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ISOLATION OF HEAVY METAL RESISTANCE BACTERIA FROM FISH GUT IN BHAVANI RIVER, TAMIL NADU

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Abstract

Rivers are playing a vital role in the advancement of human civilizations. The physicochemical features of river ecosystems will be change due to anthropogenic activities. Heavy metals are normal constituents of river environment that occur as a result of pollution, principally due to the discharge of untreated wastes into rivers by many industries. In the present study to estimate physico – chemical parameters analysis, analysis of heavy metals from water and fish sample, isolate and characterize heavy metal to resistance bacteria. The present study reveals water quality parameters of river water showed that fresh water level changes due to continuous discharge of solid waste. The ranges of heavy metals in water (µg L^{-1}) Cu, Cr, Cd, Fe, Mn, Pb and Zn were: 0.48, 0.61, 0.59, 0.38, 0.57, 0.29 and 0.22; 0.51, 0.36, 0.34, 0.54, 0.19, 0.11 and 0.18 in Bhavani river month of March and April respectively. The ranges of heavy metals in fish mussel (µg g^{-1}) Cu, Cr, Cd, Fe, Mn, Pb and Zn were 0.03, 0.02, 0.006, 0.03, 0.05, 0.02 and 0.02; 0.01, 0.006, 0.005, 0.04, 0.03, 0.02 and 0.03 in fish mussel month of March and April respectively. Eleven bacterial isolates were isolated from one samples of gut that were collected from of Bhavani river, Tamil Nadu, India. After screening, out of these 11 isolates only 3 bacterial isolates exhibited varying degree of heavy metal resistance potential against selected three heavy metals. The morphological and biochemical data assures that the isolated strain belong to the strains E1- *Vibrio cholerae*, E2 - *Bacillus sp* and E3 - *Bacillus subtilis* showed The results conclude that the isolated heavy metal resistance bacteria could be useful for the bioremediation of polluted wastewater and textile dyes.

Key words: Water Parameters, Heavy metal, Isolation and Tolerance bacteria.

1. Introduction

The study of different water parameters is very important for understanding of the metabolic events in river ecosystem. The parameters influence each other and also the water and sediment parameters, as well as they govern the abundance and distribution of the flora and the fauna. Therefore, it has become obligatory to analyze at least the important water parameters when ecological studies on river ecosystems are carried out (Nargis, 2006). Physico-chemical studies is also provides stable information with regards to incoming undesirable effluents, which eventually indicate the possible source of such effluents. Surface waters, such as rivers, dams and estuaries often serve as disposal systems for wastes coming from residential areas, industries and manufacturing plants. The consequence of increased degradative processes is the consumption of dissolved oxygen and production of ammonia from the decomposition of organic nitrogen compounds leading to impaired metabolism in fish and invertebrate communities. Among the several characteristics of water quality, the most important factors such as rainfall, atmospheric temperature, surface water temperature, turbidity, salinity, pH, dissolved oxygen, total dissolved solids, total

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alkalinity, total hardness, chloride, fluoride, biological oxygen demand, chemical oxygen demand, total organic carbon and nutrients like phosphate, silicate, nitrate, nitrites, ammonia and heavy metals viz., copper, chromium, cadmium, lead, zinc and iron will play an important role in the life of fresh water organisms (Singh and Singh, 2006).

Heavy metal bearing sediments enter river systems by discharging of domestic sewage, contaminated sites, agricultural fields, industrial waste, mine or processing waste, tailing dam failures, remobilization of mining areas and mine drainage. The metal contaminates in the river ecosystem were reported to remain either in soluble or suspension form and finally tend to settle down to the bottom sediments are taken up by organisms including fish (Oronsaye *et al*., 2010). Heavy metals in the water and 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 behaviour, transport and biological effects of these elements in river waters. Heavy metals entering the river fish have a possibility to get accumulated in different parts of the body and the residual amount can build up to a toxic level (Vinodhini and Narayanan, 2008).

Bioaccumulation and magnification is capable of leading to toxic level of these metals in fish even when the exposure is low. The presence of metal pollutant in freshwater is known to disturb the delicate balance of the aquatic systems. Fishes are notorious for their ability to concentrate heavy metals in their muscles and since the play important role in human an nutrition, they need to be carefully screened to ensure that unnecessary high level of some toxic trace metals are not being transferred to man through fish consumption (Agash *et al*., 2009).

