Influence of water quality on the bacterial contamination of resident loach, *Lepidocephalichthys guntea* (Hamilton Buchanan) and on a Terai River Lotchka of Darjeeling District, West Bengal, India

Rudra Prasad Roy and Sudip Barat*

Aquaculture and Limnology Research Unit, Department of Zoology, University of North Bengal, District Darjeeling, West Bengal, 734013 India

**To whom correspondences should be addressed*

E-mail: sudipbarat@rediffmail.com

Received August 06, 2011, Revised manuscript received November 16, 2011, Accepted November 25, 2011

Abstract

A study was performed on the water quality parameters at four different sites of a Terai River Lotchka of Darjeeling district, West Bengal and its influence on the bacterial population of water and body parts of healthy fresh water loach, *Lepidocephalichthys guntea* (Hamilton Buchanan). The water quality results did not show much significant difference between the four sites. High heterotrophic bacterial loads were found in the water body averaging from $4.1\times10^6 \pm 0.8\times10^6$ to $5.5\times10^6 \pm 1\times10^6$ cfu ml^{-1} and in gut of fish $4.5\times10^6 \pm 1.8\times10^6$ cfu g^{-1}. Five different dominating bacterial genus namely *Aeromonas spp, Pseudomonas spp, Enterobacteriacea spp, Streptococcus spp* and *Salmonella spp* were observed in the fish and river water. The study revealed a strong relationship between the bacterial contamination of healthy fish, *Lepidocephalichthys guntea* (Hamilton Buchanan), and its surrounding environment.

Keywords: Water Quality; Bacteria; River Water; Loach

1. Introduction

Water quality parameters can influence the aquatic environment and are important for the survival of aquatic flora and fauna [1]. The entire array of aquatic life and water quality are affected by pollution. Human activities and anthropogenic pressures like industrial community waste disposal, heavy use of insecticides, pesticides and fertilizers in agricultural practices are major causes of aquatic system damage. As such, water body can be contaminated with toxic chemical and pathogenic bacterial population. Therefore, the surrounding aquatic environment plays an important role in bacterial composition of fishes. Physical and chemical water quality indicators are important for assessing and/or protecting ecosystem integrity. Without the knowledge of water quality, it is difficult to understand the biological phenomenon fully, because the chemistry of water reveals much about the metabolism of the ecosystem and explains the general hydrobiological inter-relationship. The physico-chemical parameters of water and the dependence of all life process of these factors make it desirable to take water as an environment. The correlations of different water quality parameters seemed to favor the growth of the bacteria and also indicate the pollution level in the water [2]. Increasingly, monitoring efforts are focused on a holistic approach which examines the interaction of all media within a single watershed [3]. The bacterial genus mainly belonging to *Aeromonas* spp, *Enterobacteriaceae spp*, *Pseudomonas spp*, and *Streptococcus spp* are mostly present in fresh water ecosystem [4]. Aquatic microflora is associated with physiology of fish [5]. There is an indication that the type of bacteria carried in the gastrointestinal tract of fish is related to a certain degree on the level of contamination of water by enteric bacteria. The majority of these bacteria are carried in the gastrointestinal tract and they are of primary significance as a source of occupational disease in fish handlers or consumers [6]. Many pathogenic bacteria coexist with their host without causing disease under natural condition. However, stress factors such as overcrowding found in aquaculture facilities are frequently blamed for the dissemination of infections and also for the occurrence of many disease outbreaks [7].

Not much information is available on the bacteriological aspects of *Lepidocephalicthys guntea*, a fresh water loach, widely distributed in the region of Northern India, Pakistan, Nepal, Myanmar, Thailand and Bangladesh [8] and also very common in rivers of Darjeeling District, West Bengal, India. This indigenous loach has both an ornamental [9] and edible value [10] and is easily available in the local markets [11]. Inhabitants are situated on either sides of the entire stretch of the river and there are run-offs such as agricultural contaminants, domestic and human sewage especially from the traditional toilets and seepage from septic tanks into the River Lotchka. The present investigation was therefore aimed at studying the water quality status of River Lotchka and its influence on the abundance and diversity of entero- pathogenic bacteria being harboured both in the water and resident fish, *Lepidocephalicthys guntea.*

