5. DENITRIFYING BACTERIAL POPULATION ANALYSIS

5.1. INTRODUCTION

Heterotrophic bacteria play a key role in regulating accumulation, export, re-mineralization and transformation of the largest part of organic matter in aquatic ecosystems (Shiah *et al*., 2001). On account of their varied physiological properties and ability to biodegrade various organic compounds, they contribute to the reconstruction of organic matter and at the same time the self-cleaning processes of waters. Their numbers in water bodies, their developmental dynamics and changes in the population of different physiological groups are usually a reflection of the current state of the water body (Donderski and Kalwasinska, 2003)**.** Estuaries and coastal waters are exhibiting eutrophication due to the enrichment of nutrients caused by anthropogenic activities (Zillen *et al*., 2008; Cox *et al*., 2009).

Microorganisms constitute a huge and almost unexplained reservoir of resources likely to provide innovative applications useful to man and thriving in almost every habitat (Jain *et al*., 2005). Several studies were conducted on estuarine microbial diversity and some have demonstrated how freshwater and marine bacterioplankton community's mix along estuarine gradients (Biddle and Fletcher, 1995; Jay *et al*., 2000; Bouvier and Del Giorgio, 2002). Freshwater and marine populations adverted into the estuary represented a large fraction of the bacterioplankton community in all seasons. Seasonal cycles in estuaries depend on the temporal occurrence of deliveries of nutrients, the relative magnitudes of

the sources and the demands of the specific groups of organisms present (Valiela, 1995).The growth of microorganisms depends on a number of factors like nature of sediments, season, temperature, pH, salinity etc.

In estuarine environment the degree of bacterial diversity is expected to be high due to a combination of the mixture of sea water and fresh water and resuspension of sediments from benthic zones, tidal mud flats and sea grass beds. A small proportion of these bacteria may be active as consumers of detritus organic matter (Crump *et al*., 1999). Bacterial communities are structured by temporal and spatial variability of physicochemical and biotic parameters (Hewson *et al*., 2007). Importantly, bacterial communities readily respond at extremely faster rates (compared to other benthic organisms) to environmental and pollution changes. Thus reflect their micro environmental conditions and "communicate" this information to other biota in their vicinity and play key role in benthic-pelagic coupling. The estimation of bacterial abundances as well as their genetic diversity under insitu conditions is therefore the most fundamental objective of aquatic microbial ecology. Today, a major scientific challenge is to evaluate the significance of the great diversity of bacterial life in surface sediments. Surface sediment bacteria play a significant ecological and biogeochemical role in soft-bottom marine ecosystems, including tidal flats, due to their high abundance relative to the overlying water column (Thiyagarajan *et al.,* 2010).

Heterotrophic bacteria with varied physiological properties and ability to biodegrade various organic compounds, they contribute to the reconstruction of organic matter and at the same time the self-cleaning processes of waters. Heterotrophic microbes living within the sediments produce a wide range of enzymes that allow them to gain energy from the degradation of small organic molecules that are mineralized into inorganic nutrients (Nedwell and Brown, 1982). This is a respiration process if electrons flow from an organic donor to an inorganic acceptor while it is a fermentation process if both electron donor and acceptor are organic substances (Jorgensen, 2000). The complex carbohydrate composition of the ecosystem would lead to colonization of microbial communities with abilities to produce an array of complex carbohydrate degrading enzymes (Rakhee Khandeparker *et al*., 2011). The intensity of decomposition of organic macromolecules in water bodies is usually determined not only by the number of bacteria capable of carrying out those processes, but also by the level of activity of their enzymes (Mudryk and Skorczewski, 2006). Denitrification involves several semi independent steps that need not function together all the time. Denitrifiers begin the sequence with nitrate and produce varying amounts of the other products depending on the environmental conditions ((Neetha, 2012).

Facultative denitrifying bacteria which preferred low dissolved oxygen conditions are usually accumulated in the upper sediments (Fukushima *et al*., 2007). Nitrification and denitrification processes can simultaneously occur in the surface sediment thereby increasing the reduction rates of NO_3 and NO_2 (Rysgaard *et al*., 1993; Scott *et al*., 2008). The nitrate transformation to gaseous

nitrogen thus comprises a natural nutrient sink and may mitigate the increasing nitrate discharge and primary productivity in coastal environments (Jorgensen and Sorenson, 1988). As the depth increases, oxygen concentration decreases rapidly. This situation changed the microorganism from facultative aerobic phylotypes to strictly anaerobic phylotypes and different denitrifiers change with the depth (Tiquia *et al*., 2006). Concentrations of denitrification substrates, along with the dissolved inorganic nitrogen and organic matter are also changed with the sediment depth (Dalsgaard *et al*., 2005; Meyer *et al*., 2008). Nitrogen is a major element of many biological metabolites and structural molecules (amino acids, proteins, nucleic acids). In the marine environment, the availability of nitrogen often is believed to be a key in limiting growth and productivity (Ryther and Dunstan, 1971).

Nitrogen is removed from the aquatic system by microbial mediated anaerobic ammonium oxidation and denitrification process that convert the available forms of fixed nitrogen to dinitrogen gas. Coastal sediments are important sites for denitrification and often control the degree of eutrophication by acting as natural nitrogen removal filters (Voss *et al*., 2005; Seitzinger *et al*., 2006). Changes in microbial activities caused by changes in environmental conditions may cause significant impact on coastal marine ecosystems. Studying and characterizing denitrifying bacterial diversity are essential in understanding how ecosystem handles increased nitrogen loads.

Some important works related to microbial distribution in estuarine environments were Chandran and Ramamoorthi (1984c) in Vellar estuary; Coffin and Sharp (1987) in Dalaware estuary; Wright *et al*. (1987) in the Parker estuary; Alavandi (1989) in coastal waters of Cochin; Ingram and Sabh (1990) in St. Lawrence estuary; Hoch and Kirchman (1993) in temperate estuary; Narasimmalu Rajendrean *et al*. (1994) in Osaka Bay; Goosen *et al*. (1997) studied the regulation of annual variation in heterotrophic bacterial production in the Schelde estuary; Kolm *et al*. (1997) in the sediment of Antonina Bay; Prema (2000); Nallathambi *et al*. (2002) in Port Blair bay Brazil; Seshadri and Ignacimuthu (2002) in Chennai, south west coast of India; Sukumaran (2002) in Manakudy estuary; Selvamohan (2006) and Mohideen Askar Nawas (2009) in Rajakkalamam estuary; Karthikeyan *et al*. (2007) in Uppanar estuary; Yiping *et al*. (2008) in Salt Marsh Sediments; Ouseph *et al*. (2009) in southern coast of Kerala; Jalal *et al*. (2010) in Kuantan estuary of Pahang; Mahalakshmi *et al*. (2011) in fishing harbor of Cuddalore, Ramkumar *et al*. (2011) in Kottaipattinam coast; Sridevi (2011) in Champavathi Estuary; Rani *et al*. (2012) in Pazhayakayal estuary.

