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Wastewater Influencing Pathogenic Bacterial Population and Biochemical Alterations in Fish

J. N. Bhakta* and B. B. Jana
Aquaculture and Applied Limnology Research Unit,
Department of Zoology, University of Kalyani,
Kalyani-741 235, West Bengal, India,

Abstract : Experiment was conducted in sewage-fed ponds to ascertain the responses of bacterial populations and biochemical alterations in fish cultured under different stress conditions of two sewage strengths. Rohu (*Labio rohita*, 60 ±12 g) was introduced in facultative pond-1 and stocking pond-4 at the rate of 10,000 fish ha^{-1} and reared for 120 days. Water surface sediment and fish samples were collected for examination of bacterial population; biochemical and water quality analysis were made at biweekly intervals. Growth of fish was recorded at regular intervals. Load of pathogenic bacteria (Coliform and *Vibrio sp.*) was higher in facultative pond-1 than that of stocking pond-4. 41 to 100%, 43 to 96% and 25 to 105% greater value of protein, DNA and RNA were observed, respectively, in the fish of stocking pond-4 than those of the facultative pond-1. It clearly implies that fishes of facultative pond-1 must expend more energy, which is used to synthesize structural or functional proteins and biotransformer to resist the stressor's effects on homeostasis that presents in more quantity in facultative pond-1 than that of the stocking pond-4. Therefore, it can be concluded that the concentration of DNA and RNA and DNA/RNA ratio may be considered as indicators of fish growth, which is influenced by various stressors of sewage water.

Key words : Strength of sewage, stressor, fish, bacterial population, biochemical changes.

Introduction :

Sewage would become a resource or pollutant depending upon the state of treatment and their use (Okun, 2002). One of the characteristic features of raw sewage is that it contains various microorganisms like bacteria, viruses, protozoan cysts, yeasts and other moulds, algae, eggs of helminths etc. (Voznaya, 1981; Nwadiuto and Olu, 1985; Shiaris, 1985; Shashikant and Raina, 1990). Elements such as Zn, Cd, Cu, Pb, Cr, Hg, Ag and Ni are well known heavy metals; pesticides and insecticides are also present in the sewage (Leenheer *et al*., 2001). Bacterial groups reported to be capable of increasing rate of organic matter decomposition and reduction of nitrite-nitrate, ammonia and phosphorus concentrations of sewage (Boyd *et al*., 1984). Utilization of heavy loaded toxic substances containing wastewater in aquaculture poses a great threat to the aquatic organisms especially to fish species. Organisms living under polluted environments can somewhat adapt to the situation by altering their functional responses at cellular level. In maintaining homeostasis, aquatic organisms effectively compensate the changes in their biochemistry and physiology caused by the exposure to stressors. Though, the stressors available in wastewater hamper the fish growth but protein and DNA/RNA ratio alterations in fish are not clear. The present study has been attempted to ascertain the responses of coliform and *Vibrio sp*. bacterial population and alteration of protein content and DNA/RNA ratio in fish

* **Corresponding author :** J. N. Bhakta; E-mail : lsnjbhakta@rediffmail.com

cultured under different stress conditions of two sewage strengths.

Materials and methods :

Experiment was performed in a sewage-fed farm located in Kalyani township (22º58" N latitude and 88º26-58 E longitude) consisting six ponds [two facultative ponds (FP-1 and FP-2) and four stocking ponds (SP-1, 2, 3 and 4)] laid in a series. There occurs a distinct gradient in the strength of sewage effluents passing through a series of this fish ponds varying in environmental stress which tended to reduce gradually as a direct function of the distance from the source of effluent input. The distance between FP-1 and SP-4 is 300 m. Rohu (*Labio rohita*, 60 ± 12 g) was procured from a local fish farm, acclimatized for a week and introduced in two ponds (FP-1 and SP-4) at the rate of 10,000 fish ha^{-1} and reared for 120 days. No supplementary feed was provided during the culture period.