2. Materials and Methods

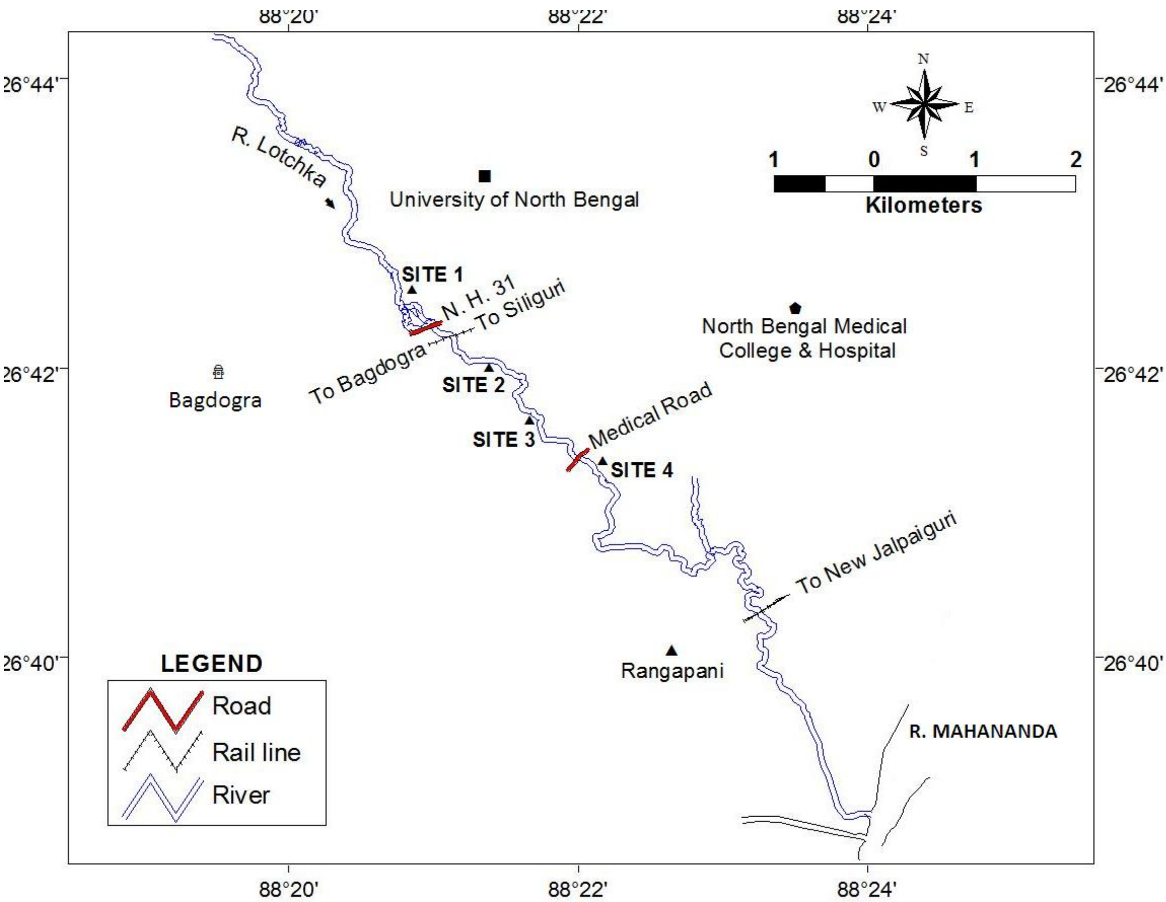
2.1. Study Area and Sampling

Water quality analysis and bacteriological study of water samples and live healthy loach, *Lepidocephalicthys guntea*, was conducted during the period December 2009 to November 2010 at four different sampling sites (S1, S2, S3, and S4) over the entire stretch of 18.2 kilometres River Lotchka of Darjeeling district, West Bengal, India located between the latitudes 26°42’39.71”N to 26°41’22.47”N and longitudes 88°20’54.78”E to 88º22’00.20”E (Fig. 1). S1 was situated on the west side of the University tea garden and received agricultural contaminants. S2 and S3 were contaminated by run-offs such as domestic and human sewage because of the inhabitants and pig farms located on either side of these two sites. S4 was located near the cremation site and agricultural farms. Water samples and live healthy loach, *Lepidocephalicthys guntea*, were collected from the river at monthly interval during the study period.

