# BACTERIAL COMMUNITY AND PHYSICO-CHEMICAL CHARACTERISTICS OF MUTHUPETTAI MANGROVE ENVIRONMENT, SOUTHEAST COAST OF INDIA

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**Abstract**

A study on bacterial community and physico-chemical parameters of water and sediment samples in mangrove environment of Muthupettai, South east coast of India was carried out in 2006-07. Six stations in an around the mangrove sites were selected for sampling and the following parameters were recorded at monthly intervals atmospheric temperature, surface water temperature, sediment temperature, salinity, water pH, sediment pH, dissolved oxygen, nitrite, nitrate , total nitrogen, inorganic phosphate, total phosphorus, ammonia, silicate and the microbial quality like total heterotrophic bacteria (THB), total coliforms (TC), faecal coliforms (FC), pathogenic bacteria as Vibrio cholerae, V. parahaemolyticus, Escherichia coli, Salmonella spp., Shigella spp., Klebsiella spp., Streptococcus spp. and Pseudomonas spp. THB strains (six hundred and seventy) and Vibrio strains (one hundred and twenty five) were isolated from mangrove environment. When compared to nutrient distribution, water nutrients were consistently higher in the mangrove environment. Partially treated aquaculture waste water are having additional sources of nutrients. Although, the mangrove habitat has been demonstrated to possess self-cleaning properties, data obtained warms possible anthropogenic pollution in the mangrove area in near future if the present conditions prevailed for a long period.

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**Key words:** Mangrove, Bacterial community, Muthupettai, Nutrients, Physico-chemical characteristics.

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## Introduction

Mangrove wetlands along the coastal zone act as barrier against cyclones, protect coastal erosion and provide good nursery ground for number of commercially important aquatic organisms (Janaki-Raman et al. 2007).In mangroves and coastal waters, factors related to water quality such as temperature, salinity, pH, dissolved oxygen and nutrients are particularly important for determining the biological factors and ecosystem functions (Paramasivam and Kannan, 2005). Many mangrove environments are receiving pollutants due to varied activities taking place around the mangrove areas and with the inflowing freshwaters, thus resulting in the degradation of water quality. Therefore, it is necessary to monitor their habitat characteristics and water quality (Paramasivam and Kannan, 2005).

The abundance and distribution of total heterotrophic bacteria have a direct bearing on other forms of nutrients in different compartments of the environment. Microbial indicators have been used world wide to indicate if human wastes have contaminated a water body and they are the most common member of faecal coliforms which indigenous to the intestinal tract of human and other warm-blooded animals. The microbes more commonly utilized are those found in elevated concentrations in human feces. The typical indicators used in the mangrove environment include total coliforms, faecal coliforms, *Esherichia coli* and *Enterococci*.

In this paper, we report on the bacterial community and physico-chemical characteristics of Muthupettai mangrove environment, Southeast coast of India.

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## Materials and methods

### Study area

Muthupettai mangroves (Lat. 10° 25'N; Long. 79° 39'E) situated 400km south of Chennai lies along the south east coast of India. It has total area of 6800ha in which the water spread area covers approximately 2720 ha (Fig.1).

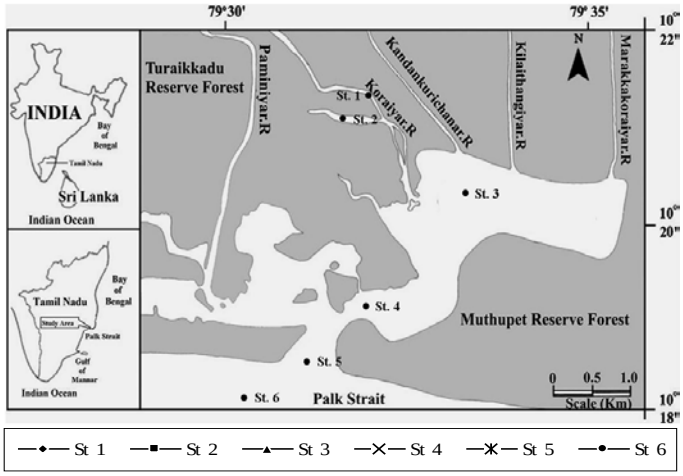



**Figure 1.** Map of Muthupettai mangrove environment showing different locations.

It has two specialized habitats viz. mangroves and lagoon. Many tributaries of the river Cauvery delta such as Paminiyar, Koraiyar, Kilaithangiyar, Kandankurichanar and Marakkakoraiyar flow through Muthupettai and nearby villages and form a lagoon before they enter into the sea, Bay of Bengal. *Avicennia marina* is the dominant mangrove species in Muthupettai and accounts for nearly 95% of the vegetative cover. The sampling areas of Station 1 (Aquaculture discharge area); Station 2 (Sethuguda); Station 3 (Lagoon); Station 4 (Sellimunai); Station 5 (Sea mouth region) and Station 6 (Open Sea) were selected for the present study.

Surface water and sediment samples at six stations were collected at monthly intervals during April (2006) to March (2007) and transported to the laboratory by keeping them in ice box and processed within 24 hours and microbial analysis were carried within 4 hours.

### Physico-chemical analyses

Initial measurements on temperature (digital thermometer), pH (pH Scan 1 Tester-Eutech Instruments) and salinity (Refractometer Atago F/mill 8901) of the water samples were made onboard and dissolved oxygen was estimated by the modified Winkler's method described by Strickland and Parsons (1972). Concentration of water nutrients such as nitrite ($NO_2$), nitrate ($NO_3$), total nitrogen, inorganic phosphate, total phosphorus ($PO_4$), ammonia ($NH_4$) and silicate ($SiO_3$) were analyzed by following the methods of Strickland and Parsons (1972) and APHA (1995). Sediment pH was measured according to Chattopadhyay (1980).Parsons correlation co-efficient was carried out for understanding the interrelationships between various physico-chemical parameters using SPSS-10.

### Microbiological analysis

### Total heterotrophic bacteria

The total heterotrophic bacteria (THB) count was determined on Zobell marine agar medium using the spread-plate technique, in triplicate. After 48h incubation the colonies were counted. Morphologically diverse colonies were isolated from each plate and sub-cultivated several times on Zobell marine agar medium. Isolated strains were stored in a fridge at 4°C in marine agar slants (prepared with filtered seawater adjusted to 20% salinity with distilled water) (Sousa et al. 2006).

