials and Methods

The larvae of *Cx. quinquefasciatus* were collected from different breeding sources, such as stagnant water in the drainage system and various watery places in the Curzon Hall campus, University of Dhaka. The larvae were collected with the help of a sieve and were taken in a large clean plastic bowl and brought to the Entomological laboratory in the Department of Zoology, University of Dhaka. They were then washed gently in tap water for several times to clean those from impurities. The larvae were then transferred to another bowl containing clean water. Ground glucose biscuit were provided as food for the larvae, which were placed on slides within a few drops of water and were examined under a compound bi-ocular microscope (Model- C8 H30 RF200). The larvae were then identified following Service (1970).

Rearing of Cx. quinquefasciatus: A colony was maintained in an ambient environment (temperature 28±6^{o}C and 60-80% RH) of the laboratory to ensure a continuous supply of different life stages of the mosquito during the experimental period. The **c**ollected larvae were reared in a plastic bowl containing tap water and provided with finely ground biscuits (commercially known as "Energy Plus" locally) as their food. The water was changed and ground biscuits were provided daily.

The 4th instar larvae were moulted into pupae which were separated daily from the larval bowl with the aid of a dropper and kept them in a plastic bowl that was previously filled

with tap water. The plastic bowl with the pupae was kept in a mosquito rearing cage for the emergence of adult mosquito. After emergence, the adults were provided with 10% glucose solution daily as their food. The glucose solution was soaked in a cotton wad and placed it on a Petri dish, which was then kept inside the cage. The male mosquito only took glucose solution as food throughout their life time. For the first two or three days of emergence, the females were also fed with glucose solution.

Blood feeding is required for the nourishment and maturation of the eggs of the mosquito. From the 3rd day after emergence, the adult female mosquitoes were allowed to feed on blood meal from a pigeon. Feathers were removed from the breast region of the pigeon and kept in a tight small iron cage which could easily be placed inside the rearing cage. The blood feeding was initiated on the 4th day after adult emergence and continued as long as the females were alive in the cage. After taking the blood meal, the females mated with the males inside the rearing cage. A plastic bowl containing tap water was placed inside the rearing cage for the mated females to oviposit on the water surface. The number of egg rafts laid per female and the number of eggs in the rafts were counted and recorded.

Egg treatment with temperature: Only the healthy, relatively large and equal sized 6 to 18 hour-old egg-rafts were used for temperature treatment in an incubator set at 40^{o}C and exposed for half an hour, one hour, two and four hours separately. The numbers of eggs per egg-raft were counted and recoded under a microscope. A thermometer was used to record the temperature of the water of Petri dish placed inside the incubator previously and when the temperature of the water was 40^{o}C, the egg rafts were transferred to the Petri dish water quickly. After the stipulated period of exposure. The Petri dishes were taken out of the incubator and the egg rafts were transferred as quickly as possible to another Petri dish containing distilled water and later placed inside the rearing cage at the ambient environment of the laboratory. The egg rafts were transferred on the tip of a triangular piece of blotting paper. Three replicates were used for each exposure period and a control treatment of three replicates was also taken, in which the eggs were not treated with temperature. The food were provided to the heat treated larvae and larvae in control in the same manner.

Data recorded: The data were recorded at each temperature exposure periods and control on the following aspects: hatching efficiency of the eggs; mortality of larvae and pupae; larval and pupal stage durations (stadia); body measurements, such as length of different instars of larvae, transverse diameter of the head capsule of different instars of larvae, length of cephalothoraxes of pupae, body length of male and female adults and fecundity of the female adults emerged.

Hatching efficiency of the eggs: The number of larvae hatched from the eggs was counted with the help of a magnifying glass. The eggs, which were not hatched, were regarded as killed due to heat treatment.

Larval mortality: The mortality of all four instar larvae was recorded sequentially from the first instar larval hatching until all the 4th instar larvae pupated. The larval mortality was counted every day. The moribund larvae were considered dead. The Abbot's formula (1925) was used when the mortality of larva in control was observed.

Pupal mortality: The mortality of pupae was recorded from the first pupation until all the adults emerged. The pupal mortality was recorded daily. The adults emerged but could not move or fly normally (deformed) were regarded as the effects of temperature and were considered dead.

Larval stage duration: This duration was recorded from the time the 1st instar larvae first hatched to the 4th instar larvae first pupated.

Pupal stage duration: The period between first pupation and first adult emergence was considered as the pupal stage duration.

Measurements of larval length and its head capsule: The lengths of the 2nd, 3rd and 4th instar larvae were only recorded. As the 1st instar larvae were very small in size, it was, therefore, difficult to measure their lengths with the instruments available in the laboratory and not attempted. In measurement, 10 larvae of each of the mentioned instars were taken into 70% ethyl alcohol and were killed. The lengths of the larvae were measured under an electronic binocular microscope by placing them on a glass slide with the help of a forceps.

Measurements of the length of pupae and their cephalothorax: The length of the cephalothorax of pupae was also measured by following the above same method.

Size of male and female adults emerged: Twenty adult mosquitoes (10 males and 10 females) were taken out from the rearing cage and were released into another empty rearing cage with the help of an aspirator. They were killed by spraying insecticide-aerosol on them. The mosquitoes were then placed on a slide and their body lengths were measured in millimeters with the aid of a compound microscope.

Fecundity: The number of female mosquitoes emerged was recorded after giving them blood meal from a pigeon, and 20 engorged females were taken in a rearing cage having a Petri dish with water for oviposition by the females. When the females completed their egg laying, the total number of egg rafts laid were counted and number of eggs in each rafts were also counted under a compound microscope.

Statistical analysis: The data obtained were reported as arithmetic mean ± standard deviation (SD). One way Analysis of Variance (ANOVA) was applied on the data to assess the treatment effect. When F-values indicated significant difference, Duncan's Multiple Range test (DMRT) was employed to discern specific difference among the treatments. All the statistical analyses were done on a computer using statistical software package SPSS. The corrected mortality was done by using Abbot's (1925) formula

whenever necessary. The formula was [(Po-Pc)/ (100-Pc)] x 100, where Po = percent mortality observed and Pc = percent mortality in control.

Results and Discussion

Hatching efficiency of the eggs: The eggs of *Cx. quinquefasciatus* hatched during the experiment showed significant difference (F=215.593, P<0.05) among different exposure periods and control (Table 1). The high percentage (96.33%) of hatching occurred in control and the lowest (74.14%) in 4-hour exposure. A trend of gradual reduction of egg hatching was observed from control to 4-hour of exposure treatment. These results indicate that the temperature at 40°C with different exposures had an impact on the egg hatching of the mosquito species. Egg mortality increased gradually with increasing exposure periods at high temperature (40°C) which is in conformity with the findings obtained by Ameen and Huda (1976) and Rahman (2006) in case of *Aedes aegypti* mosquito treated at 35°C; and by Ameen and Bhuiya (1979) in case of *Cx. pipiens fatigans* treated at temperatures ranging from 32 to 44°C. The mortality of *Ae. albopictus* eggs is higher in greater temperature (Alto and Juliano 2001). The eggs of *Cx. p. fatigans* were not hatched at 42, 40, 38 and 37°C on 10 minutes, 1-hour, 6-hour and 24-hour exposures, respectively (Ameen and Bhuiya 1979). Karamchandani (1935) however reported that the eggs of *Cx. p. fatigans* did not hatch above 39.8°C.

