

## **Ecobiology and distribution of *Streptomyces* species from distinctive metal polluted sediment of tropical estuary, Northwest coast of India**

**Najmuddin Mulani, Abhay B. Fulke<sup>#</sup>, Edna D'Souza, Aashna Khosla, Nageswar Rao M, Aayushi Maloo, Anirudh Ram**

CSIR-National Institute of Oceanography (NIO), Regional Centre,  
Lokhandwala Road, Four Bungalows, Andheri (West),  
Mumbai-400053, Maharashtra, India.

<sup>#</sup>Correspondence: Dr. Abhay B. Fulke

Email: afulke@nio.org; abhayfulke@yahoo.co.in

### **Abstract**

The present study aims to isolate diverse actinomycetes communities from the estuarine sediments of Patalganga located at Northwest coast of India. Total 40 actinomycetes were isolated from 24 sediment samples by dilution plating on six different isolation media. Among them, morphologically 18 distinct selected isolates of actinomycetes were verified by 16S rRNA gene sequencing and identified as *Streptomyces* spp. The relationship between diversity of Total Actinomycetes Population (TAP) and its antagonistic behaviour with the physico-chemical characteristics of sediment samples were investigated. Multiple regression analysis revealed that the influencing physico-chemical factors comprises of sediment temperature, sediment pH, organic carbon and heavy metals. The results of statistical analysis showed that TAP is positively correlated ( $p < 0.01$ ) with sediment organic carbon whereas, negatively correlated with Cr ( $p < 0.05$ ) and Mn ( $p < 0.01$ ). Based on Principle component Analysis (PCA) and cluster analysis results, the obtained six stations can be divided into three groups. Among them mobile fraction of metals, TAP could be the main factor reflecting the lower and middle estuary. The recovery of large number of actinomycete isolates suggests that Patalganga Estuary could be potential source of bioactive compounds with biosynthetic capabilities.

**Keywords:** Estuarine sediment, Heavy metals, PCA, Total Actinomycetes Population (TAP), *Streptomyces* spp.

## 1. Introduction

Estuary stands demarcated as a semi-enclosed coastal water body where one or more rivulets flows into it, opening into the sea and within which sea water is measurably diluted with fresh water derived from land drainage (Pritchard, 1967). They are affected by both marine and riverine influences, such as tides, waves, saline water influx, and transport of fresh water and sediment which encompasses many biological niches coupled with high variety. These are the utmost productive ecosystems jeopardized to the greatest pressures from coastal human populations. Several estuaries are eutrophicated as an outcome of human activity and consequently have strong gradients in productivity and nutrient availability (Jankowski et al., 2014). Estuarine sediments and waters are characterized by specific and complex physical, chemical and microbiological properties. These properties depend and interact with each other and collectively constitute a unique environment to the organism. Characterization of sediment quality serves as tools to define the actual status of a water body.

Actinomycetes comprise a widespread and diverse group of gram positive, frequently filamentous, free living aerobic bacteria sporulating with DNA rich in G+C nucleotide content (75%) and play an important ecological role in soil cycle (Holt et al., 1989). They are documented for their capacity to endure extreme habitats (Malviya et al., 2014). They are considered as the most valuable saprophytic prokaryotes both economically and biotechnologically known to produce array of bioactive secondary metabolites. These metabolites are known to possess antimicrobial (antibacterial, antifungal and anti- algal) neuritogenic, anticancer, antimalarial, anti-inflammatory and enzymatic activities (Ravikumar et al., 2011). Anbalagan, R. and Sivakami, R (2021) suggested that even though the actinomycetes have a wide distribution in various media, their population dynamics are influenced by the physico-chemical variables as well as the nutrient availability at that regions. According to Berdy (2005) the genus *Streptomyces* constitutes 50% of the total actinomycetes population in soil is premeditated by potential makers of metabolites with varying structures and biological activities. They are widely distributed in natural environments (soil, water, forest and mangrove) where their population and types in the ecosystem are determined by numerous physical, chemical and biological features. *Streptomyces* genus continues to be an excellent source of natural bioactive compounds (Rammali, S., et al, 2022). Although diverse forms of antibiotics have been isolated from *Streptomyces*, only a small fraction of is indexed as bioactive compounds (Berdy, 1995; Watve et al., 2001). Species of *Streptomyces*, account for more than 70% of the total antibiotic production and *Micromonospora* being at the second number with less than one-tenth as many as *Streptomyces* (Lam, 2006). The isolation of new variants of *Streptomyces* from intact natural

resources and characterization of their secondary metabolites seems to be a significant endeavor. In order to exploit more laudable actinomycetes, it is necessary to better understand the diversity of actinomycetes in a single ecosystem of the estuarine area, which covers eco tones of both fresh as well as marine water, and possesses hostile environments enriched with immense bio diverse values.

In recent years, the global scale of drug resistance calls for the need to explore marine sediments as a source of novel isolates of actinomycetes. Due to the widespread diversity of bioactive natural products that have been isolated from these microbes, their consequent application is countless in biotechnological fields (Bérdy, 2005) understanding the distribution of these microorganisms in marine environments has proven to be valuable, from both a scientific and an economical point of view (Fenical 2006). The marine sediment is well known to be rich source of actinomycetes, from which their isolation has been well documented (Walker and Colwell, 1975; Barcina et al., 1987; Goodfellow and Haynes, 1984; Helmke and Weyland, 1984; Jensen et al., 1991).

The Patalganga River runs between the Western Ghats and the Arabian Sea. It is an important source of drinking water and industrial raw water for the surrounding villages and industries (Shrivastava, A., et al, 2015). More than 13 enterprises, including those in the petrochemical, pharmaceutical, and textile sectors, have been established near the Patalganga River upstream, serving as their primary source of water supply. These industries' effluents are released back into the river, significantly degrading the quality of the water and sediment [14]. Among the 38 rivers in the nation, the Patalganga River is one of the most contaminated, according to the central pollution control board (Sadasivan, S., et al, 2019).

The present investigation involves the study of ecobiology and distribution of *Streptomyces* species in sediments from Patalganga estuary of Northwest coast of India with special emphasis on its relation to metal pollution and organic carbon. Further is also discussed about taxonomic characterization based on 16S rRNA sequence analysis in combination with the physico-chemical properties of the sediments which may redirect our bio-discovery deeds.

## **2. Materials and methods**

### *2.1 Study area*

Patalganga Estuary is a tributary of Alaknanda River originating in Kunwarikhal catchment. It rises in the Matheran uplands and in its meandering north-westward reach of about 25 miles. Nearby this estuary Patalganga industrial area has developed 172.83 ha of land in Karjat and Panvel, Maharashtra, west coast of India. These industries directly release effluents of multiple products including organic contents in the Patalganga estuary.

Sediment samples were collected from six different sampling locations, viz. mouth, lower, middle

and upper section of the estuary by using Van Veen grab during post monsoon season (October-November). The geographical locations of the stations are illustrated in Table 1 and Figure 1. Central portions of the sediment (approx. 500 g) were aseptically placed in sterilized polypropylene containers by means of sterilized gloves and preserved at 4 °C until further analysis. Samples collected from the assigned locations of the estuary are representative of spatial characteristics with regard to organic matter and texture of sediment.