### *Vibrio* counting and isolation

*Vibrio* spp. were counted and estimates of numbers made using the compendium of microbiological methods (Downes and Ito, 2001). The colonies from each Thio sulphate citrate bile salt sucrose (TCBS) plate were selected and sub-cultured in marine agar medium for isolation of the strains (Sousa et al. 2006).

### Enumeration of enteric pathogens

Total enteric pathogens were enumerated by adopting the membrane filter technique using XLD Agar (Xylose Lysine Deoxycholate Agar) medium recommended for the selective isolation of enteric pathogens especially *Salmonella* sp., *Shigella* sp. and *Klebsiella* sp. species. After 48 hours of incubation, colonies appeared with a typical pink, pink to red colours of *Shigella* sp. and with yellow colours of *Salmonella* sp. and with black colours of *Klebsiella* sp. were isolated.

### Enumeration of total coli forms

Total coliforms (TC) were counted by standard membrane filter (MF) methods using MacConkey Agar. The MF method involves filtering a sample through 0.45µg pore size membrane filter (47mm diameter membrane, Fisher, Pittsburg, PA) that retains the bacteria (Tomoyuki et al. 2004) were impregnated in the Petri plates. Appropriate volumes of samples were filtered and placed on MacConkey Agar and incubated at 41°C for 24h.

**Enumeration of faecal coliforms**

The same MF method was used for enumerating faecal coliform utilized a modified M-FC agar (Tomoyuki et al. 2004) and incubated at 44±0.5°C for 24h and colonies with various shades of blue were counted as faecal coliforms.

**Enumeration of *Escherichia coli***

M7 Hr FC Agar was used as a selective medium for *E. coli* isolation by employing membrane filtration procedure.

**Enumeration of *Enterococci* sp.**

*Enterococci* sp were also analyzed by membrane filter (MF) methods using M-*Enterococcus* Agar. After 24 hours of incubation at 41°C, the colonies of *S. faecalis* appeared with maroon colour were counted.

**Enumeration of *Pseudomonas aeruginosa***

Cetrimide Agar was used as selective medium for the isolation of *P. aeruginosa* by employing membrane filtration procedure. After 48 hours of incubation at 37°C, the colonies of *P. aeruginosa* appeared with luxuriant yellow colour were enumerated.

**Biochemical analysis**

All the strains were identified using biochemical analysis according to Bergey's manual of determinative bacteriology (Buchanan and Gibbons, 1974) without modifications. The bacteria isolated from the TCBS plates (selective for Vibrios), were further identified by biochemical tests described by Tison (1999).

**Results**

**Physico-chemical characteristics**

Atmospheric temperature, surface water temperature, sediment temperature, salinity, water pH, sediment pH, dissolved oxygen, nitrite, nitrate, total nitrogen, inorganic phosphate, total phosphorus, ammonia and silicate values are shown in Fig. 2 – 15. In general there is only very little spatial variation in most of the physical-chemical parameters recorded during the study outing to their closer geographical location. However, there is a cleat temporal variations in most of these parameters. Specifically speaking increasing amount of water nutrients recorded during the monsoon season correlating with land run off and higher river water inflow.

Regarding correlation study between the parameters of water, station 1 water temperature showed a significant negative correlation with dissolved oxygen (r = -857). Nitrate showed a significant positive correlation with total phosphorus at *p* =0.01 level. In station 2 atmospheric temperature showed a significant positive correlation with salinity (r = 915). Ammonia showed a significant negative correlation with inorganic phosphate at *p* =0.01 level. In station 3 pH showed a significant negative correlation with nitrite (r = -842). While silicate exhibited a positive correlation with total nitrogen and significant at *p* =0.01 level. In station 4 total nitrogen showed a significant positive correlation with inorganic phosphate (r = 959). Silicate showed a significant positive correlation with total phosphorus at *p* =0.01 level. In station 5 atmospheric temperature showed a significant negative correlation with total nitrogen (r = -802) and ammonia showed a negative correlation with total phosphorus at *p* =0.01 level. In station 6 salinity showed a significant negative correlation with dissolved oxygen (r = -840). Nitrate showed a positive correlation with inorganic phosphate at *p* =0.01 level.

**Microbiological analysis**

Total heterotrophic bacteria, *Vibrio cholerae, Vibrio parahaemolyticus, Escherichia coli, Salmonella* spp., *Shigella* sp., *Klebsiella* sp., *Streptococcus* sp., *Pseudomonas* sp., total coliforms and faecal coliforms values are shown in Fig. 16-37.

Regarding correlation study between the parameters of water and sediment, station 1 total heterotrophic bacteria showed a significant positive correlation with *V. parahaemolyticus* (r = 946). *Escherichia coli* showed a positive correlation with *Klebsiella* sp. and significant at *p* =0.01 level. In station 2 *V. cholerae* showed a significant positive correlation with total coliforms (r = 958). *Salmonella* spp. showed a positive correlation with *Pseudomonas* sp. and significant at p =0.01 level. In station 3 *Shigella* sp. showed a significant positive correlation with faecal coliforms (r = 922). The *Klebsiella* sp. exhibited a positive correlation with *Pseudomonas* sp. and significant at p =0.01 level. In station 4 faecal coliforms showed a significant positive correlation with *Pseudomonas* sp. (r = 963). *Shigella* sp. showed a positive correlation with faecal coliforms and significant at *p* =0.01 level. In station 5 *V. cholerae* showed a significant positive correlation with *Salmonella* spp. (r = 959). *Klebsiella* sp. showed a negative correlation with faecal coliforms and significant at *p* = 0.01 level. In station 6 *V. parahaemolyticus* showed a significant positive correlation with total coliforms (r = 921). *E.*

*coli* showed a positive correlation with *Shigella* sp. and significant at $p = 0.01$ level.

**Identification of total heterotrophic bacteria**

Totally 670 strains were isolated based on colony morphology from THB plates for identification of genus (Table 1). The majority of the isolated strains from station 2 and station 5 were identified. The lowest number of isolates was identified from station 6.

