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Bacteriological study of bottom soil of three extensive fish farm ponds

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Abstract. The study was conducted for bacteriological assessment of pond bottom soil (sediment) from three selected fish ponds of Bangladesh Agricultural University. Samples were collected both in winter and summer seasons to see the seasonal variations of some bacterial genera. Physicochemical parameters of the pond water and sediment were also estimated monthly. Sediment samples were collected from the shore and middle zone of the ponds. All sediment samples were acidic. The total viable count of bacteria in pond sediment ranged from (8.5×10^6) to (4.9×10^9) cfu/g and bacterial count were higher in summer than in winter. A total of 16 bacterial genera were identified from the sediments. The predominant microflora consisted of *Aeromonas*, *Flavobacterium*, *Cytophaga*, *Escherichia, Bacillus*, *Micrococcus*, *Staphylococcus*, *Aerococcus*, *Acinetobacter, Pseudomonas* and *Moraxella* while the first five listed the greatest abundance. *Aeromonas* was the most dominant organism. Bacterial diversification was found higher in summer than in winter.

Keywords: Bacterial count, Bacterial composition**,** Seasonal variation

Introduction

Aquatic microorganisms influence the water and sediment quality and are closely associated with fish physiology, diseases, and aquaculture as a whole. Fish take a large number of bacteria into their gut from water, sediment, and food (Sugita *et al.* 1988). Researchers (Shewan and Hobbs 1967) suggested that the bacterial flora on fish reflects the aquatic environment. Changes in bacterial sensitivity to environmental conditions might prove helpful for providing information on benthic conditions and the effects of fish farm bio-deposition (Vezzulli *et al.* 2002). Bacteria in sediments are major contributors to biogeochemical processes in benthic ecosystems, and bacterial abundance is an essential parameter for assessing the roles of bacteria in these environments (Liao *et al.* 2012). The sediment is a more appropriate medium to sample than the water column because it is less influenced by perturbations caused by rainfall and transient movements of waterborne substances (Al-Harbi and Uddin 2006).

Evidence of the previous study (Campbell and Buswell 1983, Nieto *et al.* 1984) suggests that the gastrointestinal microflora of fish is highly variable and reflects their aqueous environment, especially the food and sediments. Some common bacterial microflora in water, such as *Pseudomonas*, *Aeromonas,* and *Vibrio,* can cause major fish epizootics under stressful conditions. The bacterial content of the sediments also affects the quality of fish and fish products. Knowledge about bacterial community structure and diversity is essential to understand the relationship between environmental factors and ecosystem functions. Such knowledge can assess the effect on the ecosystem of environmental stress and perturbations like pollution, agricultural exploitation, and global changes. Therefore, studying aquatic microbiology is important for water quality management in aquaculture and public health. The

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bacteriology of pond sediments is primarily driven by diet added to ponds, ambient temperature, and oxygen levels (Smith, 1998). *Vibrio* spp in pond sediment is an important parameter to monitor because it is an indicator of bacterial load and stress on the fish (Smith 1993, Rosa *et al.* 2002). Management of pond sediments may be a key factor influencing the bacteriology of earthen fish ponds and fish health during grow-out. Conditions leading to the development of fish diseases may be recognized and corrected. Detailed information about microbial load and types of bacteria in pond sediments is essential. The present study aimed to determine the seasonal distribution of bacteria in pond sediment of extensive fish farming ponds. Seasonal variation of microbial genera in ponds and their occurrence differences in sediments taken in different position of the ponds were also studied.

Materials and Methods

Sample collection: Pond bottom soil samples for bacteriological and physicochemical analyses were collected from three BAU fish farm ponds. In all three ponds, the extensive culture of fish was practiced. The ponds were designated as No. 1, No. 2, and No. 3 ponds, respectively. Samplings were done in April (summer) and in December (winter) of the same year. In each sampling, two samples were collected from each pond, one sample from near shore (one foot from shore) and another from the middle of the pond.

Physicochemical parameters: For physicochemical studies, the water temperature was measured with a thermometer. Total organic carbon and pH of soil samples were measured in Humboldt Laboratory of Soil Science Department, Bangladesh Agricultural University, Mymensingh. Soil pH was determined by using a soil pH meter, and soil organic carbon was estimated according to Walkley-Black method which was modified by Jackson (1958).

