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Microbiological and Biochemical Analysis of Water and Soil Sample Exist In Coastal Shrimp Aquaculture Production System of Bangladesh

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Original Research Article

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Abstract: Objective of this study was to determine Biochemical condition and Microbiological load of water and soil sample of some costal shrimp Hatchery and Fisheries of Bangladesh. 8 samples were taken from 6 individual costal shrimp Hatchery. pH, temperature, Dissolved Oxygen, Biological Oxygen Demand, Ammonia, Salinity and Alkalinity of each sample was determined. The temperature and pH of the collected samples were found to vary from 29 to 32°C and 7.5 to 8.5 respectively. The Biological Oxygen Demand of the collected samples from different sources was found to range from 8.0 to 13.0 mg/L. The Dissolved Oxygen of the samples was observed to vary from 3.0 to 4.0 ppm. The ammonia content was observed to vary from 0.5 to 0.8 mg/L. The salinity was in the range of 28 to 32 ppt. The alkalinity of the samples was found to range from 120 to 130 mg/L. The total bacterial count and *Vibrio* load count of was done from collected samples. The maximum bacterial load was found to exist in the soil sample of dumping ground and at the zone of mixing point of hatchery discharged water with sea water next to it.
Keywords: Hatchery and Fisheries, Dissolved Oxygen, hazardous effects

INTRODUCTION

Shrimp farming has been one of the most important economic activities in many of the tropical countries like Bangladesh. Now a day, sustainability of shrimp culture is in danger due to disease, and disease outbreaks in shrimp farms is a common event in the south-eastern part of Bangladesh. Unlike some other aquatic animals, Shrimp are highly prone to bacterial and viral diseases.

In order to prevent disease loss, hatcheries and farms generally use huge amounts of chemical antibiotics. Such uncontrolled use of chemicals creates enormous problems in the export of shrimp, since most international consumers have modified their bio-security policies to "zero tolerance" and have specifically banned some of the antibiotics commonly used in the shrimp hatcheries and farms of Bangladesh. In Bangladesh, there are no hatchery sources of antibiotic used data. Without proper scientific investigation into treatment regimes, there has been a tendency for individual hatchery to select their own treatment regimes and to do their own experiment. Little knowledge exists among hatchery operators as to the hazardous effects of the chemicals in use. The present study is an attempt to identify the biochemical condition and bacterial load exists in natural environment as well as in hatcheries, surrounding water body (supply water and soil) and culture farm.

MATERIAL AND METHOD

Sampling site

Selected sampling sites/ points were in Cox's Bazar area both for shrimp hatchery and grow-out pond operation.

- For Shrimp Hatchery: Cox's Bazar: Kolatoli , Sonapara
- For Grow-out pond operation: Kurushkul (Beximco Fisheries Ltd.).

Sampling method

Samples from water and soil were taken at selected sampling sites at regular interval as per activity plan. In addition, feed and live samples at "different stages of production cycles' at Shrimp Hatchery and Shrimp Grow out pond were taken to check existing 'microbial load' and selected 'water quality' parameters of the respective area following Standard procedures [1,2].

Sample collection and preservation

During sample collection water quality parameters e.g.; temperature, salinity, pH etc. were recorded and Dissolved Oxygen (D.O), Biological Oxygen Demand (BOD), alkalinity and NH3 were analyzed from water at shrimp culture pond, maturation tank and water at hatchery intake and discharge /dumping drain and the mixing site at sea. Samples for bacteriological analysis (water, soil) were collected in sterilized equipment. After collection, samples were

carried by Styrofoam box with pieces of ice for preservation during transportation and brought to microbiology laboratory, CU for microbial analysis and preserved in the refrigerator at 4^0 C before and after the microbial analysis. For selected water quality analysis samples were taken to field laboratory of IMSF, CU at Cox's Bazar and analysis were done following standard procedures [3]. Alkalinity and Ammonia were tested by using Hanna test kit.

pH determination of the collected samples

pH of the samples collected from the selected industries were determined in the laboratory with an electric pH meter (pH Hanna Instrument Ltd. & 3310, pH meter Jenway, UK) immediately after collection of the samples.

