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Bacteriological Investigation in Three Different Ponds of Irongmara, Cachar District, Assam

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The physico-chemical and Microbiological assessment in water samples of three ponds of village Irongmara, Cachar district, Assam, was carried out during pre and monsoon period (March, 2010 and May, 2010) which results decrease in the quality of water. The P^H, Total alkalinity and chloride contents of water samples was within limit as prescribed by WHO. Certain nutrients like nitrate and phosphate remained above the minimum level. The bacteriological investigation showed the presence of *E. coli* and other potentially pathogenic bacteria which may cause severe water borne diseases. The heterotrophic bacteria isolated from three ponds included *Pseudomonas, Proteus, E. coli, Citrobacter* and *Bacillus*. The observations have indicated that the water of these ponds is not safe for drinking without treatment.

Key words: Physico-chemical parameters, fecal coliforms, Irongmara, Assam.

Water is essential for life. It governs the evolution and functions of universe on the earth hence water is "mother of all living world". Majority of water available on the earth is saline, only small quantity exists as fresh water. Fresh water has become a scare commodity due to over exploitation and pollution (Gupta and Shukla, 2006).rural people in southern part of Assam use community ponds water for drinking, swimming or bathing and washing clothes. Village wastes and agricultural wastes are being continuously added to these water bodies affecting the physico-chemical quality of water making them unfit for use of human beings (Dwivedi and Pandey, 2002). In recent decades, increase in human population and uncontrolled domestic waste water discharge into the nearby water bodies have resulted in eutrophication of lakes, ponds as evidenced by substantial algal bloom, dissolved oxygen depletion and large fish kill (Pandey and Pandey, 2003). Contamination of water bodies might lead to a change in their trophic status and make them unsuitable for aquaculture. Hence, regular monitoring of physico-chemical and biological water quality parameters is essential to determine the status of water bodies. Irongmara village located near Assam University, Silchar and is undergoing transformation from traditional life style to modernity in a remote area. Since there is no good drinking water facility available here, people of this place depend on the ponds for bathing, washing clothes and sometimes these ponds also serve as drinking water sources. Therefore present study was undertaken to find out status of these pond waters.

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MATERIAL AND METHODS

The present study was carried out in three ponds used by the villagers for their daily activities which were located at Irongmara village near Assam University campus, Cachar district of Assam. The study was carried from March, 2010 to May, 2010. Water samples were collected separately from each spot in one liter sterile plastic bottles. P^H, dissolved oxygen, FCO_2^-, alkalinity

Table 1. Physico-chemical properties of different pond waters in the month of March, 2010

Ponds	Physico-chemical parameters							
	P^H	Alkalinity (mg/l)	FCO_2 (mg/l)	Nitrate (mg/l)	Phosphate (mg/l)	Chloride (mg/l)	Total Hardness (mg/l)	DO (mg/l)
Pond (1)	7.20±0.54	21.0±0.68	9.0±0.30	5.3±0.46	6.1± 0.64	40.0± 0.20	50.0±0.20	9.7± 0.05
Pond (2)	6.50±0.27	12.0±0.89	9.0±0.15	5.1±0.31	3.0± 0.47	39.0± 0.60	18.0±0.35	9.2±0.08
Pond (3)	7.35±0.56	14.0±1.05	2.0±0.43	2.3±0.35	3.4± 0.16	70.0± 0.63	35.0±0.20	6.4±0.05

Table 2. Physico-chemical properties of different pond waters in the month of May, 2010

Ponds	Physico-chemical parameters							
	P^H	Alkalinity (mg/l)	FCO_2 (mg/l)	Nitrate (mg/l)	Phosphate (mg/l)	Chloride (mg/l)	Total Hardness (mg/l)	DO (mg/l)
Pond (1)	7.48±0.49	10.0±0.73	21.0±0.68	10.2±0.005	7.2±0.03	30.0±0.20	65.0±0.42	5.5± 0.005
Pond (2)	6.53±0.42	6.0± 0.14	12.0±0.89	3.0±0.43	3.8±0.53	40.0±0.20	50.0±0.20	5.6±0.02
Pond (3)	7.58±0.73	23.0±0.37	14.0±1.05	5.0±0.61	8.1±0.04	50.0±0.20	91.0±0.35	4.8±0.05

Table 3. Total fecal coliform count (MPN/100 ml) of three ponds

Months (2010)	Pond (1)	Pond (2)	Pond (3)
March	110	31	140
May	170	31	280

Table 4. Total coliform count (cfu/ml) of three ponds

Months (2010)	Pond (1)	Pond (2)	Pond (3)
March	65×10^2	33×10^2	51×10^2
May	93×10^2	41×10^2	82×10^2

Table 5. Total aerobic bacterial load (cfu/ml) of three ponds

Months (2010)	Pond (1)	Pond (2)	Pond (3)
March	21×10^5	20×10^5	34×10^5
May	31×10^5	22×10^5	42×10^5

were measured employing methods described in APHA (1995). Nitrate, phosphate, chloride, hardness were measured using portable kit provided by HiMedia. The Average (mean ± SE) for each parameter was computed considering the values from three ponds. Serial dilutions of water samples were made for the isolation of bacteria. For the enumeration of total aerobic bacteria, nutrient agar plates were used while MacConkey agar was used for total coliform count. After inoculation of water samples into the plates the plates were incubated at 37°C for 24-48 hours.

