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Isolation of Heavy Metal Resistant Bacteria from Estuarine Environs in Southeast Coast of India

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Abstract: Microbial communications with metals may have a few ramifications for the earth. Microbial Organisms may assume a substantial job in the biogeochemical cycling of lethal heavy metals likewise in tidying up or remediating metal-debased conditions. There is likewise proof of a relationship between's resilience to substantial metals and anti-infection opposition, a worldwide issue as of now compromising the treatment of contaminations in plants, creatures, and humans. Samples were made from both stations at weekly interval for a period of three months (August –October 2009) and total of 12 samples were taken for the analysis. Nutrient agar medium were used to estimate the bacterial density. Heavy metals joined media were utilized for the specific confinement of substantial metal resistant bacteria. The concentration of every heavy metal Cu2+ and Fe2+ was kept up from 10ppm to 50 ppm at 10 pmm interims in the way of life medium. The cadmium from 0.5 to 2.5ppm was maintained in the culture medium. Salinity was ranged from 32 to 18 ppt and pH was varied from 8.2 to 7.4 in these stations. THB population were biochemically identified with five species up to genus level of water and sediment in both stations. Maximum of temperaure (34^0C) was observed at the Vellar estuary in the month of Aug. 2009. Minimum of 18^0C was observed at Uppnar estuary in the month of Oct 2009. The THB colonies ranged from 3.581X10-9 CFU/ml to 4.325 CFU/ml in water samples at Station 1. The THB colonies varied from 5.712X10-9 CFU/g to 6.971 CFU/g was recorded in sediment samples at Station1. The THB colonies ranged from 2.4 X10-9 CFU/ml to 6.1 CFU/ml in water samples at Station 2. The THB colonies varied from 2.6 X10-9 CFU/g to 9.9 CFU/g was recorded in sediment samples at Station2. The maximum density of HMRB were observed 10 ppm of Cu, Fe and 0.5 ppm of Cd in both water and sediment samples at both stations. A few microbial bacteria can oppose the heavy metals at high poisonous dimensions and the obstruction might be interceded by hereditary elements, creation of chelating operators, authoritative by cell surface slime or potentially oxidative detoxification.

Keyword: Water, Sediment, Physical parameters, Medium, Bacteria, Isolation, THB, HMRB.

INTRODUCTION

Among the microorganisms, microscopic bacterial organisms, yeast and protozoa are commonly the main class to be presented to heavy metals present in the earth. Microorganisms have procured an

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assortment of components for adjustment to the nearness of dangerous heavy metals. Among the different adjustment systems, metal sorption, mineralization, take-up and aggregation, extracellular precipitation and enzymatic oxidation or decrease to a less poisonous structure, and efflux of heavy metals from the cell has been accounted for (Mergeay, 1991). The primary wellspring of heavy metals is the modern exercises, for example, metal handling, mining and electroplating, tanning, cover washing and coloring. Nearness of high centralization of harmful substantial metals in wastewater straightforwardly prompts both pollution of accepting water bodies and injurious effect on aquatic life. Microorganisms that are impervious to the two antibiotic agents and metals have been detached from nosocomial and burn wound contamination (Calomiris et al., 1984; Poiata et al, 2000).

Metal-resistant microorganisms have been separated from contaminated conditions and concentrates on the collaborations between heavy metals and microorganisms have concentrated on bacterial change and transformation of metallic particles by decrease. Metal-resistant microorganisms might be helpful as pointers of potential poisonous quality to different types of life and are significant in investigations of systems, determinants and hereditary exchange of microbial Metal-resistant (Chang et al., 1993).

When taking a gander at the microbial networks of metal-debased situations, it has been discovered that among the microscopic organisms present, there is progressively potential for special types of respiration. The oxidation condition of a metal ion particle may decide its dissolvability, numerous researchers have been attempting to utilize microbial organisms that can oxidize or decrease heavy metals so as to remediate metal-contaminated sites.

Few studies are available for heavy metal resistant or removal capacity of bacteria. Subsequently, the present work has been focused on isolation, identification of all out heterotrophic microbial bacteria and substantial heavy metal resistant microscopic bacterial organisms in these stations.

DESCRIPTION OF SAMPLING SITES

The Vellar estuary joins Bay of Bengal at Parangipettai (lat.11˚29`N, long.76˚46`) after running a distance of over 480Km. It is origin in Servarayan hills of salem district, Tamilnadu state. Vellar estuary is subjected to extended period fluctuations in saltiness coming about because of regular varieties in the amount of freshwater flow or rainfall. The tidal circulation do the exchange of elements to the estuarine systems.

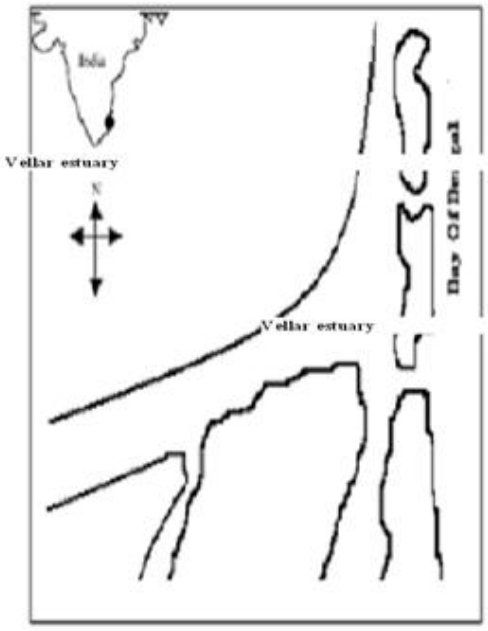


Fig. 1: Study area map

Uppanar Estuary (Station 2)

The Uppanar estuary is situated at Cuddalore (lat 11˚43'long 79˚49'). It starts from the north eastern part of the Shervarayan slopes and opens into the Bay of Bengal close Cuddalore. Aside from the civil and household sewage the Uppanar estuarine gets modern effluents from Small Industries Promotion Corporation of Tamilnadu (SIPCOT). The vast majority of businesses is wet procedure ventures and thus devour substantial amount of water.

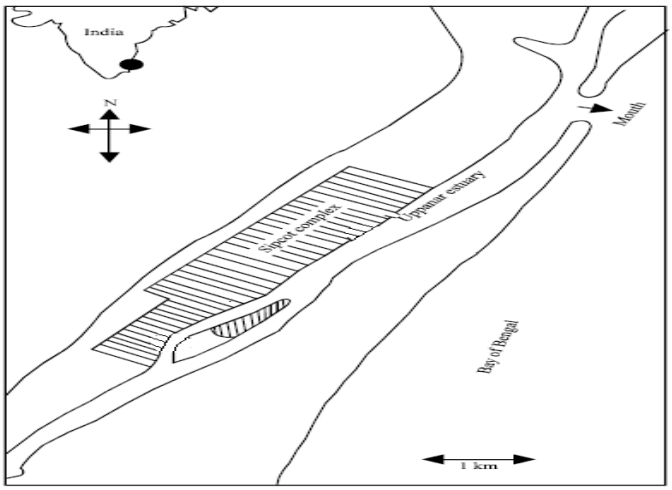


Fig. 2: Study area map

MATERIALS AND METHODS

Collection of Samples

Water and residue tests were gathered from both stations at weekly interval for a period of three months (August –October 2009) and total of 12 samples were taken for the analysis. Physical parameters as temperature, salinity and pH were analyzed in both samples. The surface water sample tests were gathered with pre-sterilized sample test bottles permitting enough air space in the containers to encourage careful blending and defensive steps were taken to limit the contamination. Silt sample tests were gathered with disinfected spatula. The focal segment of the examples was aseptically moved in to a sterile polythene sacks. Both the water and residue sample tests were exchanged to the research center in a ice box kept up at 4^{o} C for further examination.