The modern techniques approaches for heavy metal removal such as chemical oxidation of heavy metal have been associated with some limitations as they generate additional pollution and are cost ineffective. Thus, quest for alternative approach which suffices the above issues led to the development of microbial approach for remediation of heavy metal contaminated environments. As this process is cost efficient and have higher effectiveness at low metal concentrations, makes it an attractive tool compared to physiological/ conventional approaches for the remediation of metal pollution. Heavy metals and microorganisms have co-existed since early history and their survival in polluted soils depends on intrinsic biochemical and structural properties, physiological, or genetic adaptation including morphological changes of cells, as well as environmental modifications of metal speciation (Gupta Mahendra *et al*., 2014). Thus, the present study was investigated to physico – chemical parameters analysis, analysis of heavy metals from water and fish sample, isolate and characterize heavy metal to resistance bacteria from the gut samples collected from the Bhavani river, Erode District, Tamil Nadu, India.

2. Materials and Methods

Physico – Chemical parameters analysis

Plastic containers were cleaned with diluted nitric acid and rinsed several times with tap water and finally rinsed once with sample water, collected from the Bhavani River. Samples were preserved and analyzed by adopting the procedures outlined by standard methods for various parameters (APHA, 2000). Water temperature was measured using a mercury thermometer. Care was taken to obtain a constant reading and the temperature was recorded in Celsius scale. pH (Hydrogen ion concentration) was measured using digital pH meter (Elico PH-131 Digital pH meter). Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) were estimated by the standard procedures described by Parsons *et al*. (1989). Nutrients (Inorganic nitrate, nitrite, phosphate and silicate, sulphates, phosphate and free Ammonia) were estimated by titration methods as described by Parsons *et al*. (1989).

Analysis of heavy metals from water and fish sample

The Water collected in sampling bottles were pre-conditioned with 5 % Nitric acid and later rinsed thoroughly with distilled de-ionized water. At each sampling site, the polyethylene

sampling bottles were rinsed at least three times before sampling was done. Pre-cleaned polyethylene sampling bottles were immersed about 10 cm below the water surface. About 0.5 L of the water samples were taken at each sampling site. Water analysis was carried out according to the procedure described earlier (Brooks *et al*., 1967). The samples were analyzed by using ELICO's SL-176 Double Beam Atomic Absorption Spectrophotometer.

The selected freshwater fish, like *C. catla* of Bhavani River, Erode district, were caught by the local fishermen using gill net of various sizes. The fish species were ice-packed and transported to the laboratory. The selected fish organs were removed and put it in Petri dishes to dry at 120° until reaching a constant weight. The dried tissue was placed into digestion flask and ultra pure concentrated nitric acid and hydrogen peroxide [1: I V/V] [SD fine chemicals] were added. The digestion flask was heated to 130°C until all the material was dissolved (Dybem, 1983). Digest was diluted with double distilled water appropriately. The elements like Cadmium, Chromium, Copper, Lead, Manganese, Iron and Zinc were assayed using ELICO's SL-176 Double Beam Atomic Absorption Spectrophotometer.

Isolation of Nickel and Cadmium tolerant bacteria

Ten fish samples of the *C. catla* species were collected from Erode region of Bhavani River and packed in sterile plastic bags on ice. Samples were immediately transported to the laboratory and processed within 30 minutes of their collection. Fishes were externally washed with sterilized water to reduce budding contamination with skin bacteria. Intestines were taken from fish aseptically, weighed and homogenized in a sterile glass homogenizer and then transferred to a sterile vial containing 100 ml of sterile 0.85 % NaCl prepared in deoinized water and agitated at 110 rpm for 30 minutes. After allowing the homogenized suspension to stand for 5 minutes, the bacterial isolates were isolated by dilution plating of the supernatant (0.2 ml) in Nutrient Agar (NA) media plates containing varied concentrations of Cd2+ and Cr+2 as analytical grade salts of CdCl2 and $K_2Cr_2O_7$ from their sterilized stocks (100 mM). Plates were incubated at 35±2 °C for 24 hours. Cadmium and Chromium (Potassium dichromate) tolerant bacterial colonies that developed at highest concentration of Cd2+ and Cr+2 supplemented media were observed.

