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Monthly Variation of Heavy Metal and Metal Resistant Bacteria from Uppanar Estuary (South East Coast of India)

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Abstract: Attempts have been made to quantify the heavy metal and heavy metal resistant bacteria in the two stations in Uppanar estuary (Lat 11°43' Long 79° 49'). The distribution of selected heavy metals (Hg, Cd, Cu and Zn) and the level of metals in the sediment was in the order of Zn>Cu>Cd>Hg. The existence of profound seasonal variation in the distribution of metals and heavy metal resistant bacteria in the sediments was observed. The metals and their resistant bacteria were maximum during monsoon and minimum during summer. The higher level of metal concentration and bacterial population were recorded near common effluent discharge from SIPCOT industrial complex, as it receives more quantities of industrial waste.

Key words: Heavy metal, resistant bacteria, effluent discharge, estuary, sediments

INTRODUCTION

Heavy metals in the sediment are essential to assess the extent of metal pollution. The distribution of heavy metals in solution has widely been recognized as a major factor in the geochemical behavior, transport and biological effects of these elements in natural waters. Moreover, sediment has aptly been called as Trace element trap (Eugenia *et al*., 2004; Chester and Dooley, 1979) because they eventually receive almost all the heavy metals, which enter the aquatic environment (Greig and McGrath, 1977). The scavenging by suspending particles results in large concentration of pollutants being retained in estuarine sediments (Jurascic and Prohic, 1986). Sediment samples have also been widely used to monitor heavy metal pollution in coastal areas (Aksu *et al*., 1998; Shiber, 1980; Holms, 1986; Langston, 1986; Kouadio and Trefry, 1987; Lynsby and Brix, 1987; Prohic and Kniewald, 1987). Heavy metal contamination could frequently be identified and integrated more effectively through analysis.

The interaction between microorganism and heavy metal may result in either reduction or remediation of toxicity nature of the pollutant or either toxicity may be magnified. In view of reducing the toxicity by making use of the beneficial microorganisms and to prevent synergistic magnification of the toxicity, it is necessary to gain the information about the behavior of the microbes with heavy metal pollutants. Metals, like mercury and cadmium, have been extensively studied because of their effects on human health. The studies on the toxicity of copper to aquatic organisms were conducted (Steeman-Nielsen and Kamp-Nielsen, 1970; Sunda and Ferguson, 1981).

Toxicity studies have shown a wide range of tolerance to heavy metals by the aquatic microorganisms (Pekey *et al*., 2004; Hodson *et al*., 1979) and it inhibits various biological processes such as respiration (Hassall, 1963; McBrien and Hassall, 1965), photosynthesis (McBrien and Hassall, 1965; Steeman-Nielsen and Anderson, 1970) and cell division (Kanazawa and Kanazawa, 1969). Hewitt and Nicholas (1963) and Bowen and Gibbons (1979) studied the toxicity of heavy metals and found that they are strongly complexed with organic molecule and compete with transport systems of other essential metals and modify the structure of enzymes.

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The metal resistant bacteria occurring in the environment may start to detoxify the heavy metal. So it is important to study the effects of heavy metals on microorganisms. Though many works have been carried out to monitor the pollution in the estuarine environment, only few works have been carried out to understand the interaction of microorganisms and heavy metals. Hence the present study was designed to find out the interaction between microorganisms and heavy metals.

MATERIALS AND METHODS

The Uppanar estuary is located at Cuddalore (Lat 11°43' Long 79°49'). It originates from the north eastern part of the Shervarayan hills and opens into the Bay of Bengal near Cuddalore. Apart from the Municipal and domestic sewage the Uppanar estuary receives industrial effluents from SIPCOT (Small Industries Promotion Corporation of Tamil Nadu) Industrial complex. Most of industries are wet process industries and hence consume large quantity of water. In the present study two stations were selected, they are representing: Station 1- effluent discharge of an industry and Station 2- common effluents discharge site of many industries (Fig. 1).