**Identification of *Vibrio* spp.**

One hundred and twenty five strains isolated on the *Vibrio* TCBS selective medium were characterized phenotypically (Table 2). The number and names of species isolated from the TCBS plates. The greatest number of different species isolated was found at station 1. The second greatest number of species isolated was detected at station 2 and 5. The lowest number of isolates was identified from station 6.

| Identification of Genus | St.1 | St.2 | St.3 | St.4 | St.5 | St.6 |
|---|---|---|---|---|---|---|
| *Vibrio* spp. | 21 | 18 | 20 | 17 | 18 | 20 |
| *Escherichia* spp. | 16 | 14 | 16 | 13 | 15 | 17 |
| *Shigella* spp. | 9 | 13 | - | 10 | 12 | 9 |
| *Pseudomonas* spp. | 11 | 15 | 12 | - | 12 | 10 |
| *Klebsiella* sp. | - | 8 | 4 | 7 | 10 | 4 |
| *Salmonella* spp. | 10 | 12 | 8 | 12 | 14 | 11 |
| *Bacillus* sp. | 7 | 9 | 11 | 8 | 10 | 7 |
| *Flavobacterium* sp. | - | 5 | 5 | 6 | 4 | 3 |
| *Alcaligenes* sp. | 3 | 6 | 5 | - | 6 | - |
| *Aeromonas* sp. | 6 | 8 | 3 | 6 | 8 | 6 |
| *Alteromonas* sp. | 3 | 5 | 3 | 7 | 6 | - |
| *Chromobacterium* sp. | 9 | 8 | 10 | 8 | 5 | - |
| *Enterobacter spp.* | 8 | 10 | 11 | 9 | 11 | 7 |
| Total number of isolates | 103 | 131 | 108 | 103 | 131 | 94 |

**Table 1.** Heterotrophic bacteria identified from mangrove environment.

| Identification of Species | St.1 | St.2 | St.3 | St.4 | St.5 | St.6 |
|---|---|---|---|---|---|---|
| *V.cholerae* | 12 | 8 | 3 | 1 | 8 | 4 |
| *V.Parahaemolyticus* | 10 | 5 | - | 5 | 7 | 6 |
| *V.vulnificus* | 7 | 5 | 4 | - | 3 | - |
| *V. alginolyticus* | 5 | 3 | - | 4 | 4 | 2 |
| *V.fluvialis* | 1 | - | 3 | - | 3 | - |
| *V.furnissii* | - | 3 | - | 1 | - | 1 |
| *V. harveyii* | 3 | 1 | - | 2 | - | 1 |
| Total number of isolates | 38 | 25 | 10 | 13 | 25 | 14 |

**Table 2.** List of *Vibrio* spp. Identified from mangrove environment.

**Discussion**

Study attempted to correlate the native physico-chemical parameters with bioavailability of different antibiotic resistant bacteria and its population in the mangrove environment of Muthupettai, India. Temperature is one of the most important factor responsible for the regulation of physiological activities of microorganisms. In nature, each species has its own optimal temperature requirements for their growth as well as development (Paramasivam and Kannan, 2005). In the present study, the seasonal mean temperature was maximum in summer and minimum in monsoon. Temperature fluctuation was observed between 22.8 and 34.8°C. Slightly lower temperature was recorded at Station 2 and higher in station 4. All the stations recorded lower ($0^{o}/_{oo}$) salinity during the monsoon season than the other seasons reaching the maximum ($43.1^{o}/_{oo}$) during the summer when there is no fresh water flow in the rivers. Higher pH recorded (8.4) during the summer in the present study due to the removal of $CO_2$ by the photosynthetic organisms and the lower pH observed (7.2) during the monsoon season. The first two factors encourage a heating of the water during the day, provoking evaporation and an increase in the salinity values and rain reduces the temperature, pH and salinity during monsoon period.

The present study observed that the nitrite, nitrate and total nitrogen concentrations in water and sediment nitrogen in higher ranges (1.362µmol/l, 5.436µmol/l, 25.361µ mol/l and 8.59µg/g) respectively. Inorganic phosphate and total phosphorus concentration of water were observed in range between 0.36 to 1.23 µ mol/l, 1.71 to 5.36µ mol/l respectively and sediment phosphorus was in the range of 0.75 to 2.67µg/g. Regarding concentration of ammonia in water higher range in (0.077µ mol/l) monsoon season. The maximum concentration of reactive silicate (156.8µmol/l) was observed during the monsoon period and the minimum recorded during summer. The results were highly correlate with the results of Paramasivam and Kannan (2005). The increased level of nutrients in water and sediment during monsoon period due to land run off and freshwater inflow in the rivers through leaching from manured and fertilized agricultural soils, aquaculture discharge and sewage effluents from the surrounding environment.

Higher THB population density of water ($71\times10^{6}$ CFU $ml^{-1}$) and sediments ($75\times10^{7}$ CFU $g^{-1}$ dry wt) was recorded from Station 2. Kathiresan (2000) has reported that dead organic matter in the sediments of the mangrove area favors the higher bacterial growth in this environment. The lower density ($31\times10^{6}$ CFU $ml^{-1}$) in water were recorded at station 6 and ($39\times10^{7}$ CFU $g^{-1}$ dry wt) in sediment were recorded at Station 5. This could be possible because this mangrove area is open in nature as the mangrove leaves takes more than 10 days to

get decompose (Kathiresan, 2000) and more than 50% of the mangrove leaves were transported to other places before they get decomposed (Ajithkumar *et al.,* 2006).

In the present study, Higher *V. cholerae* and *V. parahaemolyticus* population density in water and sediment ($41\times10^3$ CFUml$^{-1}$) and ($45\times10^4$ CFU g$^{-1}$dry wt) were recorded from Station 1 with shrimp forms effluent pollution and indicators of sewage pollution. The lower density was recorded in the water ($16\times10^3$ CFUml$^{-1}$) and sediment ($19\times10^4$ CFU g$^{-1}$ dry wt) samples of Station 5. Higher *V. parahaemolyticus* population density in water and sediment ($38\times10^3$ CFUml$^{-1}$) and ($42\times10^4$ CFU g$^{-1}$dry wt) were observed from station 4 and station 6. The lower density was recorded in the water ($12\times10^3$ CFUml$^{-1}$) and sediment ($16\times10^4$ CFU g$^{-1}$ dry wt) samples of Station 5. The results were similar by Sousa et al, 2005.