Determination of biological oxygen demand (bod) of the sample

For the determination of the Biological Oxygen Demand (BOD) of collected samples, two bottles were filled with the sample. At first, the initial dissolved oxygen (D.O) for one BOD bottle was calculated and water from same site was remained in another BOD bottle in the dark condition for five days. After five days, the D.O for the dark bottles was calculated. Then the BOD level of the sample was determined from the difference between the initial and final D.O.To Determine the D.O for initial bottle, 200 ml of a BOD bottle was taken for sample collection. The bottle was filled up with sample and stoppered carefully. After 2 minutes settling, 2 ml of $MnSO_4$ and 2 ml of alkali-iodine azide was added in the bottle and mixed by inverting the bottle for at least 15 times. Then 2 ml of conc. H_2SO_4 was added in the bottle. The bottle was restoppered and mixed by inverting several times until dissolution observed. After that, 20 ml sample was taken from the BOD bottle to a conical flask and titrated with 0.025 (N) $Na_2S_2O_3$. A pale straw color was appeared. 1 to 2 ml starch solution was added into the conical flask and titrated with 0.025N $Na_2S_2O_3$ until the color disappeared. The initial and final reading of required $Na_2S_2O_3$ was recorded from the burette. The process repeated thrice. Average data of three titration was used to calculate the initial D.O of the sample. To Determine the D.O for Dark Bottle of the given sample, a bottle was kept in dark condition for 5 days to find out BOD. After 5 days in dark condition, 2 ml of $MnSO_4$ and 2 ml of alkaline-iodide azide solution was added in the bottle and mixed by inverting the bottle at least 15 times after 2 minutes settling. Then 2 ml of conc. H_2SO_4 was added in the bottle. The bottle was stoppered and mixed by inverting several times until dissolution observed. After that, 20 ml sample was taken from the BOD bottle to a conical flask and titrated with 0.025N $Na_2S_2O_3$. A pale straw color was appeared. 1 to 2 ml starch solution was added into the conical flask and titrated with 0.025N $Na_2S_2O_3$ until the color disappeared. The initial and final reading of required $Na_2S_2O_3$ was recorded from the burette. The process repeated thrice. Average data of three titration was used to calculate the final D.O of the sample.

Calculation

BOD (mg/l) = Initial D. O. – Final D. O

MEDIA AND TECHNIQUES FOR ENUMERATION AND ISOLATION OF BACTERIA

Media used the following media were used for the enumeration and isolation of colonies of microorganism

- Nutrient agar medium
- TCBS agar medium

Techniques employed

Three different techniques were applied for the enumeration and isolation of bacteria: dilution plate, pour plate and spread plate methods [4]. For the enumeration and isolation, serial dilution was carried out up to 10^6. The noculated media were incubated at 37^0C for 24 to 48 hours.

Enumeration of bacteria

After incubation, the plates having well-spaced colonies were selected for counting. The selected plates were placed on a colony counter (Stuart Scientific U K) and the colonies were counted. The colonies or viable bacterial count per ml were calculated by multiplying the average number of colonies per plate by reciprocal of the dilution. The calculated results would be as colony forming units (cfu) per ml of sample.

RESULTS AND DISCUSSION

Sampling site the soil and water samples were collected from different areas of Cox's Bazar including samples from a number of hatcheries (Figure 1 & Figure 2**).** General description of the samples is given in the Table 1.

Table-1: Name and place of different sampling sites at hatchery & grow out pond of Cox's Bazar

Sl. No.	Location/Hatchery	Place of Collection
1.	Kolatali, Cox'sbazar	Dumping and mixing point of water discharged from the hatchery
2.	Sonapara, Cox'sbazar	Dumping and mixing point of water discharged from the hatchery
3.	United Hatchery, Cox'sbazar	Raw water, Treated water, water from algal culture
4.	Grameen Hatchery, Cox'sbazar	Raw water, Treated water, water from algal culture
5.	Quality Hatchery, Cox'sbazar	Raw water, Treated water, water from algal culture
6.	Beximco Fisheries, Cox'sbazar	Raw water, Treated water

Collection of samples

The samples were collected aseptically from sample collection sites in sterile glass bottles and polybags, brought to the laboratory and preserved at 4^0C. The time, temperature, pH and date of the collected samples were recorded and presented in Table 2. The temperature and pH of the samples were determined by thermometer and pH meter (pH Hanna Instrument Ltd. & 3310, pH meter Jenway, UK.) respectively.

