



## International Journal of Bioscience and Biochemistry

ISSN Print: 2664-6436  
ISSN Online: 2664-6544  
Impact Factor: RJIF 5.4  
IJBB 2023; 5(2): 12-16  
[www.biosciencejournal.net](http://www.biosciencejournal.net)  
Received: 08-07-2023  
Accepted: 15-08-2023

**Chinnababu Sanapala**  
Research Scholar, Department  
of Marine Living Resources,  
Andhra University,  
Visakhapatnam, Andhra  
Pradesh, India

**K Sujatha**  
Lecturer, Department of  
Zoology, S.G.A. Govt. Degree  
College, Yellamanchili,  
Anakapalli, Andhra Pradesh,  
India

**S Nagaraju**  
Research Scholar, Department  
of Marine Living Resources,  
Andhra University,  
Visakhapatnam, Andhra  
Pradesh, India

**Paidi Sandeep**  
PG Student, Department of  
Microbiology, S.V.U. College of  
Science, Tirupati, Andhra  
Pradesh, India

**Charan Sai Suna**  
PG Student, Department of  
Microbiology, A.U. CSAT,  
Andhra University,  
Visakhapatnam, Andhra  
Pradesh, India

**Corresponding Author:**  
**Chinnababu Sanapala**  
Research Scholar, Department  
of Marine Living Resources,  
Andhra University,  
Visakhapatnam, Andhra  
Pradesh, India

## Heterotrophic bacteria in the distribution of intertidal sediments and near shore water areas of Visakhapatnam, East Coast of India

**Chinnababu Sanapala, K Sujatha, S Nagaraju, Paidi Sandeep and Charan Sai Suna**

**DOI:** <https://dx.doi.org/10.33545/26646536.2023.v5.i2a.44>

### Abstract

Density in Heterotrophic bacteria in the intertidal sediments and near shore waters was studied from June 2022 to June 2023 at fortnightly intervals from five stations. The mean values of water temperature, salinity, pH, dissolved oxygen, and sediment organic matter were 28.9 °C, 36.7 ppt, 4.75 mg/l, 7.8 and 0.75% respectively. The total bacteria density in the near-shore waters ranged from a low density of  $213 \times 10^3$  cfu/ml (Station 2; May II) to a high density of  $465 \times 10^3$  cfu/ml (Station 3; June II) and averaged to  $334 \times 10^3$  cfu/ml. The total bacteria density in the intertidal sediments fluctuated between  $174 \times 10^3$  cfu/g (Station 1; March II) and  $478 \times 10^3$  cfu/g (Station 3; July II) and averaged to  $318 \times 10^3$  cfu/g. Significant negative correlations are observed for salinity, pH and Dissolved Oxygen at Station 2. Significant positive correlations are recorded for water salinity and sediment temperature at Station 4. The paper discusses the impact of Physico-Chemical parameters on the density distribution of bacteria along Visakhapatnam coast.