Higher total coliforms population density in water and sediment ($63\times10^5$ CFUml$^{-1}$) and ($68\times10^6$ CFU g$^{-1}$dry wt) were recorded from Station 6. The total coliforms are the most widely used indicators for the microbial pollution evaluation of fresh and marine waters, which suggest that coliforms don not survive in high salinities particularly under the sunlight. The lower density was recorded in the water ($25\times10^3$ CFUml$^{-1}$) and sediment ($27\times10^4$ CFU g$^{-1}$ dry wt) samples of Station 3. The effect of sunlight is a function of the water salinity which indicates that coliforms in seawater are subjected to a lower rate of survival. Total coliforms are poorly resistant microorganisms in the natural environment.

Higher faecal coliforms population density in water and sediment ($55\times10^4$ CFUml$^{-1}$) and ($58\times10^5$ CFU g$^{-1}$dry wt) were recorded from Station 6. The presence of faecal coliforms in the seawater by the shore indicates that the source of pollution is likely to be domestic waste from the local sewage outfall, fresh water inflow and other unidentified sources of faecal pollution such as mangrove environment. The lower density was recorded in the water ($21\times10^3$ CFUml$^{-1}$) and sediment ($23\times10^4$ CFU g$^{-1}$ dry wt) samples of Station 3. Coliforms are poorly resistant microorganisms in the natural environment. However, the constant flow of domestic wastes produces high levels of pollution which permanently decreases the quality of seawater for recreational purposes. Similar results were observed by Hashim et al. 2005.

Higher population density *E. coli* in water ($32\times10^3$ CFUml$^{-1}$) and sediment ($35\times10^4$ CFU g$^{-1}$dry wt) were recorded from station 5 and 1. A monsoon season resulted in the higher loading of *E.coli* and subsequent high *E.coli* in mangrove environment. In addition, higher loading of nutrients with runoff could help the growth of *E.coli,* if any growth occurs. *E.coli* was present in sea water during all seasons in mangrove environment indicating that the water was contaminated by faecal material of humans or other warm-blooded animals and also indicates the potential for the presence of pathogenic organisms (Youn et al, 2002). The lower density was recorded in water ($15\times10^3$ CFU ml$^{-1}$) and sediment ($17\times10^4$ CFU g$^{-1}$dry wt) from station1 and 5. *E.coli* was lower in the summer season, which is lower loading of faecal material, fresh water inflow and the ecological condition of the mangrove environment. Similar results were observed by Youn et al. 2002.

Higher population density in water (28, 29, $26\times10^2$ CFU ml$^{-1}$) and sediment (30, 31, $28\times10^3$ CFU g$^{-1}$dry wt) samples were recorded from station 2, 3 and 5. The monsoon season observed in the higher loading of faecal materials and subsequent high freshwater in mangrove environment. Seawater quality criteria proposed by the world health organization and other international foundations are usually suggested enteric forms as microbiological pollution indicator. The combination of several indicators is likely to present a global picture of water quality. The lower density was recorded in water (11, 10, $6\times10^2$ CFU ml$^{-1}$) and sediment (13, 13, $9\times10^3$ CFU g$^{-1}$dry wt) from station 3 and 4. In summer season observed in the low of level of nutrients, changes in environmental parameters and decrease the faecal contamination. The results were similar by Arone and Walling, (2007).

In present study, higher population density in water ($49\times10^2$ CFU ml$^{-1}$) and sediment ($51\times10^3$ CFU g$^{-1}$dry wt) samples were recorded from station 2. Among feacal pollution indicators is a higher survival of streptococci in the marine environment. Sunlight inactivation of culturable cells of enterococci generally required 2-3 times the isolation for culturable cells of faecal coliforms. A decrease of inactivation rates of enterococci at lower temperatures was also observed. The lower density was recorded in water ($24\times10^2$ CFU ml$^{-1}$) and sediment ($26\times10^3$ CFU g$^{-1}$dry wt) from station 1. The presence indicated a recent contamination and showed that the mangrove was effectively subjected to a continuous animal pollution which decreased or disappeared in summer and similar results were reported by (Bouchriti et al. 1992).

Higher population density in water ($35\times10^2$ CFU ml$^{-1}$, and sediment ($39\times10^3$ CFU g$^{-1}$dry wt) samples were recorded from station 5 with regarded as organisms of a faecal origin. Especially higher amount of *Pseudomonas sp.* were found once in the bottom sediment of the higher number of organic

substance, phytoplankton, $PO_4$, $NO_3$, adsorbed to clay is a factor advantageous for proliferation and survival of these bacteria in the mangrove environments (Niewolak and Opieka, 2000). The lower density was recorded in water ($16\times10^2$ CFU $ml^{-1}$) and sediment ($19\times10^3$ CFU $g^{-1}$dry wt) from station 3 with less polluted in summer season. The results were similar by (Niewolak and Opieka, 2000).

Totally 680 strains of total heterotrophic bacteria were identified in genus levels at different stations, which are affected by monsoon season heavy fresh water inflow, agricultural discharges, shrimp effluent pollution with indicators of sewage pollution and this result suggests that perhaps other anthropogenic sources of pollution are present and influencing the microbial communities at all sites. The majority of the isolated strains from station 2 and 5 were identified, which are affected by aquaculture pond discharge water in mangrove environment. Similar results were observed by Hashim et al. (2005). The lowest number of isolates was identified at station 6. In the station, fresh water inflow, pollution sources and aquaculture effluent were low in marine environment.

One hundred and twenty five strains isolated were identified in species level at different stations. The greatest number of different species isolated was found at station 1. The second greatest number of species isolated was detected at station 2 and 5. In addition to the isolates which are important pathogens for humans and aquatic animals, the genus *Vibrio* also includes species involved in nutrient cycling such as *V. harveyii* not to mention others capable of breaking down chitin, aromatic polycyclical hydrocarbons which are extremely toxic for the environment (Thompson et al, 2004). Similar results were recorded by Sousa et al. 2006.