Table1. Hatching efficiency of the eggs of *Cx. quinquefasciatus* treated at 40°C in different exposure periods and control in an ambient condition of the laboratory.

Exposure period (hrs)	Mean no. of eggs per raft (mean±SD)	No. of larvae hatched per egg-raft (mean±SD)	No. of larvae hatched per egg raft (%)	F-value
Control	245.00±15.00	236.00±15.524^{a}	96.33	
0.5	230.00±24.269	202.33±24.420^{ab}	87.97	
1.0	255.00±22.913	216.00±16.523^{bc}	84.71	215.593*
2.0	233.66± 7.767	182.66±8.737^{c}	78.17	
4.0	233.33±17.559	173.02±16.523^{d}	74.14	

*Significant at 5% level; same letters in the column show significance at 5% level in DMRT

Larval mortality: The mortality of the larvae of *Cx. quinquefasciatus* is presented in Table 2. Larval mortality in control was corrected by following Abbot's formula (1925). High temperature and different exposures showed significant temperature effects (F = 73.287, P <0.05) on larval mortalities. Karamchandini (1935). reported that the lethal temperature exposed to the 4th instar larvae of *Cx. quinquefasciatus* for one hour in subtropical regions was 37.8-38°C. The effect of temperature on the mortality of *Cx. fatigans* larvae depends on the length of exposure periods (Chapman 1969). The lethal temperature at 50 percent mortality (LT_{50}) of the different stages of *Cx. pepiens fatigans* on 10 minutes exposure varied from 40.3 to 42.0 °C; on one hour exposure the LT_{50}

varied from 38.1 to 39.9 °C; 6 hours exposure from 36.1 to 37.7 °C and 24 hours exposure to 34.6 to 35.8 °C (Ameen and Bhuiya 1976). Rahman (2006) reported the effects of temperature at 35 °C on the larval and pupal

Table 2. Mortality of the larvae of *Cx. quinquefasciatus* hatched from the eggs treated at 40°C in different exposure periods and control in an ambient condition of the laboratory.

Exposure Period (hrs)	Total number of larvae hatched	Total no. of larvae died	Mean no. of larvae died (mean±SD)	Mean larval mortality (%)	Corrected larval mortality (%)	F-value
Control	708	37	12.33±1.527a	5.23	0.0	73.287*
0.5	607	117	39.00 4.583b	19.28	14.83	
1.0	648	136	45.33 2.517c	20.99	16.63	
2.0	548	156	52.00 3.606d	28.47	24.52	
4.0	519	100	33.33 2.082e	19.27	14.81	

*Significant at 5% level; the same letters in the column show insignificance at 5% level in DMRT.

mortality of *Ae. aegypti* up to 16% after half an hour to 24 hours of exposure of the eggs to the temperature mentioned. The mortality of the larvae and pupae of *Ae aegypti*, exposed to a range of 10 minutes to 24 hours, gradually increase with the rise of temperature from 33 to 46 °C, the range of lethal temperature at 50% mortality was observed at 40.2-41.8 °C (Ameen and Huda 1976). They also observed that after long exposure to the above temperatures the adults failed to emerge, failed to fly normally and manifested some deformities in body parts. Adults emerged from high temperature treatments showed deformed wings (Ameen and Bhuiya 1979, Ameen and Huda 1976, and Nayar 1968).

Table 3. Mortality of the pupae of *Cx. quinquefasciatus* developed from the eggs treated at 40°C in different exposure periods and control in an ambient condition of the laboratory.

Exposure period (hrs)	Total number of larvae pupated	Total no. of adults emerged	Total no. of pupae died	No. of pupae died (mean±SD)	Mean pupal mortality (%)	F-value
Control	661	631	30	10.00 ± 1.00^{a}	4.47	
0.5	390	340	50	16.66 ± 1.528^{b}	10.20	34.056*
1.0	512	452	60	20.00 ± 2.00^{c}	11.72	
2.0	392	328	64	21.33 ± 1.528^{cd}	16.32	
4.0	419	349	70	23.33 ± 1.528^{d}	16.71	

*Significant at 5% level; the same letters in the column show insignificance at 5% level in DMRT.

Pupal mortality: The mortality of the pupae of *Cx. quinquefasciatus* is presented in Table 3. High temperature and different exposures showed significant effects ($F=34.056$, $P<0.05$) on pupal mortalities. Temperature above 40°C killed all the pupae of *Cx. fatigans* when exposed for one hour (Karamchandini 1935). Boormann (1961) reported that the pupae of *Ae. vittatus* exposed for five minutes at 45.5°C killed 95% of pupae and exposed

for three minutes at 48°C killed all the pupae exposed. Christopher (1960) obtained total mortality of the pupae of *Ae. aegypti* after three minutes at 48°C and 15 minutes at 45°C, and 64% mortality resulted from one hour at 43°C. Service (1970) found all pupae of *Ae. vittatus* dead after 10 minutes at 46°C and one hour at 45°C.

Larval and pupal stage duration: The duration of larval and pupal periods as a result of high temperature at 40°C for various exposure periods and control were observed significantly ($F= 124.002$, $P< 0.05$ in case of larvae and $F= 10.531$, $P<0.05$ in case of pupae) (Table 4). The highest duration was observed in control (viz. 155.3 hours in larvae and 36.1 hours in pupae). The stage durations gradually decreased as the temperature exposure periods increased (viz. 136.8, 134.1, 131.5, and 127.9 hours larval durations respectively for 0.5, 1, 2 and 4 hour exposures) and 35.2, 34.4, 32.6 and 30.5 hours pupal durations respectively for 0.5, 1, 2 and 4 hour exposures. At 27°C the duration of the complete larval period of the mosquito, *Stegomyia calopus* was found 168 hours (Francis 1907). Age of pupation increased as temperature decreased from 30°C to 27°C (Lyimo *et al.* 1992). In the laboratory at 23°C, the larval development time of *Aedes (Ochlerotatus) albifasciatus* was around 216 hours and adults emerged within one week (Luduena Almeida and Gorla 1995). The time between pupation and emergence of *Stegomyia* sp. at 23-27°C was 45 hours for males and 60 hours for females, and the mean periods of the mosquito from eclosion to pupation at 27°C was 154 hours and 168 hours for this period at 23-26°C, respectively (Shannon and Putnam 1934). The larval developmental time of *Ae. albopictus* from egg hatching to pupation was inversely correlated with temperature, lasting 168 hours at 32°C and the duration of pupal period varied between 48 hours and 72 hours at that temperature (Briegel and Timmermann 2001). This indicates that with the decrease of temperature the larval and pupal period becomes lengthened. The duration of the larvae and pupae of *Ae. albopictus* depends also on the nature of containers in which they are developing; the total time from egg hatching to adult emergence in tree hole, bamboo stump and auto tyres in a temperature range between 18°C and 22°C was 19.6, 27.3 and 37.5 days, respectively (Gomes *et al*. 2001).

Table 4. Mean duration of the larvae and pupae of *Cx. quinquefasciatus* obtained from the eggs treated at 40°C in different exposure periods and control in an ambient condition of the laboratory.