Fecal coliform count

The Most Probable Number (MPN) technique using the three tube test with lactose broth was employed. Fermentation tubes were inoculated with 10 ml, 1ml, 0.1 ml aliquots of the sample in accordance with standard methods APHA (1995). The tubes were incubated at $37^{R''}$C for24h. Positive tubes producing acid and gas were used in estimating the presumptive MPN/100ml.

Confirmed test was carried out by transferring a loopful of broth from a positive tube into brilliant green lactose bile (BGLB) broth, followed by incubation at 37°C for 24-48 hrs. The tubes were observed for gas formation.

Completed test was performed by plating a loopful of broth from a positive BGLB tube onto an Eosine Methylene Blue (EMB) agar plates. The plates were incubated at 37°C for 24h and observed for dark red colonies and metallic green sheen. Stock cultures of the colonies were prepared on nutrient agar slants and colonies were used for gram staining and biochemical tests. Final fecal coliform or *E. coli* count as MPN/100 was calculated based on the completed test.

Identification of bacterial isolates

Cultural characteristics and biochemical tests used in the identification of bacterial isolates were as described by Treagan and Pulliam (1982). Identification was confirmed using Bergey's Manual of determinative Bacteriology (Holt *et al*, 1994).

RESULTS

The physico-chemical analysis of water from the three ponds is given in table (1 and 2). The P^H value was ranged from 6.30 to 7.58. Highest P^H of water was recorded in pond (3) during May,

Table 6. Confirmative biochemical characterization of bacterial isolates from water samples.

Cultural characteristics	Morphological characteristics	Biochemical parameters Gram stain	catalase	oxidase	urease	citrate	indole	Methyl red	v.p.	H_2S	motility	Sucrose	Glucose	Lactose	Maltose	Possible pathogen
Colonies were green, flat and smooth	Rods	-	+	+	-	-	-	+	-	-	+	A/-	A/-	A/-	A/-	*Pseudomonas sp.*
Flat ,irregular colonies	Rods appearing singly	-	+	-	+	-	+	+	-	+	+	A/G	A/G	-/-	A/G	*Proteus sp.*
Pink convex colonies with smooth edge	Rods	-	+	-	-	-	+	+	-	-	+	A/-	A/G	A/G	A/G	*E. coli*
Yellow smooth edge	Rods	-	+	-	-	+	+	+	-	+	+	A/-	A/G	-/-	-/-	*Citrobacter sp.*
Colonies were white entire and flat on nutrient agara	Bacilli	+	+	-	-	-	-	+	-	-	-	-	A/-	A/G	A/G	*Bacillus sp.*

+ = positive, A/G = Acid and gas production, G= gas production only, - = negative, A = Acid production only.

2010 and lowest value was recorded in pond (2) during March, 2010. Maximum alkalinity was recorded in pond (3) during May, 2010, whereas minimum in pond (2) during May, 2010. The FCO_2 value was observed maximum in pond (1) during May, 2010 and minimum was observed in pond (3) in the month of March, 2010. Nitrate level was high in pond (1) during May, 2010 while it was low in pond (3) during March, 2010. Phosphate content was recorded maximum in pond (3) during May, 2010 and minimum in pond (2) during March, 2010. Dissolved oxygen level was high in pond (1) and minimum in pond (3) during May, 2010 and March, 2010 respectively. Maximum level of hardness was recorded in pond (3) during May, 2010 and minimum in pond (2) during March, 2010. Chloride content was recorded maximum in pond (3) and minimum in pond (1) during March, 2010 and May, 2010 respectively.

Table 4 shows that the total coliform bacteria were in the range of 33×10^2 to 93×10^2 for all three ponds while fecal coliform were present in the range of 31 to 280 MPN per 100ml of water (Table 3). Table 5 shows that the total aerobic bacterial population was in the range of 20×10^5 to 42×10^5. The heterotrophic bacteria isolated from the three ponds are shown in table 6. They included species of *Pseudomonas*, *Proteus*, *E. coli*, *Citrobacter* and *Bacillus*

Table 7(a). Class limits values for microbial pollutions assessed by bacteriological standard parameters according to Kohl (1975, modified)

Classification of fecal pollution		class				
		A	B	C	D	E
Parameter	Fecal pollution	Little	Moderately	Critical	Strongly	excessively
E. coli (fecal coliforms *)	In 100 ml water	d”100	> 100-1000	>1000-10,000	>10,000-1,00,000	>100000
Intestinal enterococci	In 100 ml water	d”40	>40-400	>400-4000	>4000-40,000	>40000
Total coliform	In 100 ml water	d”500	>500-10,000	>10,000-1,00,000	>100000-1000 000	>1000000

*Fecal coliforms are mainly represented by *E. coli* as predominant species.

