

Occurrences of *Salmonella* spp. in water and soil sample of the Karnafuli river estuary

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ABSTRACT

This study was carried out to examine the presence of *Salmonella* spp. in soil and water sample along with some physio-chemical parameters of water at the Karnafuli river estuary during March-June 2012 from 3 stations. The ranges of measured parameters of water samples were 26.5- 32°C, 0.0 – 5.0‰, 7.5 – 7.9 and 2.28 – 2.91 mg/l for temperature, salinity, pH and dissolved oxygen (DO), respectively. In the present investigation, it was observed that the values of physio-chemical parameters in the estuarine area were fluctuated seasonally. Water temp, salinity and pH were within acceptable range according to EQSB and guidelines value of WHO but the concentration of DO decreased significantly indicating the deterioration of estuarine environment including water quality and soil conditions which are influenced by higher bacterial growth in the polluted estuarine zone. Maximum *Salmonella* spp. concentrations were observed 5 cells/ml in soil from one station (St-1) out of 3 total sampling stations during the month of May. The occurrences of *Salmonella* spp. was not found many of the stations they were absent. Considering the prevailing physio-chemical parameters and bacteriological conditions as detected in this study in the Karnafuli river estuarine water and soil, it appears that the estuarine environment including the water and soils were polluted. Such contaminated water is not suitable for household uses and possibly hazardous to many aquatic animals and human health.

Keywords: Karnafuli; *Salmonella* spp.; Water; Soil and Estuary

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Introduction

Bacteria have adapted to live and reproduce in a variety of environments including animals, humans, water, soils, and foods. Many *Salmonella* spp. act as a contributory representative of disease in a wide range of animals and humans. Growth of those bacteria depends on adequate supplies of water, food, nutrients and some physio-chemical parameters like proper pH, suitable oxygen, salinity, temperature etc. Aquatic environment are contaminated by limited microbial population when organic matters are scarce but higher concentration of organic matter can support a higher population of bacterial growth (Islam, 1998). *Salmonella* spp. in water and soil can lead to diseases like typhoid and dysentery. The sites of infection in animal and human body are the alimentary canal, ears, eyes, nasal cavity, skin and upper respiratory tract. Consumption of contaminated shell fishes also exposes humans to marine pathogens. In Bangladesh, pathogenic *Salmonella* spp. contamination is common leading to infectious hepatitis and dysentery. From health hazard point of view, many indicator microorganisms have been identified by Wood (1972). *Salmonella* spp. traditionally monitored by water pollution control to help limit the spread of environmental diseases (Niemi et al, 1997). Concentration levels of *Salmonella* spp. and *Vibrio* are significantly influenced by various nonpoint sources such as surface runoff, bank soils, recreational activity, and animal excreta (Geldreich, 1996; Ferguson et al., 2003; Kim et al., 2007; Servais et al., 2007; Wilkinson et al., 2006). Many studies indicate that soils can harbor much higher populations of both fecal coliforms and *Vibrio* spp. than the overlying water column (Goyal et al., 1977;

Doyle et al., 1992; Buckley et al., 1998, Crabill et al., 1999; Smith et al., 2008; Rehmann and Soupir, 2009).

Karnafuli river estuary is one of the most important estuaries in Bangladesh and hydro-biologically it is the meeting place where fresh water from upstream in continuously mixing with salt water of Bay of Bengal. Combinations of diverse fluctuating parameters are responsible for occurrences and distribution of different micro-organisms in estuarine environment. It is also blessed with estuarine water, soil, marine resources and varieties of fish species. Besides many industries fishing boats, vessels, trawlers and container ships are not uncommon on the river waters which hampers the status of water, soil and fish species of this river. Many scientists reported that the estuarine environment is polluted owing to the continuous discharge of waste material from the industries and sewage of the town. Pollution of water courses associated with industrial discharge and refuse from human settlements is a global problem (Joy et al, 1990). Everything is carried by rivers ultimately goes to the oceans through estuaries. The pollutants get dispersed by turbulence, ocean currents and tidal action firstly in the estuary, then concentrated in the food chain components through microbial action or deposited in the bottom soils (Islam, 1998). People in Chittagong city are depending on Karnafuli River for drinking water and for other household purposes. Besides, many people including a large number of fishermen live in both side of the river, use the water for bathing and drinking. They also use soil for household purposes. Many countries in the world have developed drinking water criteria and standards. Bangladesh developed the first Water Quality Standards in 1976 based on International Drinking Water Standards (WHO, 1984). The Bangladesh standard specification for drinking water (BDS 1240: 1989) was prepared and published by the Bangladesh Standard and Testing Institution (BSTI) for the control of drinking water including bacterial load. According to World Health Organization (WHO, 1993) and Environment Quality standard for Bangladesh

