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# QUALITATIVE DISTRIBUTION OF HETEROTROPHIC BACTERIA IN THE NEAR SHORE HABITATS ALONG VISAKHAPATNAM COAST, INDIA

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## ABSTRACT

Heterotrophic bacteria play an important role in food web. The diversity distribution of heterotrophic bacteria in the near shore habitats along Visakhapatnam coast was carried out for two years from May 2014 to May 2016. The study recorded the occurrence of 46 species of heterotrophic bacteria in the near shore waters and sediments along Visakhapatnam coast. They belong to *Bacillus* (16 species), *Vibrio* (11 species), *Pseudomonas* (9 species), *Staphylococcus* (6 species) and *Micrococcus* (4 species) genera of the heterotrophic bacteria. The heterotrophic bacteria diversity distribution is compared with similar investigations carried out elsewhere.

## INTRODUCTION:

Heterotrophic bacteria is a highly diverse group and the members of this group are responsible for decomposition and re-mineralization of organic molecules in nature. The heterotrophic bacteria are dominant in the marine ecosystem (Mary & Miller 2007). Heterotrophic bacteria serve as prey organisms for protozoans, invertebrate larvae and many other microscopic metazoans. They generate dissolved nutrients needed by the marine primary producers to synthesize the organic matter. As a group, the heterotrophic bacteria comprises of a wide variety of bacterial populations belonging to Gram positive bacteria, Gram negative bacteria, the unusual bacteria (fruiting, budding and lithotrophic bacteria) and the Gram positive filamentous bacteria. 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 a potential source of many commercially important bioactive compounds. Their bioremediation capabilities are also remarkable. The heterotrophic bacteria from the Indian coastal habitats were studied several researchers. They include Velankar (1957), Nair *et al* (1978), Palaniappan & Krishnamurthy (1985), Kannan & Vasantha (1986), Vasantha & Kannan (1987), Alvandi (1990), Ramamurthy *et al* (1990 ), Prabhu *et al* (1990), Rajeswari *et al* (1995), Mogal & Dube (1995), Ramaiah *et al* (1996), Chandramohan (1997, review), Cavallo *et al* (1999), Yogita & Saroj (2002), Pradeep Ram *et al* (2003), Priya *et al* (2004), Surjit Das *et al* (2006), Gouda *et al* (2006), Feng *et al* (2009), Nithya and Pandyan (2010), Vijayakumar *et al* (2010), Kumar and Kannabiran (2010), Govindasamy *et al* (2011), Govindasamy *et al* (2012), Wang *et al* (2012), Lakshmipriya and Sivakumar (2012), Murugesan *et al* (2012), Saravanan *et al* (2013), Goutham and Ramanathan (2013), Wang *et al* (2013), Zheng *et al* (2014), Basak *et al* (2014), Elisa *et al* (2015), Chen *et al* (2015), Carreira *et al* (2015) and Doghri *et al* (2015). The information available on heterotrophic bacteria, based on the earlier investigations, indicates paucity of data on the composition of heterotrophic bacteria with special reference to Visakhapatnam coast. Hence in the present study, an attempt has been made to study the fortnightly and seasonal variations in the distribution of heterotrophic bacteria in the near shore water and sediment along Visakhapatnam coast.