## 2.2 Isolation of actinomycetes

Six different media (Hi-Media, India) were used for the isolation of actinomycetes from sediment samples: (1) Starch Casein Agar (SCA); (2) Yeast extract-malt extract agar (ISP2); (3) Inorganic salts-starch agar (ISP4); (4) Glycerol-asparagine agar (ISP5); (5) Peptone-yeast extract iron agar (ISP6); (6) Tyrosine agar (ISP7). In order to suppress the growth of fungi and fast-growing Gram-negative bacteria during isolation, media were supplemented with nystatin(50 µg/ml) and nalidixic acid (20 µg/ml). The process involved adding 10 grams of wet sediment samples in 90 ml sterile saline water, homogenized for 2 hr at 100 rpm at room temperature. From serially diluted samples ( $10^{-2}$  to  $10^{-6}$ ), 200µl aliquots were plated onto the isolation media in triplicate and incubated at 28 °C for 7 to 15 days. The number of white powdery actinomycetes colonies formed on the plates was enumerated. These were purified in their original isolation media by repeated streaking (Mincer et al. 2002; Jensen et al. 2005). All the isolated strains were subjected to phenotypic and phylogenetic analysis.

## 2.3 Phenotypic and genotypic characterization

Various morphological, cultural and biochemical characterization tests were carried out using the standard procedures (Cappuccino et al., 2004; Gunasekaran, 2000; Shirling et al., 1966). The colour of mature sporulating aerial mycelium was recorded by visual observation (white, gray, pink and brown). The aerial mass (AM), spore mass (SM) and surface area (SA) were recorded. The media used were ISP2, ISP4, and SCA respectively. The mycelial characteristics such as structure, colour and arrangement of spores were observed through inverted microscope [100X, Olympus BX43, Japan]. The genomic DNA used for the PCR was prepared from single colonies grown on the Tryptone soya broth (TSB) for 2 days. Genomic DNA was extracted from the actinomycetes isolates using Qiagen DNeasy Blood & tissue kit (Qiagen, Germany) following manufacturer's instructions. 16S rRNA gene was amplified by means of universal bacterial primers: 16S F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-CGGTTACCTTGTTACGACTT-3'). Amplification was attained under standard conditions (initial denaturation at 94°C for 1 min, 30 cycles of denaturation at 94°C for 1 min, 34 cycles of annealing at 60°C for 1 min, extension at 72°C

for 1 min, and final extension at 72°C for 10 min). Amplified PCR product was purified using QIAquick PCR Purification Kit and amplicons were sequenced by Sanger's dideoxy method (SciGenom Labs. Pvt. Ltd, India). Sequences were subjected to BLAST search and compared with the NCBI database (<http://ncbi.nlm.nih.gov/blast>). Maximum likelihood method was adopted to construct the phylogenetic tree using MEGA6 software (Tamura et al., 2013).

#### 2.4 Sediment analyses

Sediment temperature were recorded immediately after collection by Celsius thermometer (Gnanam et al., 2013) and pH were determined electrochemically using glass electrode pH meter (Thermo Scientific A111) in a suspension of 1:2.5 sediment to water ratio described by Jackson (1973). The texture (sand, silt and clay) of collected sediment samples was estimated using wet-sieving method (Holme and McIntyre, 1984). Total Organic Carbon ( $C_{org}$ ) was detected by exothermic heating and oxidation with potassium dichromate (1N) and sulphuric acid, trailed by titration of surplus dichromate with 0.4N ferrous ammonium sulphate solution (Walkley and Black, 1934). For heavy metals analysis, a known quantity of sediments (0.5 g) was digested using HF- HClO<sub>4</sub>- HNO<sub>3</sub>-HCl<sup>-</sup> (Loring and Rantala, 1992). After digestion sediment samples were diluted to constant volume with MilliQ water. Heavy metals were analyzed using dual view ICP-OES (Optima 7300 DV, Perkin Elmer). Hg was analyzed by cold-vapour technique, using flow injection mercury system (FIMS-400, Perkin Elmer). All metal concentrations were expressed in µg/g on a dry-weight basis. The precision of the analytical methodology was evaluated by analyzing Standard Reference Materials (SRM) of sediment (PACS-2) obtained from National Research Council, Canada. All samples were run in triplicate batches that included known standards, reagent blanks and SRM was digested and processed in the same manner as samples. The results of SRM were found to be within ±5% of the certified values.

#### 2.5 Statistical analysis

To understand the relationships between physico-chemical parameters and total actinomycetes populations multivariate techniques were employed: Correlation co-efficient analysis and Principal Component Analysis (PCA). PCA, as a powerful multivariate analytical tool, is generally used to reduce the dimensionality of a data and to extract a smaller number of principal components (eigenvalues and eigenvectors) from the covariance matrix of original variables, routinely used in ecological studies (Maiz et al., 2000; Chabukdhara and Nema, 2012; Li et al., 2013). Principal components factors provide more structural information about the relations among metals and TAP. All mathematical and statistical analyses were computed using Statistica®, Ver 7, StatSoft Inc., SPSS

Statistics 19 for Windows and Microsoft Excel 2010.

### 3. Results

#### 3.1 Isolation and Identification of Actinomycetes

A total of 18 actinomycete strains were isolated from 24 sediment samples on the six different isolation media. All of the isolates exhibited similar growth patterns on seawater-free media, which indicated that the isolates were not exclusively dependent on seawater. The occurrence of the actinomycete isolates in Patalganga estuary according to the locations was as follows: Mouth/S1, 1 (5.6 %); Lower estuary/S2, 4 (22.22%); Middle estuary/S3, 4 (22.22%) and S4, 3(16.7%); Upper estuary/S5, 5 (27.8%) and Fresh water zone/S6, 1 (5.6%). The spatial distribution of these obtained actinomycetes has been shown in Figure 2. Morphological and cultural characteristics of the strains and their growth exhibited good growth on ISP-2, ISP6, and ISP-5 agar (Table 2). Good growth was also observed on ISP-4 and SCA. The colour of substrate and spore mycelium varied from one isolate to another. Biochemical characteristics of isolated actinomycetes are described in Table 3. None of the isolates were positive for VP test. 14 isolates showed a strong methyl red test and most of the isolates were able to hydrolyze polymeric substrate such as starch, casein, lipid and phosphate.

The Phenotypic characteristics of these isolates disclosed that they could grow well at 25°C to 37°C. Optimum temperature for growth of all actinomycetes was found to be 30°C. All actinomycetes were found to tolerate a wide range of pH 6-9. Optimum growth was exhibited at 1% and 2% NaCl by all the actinomycete isolates (Table 3). All the isolated actinomycetes utilized carbon sources glucose and xylose. Most of the isolates showed poor growth and failed to utilize rhamnose, cellbiose, melibiose, raffinose and saccharose (Table 3).

The isolates were sequenced for their 16S rRNA genes followed by PCR amplification and purification. Gene bank 139 accession numbers were as follows: KX010117, KX010119, KX010120, KX010133, KX010134, KX010135, KX010121, 140 KX010122, KX010123, KX010127, KX010124, KX010128, KX010129, KX010130, KX010131, KX010125, KX010126 and KX010132. 16SrRNA sequence data were used for BLAST analysis and construction of a phylogenetic tree (Fig. 2). Phylogenetic analyses findings as illustrated Figure 3 revealed that *Streptomyces* was the predominant actinomycete genus among the other isolated strains recovered in Patalganga estuary. Two strains were identified as *Streptomyces* sp. ABF7 was mainly closely related to *Streptomyces* sp. 10SAE (similarity index 100%) and that of *Streptomyces* sp. ABF8 to *Streptomyces griseorubens* strain ABF13 (similarity index 100%). However, remaining isolated strains showed inter-relation with each other. Characterization of sediment actinomycetes

community designated that chemical pollution may have decreased the natural variability that exists among estuary ecosystem.

