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QUANTITATIVE ABUNDANCE OF DENITRIFYING BACTERIAL DIVERSITY IN RELATION TO ABIOTIC FACTORS OF TOTAL NITROGEN AND ORGANIC CARBON IN THE SEDIMENTS OF RAJAKKAMANGALAM ESTUARY OF SOUTHWEST COAST OF INDIA

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ABSTRACT

An investigation was made on the diversity of aerobic heterotrophic denitrifying bacterial population in the sediment samples of six sampling stations (S-I to S-VI) at Rajakkamangalam estuary of southwest coast of India for the period of two years from January 2005 to December 2006. The result indicated that the distribution of denitrifying bacterial populations in the estuarine environment fluctuated much between sampling stations. Among the total denitrifying bacterial populations *Pseudomonas* was found to dominant group. The percentage of total denitrifying bacterial populations was low in the pollutant mixed stations.

KEY WORDS: Estuary, Sediment, Denitrifying bacteria, *pseudomonas.*

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INTRODUCTION

Estuary is a body of water in which river water mixes with seawater. An estuary is a semi-enclosed coastal body of water, which has a free connection with the open sea and within which the seawater is measurably diluted with fresh water derived from land drainage[1]. Microorganisms are widely distributed in the water and sediment of marine and brackish water environment. They have far reaching effects on the biological as well as geochemical systems. They also play an important role in the decomposition of organic matter, dissolution of inorganic, insoluble salts and in the regeneration of nutrients[2]. In estuarine environments, the degree of bacterial diversity is expected to be high due to a combination of the mixture of seawater and freshwater and the resuspension of sediments from benthic zones, tidal mudflats, and sea grass beds. Nonetheless, a small proportion of these bacteria may be active as consumers of detritus organic matter [3]. The sediment microbial diversity is expected to vary in type and number depending on the quantity and quality of energy manufactured by the primary producers of each sediment ecosystem [4]. Chandrika and Ramachandran Nair[5] have studied the seasonal variation of heterotrophic bacteria, generic composition, biochemical and physiological activity in the estuarine environment of Cochin and explored the correlation between seasonal variation in population density and some of the physicochemical factors such as temperature, salinity, oxygen, pH, rainfall, nutrients, organic carbon and organic nitrogen. Denitrification is mainly sustained by denitrifying bacteria, although the ability of denitrification is also found in certain fungi. The bacteria that are capable of denitrification are frequently isolated from the soil, sediments and aquatic environments[6]. Most of the denitrifying bacteria can survive in the presence of oxygen. However, denitrification is only induced in anaerobic conditions in which microorganisms use nitrogen oxides (NO_x) as electron acceptors. It is an alternative pathway to aerobic respiration [7]. Denitrifying bacteria reduce nitrate (NO_3) to nitrous oxide (N_2O) or to nitrogen gas (N_2) under the catalysis of functional enzymes, through the following steps:

$$NO_3^- \xrightarrow{Nar} NO_2^- \xrightarrow{Nir} NO \xrightarrow{Nor} N_2O \xrightarrow{Nos} N_2$$

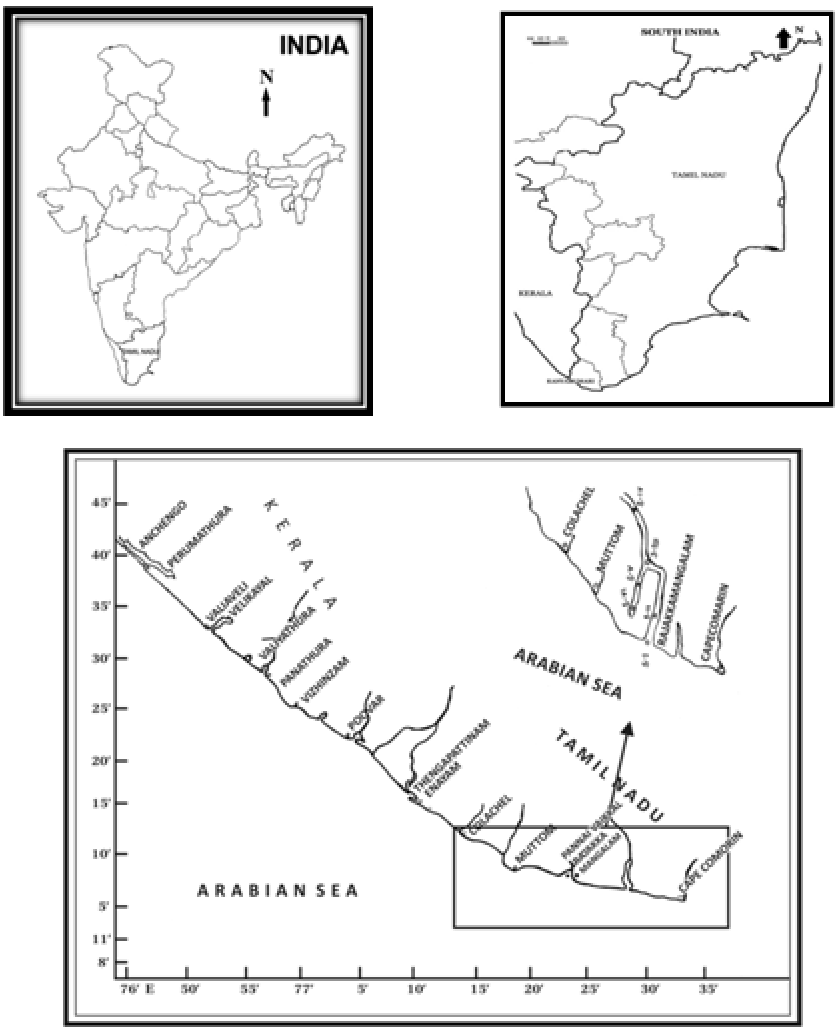
Where, *Nar* stands for nitrate reductase, *Nir* stands for nitrite reductase, *Nor* stands for nitric oxide reductase, and *Nos* stands for nitrous oxide reductase [6,8,]. Denitrification of estuarine is one of the few processes capable of counteracting eutrophication. Information available on the denitrifying bacterial diversity in the sediment samples of the estuary is relatively less. Hence the present study was proposed to enumerate quantitative abundance of denitrifying bacterial diversity in relation to abiotic factors of total nitrogen and organic carbon in the sediments of Rajakkamangalam estuary of southwest coast of India.