2.2. Water Quality Parameters

Water quality parameters like dissolved oxygen, free carbon dioxide, total alkalinity, total hardness, chloride, ammonium-N, nitrite-N, nitrate-N and phosphate-P were selected and determined following the method of APHA [12]. Temperature (in degree Celsius) was recorded by a mercury thermometer and pH using portable pH meter (Hanna Instrument Pvt. Ltd).

Figure 1. Study area map of River Lotchka.



2.3. Bacterial Enumeration and Identification

Freshly caught live healthy fishes, *Lepidocephalicthys guntea*, from the River Lotchka were brought to the laboratory in sterile plastic bags containing the river water and processed within 2 hours of the collection. Through dissection, skin, gill and gut samples of each fish were removed aseptically and collected separately in sterile containers. The organs were then homogenised in a sterile glass tissue homogenizer. Serial dilutions were prepared in sterile physiological saline and plated onto Nutrient Agar and different selective media namely, MacConkey Agar, *Pseudomonas* Agar Base, *Streptococcus* Selection Agar, *Aeromonas* Starch DNA Agar Base and Bismuth Sulphite Agar for *Salmonella spp* isolation (HiMedia laboratories Ltd, Mumbai, India) for colony count and recovery of the selective organisms using Spread Plate Technique [13]. All the plates were incubated at 37±1°C for 24–48 hours. Water samples for bacteriological analysis were collected in sterile glass bottles and subjected to serial dilution procedure as followed for the fish sample. After incubation, the selective media plates were examined and the growth of colonies on the plates were recorded for count of heterotrophic bacteria, total coliform and different entero-pathogenic bacterial genus. Growing colonies of selective media were followed by the streaking method for isolation of pure cultures. The bacterial isolates were then used for observation of cell morphology followed by Gram Staining procedure. Biochemical tests were then performed involving catalase test, IMvic tests, utilization of Simmon Citrate, acid and gas production from different carbohydrates, following the criteria as described in the Manual for the Identification of Medical Bacteria [14] and Bergey's Manual of Systematic Bacteriology [15]. Bacterial identification kits (Himedia laboratories Ltd, Mumbai, India) were also used for verification of bacteria.

2.4. Statistical Method

To verify the significance of the results of water quality and bacteriological study, statistical analysis namely, standard deviation, Karl Pearson correlation coefficient and ANOVA (one-way) were performed using SPSS software.

3. Results and Discussion

The Mean ± Standard Deviation values of selected water quality parameters during the period of sampling are summarized in Table 1. No significant variation was observed in the parameters between the four different sampling sites S1 to S4 of the river. Temperature of water at the four different sampling sites (S1, S2, S3, and S4) ranged between 26.2 ± 5.0°C to 27.0 ± 4.6°C. This temperature range of water showed appropriate condition for the higher growth of mesophilic bacteria [16]. There was a positive correlation between temperature and heterotrophic bacterial population ($r = 0.588$; $P \leq 0.001$) in the river. The average pH of water ranged between 6.8 ± 0.1 to 6.9 ± 0.3 showing slightly acidic condition. This is in conformity with other water bodies in Darjeeling district of West Bengal which are acidic in nature [17]. The dissolved oxygen content at the four sites of river water showed similar trend, irrespective of the site of sample collection. Dissolved oxygen at the four different sites ranged between 3.7 ± 1.36 to 4.1 ± 1.66 mg l^{-1}. The value of dissolved oxygen is remarkable in determining the water quality criteria of an aquatic system. With dissolved oxygen ranging between 1-5 mg l^{-1} the fish can survive, but reproduction is poor and growth is slow if exposure is continued [18]. However, when dissolved oxygen concentration is less than 5.0 mg l^{-1}, it is not considered conducive for fish growth [19]. The low values of dissolved oxygen obtained in the present study shows high rates of respiration and organic decomposition. Further, the low dissolved oxygen level could also lead to eutrophication. The addition of a variety of biodegradable pollutants from domestic and industrial sources also stimulates the growth of microorganisms, which consumes the dissolved oxygen [20]. The average free carbon dioxide in the water ranged between 12.1± 5.8 mg l^{-1} and 15.0 ± 13.0 mg l^{-1} and was present throughout the period of study as the pH remained acidic. This high concentration of free carbon dioxide may be attributed to the inflow of organic wastes in the water body from surrounding areas of the river. Total alkalinity in the form of bicarbonate alkalinity was only recorded and ranged between 17.1 ± 7.3 to 18.6 ± 8.5 mg l^{-1} indicating carbonate alkalinity was absent in the water body. pH with values greater than 8.2

