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AN ASSESSMENT OF COLIFORM BACTERIA IN THE RIVER MAHANADI SYSTEM OF SAMBALPUR

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ABSTRACT
The study evaluates the status of coliform bacteria in the River Mahanadi in Sambalpur. Water samples from 12 different sites were collected for bacteriological analysis in order to assess its potability. The data exhibited total coliform count ranging from 11 to 2400 MPN per 100mL of water at 37°C. The faecal coliform count varied from 04 to above180 MPN per 100mL of water across the sites. The higher levels of coliform counts were consistently found in almost all samples collected from different sites. Besides, *Klebsiella* sp., *Enterobacter aerogenes, Proteus vulgaris* and *Shigella* sp. were also detected along with *E. coli* in the water samples. The findings suggested that the water from different sites in Ayodhya Saro var, Mahanadi did not meet the World Health Organization Standards for drinking water and should therefore be treated before consumption.

INTRODUCTION

Water of good drinking quality is of paramount importance to human physiology as well as indispensable to man's continued existence (Lamikanra, 1999). Potable water is defined as water that is free from pathogens and chemical substances which are deleterious to health (Ihekoronye and Ngoddy, 1985). Although water is considered "*the elixir of life*", its contamination is an irrefutable concern (Szewzyk *et al.,* 2000), the most prevalent being those associated with consumable water health risks (Lemo, 2002; Omezuruike *et al.,* 2008). The main imperative concern is about the standards for drinking water, which is more stringent than those of recreational waters.

The surface water sources, in general are not acceptable for consumption as they are often contaminated with different pollutants loaded by organic, inorganic and biological constituents (Kumar *et al.,* 1996; Dahiyas and Kaur, 1999). The source of pollutants in water bodies are specifically confined to industrial, domestic or agricultural origin. Industrial pollutants involve the processed water containing heavy metal contaminants, chemicals as well as radioactive compounds (Ibe and Okplenye, 2005). Domestic pollution may involve seepage of septic tank, pit lavatories, cesspools and privies (Omezuruike *et al.,* 2008). Agricultural pollution encompasses irrigational run-offs carrying fertilizers, pesticides, herbicides and faecal matters (Ibe and Okplenye, 2005).

Indicator organisms are commonly used to assess the microbiological quality of surface waters. Faecal coliforms are the most commonly used bacterial indicator for estimating faecal pollution (DWAF, 1996; Quality of Domestic Water Supplies, 1998), as they are found in water contaminated with faecal wastes of human and animal origin. Major factors affecting microbiological quality of surface waters are discharges from sewage works and runoff from informal settlements. Faecal contaminated water, if used for domestic or recreational purposes would eventually yield to gastroenteritis, diarrhoea, dysentery, hepatitis, typhoid fever and other fulminant secondary complications (Ballester and Sunyer, 2000). Indicator microorganisms have long been used to suggest the presence of pathogens (Berg, 1978) and in order to exterminate vagueness of the term 'microbial indicator', they are often referred as 'process indicator' (organisms that demonstrate the efficiency of a process as total heterotrophic bacteria or total coliforms); 'faecal indicator' (organisms which show the presence of faecal contamination, like coliform or *E. coli*) and 'index or model organisms' (organisms, which signify the presence of other pathogenic species) (Ashbolt *et al.,* 2001).

Total coliforms comprise of bacteria from faecal origin and also other bacterial groups *i.e.* bacteria commonly occurring in soil, which have the ability to ferment lactose with β-galactosidase to acid and gas at (36 ± 2)°C within (24-48)h. The presence of thermo-tolerant coliform (coliforms that produce acid and gas from lactose at (44.5 ± 0.2)°C within (24 ± 2)h specifically confirms faecal contamination (McCrady, 1937; Mackenzie *et al.,* 1948).

According to the guidelines for drinking water, there should not be any *E. coli* or thermo-tolerant coliform bacteria detectable in water sample (WHO, 1985; 2011a, 2011b). The coliforms are indicative of the general hygienic quality of the water and potential risks of infectious diseases from water. A

number of scientific investigations have been reported. The Public Health Unit of Walkerton, Ontario reported an outbreak of *E.coli* 0157 with 5 deaths and 27 hospitalizations (WHO, 2000). The disease surveillance cell reported 44321 cases of diarrhoea with 37 fatalities in 178 blocks during Orissa floods (Anonymous, 2001). The quantum of waterborne disease outbreaks have been increasing over the years with a yearly average of 2 billion people being infected with diarrhoea and around 4 million fatalities caused owing to it (WHO/UNICEF, 2004). The World Health Organization in its "Guidelines for drinking water quality" publication has highlighted at least seventeen different and major genus of bacteria that may be found in tap water which are capable of seriously affecting human health (WHO, 2006). Such outbreaks necessitate regular monitoring of the water quality and preferably its potability.