5.2. MATERIALS AND METHODS

Water and bottom sediment samples were collected once in a month from the selected sampling stations of the experimental estuary during the early morning hours over a period of two years extending from January 2010 to December 2011.

5.2.1. SURFACE WATER

Surface water samples from selected sampling stations were collected separately in sterile bottles and brought to the laboratory in an ice box for microbiological analysis.

5.2.2. SEDIMENT SAMPLE

The sediment samples from the selected experimental stations were collected separately in sterile polythene bags for microbiological analysis and brought to the laboratory in an ice box. In the laboratory, one gram of soil from each station was aseptically and individually weighed and dissolved in 9 ml of sterile distilled water for further analysis.

5.2.3. CULTURE MEDIA PREPARATION

Bacteria require nutrients as well as favorable environment for their better growth. The medium must provide with suitable nutrients, proper pH, osmotic pressure, atmospheric oxygen and other growth factors for the better growth of microorganisms. Usually a solid medium was prepared for the culture of bacteria.

0.15 g of beef extract, 0.15g of yeast extract, 0.5g of sodium chloride and 0.5 g of peptone were weighed and dissolved in 100 ml of distilled water in a conical flask. To this dehydrated broth, 1.5 g of agar powder was added and boiled for 5 minutes till the agar becomes dissolved. The flask was plugged with cotton wool and sterilized at 15 lbs pressure for 15 minutes. After the nutrient agar medium was sterilized, it was gently swirled to disperse the agar evenly. It was cooled to about 45ºC. From this, 20 ml of liquid agar was poured into a sterile covered petridish and allowed to solidify. Contamination was avoided to ensure aseptic conditions.

5.2.4. SERIAL DILUTION PREPARATIONS

To begin with 9 ml of distilled water was taken in test tubes for water and soil samples separately and plugged by cotton. Then the test tubes were sterilized at 15 lbs pressure for 15 minutes. After sterilization each test tube was cooled at room temperature and labeled. After labeling, 1.0 ml of freshly collected sample was added in the first test tube (10^{-1}). From this, 1.0 ml of sample was taken and added into the second test tube (10^{-2}). In this method all the test tubes were serially diluted to the concentrations of (10^{-1} to 10^{-7}). This was repeated for sediment samples and the amount taken was 1.0 g.

5.2.5. INOCULATION OF SAMPLE INTO AGAR PLATES BY POUR PLATE METHOD

About 1 ml of sample was taken from each dilution and applied to the petri plate and then 20 ml of nutrient agar was added and uniformly spreaded. After inoculation, the plates were inverted and incubated at 37°C for 24 hours.

For each dilution replicates were maintained. After incubation, petriplates with 30 to 300 colonies were selected for the total viable counts. The bacterial population was expressed as number of colony forming units (CFU/ml) per ml of water for water sample and per gram of soil for soil sample.

5.2.6. ISOLATION AND IDENTIFICATION OF HETEROTROPHIC BACTERIAL FLORA OF WATER AND SOIL

Morphologically dissimilar, well isolated colonies were randomly selected and streaked on the nutrient agar slants. The selected colonies were sub-cultured in nutrient agar slants. This was done after observing the morphology and pigmentation of the colony. The slant cultured were then stored at 4°C in refrigerator. The bacterial strains isolated from water and soils were then screened for their denitrification property. After screening the denitrifying bacterial strains, the dominant bacterial strains (Two) were selected and identified up to the generic level by employing the scheme of Gunasekharan (1995) and John *et al*. (1996). After identification of dominant denitrifying bacterial genera further confirmation was made on specific media i.e, growth and appearance of opalescent pale yellow colour colony on Cetrimide agar for *Pseudomonas sp.* and appearance of pink opaque gel colour colony on *Bacillus cereus* medium for *Bacillus sp.*

5.2.7. SCREENING OF DENITRIFYING BACTERIA- NITRATE REDUCTASE TEST

A loop full of culture was taken in the inoculation needle and streaked on the nitrate broth present in the test tubes. After inoculation the tubes were

incubated for 48 to 96 hours at 37^0C in the incubator. Then 1ml of test reagent A (8.0 g sulphanilic acid in 1 litre of acetic acid) were added. The formation of red colour indicated positive result. If there is no colour change a small amount of zinc powder was added. The formation of red colour after adding zinc powder indicated the negative result. If there is no colour change after zinc powder was added, it indicated a positive result.

5.2.8. SCREENING OF ENZYMATIC BACRERIA

STARCH HYDROLYSIS

From 10^{-3} dilution of soil sample one ml was inoculated in to the petriplate and 20 ml of starch agar medium was poured. The plate was then incubated at 37°C for 24 hours in an incubator for its sufficient growth. After incubation the plate was flooded with iodine solution. Hydrolysis was indicated by clear zones around the growth and it was treated as positive colony.

CASEINOLYTIC HYDROLYSIS

From 10^{-3} dilution of soil sample one ml was inoculated in to the petriplate and then 20 ml of the casein agar medium was poured. Then the plate was incubated at 37°C for 24 hours for its sufficient growth. After incubation the plate was observed clear zones around the growth indicated the positive colony.

5.2.9. COMPOSITION OF CETRIMIDE AGAR (Brown and Lowbury, 1965)

Ingredients		**g/l**
Gelatin peptone	-	20.0
Magnesium chloride	-	1.4
Potassium sulphate	-	10.0

Glycerol	-	10.0 ml/l
Cetrimide	-	0.3
Agar	-	13.0
Final pH	-	7.0± 0.2 at 37^0C

5.3. RESULT

The total bacterial count, percentage of denitrifying bacteria, percentage of *Bacillus sp.* and *Pseudomonas sp.* and seasonal variations of total viable count and denitrifying bacteria in water and sediment samples of the sampling stations during 2010 and 2011 are given in Tables 5.1 to 5.18. There were wide fluctuations in total bacterial count and percentage of denitrifying bacteria both in sediment and water. Distribution of denitrifying bacteria in all three stations revealed that in water and sediment the dominant species involved in denitrification were *Bacillus sp.* and *Pseudomonas sp.* The percentage of *Pseudomonas sp.* was relatively more than that of *Bacillus sp.*

Tables 5.1, 5.2 and 5.3 show the total heterotrophic bacterial count, percentage of denitrifying bacterial population, *Bacillus sp.* and *Pseudomonas sp.* in the water sample at S-I during the study period. In 2010, the total bacterial count fluctuated between 16.0 x 10^{-3} CFU/ml in March and September to 26.0 x 10^{3} CFU/ml in October. The monthly average value of total bacterial count was 20.40 ± 4.0 x 10^{3} CFU/ml. The percentage of total denitrifying bacteria was minimum in November with 19.0% and maximum in March with 28.5%. The percentage of *Bacillus sp.* ranged from 2.5% in November to 6.1% in December with the monthly average was 4.47±1.21%. The percentage of *Pseudomonas sp.* was minimum in September with 11.6% and maximum in July with 16.5%. The monthly average of it was 14.1±1.67%.