Isolation of Total Heterotrophic Bacteria (THB)

Nutrient agar plates were used to estimate the bacterial density. Serial dilution samples were inoculated and kept in an incubator at 37^{0}C for 24-48 hours. Morphological characters were seen under a stage differentiate magnifying microscopic instrument and every one of the living organisms were biochemically distinguished up to the genus level by following the plan of Cappucino and Sherman (2002) and the outcomes were additionally cross checked with Bergey's Manual of determinative bacteriology (Buchanan et al., 1974).

Media for Heavy Metal Resistant Bacteria (HMRB)

Heavy metals fused media were utilized for the particular segregation of heavy metal resistant bacteria. Basal media supplement agar consolidated with heavy metals $Cu^{2+,}$ $Cd2^{+}$ and Fe^{2+}. The concentration of every heavy metal Cu^{2+} and Fe^{2+} was kept up from 10ppm to 50 ppm at 10 pmm intervals in the culture medium. The cadmium from 0.5 to 2.5ppm was maintained in the culture medium. Sequential dilution sample tests were inoculated on the agar plates and incubated at 370C for 24-48 hours. After the incubation time frame the plates were watched for any sort of development on the media. The isolated distinct colonies on these particular media were sub cultured over and over on similar media for purification. The pure cultures were distinguished based on their morphology and biochemical characters.

RESULTS

Physical parameters

The salinity was ranged from 31 to 17 ppt at Station 1. Maximum salinity (31 ppt) was recorded in the long stretch of Aug-2009 and least of 17 ppt was observed in the period of Oct-2009.The salinity was ranged from 32 to 20ppt at Station 2.

Maximum salinity (32 ppt) was recorded in the long stretch of Aug-2009 and least of 20 ppt was seen in the period of Oct-2009. pH was varied from 8.2 to 7.4 at the two stations, maximum of pH 8.2 was observed in the month of Aug-2009 and the minimum of 7.4 was observed at uppanar estuary in the month of October 2009. Maximum of temperaure (34^{0}C) was observed at the Vellar estuary in the month of Aug. 2009. Minimum of 18^{0}C was observed at Uppnar estuary in the month of Oct 2009.

Identification of total heterotrophic bacterial polulation (THB)

THB population were biochemically identified with five species up to genus level of water and sediment in both stations. They were belonged to the Genus *Alcaligenes* sp., *Bacillus* sp., *Escherichia* sp., *Pseudomonas* sp. *and Xanthomonas* sp.

Density of total heterotrophic bacterial polulation (THB)

The THB colonies ranged from 3.581X10^{-9} CFU/ml to 4.325 CFU/ml in water samples at Station 1. Maximum of THB (4.325 CFU/ml) was observed and minmum of 3.581X10^{-9} CFU/ml was recorded in this station. The THB colonies varied from 5.712X10^{-9} CFU/g to 6.971 CFU/g was recorded in sediment samples at Station1. Maximum of THB (6.971CFU/g) was observed and minmum of 5.712 X10^{-9} CFU/g was recorded in this station (Fig.3).

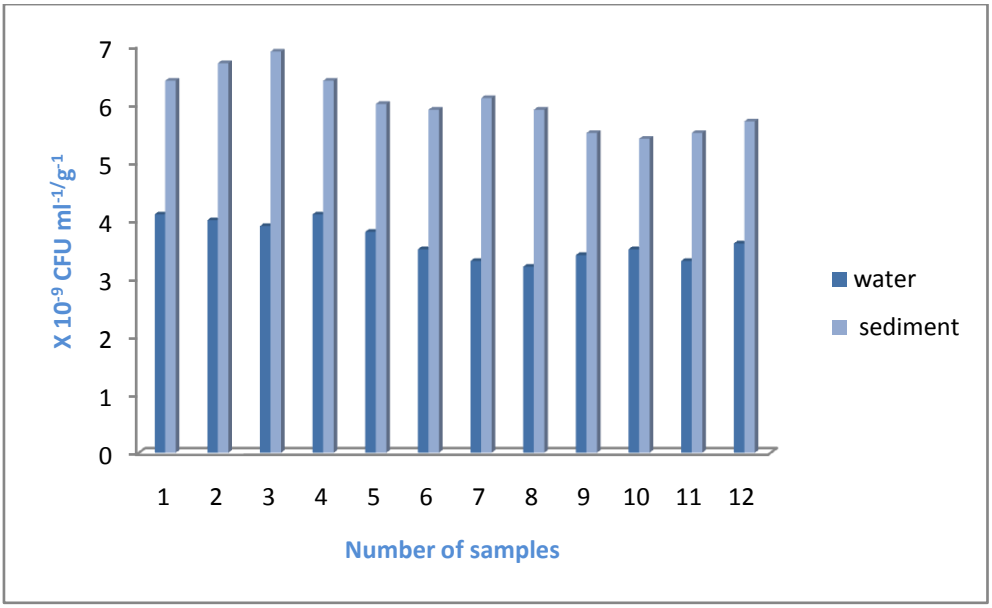


Fig. 3: THB population in water and sediment samples at vellar estuary

The THB colonies ranged from 2.4 X10^{-9} CFU/ml to 6.1 CFU/ml in water samples at Station 2. Maximum of THB (6.1 CFU/ml) was observed and minmum of 2.4 X10^{-9} CFU/ml was recorded in this station. The THB colonies varied from 2.6 X10^{-9} CFU/g to 9.9 CFU/g was recorded in sediment samples at Station2. Maximum of THB (9.9 CFU/g) was observed and minmum of 2.6 X10^{-9} CFU/g was recorded in this station (Fig.4).