## MATERIALAND METHODS:

Heterotrophic bacteria distribution in the near-shore water and sediments of Visakhapatnam coast was studied at fortnightly intervals during first year (May 2014 to May 2015) from five stations (Station I to Station V) and seasonally during second year ( 2015-2016) *i.e*. May (Summer), September (Monsoon) and January (Post-Monsoon) periods. Table 2.1 provides the Latitudes and Longitudes of the Stations I to V. Near-shore water and sediment samples were collected along Visakhapatnam coast at five stations: Station I (Coastal Battery), Station II (VUDA Park), Station III (Kailasagiri), Station IV (Sagar Nagar) and Station V (Rishi Konda). Figure 2.1 shows the location of sampling Stations I to V. The sampling information of the heterotrophic bacteria is given in Table 2.2. The water and sediment samples were collected during low tide period of Springs between 11 am and 3 pm on sampling days. The samples were kept cool until transported to laboratory for further processing. At each Station, five surface water samples and five surface sediment samples were collected for heterotrophic bacteria studies. Surface water samples, were aseptically collected using autoclaved glass bottles (100 ml). Surface sediment samples at each station were aseptically collected into sterile polythene bags. In addition, at each station samples/data were collected for physico-chemical parameters *i.e.* temperature, salinity, dissolved oxygen and pH. Temperature of air, water and sediment samples was measured using 0.1°C sensitivity hand-held mercury thermometer. Salinity and dissolved oxygen of surface water and sediment water samples, were measured by Harvey method (Harvey, 1960) and Winkler's method (Schlieper, 1972) respectively. Water samples for dissolved oxygen were treated with Winkler's A and B reagents in the field. Surface water pH and sediment water pH were measured using a digital pH meter (Elico). Besides, surface sediment samples were collected into polythene bags for sedimentary organic matter studies. The sedimentary organic matter was estimated by chromic acid digestion method (Jackson 1967). In the laboratory, each water sample (10 ml) was diluted with 100 ml aged seawater (75% seawater and 25% deionized water). This diluted sample was used for inoculations of agar media plates using ten-fold dilution. Each sediment sample (10 g) was diluted with 100 ml aged seawater. This diluted sample was used for inoculations of agar media plates using ten-fold dilution. Both the water and sediment samples were inoculated on Zobell marine agar (HIMEDIA) using spread plate technique. The inoculated media plates were kept in a bacteriological incubator for 36 to 48 hours at a temperature of 32°C (+/- 1°C). The bacteria colonies were counted using a bacteriological colony counter and densities were expressed as Nos. x $10^3$ cfu/ml for water and Nos. x $10^3$ cfu/g for sediment. Bacteria grown on the culture plates were identified up to species level using their morphological , cultural and biochemical characters (shape, motility, growth in air, swollen cell, pigmentation, spore characteristics, Gram's staining, acid fast staining, utilization of various sugars and amino acids, catalase, oxidase, starch hydrolysis, gelatin hydrolysis, glucose fermentation, H2S production, nitrate test, indole test, MR test, VP test, citrate test, ONPG test, mannitol test, sorbitol test, coagulase test, licithinase test, urea test, growth in 5% and 6.5% NaCl, growth in acidic pH, tween 80 growth at specified low and high temperatures, haemolysis, sensitivity to Novobiocin (Baumann *et al* 1972, Sneath *et al* 1987, Claus and Berkeley 1986 and Edward 1997). The physico-chemical parameters and bacteria density data were processed by ANOVA. The correlation analyses for bacteria density data and physico-chemical parameters were carried out using SPSS software XVI version.

## RESULTS AND DISCUSSION:

The distribution of air temperature, surface water temperature, sediment temperature, sediment water temperature, surface water salinity, sediment water salinity, surface water dissolved oxygen, sediment water dissolved oxygen, surface water pH, sediment water pH and sediment organic matter during study period is given in Table No. The station-wise species composition of heterotrophic bacteria is presented in Table No. The present study recorded the occurrence of 46 species of heterotrophic bacteria in the near shore waters and sediments along Visakhapatnam coast. They belong to *Bacillus* (16 species), *Vibrio* (11 species), *Pseudomonas* (9 species), *Staphylococcus* (6 species) and *Micrococcus* (4 species) genera of the heterotrophic bacteria. The *Bacillus* species include: *B. alcaliphilus, B. alvei, B. badius, B. brevis, B. circulans, B. insolitus, B. laterosporus, B. licheniformes, B. macerans, B. marinus, B. megaterium, B. mycoides, B. pasteurii, B. polymyxa, B. pumilus,* and *B. subtilis.* The genus *Vibrio* is represented by *V. anguillarum, V. alginolyticus, ?algavillarum, V. holera, V. fisheri, V. furnishii, V. harveyii, V. parahaemolyticus, V. pomeroyi, V.shigelloides* and *V. vulnificus*. The *Pseudomonas* genus is contributed by *P. aeruginosa, P. alcaligenes, P. denitrificans, P. facilis, P. florescens,P. maltophila, P. membranoformis, P. putida* and *P. saccharophila.*. The *Staphylococcus* species include *St.aureus, St. epidermidis, St. haemolyticus, St. intermedius, St. lentus* and *St. saprophyticus.* The *Micrococcus* genus is formed by the species *M.kristinae, M. luteus, M. roseus* and *M. varians.* All these reported species were observed both in water and in sediment. Of the sixteen *Bacillus* species, three species (*B. marinus, B.pasteurii* and *B. subtilis*) are recorded in all the five stations. Majority (9) of the species (*B. alvei, B. circulans, B. insolitus* (at St. I), *B.laterosporeus* (at St III), *B. licheniformes, B. macerans, B.pumilus* ( at St IV) and *B. megaterium* (at St V) are observed once only during