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(EQSB, 1991), the standard value of total load of *Salmonella* spp. for drinking water is nil/ml of water. Different researchers identified different pathogenic bacteria in respect of contaminated fish of the Karnafuli river but it is rare to find the presence of *Salmonella* spp. bacteria in water and soil in respect of this area. Considering the importance of water and soil of the estuary in daily life, the present research work aimed to estimate the presence of *Salmonella* spp. in water and soil of Karnafuli river estuary.

Materials and Methods

Description of the study area

The Karnafuli is the principal river of Chittagong district of Bangladesh. It originates in the Lushai Hills of Mizoram (India), flows through Rangamati and the port city of Chittagong and discharges into the Bay of Bengal near Patenga. The river is flashy and its length is about 131 km. Geographically this estuary located between latitude 22°53' N and longitude 92°27' E and enters the districts of Chittagong from the north eastern side. It travels about 121 miles in a zigzag ways to west and south west side and finally falls on the Bay of Bengal at latitude 22°12' N and longitude 91°47' E at near Patenga, Chittagong. The average channel depth of the basin at the river estuary is 8 meters, although the depth varies greatly in its upstream portion for strong current and siltation. Geologically, the entire catchment consists of a substratum of tertiary rocks covered with alluvial deposits. The overlying deposits show that it consists of successive layers of mud and sand (Rizbi, 1971). Hydrological parameters i.e. temperature, salinity, pH, DO fluctuate seasonally. Three sampling stations (Fig. 1) were selected for the present investigation with the basis of different types of pollution. First sampling station (St-1) located near Chaktai canal (most polluted canal of Bangladesh) receives heavy discharges from different domestic and industrial sources to the estuary. Second sampling station (St-2) located at the middle portion of the River where small city canal falls into the river and carry industrial and city wastes. Third sampling station (St-3) located at the mouth of the estuary and receives discharges composed of different kinds of city wastes through a canal which ultimately fall into the estuary.

Data collection and analysis

The present work was carried out at Institute of Marine Sciences and Fisheries, University of Chittagong, Bangladesh during March to June 2012. Water and soils samples were collected from three stations (St-1, St-2 and St-3) of the Karnafuli river estuary during this study period. In situ measurement of water temperature was measured by centigrade thermometer whereas water pH and salinity was measured by pen pH meter and refractometer, respectively. DO concentration was measured in the laboratory by following standard method (APHA, 1976). Collected water and soil samples were preserved and microbial analysis was conducted at laboratory to identify the presence of *Salmonella* spp. in the samples. *Salmonella* spp. in water and soil was identified by standard plate count (SPC) techniques (APHA, 1976). As the *Salmonella* spp. are very rare in water and soils, the investigator did the work without any serial dilution of samples before culturing in selective medium. Presence or absence of *Salmonella* spp. was estimated by using pour plate techniques. One milliliter of water or diluted soil samples (1gm: 9 ml of distilled water) were transferred by sterile pipettes onto Brilliant Green Agar (BGA) plate for *Salmonella* spp. Growth of pink opaque colonies surrounded by a bright red color were confirmed as *Salmonella* spp.