Table-2: Results of pH and Temperature of samples (water/soil) at Hatchery, source water, dumping & mixing water and grow out pond at different sampling time

SI NO	Location/ Hatchery	Type of Sample	pH	Temperature (°C)
1	Dumping water zone at Kolatali, Cox'bazar	a) Water sample b) Soil sample	8.2 8.0	30 30
2	Mixing water zone at Kolatali, Cox'bazar	a) Water sample b) Soil Sample	8.0 7.9	30 30
3	Diamond Hatchery, Cox'sbazar	a) Raw water b) Treated water c) Water from algal culture	8.2 8.0 8.3	32 32 32
4	Beximco Fisheries, Cox'sbazar	a) Raw water b) Treated water	8.1 8.0	31 31
5	Dumping zone at Sonapara, Cox'bazar	a) Water sample b) Soil sample	8.3 8.1	29 29
6	Mixing zone at Sonapara, Cox'bazar	a) Water sample b) Soil sample	8.1 8.0	28 28
7	Grameen Hatchery, Cox'sbazar	a) Raw water b) Treated water c) Water from algal culture	8.0 7.8 7.9	29 29 29
8	Quality Hatchery, Cox'sbazar	a) Raw water b) Treated water c) Water from post larval culture	8.1 7.9 8.0	28 28 28

In the present study, the temperature and pH of the collected samples were found to vary from 29 to 32°C and 7.5 to 8.5 respectively. Chen [5] demonstrated that the suitable temperature for shrimp culture is between 29 to 32°C. The temperature recorded in our present study coincides with the value specified for shrimp culture by Chen [5]. Chen *et al.* [6] and Wickins [7] reported that pH level between 7.17 and 8.34 was suitable for *P. monodon* post larvae and this coincides with the present findings. However, the pH values determined for different samples in the present study comply with the above specification.

Fig-1: Dumping Ground of Waste Water from the Hatcheries from where water and soil were collected and tested

Fig-2: Raw Water Storage Tank of "Quality Hatchery", Cox's Bazar, one of the sampling sites at Hatchery

Determination of bod, dissolved oxygen (do), ammonia, salinity and alkalinity of the samples

The analytical results of BOD, Dissolved Oxygen (D.O), ammonia, salinity and alkalinity of the collected samples are shown in Table 3. The BOD of the collected samples from different sources was found to range from 8.0 to 13.0 mg/L. The D.O of the samples was observed to vary from 3.0 to 4.0 ppm. The ammonia content was observed to vary from 0.5 to 0.8 mg/L. The salinity was in the range of 28 to 32 ppt. The alkalinity of the samples was found to range from 120 to 130 mg/L.

Table-3: The analytical results of BOD, DO, Ammonia, salinity and Alkalinity at selected sampling sites

Sl. No.	Sample	BOD (mg/L)	DO (ppm)	Ammonia (mg/L)	Salinity (ppt)	Alkalinity (mg/L)
1.	Water sample from Kolatali	8.5	3.2	0.5	31	127
2.	Water sample from Diamond Hatchery	8.7	3.3	0.5	30	126
3	Water sample from Beximco Fisheries	9.0	3.5	0.4	28	130
4.	Water sample from Sonapara location	11.5	3.8	0.5	32	129
5.	Water sample from Grameen Hatchery	12.5	4	0.5	33	125
6.	Water samplefrom Qualiy Hatchery	11.0	3.6	0.4	29	121

Water is the most vital component among the natural resources, and is crucial for the survival of all living organisms. Managing of water quality during the larval rearing phase is very important due to the sensitivity of larvae to the fluctuation of water parameters. Santhosh and Singh [8] recommended optimum BOD level for aquaculture should be less than 10 mg/L but the water with BOD less than 10-15 mg/L can be considered for fish culture. The BOD level of selected samples was found to be fall in standard value.

Dissolved oxygen affects the growth, survival, distribution, behavior and physiology of shrimps and other aquatic organisms [9]. Chen *et al.* recommended D.O level to be between 4.0-8.0 ppm for *P. monodon* post larvae rearing. The D.O values recorded during this research work were found to be less than the appropriate which is detrimental for shrimp growth and might have been influential for anaerobic bacterial infection in the larvae rearing. Bhatnagar and Singh [10] recommended the level of ammonia (<0.2 mg/L) suitable for hatchery. The calculated NH_3 value of the present study is beyond the specified limit that may have a detrimental effect on shrimp culture. Stone and Thomforde [11] suggested 50-150 mg/L (alkalinity) as desirable range; an acceptable range of above 20 mg/L and less than 400 mg/L for ponds and above 10 mg/L for hatchery water. The experimental alkalinity value (120-130 mg/L) of our present study is within the specification limit for aquaculture.