study period. At Station I, maximum (9) number of *Bacillus* species are observed. Of the eleven species of *Vibrio*, three species (*V. anguillarum, V. harveyii and V. shigelloides*) are recorded at all the five stations. *V. alginolyticus, V. furnishii* (at St III) and *V. fisheri* (at St. IV) are observed once only during the study period. Among the nine *Pseudomonas* species recorded, two species (*Ps. Aeruginosa and Ps. Alkaligenes*) are reported from all the five stations. The species *Ps. maltophila* (at St I), *Ps. saccharophila* (at St II) and *Ps. membrabranoformes* (at St V) are observed only once during the study period. Of the six species of *Staphylococcus*, *St. aureus* is only observed at all the five stations. Three species ( *St. epidermidis* (at St II), *St. intermedius* (at St IV) and *St. lentus* (at St V) are recorded once only during the study period. *Micrococcus varians* is recorded all the five stations. *M. roseus* (at St I) and *M. kristinae* (at St V) are recorded once only during the study period.

An analysis of the percentage distribution of the different genera of heterotrophic bacteria in the near shore water and sediment revealed that the genus *Bacillus* was dominant both in the water and sediment. In the surface water the abundance of *Bacillus* (46%) was followed *Staphylococcus* (20%), *Pseudomonas* (11%), *Micrococcus* (10%) and *Vibrio* (8%) (Fig. 3.35). In the sediment the abundance of *Bacillus* (48%) was followed *Staphylococcus* (15%), *Micrococcus* (12%) *Vibrio* (10 %) and *Pseudomonas* (9%), (Fig. 3.48). Earlier investigations attempted the bacterial identification up to genus level, that to, in habitats like mangroves, estuaries and shallow waters. Prabhu et al (1990) identified 7 genera (*Vibrio, Bacillus, Micrococcus, Pseudomonas, Alcaligenes, Flavobacterium* and *Photobacterium)* with the dominance of *Vibrio* (35%) in the shallow waters off Madras coast. Of them, the genera *Alcaligenes, Flavobacterium* and *Moraxella* are not recorded in the present study. Cavallo *et al* (1999) registered 5 genera (*Aeromonas, Photobacterium, Moraxella, Alkaligenes* and *Flavobacterium)* with the dominance of *Aeromonas* (35%) in the shallow ( 13 m) sediments off Mar Piccolo, Italy. All these five genera are not observed in the present investigation. Raghavendrudu (2008) identified five genera of heterotrophic bacteria in the mangrove sediments of Meghadri estuary, Visakhapatnam. They are: *Bacillus, Brevibacterium, Staphylococcus, Micrococcus and Pseudomonas.* The genus *Brevibacterium* is not recorded in the present investigation. Feng *et al* (2009) observed high density of heterotrophic bacteria in the estuarine and coastal areas of East China Sea (Proteobacteria (72.9%), Firmicutes (6.4%), Bacteroidetes (4.6%) and Actinobacteria (4.1%). In the water, Alphaproteobacteria were the most abundant, whereas in the sediment samples, Gammaproteobacteria were predominant. Govindasamy *et al* (2012) reported 5 genera (*Bacillus, Corenybacterium, Vibrio, Micrococcus* and *Pseudomonas)* from Muthupet mangrove sediments, Taminadu. The genus *Corenybacterium* is not recorded in the present study. Saravanan et al (2013) observed 9 genera in the salt marsh and 7 genera in the Vellar estuary, Tamilnadu. Of the genera reported by them, *Marinococcus, Proteus, Marinobacter, Streptococcus* and *Marinomonas* are not recorded in the present study. The variations in the generic and species composition in the present study and other studies may be due to the local conditions (physico-chemical and biological conditions) prevailing at that time.