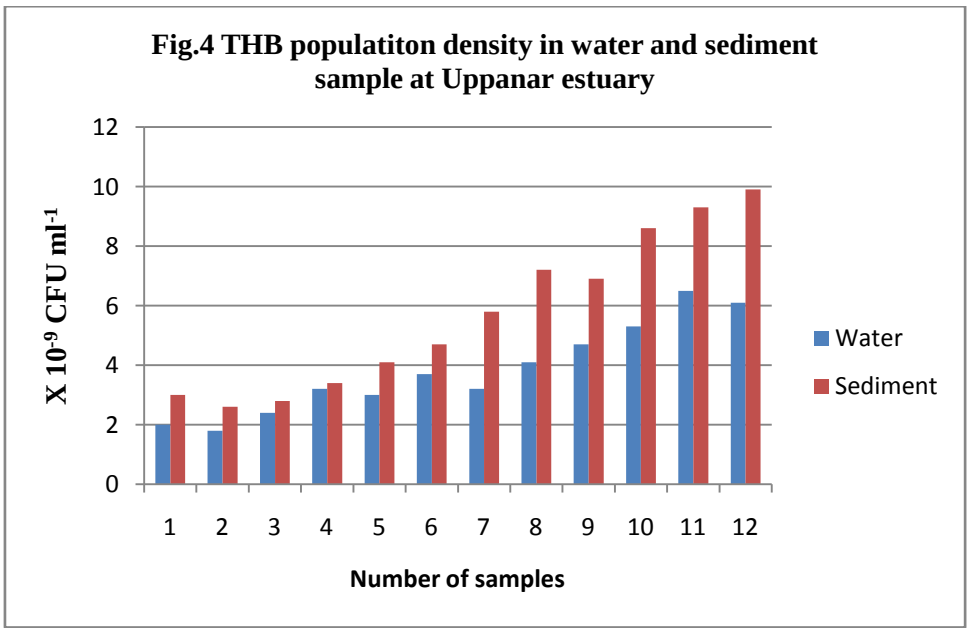
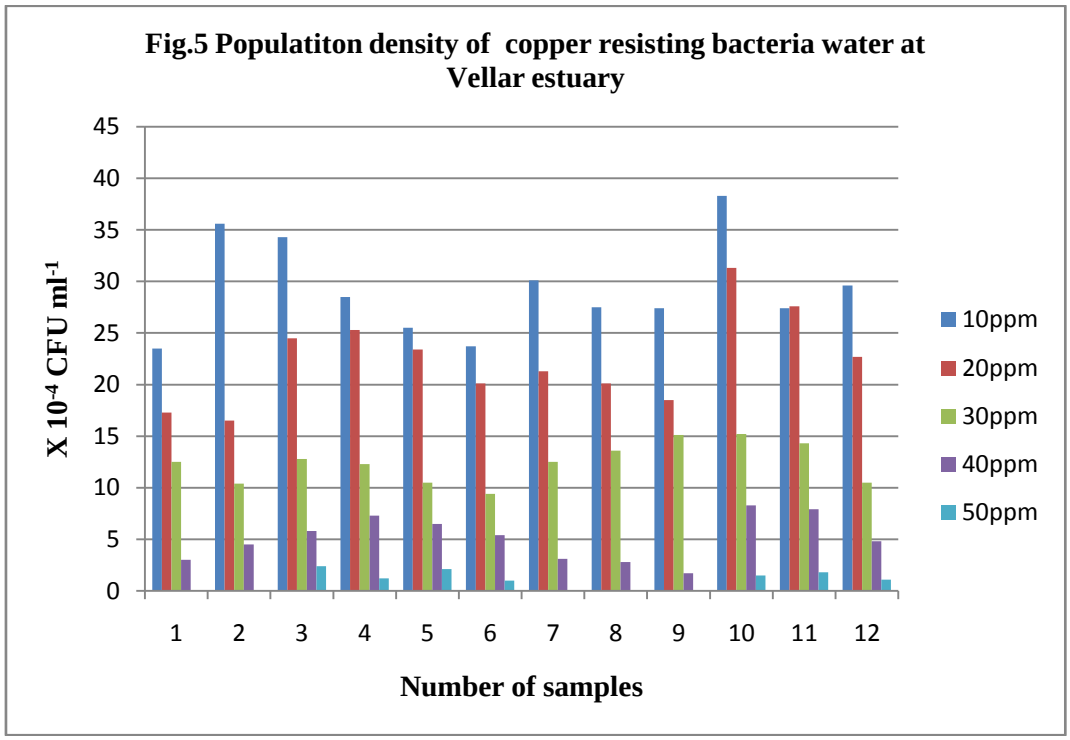


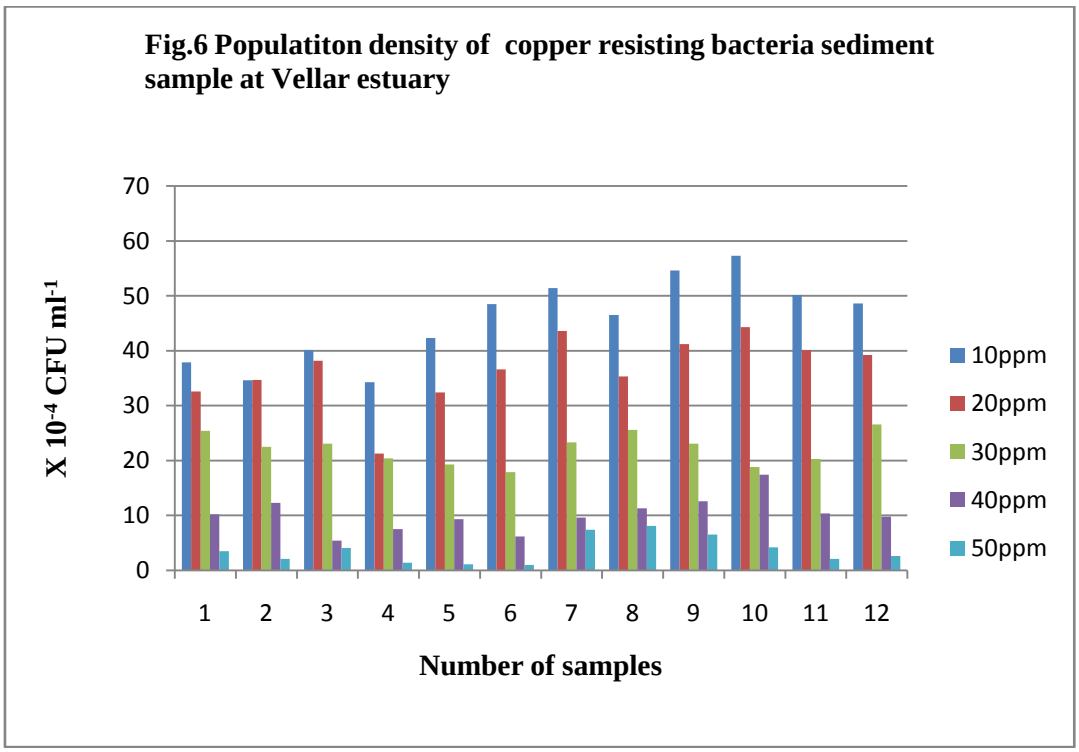
Fig. 4: Estimation of Total Heterotrophic Bacterial population in water and sediment samples at Uppanar estuary

Population density of Heavy Metal Resistant Bacteria in Vellar and Uppanar estuary