MATERIALS AND METHODS

DESCRIPTION OF THE STUDY AREA

Rajakkamangalam estuary is situated in Kanyakumari district at southern tip of Tamilnadu in the southwest coast of India. It is a bar built type. It is endowed with both southwest and northeast monsoons. Pannaivaikkal is one of the river systems that is found in this district. It originates from Vellimalai hills about 25 km northeast of Nagercoil and transverse 12 km before it joins the Arabian sea at Rajakkamangalam and forms the estuary. The villagers in and around the locality are using this estuary for their day-to-day activities. Coconut farms fringe the estuary. Beside these, there are many coconut retting fields by the side of the estuary. The effluent from the retting grounds also flows into the estuary. Hence, the estuarine part of the river is more productive as well as more destructive in nature with a great diversity of fauna and flora. For the present study six stations from the estuary were selected for sampling (Fig.1). Station I is located in the sea, whereas the Station II is located in the bar mouth region. Station III is located near the Pannaiyoor Bridge and nearly 150 m away from station II; Station IV is located 300 m away from station III and it is a freshwater zone. Station V is located 200 m away from station III and it is effluent mixing zone. Station VI is coconut husk retting zone and nearly 100 m away from station V.

FIGURE 1
MAP SHOWING STUDY AREA AND SAMPLING STATIONS (S-I TO S-VI)



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 2005 to December 2006. The sediment samples from the selected experimental stations were collected separately in sterile polythene bags for microbiological analysis and were brought to the laboratory in an icebox. In the laboratory, one gram of sediment from each station was aseptically and individually weighed and dissolved in 9 ml of sterile distilled water for used as stock. The bacterial strains isolated from water were then screened for their denitrification property. 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 37ºC in the incubator. Then 1 ml of test reagent A (8.0 g sulphanilic acid in 1 litre of acetic acid) and test reagent B (5.0 g alpha naphthalamine in 1 litre of acetic acid) were added. The formation of red colour indicated a positive result. If there is no colour change, a small amount of Zn powder was added. The formation of red colour after adding Zn powder indicated the negative result. If there is no colour change after Zn powder was added, it indicated a positive result. After screening the denitrifying bacterial strains, two dominant bacterial strains such as *Bacillus* sp. and *Pseudomonas* sp. were selected and identified up to generic level [9,10].

RESULTS AND DISCUSSION

Estuaries have ever been the changing environments as they receive water and other materials from a variety of sources. There is a significant amount of organic materials in estuaries, which are decomposed by microorganisms [11]. It is assumed that microbial diversity is linked to ecosystem function and that ecosystems with functional redundancy have an increased ability to withstand perturbations caused by pollutants [12]. Data about total bacterial count, percentage of denitrifying bacteria, percentage of *Bacillus* sp. and *Pseudomonas* sp. in sediment samples of the selected stations during 2005 and 2006 are presented in Tables 1 to 6 and Figs. 2 to 8. In all, the six stations the total bacterial count and percentage of denitrifying bacteria in sediment were highly fluctuated. Characterization of denitrifying bacteria in all the six stations revealed that the dominant species involved in denitrification were *Bacillus* and *Pseudomonas* sp. The percentage of *Pseudomonas* sp. was comparatively more than that of *Bacillus* sp. The total bacterial population, percentage of total denitrifying bacteria, *Bacillus* sp. and *Pseudomonas* sp. recorded in sediment samples at S-I were represented in Figs 2 and 3 and Table 1. At Station I, the total bacterial count was minimum (18.0 X 10^3CFU/g) in June and maximum was observed in Dec. 2005 (29.0 X 10^3CFU/g). The monthly average value was 22.72±2.97CFU/g. The percentage of total denitrifying bacteria, *Bacillus* sp. and *Pseudomonas* sp. ranged from a minimum of 16.5%, 2.0% and 11.5% to a maximum of 33.0%, 3.5% and 21.4%, respectively. The monthly average values were 24.68 ± 5.11%, 2.70 ± 0.49% and 16.26 ± 3.15%, respectively. During 2006, at Station -I, the total bacterial count fluctuated between 15.0 X 10^3CFU/g (March) and 28.3 X 10^3CFU/g (February) with the monthly average value of 23.27 ± 3.37 X 10^3CFU/g. The percentage of total denitrifying bacteria, *Bacillus* sp. and *Pseudomonas* sp. were lower in March with 20.3%, 2.4% and 16.1% and higher in February with 32.3%, 5.5% and 20.7%, respectively. The monthly mean values recorded were: 25.50 ± 3.87%, 3.61 ± 1.06% and 18.50 ± 1.46%, respectively (Table 1 and Figs. 2 & 3).

In 2005, at Station II, the total bacterial count ranged from 20.0 X 10^3CFU/g in April to 29.6 X 10^3CFU/g in January. The monthly average was 24.38 ± 3.05 CFU/g. The percentage denitrifying bacteria were minimum in April (20.0%) against the maximum in January (28.0%). The monthly average value was 24.66±2.94%. Similarly, the percentage of *Bacillus* sp. was the minimum in June with 2%, against the maximum of 3.0% in April with the average value of 2.49 ± 0.28%. Minimum percentage of *Pseudomonas* (13.9%) was noticed in April against a maximum observed in May (22.1%). In 2006, the total bacterial count ranged from 19.3 X 10^3CFU/g in December to 29.1 X 10^3CFU/g in January with the average value of 23.86 ± 3.41 X 10^3CFU/g. The percentage of total denitrifying bacteria were lower in October (20.0%) and higher (34.7%) in February. The monthly average was 25.08 ± 5.25%. The percentage of *Bacillus* sp. and *Pseudomonas* sp. was minimum (3.0% and 16.0%, respectively) in June and October, and maximum (7.0% and 23.2%, respectively) in February. The monthly mean values were 4.26 ± 1.41% and 18.38 ± 2.57%, respectively (Table.2, Figs 2 and 4). In 2005 at Station III, the total bacterial count fluctuated between 21.0 X 10^3CFU/g in July and 29.6 X 10^3CFU/g in December. The monthly average value was 26.08 ± 2.58 X 10^3CFU/g. The recorded percentage of total denitrifying bacteria, *Bacillus* sp. and *Pseudomonas* sp. was the minimum in November with 15.0%, 2.3% and 10.2% and maximum in March with 34.0%, 4.7% and 23.0%, respectively. The monthly mean average values were 25.32 ± 5.27%, 3.66 ± 0.62% and 16.66 ± 3.70%, respectively. In 2006, the total bacterial count ranged from a minimum of 20.0 X 10^3CFU/g (during November and May) to a maximum of 32.2 X 10^3CFU/g (during February) with the monthly average value of 24.73 ± 3.80 X 10^3 CFU/g. The percentage of total denitrifying bacteria (16%), *Bacillus* sp. (2.2%) and *Pseudomonas* sp. (10.9%) was minimum in November and maximum (36.8%, 6.5% and 22.5%) in February. Likewise the monthly average values were 24.85 ± 6.78%, 4.09 ± 1.54% and 15.83 ± 4.06%, respectively (Table 3; Figs 2 and 5).