Eventhough the present study recorded 46 species of heterotrophic bacteria, only 10 species (*B. marinus, B.pasteurii*, *B. subtilis, V. anguillarum, V. harveyii, V. shigelloides, Ps. aeruginosa, Ps. alkaligenes*, *Staphylococcus aureus* and *Micrococcus varians)* were observed at all the five stations indicating their uniform distribution. Nineteen species {*B. alvei, B. circulans, B. insolitus* (at St. I), *B.laterosporeus* (at St III), *B. licheniformes, B. macerans, B.pumilus* (at St IV), *B. megaterium* (at St V), *V. alginolyticus, V. furnishii* (at St III), *V. fisheri* (at St. IV), *Ps. maltophila* (at St I), *Ps. saccharophila* (at St II), *Ps. membrabranoformes* (at St V), *St. epidermidis* (at St II), *St. intermedius* (at St IV), *St. lentus* (at St V), *M. roseus* (at St I) and *M. kristinae* (at St V} were recorded only once during the study period indicating their rarity in the bacterial populations. The occurrence of these rare species in the samples may be attributed to the land drainage as there is a flood water channel entering into the coastal waters between Stations II and III.

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**Table 2.1:** Geographical location of sampling Stations 1 to 8 during study period.

| S. NO | Stations | Latitude | Longitude |
|---|---|---|---|
| 1 | Coastal Battery (St – I) | 83°18'41.15"E | 17°42'20.47"N |
| 2 | VUDA Park (St – II) | 83°20'18.77"E | 17°43'13.88"N |
| 3 | Kailasagiri (St - III) | 83°20'42.93"E | 17°44'28.12"N |
| 4 | Sagar Nagar (St – IV) | 83°21'42.42"E | 17°45'45.18"N |
| 5 | Rishikonda (St – V) | 83°23'09.08"E | 17°46'52.04"N |

**Table 2.2:** Heterotrophic Bacteria sampling dates during first year (fortnightly sampling) and during second year (Seasonal sampling)

| Sampling Stations, Points and Nature of samples | | Dates of Sampling | |
|---|---|---|---|
| Number of Stations (Station I to Station V) | : 5 | First year | 28-May-014 |
| | | | 13-Jun-2014 |
| No of Samples at each Stations | : 5 | | 27-Jun-2014 |
| | | | 12-Jul-2014 |
| Nature of Samples | : Water and Sediment | | 26-Jul-2014 |
| | | | 10-Aug-014 |
| Total No of samples (both water and sediment) | | | 25-Aug-014 |
| First year (Fortnightly) | : 1200 | | 09-Sep-2014 |
| Second year (Seasonal): 150 | | | 24-Sep-2014 |
| Total | : 1350 | | 08-Oct-2014 |
| | | | 23-Oct-2014 |
| | | | 06-Nov-2014 |
| | | | 22-Nov-2014 |
| | | | 06-Dec-2014 |
| | | | 22-Dec-2014 |
| | | | 06-Jan-2015 |
| | | | 20-Jan-2015 |
| | | | 03-Feb-2015 |
| | | | 19-Feb-2015 |
| | | | 05-Mar-2015 |
| | | | 20-Mar-2015 |
| | | | 04-Apr-2015 |
| | | | 18-Apr-2015 |
| | | | 04-May-2015 |
| | | Second Year | 27-May-2015 |
| | | | 26-Sep-2016 |
| | | | 18-Jan-2016 |