Results

Hydrographic Conditions

Results from the water samples showed distinct hydrographic conditions (Fig. 2). Water temperature varies between 26.5° C to 32.0° C. Maximum water temperature was recorded 32.0° C in June at St-1 and St-3 where minimum temperature was recorded in March at St-1. Monthly maximum average temperature was 31.67° C during May where minimum was 26.83° C during

March. On the other hand average maximum water temperature was recorded 29.25° C at St-2 and St-3, and minimum 29.13° C at St-1. No significant difference was observed in water temperature among the stations but significant difference was found among the months (F=23, P<0.01). Salinity of the present study found between 01 ppt. to 05 ppt. Maximum was recorded (1.6 ppt) in June at St-1, 2 and minimum 0.3 ppt at St-1, 4 during March and April. Monthly maximum average salinity was found 1.67 ppt during May where minimum salinity was recorded 0.33 ppt during March and April. Mean maximum salinity was observed 2.3 ppt at St-3 and no salinity was found at St-1 and 2. No significant difference was observed among the stations but have significant difference among months (F=26, P<0.001) for salinity value. DO concentration in water sample varies between 2.40 mg/l to 2.88 mg/l. The maximum DO contents were recorded in 2.91 in July at St-3 and minimum was recorded in June at St-2. Monthly maximum mean DO was found 2.71 mg/l during June and minimum 2.02 mg/l during May. On the other hand maximum mean DO 2.63 mg/l was recorded at St-1 where minimum 2.25 mg/l at St-2. No significant difference was found in DO among the stations though significant difference was observed among months (F=25, P<0.01). Water pH varies 7.5 to 8.5 and maximum pH value was recorded 8.5 at St-3 during June and minimum 7.5 during March at St-3. Monthly maximum mean pH was recorded 8.13 during May and minimum 7.2 during June. Average pH at maximum level occurred at St-1 (7.7) where minimum found at St-2 (7.6). No significant difference was found in pH values among the stations but significant difference was found among the months (F=31, P<0.01).

Status of *Salmonella* spp. colony in water and soil sample

Status of *Salmonella* spp. in water and soil sample is shown in Fig. 3. No *Salmonella* spp. was observed in water sample at station 2 and 3 during March to Jun, whereas the same was observed for St-1 samples during March and June. Only 1 cell/ml and 3 cells/ml *Salmonella* spp. was found in water sample of station 1 during April and May, respectively. In case of soil sample 2, 5 and 3 cells/ml *Salmonella* spp. was found at station 1 during March, May and June respectively but remain undetectable during the month of April. During March and April *Salmonella* spp. was absent in the soil sample of station 2 but only few (2 cells/ml and 1 cell/ml) was observed during May and June. No *Salmonella* spp. was found in soil sample of station 3 during the present study period.

Effect of environmental parameters on *Salmonella* spp. was determined by correction matrix (Table-1). With the increase of water temperature *Salmonella* spp. concentration decrease ($r = -0.388$, $P < 0.01$). Though with the increase of salinity and pH *Salmonella* spp. concentration decrease, but this is not significantly correlated ($r = -0.153$ and -0.707). On the other hand with the increase of dissolve oxygen concentration *Salmonella* spp. concentration increase ($r = 0.124$, $P < 0.01$).

Cluster analysis of *Salmonella* spp. concentration in water and soil samples in this study is shown in Fig. 4. Regarding temporal and spatial assemblage two clusters was formed at 72% similarity level. Cluster 1 consists of St3M1, St3M3, St2M3, St1M1, St1M4, St1M3, and St2M4 which showed similarity St3M4, St2M2, St1M2, St2M1 and St3M2.

Table 1. Effect of environmental parameters on *Salmonella* spp.

Parameters	<i>Salmonella</i> spp.
Temperature	-0.388**
Salinity	-0.153*
pH	-0.707*
DO	0.124**

* Not significant, **P<0.01

With the increase of water temperature *Salmonella* spp. concentration decrease ($r = -0.388$, $P < 0.01$). Though with the increase of salinity and pH *Salmonella* spp. concentration decrease, but this is not significantly correlated ($r = -0.153$ and -0.707). On the other hand with the increase of dissolve oxygen concentration *Salmonella* spp. concentration increase ($r = 0.124$, $P < 0.01$).