Stage	Control (in hrs)	Exposure period in hours (mean ± SD)				F-value
		0.5	1.0	2.0	4.0	
Larva	155.3±1.075^{a}	136.8±2.898^{ab}	134.1±2.767^{bc}	131.5±2.506^{c}	127.9±4.332^{d}	124.002*
Pupa	36.1±1.792^{a}	35.2±1.619^{ab}	34.4±2.413^{bc}	32.6±2.221^{c}	30.5±2.545^{d}	10.531*

*Significant at 5% level; the same letters in the row show insignificance at 5% level in DMRT

Measurements of larval length and its head capsule: The lengths of 2nd, 3rd, and 4th instar larvae of *Cx. quinquefasciatus* at 40°C for various exposure periods and control are presented in Table 5. Significant differences in length among different exposure periods and control were observed in all

three instars [the 2nd instar larvae (F=4.314, P<0.05); the 3rd instar (F= 3.786, P<0.05); and the 4th instar (F= 3.961, P>0.05).

Table 5. Mean length of 2nd, 3rd and 4th instar larvae of *Cx. quinquefasciatus* obtained from the eggs treated at 40°C in different exposure periods and control in an ambient condition of the laboratory.

Larval instar	Mean larval length (in mm) in exposure period					F-value P
	Control	0.5 hr	1.0 hr	2.0 hrs	4.0 hrs	
2nd	4.674±0.156[a]	4.11±0.256[b]	4.07±0.347[b]	3.93±0.25[bc]	3.822±0.23[c]	16.50* <0.05
3rd	7.195±0.254[a]	6.52±0.476[b]	6.527±0.401[b]	6.485± 0.477[b]	6.195±0.466[b]	7.558* <0.05
4th	8.025±0.437[a]	7.905±0.265[a]	7.79±0.39[a]	7.675±0.408[ab]	7.395±0.393[b]	3.961* <0.05

*Significant at 5% level; the same letters in the row show insignificance at 5% level in DMRT.

The transverse diameters of the head capsules of 1st, 2nd and 4th instar larvae were observed significant (F=8.308, P<0.05; F=4.953, P<0.05 and F=13.871, P<0.05; respectively), but of 3rd instar larvae showed insignificance (F=2.629, P<0.05) (Table 6).

Temperatures ranging from 15°C to 31°C significantly affect the size of head capsule widths of the larval instars of *Ae. albopictus* and *Ae. triseriatus* (Say) in laboratory (Teng and Apperson 1996), which is in conformity with the present results at higher temperature.

Table 6. Mean diameter of the head capsule of all four larval instars of *Cx. quinquefasciatus* obtained from the eggs treated at 40°C in different exposure periods and control in an ambient condition of the laboratory.

Larval	Mean larval length (in mm) in exposure period					F-value P
	Control	0.5 hr	1 hr	2 hrs	4 hrs	
*1st	0.384±0.025[a]	0.371±0.026[ab]	0.352±0.037[b]	0.346±0.027[b]	0.316±0.026[c]	8.308* < 0.05
*2nd	0.468±0.038[a]	0.449±0.047[a]	0.445±0.042[a]	0.432±0.033[a]	0.395±0.03[b]	4.953* < 0.05
3rd	0.910±0.044	0.704±0.047	0.692±0.05	0.675±0.043	0.652±0.045	2.629 > 0.05
*4th	0.910±0.034[a]	0.882±0.031[ab]	0.872±0.035[b]	0.855±0.032[b]	0.806±0.032[c]	13.871* < 0.05

*Significant at 5% level; the same letters in the row show insignificance at 5% level in DMRT.

Measurements of pupal length and its cephalothorax: *Size of male and female adults emerged*-- The results presented in Table 7 show that the length of the cephalothorax of the pupae of *Cx. quinquefasciatus* developed from the eggs treated with high temperature and different exposure periods showing insignificance at 5% level (F=0.662, P>0.05).

From the Table 7 it is evident that temperature showed no effect (F=0.59, P<0.05 for males and F=1.98, P<0.05 for females) on the body length of adult male and females *Cx. quinquefasciatus* emerged from the eggs treated with high temperature at 40°C. Adult size increased as temperature decreased. Alto and Juliano (2001) reported that temperature affects the size of the adults of *Ae. albopictus*; size of the adults decreased with increased temperature. In the present findings, high

temperature tended to lower the body size of the adult mosquito, but not significantly different in size in different exposure periods.

Table 7. Mean cephalothoracic length of pupae and adult body length of *Cx. quinquefasciatus* obtained from the eggs treated at 40°C in different exposure periods and control in an ambient condition of the laboratory.

Pupa	Mean larval length (in mm) in exposure period						
and Adult	Control	0.5 hr	1 hr	2 hrs	4 hrs	F-value	P
Cephalo-thorax length	2.062±0.38	1.933±0.288	1.898±0.304	1.862±0.281	1.89±0.26	0.662	>0.05
Male body length	3.58±0.27	3.44±0.299	3.41±0.296	3.42±0.308	3.49±0.26	0.59	>0.05
Female body length	4.09±0.269	3.96±0.317	3.93±0.267	3.89±0.285	3.75±0.237	1.98	>0.05

The Table 8 shows that the females laid the highest number of eggs in control (260 per female) and lowest at 4-hour exposure (230 per female). The fecundity of the mosquito deceased gradually, viz 252, 245, 237 and 230 eggs laid per female in half an hour, one, two and four hours of exposure, respectively with the increase of exposure periods. Temperature affects the production of *Ae. albopictus*; greater temperature results in greater production of the adults in mosquito (Alto and Juliano 2001). The fecundity of *Ae. cantans* (Meig.) decreased with the decrease in adult female size and also a reduction in numbers of eggs in successive ovipositions (Service 1977).

Table 8. Fecundity of the female *Cx. quinquefasciatus* obtained from the eggs treated at 40°C in different exposure periods and control in an ambient condition of the laboratory.

Exposure period (in hrs)	Total number of egg-rafts laid by ten females	Mean number of egg-rafts laid per female	Mean number of eggs per raft
Control	20	2.0	260
0.5	18	1.8	252
1.0	17	1.7	245
2.0	16	1.6	237
4.0	14	1.4	230

From the study it may be concluded that the eggs treated with high temperature at 40°C were significantly affected and the larvae hatched there-from and moulted up to the 4th instar were also affected variously; pupal and adults were apparently little affected due to the high temperature and exposures. Further work may be initiated to look into this aspect in detail as to whether high temperature (=simulating global warming) has a discernable effects on mosquito life in nature, which may be useful in formulating control strategy of arthropod-borne disease vectors, particularly mosquito species.