In 2011, at S-I the total bacterial count ranged from 16.0 x 10^3 CFU/ml in January and May to 26.3 x 10^3 CFU/ml in June with the monthly average of 20.70 ± 4.30x10^3 CFU/ml. The percentage of total denitrifying bacteria was minimum in December with 18.0% and maximum with 27.0% in May. The monthly average value was 21.59±3.20%. Among the total denitrifying bacteria the percentage of *Bacillus sp.* was minimum in December (2.9%) and maximum in May (5.9%) with the monthly average value of 4.35±0.98%. The percentage of *Pseudomonas sp.* in water was minimum in December (11.2%) and maximum in May (16.2%) with the monthly average value of 12.15±1.54%.

Data on the total bacterial population, percentage of total denitrifying bacteria, *Bacillus sp.* and *Pseudomonas sp.* recorded in water sample at S-II during 2010 are given in Tables 5.1, 5.4 and 5.5. In 2010, the bacterial count ranged from minimum of 20.3 x10^3 CFU/ml in January to a maximum of 29.3 x10^3 CFU/ml in July. The mean monthly average value was 24.12±2.68 x10^3 CFU/ml. The percentage of denitrifying bacteria ranged from 18.9% in July to 30.0% in April with the monthly average of 23.99±3.41%. The percentage of *Bacillus sp.* and *Pseudomonas sp.* was minimum in September and July with 3.9% and 11.2% and maximum in March and April with 6.7% and 18.0% respectively. The monthly average value of *Bacillus sp.* and *Pseudomonas sp*. was 5.21%±0.96% and 13.93±1.81% respectively

In 2011, the total bacterial count ranged from 29 x10^3 CFU/ml in October to 21.0 x10^3 CFU/ml in January with monthly average was 24.93±2.98 CFU/ml. The percentage of total denitrifying bacteria was minimum in July with 19.2%

and maximum in May with 27.9%. The mean monthly average value was 23.83 ±3.08%. The percentage of *Bacillus sp.* was minimum in September with 3.9% and maximum in November with 6.4%. The monthly average registered was 5.16±0.81%. But the percentage of *Pseudomonas sp.* was minimum in September with 11.2% and maximum in May with 16.2%. The monthly average value recorded was 13.74±1.79%.

Tables 5.1, 5.6 and 5.7 show the total bacterial population, percentage of total denitrifying bacteria, *Bacillus sp.* and *Pseudomonas sp.* in water at S-III during the study period (2010 and 2011). In 2010, the total bacterial count fluctuated between 20.0 $x10^3$ CFU/ml in March and 31.0 $x10^3$ CFU/ml in June with the monthly average value of 24.92 ±3.25 $x10^3$ CFU/ml. The percentage of total denitrifying bacteria was lower in July with 19.0% and higher in January with 29.5%. The monthly average value was 24 ±3.23%.The percentage of *Bacillus sp.* recorded was minimum in August with 4.8% and maximum in January with 7.3% and the monthly average was 5.72±0.83%. The percentage of *Pseudomonas sp*. varied from (10.5%) in July to (16.8%) in January. The monthly average mean value recorded was 13.70±2.05%.

In 2011, the total bacterial count ranged from a minimum of 22 $x10^3$ CFU/ml in February to a maximum of 30.0 $x10^3$ CFU/ml in October with the monthly average value of 25.11±2.08$x10^3$ CFU/ml. The minimum percentage of total denitrifying bacteria and *Pseudomonas sp.* were recorded in the month of February with 20.5%, and 11.5% respectively and minimum *Bacillus sp.* was recorded in September with 5.1%. The maximum percentage of total denitrifying

bacteria, *Pseudomonas sp.* and *Bacillus sp.* registered were in June with 29.0%, 7.0% and 16.2% respectively. The monthly mean values of them were 23.95±2.30%, 5.89±0.60% and 12.97±1.39% respectively.

Seasonal variations of bacterial population observed in water samples S-I to S-III are provided in Table 5.8. In 2010, during non monsoon period a minimum bacterial population of 19.05±4.4 CFU/ml was recorded at S-I and a maximum of 21.32±5.3 CFU/ml at S-III. The southwest monsoon period recorded a minimum of 22.77±2.5 CFU/ml at S-I and a maximum of 26.02±2.6 CFU/ml at S-II. The northeast monsoon period registered a minimum bacterial count of 22.7±2.1 CFU/ml at S-I and a maximum of 26.22±3.2 CFU/ml at S-III. In 2011, non monsoon period recorded a minimum value of 18.95±4.1 CFU/ml at S-I and a maximum (22.32±4.7 CFU/ml) at S-II. The southwest monsoon period showed a minimum of 23.5±2.8 CFU/ml at S-I and a maximum of 25.87±2.4CFU/ml) in S-II. During northeast monsoon period the minimum bacterial count was recorded at S-I (24.37 ±2.2 CFU/ml) against the maximum of 26.12 ± 2.6 CFU/ml at S-III.

Table 5.9 showed the seasonal variations in the percentage of total denitrifying bacteria in water sample at S-I to S-III. In 2010, non monsoon period recorded minimum percentage of denitrifying bacteria (21.42±3.7%) at S-III; whereas, the maximum of 23.92±4.54% was registered at S-I. The southwest monsoon period registered a minimum value of 22.85±3.2% in S-II and a maximum of 24.92±4.8% at S-I. During northeast monsoon period the minimum percentage of 23.17±4.0% was recorded at S-II and maximum (25.5±2.9%) at

S-I. In 2011, the percentage of denitrifying bacteria was low (19.17±0.68%) at S-I and high (24.5± 2.9%) at S-II during non monsoon period. The southwest monsoon period showed a minimum percentage of 22.47±2.5 percent at S-I and a maximum of 24.75±3.3% at S-III. But, in northeast monsoon season minimum percentage was noticed at S-I with (22.95±2.2%) against the maximum of 25.02±1.2% at S-II.