**Keywords:** Density, heterotrophic bacteria, near-shore water, intertidal sediment, Visakhapatnam coast

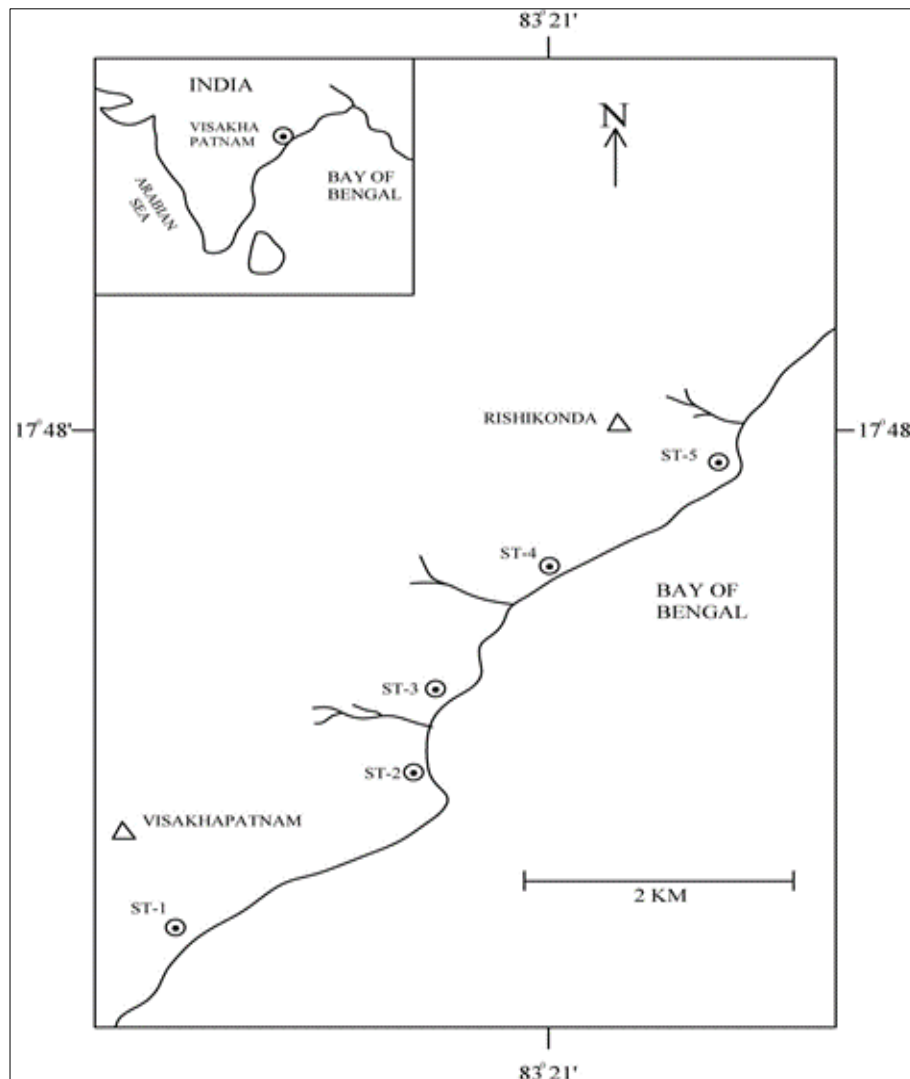
### Introduction

Heterotrophic bacteria play an important role in food web. They survive in the marine environment by obtaining nourishment from decaying organic materials and dissolved organic matter. They represent a major component of marine organisms and need nutrients like carbohydrates, proteins, lipids etc. for growth and metabolism. Marine heterotrophic bacteria are also an are gradient shores. Samples of seawater and sediment were collected during low tide time at fortnightly intervals from five stations from June 2022 to June 2023. At each station, shore water samples (Five replicates) were aseptically collected into five sterile polythene bottles. Sediment core samples (Five replicates) were aseptically collected into five sterile polythene bags. Samples of shore water and sediment water were collected for salinity and dissolved oxygen. Data on temperature were potential source of many commercially important bioactive collected for air, seawater and sediment using hand-held compounds. Their bioremediation capabilities are also remarkable. Earlier investigators studied the distribution of heterotrophic bacteria in relation to ecological parameters from thermometer (0.1 °C sensitivity). Dissolved oxygen was determined by standard Winkler's method. Salinity was determined using a digital salinometer. pH of the water samples coastal and mangrove habitats [1-15]. Studies on the heterotrophic was recorded in the field using a digital pH meter (Elico). Bacteria from Visakhapatnam coast are limited. They include Sediment organic matter was determined by chromic acid the heterotrophic bacteria from Fishing Harbour waters and from Meghadri mangroves of Visakhapatnam [16, 17]. The present study focuses on the density of different categories of digestion method 18. One ml of water sample was used for culture purposes from each replicate water samples. Each sediment replicate sample (10 g) was diluted in 100 ml aged heterotrophic bacteria in relation to the physico-chemical sterile seawater (25% distilled water and 75% seawater). Parameters in near shore waters and intertidal sediments of Visakhapatnam, east coast of India.