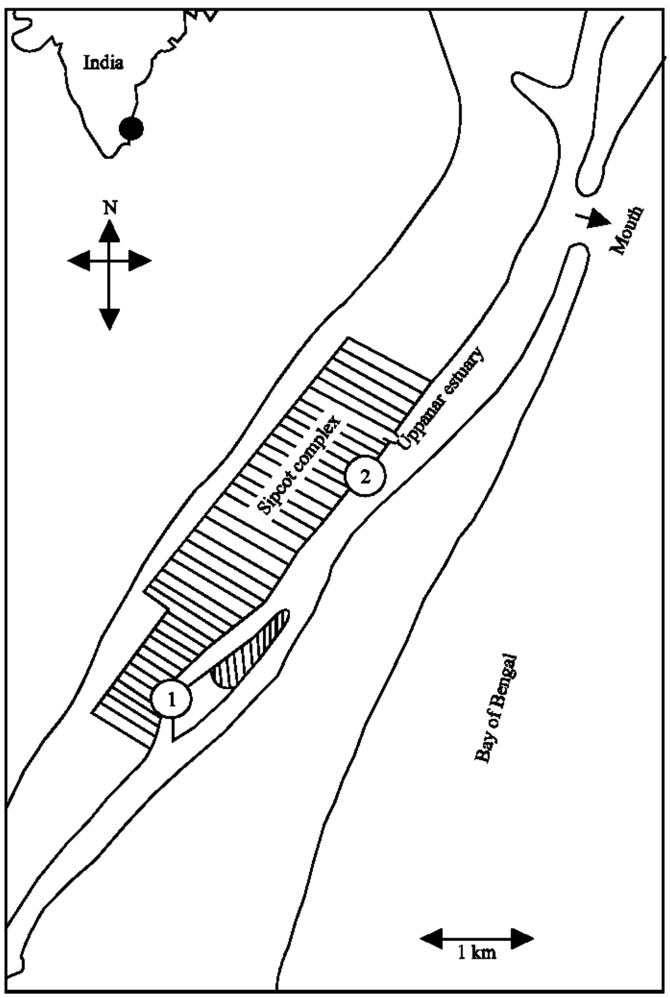

Fig. 1: Map showing the sampling site

Sediment samples were collected for a period of one year (August 2002 - July 2003) from stations I and II. Samples were collected with the aid of cleaned and dried corer and samples were transferred to clean polyethylene cover. The samples were stored frozen until analysis. The preserved sediment sub samples were dried at 110°C to constant weight for estimation of metals. For extraction of mercury, cadmium, copper and zinc from the sediments, an improved acid digestion procedure was adopted by Watling and Watling (1982). The residue formed was then dissolved in 10 mL of 10% v/v nitric acid solution and the same was then aspirated into the Atomic Absorption Spectrophotometer and finally the concentration quantified is expressed in µg/g.

For the bacteriological analysis samples were collected with sediment corer and the central portion was aseptically transferred to new polythene bags. Total Heterotrophic Bacterial (THB) population in the sediment samples were enumerated by adapting spread plate technique.

Isolation of Heavy Metal Resistance Bacteria (HMRB) from the serially diluted sample 0.1 mL of the sample was inoculated in to nutrient broth tubes which is previously amended with mercury (HgCl) (0.01, 0.1, 0.5, 1.0, 2.5 and 5.0 ppb) cadmium (CdCl) (0.1, 0.5, 1.0, 2.5, 5.0 and 10.0 ppm), copper ($CuCl_2$) (10, 20, 30, 40, 50 and 100 ppm) and zinc ($ZnSO_4$) (50,100, 200, 400, 800 and 1200 ppm). The tubes were placed in shaker at room temperature. Bacterial colonies were enumerated by spread plate method described by Austin ***et al***. (1977). Colonies were identified up to generic level with the schemes of Buchanan ***et al***. (1974).

RESULTS AND DISCUSSION

The month wise concentration of sediment fraction of heavy metals in Table 1 shows that higher level of mercury, cadmium, copper and zinc concentration were recorded at station 2 could be attributed to the Industrial effluents discharged from the SIPCOT industrial common effluent discharge and land drainage along with sewages and domestic wastes from the near by areas. The higher concentration of copper and zinc at station 2 than the station 1, could be attributed to the discharge of fertilizer, pesticide and rodenticide containing heavy metals from the near by farm lands (Haynes ***et al***., 2005). Moreover, the sediment at station 2 was clayey compared to station 1, this clayey in nature was found to adsorb higher level of metals form the water column (Seralathan, 1981; Katz and Kappan, 1981) and this would have been another possible reason for the higher level of zinc concentration in station 2. Similar findings were also reported in Swartkops river (Watling and Watling, 1982), Newport estuary (Cross ***et al***., 1970) and Abukir bay (Saad ***et al***., 1981).