3. Results

Physico – Chemical parameters

The maximum water temperature (32.54 ^{o}C) was observed during the month of April and the minimum water temperature (29.16 ^{o}C) was recorded during month of March (Figure - 1). The maximum pH (7.12) was observed during the month of April and the minimum pH (7.02) was recorded during month of March (Figure - 2). The maximum dissolved oxygen (4.28 mg/l) was observed during the month of April and the minimum dissolved oxygen (4.1mg/l) was recorded during month of March (Figure - 3). Minimum 1.44 mg/l and maximum 3.67 mg/l values of biological oxygen demand were recorded during the month of March and month of April (Figure - 4). Minimum 2.2 mg/l and maximum 2.34 mg/l values of chemical oxygen demand were recorded during the month of March and month of April (Figure - 5). The maximum nitrate (2.1 mg/l) was observed during the month of March and the minimum nitrate (1.7 mg/l) was recorded during month of April (Figure - 6). The maximum nitrite (6.34 mg/l) was observed during the month of April and the minimum nitrite (6.12 mg/l) was recorded during month of March (Figure - 7). Minimum 1.1 mg/l and maximum 3.13 mg/l values of phosphate were recorded during the month of April and month of March (Figure - 8). Minimum 19.41 mg/l and maximum 58.63 mg/l values of silicate were recorded during the month of April and month of March (Figure - 9). The maximum sulphate (984 mg/l) was observed during the month of March and the minimum sulphate (687 mg/l) was recorded during month of April (Figure - 10). The maximum free ammonia (2.71 mg/l) was observed during the month of April and the minimum free ammonia (0.9 mg/l) was recorded during month of March (Figure - 11).

