Nature & Environment
Vol. 21 (1), 2016: 46-52
Website: www.natureandenvironment.com

ISSN (Print) : 2321-810X
ISSN (Online) : 2321-8738

RESEARCH ARTICLE

Biomonitoring of Water Quality in River Asan in Murena District

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Received: 8th November 2015, Revised: 24th December 2015, Accepted: 25th December 2015

ABSTRACT

Day to day use of pesticides, detergents and other household chemical and sewage due to increasing population pose pressure on aquatic system and results in heavy water pollution. The measurement of water pollution is both in terms of chemical parameters and biological monitoring. In the present study estimation has been done on faecal coliform and planktons to assess the pollution status in river Asan at Morena district.
Key words: *Biomonitoring, River Asan, Pollution*

INTRODUCTION

In lotic habitat there is no depth in stream so temperature of water fluctuates considerably. Physical factors like temperature, rate of water flow play very important role for phytoplankton and zooplankton population. It has been observed that the zooplankton occupies a rich population dynamics between the bridge of autotrophs and heterotrophs because they are connecting link in food web of a fresh water ecosystem. Zooplankton studies are assuming of important role as many of the species as river food are very essential to good growth and survival rate of fresh water edible fishes. The costal region of Asan River has thick human population, so day by day fast deterioration of water quality is a major problem because in rural as well as industrial areas the water of river Asan is normally used for all kind of human requirements such as drinking, bathing, washing, waste disposal of industries etc.Asan River is under pressure of potential population as there is no proper sewage treatment system in many remote areas so, there is direct discharge of sewage which causes pollution in Asan River. Now, it is our moral duty that sewage and industrial waste as well as dead bodies should not be thrown in Asan River. All industrial units have not effluent treatment; they discharge their industrial effluent in the river Asan through municipal sewer drain. The sewage and domestic effluent directly mingled with river Asan without any treatment and they have direct or indirect effects on the biotic components. This alters the hydrology of the river causing great damages which might project certain interesting features useful for water quality and in turn on the population of fishes and other aquatic organisms.

MATERIALS AND METHODS

Water sampling sites are from river Asan at Murena. After each 3 months sample were collected at the each sampling stations at different times for the analysis of different parameters. Samples were collected in the middle of streams and at mid-depth in the direction of flow. Samples were storage in a low temperature (4 degree centigrade).

The study will be deal under the following head-

1. Murena will be marked out of the inlets of the pollutants.

2. Monitoring and analysis of the river water at following sampling stations-

a. Chanda Gaon **b.** Jaroni Gaon
c. Karua Gaon **d.** Girgoni Gaon

Sampling was done significantly after each three months interval for 1 year. The water samples of river Asan were collected. From all the sampling points October 2010 to July 2011 for the study of water quality. Samples from the river water were collected in five litres precleaned plastic bottles for physico-chemical analysis. One glass bottle (DO. bottle capacity 300 ml.) was filled with water at every sample point for the estimation of dissolved oxygen as referred by APHA (1992). Sample for MPN (Coliform and Faecal bacteria or Coliform) detection were collected in sterilized glass bottle and preserved in ice (APHA, 1992) water temperature was determined at the sampling point while other parameters were analyzed in the laboratory.

BIOMONITORING OF WATER QUALITY

Coliform (M.P.N.):

The multiple tube fermentation technique was used for the MPN count of coliform. The technique involves inoculating the sample or its several dilution in a suitable liquid medium after the expiry of the incubation period. The tubes were examined for gas production by the coliform bacteria. This test is known as presumptive test. Since this reaction may also be produced by the organisms other then the coliform, the positive tubes from the presumptive test were subjected to a confirmatory test. The density of bacteria was calculated on the basis of positive and negative combination of the tube using MPN table Lauryl tryptore broth which was used in the presumptive test.