In 2005 at Station IV, the minimum total bacterial count was (20.0 X 10^3CFU/g) observed in July and October and maximum count was recorded (29.0 X 10^3CFU/g) in January with a monthly average value of 23.14 ± 2.87X10^3CFU/g. The percentage of total denitrifying bacteria were minimum (20.2%) in November and maximum (36.6%) in January. The monthly average value was 26.58 ± 4.65%. In sediment sample, out of total denitrifying bacteria the percentage of *Bacillus* sp. ranged from 2.8% in November to 6.0% in January with a monthly average of 3.66 ± 0.81%. Likewise the percentage of *Pseudomonas* sp. was minimum (14.7%) in October and maximum (25.0%) in January. The monthly mean value was 19.49 ± 3.38%. In 2006, the total bacterial count ranged from a minimum of 20.0 X 10^3 CFU/g in August to a maximum of 32.8 X 10^3 CFU/g in January with the monthly average value of 24.49 ± 4.03 X 10^3CFU/g. The percentage of total denitrifying bacteria ranged from 20.8% in December to 31.5% in January with monthly average value of 24.92 ± 3.39%. Similarly the percentage of *Bacillus* sp. and *Pseudomonas* sp. recorded minimum values (2.0% and 16.2%) in December and March. Likewise, these bacterial species recorded a maximum percentage (4.7% and 20.2%) in January (Table.4; Figs 2 and 6). In 2005, total bacterial count fluctuated between 25.0 X 10^3 CFU/g in November and 33.6 X 10^3 CFU/g in August with the monthly average value of 29.36 ± 2.68 X 10^3 CFU/g. The percentage of total denitrifying bacteria was minimum (20.4%) in April and maximum (35.0%) in January. The monthly average value was 26.62 ± 4.65%. Among the total denitrifying bacteria the percentage of *Bacillus* sp. ranged from 3.2% in April to 6.0% in January with a monthly average value of 4.35 ± 0.84%. Similarly the percentage of *Pseudomonas* sp. was minimum in June (14.2%) and maximum in January (23.4%). The monthly average value was 18.18 ± 2.97%. In 2006, the total bacterial count ranged from a minimum (27.1 X 10^3 CFU/g) in October to maximum (39.4 X 10^3 CFU/g) in February. The monthly average value was 33.62 ± 3.75 X 10^3 CFU/g. The percentage of denitrifying bacteria was minimum in October (20.0%) and maximum in December (33.6%), with a monthly average of 25.08 ± 4.63%. Among the total denitrifying bacteria, the percentage of *Bacillus* sp. was minimum in October (3.7%) and maximum in December (5.3%). Likewise the percentage of *Pseudomonas* sp. was minimum in October and November (14.3%) and maximum in December (21.8%). The monthly average values of *Bacillus* sp. and *Pseudomonas* sp. were 4.34 ± 0.50% and 17.12 ± 2.65%, respectively (Table.5, Figs 2 and 7).

In 2005 at Station –VI, the total bacterial count was minimum (23.3 X 10^3 CFU/g) in June and maximum (36.3 X 10^3 CFU/g) in January with an average value of 30.71 ± 3.60X10^3 CFU/g. The percentage of denitrifying bacteria, *Bacillus* sp. and *Pseudomonas* sp. were minimum in November with 20.8%, 3.8% and 14.5%, respectively, whereas it was maximum in February with 27.2%, 5.3% and 18.4%, respectively. Likewise the monthly average values were 23.86 ± 2.29%, 4.52 ± 0.44%and 16.63 ± 1.39%, respectively. In

2006, at Station VI, the total bacterial count ranged from a minimum of 33.8 X 10^3 CFU/g in October to a maximum of 42.0 X 10^3 CFU/g in February. The monthly average mean value was 38.64 ± 2.45 X 10^3 CFU/g. Similarly the percentage of denitrifying bacteria ranged from 22.0% in November to 36.5% in February with the average value of 27.19 ± 4.53%. The percentage of *Bacillus* sp. was minimum (3.6%) in November and in September and maximum (6.0%) in June. The monthly average value was 4.69 ± 0.74%. The percentage of *Pseudomonas* sp. was minimum (15.2%) in November and maximum (24.5%) in February with the average value of 18.39 ± 3.18% (Table.6, Figs.2 and 8). In the present investigation the maximum diversity of total heterotrophic bacterial populations (THB) was observed in August, February and January during 2005 at S-III, S-V and S-VI, respectively. It may be due to less rain fall. In 2006, the maximum THB population was reported at S-V and S-VI in February. Certain marine bacteria increase in numbers from May through October followed by the gradual reduction during November and December, where it remained from January through mid March and sharp increases were noted from April to summer in the Atlantic coast[13]. In the experimental estuary the maximum monthly average values of total viable count in sediment samples were recorded at S-VI during 2005 and 2006. Likewise the minimum value was recorded at S-I during 2005 and 2006. The total viable range of sediments samples at Rajakkamangalam estuary was 6.8-37.4 x10^5 /g [14.]