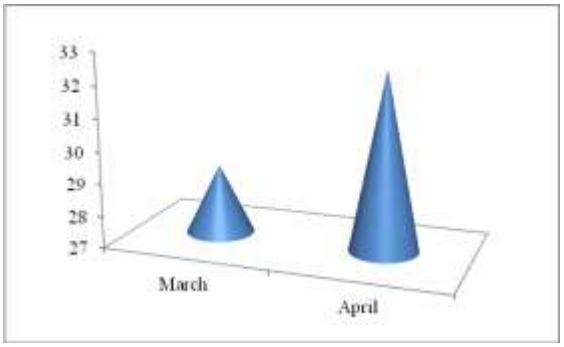

Figure – 1: Monthly variation in temperature

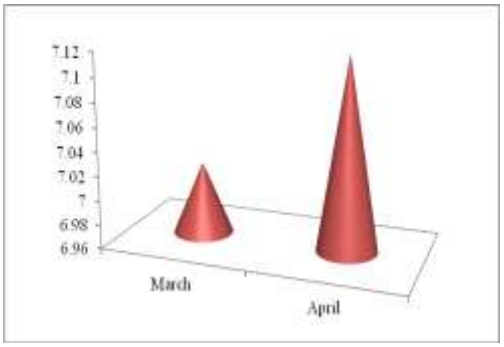

Figure - 2: Monthly variation in pH

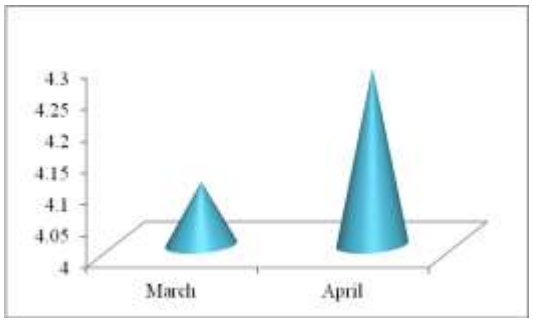

Figure – 3: Monthly variation in DO

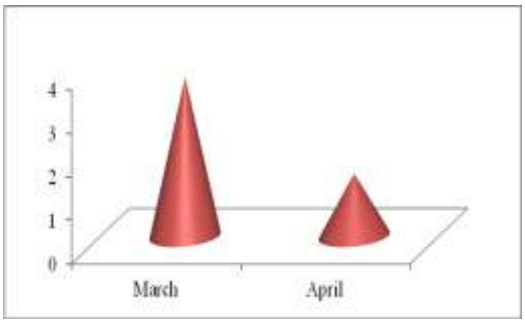

Figure – 4: Monthly variation in BOD

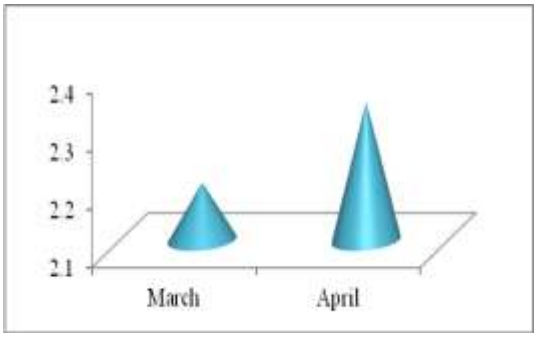

Figure – 5: Monthly variation in COD

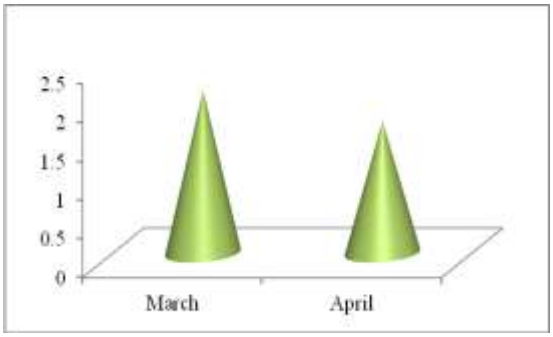

Figure – 6: Monthly variation in nitrate

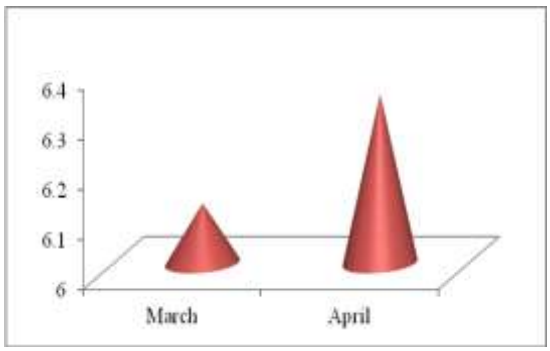

Figure - 7: Monthly variation in nitrite

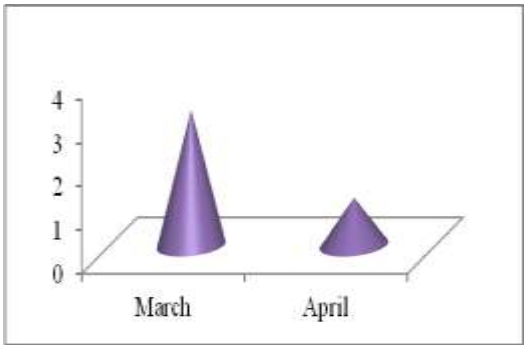

Figure – 8: Monthly variation in phosphate

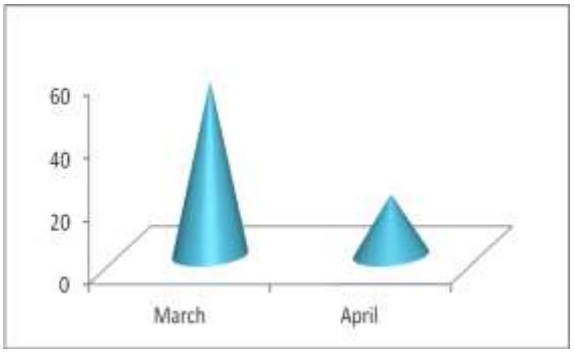

Figure – 9: Monthly variation in silicate

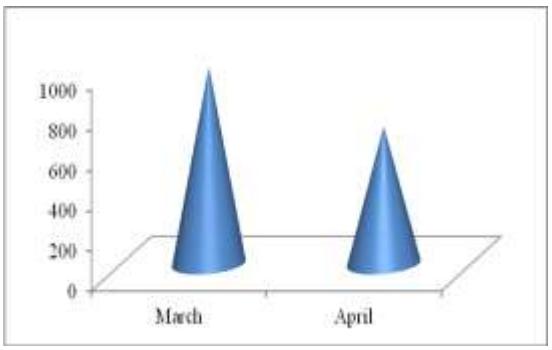

Figure - 10: Monthly variation in sulphate

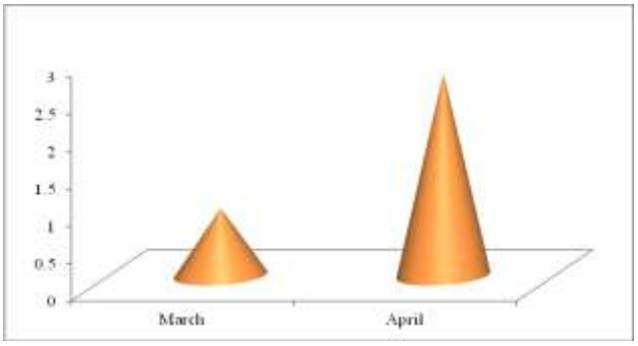

Figure – 11: Monthly variation in free ammonia

Heavy metals

The ranges of heavy metals in water (µg L^{-1}) Cu, Cr, Cd, Fe, Mn, Pb and Zn were: 0.48, 0.61, 0.59, 0.38, 0.57, 0.29 and 0.22; 0.51, 0.36, 0.34, 0.54, 0.19, 0.11 and 0.18 in Bhavani river month of March and April respectively (Figure - 12). The