Three dilution (0.10, 0.01 and 0.001) were selected for the estimation of MPN in water sample. Five test tubes with 10 ml single strength media and 0.1 ml. sample, 5 tubes with 10 ml. single strength media and 0.01 sample with 5 tubes with 10 ml. single strength media and 0.001 ml. sample were filled. The ignition tube was placed in each test tube. All the test tubes were sterilized at 120^0C for 15 minutes in autoclave. Now all the water sample were shaked immediately before removing sample to aliquots the series of test tubes, sample were added in test tube selected for the test and mixed thoroughly. Separate pipettes were used for different samples dilution. After this all these test tubes were kept in bacteriological incubator at 35^0C to 37^0C within one hour for 24 hours. After 24 hours these test tubes were examined. Those showed gas in ignition tube recorded as positive. The tubes showing positive test were subjected to confirmatory test as gas production is not the only criteria for a positive test. Total coliform M.P.N. densities were calculated with estimation of bacterial density index.

Faecal Coliform:

Multiple tube fermentation technique was used for the MPN count of faecal coliform. The faecal coliform test may be expected to differentiate between coliform of faecal origin (intestine of warm blooded animals) and coliforms from other sources. Transferred all positive presumptive tubes from the total coliform MPN test to E.C. medium. Make this examination simultaneously with the confirmatory procedure using brilliant green lactose bite broth. Used a sterile metal loop with a minimum 3 mm deam or a sterile wooden applicator to transfer from the positive fermentation tube to E.C. medium. When making such transfer, first gently shake the presumptive tube or mix by rotating. Incubate bacteriological incubator at 44.5 ± 0.2^0C for 24 ± 2 hr. Place all E.C. tubes in bacteriological incubator within 30 minutes after inoculation.

Gas production in a fermentation tube within 24 hrs or less is considered a positive reaction indicating faecal origin, failure produce gas (growth sometimes occurs) constitutes a negative reaction indicating a source other than the intestinal tract of warm blooded animals. Calculate faecal coliform densities with estimations of bacterial density index.

Total Coliform:

The following three sets of tubes are prepared for the presumptive test-

a. 5 tubes each containing 10 ml. double strength MacConkey's broth and an inverted Durham's tube (Plate 5).

b. 5 tubes each are containing 5 ml. of single strength MacConkey's broth.
c. 5 tubes each containing 5 ml. double strength MacConkey's broth and an inverted Durham's tube.

All the tubes of above three sets are sterilized by auto calving. For inoculation of water sample in the medium, the sample is thoroughly shaken, subsequently the stopper is removed and 10 ml. of the water sample is aseptically poured in each set. (i) with a sterilized pipette, using a separate sterilized pipette, 1 ml. of water sample is aseptically inoculated in each tube of set (ii) Similar 0.1 ml. of water sample is inoculated in each tube of set (iii) similarly 0.1 ml. of water sample is inoculated in each tube set. After this all the 15 tubes are inoculated at 37^0C for 48 hrs. Formulation of gas in the Durham's tube within the incubations period is an indicator of the presence of coliform bacteria (positive results). Now MPN of the total coliform is calculated by the comparing. The positive tubes from McCrady statistical take as APHA. After proper incubation if all the 15 tubes show gas formation then the water sample may be further diluted according to requirement.

Confirmatory Test:
A loop full of culture from each positive presumptive tubes is transferred to another tube containing brilliant green lactose bile broth and inverted Durham's tube. These tubes are again incubated at 37^0C for 48 hours. Gas formation (i.e. second time) is taken as the confirmatory test (APHA, 1975 page 916).

MPN of Faecal Streptococci:
In order to obtain the MPN of faecal streptococci "Azide broth" is used. Inoculation of water sample is done in a manner similar to that used for total coliform Durham's tube are not needed here. The changes in the colour from pink to yellow of the broth indicates the positive results. The MPN is obtained by comparing the number of positive tube with the McCarady's statistical table.

Density of Enterobacteria:
The enterobacteria are grown on brilliant green MacConkey's Agar. The process of isolation and counting are similar to total bacterial density.