The total heterotrophic bacterial population registered in the selected sampling stations of the experimental estuary showed a significant positive correlation with sediment total nitrogen during 2005 (r^2=0.53 to 0.81) and 2006 (r^2=0.43 to 0.88) in sampling stations (S-II to S-VI). Likewise the total heterotrophic bacterial population showed a significant positive correlation with sediment organic carbon during 2005 (r^2=0.37 to 0.88) in sampling stations S-II to S-VI; whereas, during 2006 a positive correlation was observed (r^2=0.41 to 0.71) in S-IV to S-VI (Table 7 and 8). The distribution, abundance and biology of the bacteria in aquatic environments have been reported to be closely related to the physical properties of organic contents of the ecosystem [15,16]. Denitrification is a key process and nitrogen recycling takes place in the condition of anoxia when denitrifying bacteria converts nitrate (No_3^-), one of the main activities of nitrogen, into biologically unavailable nitrogen (N_2) and small proportion of nitrous oxide (N_2O). Denitrifying bacteria are represented by members from most bacterial groups, so disturbances in these functions may pose a very serious threat to ecosystems. Aerobic denitrification were earlier studied[17-21]. A number of physicochemical parameters may determine the distribution of denitrifying bacteria in estuarine sediment. Environmental factors have effects on microbial activity and consequently control the denitrification processes. Many researchers have demonstrated that the seasonal change of environmental factors and their combination determine denitrification rate [22]. The percentage of denitrifying bacterial population in the experimental estuary was lower at S-VI. It may be due to the mixing of retting effluents in S-VI. The retting of coconut husk in the backwaters is brought about by the pectinolytic activity of microorganisms, especially bacteria and fungi, degrading the fibre binding material of the husk and liberating large quantities of organic substance into the surrounding waters. Oxidation of these organic matters liberates hydrogen sulphide. Increase in hydrogen sulphide results in nitrate reduction to ammonia, which decreases Denitrification [23].

The diversity of denitrifying populations in the experimental estuary was also less in S-VI. As a result the total nitrogen in sediment value was also increased. Denitrifying activity in relation to organic carbon in sediment was earlier studied [24]. The maximum denitrification rate appeared in the upper estuary of Galveston Bay in summer, when temperature, organic carbon content and salinity had 52%, 28% and 15% effect on denitrification, respectively [25]. In the present study total denitrifying bacterial population showed a significant positive correlation with sediment, total nitrogen (ppm) in the most of the sampling stations during 2005 (r^2=0.64 to 0.90) and 2006 (r^2=0.63 to 0.86) and the data are depicted in Table 7 and 8. Similarly the total denitrifying bacterial population also showed a significant positive correlation (r^2=0.64 to 0.73 and r^2=0.65 to 0.96) in the majority of the sampling station during 2005 and 2006. In Yangtze estuary the denitrification rates had a significant positive correlation with temperature ($P<0.01$) and total nitrogen ($P<0.05$) [26]. The regulatory effect of salinity and inorganic nitrogen on nitrification and denitrification in inertidal sandy sediments and rocky biofilms of the Douro estuary was early investigated [27]. In the present study among the diversity of the total denitrifying bacterial population, *Pseudomonas* sp. was observed as a predominant group. Similar condition was earlier observed in Rajakkamangalam estuary and Thengapatanaum estuary[14,28,29]. Bacteria reduces nitrates to nitrite also occur abundantly and is represented by genera *Aeromonas, Pseudomonas* and *Acinetobacter* as well as by the members of the enterobacteria[30]. *Pseudomonas aeruginosa* strains are typically active denitrifiers [31-34]. *Pseudomonas* has been isolated from a wide range of aquatic habitats[35,36]. *Pseudomonas aeruginosa,* a widely distributed bacterium, exhibited a high potentiality to convert nitrate to nitrites, nitric oxide, nitrous oxide and finally dinitrogen, hence they could be used as bioremedying agent in removing excess nitrate from domestic water supplies [37]. Some physiological studies showed denitrifying activity under aerobic conditions in *Pseudomonas aeruginosa*[38,39.] The pathway of anaerobic reduction of nitrite to nitrogen gas (N_2) by cell suspension of the denitrifier, *Pseudomonas aeruginosa* by using the techniques of gas chromatography and mass spectrometry[40]. *Pseudomonas* and *Bacillus* sp. are the dominant inorganic phosphorous solublizing microbes in the Port Novo waters[41]. *Pseudomonas* and *Bacillus* sp. have more solubulizing capacity than other genera[42.]

The present investigation revealed that the occurrence and distribution of bacterial population in the estuarine environment fluctuated much between sampling stations. It is generally accepted that variations in the number of THB populations are common within a location and even within a single sample[43]. The percentage of the total denitrifying bacterial populations, which was minimum in the retting effluent stations leads to an increase in the concentration of nitrogen and organic carbon. As a result more nutrient loading occurs in the estuarine water and sediment that eventually leads to the destruction of fauna and flora in the estuary. Furthermore, a detailed study on the functional efficiency of the dominant denitrifying bacterial species in *ex-situ* condition is required which in turn will be of much useful to restore the natural environment loaded with nitrogen and organic carbon.

Table 1
Denitrifying bacterial population (CFU/g X10^3) in the in the sediment sample of the Station I during 2005 and 2006

Sampling months	Percentage of denitrifying bacteria		Percentage of *Bacillus sp.* Sp.		Percentage of *Pseudomonas* sp.		Percentage of others	
	2005	2006	2005	2006	2005	2006	2005	2006
January	30.4	30.0	3.3	4.8	19.8	20.4	7.3	4.8
February	33.0	28.0	3.5	4.3	21.4	20.0	8.1	3.7
March	29.6	20.3	3.1	2.4	19.4	16.1	7.1	1.8
April	20.6	32.3	2.4	5.5	13.2	20.7	5.0	6.1
May	24.0	26.5	2.6	3.8	15.7	19.2	5.7	3.5
June	24.8	22.6	2.7	2.8	16.1	17.5	6.0	2.3
July	20.0	21.9	2.2	2.6	13.5	17.1	4.3	2.2
August	30.0	31.4	3.2	5.3	19.6	20.2	7.2	5.9
September	20.5	23.8	2.3	3.1	13.7	17.9	4.5	2.8
October	19.4	24.0	2.1	3.2	13.2	18.0	4.1	2.8
November	16.5	23.2	2.0	2.9	11.5	17.7	3.0	2.6
December	27.4	22.0	3.0	2.6	18.0	17.2	6.4	2.2
Mean ± SD	24.68 ± 5.11	25.50 ± 3.87	2.70± 0.49	3.61 ± 1.06	16.26 ± 3.15	18.50 ± 1.46	5.72 ± 1.49	3.39 ± 1.40

Table 2
Denitrifying bacterial population (CFU/g X10^3) in the in the sediment sample of the station II during 2005 and 2006