contain carbonate alkalinity [21]. In the present study pH remained below 8.2. Bicarbonate alkalinity was also recorded in the River Mahanada near Siliguri city [22]. The chloride concentration in river water plays a good indicator ion for pollution sources as well as organic waste of animal origin [23]. There is a relation between pollution level and chloride concentration in river water [24]. In the present study, chloride concentration in the water samples averaged between 19.3 ± 3.67 to 22.3 ± 3.8 mg l^{-1} thereby indicating that the concentration falls within the permissible limit of 250 mg l^{-1} [25]. Total hardness varied from 61.8 ± 7.2 to 67.8 ± 11.1 mg l^{-1}. Water with hardness value less than 60 mg l^{-1} is soft [26], as such the river water may be considered as hard. Presence of ammonium –N (NH_4-N) acts as an evidence of sewage inflow to a water body [27]. Moreover, [28] opined that the metabolism of the water body can be considered an index of environmental stress. Concentration of ammonium-N varied from 0.058 ± 0.02 to 0.100 ± 0.04 mg l^{-1}. It is observed, that the level of ammonium-N is positively correlated with heterotrophic bacterial count ($r = 0.332$; $P \leq 0.05$). Since, many heterotrophic bacteria utilize organic-N rich substrate to release ammonium and ammonium salts, it is apparent, that the rate of ammonium in the environment was determined by the heterotrophic bacterial flora. Similar observation was made by [29]. Nitrite-N (NO_2-N) averaged between 0.107 ± 0.05 mg l^{-1} and 0.142 ± 0.08 mg l^{-1} and showed higher value than permissible limit [30]. This indicates that water body was not good for aquatic animal health. Positive correlation was found between NO_2-N and heterotrophic bacterial load ($r = 0.419$; $P \leq 0.01$) of water body. The nitrate-N (NO_3-N) is one of the most oxidisable forms of nitrogen and is essential for plant nutrient and also associated with sewage and sullage discharge [22]. NO_3-N concentration in the present study varied from 0.144 ± 0.08 to 0.336 ± 0.30 mg l^{-1}. Usually Nitrate-N levels in excess of 0.2 mg l^{-1} show possible eutrophic conditions [30]. Thus, the water of the river may be considered to be eutrophic. Phosphate-P averaged between 0.295 ± 0.20 mg l^{-1} and 0.404 ± 0.28 mg l^{-1} in the four different sites and was greater than the standard limit 0.02 mg l^{-1} [30] thereby, indicating eutrophication of the river. The environmental significance of phosphorus arises out of its role as a major nutrient for both plants and microorganisms [31]. Nutrients that contribute to eutrophication are most commonly phosphorus that can be considered as a pollutant if it is present at a higher concentration than the normal limit [32]. The high concentration of nutrients in the four sites of River Lotchka indicated that the river received huge amount of sewage and waste from the surface run-off and effluent discharging from surrounding inhabitant and agriculture field.

Table 1. Mean ± standard deviation values of selected water quality parameters of four sites of River Lotchka.