The present study was designed to enumerate the coliform count and to assess the quality of water in Ayodhya Sarovar, Mahanadi.

MATERIALS AND METHODS

Sampling site

The river Mahanadi flows through the revenue district of Sambalpur (20º54' to 22º11' NL and 83º49' to 84º45' EL), Odisha. The water reservoir has been created namely as "Hirakud Dam", which exists between 21°27' 02 2 NL and 83°582 02 2 EL and draining an area of 132,100km^2. The river is the sole source of water for irrigation over 1 lakh hectors of crops, and over 1.364 million acres of water has been used for industrial purpose, electricity generation as well as for domestic uses (District statistical handbook, 2001).

The present study was carried out in the "Ayodhya Sarovar", which is an artificial lake (stretch of 3 km long), built in 1966 made for storing water from the river Mahanadi. In this investigation, 12 different sites were selected in the Ayodhya Sarovar based on the kind of water flowing into the river Mahanadi, namely: Hindalco sewage effluent (S1); Post-Hindalco sewage water (S2); Mid-river water (S3); Mid-river town sewage water (S4); Mid-river town downstream sewage water (S5); Other-side bank settlement water (S6); Mid-river no settlement water (S7); Fish ghat water (S8); Lady Lewis upstream sewage water (S9); Lady Lewis downstream sewage water (S10); Dung area sewage water (S11) and Bathing ghat water (S12). The location of sampling site has been shown in Fig. 1. The study site experiences summer from March to June (24.2°C to 52°C) and winter from October to February (14°C - 28.5°C). Average annual rainfall is recorded to be 1495.7mm.

Sampling

Twelve water samples in five replicates from each location in the Ayodhya Sarovar in the river Mahanadi were collected in sterile, opaque, plastic containers during January 2012, for overall analysis of coliform count. The water samples were collected aseptically, and subjected to specific bacteriological analysis.

Materials

Isolation and confirmation of coliform was done by using Lactose fermentation broth, EMB agar, Mac-Conkey agar, Nutrient agar, Modified Eijkman medium, Methyl red-Voges Proskauer broth, SIM agar, Simmons citrate agar and Salmonella-Shigella agar.

Microbiological analysis

Coliform test: Coliform test was initially done by presumptive test of Most Probable Number (MPN) method (Wolf, 1972; Porter, 1979; Oblinger and Koburger, 1975) and 95% confidence limit of the occurrence of probable number of microorganisms was determined by the method described by Taras *et al*. (1998). Then confirmatory and completed tests were performed using EMB agar and Brilliant Green Lactose Bile broth for confirmation. The index of total coliforms involves enumeration in lactose broth at 37°C (Ibe and Okplenye, 2005).