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BREEDING BIOLOGY OF GUPPY FISH, *POECILIA RETICULATA* (PETERS, 1859) IN THE LABORATORY

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Abstract

The breeding biology of guppy fish, *Poecilia reticulata* (Cyprinodontiformes: Poiciliidae) was studied during March 2008 to May 2009 in 'Zoological garden laboratory', Curzon Hall campus, Dhaka University. Guppy bred all over the year except in the winter months December and January with a peak period in July. They were viviparous and multiple breeders, i.e., give birth to fry several times in the breeding season. The mean egg diameter was measured to be (1.02±0.08mm) and fecundity was estimated (40-89) per gram of body weight. The gestation period ranged 25-35 days with an average of 28.1±2.12 days. Developmental stages observed under a compound microscope were classified based on the changes in the developing eye, such as optic cup, early-eyed, middle-eyed, late-eyed, very late-eyed etc. It was noticed that tail portion comes out first at birth. The number of fry per brood ranged from 12 to 60. New born fries were observed with transparent or blackish in colour having slender body with jaws developed on mouth and were fully capable of swimming, eating, and avoiding danger. Guppy grew rapidly, attained sexual maturity at 8 -10 weeks and reached full size in 6 months.

Key words: Guppy fish, *Poecilia reticulata,* Breeding biolog

Introduction

Popular aquarium fish, *Poecilia reticulata* commonly known as 'guppy' was introduced in various countries for mosquito control and often loosely called 'mosquito fish'. It has been found to establish itself in both fresh and polluted waters (Ahmed *et al.* 1985). It introduced in India as early as 1910 to control mosquito (Kaira *et al*. 1967). This larvivorous fish are quite tolerant of a variety of water conditions thus can be used as predators of mosquito larvae and they can be moved to water areas where they are needed (Travis 1957).

Synthetic insecticides are widely used for mosquito control throughout the world, and some of these has long residual activities and affect the environment adversely. Many mosquito species has developed resistance to a variety of insecticides (WHO 1986). The development of resistance in mosquitoes to insecticides, environmental pollution, the high cost of control due to short duration of action of insecticides and toxicity of newer

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insecticides and other reasons have given rise to a new thinking to find out other ways of mosquito control. Among the various vector control measures, the biological control method is favored due to its cost effectiveness. Larvivorous fish have been used in mosquito control on and off for many years in different parts of the world (Ahmed *et al.* 1985). Guppies have the capacity to survive and multiply in both fresh and polluted waters to solve this problem. An understanding of the breeding biology of *P. reticulata* is a basic requirement for the successful proliferation of the fish, hence successful mosquito control. The guppy is suitable for mosquito control due to its flattened head, protruded mouth, small size and its voracious appetite for living on prey, especially insect larvae (Bay 1967). It is a viviparous fish and is capable of increasing its population in shallow or polluted water (Menon and Rajagopalan 1977). In Bangladesh no published information on the breeding of *P. reticulata* is available. Researches have been carried out on the bio-control of mosquito larvae by guppies (Ahmed *et al.* 1985, Khanum *et al.* 2002). Therefore, the present investigation was carried out on the breeding biology of *P. reticulata* in a controlled aquarium condition.

Materials and Methods

The experiments were conducted in Curzon Hall area of Dhaka University campus. A total of 40 fishes was collected from Kataban fish market and from different drains of Curzon Hall area and was transferred to rearing aquaria. Males were clearly distinguished by having modified anal fin taking the form of a gonopodium and in females, body colour was less bright and had swollen abdomen. Each of the aquaria contained 8-10 liters of water. Water was changed manually every two alternate days in the afternoon when the temperature of the aquarium water was close to that of the tap water. The guppy prefers hard water and can withstand salinity up to one ppt. So, half a tablespoon (8g) of salt was mixed with 20 liter of water every time during water change. The broods were provided with bloodworms, *Chironomus* sp., mosquito larvae and commercial pallet feed as their food and given twice a day in the morning and at afternoon.

Diameter of eggs was measured for the estimation of the fecundity. Twelve ripe females of *P. reticulata* were randomly sampled and the fecundity was computed by counting the number of ova and the developing embryonic stages by dissecting the abdomen of the gravid female specimens. The developmental stages of the embryos inside the gravid females were observed by dissecting the gravid female's abdomen. Different embryonic stages found in the same mother fish at a time were observed with naked eye and under a compound microscope (10X). The newly born baby fish (fry) were observed in ten occasions, viz. (1hour, 1, 7, 14, 21, 28, 35, 42, 49 and 56 days) of age, till up to 8 weeks.

Results and Discussion

The month wise reproduction of *P. reticulata* was observed throughout the year except in December and January (Fig. 1). Guppy began fry birth in February and ended in November with a peak in July. In February, comparatively few mother guppies gave birth. Hidebrand (1921) reported that the breeding season of guppy in the Southeastern U. S. begins in May and ends in September and October. Davis (1978) reported that in South-central Texas, the breeding season of *Gambusia affinis,* a closely related fish of guppy ranges from March to October with a peak in April.

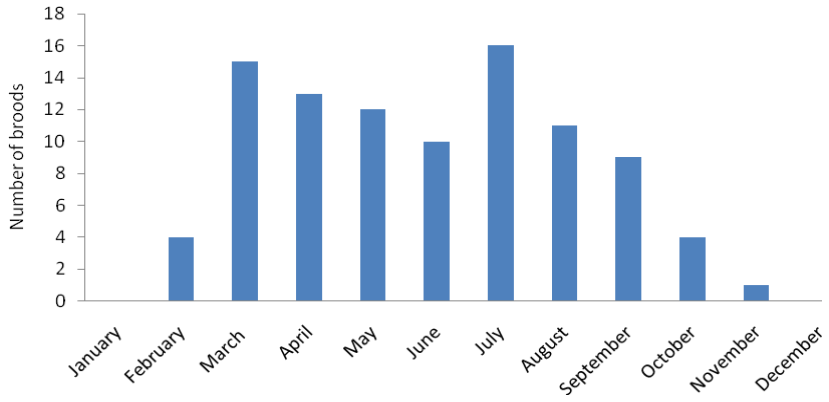

Fig. 1. Number of broods of *Poecilia reticulata* in different months of the year 2008-2009.

In the present study, it was observed that the gestation period of guppy was 25-35 days with an average of 28 days (Table 1). Guppy was reported to bring out broods at approximately four-week intervals (Ahmed *et al.* 1985). Krumholz (1948) reported that the average gestation period of *G. affinis*, a closely related fish of guppy (*P. reticulata*), was 23-24 days. In guppy, fertilization is internal and takes place through mating of couples showing specific mating behavior. Male transfers sperms into female body by a modified anal fin called gonopodium. Male perform an S-shaped posture known as 'sigmoid display' and orientates himself in front of the females at the beginning of courtship. Collier (1936) and Paden (1975), obsereved courtship behaviour in mosquito fish, that was similar to the present study. Houde and Endler (1995) observed that females exhibit sexual preferences for males with larger color spots, which is displayed during elaborate "sigmoid" courtship displays.

The egg weight of 12 gravid females was 0.60 ± 0.04 g (range, 0.54-0.68 g) and mean egg diameter was: 1.02 ± 0.08 mm (range, 0.9-1.2mm) (Table 1). Fecundity was expressed as the total number of eggs produced by fully mature gravid female of *P.*

reticulata during the peak period of their breeding. The fecundity of guppy under different body sizes as well as per gm estimated body weight has been shown in Table 1. It was observed that total fecundity of various body sizes (range, 0.54- 0.68 g) was 22-52, while estimated mean fecundity per gm body weight was estimated 63 ± 13.45 (range, 40-89). The results indicate that the fecundity was directly proportional to the body weight of the fish, i.e., the fecundity increased with the increase in body weight. It was found that the number of the fry per brood ranged from 12-60 and the number also varied with the size of female. Larger females produce large number of offspring than smaller fish. Shikano and Fujio (1997) reported that the female guppy gave birth 2 to100 fry, but the typical range is between 5 and 30. Nutritional balance, water condition or differences in rearing procedure might be the cause of the variation.