Sampling months	Percentage of denitrifying bacteria		Percentage of *Bacillus sp.* sp.		Percentage of *Pseudomonas* sp.		Percentage of Others	
	2005	2006	2005	2006	2005	2006	2005	2006
January	28.0	33.5	2.7	6.8	21.8	22.0	3.5	4.7
February	27.2	34.7	2.5	7.0	22.0	23.2	2.7	4.5
March	26.1	20.8	2.4	3.2	21.6	16.6	2.1	1.0
April	20.0	29.4	3.0	4.6	13.9	20.0	3.1	4.8
May	27.0	30.9	2.4	5.8	22.1	22.0	2.5	3.1
June	23.4	20.2	2.0	3.0	18.7	16.0	2.7	1.2
July	20.3	22.0	2.8	3.5	14.6	16.7	2.9	1.8
August	27.5	24.5	2.5	4.0	21.2	18.2	3.8	2.3
September	23.8	22.5	2.1	3.7	18.9	17.0	2.8	1.8
October	24.7	20.0	2.2	3.0	19.5	16.0	3.0	1.0
November	20.3	22.1	2.7	3.4	14.4	16.8	3.2	1.9
December	27.6	20.4	2.6	3.1	21.6	16.1	3.4	1.2
Mean ± SD	24.66 ± 2.94	25.08 ± 5.25	2.49 ± 0.28	4.26 ± 1.41	19.19 ± 3.04	18.38 ± 2.57	2.97 ± 0.44	2.44 ± 1.40

Table 3
Denitrifying bacterial population (CFU/g X10^3)
in the in the sediment sample of the station III during 2005 and 2006

Sampling months	Percentage of denitrifying bacteria		Percentage of *Bacillus sp.* sp.		Percentage of *Pseudomonas* sp.		Percentage of Others	
	2005	2006	2005	2006	2005	2006	2005	2006
January	32.7	32.5	4.4	5.8	22.3	20.5	6.0	6.2
February	31.0	36.8	4.2	6.5	21.0	22.7	5.8	7.6
March	34.0	27.0	4.7	5.7	23.0	17.3	6.3	4.0
April	20.3	35.1	3.2	6.0	13.3	21.9	3.8	7.2
May	23.6	19.0	3.5	2.8	14.8	12.4	5.3	3.8
June	27.8	27.7	3.8	2.9	18.4	19.0	5.6	5.8
July	24.6	17.6	3.7	2.5	15.8	12.0	5.1	3.1
August	20.6	20.2	3.1	3.1	13.5	12.8	4.0	4.3
September	24.0	20.8	3.5	3.3	15.5	13.0	5.0	4.5
October	23.4	19.5	3.4	3.0	15.2	12.5	4.8	4.0
November	15.0	16.0	2.3	2.2	10.2	10.9	2.5	2.9
December	26.8	26.0	4.1	5.3	17.0	15.0	5.7	5.7
Mean ± SD	25.32 ± 5.27	24.85 ± 6.78	3.66 ± 0.62	4.09 ± 1.54	16.66 ± 3.70	15.83 ± 4.06	4.99 ± 1.04	4.92 ± 1.48

Table 4
Denitrifying bacterial population (CFU/g X10^3) in the in the
sediment sample of the station IV during 2005 and 2006

Sampling months	Percentage of denitrifying bacteria		Percentage of *Bacillus sp.* sp.		Percentage of *Pseudomonas* sp.		Percentage of Others	
	2005	2006	2005	2006	2005	2006	2005	2006
January	36.6	31.5	6.0	4.7	25.0	20.2	5.6	6.6
February	31.8	28.5	4.0	4.3	23.1	20.0	4.7	4.2
March	28.2	21.0	3.5	2.2	22.0	16.2	2.7	2.6
April	25.1	27.4	4.0	4.0	18.1	19.6	3.0	3.8
May	29.0	24.9	3.3	3.2	22.2	18.2	3.5	3.5
June	23.2	25.5	3.2	3.7	16.3	18.3	3.7	3.5
July	24.2	28.8	3.6	4.5	17.8	20.0	2.8	4.3
August	27.1	21.0	3.0	2.1	21.0	16.5	3.1	2.4
September	23.5	21.7	4.1	2.5	16.2	16.6	3.2	2.6
October	20.8	23.6	3.1	2.8	14.7	17.8	3.0	3.0
November	20.2	24.3	2.8	3.0	15.0	18.0	2.4	3.3
December	29.3	20.8	3.4	2.0	22.5	16.3	3.4	2.5
Mean ± SD	26.58 ± 4.65	24.92 ± 3.39	3.66 ± 0.81	3.25 ± 0.93	19.49 ± 3.38	18.14 ± 1.46	3.42 ± 0.86	3.52 ± 1.11

Table 5
Denitrifying bacterial population (CFU/g X10^3) in the in the sediment sample of the station V during 2005 and 2006

Sampling months	Percentage of denitrifying bacteria		Percentage of *Bacillus sp.* sp.		Percentage of *Pseudomonas* sp.		Percentage of Others	
	2005	2006	2005	2006	2005	2006	2005	2006
January	35.0	28.2	6.0	4.3	23.4	19.1	5.6	4.8
February	34.1	32.8	5.8	5.0	23.0	21.5	5.3	6.3
March	29.6	26.3	4.7	4.1	19.8	18.0	5.1	4.2
April	20.4	28.8	3.2	4.8	14.5	19.4	2.7	4.6
May	29.5	20.5	4.8	3.8	20.0	14.5	4.7	2.2
June	20.9	21.0	3.6	4.0	14.2	14.6	3.1	2.4
July	24.8	22.8	4.1	4.4	17.1	15.6	3.6	2.8
August	27.0	22.8	4.2	4.4	18.5	15.6	4.3	2.8
September	23.0	22.4	3.7	4.1	16.0	15.7	3.3	2.6
October	25.3	20.0	4.0	3.7	17.5	14.3	3.8	2.0
November	21.7	20.2	3.5	3.8	14.7	14.3	3.5	2.1
December	28.1	33.6	4.6	5.3	19.5	21.8	4.0	6.5
Mean ± SD	26.62 ± 4.65	25.08 ± 4.63	4.35 ± 0.84	4.34 ± 0.50	18.18 ± 2.97	17.12 ± 2.65	4.08 ± 0.88	3.62 ± 1.55

Table 6
Denitrifying bacterial population (CFU/g X10^3) in the in the sediment sample of the Station VI during 2005 and 2006