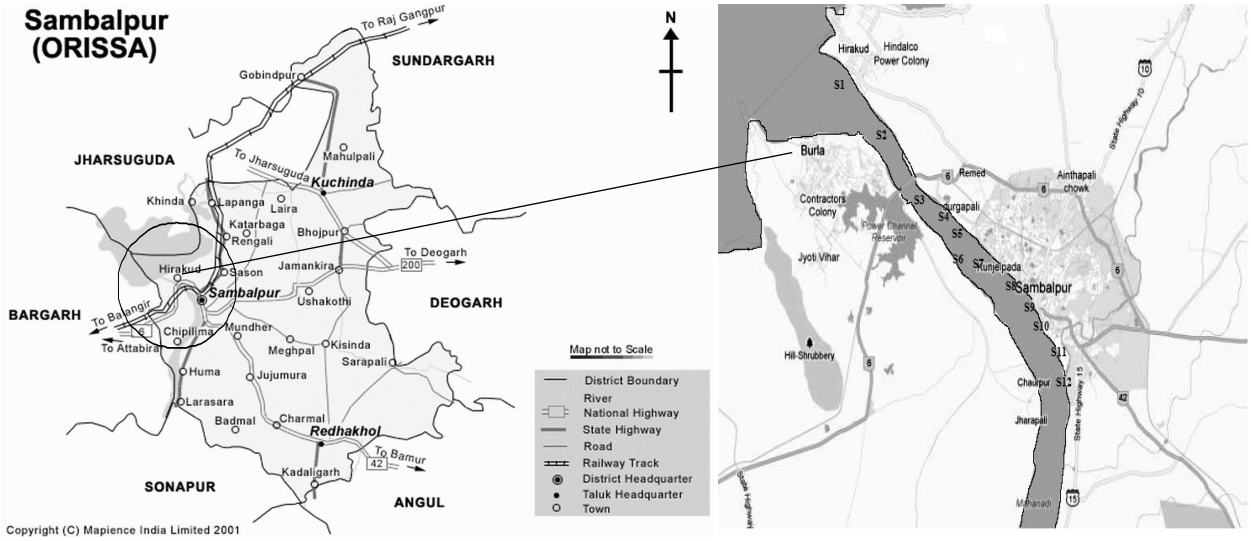

Figure 1: Map of the River Mahanadi close to Sambalpur showing sampling sites

Test for thermo-tolerant coliform: Test for thermo-tolerant coliform was performed by modified Eijkman test (McCrady, 1943; WHO, 2011). The index of thermo-tolerant coliforms involves initially increasing the numbers of coliform in lactose broth at 37°C and then incubating them at a temperature of 44.5°C for faecal coliforms (Hajna *et al.,* 1943; Ibe and Okplenye, 2005).

Test for *Escherichia coli*: *E. coli* was confirmed by plating on EMB agar and through different biochemical tests such as Indole test, Methyl Red-Voges Proskauer test and citrate utilization test (Cappuccino and Sherman, 2009).

Heterotrophic plate count: Total heterotrophic bacterial count was done by serial dilution method using Nutrient Agar.

Test for culture and isolation: All water samples were tested for coliforms, and cultured on EMB agar for isolation and sub-culturing. The identities of the requisite bacterial isolates were further confirmed by biochemical tests.

RESULTS

In the present study, all the 12 different water samples collected from different sites showed the presence of coliform. The variation in the MPN index per 100mL with respect to twelve different sites at 37°C is presented in Table 1.

The data suggested that the coliform counts in mid-river no settlement water (S7) was found to be minimum *i.e.* 11 per 100mL of water, with a 95% probability that there are between 05 and 35 microorganisms present at 37°C. However, the highest level of coliform count *i.e.* 2400 was exhibited per 100mL of water in Lady Lewis upstream sewage (S9); Lady Lewis downstream sewage (S10) and Dung area sewage water (S11) at 37°C.

Similarly, the probable number of faecal coliform count in 12 different water samples collected from different sites at 44.5°C was determined as per McCrady Table. The faecal coliform count varied from 4 (S3: Mid-river water) to >180 (S6: Other-side bank settlement water) per 100mL water sample at 44.5°C (Table 2). It is evident from the MPN index data that the faecal coliform count at 44.5°C was found be comparatively less as compared to the MPN index determined at 37°C.

The heterotrophic plate count with respect to 12 different water samples collected from Ayodhya Sarovar, Mahanadi ranged from $1x10^7$ to $7x10^7$ CFU per ml with minimum in S3 (mid-river water), S7 (mid-river no settlement), S12 (bathing ghat water), and maximum in S6 (other-side bank settlement), S9 (Lady Lewis upstream sewage) (Table 3).

Besides, *Klebsiella* sp., *Enterobacter aerogenes, Proteus vulgaris* and *Shigella* sp. were also detected along with *E. coli* in almost all water samples collected from Ayodhya Sarovar, Mahanadi.

Table 2: MPN index at 44.5°C of different sites located in Ayodhya Sarovar, Mahanadi

Sites	Location	MPN Index at 44.5°C (As per McCrady's Table)
S1	Hindalco sewage effluent	09
S2	Post-Hindalco sewage	05
S3	Mid-river water	04
S4	Mid-river town sewage	10
S5	Mid-river town downstream sewage	09
S6	Other-side bank settlement	>180
S7	Mid-river no settlement	05
S8	Fish ghat water	05
S9	Lady Lewis upstream sewage	14
S10	Lady Lewis downstream sewage	14
S11	Dung area sewage	10
S12	Bathing ghat water	05

Table 3: Heterotrophic plate count of different sites in Ayodhya Sarovar, Mahanadi