Figure 3. Molecular Phylogenetic analysis by Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei., 1993). The percentage of trees in which the associated taxa clustered together is shown next to the branches. Matrix of pair wise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

### 3.2 *Physico-chemical Characterization*

Data on physico-chemical properties of six different sediment samples collected from various study locations is presented in Fig. 1. The temperature of sediment ranged from 25.5 to 29.3°C. The maximum temperature was observed at station S4 and minimum was recorded at station S6 (Fig. 4a). The pH value was maximum (7.8) at station S2 and minimum (7.0) was recorded at station S6 (Fig. 4b). The maximum contents of Aluminium (Al) 6.7%, Organic Carbons ( $C_{org}$ ) 2.2% and Iron (Fe) 9.2% were established in the sediment samples at station S5, and S6 respectively (Fig. 4c). The micronutrients such as Chromium (Cr), Manganese (Mn) and macronutrient viz Phosphorous (P) were recorded at all stations (Fig. 4d). Trace metals viz, Cobalt (Co), Copper (Cu), Nickel (Ni), Zinc (Zn) and Mercury (Hg) observed highest concentration in sediments of upper region of the estuary at station S5 (Fig. 4e, Fig. 4f).

### 3.3 *Correlation co-efficient analysis*

In this present investigation, the correlation co-efficient between physico-chemical parameters of sediments and TAP was analyzed by Pearson method. The Pearson coefficient is a statistical formula that measures how closely the data points combining the two variables approximate the line of best fit. Regression analysis can be used to determine the best fit line. The greater the distance between the coefficient and zero, whether positive or negative, the better the fit and the greater the correlation. TAP was positively correlated with organic carbon ( $C_{org}$ ) ( $r = 0.9611$ ,  $p < 0.01$ ) while negatively correlated with Cr ( $r = -0.8331$ ,  $p < 0.05$ ) and Mn ( $r = -0.9273$ ,  $p < 0.01$ ) (Table 4).

### 3.4 *Principle component Analysis*

By data mining the eigenvalues and eigenvectors from the correlation matrix; the sum of significant factors and the percent of variance elucidated by each of them are given in Table 5. Rotated component matrix is given by Table 6.

Table 6. Principal component matrix

	Component Matrix <sup>a</sup>			Rotated Matrix <sup>b</sup>		
	PC1	PC2	PC3	PC1	PC2	PC3
Temp	-.867	-.260	.314	-.935	.184	-.104
pH	-.630	-.589	.357	-.874	-.183	-.271
Al	-.495	.637	-.579	.108	<b>.986</b>	-.020
Cr	.994	-.053	.069	<b>.739</b>	-.651	.163
Mn	.760	-.527	.354	.253	-.957	-.015
Fe	.958	.131	-.135	<b>.874</b>	-.409	.148
Co	.788	.418	.448	.532	-.432	<b>.726</b>
Ni	.951	.097	-.234	<b>.908</b>	-.376	.055
Cu	-.026	.971	.235	.171	.500	<b>.848</b>
Zn	.750	.610	-.232	<b>.912</b>	.061	.390
Hg	-.347	.897	.195	-.088	<b>.662</b>	.719
Corg	-.906	.395	.139	-.665	<b>.706</b>	.237
P	.423	.705	.558	.277	-.096	<b>.949</b>
TAP	-.816	.535	-.012	-.471	<b>.818</b>	.246

Extraction method: principal component analysis

Bold indicates correlation is significant at the 0.01 level (2-tailed)

<sup>a</sup> 3 components extracted

<sup>b</sup> Rotation method: varimax with Kaiser normalization

In accordance to Table 5 results, three total eigenvalues are >1.4 and elucidate 96 % of cumulative variance. By associating the initial and rotated eigenvalues and their corresponding involvement towards clarification percent of variance, it shows that mutually the eigenvalues and their corresponding contribution towards the explanation of cumulative percent of variance have not changed substantially (Table 5). Collectively the tables 5 and 6 presents the first component (PC1), which explains 41.0 % of the system variance, has high positive factor loadings on mobility factors of Zn and Ni, high negative loadings on temperature. The subsequent component (PC2) accounted for 33.9 % of the total variance and is characterized by highly associated positive loadings on Al and TAP and moderate loadings on C<sub>org</sub>. The third component (PC3) accounted for 21.9 % of the total variance and is mainly characterized by high levels of P (0.949) and moderate loadings on Co mobile factor. Based on the factor scores obtained from PCA, a hierarchical cluster analysis by means of nearest neighbour method was achieved by means of Euclidean distances as a degree of similarity and results were represented in a dendrogram. Cluster analysis was performed to identify the distinguishing groups as illustrated in the Fig. 4. Among of them, S2, S3 and S4 (Group 1) were significantly correlated with each other and formed a clade. All the three samples were obtained from lower and middle estuary. The cluster (Group 2) comprised of S1 and S5. Except



sample S6 (Group 3), the rest samples were allotted into Group 2 which indicates no influx of sea water. The PCA loadings for the first three rotated components are plotted in Fig. 5, which visually demonstrates the associations among the basic physico-chemical characteristics, metal mobile factors and TAP. According to the 3-dimension loading plot with PC1, PC2 and PC3, mobile factors of metals, Zn, Fe, Co, Ni, Cr and Mn can be classified in a cluster because of their closed connection which presents the basic characteristics of sediment (Fig 6). However, TAP is separated from five type metals mobile factor by a long distance, which suggests negative correlation. On the other hand, TAP with organic carbon shows close connection.

#### 4. Discussion

The methods utilised in this study shows only a part of total actinomycetes population of the Patalganga estuary, where microbial ecology at the sampling sites is influenced by the chemical nature of the sediment. High TAP was observed in the upper region of estuary at station (S5). It might be due to the discharge of industrial effluents that led to shift in sediment bacterial composition (Lu et al., 2014), while low TAP were recorded in the mouth of the estuary and fresh water zone at stations (S1 and S6) respectively. Overall, phylogenetic results showed that *Streptomyces* was the most predominant genera in the Patalganga Estuary. Zhang et al., (2012) investigated that the microbial communities can be stratified based on the surrounding geological background that strongly affects community structure. Studies done by several researchers (Moncheva et al., 2002; Jensen et al., 1991; Peela et al., 2005) stated that *Streptomyces* dominates among other actinomycetes especially in soils. These organisms are said to be resistant to high and low temperatures, osmotic stress, and various proteolytic enzymes and organic solvents (Rani, A., et al, 2021). Of all the six different culture based medium, SCA medium with addition of specific antibiotic supplements, provided better retrieval of actinomycetes on direct inoculation of wet sediment samples after serial dilution than the rest. Also, Patil et al., (2001), Ellaiah et al., (2001), Kokare et al. (2004), Kathiresan et al., (2005), Sujatha et al., (2006) and Saha et al., (2006) stated that starch-casein agar was the most effective in the isolation of marine actinomycetes.

The important physico-chemical parameters influencing the distribution of actinomycetes were sediment temperature, sediment pH, organic carbon and heavy metals. Temperature is one of the crucial factors regulating the physiological activities of tropical marine organisms. The sediment pH was ranging from slightly alkaline to neutral (pH range 7.8 to 7.0). Goodfellow and Williams (1983) testified that soil actinomycetes behave as neutrophiles in culture, growing between pH 5 and 9 with an ideal close to neutrality. Thus, it is a major factor determining their distribution and activity. Similarly, the results indicated that the sediment pH was slightly alkaline or neutral in the study area.

An understanding of the distribution of organic carbon and its impacts on other physico-chemical and biological parameters is a prerequisite for nutrient input and biological alteration of major and minor elements of the system. Sediment nutrients especially organic carbon ( $C_{org}$ ) can significantly influence the TAP can survive well as they are saprophytic organisms, depending on the availability of carbon. Here, higher concentration of sediment  $C_{org}$  (2.2%) was recorded upper region at station S5. This might be attributed to discharge of industrial effluents and other land-based activities. In addition to this, large amount of dead planktonic matter that settled at the bottom may get oxidized and decomposed into organic carbon and laid in the sediments (Gnanam et al., 2013).