Discussion

From the present physio-chemical study of the water quality of the Karnafuli shows that the condition of the Karnafuli river is critical. Decreasing trend of DO of the Karnafuli river water was observed in the present and previous studies (Majid et al, 1977). Minimum DO value found for the Karnafuli was as low as 2.40 mgL^{-1} which reflects the critical condition of this River. Similar situation was observed by Gasim, 2007. Alam et al. 1996, reported DO values in the range ($2.74\text{-}5.12 \text{ mgL}^{-1}$) and ($3.95\text{-}5.97 \text{ mgL}^{-1}$) for river water samples and ($3.73\text{-}5.01 \text{ mgL}^{-1}$) and ($5.04\text{-}5.49 \text{ mgL}^{-1}$) for lake water samples in the dry and rainy seasons, respectively. Mean pH value of the Naf River water was found in the alkaline range. Similar pH values 7.5-8.5 were reported by Chowdhury et al. 2009. The Naf River findings are somewhat similar to the present findings. McLusky (1971) stated that salinity of an estuary ranged between 0.50 ppt to 35 ppt and Ahammad (2004) showed the salinity ranged between 14.43 ppt to 25.92 ppt salinity. Our current study shows similar results.. Alam (1993) also reported variation of water temperature for seasonal change. Kamal (1992) and Zaman (1994) observed seasonal variation of water temperature ranged between 7.54°C and 10°C . Belaluzzaman (1995) also found 10°C water temperature variations at Bangladesh coast. During the present study monthly variation of water temperature was observed.

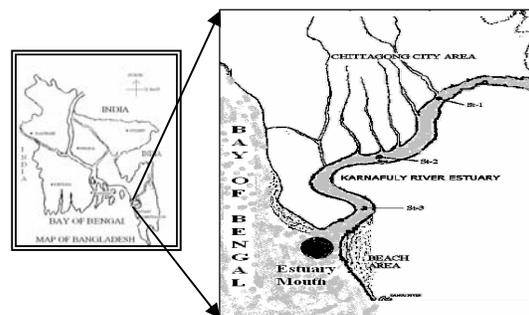


Fig. 1. Geographical location of the study area showing different sampling stations. Three sampling stations were selected for the present investigation with the basis of different types of pollution. First sampling station (St-1) located near Chaktai canal (most polluted canal of Bangladesh) receives heavy discharges from different domestic and industrial sources to the estuary. Second station (St-2) located at the middle portion of the river where small city canal falls into the river and carry industrial and city wastes. Third station (St-3) located at the

mouth of the estuary and receives discharges composed of different kinds of city wastes through a canal which ultimately fall into the estuary.

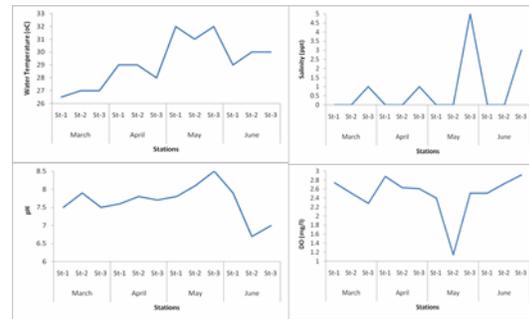


Fig. 2. Physico-chemical parameters of Karnafuli River at different stations in different months. Hydrological parameters i.e. temperature, salinity, pH, DO fluctuate seasonally. Water temperature varies between 26.5°C to 32.0°C . Maximum water temperature was recorded 32.0°C in June at St-1 and St-3 where minimum temperature was recorded in March at St-1. Salinity found between 01 ppt. to 05 ppt. Maximum was recorded (1.6 ppt.) in June at St-1,2 and minimum 0.3 ppt. at St-1,4 during March and April. Dissolved Oxygen (DO) concentration in water sample varies between 2.40 mg/l to 2.88 mg/l. The maximum D.O. contents were recorded in 2.91 in July at St-3 and minimum was recorded in June at St-2. Water pH varies 7.5 to 8.5 and maximum pH value was recorded 8.5 at St-3 during June and minimum 7.5 during March at St-3.