Table 7(b).

Classification of organic pollution		class				
		A	B	C	D	E
Heterotrophic plate count	In 1 ml water	≥500	> 500-10,000	>10,000-100,000	>100,000-7,50,000	>7,50,000

DISCUSSION

High value of P^H in all water samples of different ponds was recorded in the month of May (summer) which might be due to utilization of bicarbonate and carbonate buffer system (Bohra, 1976). However the P^H values were within highest desirable limit prescribed by WHO (6.5-8.5). Water nutrients like phosphate and nitrate, determines the productivity of the water body. More than 0.5 mg/l of phosphate is an indicator of water pollution (Jain *et al*, 1996). In our study, nitrate and phosphate values remained well above the minimum level indicating the pollution of these ponds. Alkalinity is an estimate of the ability of water to resist change in P^H upon addition of acids. Total alkalinity values recorded throughout the studied months were lower than levels required for fish culture. Higher

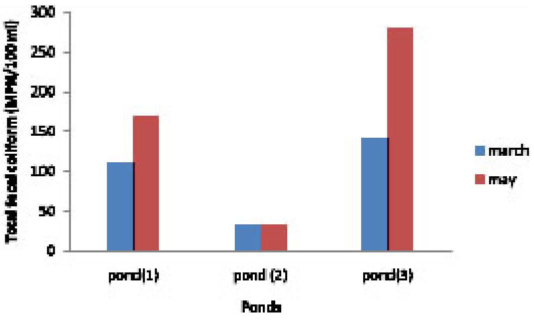

Fig. 1. Total fecal coliform count (MPN/100 ml) of three ponds during March 2010 and May 2010

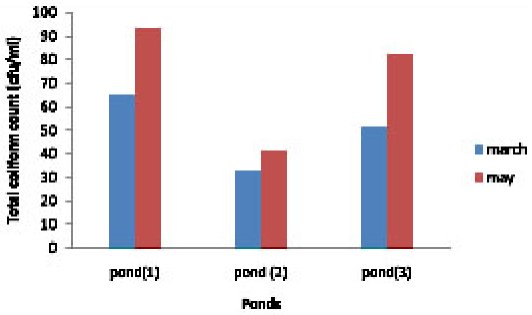

Fig. 2. Total coliform count (cfu/ml) of three ponds during March 2010 and May 2010

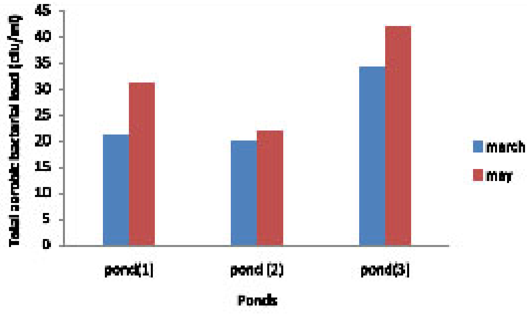

Fig. 3. Total aerobic bacterial load (cfu/ml) of three ponds during March 2010 and May 2010

concentration of chloride in water bodies may be due to organic wastes of animals or industrial origin and are also detrimental for irrigation and aquaculture (Rajkumar *et al*, 2004). The levels of chlorides in the present study ranged from 30 ± 0.20 to 70 ± 0.63 mg/l which were well within prescribed limit (250 mg/l). In the present study total hardness showed maximum value during May (summer), which might be due to reduced inflow of water and rate of evaporation. Dissolved oxygen content is one of the most important factors in aquatic ecosystem. DO values were maximum during March and minimum during May which might be due to higher algal productivity due to higher rate of photosynthesis (Rajkumar *et al*, 2004). Lower values of FCO_2 in water samples in the month of March, 2010 might be due to less suspended microbes on suspended particles and algal masses (Michael, 1984).

The presence of *E. coli* and other potentially pathogenic species may cause waterborne diseases in the local inhabitants. The number of fecal coliform was high during May, 2010 and low during March, 2010. High level of fecal pollution was found in pond (1) and pond (3) in both the periods. Both total and fecal coliforms exhibited more cfus during May (summer). Summer maxima might be due to discharging of domestic wastes containing fecal matters to the ponds. Lower values during March may be due to cold climatic condition, inhibiting the bacterial growth. Based on the Kohl model pond (1) and pond (3) has been classified as moderately polluted and pond (2) as little polluted pond.

CONCLUSION

Villagers of Irongmara use highly contaminated water by fecal coliform bacteria for their daily activities like bathing, washing clothes and not suitable for drinking purpose without pretreatment. These water bodies do not meet the WHO standard of zero fecal coliform per 100 ml. There is need to improve the quality of water through continuous monitoring of the pollution level of these ponds. Water treatment and awareness programs must be taken to educate local villagers to safeguard the precious water and protect them against water borne diseases.

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