## Materials and Methods

Visakhapatnam coast the investigations were carried out along (Lat 17°42'N; Long 83°18'E). The coast of is principally formed by sandy shores. The rocky shores are also present here and there. Five stations (St1: Coastal Battery, St 2: VUDA Park, Inoculations were prepared using 10 fold dilutions. The heterotrophic bacteria were cultured on Zobell Marine Agar (Himedia) at 33 °C for 36 hours. The bacterial colonies were counted using a bacteriological colony counter and the densities were expressed as  $\times 10^3$  cfu per ml or per g. The density data were presented as mean

data of the station. Bacteria were identified upto genus level using cultural, morphological and biochemical characters using Bergey's manual 2010. Pearson correlations were calculated to determine the relation between St 3: Kailasagiri, St 4: Sagar nagar and St 5: Rishikonda) were selected for the present study. An open sewer enters the Bay between St 2 and St 3. Rocky shores are present between St 1 and St 2 and also between St 3 and St 4. All the sampled shores physicochemical parameters and bacterial densities. The obtained correlations were tested for their significance using 't' test.



**Fig 1:** The location of sampling Stations 1 to 5 along Visakhapatnam coast

## Results and Discussion

The locations of sampling Stations 1 to 5 along Visakhapatnam coast are shown in Figure-1. Figure-2 gives the fortnightly data on temperature of air (2a), near-shore water (2b), and sediment (2c) and sediment water (2d) at Stations 1 to 5 from June 2022 to June 2023. Figure-3 presents the fortnightly data on near-shore water salinity (3a), near-shore water dissolved oxygen (3b), near-shore water pH (3c), sediment water salinity (3d), sediment water dissolved oxygen (3e), sediment water pH (3f) and sediment organic matter (3g) at Stations 1 to 5 during the study period. Table-1 shows the mean ( $n=5$ ) density distribution ( $\times 10^3$  cfu/ml) of heterotrophic bacteria in the near-shore waters at Stations 1 to 5 from June 2022 to June 2023. The mean ( $n=5$ ) density distribution ( $\times 10^3$  cfu/g) of heterotrophic

bacteria in the sediment at Stations 1 to 5 from June 2022 to June 2023 is presented in Table-2. Table-3 presents Pearson correlation coefficients ( $p=0.05$ ) between physico-chemical parameters and bacterial densities of near-shore water and intertidal sediments at Stations 1 to 5 during the study period.

The temperature distribution of air, near-shore water, sediment and sediment water showed in general similar trends during study period. High temperatures were recorded during April and May months. Low temperatures were observed during January month. The mean values of temperature for the air, near-shore water, sediment and sediment water were 31.5, 28.6, 29.2 and 28.6 °C respectively. The salinity distribution of near-shore water and sediment water exhibits minor fluctuations between

stations. High salinities were recorded in April and May months and the low values were observed from August to January. The mean salinity values for near-shore water and sediment water 34.9 and 35.1 ppt respectively and they were more or less similar for all the five stations investigated. The dissolved oxygen (D.O.) values showed irregular fluctuations and they did not represent any trend either in the near-shore water or in the sediment water in all the five stations. The D.O. values for the near-shore waters were slightly higher than the values of sediment water. The mean values of D.O. for the near-shore and sediments waters were 4.73 and 4.44 mg/l respectively. Minor irregular fluctuations were observed for pH, both in the near-shore and sediment waters, in all the stations. The mean values of pH for near-shore and sediment waters were 7.7 and 7.7 respectively. The sediment organic matter is relatively low in all the stations and did not exhibit any seasonal trend. The mean value of sedimentary organic matter was 0.73%.