MPN of Pathogenic:
(Escherichia coli) Examination of enteropathogenic Escherichia coli organisms are confirmed by positive indication of indole test from the tubes which had given positive test for total coliform, two loops full of liquid both are taken out and inoculated to the peptone water which is incubated at $44+1-0.5^0$C for 24 hrs. to 28 hrs. After incubation 0.5 ml. Kovac's reagent is added. Tubes containing E. coli give a red ring which is a positive indicator of E. coli. MPN is calculated by comparing McCardy's statistical table.

Salmonella and Shigella:
Liquid enrichment medium is used for isolation of Salmonella and Shigella from water samples containing mixed bacterial flora 1 ml. of water samples is inoculated in to selenite F. broth i.e. Tetrathionate broth and incubated at 37^0C for 48 hrs. During the incubation the Salmonella (if any) grow rapidly while E. coli and most other bacteria inhabited. Tetrathionate broth with brilliant green increases the selectivity for most Salmonella but inhibits Salmonella typhi and Shigella selenite. F. broth is also used or Salmonella and Shigella deoxycholate citrateagar may also be used as selective plating medium for Shigella (Cruick Shank et al., 1975, page 420).

PHYTOPLANKTON AND ZOOPLANKTON

(Sample collected at mid– stream 0.5 to 1 meter below the surface).
In general practice it is only the net plankton that is collecting for determining the density and species diversity in on water body. The most standard convenient and widely used plankton net is made up of bolting silk no. 25.

Plankton samples should be collected from different cross sections of the river between 8.00-9.30 am. Plankton net (Plate 1) comprises a metal ring of 15 cm diameter attached to a wooden/metal handle of one meter length, bolting silk cloth is tightly stitched around the metal ring in a manner to shape it like a truncated cone.

At the tapering end of the cone a specimen tube (10 ml) with a rim at the top is strongly tied. At the time of collection the net is dipped in the water, so that the ring is completely immersed below the water surface.

This dipping may be done from any of the sides of the boat, subsequently the dipped net moved against the water current about a meter and it should be quickly lifted (Plate 1) in this manner that two to six successive hauling should be made. It will be appropriate if haulings are made from both the sides of the boat.

The contents of specimen tube are subsequently transferred to other specimen tube having 5% formalin, one fraction of the sample is left for the microscopic examination and identification of plankton density. Usually the plankton density is determined by micro transect method (Lackey 1938) and subsequently modified by Edmondson (1974). The details of this method are as follows:

The concentrate of zooplankton is thoroughly mixed, then one drop of it is put in the clean slide by a pipette (one drop = 0.1 ml) and covered with 22x22 mm glass coverslip. These counting of organism should be made in five strips along the length or breadth of the coverslip under the microscopic (Plate 2). Five such drops are examined.

The width of microscopic field is measured with the help of stage micrometer. Each transect will represent a define fraction of area under coverslip, hence a definite volume of sample. The number of plankton per drop can be calculated as follow:

$$\text{Total number per drop} = \frac{\text{Area of coverslip x Average number of plankton per transect or focus}}{\text{Area of transect}}$$

The number of zooplankton per litre may be calculated by the following formula:

$$\text{Number of Zooplankton per litre} = \frac{a \times v'}{v''}$$

Where a = number of plankton per ml.

v' = volume of concentrate

v'' = volume of water filtered through the net

The volume of water filtered through the net can be calculated by using following formula:

$$V = iirl$$

Where V = Volume of water filtered

r = Radius of plankton net ring

l = Column of water filtered

Photograph 1: Front view of river Asan

Photograph 2: Polluted site view of river Asan

RESULTS AND DISCUSSION

COLIFORM (MPN) AND FAECAL COLIFOM:

The coliform (MPN) and faecal colifom values have been observed to be significantly increased throughout study period at down stream site (D) as compared to up stream site (A). However, a non -significant increase in coliforms have been observed during July (2011) to against. This may probably be due to rainy season on account of dilution of Asan water. Significant increase in MPN values may be due to discharge of industrial wastes and untreated municipal sewage in Asan River.