Table 1. Fecundity, size of ova and gestation period of the gravid females of *Poecilia reticulata* (n=12).

Body length (cm) (mean ±SD)	Body weight (g) (mean ±SD)	Total fecundity (mean ±SD)	Estimated fecundity/g body weight (mean ±SD)	Egg diameter (mm) (mean ±SD)	Gestation period (days) (mean ±SD)
(3.98±.208)	(0.60±.04)	(39.58±7.76)	(63±13.45)	(1.02±.08)	(28.1±2.12

Embryonic development (in womb): As guppy can store the sperm of their mates, eggs may become fertilized at different times facilitating the availability of several successive embryonic stages at a time in a single mother fish. Studies on embryogenesis have been limited by the fact that fertilization is internal and guppies are live-bearers. Normal development of guppy embryos was observed at various times after dissecting gravid mother guppies. Development of each batch of eggs was found to be slightly asynchronous, most likely due to asynchronous fertilization. Embryos were withdrawn from the gravid guppy and were observed. Eleven stages (Plate 1a-k) could be observed during the study.

Fertilized eggs: Fully swollen fertilized eggs were observed as brownish yellow in colour, rounded in shape and translucent (Plate 1a). The mature ovum contains oil droplets that are evenly distributed over the yolk surface similar to the observation stated by Martyn *et al.* (2006).

Blastodisc: After fertilization, the oil droplets coalesce underneath the embryo proper, which forms a blastodisc (Plate 1b).

Gastrula: Gastrula was viewed under microscope with clearly visible archenteron (Plate 1c).

Optic cup stage: At the optic cup stage (Plate 1d), the eyes remain unpigmented, blood vessels of the portal system are visible in the lower part of the yolk sac.

Early-eyed stage: During the early-eyed period, pigmentation of the eye, including the choroid, gradually increases, the pectoral fin buds emerge, and somitic as well as nonsomitic muscles differentiate (Plate 1e).

Middle-eyed stage: During the middle-eyed stage, melanophores first appear above the midbrain and subsequently behind the midbrain-hindbrain boundary.

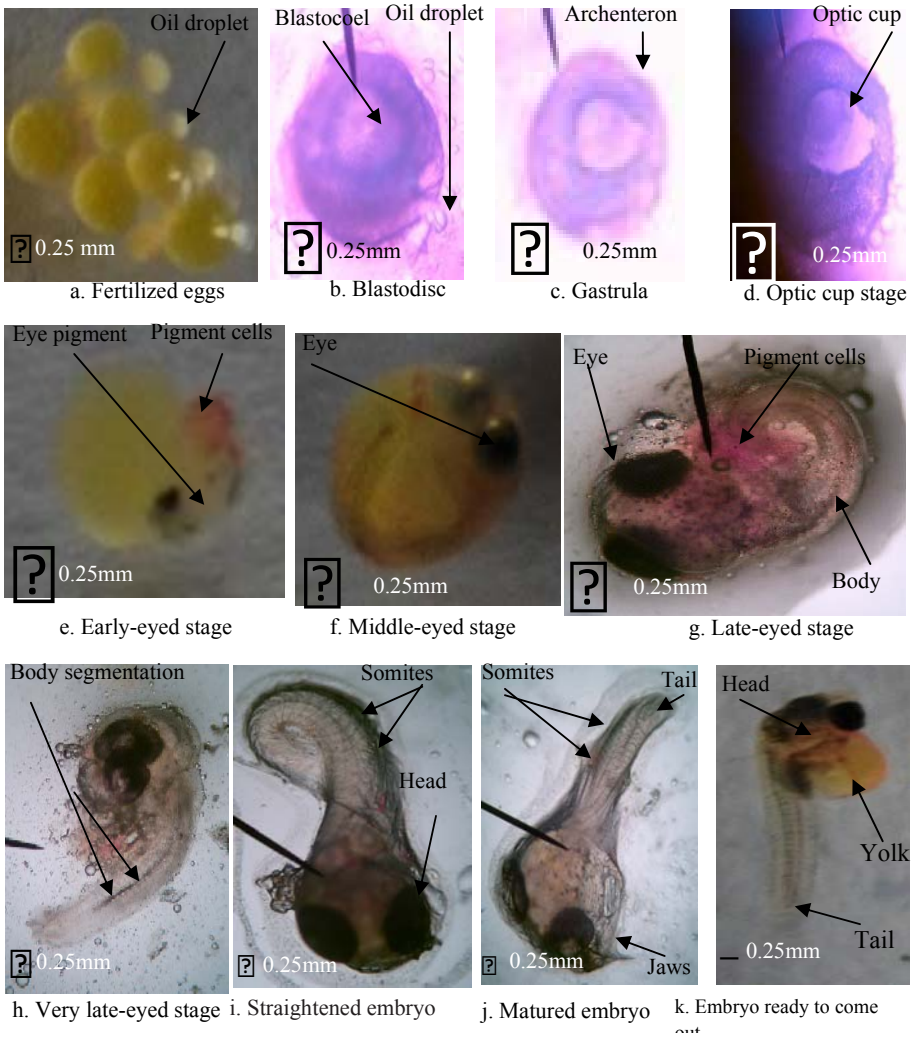

Plate. 1. Successive stages of embryo found in gravid guppy, *Poecilia reticulata*. The stages b,c,d,g,h,i and j were observed by compound microscope (10X) whereas a,e,f and k were taken directly by 13 megapixels camera. Name of the stages were according to Martyn *et al.* 2006.

Late-eyed stage: In the subsequent late-eyed stage, a line of dark pigment cells appears that demarcates the horizontal midline, and the mostly stellate black pigment cells on the head increase in number, size and density, and become more dendritic in their appearance. Head bent with chromatophores on it, eyes were large and huge blood vessels appeared in yolk sac. Different regions of the embryo are often covered by melanophores of different shapes (Goodrich *et al.* 1944 and Tavolga 1949).

Very late-eyed stage: During very late-eyed stage (Plate 1h), clear segmentation of the embryo was visible. The almost rectangular flexure between the head and the trunk was gradually straightened.

Straightened embryo: The myotome consists of approximately 22 somites (Plate 1i).

Matured embryo: Embryo was then observed with developing jaws and became mature (Plate 1j). Matured embryo was ready to come out (Plate 1k). Some of the mature embryo absorbed its yolk completely and retracted the yolk sac, but the rest came out with a small amount of yolk. The developmental stages in present observation were similar to those of Goodrich *et al.* (1944) Tavolga (1949) and Martyn *et al.* (2006).

The neonate fry was knocked by the mother with her head to learn swimming and they floated and moved around the aquarium. The fries took birth with jaws developed on mouth. So, they could easily take food immediately after birth. It was observed that from the moment of birth, each fry was fully capable of swimming, eating and avoiding danger. Shikano and Fujio (1997) also made similar observation.