The qualitative analyses of the bacterial samples, both in the water and sediment, revealed the occurrence of five genera along Visakhapatnam coast and their density distribution will be discussed elsewhere. Quantitatively, the total bacteria in the near-shore waters exhibited irregular fluctuations in all the stations investigated during the study period. The total bacteria density in the near-shore water ranged from a low density of  $213 \times 10^3$  cfu/ml (Station 2; May II) to a high density of  $463 \times 10^3$  cfu/ml (Station 3; November II) and averaged to  $334 \times 10^3$  cfu/ml. Among the

stations, the mean total bacterial densities were lowest ( $322 \times 10^3$  cfu/ml) and highest ( $356 \times 10^3$  cfu/ml) at St. 4 and St. 3 respectively and averaged to  $334 \times 10^3$  cfu/ml. The quantitative distribution of total bacteria in the intertidal sediments also showed irregular fluctuations, throughout the study period, in all the five stations. The total bacteria density in the sediment fluctuated between  $172 \times 10^3$  cfu/g (Station 1; March II) and  $475 \times 10^3$  cfu/g (Station 3; December II) and averaged to  $316 \times 10^3$  cfu/g. Among the stations, the mean lowest ( $286 \times 10^3$  cfu/g) and highest ( $353 \times 10^3$  cfu/g) total bacteria densities were observed in St.4 and St. 5 respectively and averaged to  $316 \times 10^3$  cfu/g. Variations in bacterial density fluctuations at St. 3 and St.5 were relatively low in the near-shore waters when compared with the intertidal sediments. The Pearson correlation analyses between physico-chemical parameters and bacterial densities revealed both the negative and positive correlations. Majority of these correlations were insignificant ( $p=0.05$ ). At Station 2, significant negative correlations were observed in the intertidal sediments for salinity, dissolved oxygen and pH. At Station 4, the total bacteria densities showed significant positive correlations for near-shore water salinity and sediment temperature. At Station 3, a significant negative correlation was observed between near-shore water temperature and total bacteria density. Sediment organic matter, which was present in low quantities during the present study, showed insignificant negative correlations at all stations except at St.1.

**Table 1:** The mean (n=5) density distribution ( $\times 10^3$  cfu/ml) of heterotrophic bacteria at fortnightly intervals in the near-shore waters at Stations 1 to 5 from June 2022 to June 2023

S1 Water			S2 Water		S3 Water		S4 Water		S5 Water	
Fortnig HTS	T.B.C in $10^3$ cfu/ ml	SD	T.B.C in $10^3$ cfu/ ml	SD	T.B.C in $10^3$ cfu/ ml	SD	T.B.C in $10^3$ cfu/ ml	SD	T.B.C in $10^3$ cfu/ ml	SD
M-2	247	142.419	215	67.61	291	75.623	241	80.566	298	16.500
J-1	282	41.020	303	143.60	328	131.567	340	74.287	303	139.041
J-2	247	146.293	264	112.37	253	148.928	434	85.431	346	131.333
J-1	376	140.520	307	134.14	356	72.772	422	87.460	338	86.987
J-2	356	156.090	488	78.99	393	122.469	285	141.656	259	86.261
A-1	271	106.214	331	99.09	356	83.242	364	128.332	285	93.859
A-2	334	110.646	334	160.85	419	220.714	375	70.235	292	188.293
S-1	284	147.098	260	162.39	362	180.283	256	85.587	367	105.833
S-2	330	176.382	364	88.20	305	127.248	347	88.519	334	112.350
O-1	348	121.106	301	107.35	301	107.670	233	111.747	367	107.321
O-2	421	200.766	230	73.37	403	262.809	353	108.146	343	201.173
N-1	345	132.926	341	93.25	403	82.707	302	143.954	374	83.306
N-2	285	108.787	331	65.40	463	173.447	271	87.426	401	106.872
D-1	241	126.379	341	164.56	411	246.127	291	71.143	302	153.462
D-2	383	59.781	380	150.79	409	80.739	345	95.366	334	91.212
J-1	401	161.214	281	81.37	361	84.769	462	76.292	293	146.114
J-2	363	128.778	257	102.71	418	60.632	233	112.713	393	72.178
F-1	320	96.831	202	99.60	348	100.979	300	103.640	345	61.344
F-2	313	120.020	300	106.98	431	221.301	252	105.398	361	68.253
M-1	356	143.278	381	120.26	349	137.393	368	178.806	371	169.377
M-2	349	35.473	406	113.80	328	88.990	361	156.857	292	72.207
A-1	320	128.632	414	219.48	243	81.509	266	122.453	294	60.554
A-2	325	116.622	320	105.90	250	121.826	262	95.578	321	96.231
M-1	386	109.017	411	88.81	376	84.621	354	50.499	282	121.861