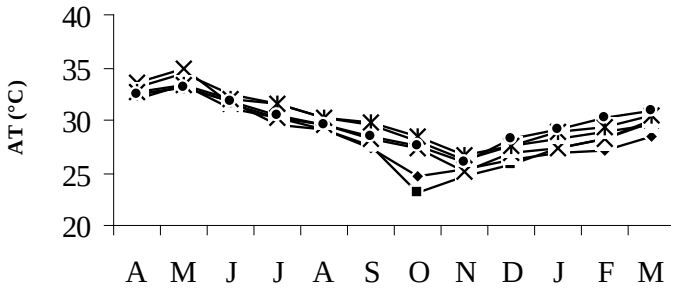



**Figure 2.** Atmospheric temperature

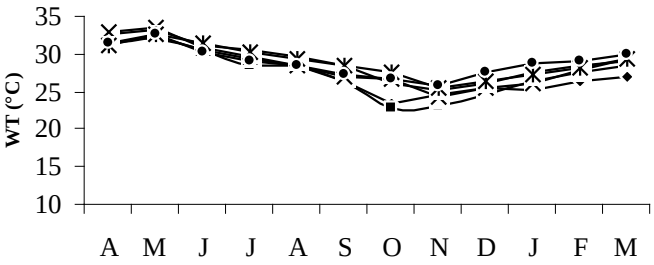



**Figure 3.** Surface water temperature

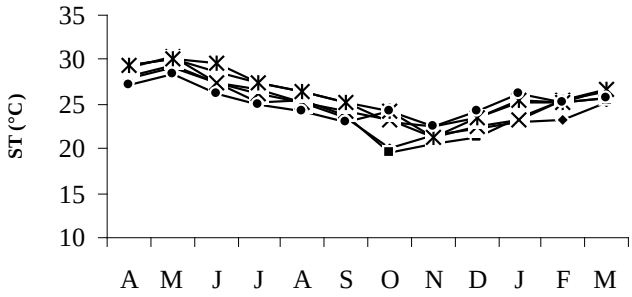
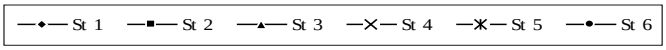



**Figure 4.** Sediment temperature



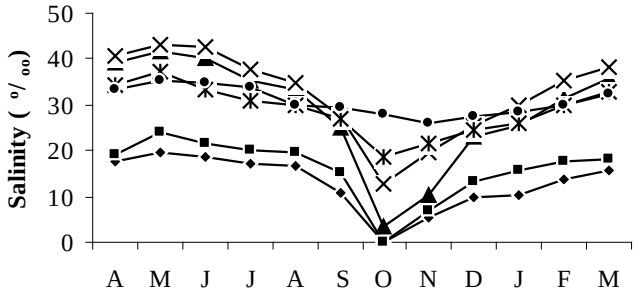




**Figure 5.** Salinity

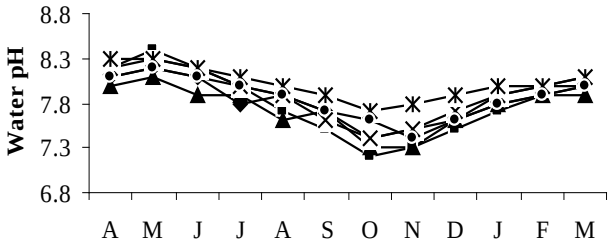



**Figure 6.** Water pH

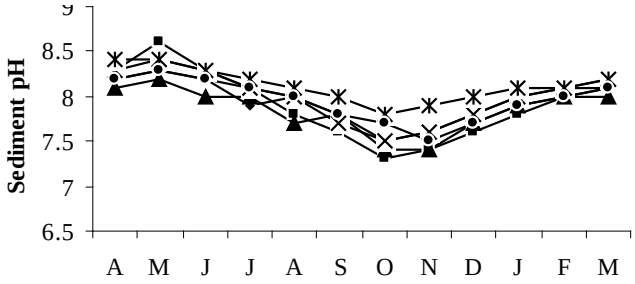
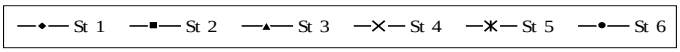



**Figure 7.** Sediment pH



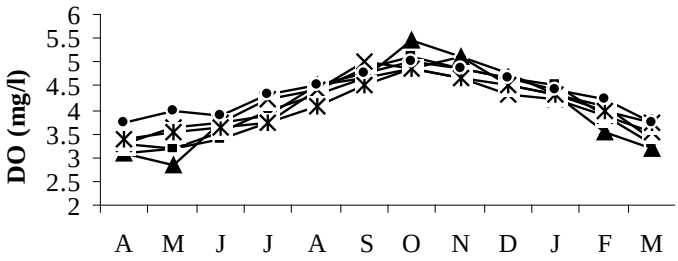




**Figure 8.** Dissolved oxygen

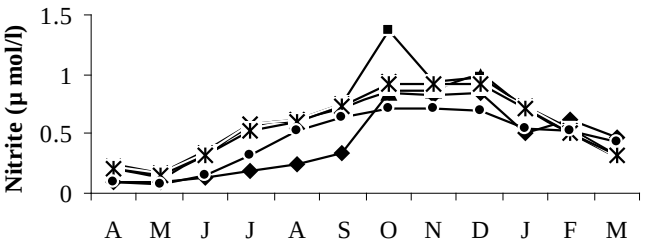



**Figure 9.** Nitrite

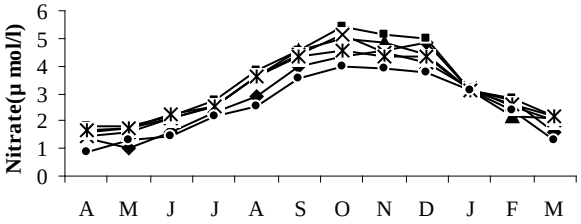
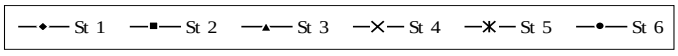