Parameters	S1	S2	S3	S4
Temperature (°C)	27.00 ± 4.60	26.75 ± 5.20	26.25 ± 4.90	26.91 ± 4.70
pH	6.83 ± 0.10	6.85 ± 0.20	6.89 ± 0.30	6.85 ± 0.30
Dissolved oxygen (mg l^{-1})	4.15 ± 1.66	4.08 ± 1.25	3.88 ± 1.42	3.68 ± 1.36
Free carbondioxide (mg l^{-1})	12.16 ± 5.84	13.33 ± 6.87	13.58 ± 6.63	15.00 ± 13.04
Total alkalinity (mg l^{-1})	18.16 ± 9.23	17.50 ± 7.58	17.16 ± 7.27	18.58 ± 8.50
Total hardness (mg l^{-1})	61.83 ± 7.20	64.00 ± 9.18	66.00 ± 9.02	67.83 ± 11.13
Chloride (mg l^{-1})	19.33 ± 3.67	20.25 ± 3.91	20.50 ± 5.03	22.33 ± 3.86
Ammonium-N (mg l^{-1})	0.05 ± 0.02	0.05 ± 0.02	0.09 ± 0.04	0.10 ± 0.04
Nitrite-N (mg l^{-1})	0.11 ± 0.05	0.11 ± 0.05	0.13 ± 0.05	0.14 ± 0.08
Nitrate-N (mg l^{-1})	0.14 ± 0.08	0.21 ± 0.07	0.24 ± 0.12	0.33 ± 0.30
Phosphate (mg l^{-1})	0.30 ± 0.15	0.29 ± 0.20	0.40 ± 0.28	0.39 ± 0.22

Heterotrophic bacteria play an important role in altering water quality and leads to the transformation of organic matter in the water column [29]. The heterotrophic bacterial count in the four sites of river ranged from $4.1 \times 10^6 \pm 0.8 \times 10^6$ to $5.5 \times 10^6 \pm 1 \times 10^6$ cfu ml l^{-1}. This probably indicates, that the luxuriant growth of heterotrophic bacteria in the river is the outcome of the influx of waste organic matter from the surrounding areas. The total coliform counts in water recorded ranged from $1.5\times10^6 \pm 1.5\times10^6$ to $2.0\times10^6 \pm1.8\times10^6$ cfu ml^{-1} and count of different bacterial genus are presented in Table 2. Presence of coliform organisms in water is regarded as evidence of pathogenic bacterial contamination in water body. This clearly suggests, that the bacterial contamination in the water body is chiefly caused by surrounding effluents. The major water quality parameters were highly correlated with heterotrophic bacteria load (Table 3), indicating that the water quality parameters provided favourable condition for bacterial growth in the river water. Physico-chemical constituents of River Damodar provided favourable microhabitat for the growth of bacteria [2].

Table 2. Mean ± standard deviation values of bacterial count (cfu ml^{-1}) of four sites of River Lotchka.

Sites	Heterotrophic Bacteria ($\times10^6$)	Total Coliform ($\times10^6$)	*Pseudomonas spp* ($\times10^6$)	*Streptococcus spp* ($\times10^6$)	*Aeromonas spp* ($\times10^6$)	*Salmonella spp* ($\times10^4$)
S1	4.1 ± 0.8	1.5 ± 1.5	2.5 ± 1.6	1.9 ± 1.5	2.2 ± 1.4	0.04 ± 0.1
S2	4.9 ± 1.1	1.8 ± 1.6	2.8 ± 1.6	2.4 ± 1.5	2.1 ± 1.5	0.06 ± 0.2
S3	5.2 ± 1.2	1.9 ± 1.7	3.1 ± 1.7	2.5 ± 1.6	2.3 ± 1.6	1.3 ± 7.7
S4	5.5 ± 1.0	2.0 ± 1.8	3.4 ± 1.8	2.7 ± 1.7	2.6 ± 1.6	8.8 ± 5.3

Table 3. Correlation and coefficient values of selected water quality parameters and heterotrophic bacteria counts of four sites of River Lochka. Asterisks show significant values (*P < 0.05, **P < 0.01, ***P < 0.001).

Parameters	T	pH	DO	NH_4-N	NO_2-N	NO_3-N	PO_4-P	HB
T	1	-0.377**	0.419**	0.376**	0.072	0.035	0.519***	0.588***
pH		1	0.152	0.276	0.003	0.168	0.275	0.115
DO			1	0.110	0.119	0.110	0.083	0.458**
NH_4-N				1	0.169	0.306*	0.172	0.332*
NO_2-N					1	0.163	-0.147	0.419**
NO_3-N						1	0.214	0.267
PO_4-P							1	0.447**
HB								1