Organic phosphorus usually accumulated in the sediments and also chemically bonded to shells and bones of invertebrate animals and after decomposition phosphorus is released into the sediments and water (Vinithkumar et al., 1999). Presently, higher phosphorus content (2250  $\mu\text{g/g}$ ) was observed in the upper region of estuary at station S5 which is much higher than reported by Rajasegar et al., 2002 (concentration of total sediment phosphorus was about 770  $\mu\text{g/g}$  in the Vellar Estuary). Concentration of trace elements followed by texture of sediments, soil particles of all sizes from clay to sand play a vital role in the deposition of sediments in estuaries, particularly in semi closed or bar-built estuaries (Mohan, 2000). The order of the abundance of average concentrations of metals in the Patalganga estuarine sediments were as follows:  $\text{Fe} > \text{Al} > \text{Mn} > \text{Cr} > \text{Cu} > \text{Zn} > \text{Ni} > \text{Co} > \text{Hg}$ . Availability of major trace elements (Al, Fe and Mn) was 9.2% in station S6. Alagarsamy (2006) also observed that the concentrations of Fe varied from 2.2% to 49.7% on the surface sediments of the Mandovi estuary. The Al content in the sediment samples ranged from 4.3 % to 6.7 % higher than the Weisberg et al., (2000) stated 4.6% of Al content from Gulf coasts of the United States and lower than the major part of Han River, Korea (Kim et al., 2011). The Mn content was higher in station S1 that can be attributed to Mn oxide coatings on the sand grains (Badr et al., 2009). The values of mean concentrations of trace metals Cr, Cu, Zn, Ni, Co and Hg in upper region of the estuary (station S5) sediment were highest as compared to other stations selected for the study which indicated that the discharge of various pollutants by anthropogenic activities (Kim et al., 2011).

Statistical analysis revealed that there is a positive relationship between organic carbon and number of actinomycetes at ( $p < 0.01$ ) significance level. Ghanem et al., (2000) also reported similar kind of observation in soil samples collected from Alexandria, Egypt. Similar type of studies was reported by Radhakrishnan et al., 2016; Singh et al., 2013; Zhong and Cai, 2007; Singh et al., 2012. Moreover, our results indicated a negative correlation amid actinomycetes load and heavy metal pollution levels. Our findings agree with other reports in which some heavy metals have high affinity to bind organic matter implying a decrease of their bioavailability (Polti et al, 2007; Laxman et al., 2002) Ghanem

et al., (2000) reported that the disparity in temperature, pH and dissolved phosphate showed inconsequential values, but variation in organic matter was significant in the population similar to our findings. TAP were significantly and negatively correlated with chromium ( $p < 0.01$ ) and manganese ( $p < 0.05$ ) levels of significance, respectively. Similarly, strong negative correlation exists between TAP and chromium reported by Sheik et al., (2012); Nemecek et al., (2014). In other hand Juan et al., (2008); Kandavel and Sekar, (2015) observed negative correlation exists between TAP and manganese. Hence it might be concluded that though actinomycetes are ubiquitous, their population dynamics are often influenced by the available nutrients and the physico-chemical conditions of the ecosystems. The distribution of *Streptomyces* species throughout the estuary indicates their plasticity and adoptability to varied environment.

The principal component analyses of TAP and physico-chemicals parameters of the sediment samples were computed and relations between them were studied. The principal component analysis includes loading for the rotated component matrix, eigenvalues for each component, percent of variance and cumulative percent of variance explained by each component indicating the portion of variance of each variable controlled by the set of components. This is also supported by the Nowak's theory which confirmed that the factor loadings of PCA  $> 0.71$  are considered exceptional, while those  $< 0.32$  are regarded as very poor (Nowak 1998). Based on the PCA the correlation between the heavy metal content in sediment and TAP, a negative correlation was found between the concentration of the Cr and Mn as shown in Fig 6, i.e. the abundance of actinomycetes seemed to decrease with the increase in the concentration of these metals. Similarly, He et al., 2016 reported a weak correlation between abundance of actinomycetes and concentration of these two metals. Also, in this study we observed a weak correlation between TAP and the concentration of four types of metals (Zn, Co, Ni and Fe) and this result is in agreement with that of Lenart and Wolny-Koladka, (2013).

## 5. Conclusion

The present investigation proved that among the estuarine actinomycete isolates, *Streptomyces* spp. found as the dominant species in Patalganga estuary of west coast of India. These isolates were confirmed by 16S rRNA gene sequencing and identified as *Streptomyces* spp. which is known for the potential sources of secondary metabolites/value added compounds. Principle component Analysis (PCA) was implemented to assess the likely contributing factors such as the metal and other environmental factors along with actinomycetes population in the sediment samples from six stations of mentioned estuary. The pH of the area ranges towards an alkaline/neutral side. All the metals were found in the sample with the highest average concentration of and with least abundance of Hg. Overall, the present findings may contribute towards enhanced understanding of marine

actinomycetes which could lead to attempts commercial applicability and development in future.

## Acknowledgements

Authors are grateful to Director, CSIR-National Institute of Oceanography (CSIR-NIO), Goa, India and CSIR-NIO, Scientist-in-Charge, Regional Centre, Mumbai for their encouragement and support.

## Conflict of Interest

The authors report no potential conflict of interest.

## References:

1. Alagarsamy, R., (2006). Distribution and seasonal variation of trace metals in surface sediments of the Mandovi estuary, west coast of India. *Estuar. Coast Shelf Sci.* 67, 333–339.
2. Anbalagan, R. and Sivakami, R (2021). Enzymatic and antimicrobial activities of Actinomycetes species obtained from Mayanur Dam, Tamil Nadu, India. *Plant Archives*, 21(No 1).
3. Badr, N.B., El-Fiky, A.A., Mostafa, A.R., Al-Mur, B.A., (2009). Metal pollution records in core sediments of some Red Sea coastal areas, Kingdom of Saudi Arabia. *Environ.Monit. Assess.* 155, 509–526.
4. Barcina, I., Iriberry, J., Egea, L., (1987). Enumeration, isolation and some physiological properties of actinomycetes from sea water and sediment. *Syst. Appl. Microbiol.*10, 85-91002E.
5. Berdy, J., (1995). Are actinomycetes exhausted as source of secondary metabolites? In Proceedings of the 9th International Symposium on the Biology of Actinomycetes, 13-34.
6. Berdy, J., (2005). Bioactive microbial metabolites. Review article. *J.Antibiot.* 58,1.
7. Cappuccino, J.G., Sherman, N., (2004). Microbiology, Laboratory manual. Person education. INC, New Delhi, 282-283.
8. Chabukdhara, M., Nema, A.K., ( 2012). Assessment of heavy metal contamination in Hindon River sediments: a chemometric and geochemical approach. *Chemosphere* 87, 945–953.
9. Ellaiah, P., Raju, K.V., Adinarayana, K., Adinarayana, G., Prabhakar, T., Premkumar, J., ( 2001). Bioactive rare actinomycetes from indigenous natural substrates of Andhra Pradesh. *Hindustan Antibiot. Bull.* 44, 17-24.
10. Fenical, W., (2006). Developing a new resource for drug discovery: marine actinomycete bacteria. *Nature* 2, 666–673.
11. Ghanem, N.B., Sabry, S.A., El-Sherif, Z.M., Abu El-Ela, G.A., (2000). Isolation and enumeration of marine actinomycetes from seawater and sediments in Alexandria. *J. Gen.ApplMicrobiol.* 46, 105–111.
12. Gnanam, S., Sivakumar, K., Vijayalakshmi, S., Balasubramaniyam, T., (2013). Ambience of physico chemical characteristics to actinobacterial density in the Chennai coast of the Bay of Bengal,