The higher concentration of metals observed during monsoon could be attributed to the heavy rain fall and subsequent river runoff, bringing much industrial and land derived materials along with domestic, municipal and agricultural wastes, which include residues of heavy metal containing

Table 1: Month wise distribution of heavy metals concentration (µg L^{-1}) from station 1 and 2

	Mercury		Cadmium		Copper		Zinc	
Months	St. 1	St. 2	St. 1	St. 2	St. 1	St. 2	St. 1	St. 2
Aug.	3.06	3.49	2.59	2.76	21.39	36.43	31.72	42.44
Sep.	3.14	4.17	5.62	6.23	46.32	49.07	59.91	96.09
Oct.	3.37	5.49	6.73	6.93	49.32	61.27	61.07	111.29
Nov.	3.41	5.97	6.72	8.09	61.03	71.31	73.12	127.76
Dec.	3.49	6.93	7.01	9.62	67.29	85.70	86.21	141.25
Jan.	3.17	5.01	6.17	6.21	46.01	62.17	58.36	101.63
Feb.	3.01	4.93	5.23	4.91	40.83	53.19	50.93	83.76
Mar.	2.92	4.72	5.07	4.69	35.92	47.96	58.36	77.29
Apr.	2.81	3.97	4.72	3.72	33.91	39.27	42.36	72.96
May	1.63	3.16	1.67	2.39	20.17	30.92	23.83	34.51
Jun.	0.21	2.72	0.39	0.42	12.00	15.06	16.81	17.39
Jul.	2.93	3.21	0.58	3.91	17.31	21.91	19.76	29.21

pesticides. The load of metals was found to be lower during summer months and this could be due to the meager metal rich freshwater influx. This observation was supported by earlier reports in the Kodiakkarai coastal area by Pragatheeswaran *et al.* (1988).

In the present study, total heterotrophic bacterial population in the sediments showed higher density value during monsoon and premonsoon seasons at both stations ranged from 46.5 to 71.3×10^4 CFU g^{-1} at station 1 and 27.2 to 79.8×10^5 CFU g^{-1} at station 2. An increase in population density was noticed during the monsoon season (71.3×10^4 CFU g^{-1}) at station 1 and 79.8×10^5 CFU g^{-1}) at station 2 (Fig. 2).

Kannan and Vasantha (1986) has reported viable counts of THB ranging from 1.9 to 10.9×10^3 CFU g^{-1} in Vellar estuary. The estimated THB from the Uppanar estuary was higher than the previous work. This work was supported by Sathyamurthy *et al.* (1990) from Pichavaram mangrove. The higher population was attributed to industrial discharge and terrigenous materials through land run-off, carrying high bacterial population (Timoney, 1998).

In present study THB (in control sediment) varied from 22.2 to 60.3×10^4 CFU g^{-1} and at station 1 and station 2, respectively (9.7 to 65.5×10^4 CFU g^{-1}) (Fig. 3). Heavy metal resistant bacterial population showed maximum occurrence during pre monsoon and monsoon seasons at both stations. The maximum growth was attributed to higher heavy metal discharge through industrial effluent or land run-off (Table 2-5).

The higher population of HMRB at station 2 than the station 1 this is due to high heavy metal input form the common effluent discharge (mercury 6.93, cadmium 9.62, Copper 85.7, Zinc 48.536 µg g^{-1} maximum value at station 2). These results corroborates with Tanaka *et al.* (1974). Form

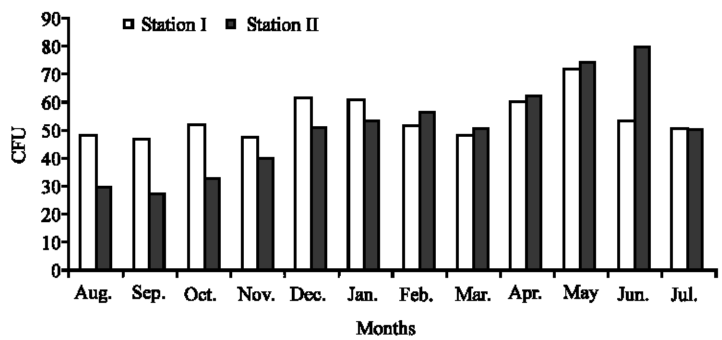

Fig. 2: Number of total heterotrophic bacteria in the sediment samples of station I and II (X 10^4 CFU g^{-1})