**Table:** Distribution environmental parameters along Visakhapatnam coast during study period

| Month | AT (°C) | SuW T (°C) | Se T (°C) | SeW T (°C) | Su W S‰ | SeW S‰ | SuW DO | SeW DO | SuW pH | SeW pH | Se OM% |
|---|---|---|---|---|---|---|---|---|---|---|---|
| May II | 37.4 | 31.1 | 31.3 | 31.1 | 36.2 | 35.6 | 4.3 | 4.1 | 7.0 | 7.6 | 0.62 |
| Jun I | 37.4 | 30.6 | 33.2 | 32.3 | 36.4 | 35.2 | 4.4 | 4.2 | 7.6 | 7.6 | 0.69 |
| Jun II | 37.3 | 32.2 | 33.6 | 33.3 | 35.0 | 35.0 | 4.3 | 4.1 | 7.7 | 7.7 | 0.69 |
| Jul I | 30.8 | 29.8 | 30.2 | 29.8 | 34.9 | 35.0 | 4.5 | 4.2 | 7.7 | 7.8 | 0.65 |
| Jul II | 30.9 | 29.3 | 30.1 | 29.2 | 34.6 | 35.3 | 4.8 | 4.5 | 7.5 | 7.7 | 0.76 |
| Aug I | 28.9 | 28.4 | 28.2 | 28.2 | 34.0 | 34.4 | 4.9 | 4.6 | 7.7 | 7.8 | 0.76 |
| Aug II | 32.7 | 30.7 | 30.9 | 29.9 | 34.8 | 34.4 | 4.4 | 4.1 | 7.6 | 7.7 | 0.74 |
| Sep I | 33.6 | 30.3 | 31.4 | 31.3 | 33.8 | 34.1 | 4.6 | 4.4 | 7.8 | 7.7 | 0.76 |
| Sep II | 33.6 | 30.8 | 31.5 | 30.9 | 33.5 | 34.1 | 4.7 | 4.5 | 7.6 | 7.6 | 0.70 |
| Oct I | 34.6 | 30.7 | 32.1 | 30.3 | 33.9 | 35.1 | 4.5 | 4.2 | 7.7 | 7.7 | 0.74 |
| Oct II | 33.7 | 30.6 | 31.8 | 30.4 | 33.7 | 34.6 | 4.6 | 4.3 | 7.6 | 7.6 | 0.67 |
| Nov I | 33.1 | 30.5 | 31.0 | 30.0 | 33.4 | 34.5 | 4.6 | 4.3 | 7.6 | 7.5 | 0.72 |
| Nov II | 30.9 | 29.0 | 29.6 | 28.8 | 34.5 | 34.9 | 5.0 | 4.8 | 7.7 | 7.5 | 0.65 |
| Dec I | 29.4 | 27.3 | 28.6 | 27.6 | 35.2 | 35.1 | 4.9 | 4.5 | 7.6 | 7.5 | 0.67 |
| Dec II | 25.9 | 24.1 | 25.6 | 24.5 | 34.3 | 33.9 | 5.2 | 4.8 | 7.5 | 7.5 | 0.76 |
| Jan I | 24.3 | 23.1 | 24.0 | 23.1 | 33.8 | 34.1 | 4.4 | 4.1 | 7.5 | 7.4 | 0.77 |
| Jan II | 22.8 | 21.8 | 22.5 | 22.2 | 34.2 | 34.3 | 3.9 | 3.6 | 7.5 | 7.4 | 0.76 |
| Feb I | 23.1 | 22.3 | 22.8 | 22.4 | 34.4 | 34.7 | 4.3 | 4.0 | 7.4 | 7.5 | 0.72 |
| Feb II | 26.9 | 24.4 | 25.6 | 24.8 | 35.1 | 35.9 | 4.7 | 4.4 | 7.7 | 7.6 | 0.76 |
| Mar I | 28.5 | 26.8 | 25.6 | 25.2 | 35.3 | 35.7 | 5.0 | 4.8 | 7.7 | 7.7 | 0.82 |
| Mar II | 31.8 | 29.5 | 28.7 | 28.5 | 36.0 | 36.0 | 5.1 | 4.8 | 7.7 | 7.7 | 0.74 |
| Apr I | 35.4 | 31.2 | 30.3 | 29.4 | 36.3 | 36.9 | 5.0 | 4.8 | 7.7 | 7.7 | 0.76 |
| Apr II | 36.4 | 31.3 | 30.5 | 30.2 | 36.3 | 36.3 | 5.3 | 4.9 | 7.6 | 7.7 | 0.78 |
| May I | 35.6 | 30.3 | 30.7 | 30.7 | 36.8 | 36.5 | 5.1 | 4.8 | 7.6 | 7.7 | 0.69 |
| May | 35.2 | 30.4 | 31.6 | 30.0 | 34.1 | 34.6 | 4.7 | 4.6 | 7.6 | 7.7 | 0.76 |
| Sep | 32.2 | 29.4 | 31.1 | 29.3 | 33.6 | 33.9 | 4.5 | 3.9 | 7.7 | 7.8 | 0.75 |
| Jan | 28.4 | 27.2 | 28.5 | 27.5 | 33.3 | 33.6 | 4.1 | 4.0 | 7.7 | 7.7 | 0.75 |
| Mean | 31.5 | 28.7 | 29.3 | 28.5 | 34.5 | 34.9 | 4.7 | 4.4 | 7.7 | 7.6 | 0.73 |