India. *Indian J. Geo-Mar. Sci.* 42, 254- 305

13. Goodfellow, M., Haynes, J.A., (1984). Actinomycetes in marine sediments, In Biological, biochemical and biomedical aspects of actinomycetes (eds. L. Ortiz-Ortiz; C. F. Bojali; & V. Yakoleff), 453–463, *Academic press, New York, London*
14. Goodfellow, M., Williams, S.T., (1983). Ecology of actinomycetes. *Ann. Rev. Microbiol.* 37, 189-216.
15. Gunasekaran, P., (2000). Laboratory Manual in Microbiology. *New Age International, New Delhi.*
16. He, H., Hong, F.F., Tao, X.X., Huang, G.H., Leng, Y.W., Shao, J.F., Zhao, Y.D., (2016). A study on soil basic characteristics, main microbial flora and typical metal fraction surrounding coal gangue dump in Xiangtan Hunan Province, south of China. *Environ. Earth Sci.* 75, 1-9.
17. Helmke, E., Weyland, H., (1984). *Rhodococcus marinonascens* sp. Nov., an actinomycetes from the sea. *Int. J. Syst. Bacteriol.* 34, 127-138.
18. Holme, N.A., McIntyre, A.D., (1984). Methods for the study of marine benthos. Blackwells
19. Holt, J.G., Williams, S.T., Sharpe, M.E., *Bergey's manual of systematic bacteriology*, vol 4. Williams and Wilkins, 325 London. ISBN 0-68309-061-5.
20. Jackson, M.I. L., (1973). Soil Chemical Analysis. Prentice Hall (India) Pvt. Ltd. New Delhi 327
21. Jankowski, K., Schindler, D.E., Horner-Devine, M.C., (2014). Resource availability and spatial heterogeneity control bacterial community response to nutrient enrichment in lakes. *PLoS One* 9, e86991.
22. Jensen, P.R., Dwight, R., Fenical, W., (1991). Distribution of Actinomycetes in near-shore tropical marine sediments. *Appl. Environ. Microbiol.* 57, 1102-1108.
23. Jensen, P.R., Gontang, E., Mafnas, C., Mincer, T.J., Fenical, W., (2005). Culturable marine actinomycete diversity from tropical Pacific Ocean sediments. *Environ. Microbiol.* 7, 1039–1048.
24. Juan, Z.H., Weihua, G.U., Renqing, W.A., Xuemei, H.A., Qiang, W.A. (2008). Microbial community diversity in the profile of an agricultural soil in northern China. *J. Environ. Sci.* 20, 981-988.
25. K Tamura, M Nei., (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 10, 512–526
26. Kandavel, D., Sekar, S., (2015). Impact of certain biotic and abiotic factors on phyllanthin and hypophyllanthin content of phyllanthus amarus schum. & thonn. from three different habitats. *Int. J. Pharm. Pharm. Sci.* 7, 258-265.
27. Kathiresan, K., Balagurunathan, R., Selvam, M.M., (2005). Fungicidal activity of marine actinomycetes against phytopathogenic fungi. *Indian J. Biotechnol.* 4, 271-276.

28. Kim, K.T., Ra, K., Kim, E.S., Yim, U.H., Kim, J.K., (2011). Distribution of Heavy Metals in the Surface Sediments of the Han River and its Estuary, Korea. *J. Coastal Res.* 64, 903-907.
29. Kokare, C.R., Mahadik, K.R., Kadam, S.S., Chopade, B.A., (2004). Isolation of bioactive marine actinomycetes from sediments isolated from Goa and Maharashtra coastlines (west coast of India). *Indian J. Mar. Sci.* 33, 248-256.
30. Lam, K.S., (2006). Discovery of novel metabolites from marine actinomycetes. *Curr.Opi.Microbiol.* 9,245- 353.
31. Laxman, R.S., More, S. (2002). Reduction of hexavalent chromium by *Streptomyces griseus*. *Miner. Eng.* 15, 831–837.
32. Lenart, A., Wolny-Kołodka, K., (2013).The effect of heavy metal concentration and soil pH on the abundance of selected microbial groups within Arcelor Mittal Poland steelworks in Cracow. *Bull. Environ.Contam.Toxicol.* 90, 85-90.
33. Li, X., Liu, L., Wang, Y., Luo, G., Chen, X., Yang, X., Hall, M.H., Guo, R., Wang, H., Cui, J., He, X., (2013). Heavy metal contamination of urban soil in an old industrial city (Shenyang) in Northeast China. *Geoderma.* 192, 50–58.
34. Loring, D.H., Rantala, R.T., (1992). Manual for the geochemical analyses of marine sediments and suspended particulate matter. *Earth-Sci. Rev.* 32, 235-283.
35. Lu, X.M., Lu, P.Z., (2014). Characterization of bacterial communities in sediments receiving various wastewater effluents with high-throughput sequencing analysis. *Microb.Ecol.* 67, 612-623.
36. Maiz, I., Arambarri, I., Garcia, R., Millan, E., (2000). Evaluation of heavy metal availability in polluted soils by two sequential extraction procedures using factor analysis. *Environ.Pollut.* 110, 3–9.
37. Malviya, N., Yandigeri, M.S., Yadav, A.K., Solanki, M.K., Arora, D.K., (2014). Isolation and characterization of novel alkali - halophilicactinomycetesfromChilika brackish waterlake, India. *Ann.Microbiol.* 64, 1829-1838.
38. Mincer, T.J., Jensen, P.R., Kauffman, C.A., Fenical, W., (2002). Widespread and persistent populations of a major new marine actinomycete taxon in ocean sediments. *Appl. Environ.Microbiol.* 68, 5005–5011.
39. Mohan, P.M., (2000). Sediment transport mechanism in the Vellar estuary, east coast of India. *Indian J. Mar. Sci.* 29, 27-31.
40. Moncheva, P., Tishkov, S., Dimitrova, N., Chipeva, V., Antonova-Nikolova, S., Bogatzevska, N., (2002). Characteristics of soil actinomycetes from Antarctica. *J. Cult. Coll.* 3, 3-14.
41. Nemecek, J., Lhotský, O., Cajthaml, T., (2014). Nanoscale zero-valent iron application for in situ reduction of hexavalent chromium and its effects on indigenous microorganism populations. *Sci. Total Environ.* 485, 739-747.
42. Nowak, B., (1998). Contents and relationship of elements in human hair for a non-industrialised population in Poland. *Sci. Total Environ.* 209, 59–68.