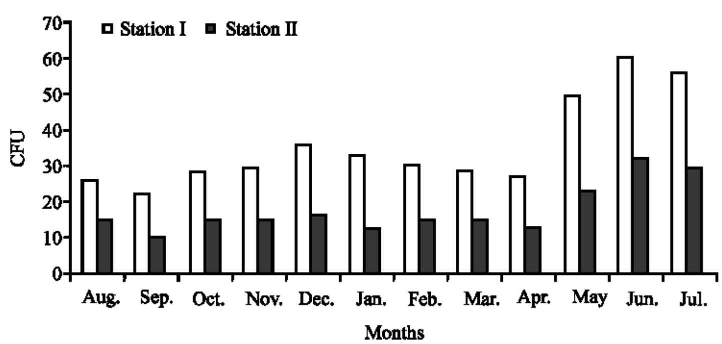

Fig. 3: Number of bacteria in the Controls of station I and II (X 10^4 CFU g^{-1})

Table 2: Number of mercury resistant bacteria in the sediment samples of station I and II (X 10^4CFU g^{-1})

	0.01 ppm		0.1 ppm		0.5 ppm		1.0 ppm		2.5 ppm		5.0 ppm	
Months	St. 1	St. 2	St. 1	St. 2	St. 1	St. 2	St. 1	St. 2	St. 1	St. 2	St. 1	St. 2
Aug.	14.9	15.2	7.3	8.3	4.0	1.2	1.7	0.9	0.5	0.4	0.2	0.3
Sep.	10.2	6.3	3.9	3.1	1.8	1.9	1.2	1.3	0.4	0.5	0.1	0.2
Oct.	14.7	13.3	7.1	6.8	3.8	2.8	1.3	1.0	0.6	0.5	0.2	0.2
Nov.	14.5	12.2	6.8	6.4	3.0	3.1	1.1	1.2	0.3	0.6	0.1	0.3
Dec.	16.3	13.9	7.9	5.8	2.9	2.6	1.2	1.3	0.5	0.4	0.2	0.1
Jan.	12.5	16.2	7.2	7.4	4.0	4.1	1.9	1.9	0.9	0.8	0.1	0.3
Feb.	14.8	8.1	7.0	5.2	2.9	2.6	1.2	1.1	0.5	0.8	0.2	0.2
Mar.	14.5	21.8	6.3	11.5	3.4	6.0	1.7	2.8	1.0	1.2	0.5	0.9
Apr.	12.9	7.2	5.8	3.0	3.0	3.9	1.3	1.8	0.6	0.3	0.2	0.1
May	22.8	19.8	10.5	8.7	4.9	4.5	2.2	2.0	1.0	0.9	0.5	0.4
Jun.	32.1	41.3	14.6	23.1	6.3	16.0	2.3	12.9	1.1	1.2	0.8	0.9
Jul.	29.5	29.1	11.8	12.9	5.2	5.9	2.4	2.5	1.0	11.3	0.4	0.6

Table 3: Number of cadmium resistant bacteria in the sediment samples of station I and II (X 10^4CFU g^{-1})

	0.1 ppm		0.5 ppm		1.0 ppm		2.5 ppm		5 ppm		10 ppm	
Months	St. 1	St. 2	St. 1	St. 2	St. 1	St. 2	St. 1	St. 2	St. 1	St. 2	St. 1	St. 2
Aug.	11.5	11.9	5.3	5.4	2.2	2.6	1.0	1.1	0.5	0.4	0.2	0.2
Sep.	7.4	7.9	3.5	3.4	3.1	1.6	1.5	0.9	0.6	0.6	0.3	0.2
Oct.	10.5	10.5	4.6	5.0	1.9	2.2	1.2	1.0	0.7	0.4	0.3	0.2
Nov.	13.3	12.3	6.5	6.1	2.9	3.1	1.0	1.3	0.4	0.5	0.2	0.2
Dec.	14.2	16.3	6.9	7.1	3.1	3.8	1.5	1.6	0.6	0.7	0.3	0.4
Jan.	12.1	13.2	6.0	6.3	2.8	4.0	1.2	1.2	0.6	0.7	0.2	0.3
Feb.	13.1	9.3	6.8	4.8	3.0	2.6	1.9	1.2	0.9	0.8	0.3	0.4
Mar.	10.2	16.0	6.2	8.4	3.0	3.1	1.4	1.2	0.8	0.6	0.2	0.3
Apr.	14.3	8.6	6.3	5.2	3.1	2.4	1.2	1.0	0.7	0.4	0.2	0.1
May	13.1	14.9	6.5	6.8	3.0	3.1	1.3	1.5	0.9	0.9	0.4	0.3
Jun.	15.9	16.2	7.4	8.3	3.2	4.2	1.2	11.3	0.7	0.8	0.5	0.5
Jul.	20.5	27.6	10.1	21.5	4.3	14.9	2.0	12.4	1.0	1.1	0.4	0.4