T: Temperature; DO: dissolved oxygen; HB: heterotrophic bacteria

The heterotrophic bacterial count, total coliform count and count of different bacterial genus found in fish are represented in Table 4. Results show that bacterial counts in both river water and fish are relatively higher, this indicates that bacterial load in fish is the reflection of environment. Higher bacterial counts were also observed by earlier workers in river water and fish organs [33]. The maximum counts of bacteria in the above category; heterotrophic bacteria $4.5\times10^6 \pm 1.8\times10^6$ cfu g^{-1} and total coliform $2.2\times10^6 \pm 2.5\times10^6$ cfu g^{-1} has been found to be present in the gut followed by gill and skin in *Lepidocephalicthys guntea*. Highest count of *Aeromonas spp, Pseudomonas spp* and *Streptococcus spp* were also observed in the gut than gill and skin (Table 4). The intestinal bacterial load of fresh water healthy Tilapia in Saudi Arabia was higher than gill filaments. The presence of a high bacterial load in intestine of fish might be due to high metabolic activity of fish associated with increased feeding rates of organic matter at higher temperatures [34].

Random colonies of selective medium were isolated to confirm the above mentioned bacterial genus through morphological and biochemical procedures as represented in Table 5. These different bacterial genus are opportunistic pathogens and may have risk on environment. It is reported earlier that similar bacterial genus were dominant in the intestine of river water fish and also in river and pond water as well [35,36]. The leading genus belonging to

Pseudomonas, *Aeromonas*, *Enterobacteriaceae* and *Streptococcus* are also found in the internal organ of fourteen different fishes of River Congonhas in Brazil [36]. Several of these bacteria are opportunistic pathogens and may cause diseases when the fish are under unfavourable condition. The genera *Pseudomonas*, *Aeromonas* and *Vibrio* are commonly isolated from normal healthy fish, but only certain strains of these bacteria possess the virulence factors necessary to induce disease. The incidence of virulence factors in strains associated with populations of healthy fish could be determined for epidemiological purposes [37]. The investigation, therefore, clearly indicates that opportunistic pathogenic bacterial population increases down the stretch of the river with deteriorating water quality, thus indicating human activity and anthropogenic pressures to be contributing to pollution of River Lotchka. Lotic aquatic ecosystem revealed, bacterial population flourishes with release of organic wastes. As bacterial load increases dissolved oxygen level declines, causing trouble for fish and other aquatic organisms by either inciting fish to migrate to new environment or result in fish kills [38].

Table 4. Mean ± standard deviation values of bacterial count (cfu g^{-1}) of fish, *Lepidocephalicthys guntea*, collected from River Lotchka.

Sources	Heterotrophic Bacteria ($\times 10^6$)	Total Coliform ($\times 10^6$)	*Pseudomonas spp* ($\times 10^6$)	*Streptococcus spp* ($\times 10^6$)	*Aeromonas spp* ($\times 10^6$)	*Salmonella spp* ($\times 10^4$)
Skin	3.2 ± 1.5	2.0 ± 2.2	1.5 ± 1.7	1.2 ± 1.5	1.7 ± 1.8	1.4 ± 0.5
Gill	4.1 ± 1.6	2.0 ± 2.3	2.0 ± 2.1	1.4 ± 1.8	2.1 ± 2.2	1.9 ± 0.6
Gut	4.5 ± 1.8	2.2 ± 2.5	2.2 ± 2.3	1.6 ± 1.9	2.3 ± 2.4	2.2 ± 0.8

Table 5. Results of biochemical characterization of bacterial isolates from four sites of River Lotchka and fish, *Lepidocephalicthys guntea.*

Biochemical characteristics	*Pseudomonas sp*	*Aeromonas sp*	*Streptococcus sp*	*Salmonella sp*	*Enterobacteriaceae sp*
Gram stain	-ve	-ve	+ve	-ve	-ve
MR	nd	-ve	+ve	+ve	-ve
VP	nd	+ve	-ve	-ve	+ve
Indole	nd	+ve	nd	-ve	-ve
Aesculine hydrolysis	nd	+ve	-ve	nd	nd
Arginine utilization	nd	+ve	+ve	nd	nd
Citrate utilization	+ve	nd	nd	+ve	+ve
Catalase	-ve	nd	nd	nd	nd
Urease	-ve	nd	nd	nd	nd
Glucose	+ve	+ve	+ve	+ve	+ve
Lactose	+ve	nd	+ve	-ve	+ve
Sorbitol	+ve	nd	-ve	+ve	+ve
Mannitol	nd	nd	-ve	+ve	+ve
Raffinose	nd	nd	-ve	+ve	+ve
Sucrose	nd	+ve	+ve	-ve	+ve
Salicin	nd	+ve	nd	nd	nd
Maltose	nd	+ve	nd	nd	+ve
Arabinose	+ve	-ve	-ve	+ve	+ve
Inositol	nd	+ve	nd	nd	nd

