

Microbial Pathogens Diversity in Sewage Fed Bheris and Flood Plain Wetlands of West Bengal, India in Relation to Public Health

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Abstract: In sewage fed fishery the sewage water is purified through a series of sedimentation tanks and re- use the water for pisciculture and agriculture. Possible health hazard from these sewage-fed aquaculture systems has become a subject of serious debate probably since the inception of such fishery. Fish and water samples from different sewage-fed bheries and pond were analyzed using standard microbiological techniques and physico-chemical properties of water were analyzed as per APHA1995. The study was undertaken to evaluate the microbiological quality of sewage fed fishes and their hazardous impact on human health. Different species of bacteria were identified as associated with the fish culture systems in this study. The identified bacteria included *Escherichia* sp., *Salmonella* sp. and *Vibrio* sp which might cause zoonotic infection to human. It was concluded that cooking and boiling of fish prior consumption would eliminate the chance of infection even if they are contaminated.

Key words: Sewage fed fishery % Pathogens % Floodplain wetlands % Public health

INTRODUCTION

Application of sewage in pisciculture in order to augment fish production has been an ancient practice in India and other countries like i.e. China, Egypt and Europe. In West Bengal East Kolkata Wetlands is very significant for the wise use of sewage water, especially for pisciculture, irrigation for garbage farming and harbouring the biological diversity. In West Bengal scenario the floodplain wetlands of West Bengal, (Beels) cover an area of 42000 ha and constitute one of the important fishery resources. Possible health hazard from these sewage-fed aquaculture systems or bheries has become a subject of serious debate probably since the inception of such fish culture system. Fish may passively carry human pathogens in and on their bodies and these pathogens may subsequently cause health hazards to fish handlers and consumers[1].

Considering the importance, the present study was undertaken with the objective to evaluate microbiological quality of sewage fed fishes and their impact on human health after consumption.

MATERIALS AND METHODS

Water and fish samples were collected from different bheries of East Calcutta Wetlands, sewage-fed freshwater farm of Rahara, flood plain wet lands like, Mathura beel, Kancharapara (District-N, 24 Parganas), Kulia beel, Kalyani and Amda beel, Ranaghat (Nadia District). Water samples from above sewage-fed farm were collected from the inlet point of the pond storing raw sewage (W1) and collection made from settling tank (W2) and finally the samples were collected from the fish pond (W3) for comparative study of microbiological and chemical characteristics of the samples. The study was conducted during June to November 2009.

All fish samples were collected in sterile polythene bags, brought to laboratory under ice -cover and processed within 1 to 2 h. Further, sections of muscles with skin of fish were cut and homogenized in PBS [Phosphate Buffer Solution and pH is adjusted to 7.3-7.4] to 10 % (w/v) suspension. The homogenized tissue samples were serially diluted in PBS and plated on to Tryptic soy agar (DIFCO, Becton Dickson and Company, Sparks Md., USA) for total plate count (TPC) of bacteria

and pour plated on to mFC agar (DIFCO) for enumeration of faecal coliforms. The plates were incubated at 37°C for 24 h and at 44.5°C for 24h for TPC and coliforms respectively. The homogenized tissue samples were enriched in Alkaline peptone water (APW, DIFCO), EC broth and Tetrathionate broth at 37°C for 6-8, 18h and 24h for isolation of *Vibrio cholerae*, *E.coli* and *Salmonella* respectively. The enriched culture media were plated on to Thiosulfate Citrate Bile Salt Sucrose Agar (TCBS Difco), Eosin Methylene Blue agar (EMB agar, DIFCO) and Xylose Lysine Deoxycholate Agar (XLDA, DIFCO). respectively. Following incubation at 37°C for 24h, the presumptive *Vibrio cholerae*, *E.coli* and *Salmonella* were isolated in pure culture and identified by cultural and biochemical characters. The physicochemical properties of water samples collected from sewage fed ponds, beels and raw sewage were analyzed as per APHA[4]. Analyses of mean and Standard deviation (SD) were calculated using Excel2003 (Microsoft Corp. Seattle, W.A).