***Bacterial viable count*:** Bacterial viable count was done by the consecutive decimal dilution method. All plating was done on plate count agar in duplicate. The plates so treated were kept in an incubator at 30°C for 24 to 72 h in an inverted position. Following incubation, plates exhibited 30 to 300 colonies were counted. The viable count was calculated according to International Standard Organization (ISO, 1965) by the following formula:

Bacterial count (cfu/g) $= C \times D \times 10$

Where, C= Nos. of colonies found; D= Dilution factor, cfu/g= Colony forming unit/ gram.

***Isolation and identification of bacteria*:** Bacterial strains were isolated from the sediments of three ponds at each sampling. The bacterial colonies were divided into different types according to the colony characteristics of shape, size, elevation, structure, surface, edge, color, and opacity. The number of colonies of each recognizable type was counted. Three to five representatives of each colony type were then streaked onto plate count agar repeatedly until pure cultures were obtained. Cultures on plate count agar slants were kept at 4°C as stock, and these were transferred to new slants at every six weeks. For identification of the bacterial isolates, it was first taken for the Gram's staining. Then a series of tests were done to identify the bacterial isolates up to genus level. Different types of colonies that grew on the surface of different media were identified by studying their morphological and biochemical characteristics. It was done according to the method suggested in Cowan and Steel's manual for the identification of medical bacteria, which was edited by other microbiologists (Barrow and Feltham 1993).

Results

***Physicochemical parameters*:** The study of physicochemical parameters (temperature, pH, and organic carbon) of water and bottom soil samples were taken from three ponds on both the summer and winter seasons on a monthly basis (Table I). The temperature ranged from 19.8 to 22.8°C; pH ranged from 5.45 to 6.22. The highest organic carbon was found 2.20±0.07 in the sediment from the middle in winter and 0.31±0.03 in the shore sediment from pond 1 in summer.

Table I. The temperature (°C), pH, and total organic carbon (%) of pond sediments

Pond	Temperature (°C)		pH				Organic carbon (%)			
	Summer (March-May)	Winter (Dec. - Feb)	Summer (March- May)		Winter (Dec. - Feb.)		Summer (March- May)		Winter (Dec. – Feb.)	
			Shore	Middle	Shore	Middle	Shore	Middle	Shore	Middle
1	21.8± 1.5	20.5± 1.3	5.66± 0.03	5.77± 0.02	5.83± 0.04	5.68± 0.04	0.31± 0.03	0.81± 0.01	1.77± 0.02	0.350± 0.04
2	22.6± 2.1	19.8± 2.0	5.50± 0. 03	5.45± 0.04	5.66± 0.28	6.22± 0.10	0.84± 0.02	1.82± 0.02	0.46± 0.03	2.20± 0.07
3	22.8± 1.9	21.2± 1.5	5.58± 0. 03	5.68± 0.08	6.07± 0.01	5.76± 0.03	0.62± 0.03	0.350± 0.04	0.41± 0.01	0.33± 0.02

***Aerobic plate count*:** Results of the seasonal quantitative estimation of aerobic heterotrophic bacteria in pond sediments are given in Table II. Each count was the mean value of viable colonies that appeared in duplicate agar plates made per individual sample. APC varied from 1.87×10^7 to 1.31×10^9 cfu/g in summer whereas 8.5×10^6 to 4.9×10^7 cfu/g in winter (Table II).

Table II. Bacterial load (cfu/g) in the sediments in two seasons

Pond	Bacterial load in Summer (April) (cfu/g)		Bacterial load in Winter (December) (cfu/g)	
	Shore	Middle	Shore	Middle
1	2.60×10^8	1.87×10^7	1.51×10^7	4.90×10^7
2	1.31×10^9	6.30×10^8	1.27×10^7	1.83×10^6
3	4.80×10^8	1.12×10^8	8.50×10^6	1.12×10^7

A total of 14 bacteria genus have been identified from the sediments of the three experimental ponds in winter and summer. No marked differences in bacterial composition were found in different ponds but there observed some variations between winter and summer samples. In winter *Vibrio, Streptococcus*, and *Corynebacter* were absent in all three ponds (Fig. 1). In pond 1, Pseudomonas was absent both in the middle and shore sediments. In pond 2, *Staphylococcus* was absent in shore sediments, and *Acinetobacter*, *Moraxella*, and *Salmonella* were not present in the middle sediments during winter In pond 3, *Pseudomonas* was absent in shore and *Micrococcus* in middle during winter (Fig. 1).