**Table 2:** The mean (n=5) Density distribution ( $\times 10^3$  cfu/g) of Heterotrophic Bacteria at fortnightly intervals in the intertidal sediments at Stations 1 to 5 from June 2022 to June 2023

S1 Sediment			S2 Sediment		S3 Sediment		S4 Sediment		S5 Sediment	
Fort Nights	T.B.C $10^3$ cfu/ml	SD	T.B.C $10^3$ cfu/ml	SD	T.B.C $10^3$ cfu/ml	SD	T.B.C $10^3$ cfu/ml	SD	T.B.C $10^3$ cfu/ml	SD
M-2	211	70.151	329	4.230	231	85.522	187	32.166	254	62.7182

J-1	220	133.813	212	21.621	243	81.230	251	117.127	317	137.9022
J-2	212	100.071	302	15.601	327	86.732	477	83.741	433	168.9221
J-1	301	167.563	232	32.172	2547	122.061	423	184.211	226	69.5976
J-2	391	123.607	222	11.519	411	243.200	365	73.312	254	15.2125
A-1	279	112.457	244	18.104	284	151.014	287	119.515	332	34.3243
A-2	211	86.876	344	23.191	416	176.324	279	103.166	413	110.7610
S-1	251	178.302	267	21.672	366	81.210	187	73.454	321	122.3561
S-2	354	167.228	401	12.756	343	80.331	389	56.741	367	91.7141
O-1	347	122.633	301	23.133	221	64.062	276	81.243	337	54.7712
O-2	331	89.231	354	8.434	254	71.906	319	51.423	313	132.7123
N-1	320	178.077	346	09.354	231	62.612	231	81.564	454	72.9043
N-2	355	91.253	276	8.221	351	154.227	387	89.963	423	153.1521
D-1	311	141.521	321	4.203	400	98.761	311	910.250	275	94.2911
D-2	210	56.417	303	16.017	421	60.181	242	102.610	243	60.0433
J-1	319	102.345	325	21.528	347	154.327	432	81.501	419	61.4143
J-2	341	148.743	334	20.755	382	111.762	190	107.754	475	60.0541
F-1	391	177.261	387	19.551	322	42.876	221	54.221	378	61.2187
F-2	340	160.451	264	32.432	257	141.331	287	71.912	476	132.0111
M-1	329	130.066	342	20.443	310	87.944	261	60.124	336	68.2232
M-2	152	112.943	381	33.118	347	61.496	197	54.341	257	52.5322
A-1	274	152.982	277	22.077	261	42.543	415	79.267	286	87.1361
A-2	285	143.977	316	18.116	332	143.821	276	132.576	411	99.3461
M-1	341	110.676	292	7.176	304	161.123	365	51.187	344	165.5212

**Table 3a:** Pearson correlation coefficients ( $p=0.05$ ) between physico-chemical parameters and bacterial densities of near-shore water at Stations 1 to 5 during the study period

	St.1	St.2	St.3	St.4	St.5
Temperature	-0.321	0.141	-0.401*	0.005	-0.245
DO	-0.027	0.321	-0.0148	0.166	0.082
pH	-0.228	-0.148	-0.306	-0.121	-0.171
Salinity	-0.090	0.213	-0.0492	0.366*	-0.412

**Table 3b:** Pearson correlation coefficients ( $p=0.05$ ) between physico-chemical parameters and bacterial densities of intertidal sediment at Stations 1 to 5 during the study period