**Figure 10.** Nitrate



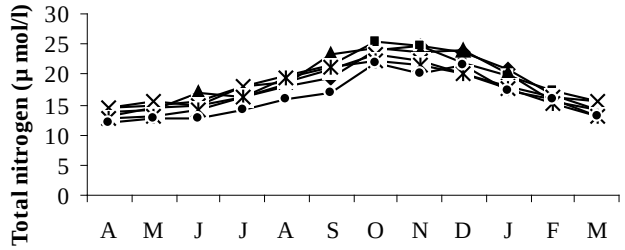




**Figure 11.** Total nitrogen

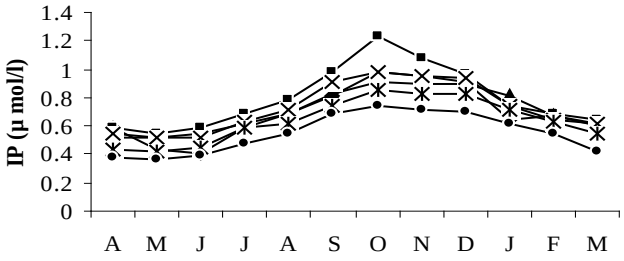



**Figure 12.** Inorganic phosphate

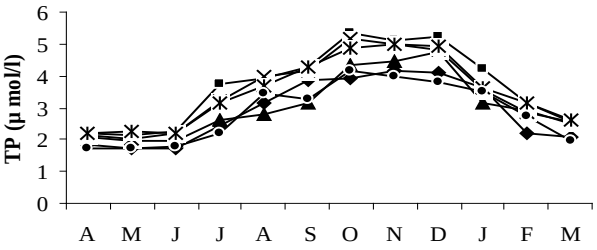



**Figure 13.** Total phosphorus

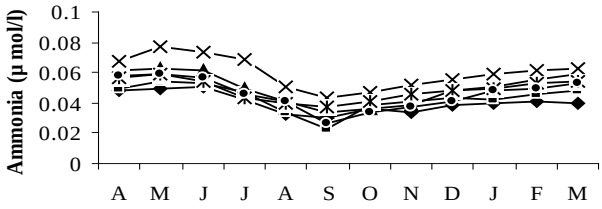



**Figure 14.** Ammonia

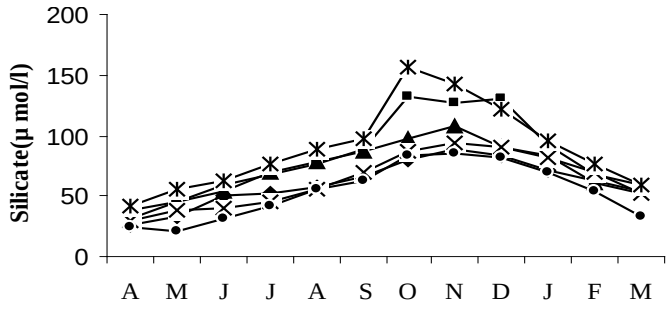



**Figure 15.** Silicate

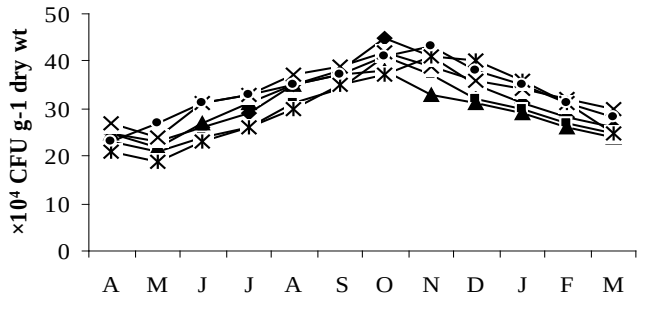
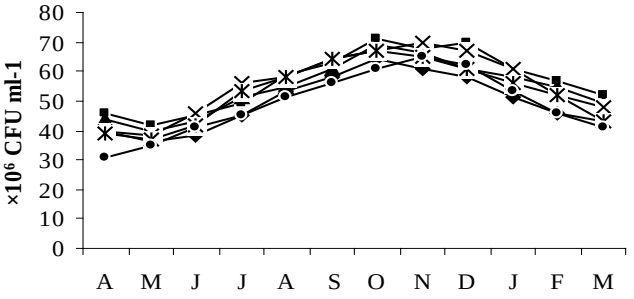