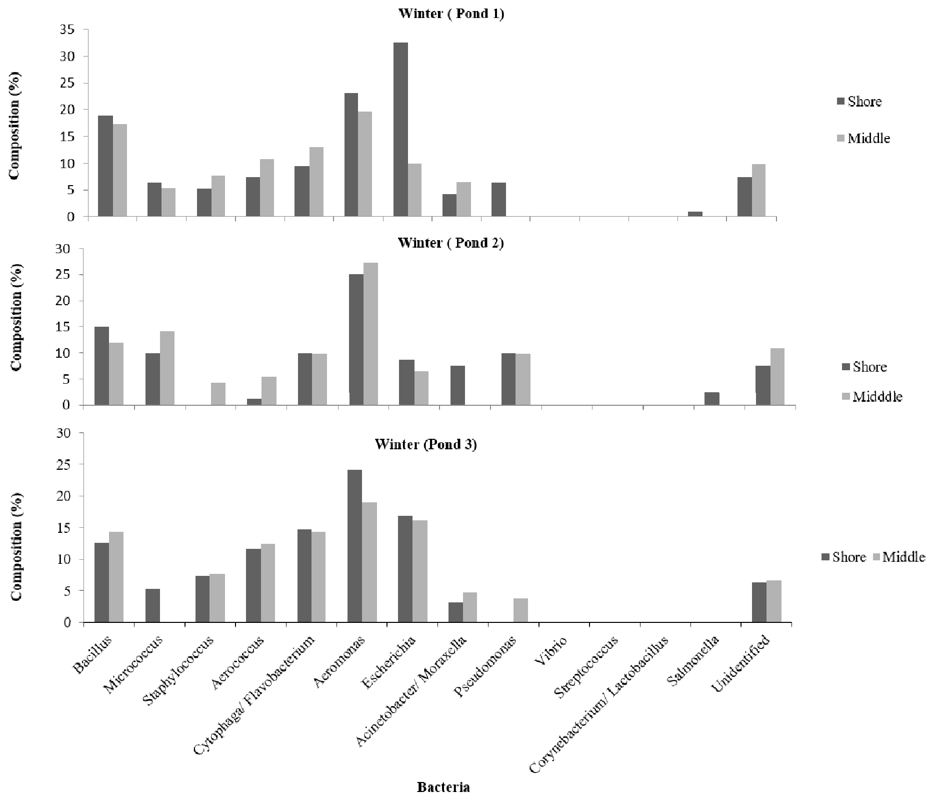

Fig. 1. Bacterial distribution (%) in the shore and middle sediment of ponds in winter (December).

Micrococcus and *aerococcus* were not found in pond shore and *Salmonella* was absent in the shore and middle sediments in the summer season (Fig. 2). *Micrococcus* and *Acinetobacter/ Moraxella* were not found in the pond middle during the summer season (Fig. 2). *Salmonella* was not found during the summer season. The most prevalent bacteria were *Aeromonas* in both the winter and summer season.

Discussion

Bacterial load varied seasonally in the studied pond sediments. In winter, bacterial load in all sediment samples was lower than that of the warmer month, which could be partly explained by the low temperature of pond waters (20.5±0.7ºC). The rest of the seasons had higher bacterial growth, probably due to the prevailing higher temperatures, close to the optimum growth temperature of many mesophilic bacteria in natural systems (Rheinheimer 1985).