*A = Air, T= Temperature, Su = Surface, Se = Sediment, W = Water, S = Salinity, DO= Dissolved Oxygen, OM = Organic Matter.*

**Table 3.23f:** Station-wise distribution of heterotrophic bacteria species recorded during the present study (2014-16).

| | Species | St I | St II | St III | St IV | St V |
|---|---|---|---|---|---|---|
| | Bacillus | | | | | |
| 1 | alcalophilus | + | + | + | - | - |
| 2 | alvei | + | - | - | - | - |
| 3 | badius | + | + | - | - | - |
| 4 | brevis | - | - | + | + | - |
| 5 | circulans | + | - | - | - | - |
| 6 | insolitus | + | - | - | - | - |
| 7 | laterosporus | - | - | + | - | - |
| 8 | licheniformis | - | - | - | + | - |
| 9 | macerans | - | - | - | + | - |
| 10 | marinus | + | + | + | + | + |
| 11 | megaterium | - | - | - | - | + |
| 12 | mycoides | - | + | + | - | - |
| 13 | pasteurii | + | + | + | + | + |
| 14 | polymyxa | + | - | - | - | + |
| 15 | pumilus | - | - | - | + | - |
| 16 | subtilis | + | + | + | + | + |
| | Total | 9 | 6 | 7 | 7 | 5 |
| | Micrococcus | | | | | |
| 1 | kristinae | - | - | - | - | + |
| 2 | luteus | - | + | + | - | - |
| 3 | roseus | + | - | - | - | - |
| 4 | varians | + | + | + | + | + |
| | Total | 2 | 2 | 2 | 1 | 2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Psuedomonas | | | | | |
| 1 | aeruginosa | + | + | + | + | + |
| 2 | alcaligenes | + | + | + | + | + |
| 3 | denitrificans | + | + | + | - | - |
| 4 | facilis | - | - | - | + | - |
| 5 | fluorescens | + | - | - | + | + |
| 6 | maltophila | + | - | - | - | - |
| 7 | membranoformis | - | - | - | - | + |
| 8 | putida | - | - | - | + | - |
| 9 | saccharophila | - | + | - | - | - |
| | Total | 5 | 4 | 3 | 5 | 4 |
| | Staphylococcus | | | | | |
| 1 | aureus | + | + | + | + | + |
| 2 | epidermidis | - | + | - | - | - |
| 3 | haemolyticus | - | + | + | - | - |
| 4 | intermedius | - | - | - | + | - |
| 5 | lentus | - | - | - | - | + |
| 6 | saprophyticus | - | + | + | - | + |
| | Total | 1 | 4 | 3 | 2 | 3 |
| | Species | St I | St II | St III | St IV | St V |
| | Vibrio | | | | | |
| 1 | aerogenes | + | + | + | + | + |
| 2 | alginolyticus | - | - | + | - | - |
| 3 | anguillarum | + | + | + | + | + |
| 4 | cholerae | - | + | + | - | - |
| 5 | fisheri | - | - | - | + | - |
| 6 | furnishii | - | - | + | - | - |
| 7 | harveyi | + | + | + | + | + |
| 8 | parahaemolyticus | - | + | + | - | - |
| 9 | pomeroyi | + | - | - | + | - |
| 10 | shigelloides | + | + | + | + | + |
| 11 | vulnificus | + | - | - | - | + |
| | Total | 6 | 6 | 8 | 6 | 5 |
| | Grand Total | 23 | 22 | 23 | 21 | 19 |