Table 4: Number of copper resistant bacteria in the sediment samples of station I and II (X 10^4CFU g^{-1})

	10 ppm		20 ppm		30 ppm		40 ppm		50 ppm		100 ppm	
Months	St. 1	St. 2	St. 1	St. 2	St. 1	St. 2	St. 1	St. 2	St. 1	St. 2	St. 1	St. 2
Aug.	9.5	9.3	10.6	12.3	7.2	11.7	3.0	29.4	9.3	27.1	3.2	31.2
Sep.	10.3	8.1	8.2	12.0	3.1	11.3	12.1	25.1	12.3	26.9	4.9	32.5
Oct.	15.8	17.1	19.9	20.5	14.1	23.4	30.5	49.1	19.5	50.3	12.0	57.4
Nov.	17.1	19.3	18.2	22.9	15.3	25.6	32.5	48.3	20.3	47.1	13.4	59.3
Dec.	18.9	19.1	20.3	19.5	14.9	14.9	31.6	7.0	20.9	49.1	15.9	53.9
Jan.	12.4	15.2	14.1	19.0	12.9	15.4	29.2	47.9	18.3	47.0	10.3	52.2
Feb.	13.0	12.5	14.0	20.5	12.6	23.0	28.9	38.5	22.5	42.7	8.5	57.2
Mar.	9.5	13.0	15.9	19.5	9.7	22.3	19.3	47.0	24.3	43.9	9.1	51.9
Apr.	12.0	15.1	12.8	19.3	12.0	15.6	27.3	39.1	23.0	47.3	10.5	52.7
May	11.3	13.6	13.1	20.3	12.8	21.0	28.0	42.9	9.0	48.1	10.2	52.2
Jun.	12.5	14.1	14.3	18.1	12.5	14.9	23.0	46.4	20.9	45.9	8.3	52.1
Jul.	8.3	12.0	9.1	14.9	7.2	13.1	12.7	34.1	10.8	39.3	3.7	47.1

their results the availability of heavy metals is an important factor governing the distribution of HMRB population. The present investigation was also supported by findings of Timoney (1998) at Hudson Shelf Valley of New York in metal resistant *Bacillus* sp. Similarly Austin *et al*. (1977) have also observed larger population of metal resistant bacteria in heavily polluted sites in Chesapeake Bay.

No changes occur on the growth and multiplication of bacteria at the lower concentration of heavy metals (Hg, Cd and Zn). Thus maximum population densities were recorded at 0.01 ppm (Hg), 0.1 ppm (Cd), 40 ppm (Cu) and 200 ppm (Zn) concentrations. The minimum was recorded at 5 ppm (Hg), 10 ppm (Cd), 10 ppm (Cu) and 50 ppm (Zn) concentrations. Hence, the bacteria grown in the medium containing higher concentration of metals were considered as metals resistant bacteria.