Water, surface sediment and gut content of fish samples were collected aseptically at a fixed hour of the day (09.00 h) at regular intervals for microbial examinations. Aliquots of ten-fold dilution 10^{-2} to 10^{-3} for water and 10^{-3} to 10^{-4} for sediment and gut content samples were made in sterilized distilled water. Conventional spread plate technique under aerobic conditions was used to enumerate viable counts of coliform bacteria and *Vibrio sp.*, following the methods described by Rodina (1972) and Austin (1990) at an incubation temperature of 35° C for three days. Each dilution of the sample was plated in triplicate and arithmetical means of the three petri plates was used in the present study. Parallely, water quality parameters (temperature, pH, orthophosphate and ammonium-N) were also monitored following the standard methods of APHA (1995). Changes in growth of test fish were determined over the time. Total protein content was quantified with standard method bovine serum albumin (Lowry *et al.*, 1951). Quantitative analysis of DNA and RNA was done according to procedure described by Cherry (1962).

All data were statistically analyzed using one-way ANOVA. If the main effect was found significant, the ANOVA was followed by a LSD (least significance difference) test. All statistical tests were performed at a 5% probability level using statistical package EASE and M-STAT.

Results :

Bacterial population : The counts of coliform varied from 65 to 180 x 10^3 ml^{-1} in water and 60 to 178 × 10^4 g^{-1} in soil. The mean count showed 170% and 192% higher value in water and soil of FP-1, respectively over SP-4 (Fig. 1a). In gut content, the number of coliform ranged from 42 to 68 × 10^4 g^{-1}. Fish of FP-1 contained 50% higher number of coliform in the gut than that of SP-4.

The number of *Vibrio sp.* ranged from 30 to 196 × 10^2 ml^{-1} and 40 – 180 × 10^3 g^{-1} in water and soil, respectively. A significant count difference was observed in both FP-1 and SP-4 (ANOVA, $P < 0.05$). The mean count of *Vibrio sp.* in FP-1 was 480% (in water) and 285% (in soil) higher than SP-4 (Fig. 1b). In the gut content of fish, the counts of *Vibrio sp*. showed a clear-cut difference and ranging from 55 to 116 × 10^3 g^{-1} in FP-1 and 13 to 28 × 10^3 g^{-1} in SP-4.

Total protein : The total protein content varied from 6.85 to 17.25 mg g^{-1} in FP-1 and 12.65 to 28.10 mg g^{-1} in SP-4. The mean value of total protein in the fish of SD-4 was 41 to 100% higher than that of the FP-1 (Fig. 2a).

DNA and RNA : There was a marked difference in the concentration of DNA and RNA of fish cultured in FP-1 and SP-4 (ANOVA, $P < 0.05$). The values ranged from 0.215 to 4.610 mg g^{-1} and 0.176 to 1.985 mg g^{-1} in the concentration of DNA and RNA, respectively (Fig. 2a). Fish of SP-4 showed 43 to 96% and 25 to 105% greater value in DNA and RNA, respectively than that of the FP-1 (Fig. 2a).

DNA/RNA : The ratio of DNA and RNA revealed a significantly higher value (ANOVA, $P < 0.05$) in FP-1 than that of SP-4 (Fig. 2b).

Water quality parameters : Temperature and pH of water ranged from 30.5 to 32.5ºC and 7.1 to 7.88, respectively, in both the ponds. The pH of SP-4 showed slightly higher over the value of FP-1. Orthophosphate (0.17 – 0.466 mg l^{-1}) and Ammonium-N (0.5 – 3.15 mg l^{-1}) also exhibited 46 to 55% and 61 to 78% reduced concentration, respectively, in SP-4 over FP-1 (Fig. 2a).

Fish Growth : The growths of fish ranged from 241 to 475 g in both FP-1 and SP-4 (Fig. 2b). The net weight gain ranged 186 – 194 g in FP-1 and 399 – 414 g in SP-4. Daily growth rate of FP-1 (1.28 - 2.82) showed a markedly lower value than SP-4 (2.87 - 3.97).