Sampling months	Percentage of denitrifying bacteria		Percentage of *Bacillus sp.* sp.		Percentage of *Pseudomonas* sp.		Percentage of Others	
	2005	2006	2005	2006	2005	2006	2005	2006
January	26.8	32.0	5.1	4.6	18.3	22.2	3.4	5.2
February	27.2	36.5	5.3	5.3	18.4	24.5	3.5	6.7
March	24.2	30.2	4.5	4.5	17.0	20.6	2.7	5.1
April	21.4	31.7	4.2	4.8	15.0	22.0	2.2	4.9
May	21.3	25.9	4.0	5.4	15.3	16.2	2.0	4.3
June	20.8	25.3	4.1	6.0	14.6	16.8	2.1	2.5
July	24.0	23.8	4.6	4.5	16.8	15.8	2.6	3.5
August	26.5	23.0	5.0	5.6	18.2	15.4	3.3	2.0
September	24.5	22.3	4.5	3.6	17.2	15.5	2.8	3.2
October	23.0	23.5	4.3	3.9	16.5	16.0	2.2	3.6
November	20.8	22.0	3.8	3.6	14.5	15.2	2.5	3.2
December	25.8	30.1	4.8	4.5	17.8	20.5	3.2	5.1
Mean ± SD	23.86 ± 2.29	27.19 ± 4.53	4.52 ± 0.44	4.69 ± 0.74	16.63 ± 1.39	18.39 ± 3.18	2.71 ± 0.51	4.11 ± 1.29

Table 7

Regression co-efficient for the relationship between sediment TVC (CFU/g) and total nitrogen (ppm), total denitrifying bacterial population (%) and total nitrogen, TVC (CFU/g) and organic carbon (%) and Total denitrifying bacterial population (%) and organic carbon (%) during 2005.

Sampling stations	Parameters compared	Regression co-efficients		
		a	bx	R^2
I	TVC (CFU/g) and total nitrogen (ppm)	21.157	0.053	0.0562
	Total denitrifying bacterial population (%) and total nitrogen (ppm)	13.75	0.3696	0.9016
	TVC (CFU/g) and organic carbon (%)	20.477	67.108	0.14
	Total denitrifying bacterial population (%) and organic carbon (%)	14.985	289.5	0.8589
II	TVC (CFU/g) and total nitrogen (ppm)	21.97	0.0573	0.0961
	Total denitrifying bacterial population (%) and total nitrogen (ppm)	17.811	0.1627	0.8317
	TVC (CFU/g) and organic carbon (%)	21.508	63.886	0.0771
	Total denitrifying bacterial population (%) and organic carbon (%)	16.305	185.64	0.6997
III	TVC (CFU/g) and total nitrogen (ppm)	25.171	0.0052	0.0525
	Total denitrifying bacterial population (%) and total nitrogen (ppm)	17.877	0.0422	0.8372
	TVC (CFU/g) and organic carbon (%)	23.621	6.609	0.1060
	Total denitrifying bacterial population (%) and organic carbon (%)	14.275	29.643	0.5113
IV	TVC (CFU/g) and total nitrogen (ppm)	19.125	0.0181	0.8059
	Total denitrifying bacterial population (%) and total nitrogen (ppm)	19.936	0.030	0.8849
	TVC (CFU/g) and organic carbon (%)	18.246	20.284	0.7131
	Total denitrifying bacterial population (%) and organic carbon (%)	18.747	32.469	0.7327
V	TVC (CFU/g) and total nitrogen (ppm)	25.75	0.0129	0.5281
	Total denitrifying bacterial population (%) and total nitrogen (ppm)	19.419	0.0258	0.6966
	TVC (CFU/g) and organic carbon (%)	22.708	14.749	0.8857
	Total denitrifying bacterial population (%) and organic carbon (%)	16.793	21.787	0.6406
VI	TVC (CFU/g) and total nitrogen (ppm)	24.662	0.0059	0.7072
	Total denitrifying bacterial population (%) and total nitrogen (ppm)	20.194	0.0036	0.6400
	TVC (CFU/g) and organic carbon (%)	21.789	4.9651	0.3732
	Total denitrifying bacterial population (%) and organic carbon (%)	17.128	3.7364	0.5248

Table 8

Regression co-efficient for the relationship between sediment TVC (CFU/g) and total nitrogen (ppm), total denitrifying bacterial population (%) and total nitrogen, TVC (CFU/g) and organic carbon (%) and Total denitrifying bacterial population (%) and organic carbon (%) during 2006.

Sampling stations	Parameters compared	Regression co-efficients		
		A	Bx	R^2
I	TVC (CFU/g) and total nitrogen (ppm)	20.002	0.1205	0.2723
	Total denitrifying bacterial population (%) and total nitrogen (ppm)	20.377	0.1886	0.5067
	TVC (CFU/g) and organic carbon (%)	22.658	18.024	0.0077
	Total denitrifying bacterial population (%) and organic carbon (%)	18.999	189.81	0.6507
II	TVC (CFU/g) and total nitrogen (ppm)	18.402	0.1903	0.7332
	Total denitrifying bacterial population (%) and total nitrogen (ppm)	19.560	0.1927	0.3175
	TVC (CFU/g) and organic carbon (%)	22.935	22.517	0.0115
	Total denitrifying bacterial population (%) and organic carbon (%)	13.443	283.90	0.7696
III	TVC (CFU/g) and total nitrogen (ppm)	20.866	0.0218	0.6233
	Total denitrifying bacterial population (%) and total nitrogen (ppm)	16.739	0.0458	0.8579
	TVC (CFU/g) and organic carbon (%)	23.366	3.2667	0.0347
	Total denitrifying bacterial population (%) and organic carbon (%)	23.288	3.7537	0.0143
IV	TVC (CFU/g) and total nitrogen (ppm)	17.186	0.0337	0.8894
	Total denitrifying bacterial population (%) and total nitrogen (ppm)	19.538	0.0248	0.6807
	TVC (CFU/g) and organic carbon (%)	18.221	25.859	0.4476
	Total denitrifying bacterial population (%) and organic carbon (%)	18.243	27.521	0.7157
V	TVC (CFU/g) and total nitrogen (ppm)	26.182	0.0187	0.5489
	Total denitrifying bacterial population (%) and total nitrogen (ppm)	16.671	0.0238	0.6257
	TVC (CFU/g) and organic carbon (%)	28.429	11.314	0.4082
	Total denitrifying bacterial population (%) and organic carbon (%)	17.092	20.725	0.9639
VI	TVC (CFU/g) and total nitrogen (ppm)	35.506	0.0026	0.4362
	Total denitrifying bacterial population (%) and total nitrogen (ppm)	22.264	0.0052	0.6382
	TVC (CFU/g) and organic carbon (%)	32.415	3.6223	0.7069
	Total denitrifying bacterial population (%) and organic carbon (%)	22.873	3.2681	0.2134