**Figure 19.** *Vibrio cholerae* in sediment



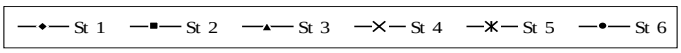




**Figure 16.** Total heterotrophic bacteria in water

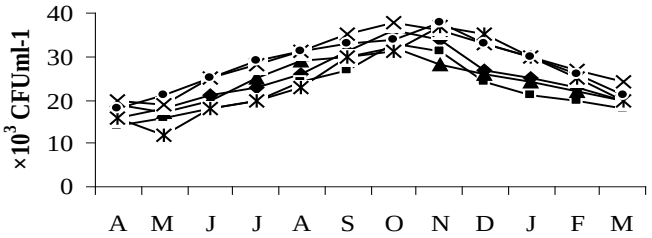



**Figure 20.** *Vibrio parahaemolyticus* in water

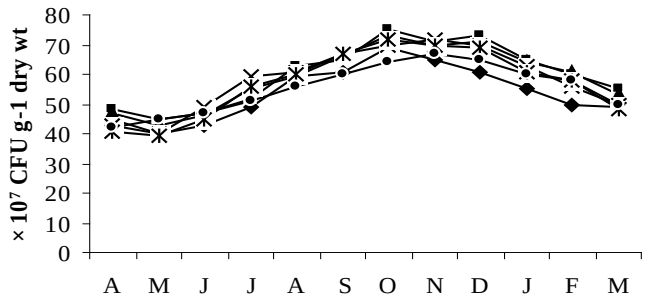



**Figure 17.** Total heterotrophic bacteria in sediment

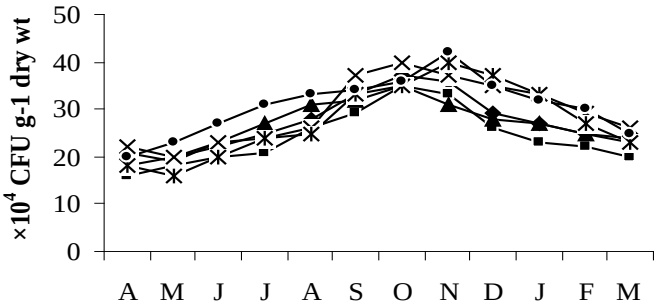



**Figure 21.** *Vibrio parahaemolyticus* in sediment

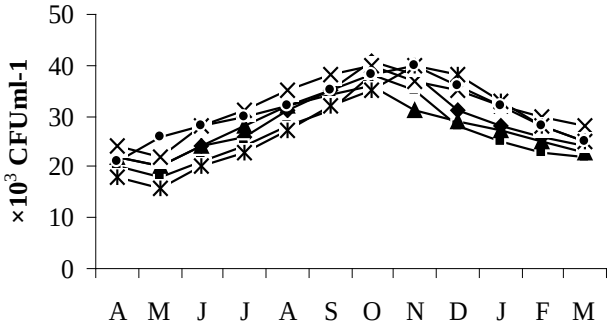



**Figure 18.** *Vibrio cholerae* in water

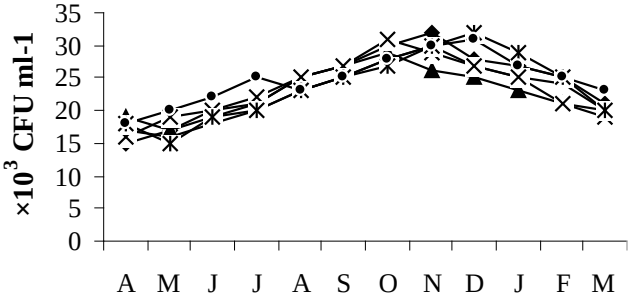
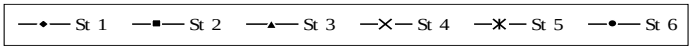



**Figure 22.** *Escherichia coli* in water



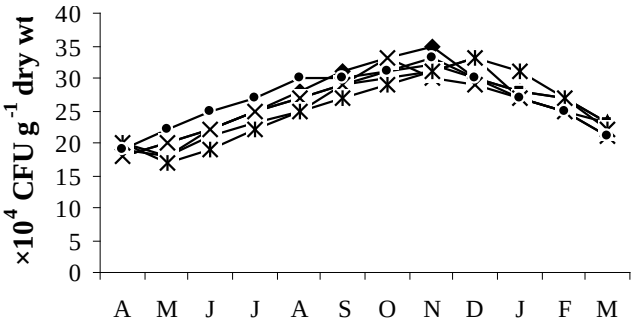



**Figure 23.** *Escherichia coli* in sediment

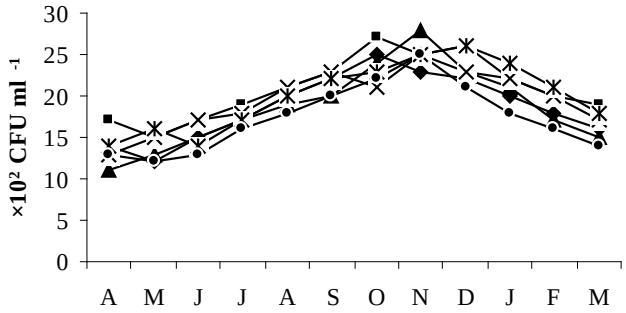



**Figure 24.** *Salmonella* spp. in water

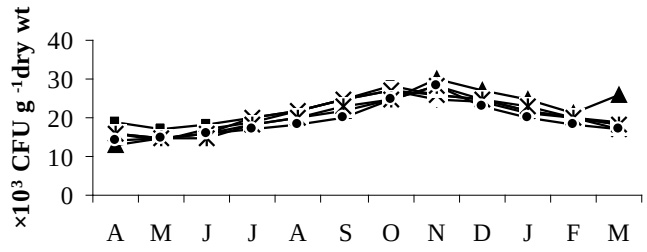
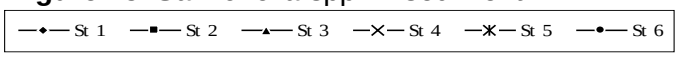



**Figure 25.** *Salmonella* spp. in sediment



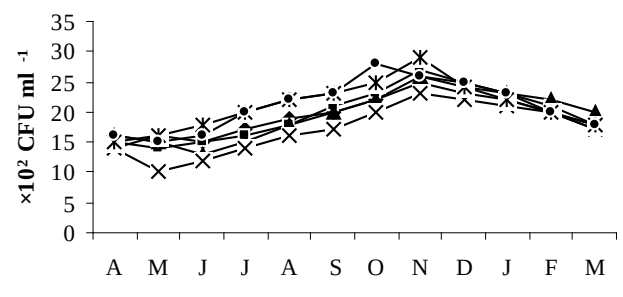




**Figure 26.** *Shigella* sp. in water

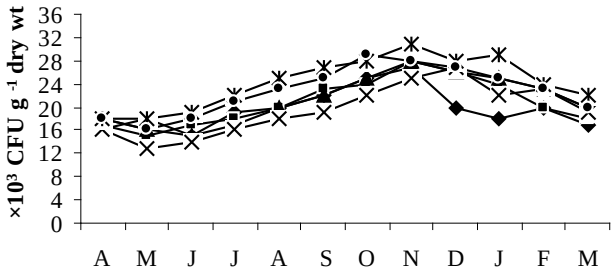



**Figure 27.** *Shigella* sp. in sediment

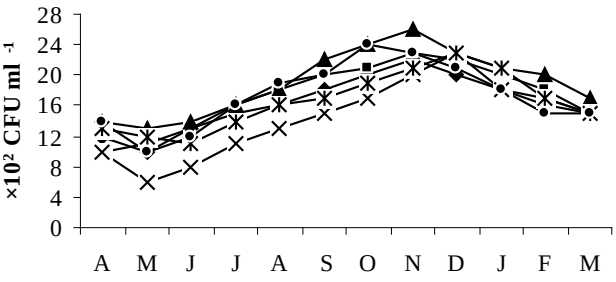
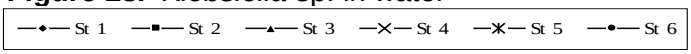