Discussion :

Results of the present experiment showed that the load of pathogenic bacteria (Coliform and *Vibrio sp.*) was higher in FP-1 than that of SP-4, whereas fish growth showed a reverse response which indicating that pathogenic bacterial acts as a stressor in fish growth. Growth difference of fish cultured in FP-1 and SP-4 was attributable to the differences in the water quality parameters along the gradients of sewage effluents. Maximum growth of fish in the SP-4 was the result of better water quality expressed in terms of low concentrations of nutrients congenial for growth of diversity of plankton that were favourable for fish growth. The physico-chemical characteristics of pond indicated eutrophic state resulting in massive algal bloom (Saha, 1999). This was evident from the fact that the concentrations of major nutrients such as ammonium-N (61 to 78%) and orthophosphate (46 – 55 %) declined substantially in SD-4 than the FD-1 (Fig. 2a). The reduced level of ammonium – N recorded in the SP-4 was conductive to fish growth as high concentrations were inhibitory to their growth because of poor fish growth was associated with high concentration of ammonium – N and orthophosphate in FP-1. This implied that environmental stresses of the swage effluent were gradually reduced as the effluents passed away from the source. It has been proposed that chemical contaminants are considered as sources of physiological stress to fish (Beyers *et al.*, 1999; Hodson, 1990).

Concentration of DNA and RNA per unit weight of fish exhibited an increasing trend from FP-1 to SP-4 indicating the direct relationship between the fish growth with DNA and RNA concentration of fish tissue. Further, fish growth showed an inverse correlation with DNA/RNA ratio (Fig. 2b), pathogenic bacterial population

Fig. 1. Responses of coliform (a) and Vibrio sp. (b) bacterial population in two ponds of different sewage strength used for fish culture

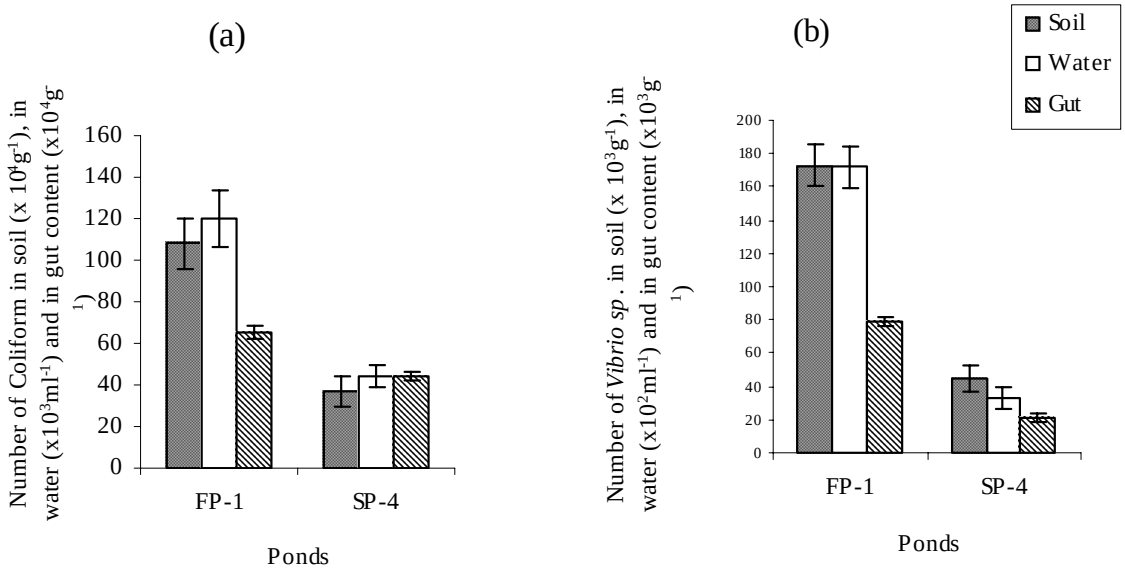

Fig. 2 : Responses of protein, DNA, RNA, ammonium-N, and orthophosphate (a) and relationship between growth and DNA/RNA ratio of fish (b) in two ponds of different sewage strength used for fish culture