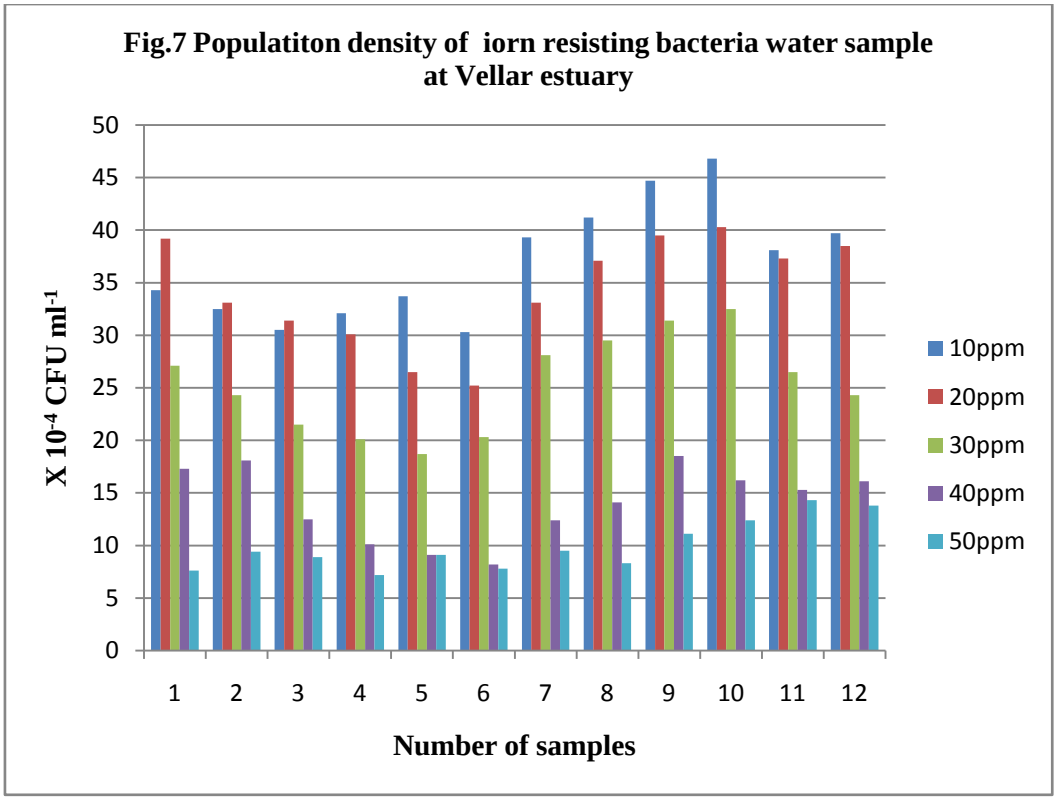
The HMRB colonies ranged from 23.5 X10^{-4} CFU/ml to 38.3 X10^{-4} CFU/ml was observed at 10 ppm of Cu at water samples in station1. Maximum of HMRB (38.3 X10^{-4} CFU/ml) was observed and minmum of 23.5 X10^{-4} CFU/ml was recorded in this station (Fig.5).



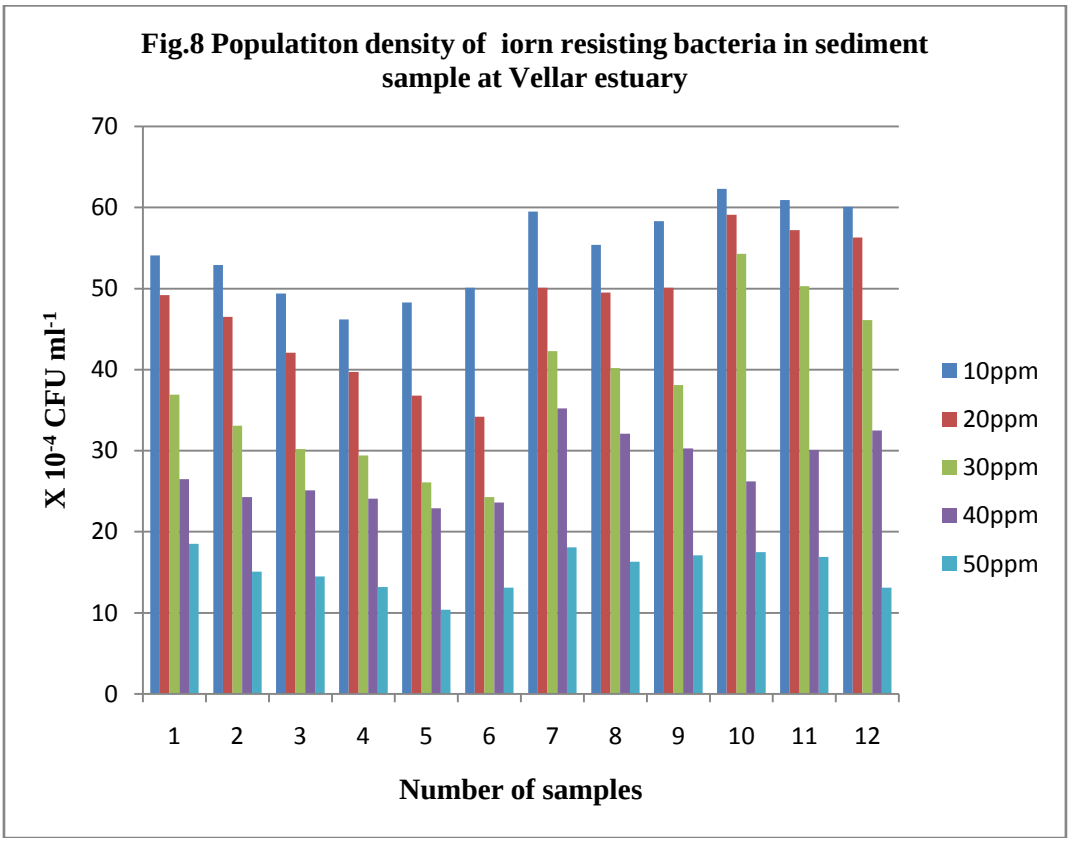
The HMRB colonies varied from 34.3 X10^{-4} CFU/g to 57.9 X10^{-4} CFU/g was recorded at 10 ppm of Cu at sediment samples at Station 1. Maximum of HMRB (34.3 X10^{-4} CFU/ml) was observed and minmum of 34.3 X10^{-4} CFU/ml was recorded in this station (Fig.6).