RESULTS

The microbial load of various micro-organisms in water and fish samples, collected from different sewage fed ponds and beels are given in Table 1. In the inlet point (W1) of sewage fed bheri and farm total microbial load were 96×10^6 and 86×10^6 cfu/g and total bacterial load in settling tank (W2) of the said water bodies were 65×10^6 and 45×10^6 cfu/g respectively. The total bacterial load in ponds (W3) of sewage fed bheries and farms varied from 38×10^5 to 52×10^5 cfu/g of fish. The total bacterial count in

fish skin of sewage fed bheri and farms varied in between 25×10^5 and 36×10^5 cfu/g whereas for beels varied in between 65×10^3 and 84×10^3 cfu/g. But in fish muscle of sewage fed bheri and farm total bacterial count were 11×10^5 and 18×10^5 cfu/g respectively and of fish muscle of beel fish samples the total bacterial load varied from 2.1×10^3 to 4×10^3 cfu/g. The faecal coliform count in the pond of sewage fed bheri and farm varied from 49×10^5 to 93×10^5 cfu/g. Screening of fishes grown in sewage fed ponds for the presence of different pathogenic bacteria are given in Table 2. *Vibrio* sp. and *Salmonella* sp. were found in the skin and gut of the fish exposed to sewage fed bheri and farm but they are not found in the fish muscle where as *E.coli* was found in the fish muscle of all samples

The details of physico-chemical parameters of sewage fed ponds and beels in comparison to raw sewage are given in Table 3. BOD(Biological Oxygen Demand) level in sewage fed Bheri had a range of variation between 30 and 37 mg/l and the BOD level was recorded in sewage fed farm, Rahara in the range between 26 and 32 mg/l whereas in raw sewage BOD values ranged from 195 to 200 mg/l. Similarly COD(Chemical Oxygen demand) values in sewage fed bheri and sewage fed farm were 58 to 65 mg/l and 52 to 57 mg/l. But in case of raw sewage COD values ranged from 345 to 350 mg/l. The Dissolved oxygen (D.O) was higher in freshwater beels (6.2-7.3 mg/l) as compared to sewage fed bheri and farms (3-3.6 and 3.1-4 mg/l). But in case of raw sewage D.O was recorded in the range of 1-1.5 mg/l indicating very low concentration compared to other water bodies.

Table 1: Estimation of Microbial load of various microorganisms in water (Per ml) and fish samples (Per gram) collected from different sewage fed ponds and beels

	Sewage fed bheri		Sewage fed fish farm		Kulia beel		Muthura Beel		Amda Beel	
	A	B	A	B	A	B	A	B	A	B
W1	95×10^6	280×10^5	86×10^6	254×10^5	—	—	—	—	—	—
W2	65×10^6	180×10^5	45×10^6	168×10^5	—	—	—	—	—	—
W3	52×10^5	93×10^5	38×10^5	49×10^5	48×10^5	63×10^4	22×10^5	73×10^4	15×10^5	26×10^4
Fish Skin	36×10^5	16×10^5	25×10^5	8×10^5	84×10^3	12×10^3	73×10^3	23×10^3	65×10^3	16×10^3
Fish Muscle	11×10^5	3×10^5	18×10^5	3×10^5	4×10^3	—	6×10^3	—	2.1×10^3	—

A = Total bacterial count and B = Faecal Coliform count CFU/g of fish

Table 2 Screening of fishes grown in sewage fed ponds for the presence of different pathogenic bacteria

Organism	Fish sample															Water sample				
	Skin					Muscle					Gut									
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
<i>Vibrio sp.</i>	+	+	+	–	–	–	–	–	–	–	+	+	+	–	–	+	+	+	–	–
<i>Salmonella sp.</i>	+	+	–	–	–	–	–	–	–	–	+	+	–	+	–	+	+	–	–	–
<i>E.coli</i>	+	+	–	+	–	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+