It was observed that females typically mate with more than one male. Evans and Magurran (2000) noted the similar facts in guppies during their sexually receptive phase. Females have the ability to store sperm, so that they can give birth many times, after mating with a male only once. Three to five times hatching was observed after a single mating in several couples during this study. Constantz (1989) also observed that female guppies store sperm for several months. It was observed that *P. reticulata* hatch its first brood at the age of 3-4 months. Menon and Rajagopalan (1977) also reported that this fish can reproduce at the age of 90 days. After giving birth, the female became ready for conception again within only a few hours.

Development after birth: Newly hatched fries were blackish or transparent and slender with chromatophore on head measuring from 6.5 to 7.5 mm in length. They settled down at the bottom of the aquarium and searched food in the stones within 1-2 hours. Characteristics of the different stages of fry development are briefly pointed below-
Fry, just after birth: Transparent, some are blackish or grayish in colour. Body slender, about 6.5~7.5 mm long. Pectoral fins were larger than caudal.

One hour after hatching: All of the fry appeared transparent within few hours of birth. Approximate body size was 6.5~8.0 mm.

One day old fry: Fins clearly observed, pelvic fin is smaller than pectoral. Caudal fin with dark spot. Length of the body was 6.8~ 8.5 mm.

7-day old fry: Caudal fin somewhat flat clearly appeared as a tail. A longitudinal thread like structure, the alimentary canal was observed in the transparent body. Body length was 7.0~9.0 mm.

14-day old fry: A primary concept might be achieved of sex differences. Female abdomen was somewhat wider than male. Body measured 7.5~9.5 mm in length. Anal fins of both sexes were similar.

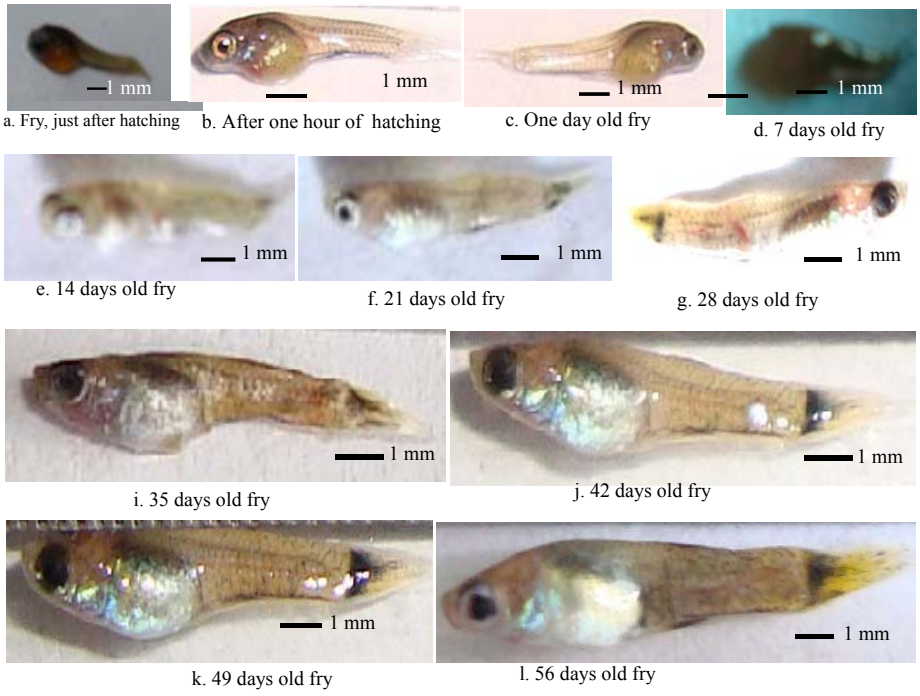

Plate 2. Successive stages of fry development of *Poecilia reticulate.*

21-day old fry: The anal fin of male becomes elongated and tube shaped while in the female it becomes small and rounded. Fin rays observed in male but not in female.

28-day old fry: Caudal fin of the male becomes coloured, especially appeared as brownish. Female caudal fin was blackish in colour. Size of female became larger compared to that of the male. The range of the body length was 8~9.5 mm.

35-day old fry: Male and female were clearly distinguished according to their size, tail and anal fin. The abdomen of female became larger and flatter compared to that of the male. Body size ranged 8.5~10 mm on average.

42-day old fry: Blacked tail clearly appeared in female. Body length was 91~0.5 mm.

49-day old fry: Male initiate their sexual traits and moved behind female. In some cases, a dark spot surrounding the anus and urinogenital area known as 'gravid spot' present in females while that was absent in males. Approximate body length was 9.5~12 mm.

56-day old fry: The male and female become sexually mature. Fins were fully developed. Approximate body length was 11.5~14 mm. Body and fins were brightly coloured in male.

The full size of male ranged 3~5 cm and female ranged 4~7 cm and reached full size at about 6 months.

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— Short Communication

ENHANCEMENT OF HETEROTROPHIC ACTIVITIES IN POLLUTED WATER BY *BACILLUS COAGULANS* BW-25

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Unsafe water is a global public health threat, placing persons at risk for a host of diarrhoeal and other diseases as well as chemical intoxication (Hughes and Koplan 2005). With the increase in interest in the microbial ecology of aquatic environments, it is important to have an understanding of the autochthonous micro flora. Since the bacteria are well known agents of mineralization and transformation of organic and inorganic matters in aquatic ecosystem, it was considered useful to determine the prevalence of some selected heterotrophic bacteria in the river. The objectives of the present study were, (i) isolation of indigenous bacteria possessing high metabolic activities, and (ii) improvement of the quality of water by enhancement of heterotrophic activities.

The area in and around Farashganj, Dhaka at the bank of the river Buriganga is a whole sell market of daily commodities. Residents around the river and people of the market take bath in the river and use the water for washing of vegetables and fruits were selected for the study site. Water samples were collected from surface area (5 cm depth) with a sterile 2.0 L plastic bottle and were transferred to the laboratory within an hour of sampling. Temperature of water samples was measured at the time of sampling with the help of a mercury thermometer. The pH of water samples was measured by digital pH meter (Jenway 3310, UK) immediately after bringing the sample in the laboratory. Dissolved Oxygen (DO) of water sample was determined by DO meter (Jenway 970 DO_2, UK), while, conductivity was measured by conductivity meter (Hanna, MODEL-HI 9033). Total Dissolved Solids (TDS) was measured by TDS meter (Hanna, MODEL-HI 9034), and alkalinity was determined by titrimetric method.

One ml of sample water was transferred into 10 ml of nutrient broth medium and incubated at 37ºC. After 48 hours of incubation, 0.1 ml of bacterial culture from each tube was inoculated onto nutrient agar plates and incubated at 37ºC. Coagulated egg albumin medium, alkaline egg medium and skim milk agar were used for the screening of the proteolytic activity of the isolates (Wandersman *et al*. 1986). Considering fast growing and high proteolytic activity bacterial isolate BW-25 was selected for biotechnological application.