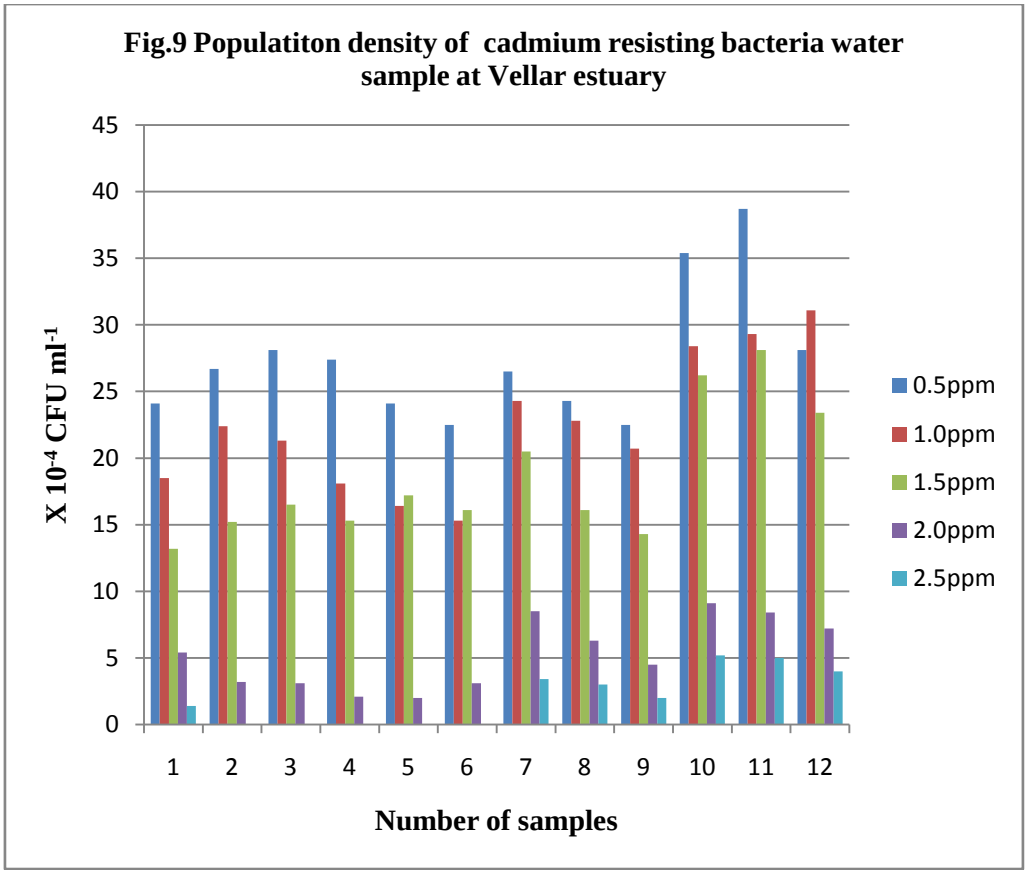
The HMRB colonies ranged from 30.5 X10^{-4} CFU/ml to 46.8 X10^{-4} CFU/ml was observed at 10 ppm of Fe at water samples in station1. Maximum of HMRB (46.8 X10^{-4} CFU/ml) was observed and minmum of 30.5 X10^{-4} CFU/ml was recorded in this station (Fig.7).



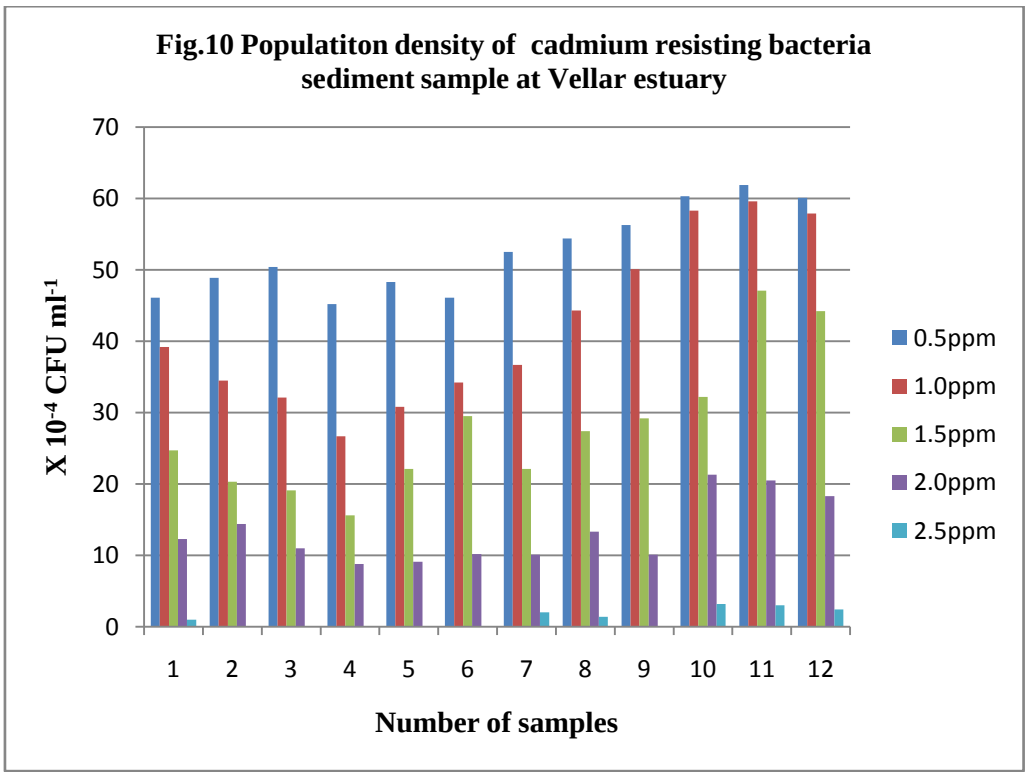
The HMRB colonies varied from 46.2 X10^{-4} CFU/g to 62.3 X10^{-4} CFU/g was recorded at 10 ppm of Fe at sediment samples at Station 1. Maximum of HMRB (62.3 X10^{-4} CFU/ml) was observed and minmum of 46.2 X10^{-4} CFU/ml was recorded in this station (Fig.7).



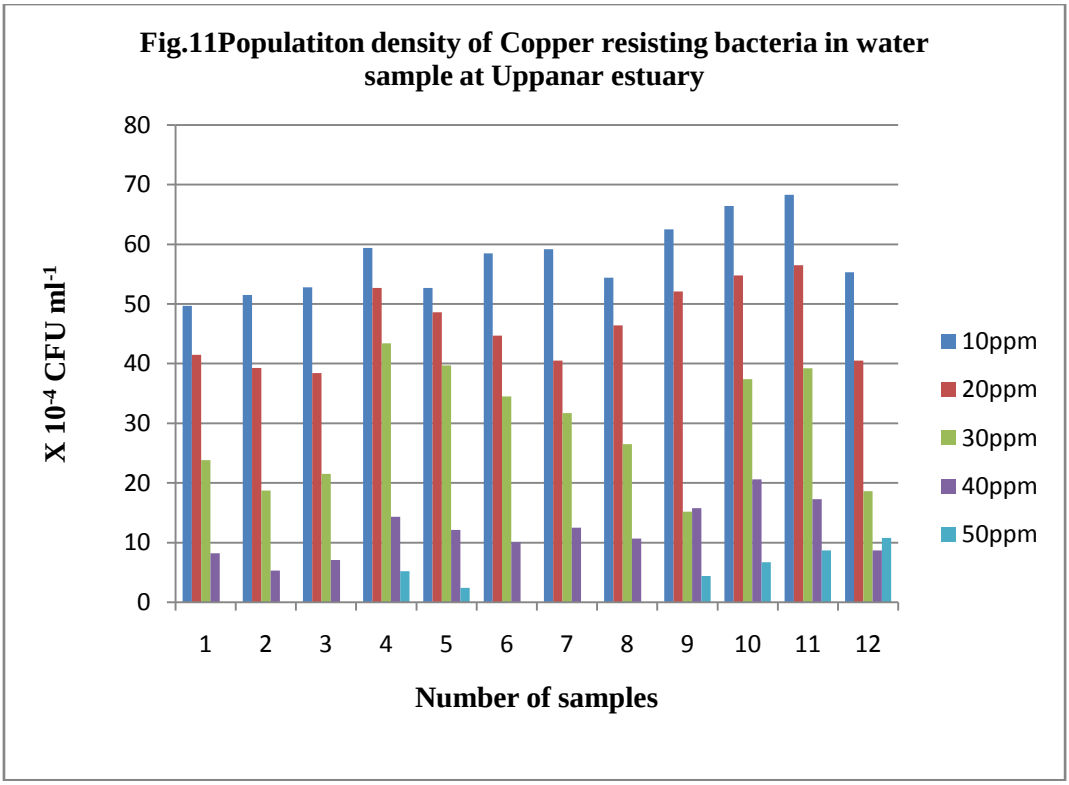
The HMRB colonies ranged from 22.5 X10^{-4} CFU/ml to 38.7 X10^{-4} CFU/ml was observed at 0.5 ppm of Cd at water samples in station1. Maximum of HMRB (38.7 X10^{-4} CFU/ml) was observed and minmum of 22.5 X10^{-4} CFU/ml was recorded in this station (Fig.9).