The total bacterial population, percentage of total denitrifying bacteria, *Bacillus sp.* and *Pseudomonas sp.* recorded in sediment samples at S-I are represented in Tables. 5.10 5.11 and 5.12. During 2010, at S-I the total bacterial count was a minimum of 21.9 $x10^3$ CFU/g in August and maximum of 31.7$x10^3$ CFU/g in October. The monthly average value was 26.55±2.86 $x10^3$CFU/g. The minimum percentage of total denitrifying bacteria was recorded in March with 20.5% and maximum 28.4% in October with the monthly average of 25.64 ±2.07%.The percentage of *Bacillus sp.* was minimum in March (2.1%) and maximum in February (3.6%) with the monthly average of 3.08 ±0.52%. The percentage of *Pseudomonas sp.* was minimum in March (14.5%) and maximum in October (21.2%) with the monthly average of 19.01±1.68%.

During 2011, at S-I the total bacterial count in sediment fluctuated between 20.4$x10^3$ CFU/g in January and 28.3$x10^3$ CFU/g in June with the monthly average value of 25.06 ±2.81 $x10^3$ CFU/g. The percentage of total denitrifying bacteria ranged from 20.7% in January to 29.0% June and the monthly average was 22.68 ±3.04%. The minimum percentage of *Bacillus sp*. was recorded in February (2.1%) and maximum (4.6%) in April with the

monthly average of 3.52 ±0.86%.The percentage of *Pseudomonas sp.* ranged from 14.8% in January to 20.7% in July with the monthly average of 16.68 ±1.95%.

Fig 5.1 and 5.2 depict the correlation between the total viable count and percentage of denitrifying bacterial population with total soil nitrogen at S-I during the study period. In 2010, the total viable count (r^2 = 0.570) and total denitrifying bacterial population (r^2 = 0.722) showed a significant positive correlation ($P < 0.05$) with total nitrogen. Similarly the total viable count (r^2 = 0.863) and total denitrifying bacterial population (r^2 = 0.815) showed a significant positive correlation ($P < 0.05$) with total nitrogen during 2011.

Tables 5.10, 5.13 and 5.14 show the total bacterial population, the percentage of denitrifying bacteria, *Bacillus sp.* and *Pseudomonas sp.* in sediment at S-II during the study period (2010 and 2011). In 2010, the total bacterial count ranged from 21.6 $x10^3$ CFU/g in March to 32.2 $x10^3$ CFU/g in October. The monthly average value was 28.33±2.79 CFU/g. The minimum percentage of denitrifying bacteria was recorded in January with 21.2% against the maximum in October with 34.6%. The monthly average value was 26.08±4.03%. The percentage of *Bacillus sp.* and *Pseudomonas sp.* was minimum in March with 3.1% and 14.6% respectively against the maximum of 5.6% and 23.5% in October respectively The monthly value registered were; 4.05±0.74% and 18.26 ±2.68% respectively.

In 2011, the total bacterial count ranged from a minimum of 24.5 CFU/g in March to a maximum of 32.8 CFU/g in July with the monthly average value was 28.92±2.41x10^3 CFU/g. The percentage of total denitrifying bacteria was lower in March (20.5%) and higher in July (31.8%). The monthly average value recorded was 24.24±3.72%. The percentage of *Bacillus sp.* and *Pseudomonas sp.* was minimum in March with 3.20% and 13.6% respectively and maximum in July with 4.9% and 22.0% respectively. The monthly mean values registered were; 3.82±0.53% and 16.70±2.60% respectively.

The regression analysis for the relationship between total viable count and sediment nitrogen showed a positive correlation in 2010 (r^2 = 0.689) and in 2011 (r^2 = 0.828) at S-II. Likewise the relationship between the percentage of denitrifying bacteria and sediment nitrogen also showed a positive correlation (r^2 = 0.908 and r^2 = 0.869) during 2010 and 2011 respectively (Fig.5.3 and 5.4), and it was statistically significant ($P < 0.05$).

Tables 5.10, 5.15 and 5.16 show the total bacterial population, percentage of total denitrifying bacteria, *Bacillus sp*. and *Pseudomonas sp.* in the sediment sample at S-III during the study period. In 2010, the total bacterial count fluctuated between 23.0 x10^3 CFU/g in September and 32.5 x10^3 CFU/g in June. The monthly average value was 29.23±.3.20 x10^3 CFU/g. The percentage of total denitrifying was minimum in March with 21.5% and maximum in July with 30.5%. The percentage of *Bacillus sp.* ranged from 5.8% in September to 7.8% in April and the monthly mean average was 6.39 ±0.64%. The percentage of

Pseudomonas sp. was low in March with 11.2% and high in July with 16.8%.The monthly mean average value was 13.43±1.91%.

In 2011, the total bacterial count ranged from a minimum of 20.0 $x10^3$ CFU/g in February and to a maximum of 32.0 $x10^3$ CFU/g in October with the monthly average value of 27.90±2.9$x10^3$ CFU/g. The percentage of total denitrifying bacteria was minimum in March with 22.2% and maximum in April with 30.0% and the monthly average value was 24.44 ±2.71%.The percentage of *Bacillus sp.* was minimum (5.4%) in March and maximum (7.8%) in June with the monthly average was 6.25 ±0.83%. The percentage of *Pseudomonas sp.* ranged from 11.2% in March to 16.9% in April and the monthly average value was 12.71 ±1.86%.

Seasonal variations of bacterial population observed in sediment samples are provided in Table 5.8. In 2010, during non monsoon period a minimum total viable count of 25.7±2.9 CFU/g was recorded at S-II and a maximum of 28.0±3.7 CFU/g at S-III. The southwest monsoon period showed a minimum of 26.92±4.1 CFU/g at S-I and a maximum of 29.52±0.67 CFU/g at S-II. The northeast monsoon period registered a minimum bacterial population of 28.5±4.2 CFU/g at S-III and a maximum of 30.62±1.93CFU/g at S-III. In 2011, non monsoon period recorded a minimum bacterial count of 23.65±3.3 CFU/g at S-I and a maximum of 26.02±1.3 CFU/g at S-III. The southwest monsoon period recorded a minimum of (27.7±1.3 CFU/g) at S-III and a maximum of (30.6±1.7CFU/g) in S-II. During northeast monsoon period the minimum

bacterial count recorded was at S-I (25.52 ±3.6 CFU/g) against the maximum of 29.45 ± 1.9 CFU/g at S-III.