43. Patil, R., Jeyasekaran, G., Shanmugam, S.A., Shakila, R.J., (2001). Control of bacterial pathogens associated with fish diseases by antagonistic marine actinomycetes isolated from marine sediments. *Indian J. Mar. Sci.* 30, 264-267.
44. Peela, S., Kurada, V.B., Terli, R., (2005). Studies on antagonistic marine actinomycetes from the Bay of Bengal. *World. J. Microbiol. Biotechnol.* 21, 583-585.
45. Polti, M.A., Amoroso, M.J., Abate, C.M., (2007). Chromium (VI) resistance and removal by actinomycete strains isolated from sediments. *Chemosphere* 67, 660–667.
46. Pritchard, D.W., (1967). What is an estuary: a physical viewpoint. *American Association for the Advancement of Science* 83, 3–5.
47. Radhakrishnan, S., Varadharajan, M., (2016). Status of microbial diversity in agroforestry systems in Tamil Nadu, India. *J. Basic. Microbiol.* 56, 662-669.
48. Rajasegar, M., Srinivasan, M., Khan, S.A., (2002). Distribution of sediment nutrients of Vellar estuary in relation to shrimp farming. *Indian J. Mar. Sci.* 31, 153-156.
49. Rammali, S., Hilali, L., Dari, K., Bencharki, B., Rahim, A., Timinouni, M., Gaboune, F., El Aalaoui, M., & khattabi, A. (2022). Antimicrobial and antioxidant activities of streptomyces species from soils of three different cold sites in the fez-meknes region Morocco. *Scientific Reports*, 12(1).
50. Ravikumar, S., Inbaneson, S.J., Uthiraselvam, M., Priya, S.R., Banerjee, M.B., (2011). Diversity of endophytic actinomycetes from Karangkadu mangrove ecosystem and its antibacterial potential against bacterial pathogens. *J. Pharm. Res.* 4, 294 – 296.
51. Sadasivan, S., Gurjar, UR., Shukla, SP., Jaiswar, AK., Shenoy, L., Deshmukhe, G., (2019). Zooplankton abundance and its seasonal distribution in Patalganga estuary, Maharashtra, India. *Journal of Entomology and Zoology Studies*; 7(1): 1156-1160.
52. Rani, A., Saini, K. C., Bast, F., Varjani, S., Mehariya, S., Bhatia, S. K., Sharma, N., & Funk, C. (2021). A review on microbial products and their perspective application as antimicrobial agents. *Biomolecules*, 11(12), 1860. <https://doi.org/10.3390/biom11121860>
53. Saha, M., Jaisankar, P., Das, S., Sarkar, K.K., Roy, S., Besra, S.E, Vedasiromani, J.R., Ghosh, D., Sana, B., Mukherjee, J., (2006). Production and purification of a bioactive substance inhibiting multiple drug resistant bacteria and human leukemia cells from a salt-tolerant marine Actinobacterium sp. isolated from the Bay of Bengal. *Biotechnol.Lett.* 28, 1083-1088.
54. Sheik, C.S., Mitchell, T.W., Rizvi, F.Z., Rehman, Y., Faisal, M., Hasnain, S., McInerney, M.J., Krumholz, L.R., (2012). Exposure of soil microbial communities to chromium and arsenic alters their diversity and structure. *PloS one* 7, e40059.
55. Shirling, E.T., Gottlieb, D., (1966). Methods for characterization of Streptomyces species. *Int. J. Syst. Bacteriol.* 16, 313- 423
56. Shrivastava, A., Tandon, S. A., & Kumar, R. (2015). Water Quality Management Plan for Patalganga river for drinking purpose and human health safety. *International Journal of Scientific Research in Environmental Sciences*, 3(2), 71–87.

57. Singh, A.K., Singh, M., Dubey, S.K., (2013). Changes in Actinomycetes community structure under the influence of Bt transgenic brinjal crop in a tropical agroecosystem. *BMC Microbiol.* 13, 1.
58. Singh, R.J., Ahlawat, I.P., Singh, S., (2012). Effects of transgenic BT cotton on soil fertility and biology under field conditions in sub-tropical Inseptisol. *Environ.Monit. Assess.* 185, 485–495.
59. Sujatha, P., Raju, K.B., Ramana, T., (2005). Studies on a new marine streptomycete BT-408 producing polyketide antibiotic SBR-22 effective against methicillin resistant *Staphylococcus aureus*. *Microbiol. Res* 160, 119-126.
60. Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol.* 30, 2725-2729.
61. Vinithkumar, N.V., Kumaresan, S., Manjusha, M., Balasubramanian, T., (1999). Organic matter, nutrients and major ions in the sediments of coral reefs and seagrass beds of Gulf of Mannar Biosphere Reserve, South East Coast of India. *Indian J. Mar. Sci.* 28, 383-393.
62. Walker, J.D., Colwell, R.R., (1975). Factors affecting enumeration and isolation of actinomycetes from Chesapeake Bay and Southeastern Atlantic Ocean sediments. *Mar. Biol.* 30, 193-201.
63. Walkley, A., Black, I.A., (1934). An examination of the Degtjareff method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents. *Soil Sci.* 63, 251-263.
64. Watve, M.G., Tickoo, R., Jog, M.M., Bhole, B.D., (2001). How many antibiotics are produced by the genus *Streptomyces*?. *Arch.Microbiol.* 176, 386–390.
65. Weisberg, S.B., Wilson, H.T., Heimbuch, D.G., Windom, H.L., Summers, J.K., (2000). Comparison of sediment metal: aluminum relationships between the eastern and gulf coasts of the United States. *Environ.Monit. Assess.* 61, 373-385.
66. Zhang, Y., Su, X., Chen, F., Wang, Y., Jiao, L., Dong, H., Huang, Y., Jiang, H., (2012). Microbial diversity in cold seep sediments from the northern South China Sea. *Geoscience Frontiers* 3, 301-316.
67. Zhong, W.H., Cai, Z.C., (2007). Long-term effects of inorganic fertilizers on microbial biomass and community functional diversity in a paddy soil derived from quaternary red clay. *App. Soil Ecol.* 36, 84–91.



## Figure Caption

1. Figure 1. Sampling site location of Patalganga Estuary.
2. Figure 2. Spatial distribution of actinomycetes throughout the location.
3. Figure 3. Molecular Phylogenetic analysis by Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Matrix of pair wise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.
4. Figure 4. Physico-chemical properties of different sediment samples from Patalganga estuary.
5. Figure 5. Dendrogram derived from the hierarchical cluster analysis of soil samples.
6. Figure 6. The sample variable loading 3-D plot of PCA.

## Table Captions

1. Table 1. Sampling Locations, actinomycetes population, and texture of sediments of Patalganga estuary
2. Table 2. Morphological and cultural tests of actinomycetes isolates from Patalganga estuary.
3. Table 3. Biochemical and physiological characteristics of actinomycetes isolates from Patalganga estuary.
4. Table 4. Pearson correlation co-efficient analysis of physico-chemical properties of soil and total actinomycetes population.
5. Table 5. Total Variance Explained
6. Table 6. Principal component matrix