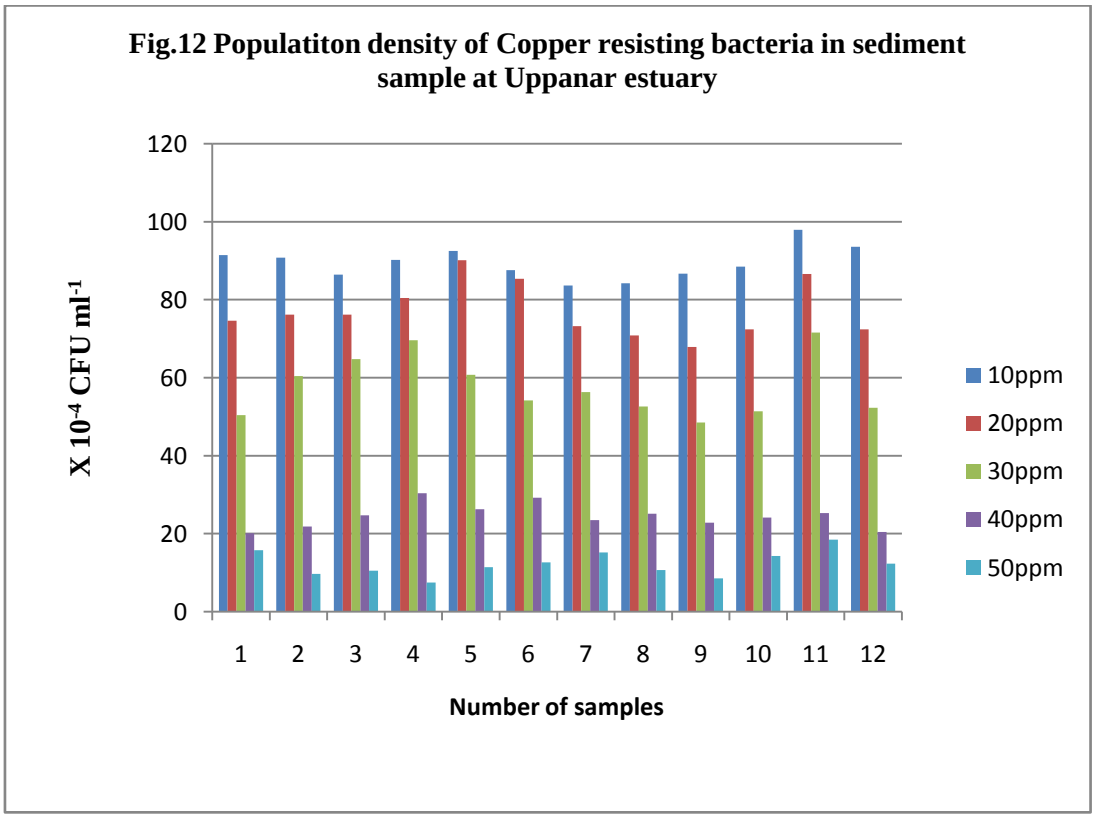
The HMRB colonies varied from 45.2 X10^{-4} CFU/g to 61.9 X10^{-4} CFU/g was recorded at 0.5 ppm of Cd in sediment samples at Station 1. Maximum of HMRB (45.2 X10^{-4} CFU/ml) was observed and minmum of 61.9 X10^{-4} CFU/ml was recorded in this station (Fig.10).



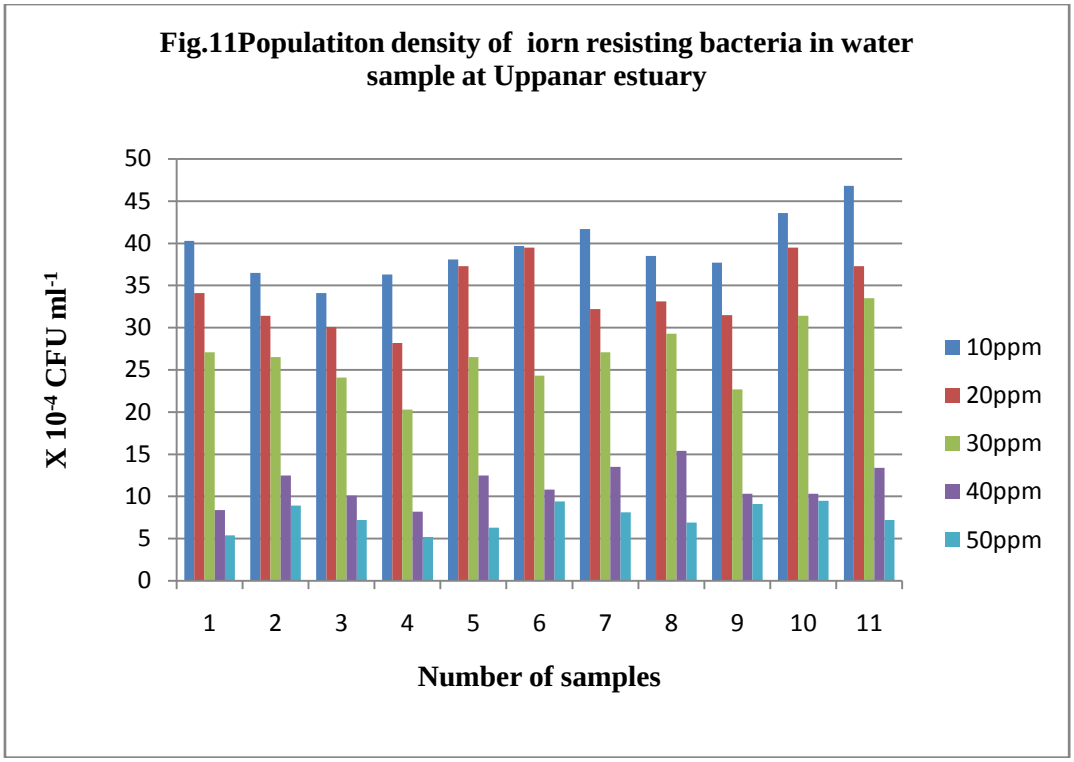
The HMRB colonies ranged from 49.7 X10^{-4} CFU/ml to 68.7 X10^{-4} CFU/ml was observed at 10 ppm of Cu at water samples in station1. Maximum of HMRB (68.7 X10^{-4} CFU/ml) was observed and minmum of 49.7 X10^{-4} CFU/ml was recorded in this station (Fig.11).