Sites	Location	CFU/mL
S1	Hindalco sewage effluent	$2 x10^7$
S2	Post-Hindalco sewage	$2 x10^7$
S3	Mid-river water	$1 x 10^7$
S4	Mid-river town sewage	$4 x10^7$
S5	Mid-river town downstream sewage	$2 x10^7$
S6	Other-side bank settlement	$7 x10^7$
S7	Mid-river no settlement	$1 x10^7$
S8	Fish ghat water	$2 x10^7$
S9	Lady Lewis upstream sewage	$7 x10^7$
S10	Lady Lewis downstream sewage	$5 x10^7$
S11	Dung area sewage	$5 x10^7$
S12	Bathing ghat water	$1 x10^7$

DISCUSSION

The use of bacteria as water sanity indicators dates back to 1880 when *Klebsiella pneumonia* and *K.rhinoscleromatis* were characterized in human faeces (Geldreich, 1978). Routine bacteriological analysis of water gained paramount sanitary importance ever since 1885 and has been piously followed

Table 1: MPN index at 37°C of different sites located in Ayodhya Sarovar, Mahanadi

Sites	Location	MPN Index at 37°C (As per Standard MPN Index)	95% Confidence Limit	
			Lower	Upper
S1	Hindalco sewage effluent	350	100	710
S2	Post-Hindalco sewage	140	52	400
S3	Mid-river water	140	52	400
S4	Mid-river town sewage	1600	400	4600
S5	Mid-river town downstream sewage	220	70	440
S6	Other-side bank settlement	33	10	100
S7	Mid-river no settlement	11	05	35
S8	Fish ghat water	280	100	710
S9	Lady Lewis upstream sewage	2400	700	—
S10	Lady Lewis downstream sewage	2400	700	—
S11	Dung area sewage	2400	700	—
S12	Bathing ghat water	12	03	28

since then (Hutchinson and Ridgway, 1977). The coliform group consists of several genera of bacteria in the family *Enterobacteriaceae*. *E. coli* is a normal inhabitant of the intestinal tract of humans and thus is regarded as faecal coliform (Atlas and Bertha, 1997). The *E. coli* are not generally present in environments other than intestinal tract of humans (warm blooded animals), which supports their use as sensitive indicator of faecal pollution (Edberg *et al.,* 2000).

The present study revealed that the coliform MPN index ranged from 11 to 2400 per 100mL of water samples, which is beyond the permissible limits (WHO, 1985, 2011a, 2011b). The increased coliform counts can be attributed to the unrestricted inflow of domestic as well as industrial sewage effluents, unwise domestic use, livestock waste run-offs and public defecation along the banks. Therefore, the coliform counts are higher near the settlement area and sewage discharge sites. However in Bathing ghat (S12) site, the discharge of detergents and soaps partially disinfects the zone, and hence the coliform and heterotrophic plate counts are considerably lower in that site (Dvorak, 2008; Aiello *et al.,* 2007).

The number of total coliform count determined as MPN index at 37°C with respect to different water samples collected from Ayodhya Sarovar, Mahanadi is comparatively higher than the number of thermo-tolerant faecal coliform in MPN index at 44.5°C, which may be due to the inclusion of certain soil intermediates (Parr, 1938). However, the Other-side bank settlement site (S6) showed a variation with decreased MPN index at 37°C and increased MPN index 44.5°C. Such deviation in site (S6) may be due to the fact that enormous amount of acid is produced by the coliform in lactose fermentation broth in the absence of a buffer, which led to its elimination at 37°C. However, the presence of a suitable buffer such as K_2HPO_4 used in the modified "Eijkman medium" averts similar consequences (Hajna, 1943). The result was found to be consistent with the high heterotrophic plate count *i.e.* 7 x10^7 CFU/mL water sample. The results obtained also indicated that there was high heterotrophic count in the sites with close proximity to waste disposal point source.

Further, the presence of *Klebsiella* sp., *Enterobacter aerogenes, Proteus vulgaris* and *Shigella* sp. in different sites of the Ayodhya Sarovar, Mahanadi ascertains that the water is unfit for consumption (WHO, 2011). The presence of coliform bacteria prophesize the probable incidence of other enteric pathogens (Ashbolt *et al.,* 2001). Hence, the utilization of such water would cause dysentery, diarrhoea, typhoid, cholera, jaundice, gastroenteritis, shigellosis, enteric fevers and other ailments (Manja *et al*., 1982).

The present investigation conclusively indicates that the water samples collected from Ayodhya Sarovar of the River Mahanadi in Sambalpur has high contamination with coliform bacteria and other pathogenic microbes of faecal, domestic sewage and industrial origin. Therefore it is unsafe and poses significant risk to human health. This mandates further bacteriological investigation of the river stretch for recommending remedial measures for the issue.

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