Five ml of bacterial cells suspension was used as inocula and it was inoculated into 250 ml treatment bottles (Schott, Duran, Germany). The glass bottles were incubated in a

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light condition *in situ* (in duplicate) at 32°C temperature for 15 days. Bacterial activities were evaluated by the changes of inorganic nutrient concentration into the water during incubation. Just after adding bacteria samples were taken as 0-day and it was continued until 15 days of incubation at 5 days interval. For chemical analysis, concentrations of inorganic nitrogen (DIN: ammonium, nitrate and nitrite) and phosphorus (DIP) in the experimental bottles were measured following the methods described in the previous paper (Karim *et al*. 2013). Important physiological and biochemical characteristics were studied for the identification of the selected bacterial isolate (Sneath *et al*. 1986 and SAB 1957).

The physico-chemical properties of water sample presented in Table 1 showed that in June, water temperature and pH were at 32°C and 6.7, respectively, that indicate the favourable conditions for bacterial growth. But in case of DO, it was 0.76 (mg/l), COD was 18.26 (mg/l) and the BOD was consisted with water temperature as 0.59 (mg/l). The alkalinity, conductivity and TDS of sample water were 2.18 (meq/l), 299.15 (µs/cm) and 145 (mg/l), respectively. In regards to proteolytic activities, BW-25 possessed high activities by clear zone into the medium and increased with incubation period.

Table 1. Chemical properties of the water sample collected from Buriganga river.

Date of Sampling	Air temperature	Water temperature	pH	DO (mg/l)	BOD (mg/l)	COD (mg/l)	Alkalinity (meq/l)	Conductivity (µs/cm)	TDS (mg/l)
June 6, 2011	31ºC	32ºC	6.70	0.76	0.59	18.26	2.18	299.15	145.00

The isolate BW-25 showed positive results in gram reaction, catalase, oxidase, utilization of citrate, protease, KOH, nitrate reduction, motility, hydrolysis of starch, gelatin and casein. However, it showed negative results in VP, MR, levan, phenayl alanine deaminase, tyrosine degradation, egg yolk lecithinase, egg yolk lipase, degradation of urea, propionate utilization, indole formation and dihydroxy acetone production. In case of hydrolysis of different carbohydrates the strain could produce acid from D-glucose with no gas, L-arabinose, D-xylose and D-mannitol. Considering morphological and physiological characteristics the isolate BW-25 was identified as *Bacillus coagulans* (Sneath *et al*. 1986 and SAB 1957).

One experiment was carried out using *Bacillus coagulans* BW-25 with water sample collected on June 6, 2011 and incubated at 32°C, the initial concentration of DIP in control was 0.06 mg/l that increased to 0.32 mg/l after adding bacteria (Fig. 1). Net releases of DIP in control were 0.13 mg/l, while after adding bacteria it was increased to

0.26 mg/l. In the result, net releases of DIN in control were 2.52 mg/l and it was increased to 14.82 mg/l with bacteria (Fig. 1). So, net releases of DIN after adding bacteria were 12.02 mg/l (Fig. 1).

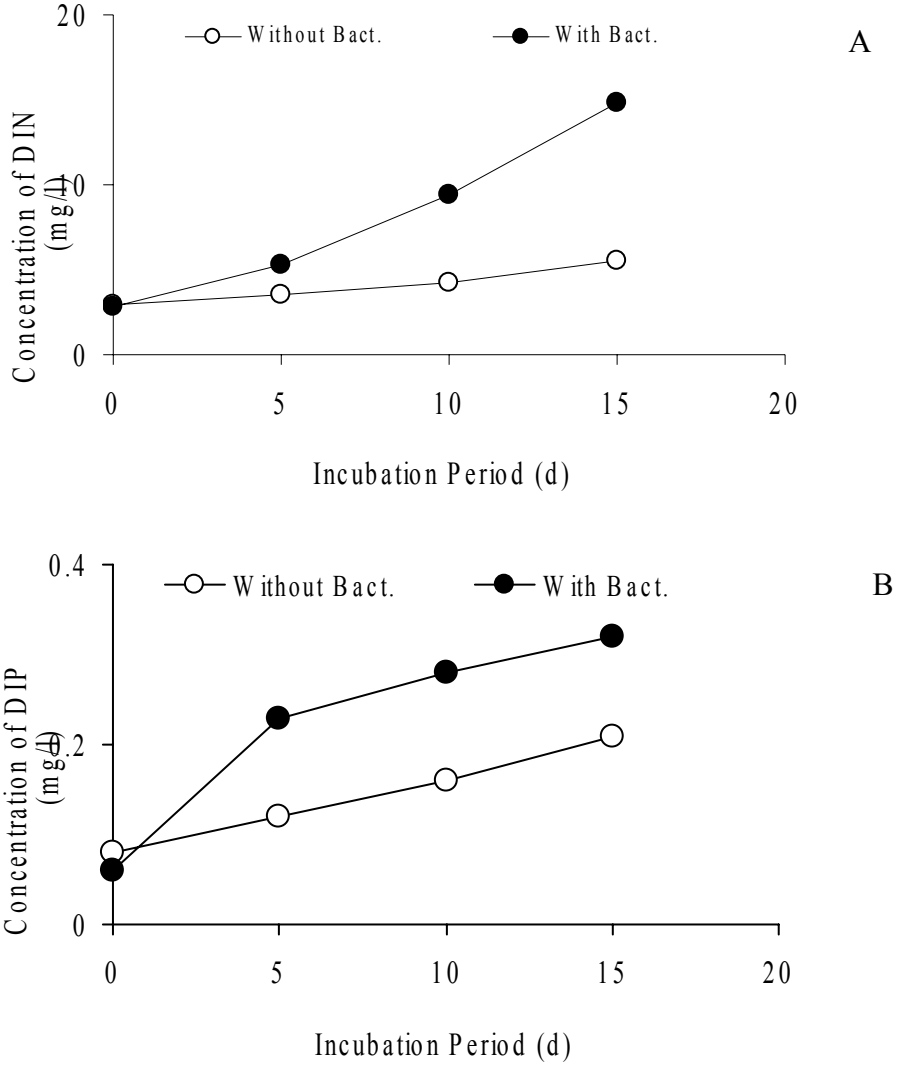

Fig. 1. Changes in concentration of dissolved inorganic nutrients by adding *Bacillus coagulans* BW-25. A & B, incubation temperature was 32°C; sampling on June 6, 2011. Without *Bacillus coagulans* BW-25 (o) and with *Bacillus coagulans* BW-25 (•).

Due to presence of oxygen demanding pollutants like organic wastes, there existed a rapid depletion of dissolved oxygen from this river water and thus creates problem for survival of fish. Even after treatment, domestic effluents are sources not only of chemical compounds but also of microorganisms that can be very active in the river, and play a fundamental role in the ecological functioning of the system (Garnier *et al*. 1992).

To stimulate heterotrophic activities in the specific limiting environmental conditions, addition of effective bacterial isolate which is capable of high proteolytic activities might be one useful way to control water pollution. Inducing the growth of particular bacteria could facilitate the biodegradation process of organic wastes or control fish pathogens even in large area of water (Rijn *et al*. 1995). In present study we tried to apply this method in a eutrophic ecosystem and monitored the effects of adding bacteria to enhance the heterotrophic activities. After adding *Bacillus coagulans* BW-25, net amounts and rates of DIN and DIP regeneration into water were markedly enhanced and it was about two folds higher than the control (Fig. 1). Probably it happened due to availability of labile organic matter in the water. It is necessary to conduct further study at the next stage regarding the removal of enhanced inorganic nutrients from the water.