Seasonal variation in the pooled data (Table 5.17) showed a minimum percentage of denitrifying bacteria (24.45±2.8%) at S-I and the maximum of 26.57±1.5% at S-III during non monsoon period. The southwest monsoon season registered a minimum value of 25.6±5.1% in S-I and a maximum of 29.85±4.01% at S-III. During northeast monsoon period the minimum percentage of 25.37±2.86% was recorded at S-I and maximum (28.1±2.3%) at S-II. In 2011, the percentage of denitrifying bacteria was low (23.52±3.2%) at S-I and high (25.37± 3.9%) at S-II during non monsoon period. The southwest monsoon period showed a minimum percentage of 24.52±2.6%t at S-III and a maximum of 28.45±3.0% at S-II. In northeast monsoon season minimum percentage was noticed at S-I with 25.05±3.4% against the maximum of 26.87±2.5% at S-II.

The regression analysis between total viable count and sediment nitrogen showed a positive correlation ($r^2 = 0.587$ and $r^2 = 0.588$) at S-III during the study period. The sediment nitrogen and total denitrifying bacteria also showed a significant ($P < 0.05$) positive correlation ($r^2 = 0.758$ and $r^2 = 0.830$) during 2010 and 2011 respectively (Fig. 5.5 and 5.6).

The data on starch hydrolyzing bacterial population recorded at the sampling stations during 2010 and 2011 are given in Table 5.19 and it showed much variation between sampling stations. In 2010, at S-I the starch hydrolyzing bacterial population ranged from a minimum of 3.5×10^3 CFU/g in March to a maximum of 24.5×10^3CFU/g in November. In S-II, it fluctuated between 6.5×10^3

CFU/g in February to 40.2×10^3 CFU/g in November and the monthly average value was $20.2\pm11.4\times10^3$ CFU/g. In S-III, it was maximum (44.5×10^3 CFU/g) in October and minimum (9.5×10^3CFU/g) in January. During 2011 the starch hydrolyzing bacterial population fluctuated between 3.0×10^3 CFU/g in February to 30.2×10^3 CFU/g in June, 4.0×10^3 CFU/g in September to 36.0×10^3 CFU/g in July and 5.5×10^3 CFU/g in January to 35.0×10^3 CFU/g in October at S-I, S-II and S-III respectively. In 2010, the monthly mean starch hydrolyzing bacterial population was maximum ($22.4\pm11.2\times10^3$ CFU/g) at S-III and minimum ($14.2\pm6.7\times10^3$ CFU/g) at S-I. In 2011 the maximum mean value of $20.4\pm10.6\times10^3$ CFU/g was registered at S-III against the minimum of $14.8\pm8.2\times10^3$ CFU/g at S-I.

Seasonal changes in starch hydrolyzing bacterial population indicated that, it was more during monsoon season (Table 5.21). In the non monsoon season, the maximum starch hydrolyzing bacterial population of $19.0\pm8.9\times10^3$ CFU/g at S-III and $18.7\pm10.5\times10^3$ CFU/g at S-II were noticed during 2010 and 2011 respectively. Likewise minimum starch hydrolyzing bacterial population of $11.8 \pm 5.58\times10^3$ CFU/g and $8.25 \pm 4.9\times10^3$ CFU/g were noticed at S-I during 2010 and 2011 respectively. In southwest monsoon period, the maximum starch hydrolyzing bacterial population of $24.38 \pm 8.2\times10^3$ CFU/g at S-III and $22.83 \pm11.88\times10^3$ CFU/g at S-II were noticed during 2010 and 2011 respectively. At the same time, minimum starch hydrolyzing bacterial population were observed at S-I with $12.68 \pm 8.9\times10^3$ CFU/g and $19.8 \pm 10\times10^3$ CFU/g during 2010 and 2011 respectively. In northeast monsoon period, the maximum level of starch hydrolyzing bacterial population of $23.47\pm15.4\times10^3$ CFU/g at S-II and

$24.68\pm9.22\times10^3$ CFU/g at S-III were recorded during 2010 and 2011 respectively. The minimum value registered were $18.0\pm5.0\times10^3$ CFU/g and $16.35\pm5.4\times10^3$ CFU/g at S-I in 2010 and 2011 respectively.

Fig 5.7 and 5.8 provide the regression analysis for the relationship between sediment nitrogen and starch hydrolyzing bacteria at the sampling stations. The relationship showed a positive correlation (r^2 = 0.487, 0.586 and 0.492) at S-I, S-II and S-III respectively during 2010. Similarly in 2011, sediment nitrogen and starch hydrolyzing bacteria also showed a positive correlation (r^2 = 0.498 at S-I, r^2= 0.553 at S-II and r^2= 0.507 at S-III). The correlation was statistically not significant ($P > 0.05$) during the study period.

The data on casein hydrolyzing bacterial population recorded at the sampling stations during 2010 and 2011 are shown in Table 5.20. It showed much variation between sampling stations. In 2010, at S-I the casein hydrolyzing bacterial population fluctuated from a minimum of 5×10^3 CFU/g in January to a maximum of 25.8×10^3 CFU/g in October and the monthly average value was 11.88 ± 10^3 CFU/g. In S-II it varied from 6.8×10^3 CFU/g in March to 36.0×10^3 CFU/g in October and the monthly average value was $18.73\pm9.44\times10^3$ CFU/g. In S-III, it was maximum (34.0×10^3 CFU/g) in July and minimum (11.0×10^3 CFU/g) in March and the monthly average value was $19.53\pm0.7\times10^3$ CFU/g. During 2011 the casein hydrolyzing bacterial population ranged from 3×10^3 CFU/g in May to 35.8×10^3 CFU/g in June, 3×10^3 CFU/g in March to 44×10^3 CFU/g in July and 3.5×10^3 CFU/g in February to 40.2×10^3 CFU/g in July at S-I, S-II and S-III respectively. The monthly mean casein hydrolyzing bacterial

population during 2010 ranged from 11.88 ±7.3 $\times 10^3$ CFU/g at S-I to 19.53±9.7$\times 10^3$ CFU/g) at S-III. In 2011 the minimum mean value of 14.96±10.7$\times 10^3$ CFU/g was registered at S-I against the maximum of 22.99±13.2$\times 10^3$ CFU/g at S-III.