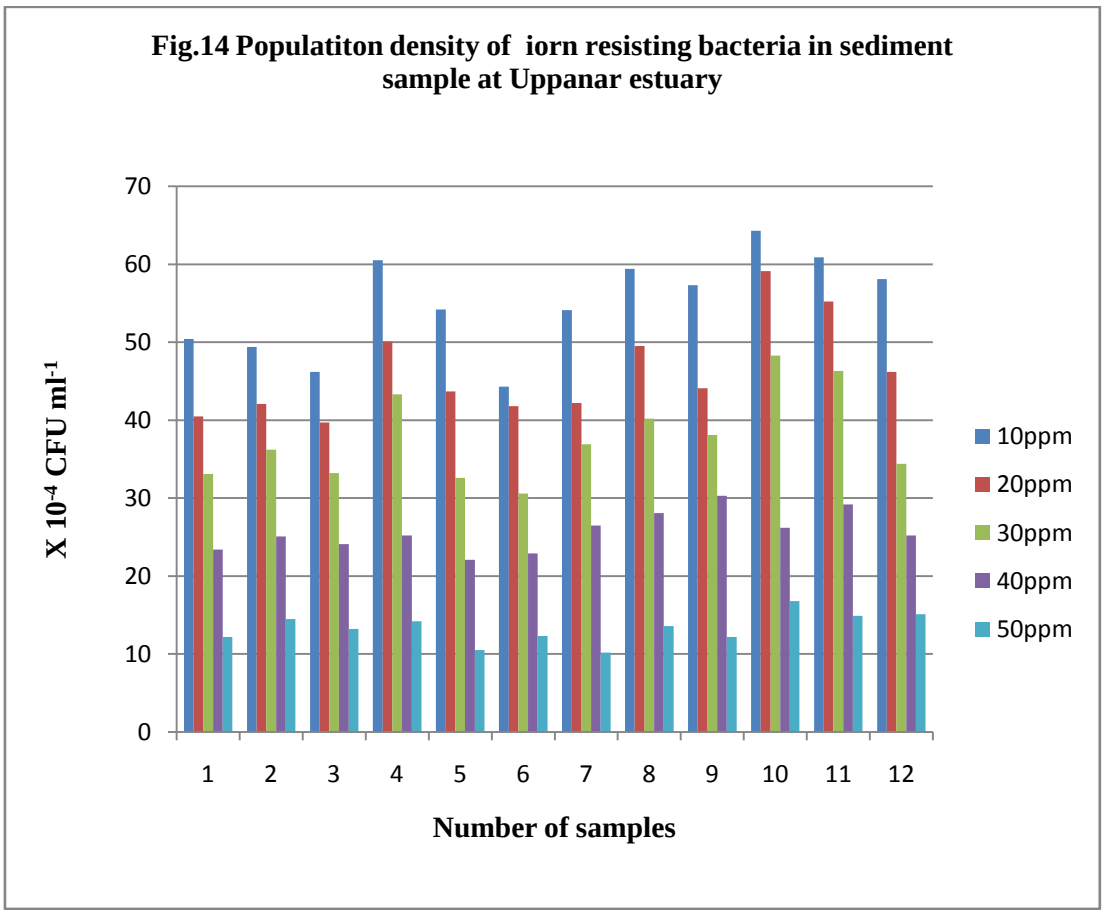
The HMRB colonies varied from 83.6 X10^{-4} CFU/g to 97.9 X10^{-4} CFU/g was recorded at 10 ppm of Cu in sediment samples at Station 1. Maximum of HMRB (83.6 X10^{-4} CFU/ml) was observed and minmum of 97.9 X10^{-4} CFU/ml was recorded in this station (**Fig. 12).**



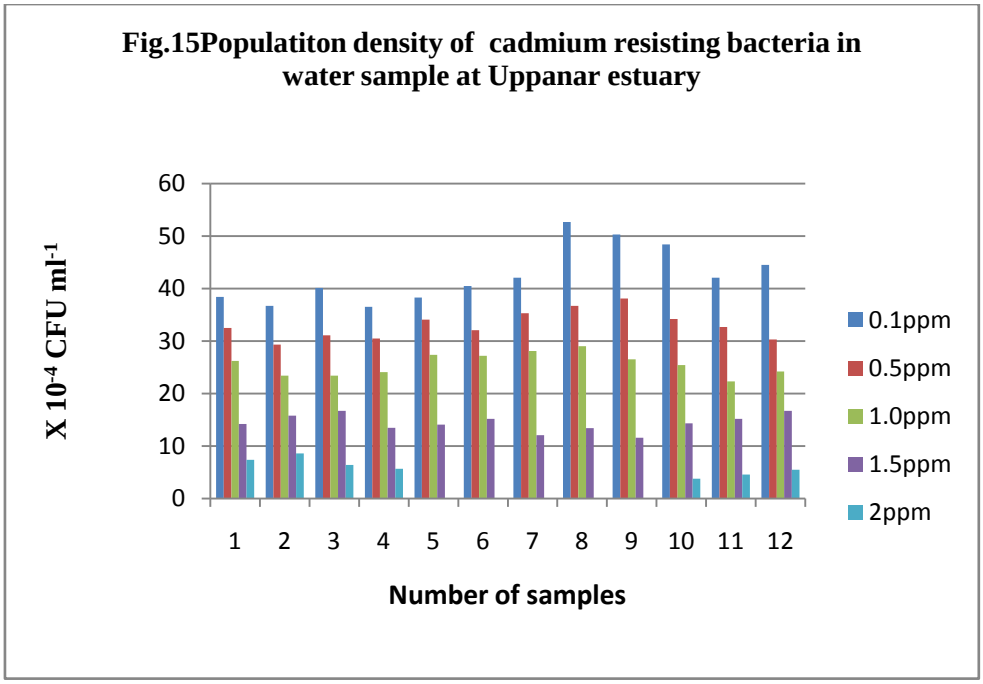
The HMRB colonies ranged from 34.1 X10^{-4} CFU/ml to 46.8 X10^{-4} CFU/ml was observed at 10 ppm of Fe at water samples in station1. Maximum of HMRB (46.8 X10^{-4} CFU/ml) was observed and minmum of 34.1 X10^{-4} CFU/ml was recorded in this station (Fig.13).