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— Short Communication

STUDY ON THE INSECT INFESTATION OF DRY FISHES AT SINGRA

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Drying is regarded as a traditional, cheapest and simplest method of preservation of fishes. It plays a vital part in the developing countries of the world like Bangladesh. Bala (2000) reported that about 30% of the freshly harvested fish is spoiled every year due to lack of proper preservation facility in the country and this amount is 0.308 million metric ton and about 40% of the remaining harvested fish was sun dried and this amount was 0.072 million metric ton. Significant portion of dried fish approximately 622 tons were exported that earns a good amount which is 25.06 crore taka of foreign currency (DoF 2011). Dried fishes are not only economically important but also an important source of animal protein in Bangladesh which supplements 60% of animal protein (DoF 2012). Graikoski (1998) also reported that, dried fish products are the pre-dominant food bringing vital protein to people in rural areas. Besides protein source dried fishes are also rich in vitamins and minerals, which are often overlooked in developing countries (Hossain and Afroze 1991, Nettleton 1992, Basu and Gupta 2004 and Ross *et al.* 2007). Dried and drying fishes are susceptible to many types of spoilage which can affect the quality and shelf life. Physical and organoleptic qualities of many traditional sun-dried products are un-satisfactory for human consumption (Nowsad 2005). Damages occurring due to flies and insects are of great significance in open drying under the sun and this is a serious problem in traditional drying. A good number of researchers worked scattered on insect infestation and protection policy (Azam 2002 and Samad *et al.* 2009) but there is little work particularly in Chalan beel area. Chalan beel is an extensive low land area at the lower *Atrai* basin in the northeastern region of Bangladesh and spread across the districts of Natore, Naogaon, Pabna and Sirajgang (Samad *et al*. 2009). It consists of a series of *beels* connected to one another by various channels during the rainy season. A very dense water network over the entire Chalan beel is formed by rivers and their tributaries. The total area covered being slightly above 150 square miles (375 sq. km.). Since this area is a great source of fresh water fishes of north-western region of Bangladesh and many people engage with drying activities the present investigation was conducted in different dry fish yards at Singra in Chalan beel area of Natore district to study the conditions of dry fish infestation and the protection policy which are normally used. The studied fish drying yards and their detail information are shown in Table 1. A questionnaire was developed in logical sequence of information include both qualitative and quantitative values of findings, so that the respondents could answer easily and chronologically. A total of 25 dry fish professionals in different drying points was interviewed.

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Table 1. Detail description of the study area.

Location of the Yards	Area (Decimal)	Number of fish drying points	Number of man power engaged	No. of interviewed professionals
Dakin Domdoma	33	3	7-8	4
Zolar Bata	38	3	8-9	5
Ningoil	69	4	12-15	6
Baria	18	1	5-6	2
Chaugram	15	1	4-5	3
Kalam Nazarpur	16	1	3-4	2
Dahia	19	2	4-5	3
Total	208	15	43-52	25

Insect Infestation: During rainy season, humidity levels are high, sufficient drying cannot be achieved using traditional methods, processed and stored dried fishes re-absorb moisture and become susceptible to insect attack. Losses also result during storage from attack by pests which can gain access. The amount of quantitative loss by insect infestation was nearly 10%. This amount increases during the rainy season (15-20%) at Singra drying yards. It was noticed that two major infestations damage the dry fish products such as larvae (maggots) of several species of fly (*Diptera*) during the early stages and Beetle (both larvae and adult). Mite also infests during storage and in distribution. Mainly adult females lay their eggs on fish flesh. After hatching young larvae then feed fish muscle vigorously. Most of the damage in dry fishes is caused by the larval stage. More or less same results were reported by FAO (1981) and Nowsad (2007). They opined that insect infestations are the real problems in dry fish in Bangladesh. Bala (2000) reported that, in tropical climates under highly humid conditions, heavy infestation of unsalted dry fish by beetles may cause up to 30% loss of the products. Doe (1977) and Ahmed (1978) reported that both quantitative and qualitative losses occurred through spoilage and insect attack in dry fish processing.

Protection Policy: In the fish drying point (Singra, Chalan beel) there was no fly proof netting system (Plate 1). For this reason infestation by flies and beetles was very much common problem (Plate 2. A and B). To protect the dry fishes from the insects, dry fish professionals used different insecticides in different doses. Name of the insecticides and its doses are presented in Table 2.

Plate 1. Showing open drying activity in the study area.

A B

Plate 2. Showing the infestation of dry fish by cheese flies (A) and houseflies (B).

Table 2. Name and doses of the insecticides.

Sl. No	Name of the insecticides	Name of the company	Price Tk.	Doses		
				Amount of medicine	Amount of water mixed with medicine	Amount of fish spread by medicine
01.	Basudin	Sinzenta, Bangladesh	315 (2kg)	0.5 kg	---	200 kg
02.	Finish	Standard finish oil company, Dhaka	27 (100g)	No actual amount	No actual amount	No actual amount
03.	Booster	Padma oli company, Chittagong	432 (400ml)	400 ml	5 liter	500 kg
04.	Ripcord	National agricare import and export limited	465 (400ml)	400 ml	5 liter	500 kg
05.	Diazinon	The limit company, Chittagong	300 (400ml)	400 ml	5 liter	500 kg
06.	Camcrone	---	60 (50 ml)	50 ml	2 liter	200 kg

Cypermethrin

Often the extent of pesticide use was sharply reduced in sunny days. If the storage time prolongs, processors check the condition of the stored products at certain intervals. If further infestation was found, the product was treated with the pesticides again after a day of drying. There was no indigenous method of protection of dried fishes from insects but salting. Most dry fish professionals use salts to protect their products from insects. Some fish traders use additional salt to increase the weight of dry fish but the quality of salts is very poor and not proper ratio of salt and fish is maintained.

In the study area the dry fish processors or labourers have no knowledge on pesticide action, dose limit and residual effects. Nowsad (2007) worked on tolerance limit of pesticides in dried fishes. For example 100 g Basudin (active ingredient: 100 g Diazinon in 1 kg) is applied to 100 kg dried fish in gunny sacks during storage. He also reported, during processing *Nogos*, *Nuvacron, Endrin, Malathion, Dimacron* etc. are popularly used, while in storage of the product, DDT*, Basudin* and *Malathion* were preferred ones. Both insects and insecticides comprise about 60% of the total dried product that is considered to be unfit for human consumption (Nowsad 2005). Fish processors in Thailand were found to rely on the application of illegal insecticides to control blowfly infestation (Esser 1992). Clucas and Ward (1996) recommended insecticides applications by properly instructed trained people. Samad *et al* (2009) reported that generally mixed 25-50 kg commercial salt for 1 kg of fishes. Mushi and Chiang (1974) suggested that dried fish containing 13% or more salt could prevent the growth of insect at all developmental stages. Doe (1977) and Ahmed (1978) also reported that damage can be heavy where salt is not used and drying condition is poor, as much as 25-30% under very humid conditions in Bangladesh.

To keep the dried product free from the insect infestation proper training should be necessary for improvement of traditional sun-drying, good handling, sanitation and public health. Use of insecticides in dried fishes must be stopped and tent or funnel sun drier developed by AERC and BCIRL must be ensured.

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