ranges of heavy metals in fish mussel (µg g^{-1}) Cu, Cr, Cd, Fe, Mn, Pb and Zn were 0.03, 0.02, 0.006, 0.03, 0.05, 0.02 and 0.02; 0.01, 0.006, 0.005, 0.04, 0.03, 0.02 and 0.03 in fish mussel month of March and April respectively (Figure -13).

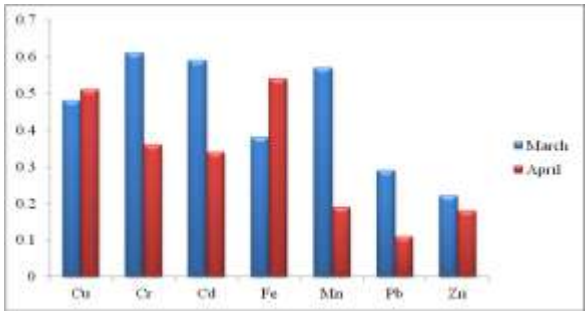

Figure – 12: The ranges of heavy metals in water and fish gut

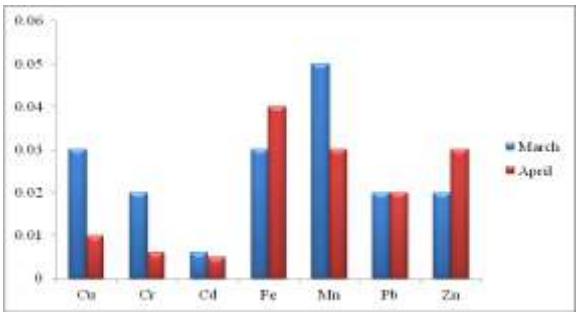

Figure – 13: The ranges of heavy metals in water and fish gut

Isolation and screening of heavy metal resistant bacteria

Eleven bacterial isolates were isolated from one samples of gut that were collected from of Bhavani river. After screening, out of these 11 isolates only 3 bacterial isolates exhibited varying degree of heavy metal resistance potential against selected three heavy metals (Table - 1 and Table - 2). Hence, these isolates were selected for further study.

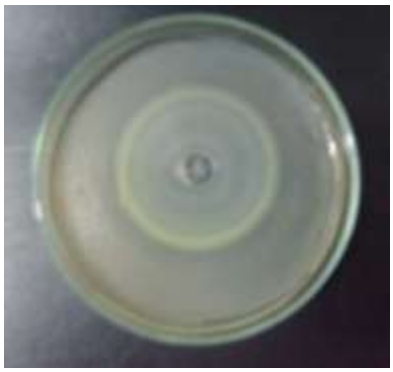

Figure – 14: Heavy metal resistant bacteria

Table – 1: Comparative Cd2+ tolerant assay between the three isolates

Bacteria	B	0.5	1	2	3	4	5	6	7	8	9	10
E1	-	-	+	+	+	+	-	-	-	-	-	-
E2	-	+	+	+	+	+	+	+	-	-	-	+
E3	-	+	+	+	+	+	+	+	+	+	+	+

(+ denotes growth, - denotes no growth)