Figure 2

Monthly mean distribution of total heterotrophic bacterial population (CFU/g) in sediment samples of the experimental stations (S-I to S-VI) during 2005 and 2006. Each value is the mean (X) of three individual estimates

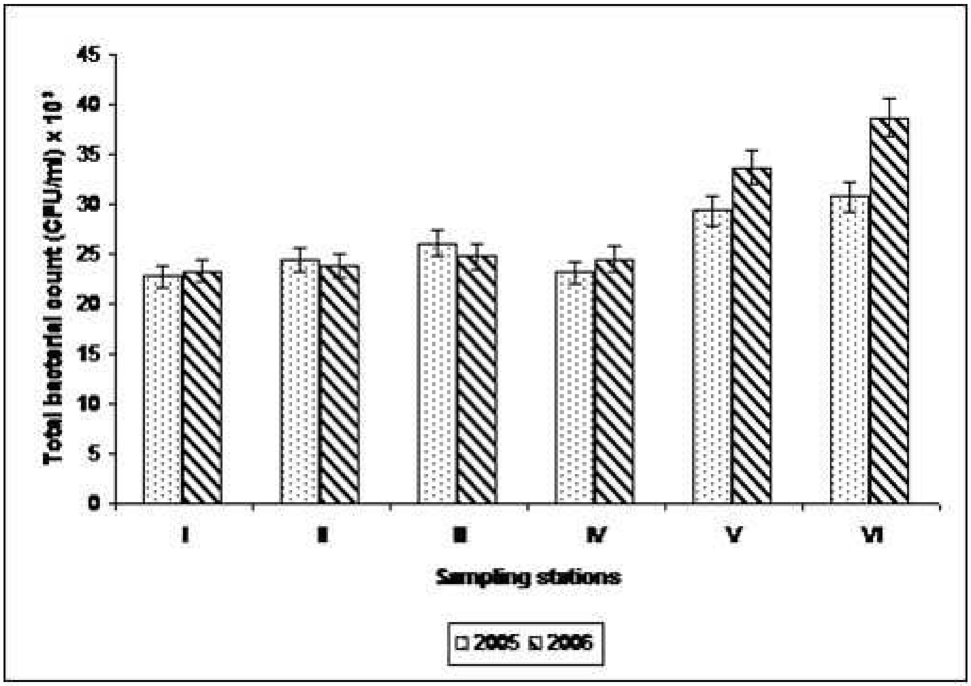


Figure 3

TVC (CFU/g X 10^3) in the sediment sample of the station I during the study period

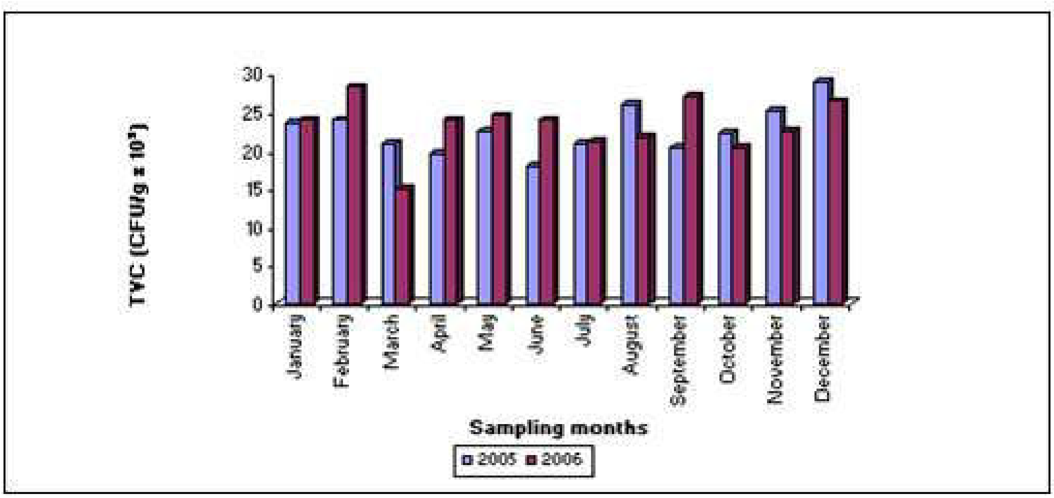


Figure 4
TVC (CFU/g X 10^3) in the sediment sample of the station II during the study period

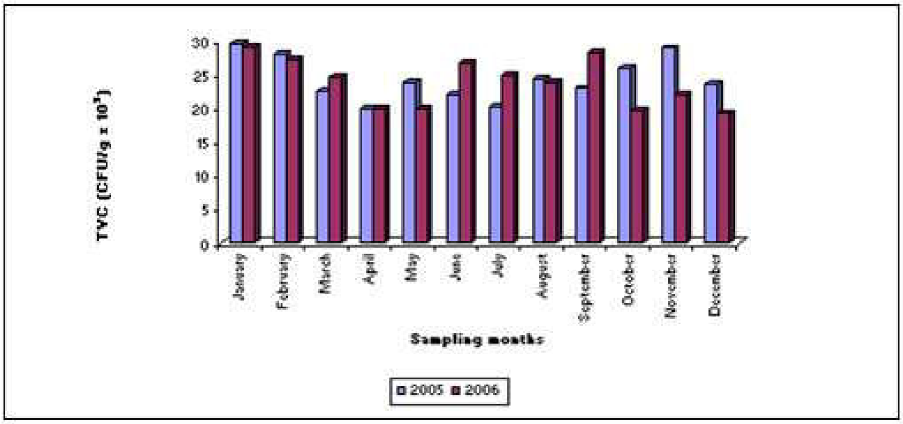


Figure 5
TVC (CFU/g X 10^3) in the sediment sample of the station III during the study period