Seasonal fluctuations in casein hydrolyzing bacterial population indicated that, it was more during monsoon season (Table 5.22). In the non monsoon season, the minimum casein hydrolyzing bacterial population of 6.66±4.22$\times 10^3$ CFU/g and 7.68 ± 5.39$\times 10^3$ CFU/g were noticed at S-I during 2010 and 2011 respectively. In the same season maximum casein hydrolyzing bacterial population of 14.17± 7.06$\times 10^3$ CFU/g and 18.60±14.74$\times 10^3$ CFU/g were noticed at S-III during 2010 and 2011 respectively. In southwest monsoon period, the maximum casein hydrolyzing bacterial population of 21.26±10.98$\times 10^3$ CFU/g at S-III and 28.85±15.01$\times 10^3$ CFU/g at S-II were noticed during 2010 and 2011 respectively. At the same time, minimum casein hydrolyzing bacterial population were observed at S-I with 11.14± 5.02$\times 10^3$ CFU/g and 19.95±15.74$\times 10^3$ CFU/g during 2010 and 2011 respectively. In northeast monsoon period, the maximum level of starch hydrolyzing bacterial population (21.39± 10.46$\times 10^3$ CFU/g) at S-II and (27.0±15.43$\times 10^3$ CFU/g) at S-III were recorded during 2010 and 2011 respectively. The minimum value registered were 14.59±10.26$\times 10^3$ CFU/g and 17.75±6.51$\times 10^3$ CFU/g at S-I in 2010 and 2011 respectively.

The regression analysis for the relationship between sediment nitrogen and casein hydrolyzing bacteria in the selected sampling stations during the study period are depicted in Fig. 5.9 and 5.10. The casein hydrolyzing bacteria showed

a significant positive correlation (r^2 = 0.937, 0.896 and 0.874) at S-I, S-II and S-III respectively during 2010. Likewise during 2011, the casein hydrolyzing bacteria also showed a positive correlation (r^2= 0.940, 0.893 and 0.901) in S-I, S-II and S-III and it was statistically highly significant ($P < 0.05$).

5.4. DISCUSSION

Microorganisms present in coastal waters carry out many ecologically important functions. Heterotrophic bacteria play an important role as an intermediate group between phytoplankton and zooplankton in transforming dissolved organic matter into biomass (Sherr and Sherr, 1994). Heterotrophic bacteria are known to efficiently decompose organic matter and regenerate minerals in aquatic ecosystems, and their abundance represents an index of heterotrophic activity (Henssen and Tranvik, 1998). They play an important role in the structure and functioning of the microbial food web, in relation to environmental conditions such as temperature (Simon and Wunsch, 1998), resource availability (Pace and Cole, 1996), and predation pressure (Thouvenot *et al*., 1999). Denitrifiers utilize nitrate and nitrite as respiratory substrates they can respire using these oxides of nitrogen as electron acceptors in place of oxygen, in the process of converting, nitrate and nitrite to nitric oxide and nitrous oxide, and finally to dinitrogen gas (Tiedje, 1988).

Denitrification involves several semi independent steps that need not function together all the time, denitrifiers begin the sequence with nitrate and produce varying amounts of the other products depending on the environmental conditions (Ferguson, 1994).It is assumed that microbial diversity is linked with ecosystem function, and that ecosystem with functional redundancy have an increased ability to withstand perturbations caused by pollutants (Girvan *et al*., 2005). The progressive increase in nitrogen loading and reduction of fresh water discharge into estuarine systems represent worldwide problems (Jickells, 1998;

De Jonge *et al*., 2002). Microorganisms are considered the main primary producers as well as being secondary producers and consumers and bacteria are known to play a major role in the organic matter degradation in marine sediments (Deming and Baross, 1993).

In water samples, in the year 2010, the bacterial population were ranged from 16.0 x 10^3 CFU/ml (March and September) to 26.0 x 10^3 CFU/ml (October) at S-I, 20.3 x 10^3 CFU/ml (January) to 29.3 x 10^3 CFU/ml (July) at S-II and 22.5x 10^3 CFU/ml (January and September) to 31.0 x 10^3 CFU/ml (June) at S-III. In 2011, it was ranged from 16.0 x 10^3 CFU/ml (January and May) to 26.3 x 10^3 CFU/ml (June) at S-I, 21.0 x 10^3 CFU/ml (January) to 29.0 x 10^3 CFU/ml (October) at S-II and 22.0x 10^3 CFU/ml (February) to 30.0 x 10^3 CFU/ml (October) at S-III. In sediment samples, in the year 2010, the THB population were ranged from 21.9 x 10^3 CFU/g (August) to 31.7 x 10^3 CFU/g (October) at S-I, 21.6 x 10^3 CFU/g (March) to 32.2 x 10^3 CFU/g (October) at S-II and 23.0 x 10^3 CFU/g (September) to 32.5 x 10^3 CFU/g (June) at S-III. In 2011, it was ranged from 20.4 x 10^3 CFU/g (January) to 28.3 x 10^3 CFU/g (June) at S- I, 24.5 x 10^3 CFU/g (March) to 32.8 x 10^3 CFU/g (July) at S-II and 20.0x 10^3 CFU/g (February) to 32.0 x 10^3 CFU/g (October) at S-III.

The monthly averages of water and sediment samples revealed that, the THB populations were higher in sediment (29.23±3.20 in 2010 and 28.92±2.41 in 2011) than water (24.92±3.25 in 2010 and 25.11±2.08 in 2011) samples. The reason for that may be the circulation of water and proliferation of microbes on the surface of sediments might have enhanced the THB population in sediments.

In the previous studies also the same results were recorded. Wollast (1991) reported that the coastal and shelf sediments play a significant role in the demineralization of organic matter which supports the growth of microbes. Anon (1997) also reported the higher bacterial population density in the sediments than water is generally due to the rich organic content of the former and the lesser residence time of the microorganisms in the water column than the sediments. Maria Jesus Ferrara-Guerrero *et al*. (2007) reported higher bacterial population in sediment than in water in Coyuca de Benitez coastal lagoon. Prema (2000) reported a total viable count of 8.6-3.5×10^5/ml in water samples and 6.8 to 37.4×10^5/g in sediment sample at Rajakkamangalam estuary. Sukumaran (2002) registered that the bacterial count varied from 1.35×10^5/ml in water to 1.91×10^5/g in sediment. In Port Blair Bay THB population ranged from 0.6× 10^5/ml to 375×10^3/g (Nallathambi *et al*. 2002). Devendran *et al*. (1987) recorded a population range from 0.001 to 5.2×10^5 CUF/ml in water and 1.21 to 3.32×10^5CFU/g in sediments. David Omiema and Ideriah, (2012) reported THB range of 1.8x10^3 to 3.4x10^3 unit/ml in water and 1.93x10^4 to 6.12x10^4 unit/ml in sediment of eastern Niger Delta. Surajitdas *et al.* (2007) reported 1.01 x10^3 to 37.98 x 10^3 CFU.g-1 heterotrophic bacteria in the slope sediments of western Bay of Bengal.