Table – 2: Comparative Cr+2 tolerant assays between the three isolates

Bacteria	B	0.5	1	2	3	4	5	6	7	8	9	10
E1	-	-	+	+	+	-	-	-	-	-	-	-
E2	-	-	-	+	+	+	+	+	-	-	-	-
E3	-	-	+	+	+	+	+	+	+	+	-	-

(+ denotes growth, - denotes no growth)

Isolation and identification of gut bacteria

In the present study five bacterial strains were recovered from *Catla catla* gut region of healthy fish. After analysis of the samples (Fish gut), the total heavy metals tolerant bacterial count in fish gut was found to be 6.7×10^7, 5.7×10^6 and 5.4×10^6 CFU/g respectively. The morphological and biochemical data assures that the isolated strain belong to *Bacillus* sp. by comparing characteristic features as given in the seventh edition of Bergey's manual of deterministic bacteriology. The strains E1 - *Vibrio cholerae*, E2 - *Bacillus* sp. and E3 - *Bacillus subtilis* showed in Table - 3. The isolated strains were cultured and extracted to against the heavy metals tolerant strains were selected for further resistance study.

Table – 3: Biochemical characteristics of isolated gut bacteria

Biochemical test	E1	E2	E3
Gram reaction	-	+	+
Shape	Rod	Rod	Rod
Methyl red	+	-	-
Catalase	+	+	+
Indole motility	+	-	-
Motility	-	+	+
Voges Proskauer test	+	+	+
Nitrate reduction	+	+	+
Lactose	+-	-	+
Manitol	+	-	+
Glucose	+	+	+
Maltose	+	+	+
Arabinose	-	-	+
Oxidase	+	+	+
Nylose	-	-	+
Citrate	+	-	-
Skim milk	-	-	+
Starch	+	+	+

(+) = positive, (-) = negative

4. Discussion

The effect of exposure to the atmospheric temperature varies from one area to another depending on the time of the day during which low tide occurs. In the present investigation, the maximum water temperature (32.54 ^oC) was observed during the month of April and the minimum water temperature (29.16 ^oC) was recorded during month of March. During summer, solar radiation and clear sky enhanced

the atmospheric temperature to the maximum and during monsoon season, rainfall and cloudy sky brought down the atmospheric temperature. In general, pH was low during the monsoon season associated with low salinity due to dilution of seawater by freshwater. In the present investigation, the maximum pH (7.12) was observed during the month of April and the minimum pH (7.02) was recorded during month of March. High values of pH during summer might be low water levels and concentration of nutrients in water. The decrease pH values were due to dilution caused by the rain water during monsoon.

The amount of dissolved oxygen in water depends on several factors including temperature, volume and velocity of water flow in the water body and the number and diversity of organisms using oxygen for respiration. In the present investigation, the maximum dissolved oxygen (4.28 mg/l) was observed during the month of April and the minimum dissolved oxygen (4.1mg/l) was recorded during month of March. In the present investigation, the Minimum 1.44 mg/l and maximum 3.67 mg/l values of biological oxygen demand were recorded during the month of March and month of April. The higher levels of BOD in the polluted water indicate the presence of higher concentration of organic matter. In the present studies minimum 2.2 mg/l and maximum 2.34 mg/l values of chemical oxygen demand were recorded during the month of March and month of April. The chemical oxygen demand is a measure of the oxidation of reduced chemicals in water.

The presence of nitrate in fresh water bodies depends mostly upon the activity of nitrifying bacteria, domestic and agricultural source. In the present investigation, the maximum nitrate (2.1 mg/l) was observed during the month of March and the minimum nitrate (1.7 mg/l) was recorded during month of April. These changes commonly associated with erosion and transportation of nitrogenous rich fertilizers, soil and local sources into the water. Nitrite is formed from ammonia and may be accumulated in aquatic systems as a result of imbalances of nitrification process by bacterial activity. High level of nitrite in water is a potential factor triggering stress in aquatic organisms. In the present investigation, the maximum nitrite (6.34 mg/l) was observed during the month of April and the minimum nitrite (6.12 mg/l) was recorded during month of March.