	St.1	St.2	St.3	St.4	St.5
Temperature	-0.298	-0.194	-0.229	0.340*	-0.261
DO	0.085	-0.360*	0.297	0.185	0.063
pH	-0.264	-0.477*	-0.157	0.254	-0.290
Salinity	-0.128	-0.469*	-0.214	0.055	-0.007
OM	0.113	-0.214	-0.066	-0.279	-0.172

## Discussion

It is observed that, in all the stations investigated, the total bacteria densities are relatively higher in shore water than in intertidal sediments. The mean total bacterial densities for the near-shore water ( $334 \times 10^3$  cfu/ml) and intertidal sediment ( $316 \times 10^3$  cfu/g) also indicate that shore water support *ca.* 6% high load of bacterial density than the intertidal sediments. The high bacterial density in the shore water may due to land drainage, which enters the Bay waters between St. 2 and St. 3. The occurrence of high bacterial densities at Station 3 ( $356 \times 10^3$  cfu/ml; near-shore water) and at Station 5 ( $352 \times 10^3$  cfu/g; intertidal sediment) may be due to anthropogenic factors as these two stations are used by tourists.

It is interesting to note that at Station 4 the bacterial densities exhibited significant positive correlations with near-shore water salinity and sediment temperature. At Station 2, the sediment bacteria showed significant negative correlations with dissolved oxygen, pH and salinity. At Station 3, the near-shore water bacteria density recorded a significant negative correlation with water temperature. Heterotrophic bacteria densities in the marine zones of Vellar estuary and Killai were reported as  $10.9 \times 10^3$  cfu/ml

and  $100 \times 10^3$  cfu/ml respectively [5, 7]. In Cochin coastal waters,  $750 \times 10^3$  cfu/ml of heterotrophic bacteria were recorded [9].

He registered two peaks: the major one in January and February and the minor one in August and September. He attributed the evaporation of surface water, suitable temperature and low variations in salinity as the reasons for a major peak during summer. The minor peak is attributed to the DOM-exudates of microalga *Noctiluca*. The *Noctiluca* releases DOM-containing exudates, which favour the growth of bacteria. Prabhu *et al.* recorded highest density  $26.8 \times 10^3$  cfu/ml of bacteria in the coastal waters of Madras [19]. He observed very minimal fluctuations in the monthly density distribution of bacteria.

He attributed the high density distribution to sewage pollution. Even though anthropogenic impact is noted, the present study did not cover on the pollution aspect. From Dandi coastal waters, bacterial density of  $720 \times 10^3$  cfu/ml was recorded [20]. The coastal waters of Tamilnadu supported very low ( $1.0 \times 10^3$  cfu/ml) bacterial densities [21]. Mandovi-Zuari estuaries harboured  $18.9 \times 10^3$  cfu/ml [11]. Visakhapatnam fishing harbour registered  $2.23 \times 10^3$  cfu/ml [16]. Meghadri estuary at Visakhapatnam recorded  $7.93 \times 10^3$  cfu/ml [17].

## Conclusion

The heterotrophic bacterial densities recorded in the near-shore waters of the present study are relatively higher than the earlier reports except from Cochin coastal waters [9]. The sediment bacterial density recorded in Madras coastal sediments ( $2460 \times 10^3$  cfu/g) is relatively higher than the present study report [19]. In the present investigation, only some of the observed fluctuations in the bacterial densities could be explained with the observed temperature, salinity, dissolved oxygen and sedimentary organic matter. The recorded variations in bacterial densities may be due to some other physico-chemical and biological factors besides regional variations in the habitats.

**Acknowledgement:** The authors are thankful to the Head, Department of Marine Living Resources, Andhra

University, Visakhapatnam for providing facilities to carry out this work.