PHYTOPLANKTON AND ZOOPLANKTON:

Phytoplankton & Zooplankton count have been observed significantly in Asan River water during study period at Up stream site (A). down stream site (D). All time is may be probably be due to rainy season on account of dilution of Asan water. Significant increases are all site values of phytoplankton & Zooplankton due to discharge of industrial wastes and untreated municipal sewage in Asan River.

The Coliform water samples from different four stations have been observed. However, the Coliform (MPN) of Water of Asan River varies significantly after each three months intervals.

Table 1: Average Coliform

Month	Coliform/100 ml			
	Site A	Site B	Site C	Site D
Oct-10	300.00	320.00	321.00	432.00
Jan-11	283.00	350.00	368.00	1200.00
April-11	500.00	500.00	700.00	865.00
July-11	200.00	230.00	358.00	565.00

The Faecal Coliform of water samples from different four stations has been observed. However, the Faecal Coliform of Water of Asan River has no significant variation after each three months intervals.

Table 2: Average Faecal Coliform

Month	Average Faecal Coliform			
	Site A	Site B	Site C	Site D
Oct-10	134.78	198.68	210.00	249.00
Jan-11	194.00	243.00	280.00	390.00
April-11	200.00	262.00	400.00	400.00
July-11	193.00	197.00	284.34	374.36

Site A= Chanda Gaon, Site B= Jaroni Gaon, Site C= Karua Gaon, Site D= Girgoni Gaon

For collection of Plankton Von Dorn Sampler was used.The Planktons in water belong to two groups- Phytoplankton and Zooplankton.The Zooplankton in water belong to four main groups (Rotifera, the Cladocera, the Cyclopoid and the Colanoid, Copipoda). There are only few species occur in abundant in Asan River.The Phytoplanktons like Cladosponum, Aspergillus, Alternaria, Tricoderma significantly increase almost each side of the stations due to increasing the temperature from Oct. 2010 to July 2011. Zooplanktons like beetles, aquatic moths, Hellgrammites, Spring-tails, minute beetles have been observed.The increase in Phytoplankton and Zooplankton population was probably due to algal forms which confined to sewage discharge and mixing points only which can be treated as pollution tolerant form. The density of Planktons was maximum during summer all the each site Upstream site A and down stream site D and minimum population was during winter all the each sites of Upstream site A and down stream site D.

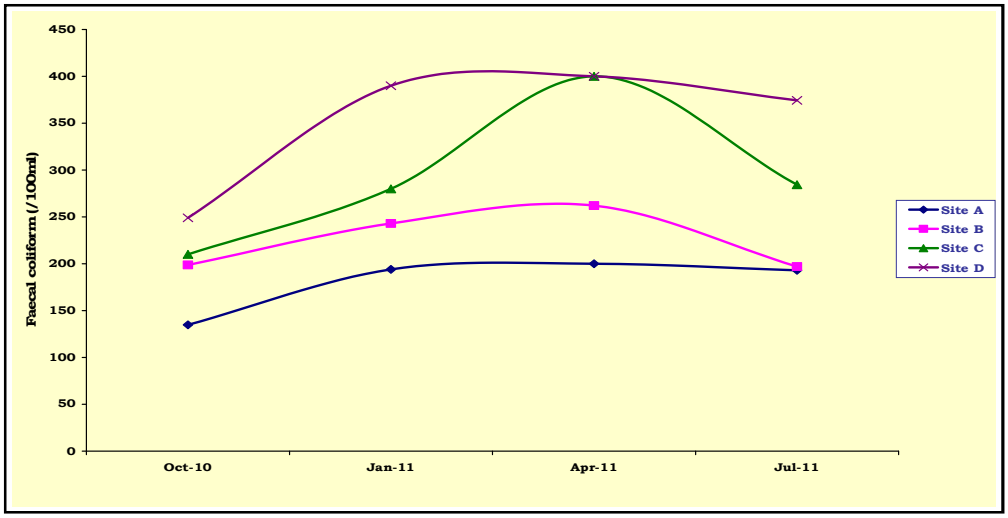

Fig. 1: Coliform of water sample at the four different stations at three months interval