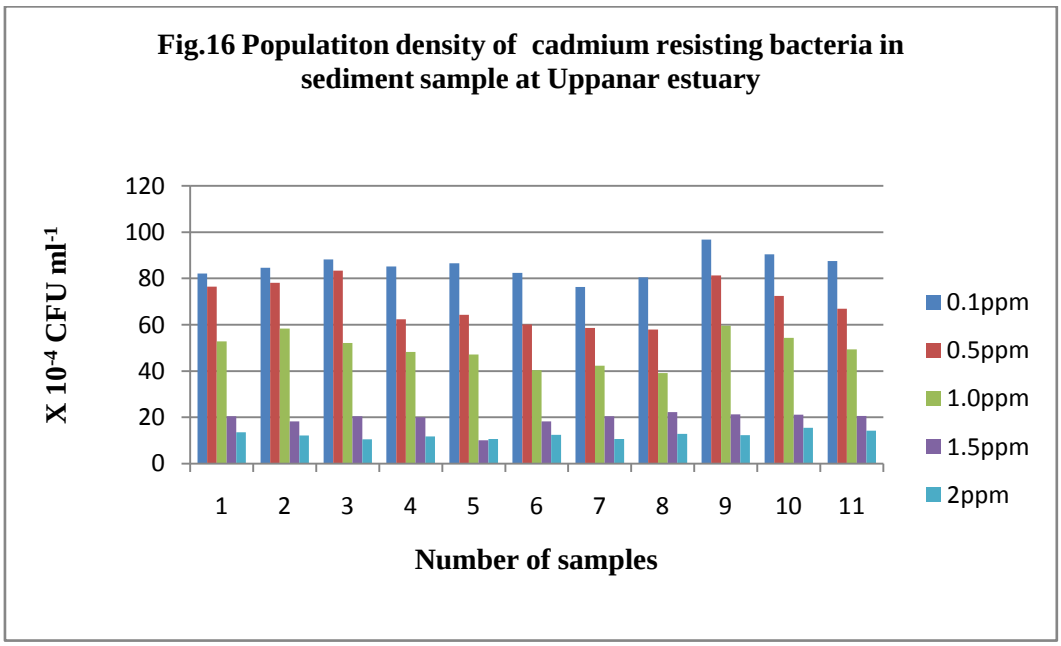
The HMRB colonies varied from 44.3 X10^{-4} CFU/g to 64.3 X10^{-4} CFU/g was recorded at 10 ppm of Fe in sediment samples at Station 1. Maximum of HMRB (64.3 X10^{-4} CFU/ml) was observed and minmum of 44.3 X10^{-4} CFU/ml was recorded in this station (**Fig. 14).**



The HMRB colonies ranged from 36.5 X10^{-4} CFU/ml to 48.4 X10^{-4} CFU/ml was observed at 0.5 ppm of Cd at water samples in station1. Maximum of HMRB (48.4 X10^{-4} CFU/ml) was observed and minmum of 36.5 X10^{-4} CFU/ml was recorded in this station (Fig.15).



The HMRB colonies varied from 76.3 X10^{-4} CFU/g to 96.8 X10^{-4} CFU/g was recorded at 0.5 ppm of Cd in sediment samples at Station 1. Maximum of HMRB (96.8 X10^{-4} CFU/ml) was observed and minmum of 76.3 X10^{-4} CFU/ml was recorded in this station (**Fig.16).**



DISCUSSION

Salinity is of the important factors which influences the wealth and dissemination of the microorganisms in the estuarine environmental condition. Further in flow of fresh water and prevailing air temperature assume a noteworthy job in the varying estuarine biotope. Low concentration of salinity was recorded amid the rainstorm season because of the overwhelming rainfall and freshwater runoff at both stations. The seasonal variations in pH might have arisen due to the changes in temperature, salinity and nature of effluents discharges. The sediment pH has showed the wide fluctuations due to $CaCO_3$ content, organic matter, temperature and salinity besides bacteria action.

The THB population of bacterial genera *Alcaligenes, Pseudomonas, Bacillus, E.coli and Xanthomonas* were recorded in both stations. Among the five genera the *Pseudomonas* was dominant form followed by *Alcaligens* sp., *Bacillus*, *E.coli* and *Xanthomonas sp.* Conversely Austin et al., (1977) have likewise recorded bigger populace of metal resistant Bacillus in intensely dirtied destinations in Chesapeake Bay. Timoney *et al*., (1978) reported higher populations of *Bacillus* strains were highly resistant to metals and grow up

to the maximum level. It has been reported that *Bacillus* strains produce the property of metal reductase might be the possible reason for the resistant to metals and grow in maximum numbers compared to the other bacterial forms in the polluted environmental conditions.

In the current research, THB population density was high in station 2 than the station1. It ranged from 1.9 $X10^{-9}$ to 6.5 $X10^{-9}$ CFU/ml in water sample and 2.4$X10^{-9}$ CFU/gto 9.9 $X10^{-9}$ CFU/g in sediment sample at station 2. In station 1, THB ranged from 3.5 $X10^{-9}$ to 4.3 $X10^{-9}$CFU/ml in water samples and it ranged from 5.4 $X10^{-9}$ to 6.9 $X10^{-9}$ CFU/g in sediment samples. The maximum density of THB was obtained from station 2. THB density ranged from 0.23 to 59.6$x10^{4}$ CFU/g has recorded at east coast of India. Lakshmanaperumalsamy and Ramamurthy (1984) have observed the range of 3-47$x10^{4}$ CFU/g in Vellar estuary. The higher populace of THB was credited to mechanical release of natural based materials and terrigenous materials through land run-off, conveying high bacterial populace.

The high concentration of copper, iron and cadmium concentrations were recorded at Uppanar estuary due to common effluents (manure, pesticide and rodenticide from the close by horticultural fields) discharge, land seepage alongside sewages and local squanders. Increasingly over the residue at Uppanar estuary was clayey contrasted with Vellar estuary which was found to ingest more elevated amount of metals from the water segment and soil (Katz and Kappar 1981). Comparable discoveries were likewise announced in Swarkops stream (Walting and Walting, 1982) and Newport estuary(Cross et al.,1970).

Population densities of HMRB on Cu, Fe and Cd in water and residue test samples were enumerated in both stations. The maximum of HMRB colonies were recorded at 10 ppm of Cu, Fe and 0.5 ppm of Cd in both samples. The increasing concentration of heavy metal with decreasing colonies were found in both samples due to toxic effect inhibit the development of colonies even at the lowest concentration. The HMRB were found to differ with two estuaries. The result indicates that, the maximum HMRB colonies were observed from station 2 than the station1. The station 2 has highly considered as a heavy metal polluted environment in which the HMRB, maximum concentrations with maximum colonies were recorded due to the capable of heavy metals tolerance than the station 1. HMRB minimum colonies were recorded from station 1, which is considered as pollution free environment.

HMRB is capable to tolerate in minimum concentrations of heavy metals. Increase in the concentration of Cu, Fe and Cd resulted in the decrease in the density of the population at both station. The distribution of HMRB in any aquatic environment is governed by biotic and abiotic factors. These heavy metals might be in charge of arrangement of metal resistant bacteria in the estuaries (Kan-Atireklap *et al*., 1997). Heavy metal resistant bacteria were specified by the strategy for Austin *et al*., (1977). Recent investigations pointed out the existence of various pollutants like heavy metals and pesticides in estuary (Sathyamurthy *et al*., 1990).

In the present examination heavy metal resistant microscopic bacterial organisms were recorded in expansive number at lower centralization of heavy metals Iron and Copper. In this way most extreme populace densities were recorded at 10ppm of Cu, Fe and 0.5ppm of Cd focuses. The minimum number of resistant bacteria was recorded at 50 ppm of Cu, Fe and 0.5 ppm of Cd concentrations. Bacterial microorganisms developed in the medium containing higher centralization of heavy metals were considered as metal resistant bacteria. The THB population decreased with increasing concentration of heavy metals (Babich and Stotzky, 1979 and Clark *et al*., 1977).

CONCLUSION

Estuarine ecosystems are highly productive but ecologically sensitive due to multifarious anthropogenic activities. Among the stress causing variables, the heavy metals are posing serious threat to these ecosystem. The occurrence, distribution pattern of metals produce deleterious effects to the biological and ecological factors. In the present examination has been done the microbial organisms resist heavy metal toxic danger and endeavors have been made for distinguish the amount of THB and HMRB in these two stations.

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