and concentration of ammonium – N and orthophosphate of water which indicating that the rate of RNA synthesis was proportionately higher in the fish of SP-4 than that of the FP-1 for more protein synthesis resulting in the higher rate of fish growth due to low pathogenic bacterial population and concentration of ammonium–N and orthophosphate. From this relationship, it clearly implies that fishes of FP-1 must expend more energy, which is used to synthesize structural or functional proteins and biotransformer to resist the stressor's effects on homeostasis that presents in more quantity in FP-1 than that of the SP-4. Differences in pollution states of the streams were reflected by different biomarker responses of the trout (Triebskorn, 1997). Further more a change in pH and osmtics changes may lead to transformed physiology of bacterial species. For example, in response to increasing external osmotic strength halophilic bacteria accommodate certain compatible solutes. These are synthesized *de novo* with the help of inducible enzymes. The protein profile expression of halophilic bacteria grown under different stress conditions of salinity and temperature showed that a novel low molecular weight protein was found to be synthesized by bacteria when grown at (a) 23% salt and 45º C temperature, and (b) 18% and 23% salt and 55º C temperature (Vashishtha *et al*.**,** 2006). Therefore, it can be concluded that the concentration of DNA and RNA and DNA/RNA ratio may be considered as indicators of fish growth, which is strongly influenced by various strength of sewage and stress factors.

To investigate the influence of sewage plant effluents, brown trout as well as rainbow trout *Oncorhynchus mykiss* were exposed to 10% diluted waste water over a period of 12 months. The mortality rate was low and no pathogenic bacteria or viruses were recorded in exposed and tap-water animals. Parasitological examination revealed a mild infestation with *Gryodactylus* sp. in all groups. Macroscopically and histologically, only minor changes in gills, skin, and kidney of exposed animals were found when compared to fish kept in tap water. (Schmidt *et al.,* 1999). Trout kept in two cages in a moderately polluted river at a site where a sewage plant discharges were studied. After 66 days the infectious agent of furunculosis (*A. salmonicida*) and another bacterial species (*A. hydrophila*) were isolated. Parasites were found in fish from both cages, whereby there were differences in species composition and degree of infestation between the groups (Escher *et al*., 1999).

Poor water quality has been discussed as a major factor causing a decline of brown trout populations in Swiss rivers, a river in the Swiss midlands, where the brown trout population has decreased dramatically during the last 10 yr and where feral fish have shown distinctive pathological alterations. The river water may be responsible for impaired fish health leading to an increased mortality in the river. Groups of brown and rainbow trout exposed to polluted river water for 24 months high mortality rates and severe pathological alterations of the inner organs were observed in fish held in river water. Especially gills, liver and kidney of these fish showed significantly higher changes than fish from tap water. These changes were dominated by degenerative and inflammatory reactions. Additionally,

several infectious agents were diagnosed in fish exposed to river water. The most important findings were furunculosis and proliferative kidney disease. Brown trout seemed to be more sensitive than rainbow trout to environmental stress and infectious agents (Schmidt-Posthaus *et al*., 2001).

During the interim, end-of-pipe technologies, particularly biotreatment, will remain the primary defence against the ravages of environmental pollution. In addition, questions of effective biodegradation for the *in situ* amelioration of historical pollution are of increasing concern. Unlike microbially mediated production processes, microbially mediated environmental protection and restoration processes involve process cultures comprising multiple microbial consortia and it is individual consortium performance, rather than individual strain performance, that is critical as far as both process efficiency and economics are concerned. For its first hundred years as an independent discipline, microbiology was very largely restricted to the study of pure mono-cultures growing on single carbon energy substrates. Biotreatment processes involve multiple substrates (pollutants) and highly complex mixed microbial cultures, irrespective of whether it is wastewater, waste gas or waste slurry streams that are undergoing treatment. Furthermore, biotreatment processes function in the continuous flow mode, frequently under unsteady state conditions, and involve multiple elemental (biogeochemical) cycles, whereas most physiological data have been generated under either batch or steady state continuous culture conditions. Such data have often been used to provide an erroneous basis for biotreatment process design (Hamer, 1997).

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