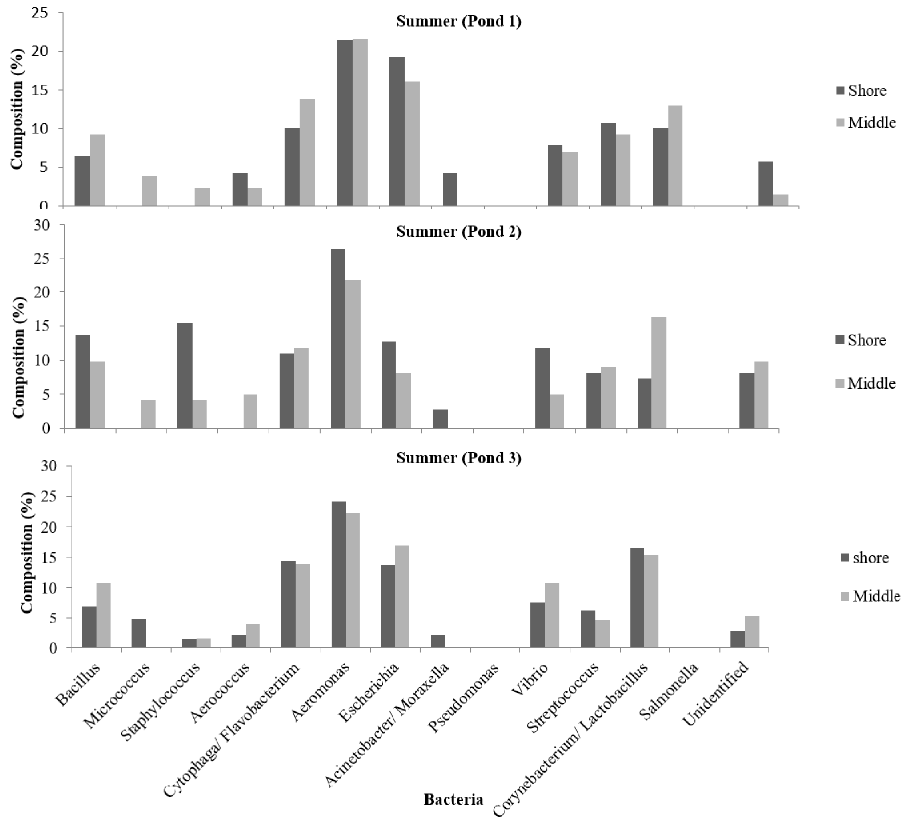

Fig. 2. Bacterial distribution (%) in the shore and middle sediment of ponds in summer (April).

In sediments, organic enrichment and the consequent modification of the characteristics of the benthic environment determined an increase in bacterial population (Rosa et al., 2002). The organic matter influences the size and composition of the microbial population (Rheinheimer 1985). Higher organic carbon ($1.331 \pm 0.817\%$) in pond two results in higher bacterial load in that pond. Others (Okpokwasili and Alapiki 1990) reported that the sediment isolates were made up entirely of gram-positive bacteria, and the present study also found similar results.

Bacillus, *Aeromonas*, *Flavobacterium*, *Cytophaga*, *Escherichia, Micrococcus*, *Staphylococcus*, *Aerococcus*, *Acinetobacter, Pseudomonas*, *Moraxella* were found throughout the study period. All other isolated bacteria occurred at too low frequency to assess their seasonal prevalence. The genera *Achromobacter, Flavobacterium, Brevibacterium, Vibrio, Micrococcus, Sarcina, Bacillus, Pseudomonas, Nocardia, Streptomyces* and *Cytophaga* occur widely in lake water (Rheinheimer 1985). The high prevalence of *Aeromonas*, *Flavobacterium*, *Cytophaga*, *Escherichia, and Bacillus* throughout the study period suggests that these bacteria may be common in pond sediments. There were inputs for sources of rainfall run-off and human or other animal feces directly to the ponds and Coliform bacteria were found in all ponds. Fecal Coliforms could have been imported to the present ponds via those inlets and also birds. In the present study, fecal coliform *E. coli* was detected in larger numbers during summer and winter. *Pseudomonas* was present at cooler temperatures in winter, which perhaps reflects its lower optimum temperature for growth. The presence of fecal coliform bacteria in the sediments suggests that this water is not safe for household uses, and care should be practiced during processing the fish caught from these waters to prevent contamination of edible meat.

There was little effect on fish disease prevalence of the bacteria detected in pond sediments except for *Aeromonas,* which reflected to cause EUS in fish in winter. The water quality of the fish ponds appeared to be sufficient to support the good growth of fish, these fish were not exposed to significant stress.

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