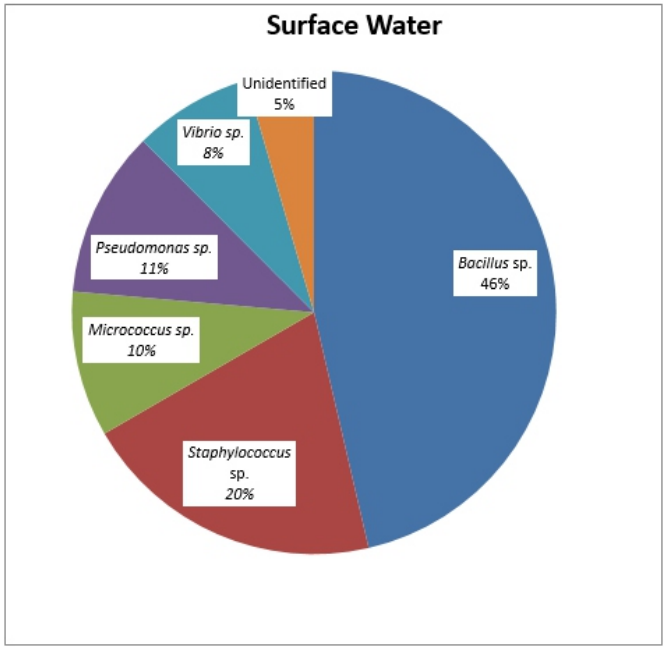


**Figure 1:** Abundance (%) of heterotrophic bacteria species in surfaces water along Visakhapatnam coast during 2014-2016

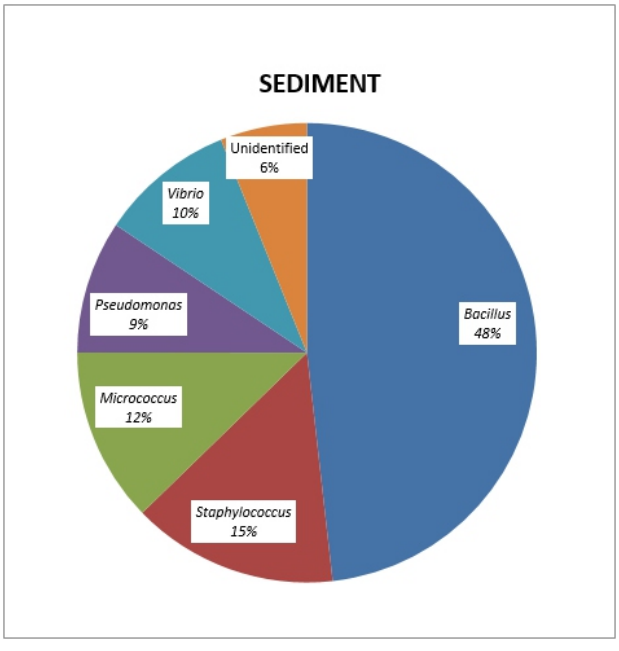


**Figure 2:** Abundance (%) of heterotrophic bacteria Species in near shore sediment along Visakhapatnam coast during 2014-2016

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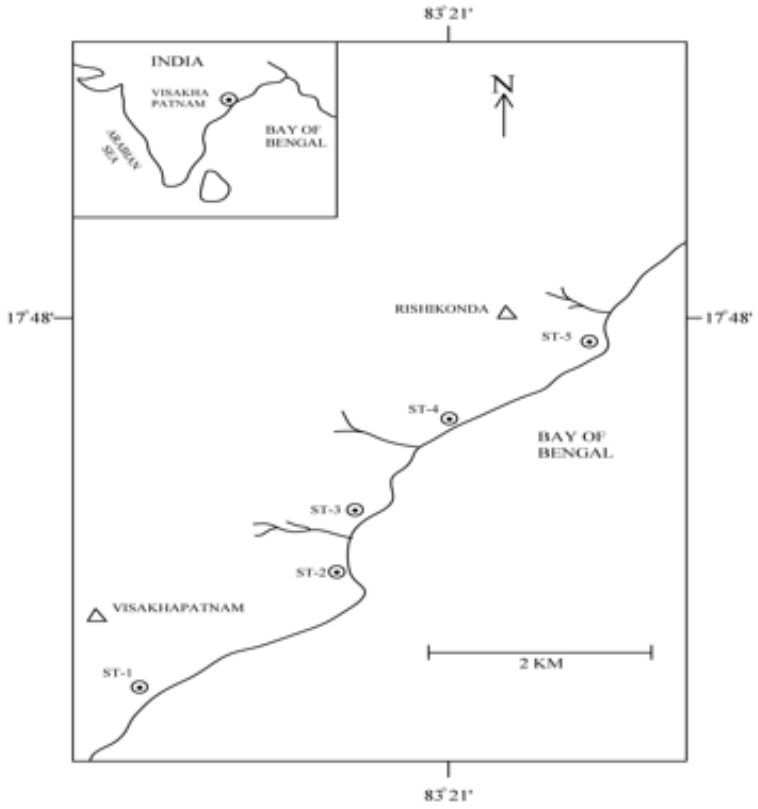


**Location of Sampling Stations I to V**