### References

1. Verlankar NK. Bacteria isolated from sea water and marine mud of Mandapam (Gulf of Mannar and Palk Bay). *Indian Journal of Fisheries*. 1957;4:208-227.
2. Stevenson HL, Millwood CE, Hebel BH. Aerobic, heterotrophic bacterial populations in estuarine water and sediments. R.R. Colwell & Morita R.Y, (Dds). *Effect of the Ocean environment on microbial activities*, University Park press, Baltimore, U.S.A; c1974. p. 268-285.
3. Nair S, Lokabharathi P, Achutankutty CK. Distribution of heterotrophic bacteria in marine sediments [India]. *Indian J Mar. Sci.* 1978;7:18-22.
4. Palaniappan R, Krishnamurthy K. Heterotrophic bacteria of near shore waters of the Bay of Bengal and Arabian Sea. *Indian J Mar. Sci.* 1985;14:110-113.
5. Kannan L, Vasantha K. Distribution of Heterotrophic bacteria in Vellar estuary east coast of India. *Indian J Mar. Sci.* 1986;15:265-267.
6. Shiaris MP, Rex AC, Pettibone GW, Keay K, McManus P, Rex MA, *et al.* Distribution of indicator bacteria and *Vibrio parahaemolyticus* in sewage-polluted intertidal sediments. *Appl. Environ. Microbiology*. 1987;53:1756-1761.
7. Vasantha K, Kannan L. Distribution of heterotrophic bacteria in the Killai backwaters, Porto Novo, south east coast of India. *Mahasagar*. 1987;20:32-35.
8. Choudhary A. Multidisciplinary research project on Mangroves of Sundarbans. Project Report, University of Calcutta; c1987, 87.
9. Alavandi SV. Heterotrophic bacteria in the coastal waters of Cochin. *Indian J Mar. Sci.* 1989;18:174-176.
10. Chandramohan D. *Marine microbiology: challenges and future directions*. Citeseer; c2004. p. 7-13.
11. Priya MD, Kalekar S, Bhosle S. Diversity of free-living and adhered bacteria from mangrove swamps. *Indian J Microbiol.* 2004;44:247-250.
12. Surajit D, Lyla PS, Ajmalkhan S. Spatial variation of aerobic culturable heterotrophic bacterial population in the sediments of the continental slope of western Bay of Bengal. *Indian J Mar. Sci.* 2007;36:51-58.
13. Azam F, Malfatti F. Microbial structuring of marine ecosystems. *Nature Reviews on Microbiology*. 2007;5:782-794.
14. Patra AK, Acharya BC, Mohapatra A. Occurrence and distribution of bacterial indicators and pathogens in coastal waters of Orissa. *Indian J Mar. Sci.* 2009;38:474-480.
15. Chen M, Li H, Li G, Zheng T. Distribution of *Vibrio anguilyticus*-like species in Shenzhen coastal waters, China. *Braz. J Microb.* 2011;42:884-896.
16. Sreedevi P, Kondalarao B. Density distribution of heterotrophic bacteria in the surface waters at Visakhapatnam Fishing Harbour. *J Mar. Biol. Ass. India.* 2006;48:237-240.
17. Raghavendrudu G, Kondalarao B. Density of heterotrophic bacteria in Meghadri mangrove ecosystem, Visakhapatnam, east coast of India. *J Mar. Biol. Ass. India.* 2008;50:1-4.
18. Jackson ML. *Soil chemical analysis*. Prentice Hall of India Ltd, New Delhi; c1967, 498.
19. Prabhu SK, Subramanian B, Mahadevan A. Occurrence and distribution of heterotrophic bacteria of Madras coast (Bay of Bengal). *Indian J Mar. Sci.* 1991;20:130-133.
20. Mogal HF, Dube HC. Heterotrophic bacterial population of waters of Dandi Sea coast. *Indian J Microbiology*. 1995;35:43-46.
21. Ramaiah N, Raghukumar C, Sheelu G, Chandramohan D. Bacterial abundance, communities and heterotrophic activities in coastal waters of Tamil Nadu. *Indian J Mar. Sci.* 1996;25:234-239.