nd: not done; +ve: positive; -ve: negative

4. Conclusion

The present study reveals that the river water was probably in eutrophic condition due to loss of essential nutrients and high bacterial loads in water which has ultimately influenced greater harbour of entero-pathogenic bacteria in resident fish, *Lepidocephalicthys guntea.*

It is recommended for River Lotchka to remain self-sustaining and allowing of conservation of endemic fish, *Lepidocephalicthys guntea*, the release of anthropogenic substances and human activities should be controlled.

Acknowledgements

This investigation was carried by the award of a Fellowship sponsored by the University Grants Commission, New Delhi, India to the first author. The authors are thankful to Dr. C. R. Nayak Computer Centre, University of North Bengal for his help in statistical analysis. First author is also thankful to Dr. Prithwiraj Jha, Lecturer, Department of Zoology, Surendranath College, Raiganj and Miss Ruksa Nur, Research Scholar, Aquaculture and Limnology Research Unit, University of North Bengal for their time to time assistance.

References

[1] Deekae SN, Abowei JFN, Alfred-Ockiya JF (2010) Seasonal variation of some physical and chemical parameters of Luubara Creek, Ogoni Land, Niger Delta, Nigeria. *Res J Environ Earth Sc* 2(4):208-215.

[2] Chatterjee SK, Bhattacharjee I, Chandra G (2010) Water quality assessment near an industrial site of Damodar River, India. *Environ Monit Assess* 161:177-189.

[3] Adedokun OA, Adeyemo OK, Adeleye E (2008) Seasonal limnological variation and nutrient load of the river system in Ibadan Metropolis, Nigeria. *European J Sc Res* 23(1):98-108.

[4] Toranzo AE, Baya AM, Romalde JL, Hetrick FM (1989) Association of *Aeromonas sobria* with mortalities of adult gizzard shad, *Dorosoma cepedianum* Lesueur. *J. Fish Dis* 12:439-448.

[5] Al-Harbi AH (2003) Faecal coliforms in pond water, sediments and hybrid tilapia (*Oreochromis niloticus* X *Oreochromis aureus*) in Saudi Arabia. *Aquat Res* 34:517-524.

[6] Apun K, Yusof AM, Jugang K (1999) Distribution of bacteria in tropical freshwater fish and ponds. Int *J Environ Health Res* 9:285-292.

[7] Mcvicar AH (1997) Interactions of pathogens in aquaculture with wild fish populations. *Bull. Eur Ass Fish Pathol* 17(6):197-200.

[8] Talwar PK, Jhingran AG (1991) Inland Fishes of India and Adjacent Countries, Vol. I, Oxford & IBH Publishing Co. Pvt. Ltd. New Delhi, Calcutta.

[9] Panigrahi AK, Dutta S, Ghosh I (2009) Selective study on the availability of indigenous fish species having ornamental value in some districts of West Bengal. *Sus Aqua* 14(4):13-15.

[10] Mondal D, Barat S, Mukhopadhyay MK (2007) Toxicity of neem pesticide on a fresh water loach, *Lepidocephalichthys guntea* (Hamilton Buchanan) of Darjeeling district in West Bengal. *J Environ Biol* 28(1):119-122.

[11] Jha P, Roy RP, Barat S (2010) Application of sensory and microbial analysis to assess quality of fish in Siliguri city of West Bengal, India. *J Environ Biol* 31:587-594.

[12] APHA (2005) Standard methods for the examination of the water and wastewater, 21st edition. American Public Health Association, Washington D.C.

[13] Okpokwasili GC, Alapiki AM (1990). Bacterial flora associated with a Nigerian freshwater fish culture. *J. Aquac. Trop* 5:87-90.

[14] Barrow GI, Feltham RKA (1993) Manual for the identification of medical bacteria, 3rd edition. Cambridge University press, Great Britain.