A- Sewage fed bheri B – Sewage fed fish farm Rahara C– Kulia Beel D– Mathura Beel E– Amda Beel

Table 3: Physio-chemical parameters of Sewage-fed ponds and Beels in comparison to raw sewage

Sl No.	Parameters	Sewage-fed Bheri	RaharaSewage fed pond	KuliaBeel	MathuraBeel	AmdaBeel	RawSewage
1.	BOD (mg/l)	33.8±2.86(30-37)	28.4±2.60(26-32)	4.52±0.75(3.6-5.5)	7.08±0.27(6.9-7.5)	3.17±0.54(2.48-3.9)	197.6±2.07(195-200)
2.	COD (mg/l)	61±2.82(58-65)	54.2±1.92(52-57)	7.8±1.19(6.6-9.5)	13.86±1.10(12.2-15.1)	7.36±0.78(6.2-8.1)	347.4±1.81(345-350)
3.	TSS (mg/l)	77.4±2.07(75-80)	76.8±1.92(74-79)	-	-	-	283.8±2.77(280-320)
4.	DO (mg/l)	3.3±0.24(3-3.6)	3.52±0.42(3.1-4)	6.9±0.41(6.2-7.3)	7.56±0.64(6.9-8.4)	6.4±0.48(5.8-6.9)	1.21±0.20(1-1.5)
5.	Temperature°C	29.54±0.45(29-30)	30.04±0.68(30-31)	28.48±0.47(28-29)	29.54±0.45(29-30)	30.02±0.80(29-31)	32.5±0.47(32-33)
6.	pH	8.06±0.06(8.05-8.2)	7.45±0.04(7.4-7.5)	7.46±0.27(7.5-7.7)	7.68±0.08(7.6-7.8)	8.02±0.08(7.9-8.1)	7.58±0.14(7.4-7.8)
7.	Nitrite (mg/l)	0.13±0.02(0.1-0.15)	0.10±0.01(0.1-0.12)	-	-	-	0.26±0.12(0.2-0.3)
8.	Ammonium (mg/l)	1.53±0.04(1.5-1.6)	1.46±0.09(1.3-1.5)	-	-	-	4.15±0.10(4-4.25)
9.	Phosphate (mg/l)	0.17±0.01(0.16-0.18)	0.10±0.01(0.10-0.12)	-	-	-	0.25±0.01(0.24-0.26)

Values are mean ± S.D.; Values in parentheses represent range

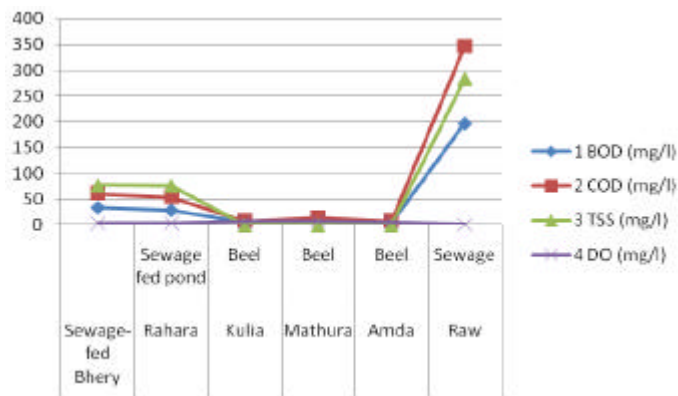


Fig. 1: Graphical representation of water quality parameters of various sewage fed bheries and beels in comparison to raw sewage

The values of pH, nitrite, ammonium and phosphate were also studied in sewage fed bheri, sewage fed farm and different beels and it was found that the pH ranged from 7.4 to 8.05 which indicated that there was no abrupt change in pH values. Similarly nitrite, ammonium, phosphate showed no mark variation during the study except in raw sewage and the range was in the increasing trend in comparison to beels and sewage fed bheri.