Determination of total bacterial count and vibrio load count of the collected samples. The total bacterial count and *Vibrio* load count of collected samples are shown in the Table 4. There is a variation in the bacterial count and *Vibrio* load count among different types of samples. (Figure 3 & Figure 4)

Table-4: Total bacterial count and Vibrio load count of the collected samples at investigated sites

Sl. No.	Location	Type of Sample	Total Bacterial Count (cfu/ml)	*Vibrio* Load Count (cfu/ml)
1.	Dumping water zone at Kolatali, Cox'bazar	Water sample	7.06×10^5	4.58×10^5
		Soil sample	7.27×10^6	4.6×10^6
2.	Mixing water zone at Kolatali, Cox'bazar	Water sample	1.15×10^3	5.07×10^2
		Soil sample	5.04×10^3	2.74×10^3
3.	Diamond Hatchery, Cox'sbazar	Raw water	3.22×10^2	1.6×10^2
		Treated water	4.49×10^1	3.21×10^1
		Water from algal culture	2.94×10^3	1.52×10^3
4.	Beximco Fisheries, Cox'sbazar	Raw water	2.14×10^3	1.03×10^3
		Treated water	5.31×10^2	2.76×10^2
5.	Dumping water zone at Sonapara, Cox'bazar	Water sample	1.56×10^6	9.82×10^5
		Soil sample	1.18×10^7	6.84×10^6
6.	Mixing water zone at Sonapara, Cox'bazar	Water sample	6.54×10^3	3.99×10^3
		Soil sample	1.17×10^4	6.55×10^4
7.	Grameen Hatchery, Cox'sbazar	Raw water	3.37×10^2	1.65×10^2
		Treated water	5.6×10^1	3.62×10^1
		Water from algal culture	3.09×10^3	1.61×10^3
8.	Quality Hatchery, Cox'sbazar	Raw water	2.98×10^2	1.44×10^2
		Treated water	3.05×10^1	0
		Water from post larval culture	1.03×10^2	4.39×10^1

Fig-3: Total Bacterial Count (TBC) on Nutrient Agar Medium

Fig-4: *Vibrio* Load Count on TCBS Agar Medium

The maximum bacterial load was found to exist in the soil sample of dumping ground and the total bacterial load at the zone of mixing point of hatchery discharged water with sea water was next to it. The waste water of hatchery discharged with poor or no treatment is supposed to be responsible for making the raw sea water contaminated. A revious study on enumeration of bacteria associated with environment and body parts of hatchery reared juvenile *Penaeus monodon* counts for 2.9×10^4 cfu/ml [12]. There are quite large amount of information about total plate count of shrimp rearing water for different shrimp species at different stages under rearing conditions. Wang and his co-workers published their studies on total bacterial count of new and 3 years old grow out pond which was under cultivation of *Liptopenaeus vannamei*. Their finding revealed that total bacterial count of recently constructed pond was 1.11×10^6 cfu/ml, while it was 6.25×10^6 cfu/ml for 3 years old pound. According to Sung *et al.*[13] total bacterial count of three *Penaeus monodon* grow out ponds ranges from 2×10^3 to 3×10^6 cfu/ml. Total plate count of shrimp rearing water in a number of *Penaeus. monodon* hatcheries in India was reported in ranges of 10^2 to 10^4 cfu/ml and 10^4 to 10^7 cfu/ml by Otta [14]. Yasuda [15] exhibited that total plate count of rearing water of juvenile *Penaeus japonicus* was lower than 10^4 cfu/ml. In the present study, the bacterial count in hatchery system was found to be less than what was demonstrated in earlier studies. Though the number bacteria represent a lower value comparing with the previous research, the possibility of presence of pathogenic microorganisms in the shrimp culture system is not out of consideration.

CONCLUSION

Waste water from discharged from shrimp hatchery and aquaculture without any or proper treatment is a potential source of microbial contamination within the shrimp culture. The untreated waste water gets mixed with sea water which is further used for hatchery operation. The biochemical condition of this water is not satisfactory. This worse environmental condition is responsible for deterioration of shrimp quality as well as emergence of many shrimp diseases. Proper treatment of raw water before use, maintenance of personnel hygiene, treatment of waste water before discharge, use of appropriate dosage of antibiotic and probiotic treatment instead of antibiotic- are the requisites for a disease free shrimp culture system.

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