[15] Holt JG, Krieg NR, Sneath PHA, Williams ST (Eds.), (1994) Bergey's Manual of Determinative Bacteriology, 9th edition. Williams and Wilkins, Baltimore.

[16] Rheinheimer G (1985) Aquatic Microbiology, 3rd ed. University of Kiel, West Germany. John Wiley and Sons, Chichester, New York, Brisbane, Toronto.

[17] Chakraborty D (1998) A study on the water and sediment quality as well as macropopulation of a natural hill River in the

Darjeeling hill. *Indian J. Env and Ecoplan* 1:69-72.

[18] Swingle HS (1967) Standardization of chemical analysis of water and pond muds. FAO Fish Rep 4:396-421.

[19] Mondal D, Barat S (2004) Effect of varying stocking density of sword tail (*Xiphophorous helleri* Heckel) on the water quality and growth rate. *J Inland Fish Soc Ind* 36(2):25-30.

[20] Moundiotiya C, Sisodia R, Kulshreshtha M, Bhatia A L (2004) A case study of the Jamwa Ramgarh wetland with special reference to physico-chemical properties of water and its environs. *J Environ Hydrol* 12:24.

[21] Trivedy RK, Goel PK (1984) Chemical and biological methods for water pollution studies. Environmental publications, Karad, India.

[22] Barat S, Jha P (2002) Changes in the water quality and total coliform bacterial load in a stretch of river Mahananda at Siliguri city, West Bengal, India. *Asian J Microbiol Biotech Env*. Sc 4(4):571-575.

[23] Kegley SE and Andreus J (1997). The chemistry of water University Science Books. California.

[24] Munawar M (1970) A limnological study of fresh water ponds of Hyderabad, India. 1-The biotope. *Hydrobiol* 35:127-162.

[25] WHO (2003) The World Health Report 2003: Shaping the Future, World Health Organization, 1211 Geneva 27, Switzerland.

[26] Kannan K (1991) Fundamentals of environmental pollution. S. Chand and Co. Ltd, New Delhi, India.

[27] Saksena DN, Garg RK, Rao RJ (2008) Water quality and pollution status of Chambal river National Chambal sanctuary, Madhya Pradesh. J Environ Biol 29(5):701-710.

[28] Jana BB, Chakrabarti R (1993) Life table responses of zooplankton (*Moina micrura* King and *Daphnia carinata* King) to manure application in a culture system. Aquaculture 117:274-285.

[29] Jana BB, Barat S (1983) Development of heterotrophic and ammonifying bacterial populations as affected by the fish *Clarias bactrachus* (Linn.) under different experimental conditions. *Acta Hydrochim et Hydrobiol* 11(5):569-576.

[30] WHO (1992) Water quality assessments: a guide to use of biota, sediments and water in environmental monitoring, 2nd edition, WHO, Geneva.

[31] Vanloon GW, Duffy SJ (2000) Environmental chemistry. A Global Perspective, Oxford, New York.

[32] Saad MAH (1973) Distribution of phosphate in lake Mariut, a heavily polluted lake in Egypt. *Water air and soil poll* 2:515.

[33] Sugita H, Oshima K, Tamura M, Deguchi Y (1983) Bacterial flora in the gastrointestine in the freshwater fishes in the river. Bull. *Japanese Soc Sci Fish* 49(9):1387-1395.

[34] Al-Harbi AH, Uddin N (2004) Seasonal variation in the intestinal bacterial flora of hybrid tilapia (*Oreochromis niloticus* X *O. aureus*) cultured in earthen ponds in Saudi Arabia. *Aquaculture* 229:37-44.

[35] Al-Harbi AH, Uddin N (2005) Bacterial diversity of tilapia (*Oreochromis niloticus*) cultured in brackish water in Saudi Arabia. *Aquaculture* 250:566-572.

[36] Sousa JAD, Silva-Souza AT (2001) Bacterial community associated with fish and water from Congonhas River in Sertaneja, Parana, Brazil. *Brazilian archives of biology and technology* 44:373-381.

[37] Cahill MM (1990) Bacterial flora of fishes: a review. *Microbiol Ecol* 19:21-41.

[38] Chiras DD (2010) Environmental Science, 8^{th} edition. Jones and Bartlett India Pvt. Ltd, New Delhi.