DISCUSSION

According to Buras *et al.* [2] total aerobic bacterial concentration in fish muscle in the range of 0-10, 10-30 and >50 bacteria/g indicated, very good, medium and unacceptable quality for human use. In the present study results were corroborated well with the findings of Nguyen *et al.* [3]. The current results indicated that these fish flesh qualities were satisfactory in terms of their faecal bacterial indicator counts and complied with the recommendations of the APHA [4] and the International Commission on Microbiological Specifications for Foods [5]. In the study it was observed that *Vibrio* sp and *Salmonella* sp were found in the skin and gut of fish exposed to the sewage fed bheri and sewage fed farm but they were absent in the fish muscle whereas *E.coli* was

present in the fish muscles of all samples. According to Buras *et al.* [1-2] faecal bacteria could penetrate the fish flesh when the fish is raised in highly polluted water. However the said bacteria were also present in the pond water of sewage fed bheri and farm. The organisms were potential entero pathogenic and any mishandling of the fish can lead to the transmission of the pathogen to humans. For human consumption, fish gut was not our main concern because during preparation of fish based products gut portion was removed though huge no of bacteria were present in the gut of fish. The majority of bacteria were washed off from the skin during cleaning with potable water. The presence of bacteria in muscle was a great importance for human health. In India, especially Bengalis eat fish by adding salt and turmeric powder and proper cooking (100°C) and frying in mustered oil at 180°C. The cooking and frying temperature kill most of the pathogens. Moreover, salt and turmeric powder have bactericidal effect.

In this study the pH of all water bodies were in alkaline side indicating suitability for pisci-culture. The pH of all freshwater beels fluctuated between 7.5 and 8.1 and for sewage fed bheries varied from 8 to 8.2. Similar observations were also recorded by Bhowmik *et al.* [6]. and Ghosh *et al.* [7]. respectively. The BOD values were recorded in the range from 30-37mg/l in sewage fed bheri

and 26-32 mg/l in Rahara sewage fed farm. The values were within limit for waste water aquaculture as Forsberg *et.al* [8] reported that the requirement of BOD concentration for aquaculture practices was <20mg/l. Whereas, Chattopadhyay *et.al.* [9] indicated 10-20 mg/l BOD as optimum range for fish culture in effluent and polluted water. COD gives valuable information about the pollution potential of sewage effluent to the aquatic system. In the present study, COD values varied from 58 to 65 mg/l in sewage fed bheries and 52-60 mg/l in Rahara Fish farm and values of TSS were 75-80 mg/l, 74-79 mg/l sewage fed bheries and Rahara farm respectively. Similar results were also reported by Bansal *et al.* [10]. In the present study the ammonium, nitrate and phosphate of Sewage fed bheries and Rahara sewage fed farm were found in the permissible limit for aquaculture. Similar observations were recorded by Bansal *et al.* [10]. From the result it was clear that dissolved oxygen concentration in all the fresh water beels were more than 4mg/l which was within limit the growth of fish. But DO levels were low in sewage fed Bheries due to pollutional load resulting from sewage disposal. During the study it was also observed that the temperature of all the water bodies fluctuated from 29 to 33°C which were in conformity with the findings of Bhowmik *et al.* [11].

Finally, it can be concluded that the microbiological quality of fish grown in sewage fed fishery has immense importance in relation to human health. There is a chance of zoonotic infection to human from fish which is raised in sewage fed farm. Accumulation of pathogenic bacteria is passively carried out on the surface of fish but they are rarely penetrated in to edible fish flesh or muscle. But in Bengal during fish preparation cooking and boiling temperature would eliminate the possibility of any pathogen transmission even if they are contaminated. However, further investigations involving microbiological quality of fishes which are raised in sewage fed farms for human consumption need more research.

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