Phosphates are essential for the growth of organism and a nutrient that limits primary productivity of the water body. In the present investigation, the minimum 1.1 mg/l and maximum 3.13 mg/l values of phosphate were recorded during the month of April and month of March. The low content of phosphate in summer season may be due to utilization of phosphate by the phytoplankton. In the present studies, minimum 19.41 mg/l and maximum 58.63 mg/l values of silicate were recorded during the month of April and month of March. The silicate content was higher than that of the other nutrients and the recorded high April values could be due to large influx of fresh water derived from land drainage carrying silicate leached out from rocks and also from the bottom sediment.

Sulfates can interfere with the disinfection efficiency by scavenging residual chlorine in the distribution system. In the present investigation, the maximum sulphate (984 mg/l) was observed during the month of March and the minimum sulphate (687 mg/l) was recorded during month of April. Sulphate content in the water is strongly influenced by the geological nature of the underlying rocks. Free ammonia in natural waters is due to the presence of dissolved ammonia in water. In the present determination, The maximum free ammonia (2.71 mg/l) was observed during the month of April and the minimum free ammonia (0.9 mg/l) was recorded during month of March. Even at low concentration, ammonia is toxic especially to aquatic organisms and the increased level of ammonia from Bhavani river shows its polluted nature.

Heavy metal pollution exerts adverse effects on environment and living organisms. Inadequate metropolitan sanitary transportation, lack of well–organized implementation of desirable pollution control measures has complicated the condition. The heavy metals and

inorganic pollutants of stream water have gain the attention of scientists of all over world due to their non–degradable nature which creates a lethal biological effect on living organisms when added frequently through tropic level. Bhavani River is also found highly contaminated like other mentioned rivers due to the rapid anthropogenic expansion around it. In the present investigation the ranges of heavy metals in water (µg L^{-1}) Cu, Cr, Cd, Fe, Mn, Pb and Zn were: 0.48, 0.61, 0.59, 0.38, 0.57, 0.29 and 0.22; 0.51, 0.36, 0.34, 0.54, 0.19, 0.11 and 0.18 in Bhavani river month of March and April respectively. The ranges of heavy metals in fish mussel (µg g^{-1}) Cu, Cr, Cd, Fe, Mn, Pb and Zn were 0.03, 0.02, 0.006, 0.03, 0.05, 0.02 and 0.02; 0.01, 0.006, 0.005, 0.04, 0.03, 0.02 and 0.03 in fish mussel month of March and April respectively. Cadmium was found to be slightly higher (0.02 µg L) in the T5 and T7 sites of the river Thamirabarani (Raghavan Kuppu *et al*., 2018).

Prabudoss kumar *et al*. (2019) studied that the higher concentration is 13.93 µg g^{-1} in Ambasamuthiram and Vickramasingapuram and lower concentration 10.58 µg g^{-1}. Zarith Sufiani Baharoma and Mohd Yusoff Ishak (2015) reported that the heavy metal accumulation in important tilapia fish species, *Oreochromis niloticus* (µg L^{-1}) (Zn 0.434, Cu 0.03, Pb 0.053, Cd 0.016) was analysed river in Malaysia. The heavy metal concentrations in the downstream indicated an increase in the pollution load due to movement of suspended and dissolved industrial effluents and anthropogenic wastes. Thus, the present study defines a warning alarm for the proper remediation steps to be taken to safeguard the natural water resources as well as the aquatic ecosystem.

Elevated concentration of heavy metals in the environment builds selective pressure on the microorganism for the endurance in a polluted environment. The river Mula is highly contaminated with various industrial and domestic discharges (Eknath *et al*, 2013) including organic and metal containing pollutants. Previous studies have documented an isolation of heavy metal tolerant organisms from river Mula (Daware *et al*, 2012). In the present investigation contamination in study area of Bhavani River s water samples and chromium and cadmium resistance in the bacteria from the fish samples were also found. Hence a more intensive study is needed in order to determine the bioaccumulation and bioremediation of heavy metals in water and fishes from the study area.

The present study reveals water quality parameters of river water showed that concentrations of physico-chemical parameters were above the fresh water level due to continuous discharge of solid waste due to garbage, cleaning of vehicles, aquaculture waste water, domestic sewage and industrial effluents and the river is severely polluted. Pollution caused by a plethora of human activities primarily affects physicochemical characteristics and heavy metal of water leading to the destruction of community disrupting delicate food webs, deteriorating aquatic environment. In the present report pollution levels in surface water often show marked monthly fluctuations with higher concentrations being found during month of March compared to month of April.

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