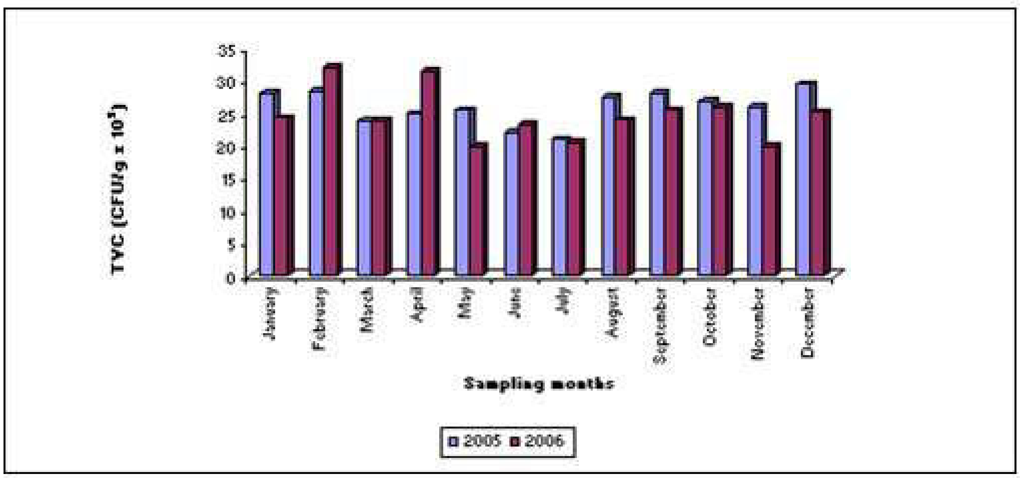


Figure 6
TVC (CFU/g X 10^3) in the sediment sample of the station IV during the study period

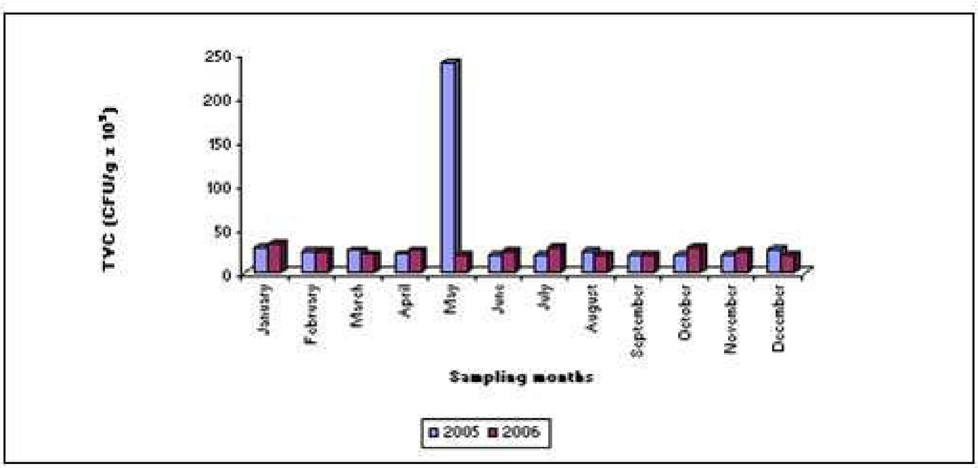


Figure 7
TVC (CFU/g X 10^3) in the sediment sample of the station V during the study period

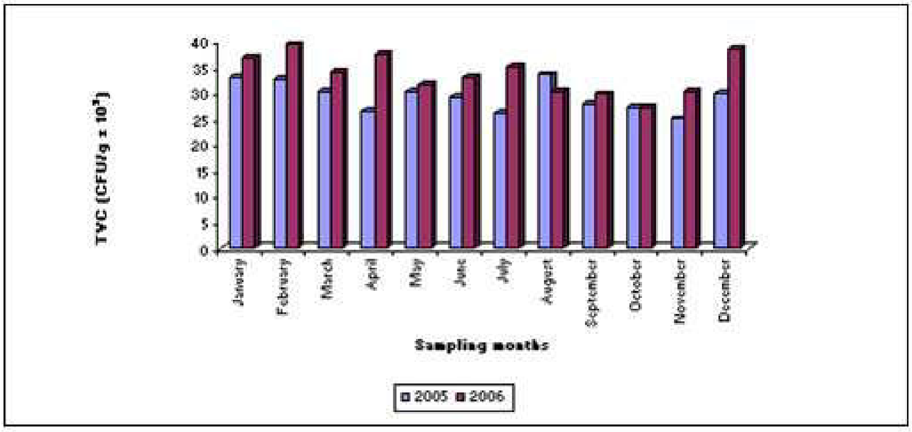
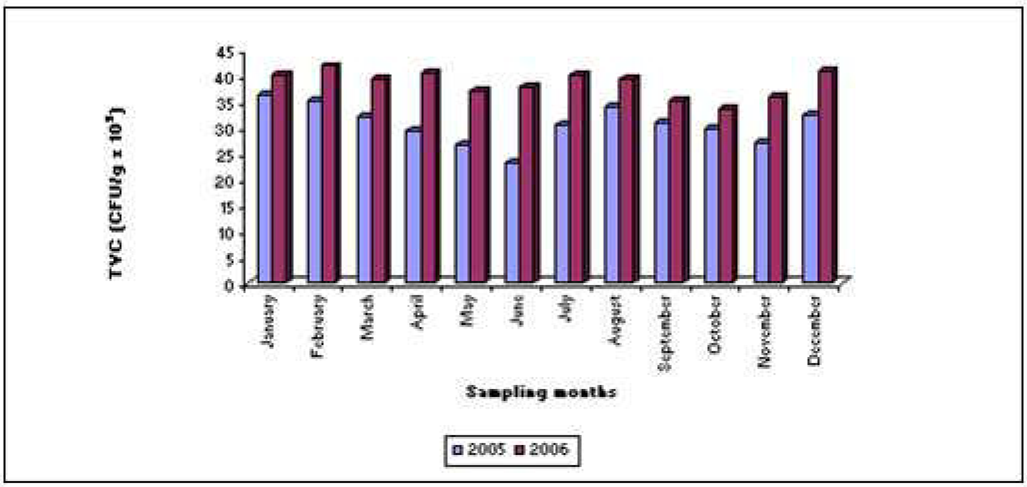


Figure 8
TVC (CFU/g X 10^3) in the sediment sample of the station VI during the study Period



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