In the present study, the higher populations of THB were recorded in both water and sediment samples during monsoon months. This could be attributed to the influence of terrigenous material brought in by the fresh water which carried the high density of bacterial population from the land. Similar results were observed by Chadran and Ramamoorthy (1984c) in Vellar estuary. During

summer the population level was maintained at low in water. (Velammal (1993), Natarajan *et al*. (1980) also observed very low levels of pathogens in estuarine and marine waters during summer season. High bacterial population during monsoon may be due to the rain water flow which brings huge quantities of nutrients (Martin, 1981). The primary peak of THB was noticed during the monsoon season when higher concentration of water nutrients was observed. This might be due to continuous availability of organic matter through decomposition of seaweeds and seagrasses and also from the land run-off (Kannapiran *et al*., 2008). Swarnakumar *et al*. (2008); Mahalakshmi *et al*. (2011) have reported sediment microbes are lower in summer and higher in monsoon. Rani *et al*. (2012) also recorded maximum THB during northeast monsoon might be due to the bulk rainfall and freshwater input which brings more organic matter in turn greatly influenced the diversity of heterotrophic bacteria.

The high organic load at S-II and S-III resulting from discharge of retting and domestic wastes may be the reason for more bacterial population in S-II and S-III. The higher distribution of microorganisms may be as a result of high decomposable organic load and low concentration of predators like protozoa along the shoreline (Balba and Bewley, 1991). The distribution, abundance and biological activity of the bacteria in aquatic environment have been reported to be closely related to the physical properties and organic carbon content of the ecosystem (Meyer-Reil, 1979; Martinez, 1996). In the present study showed that the sandy sediment of S-I could withstand low nutrients and this may be the reason for low bacterial counts. Similar observations were made by Prabhu *et al*. (1991) along the Madras coast, Sukumaran (2002) in one of the station in

Manakudy and Goosen *et al*. (1995) in Scheldt estuary. The muddy sediments of Lake Ohrid, containing more organic matter were characterized by a greater number of bacteria in comparison with sandy sediments (Lokoska, 2012).

The period of low bacterial density among the different groups could be associated with an increase in the number of detritivores such as microfauna which feed mainly on bacteria (Wahbeh and Mohasneh, 1984; Berdjeb *et al*., 2011). THB recorded in most of the sampling stations showed a significant positive correlation with sediment total nitrogen (r^2 = 0.570 to 0.908) during the study period. Changes in microbial activities caused by changes in environmental conditions may therefore cause significant impact on coastal marine ecosystems. The negative correlation of temperature with total heterotrophic bacteria was observed by Raghavendrudu and Kondalarao (2008). Kumaraswamy (2005) reported a suitable temperature range for increasing bacterial population was 26-29ºC. Sukumaran (2002) reported the annual mean temperature of 28 ºC is responsible for maximum number of bacterial population. The water temperature has more influence on bacterial distribution compared with other environmental factors such as salinity, pH and dissolved oxygen in Bahrain pelagic and near shore waters (Mahasneh and Sayed, 1997).

Agricultural and other anthropogenic activities result in increasing amount of nitrogen compounds entering the freshwater and marine systems, causing eutrophication problems. In the microbial process of denitrification, nitrate (NO3−) is converted to gaseous forms then lost from the systems (Canfield *et al*., 2010). Therefore, the denitrification process should help to

minimize the eutrophication problems. Denitrification is commonly used to remove nitrogen from sewage and municipal wastewater. Denitrifying microbial communities and denitrification in salt marsh sediments may be affected by many factors, including environmental conditions, nutrient availability, and levels of pollutants (Yiping *et al*., 2008).

The monthly mean percentage of denitrifying bacteria in water samples at S-I in the study period 2010 and 2011 were 23.05±3.65 and 21.59±3.20 respectively. In S-II it was 23.99±3.41 and 23.83±3.08; in S-III it was 24.0±3.23 and 23.95±2.30 in respective years. In sediment samples, the percentage monthly mean of denitrifying bacteria in study period 2010 and 2011 were 25.64±2.07% and 22.68±3.04% in S-I; 26.08±4.03% and 24.24±3.72% in S-I; 24.87±2.81% and 24.44±2.71% in S-III in respective years.

Maximum monthly average of denitrifying bacterial population recorded in sediment of S-II is due to mixing of sewage and retting effluents. Jorgensen and Sorenson (1988) found that the diversity of the denitrifier communities was high in the sediment because the river sediment was affected by both domestic sewage inputs and irregular tides. The denitrification rates were always found in organically polluted low land rivers where sediment tends to be covered by decomposing algae and other organic debris (Gracia-ruiz *et al*., 1998). The high rates of denitrification occurred in water bodies receiving massive amounts of anthropogenic nutrients from overland runoff and sewage treatment discharges, and no denitrification was observed in pristine streams Seitzinger (1988). Kemp

et al. (1990) have identified a decrease in nitrogen loading, which increases denitrifying efficiencies that may improve water quality.

The denitrification rates are higher during autumn and early winter, when higher concentrations of nitrate enter the system (Dong *et al*., 2000). The head of the estuary the rates of denitrification were low in summer and high in the early winter (Ogilvie *et al*., 1997). Oxygen and nitrate concentrations, temperature, pH, and the availability of suitable electron donor substrates (mainly organic carbon compounds) are considered the key factors controlling the occurrence and rate of denitrification (Nielsen *et al*., 1990). Denitrifiers are facultative heterotrophic anaerobes including representatives of common genera such as Escherichia, Bacillus and Pseudomonas (Fenchel *et al*., 1998). The high diversity of facultative denitrifiers was consistent with the high permeability, deeper oxygen penetration, and high rates of aerobic respiration determined in the sediments (Mills *et al*., 2008).