Table 5: Number of zinc resistant bacteria in the sediment samples of station I and II (X 10^4CFU g^{-1})

	50 ppm		100 ppm		200 ppm		400 ppm		800 ppm		1200 ppm	
Months	St. 1	St. 2	St. 1	St. 2	St. 1	St. 2	St. 1	St. 2	St. 1	St. 2	St. 1	St. 2
Aug.	2.1	6.5	6.3	35.9	15.0	39.0	19.7	43.1	38.1	31.6	13.1	2.6
Sep.	2.3	3.9	5.2	32.6	19.1	42.1	17.3	49.1	22.5	50.7	9.3	3.9
Oct.	11.9	15.6	15.7	52.1	26.9	57.4	40.3	69.1	72.9	59.3	23.1	7.8
Nov.	12.3	17.3	17.9	53.5	23.5	59.3	39.8	70.1	71.2	60.3	25.1	9.3
Dec.	9.0	16.5	12.7	47.3	25.1	56.0	39.0	67.5	62.1	59.6	15.7	7.2
Jan.	5.7	14.9	11.7	46.4	22.4	52.5	39.7	66.0	62.4	57.2	22.4	6.9
Feb.	6.9	12.5	9.9	45.4	23.5	53.2	37.0	62.1	63.9	50.7	17.0	5.2
Mar.	4.5	13.7	10.5	39.2	22.0	47.9	38.1	67.3	61.9	55.6	17.9	2.3
Apr.	5.0	14.0	11.2	43.5	20.7	49.1	30.6	65.2	59.5	56.1	22.1	7.2
May	2.3	15.9	9.6	45.3	23.9	53.1	32.9	62.4	56.1	52.9	20.1	6.2
Jun.	5.6	9.1	11.3	38.4	22.6	47.1	37.3	67.4	60.7	59.1	22.1	2.9
Jul.	1.6	7.3	7.2	36.2	17.5	45.3	22.4	51.6	47.3	50.3	15.3	3.0

Table 6: Percentage composition of heavy metal resistant bacteria from sediment samples at station I and II

Name of genera	Percentage
Aeromonas	13.10
Micrococus	23.60
Flavobacterium	5.20
Achromobacter	10.50
Corenebacterium	7.89
Bacillus	26.30
Vibrio	7.89
Pseudomonas	5.20
Total	98.02

Increasing the concentration of heavy metals (Hg, Cd and Cu) led to decreased bacterial population. But in the case of zinc maximum population density of THB was recorded at 800 ppm and minimum was recorded at 50 ppm concentration (Table 2-5).

The microorganisms have been extensively demonstrated as sensitive to metal pollutants. These heavy metals may be responsible for occurrence of metal resistant bacteria in the estuaries (Kan-atireklap *et al*., 1998). The higher percentage of heavy metal resistant bacteria in Cochin backwater was attributed to the input of heavy metal wastes from near by industry (Lakshmanaperumalsamy and Purushothaman, 1982).

Totally 51 isolates were collected, of which, the *Bacillus* constituted 26.3% and followed by *Aeromonas* (13.1%) at station 1 and 2. At both station *Bacillus* was dominant form (Table 6). Among all the above genera *Bacillus* is predominantly recorded, which was supported by Audus (1970), he reported herbicide resistant *Bacillus* sp. in the soil. Lakshmanapurumalsamy and Purushothaman (1982) described the *Bacillus* dominance in Vellar estuary and Nelson and Colwell (1975) reported eight genera of metal resistant bacteria from the Chasapeake Bay. Timoney (1998) isolated metal resistant *Bacillus* bacterial strains form the polluted site and Clark *et al*. (1977) also isolated the mercury resistant bacteria from coastal water and sediments.

In the present observation a comparison was made between the heavy metals concentration and HMRB in sediment. The station 2 was found to have higher level of heavy metals and heavy metal resistant bacteria. At both stations, *Microccous*, *Flavabacterium*, *Achromobacter*, *Pseudomonas*, *Bacillus*, *Vibrio* and *Corynebacterum* and *Bacillus* were the dominant genera, which were resistant to heavy metals.

CONCLUSIONS

In the present study, the microbes resist heavy metal toxicity. Attempts have been made therefore to quantity the THB and heavy metals resistant bacteria in the two stations in the Uppanar estuary. The sediment heavy metals have also been monitored. The results showed the existence of profound seasonal variation in the physio-chemical condition in the estuarine environment. Further, the station 2 was found to be moderately polluted with heavy metal compared with station 1, as it receives more quantities of municipal and Industrial effluent.

The sediment quality parameters were found to have compared with the distribution pattern of microbial population. The station 2 was found to have higher number of total heterotrophic bacteria and heavy metal resistant bacteria. Totally 51 isolates were collected, of which, the *Bacillus* constituted and followed by *Aeromonas* at station 1 and 2. At both station *Bacillus* was dominant form. Among all the above genera *Bacillus* is predominantly recorded.

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