Fig 1



Fig 2

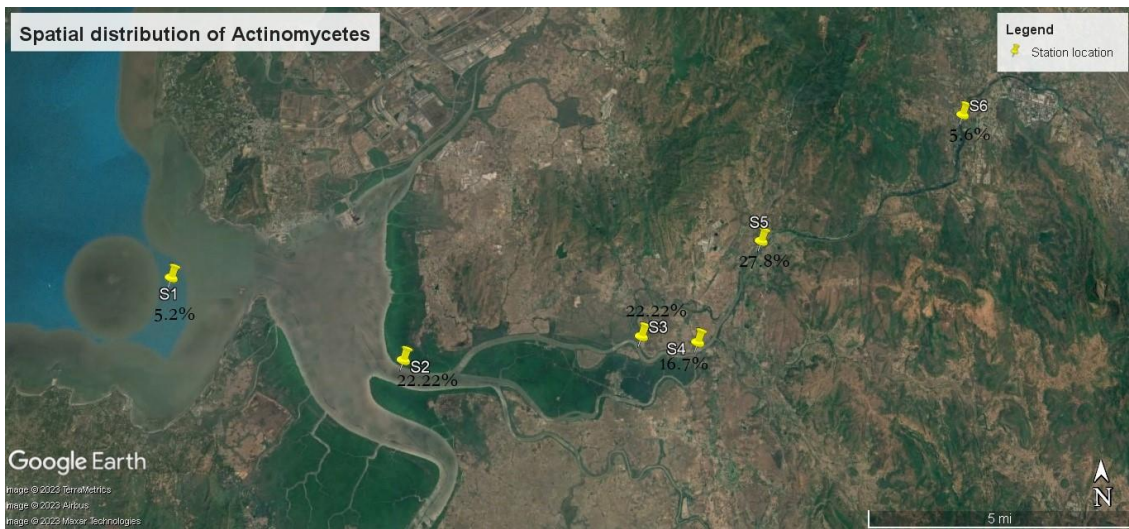


Fig 3

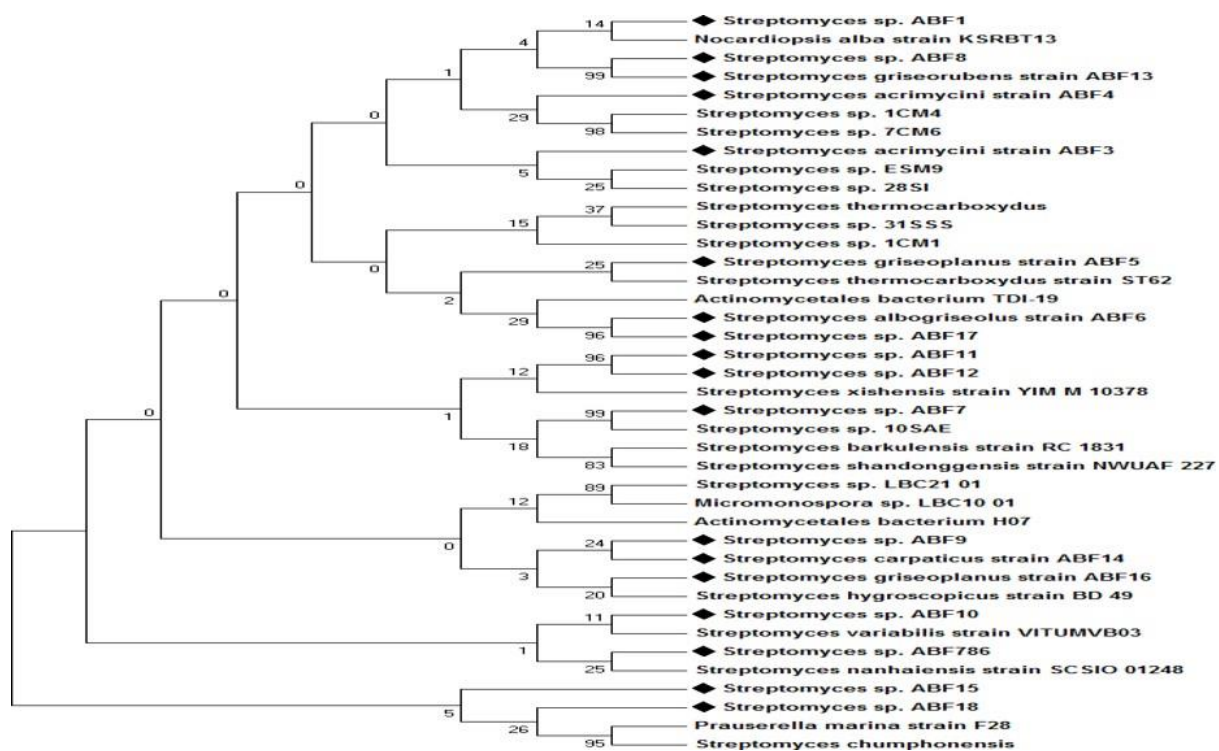


Fig 4

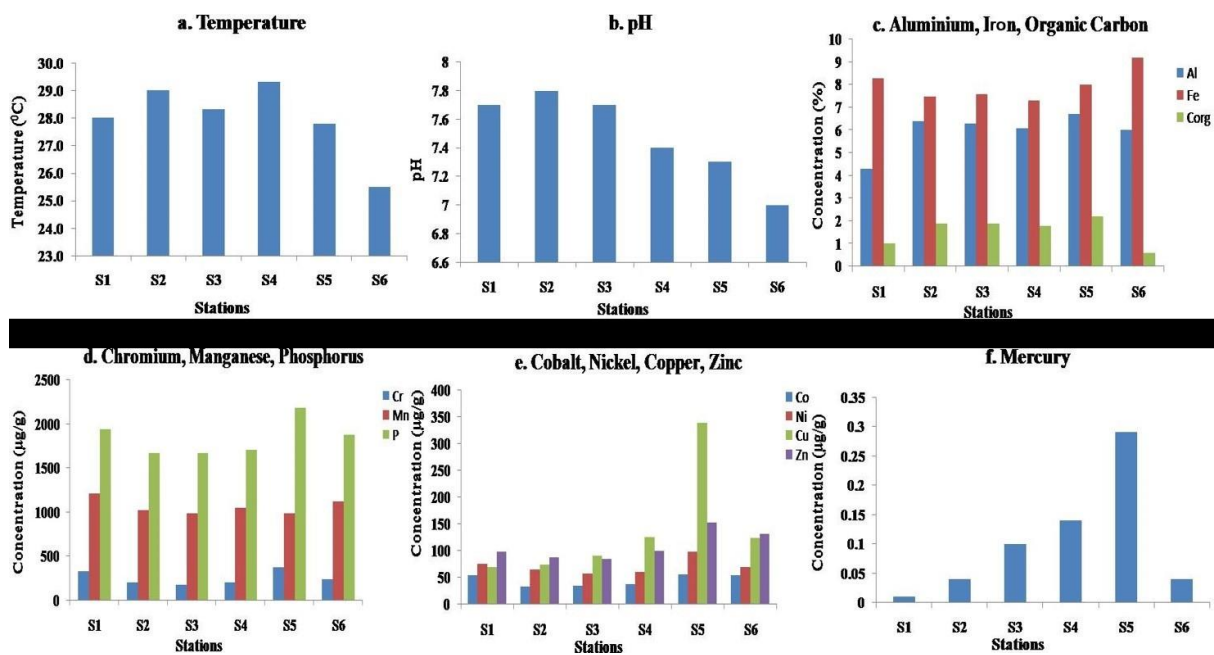


Fig 5

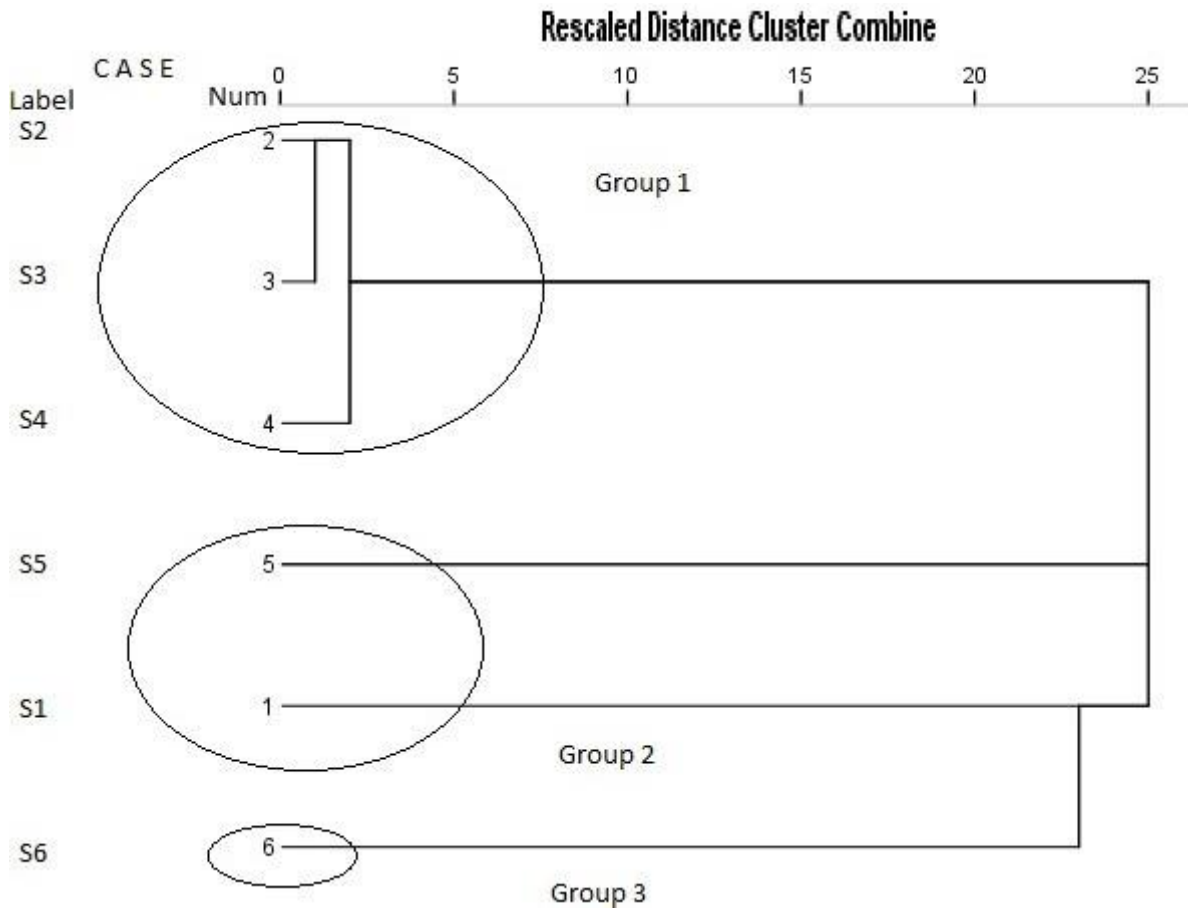


Fig 6

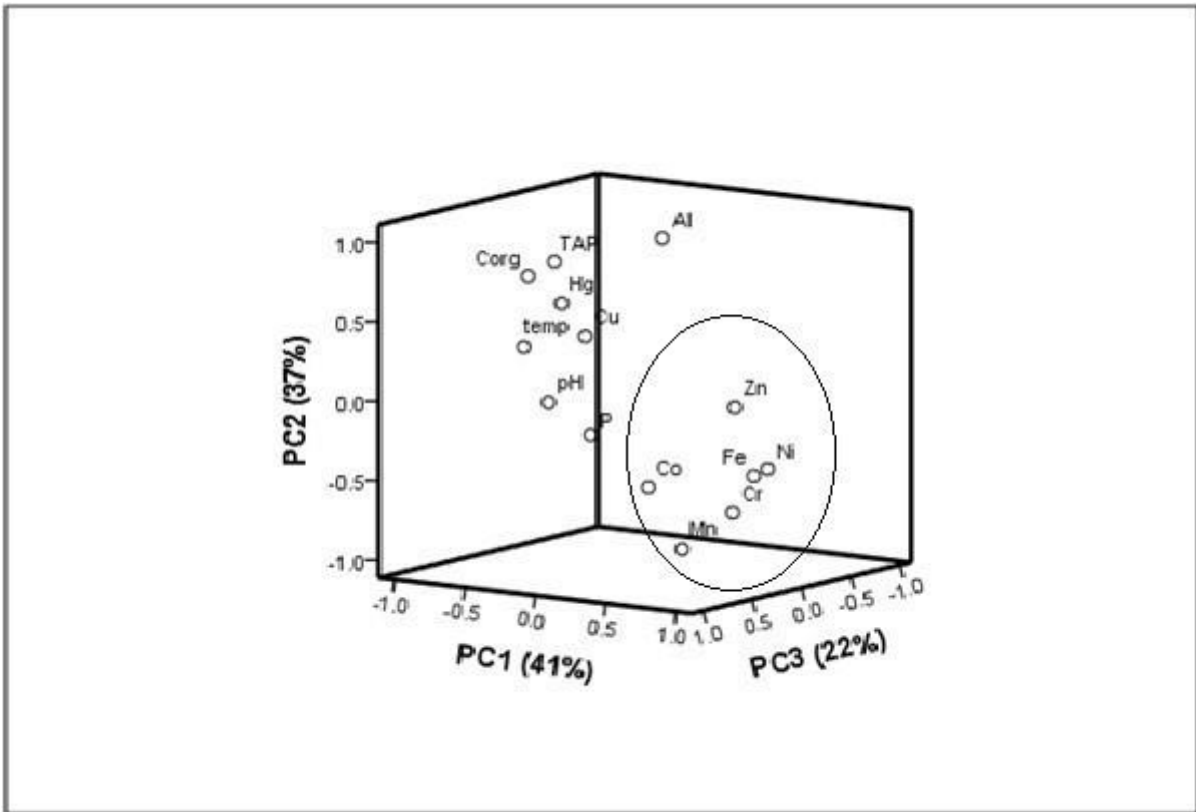


Table 1. Sampling Locations, actinomycetes population, and texture of sediments of Patalganga estuary

Where; SCA= Starch Casein Agar, ISP= International Streptomyces Project

No	Location/Code	Coordinates	Depth	No. of sediment samples	Isolation Medium and Total isolates of actinomycetes						No. of actinomycetes screened for current study	Texture of the sediment samples		
					SCA	ISP2	ISP4	ISP5	ISP6	ISP7		Sand(%)	Silt(%)	Clay(%)
1	Mouth/S1	18°49'52.87" N 72°54'50.26" E	6.5	4	2	-	1	-	-	-	1	68.2	18	13.8
2	Lower estuary/S2	18°48'23.70" N 72°59'2.70" E	7.0	4	3	-	2	-	1	1	4	54	41.8	4.2
3	Middle estuary/S3	18°48'42.90" N 73°03'17.19" E	4.5	2	4	1	1	1	2	-	4	9.6	50.2	40.2
4	Middle estuary/S4	18°48'35.80" N 73°04'17.20" E	4	4	2	1	-	2	1	1	3	3.5	41.8	54.7
5	Upper estuary/S5	18°50'19.20" N 73°05'31.40" E	Shore collection	4	4	2	2	-	-	2	5	8.1	85.1	6.8