In the present study among the diversity of the total denitrifying bacterial population *Pseudomonas sp.* and *Bacillus sp.* were recorded as a predominant genus. Similar observations were made by Prema (2000), Selvamohan (2006) and Mohiddeen (2009) in Rajakkamangalam estuary, Alavandi (1989) in coastal waters of Cochin. Ramkumar *et al*. (2011) recorded *Pseudomonas* and *Bacillus* dominates both in water and sediment of Kottaipattinam coast. But Kannapiran *et al*. (1999) isolated bacterial strains from predominance of *Bacilus sp.* followed by *Pseudomonas sp.* and *Spirllum* in Gulf of Mannar. Raghavendrudu and Kondalarao (2008) recorded *Bacillus, Pseudomonas, Staphylococcus and*

Micrococcus are the common heterotrophic bacteria in the mangrove habitats of Meghadri. Venkateswarwn and Natarajan (1983) during their study about the Port Nova waters reported that *Pseudomonas* and *Bacillus sp.* are dominant inorganic phosphorous solublizing microbes. Parvetheesam and Bulchandani, (2005) reported that *Pseudomonas aeruginosa* a widely distributed bacterium, exhibited a high potentiality to convert nitrate to nitrates, nitric oxide, nitrous oxide and finally dinitrogen, hence they could be used as a bioremedying agent in removing excess nitrate from domestic water supplies. *Pseudomonas* has been isolated from wide range of aquatic habitats (Baumann *et al*., 1982; Cook and Goldman, 1976). Dunn *et al*., 1980) reported that bacteria reducing nitrates to nitrite also occurs abundantly and is represented by genera *Aeromonas, Pseudomonas and Acinetobacter* as well as the members of *enterobacteria.*

In the present investigation the total denitrifying bacterial population showed a significant positive correlation with sediment nitrogen in most of the sampling stations during the study period. Balbina *et al*. (2002) observed higher denitrification rates in the sediments at the head of estuary, correlating with the higher concentration of nitrate in river water. Ogilvie *et al*. (1997) recorded strong correlations between denitrification rates and water column $NO3^-$ concentrations in river Colene estuary. Wang Dong *et al.* (2007) reported that the denitrification rates had a significant positive correlation with temperature ($P < 0.01$) and total nitrogen ($P < 0.05$). Muller *et al*. (1980) reported that the optimum pH range of denitrification was 7.0- 8.0. At low pH values, the nitric oxide reductase which reduces N_2O is inhibited and hence the overall denitrification decreases (Nommik, 1956). Magalhaes *et al*. (2003) investigated the regulatory

effect of salinity and inorganic nitrogen on nitrification and denitrification in intertidal sandy sediments and rocky biofilms of Douro estuary. A positive correlation between the temperature and overall denitrification was recorded in temperate marsh sediments (Smith *et al*., 1985). The distribution of denitrifying genes in sediments are affected by many factors, including concentration gradient of the dissolved inorganic nitrogen (DIN) (including NO^{-3}, NO^{-2} and NH^{+4}), organic matter content, dissolved oxygen, and redox potential (Dong *et al*., 2009; Reyna *et al*., 2010; Huang *et al*., 2011).

Season wise pooled data showed that the denitrifying bacterial population was high in southwest monsoon and low in non monsoon period. Daoming *et al*. (2013) recorded that sediment denitrification rates were high in the spring and low in the summer and early autumn, primarily due to seasonal differences in nitrate concentration and water temperature and positive and linear relationships were regularly observed between denitrification rate and the number of denitrifying bacteria. Lin *et al*. (2009) observed a significant correlation between denitrification with the quantity of denitrifying bacteria ($P = 0.950$). Bacterial denitrification played a significant role in the cycling and removal of nitrogen.

The variations in the distribution and abundance of starch and casein hydrolyzing bacteria in the sampling stations of the estuarine environment may be due to the environmental conditions prevailed in those stations. Among the three stations S-I is bar mouth region with variables of marine nature and hence harbored with less enzymatic bacterial population. Similar observations were made by Selvamohan (2006) in Rajakkamangalam estuary. Bezeuevort *et al*.

(1998) reported concomitant evolution of ectoprotease producers with phytoplankton bloom in coastal water of the North Sea. Colwell and Mortia (1972) reported higher proteolytic bacteria in marine habitat as compared to fresh water and sediment habitats. The type of bacterial population found at particular station can also be influenced by salinity changes (Rakhee Khandeparker *et al*., 2011). Nair *et al*. (1978) reported that marine sediments on the west coast of India contained high numbers of cellulase, lipase, protease, amylase, and urease producing bacteria. The variation in the abundance of amylolytic and proteolytic bacterial groups was found to be parallel to the variation in soluble carbohydrates and free amino acids (Gouda e*t al*., 2006).

In the present study starch and casein hydrolyzing bacteria are more in S-II and S-III; this may be due to the availability of starch and casein substrate in the sediments and mixing of sewage and retting effluents. It has also been reported that in most of the aquatic environments there is a significant relationship between extracellular enzyme activities, their corresponding substrates (polymers) and their hydrolysis products (monmers) (Munster *et al.,* 1992; Chrost, 1991; Vetter and Deming, 1999). Domestic sewage effluents and wastes from the food vendors and workshops can contribute greatly to the organic pollution which increases microbial bloom (Atobatele, and Owoseni, 2012). Heterotrophic bacteria plays an important role in the decomposition of organic matter of the sediment and protein decomposition is done by proteolytic *Pseudomonas* and other eubacteria, chitin by chitinolytic or chitinoclastic *Bacillus*, *Pseudomonas* and *Vibrio* (Neetha, 2012).

The seasonal variation of the chosen enzymatic bacterial population indicated that the minimum bacterial count of the different groups was found during non monsoon period and maximum counts in the monsoon period. Among the sampled stations, S-II recorded the highest value during southwest monsoon period and S-I registered the lowest value during non monsoon season. Seasonal fluctuations in water temperature may participate in inducing such variation in bacterial counts. Studying protease and lipase producers among pelagic marine bacteria Martinez *et al*. (1996) inferred that the broad range of enzyme activity suggesting shifts in the dominant species of bacteria at a given time and space, could strongly influence the rates and pattern of polymer and particle hydrolysis in sea water. This agrees with the notion that only small part of the microbial community is active at any given time (Biddle and Fletcher, 1995).

Regression analysis reveals that the starch hydrolyzing bacterial population in the selected sampling stations of the estuary showed a positive correlation with total nitrogen. The r^2 value of the starch hydrolyzing against total nitrogen ranged from 0.487 to 0.586 and from 0.498 to 0.533 during 2010 and 2011 respectively and it was not statistically significant ($P > 0.05$). On the other hand a significant positive correlation was found to exist between casein hydrolyzing bacteria and total nitrogen and the correlation ranged from 0.874 to 0.937 and from 0.893 to 0.940 during 2010 and 2011 respectively and it was statistically highly significant ($P < 0.05$). These results inferred that, the concentration of the substrate in the immediate environment controlled the population dynamic of the enzymatic bacteria.