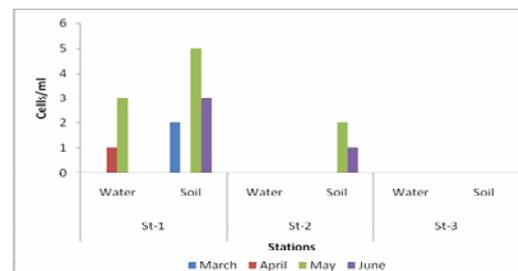


Fig. 3. *Salmonella* spp. concentration (cells/ml) in water and soil sample at different stations during sampling period. 1 cell/ml and 3 cells/ml *Salmonella* spp. was found in water samples of station 1 during April and May, respectively. In soil samples, 2, 5 and 3 cells/ml *Salmonella* spp. were detected from station 1 during March, May and June respectively. No *Salmonella* spp could be detected during the month of April.

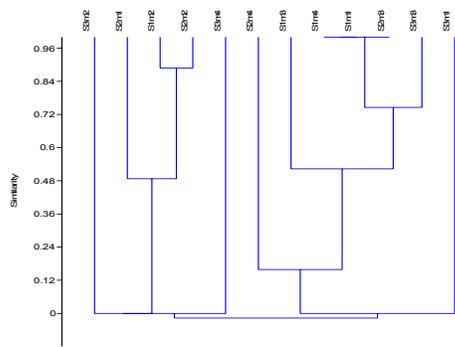


Fig. 4. Temporal and spatial similarity of *Salmonella* spp. occurrence at Karnafuli river estuary. Regarding temporal and spatial assemblage two clusters was formed at 72% similarity level. Cluster 1 consists of St3M1, St3M3, St2M3, St1M1, St1M4, St1M3, and St2M4 which showed similarity St3M4, St2M2, St1M2, St2M1 and St3M2.

Several non-point and point sources can contribute to the presence of *Salmonella* spp. in aquatic systems that can be detrimental to public health such as: humans, agriculture, water run-off, tidal actions, animal traffic, sustained winds, boats, dredging and polluted groundwater and environmental sources such as soil. Toxic metals pollution is predominant in the Karnafuli river water as the toxic metals were included from drainage of domestic waste. A large number of chemical and fertilizer industries have been established since the independence on both the banks of the Karnafuli. Effluents from these industries are reportedly being directly discharged into this river. Huge amount of solid wastes and effluents are discharged through Chaktai, Sundari, Noakhali, Mazirghat, Gupta, Mohesh, Shikalbaha and Ferighat canals into the Karnafuli river, as a result pollution of this river is increasing day by day. Leaking and leaching of oil from ships and boats are also polluting the water of the Karnafuli estuary. Due to the climate change during flood, soil erosion and land slide increases the suspended solids, metallic and other pollutants. Polluted water and soil create a favorable condition for *Salmonella* spp. multiplication.

In Karnafuli estuarine area, a lot of people used estuarine water for drinking, bathing etc. and soil various houses hold purposes. Estuarine resources and fish species are contaminated by some pathogenic bacteria because water and soils are the potential carrier of such types of bacteria. Estuarine water and soils should be free from health hazard causing micro organisms. During the study period (2012), it was observed that *Salmonella* spp. was very rare and sometimes absent in different station.

The presence or absence of *Salmonella* spp. was also observed in all the sampling stations during the study period. The presence of these health hazard organisms indicate truly polluted aquatic environments because of producing many serious diseases in animal and human body. In the present investigation, *Salmonella* spp. was not found both in water and soils at St-3 during the four months study period. *Salmonella* spp. was found more frequently in the investigation of Kanti (1988) during a study in the estuary. He reported that, a great number of *Salmonella* spp. present in the estuarine environment. Seafood processors also reported *Salmonella* spp. contaminates in their sea food harvested from the offshore water (HACCP, 1995). Huq *et al.* (1980), Rahim (1992) and Riad (1997) also reported the occurrence of *Salmonella* spp. in the aquatic environment of Bangladesh.

Conclusion

Waters of Karnafuli river estuary contains *Salmonella* spp as detected in this study. However, the estuary soil samples even contained higher concentration of the bacteria. In Karnafuli river

area, people are suffering from various health hazards that may be directly related to the uses of water for drinking, bathing, washing and soil for various houses hold purposes. During the study period, the detected *Salmonella* spp. bacterial concentrations in water and soil samples exceed the standard level in some stations - both in water and soil. Therefore, this contaminated water is unsuitable for drinking or for even washing without appropriate water treatment for humans.

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