**Figure 28.** *Klebsiella* sp. in water



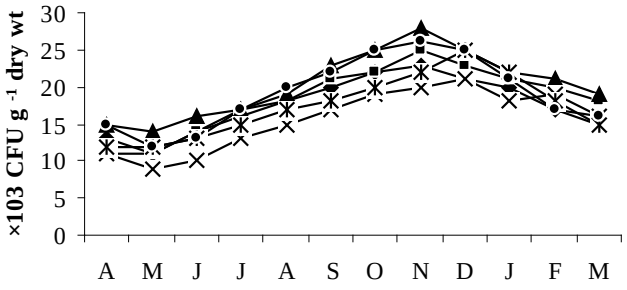




**Figure 29.** *Klebsiella* sp. in sediment

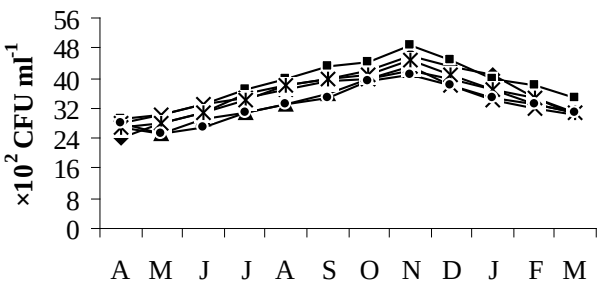



**Figure 30.** *Streptococcus* sp. in water

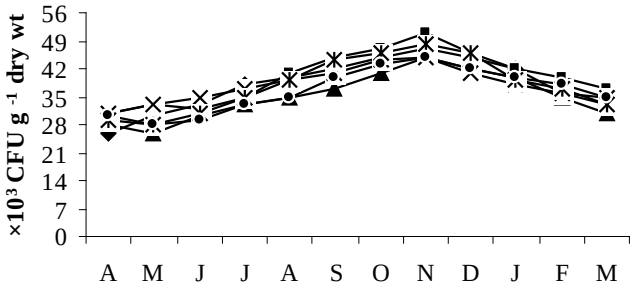
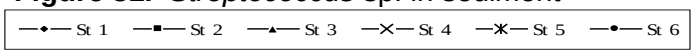



**Figure 31.** *Streptococcus* sp. in sediment



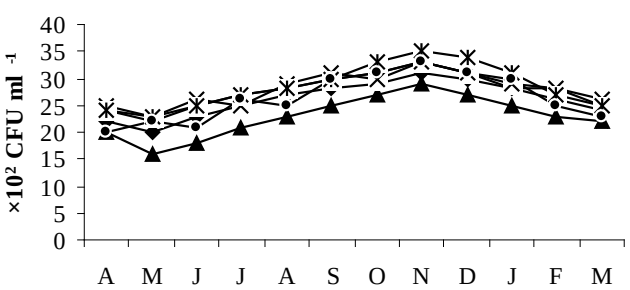




**Figure 32.** *Pseudomonas* sp. in water

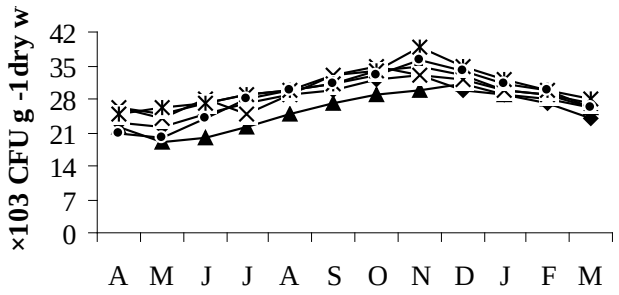



**Figure 33.** *Pseudomonas* sp. in sediment

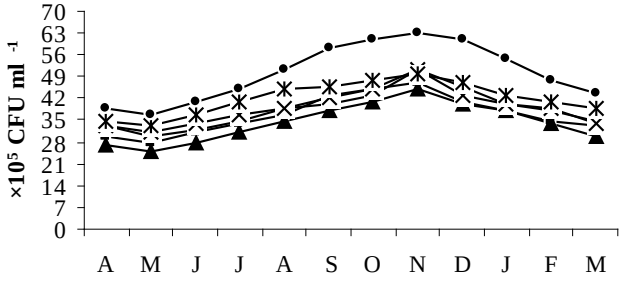
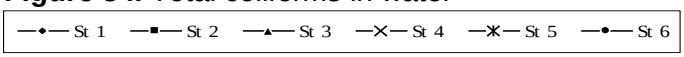



**Figure 34.** Total coliforms in water



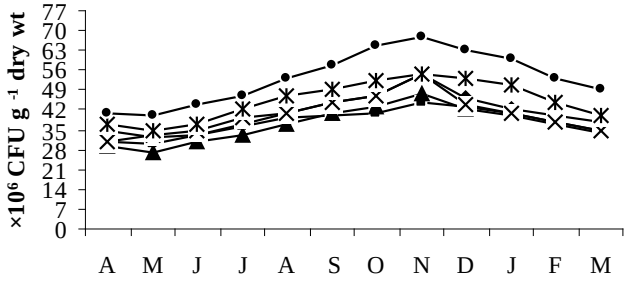




**Figure 35.** Total coliforms in sediment

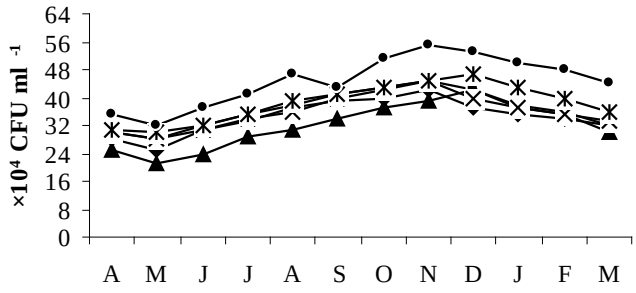



**Figure 36.** Fecal coliforms in water

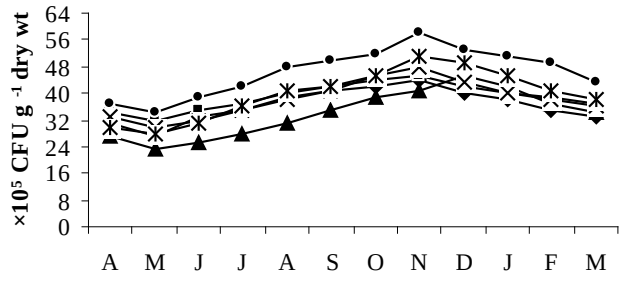



**Figure 37.** Fecal coliforms in sediment

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