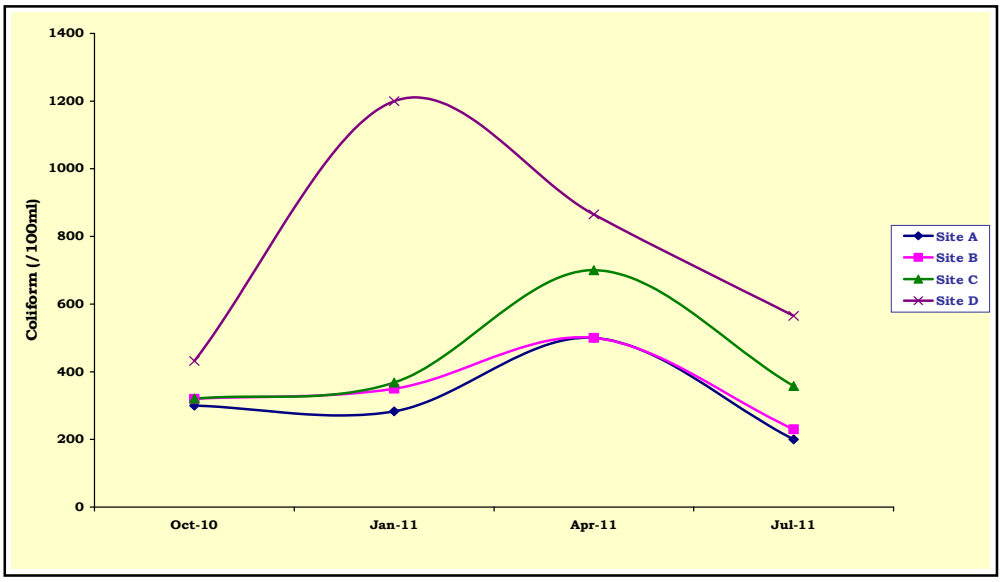

Fig. 2: Faecal coliform of water sample at the four different stations at three months interval

It is actually the microscopical examination of water the method include a qualitative analysis of the types of coliform, faecal coliform and planktons, and a quantitative estimation of their number. Most popular and simplest method of biomonitoring called "Most probable number".In the present investigation measuring datas indicate that the number of coliform (MPN) as well as faecal coliform continuously increase throughout study period from upstream site 'A' to down stream site 'D'. Depletion in number coliform (MPN) and Faecal coliform is an index of increased organic pollution at down stream site 'D' as compared to up stream site 'A' increased of coliform and faecal coliform population are due to increase of pollutants. It is clear that the population of coliform and Faecal coliform increased due to domestic waste and sewage and industrial waste in affirmation to Aranzo, *et al.* (1989), Yousuf, *et al*. (1989), Rai and Sharma (1990); Jain, *et al*. (2000), Vijendra (2006).

The presence of coliform does not mean that pathogens are present, it simply means that there might be presence of high coliform & faecal coliform count suspicious and the water should not be consume. Presence of phytoplanktons recorded positive correlation with free CO_2, temperature and

chloride whereas negative correlation exhibited in D. O., Phosphate, pH and total alkalinity. Zooplanktons correlated positively with chloride and D. O. while show negative correlation with pH and total alkalinity. In the present investigation both phytoplankton and zooplankton population significantly increase which indicate that it is due to algal forms which confirmed to sewage discharge.

Another group of algae which are more sensitive to toxic pollutant and were found in clean water. Such algae which are indicator of organic pollutions they are monitoring for coliform and faecal coliform which are responsible for organic pollution in water. Zooplankton density continuously increases at all collecting stations which indicate that there is continuously discharge of sewage. Zooplankton and phytoplankton recorded maximum during summer in each sites upstream and down stream and minimum population was recorded during winter at all the sites of upstream and down stream. Algae of Rhodophycae group, blue green algae clearly indicate that in Asan River organic pollution is going to increase. In the month of July 2011 when temperature is more than 30^0C and pH more than 7, there is presence of abundant Protozoans. Besides presence of Protozoans, zooplanktons the rotifires also indicator of organic pollution in Asan River of Murena region.

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