6	Fresh water zone/S6	18°52'31.40" N 73°09'24.90" E	Shore collection	6	1	-	-	1	1	1	1	66.2	25.6	8.2

Table 2. Morphological and cultural tests of actinomycetes isolates from Patalganga estuary.

Isolate no.	Sequence length (bp)	16S rRNA BLAST results	Morphological Characteristics			Cultural Characteristics					
			AM	SM	SA	SCA	ISP2	ISP4	ISP5	ISP6	ISP7
S101	867	<i>Streptomyces</i> sp. ABF1	Whitish	Whitish	Smooth	+++	++	+++	++	PG	+
S201	339	<i>Streptomyces acrimycini</i> strain ABF3	White gray	White gray	Smooth	+++	+	+++	++	++	++
S202	340	<i>Streptomyces acrimycini</i> strain ABF4	Gray colour	Leathery	Wrinkled	++	++	+++	PG	+	++
S203	874	<i>Streptomyces griseoplanus</i> strain ABF16	Cottony white	White	Smooth	+++	+	++	++	+++	+
S204	857	<i>Streptomyces</i> sp. ABF17	Whitish	Whitish	Straight	+++	++	+++	++	+	++
S301	866	<i>Streptomyces</i> sp. ABF18	Whitish	Whitish	flexuous	+++	+++	++	++	+	++
S302	716	<i>Streptomyces griseoplanus</i> strain ABF5	Whitish	Whitish	Smooth	+++	+	+++	PG	++	+
S303	873	<i>Streptomyces albogriseolus</i> strain ABF6	Greyish	Whitish	Smooth	+++	++	+++	+	++	+
S304	892	<i>Streptomyces</i> sp. ABF7	Greyish	Whitish	Smooth	++	+	+++	++	+	++
S401	880	<i>Streptomyces</i> sp. ABF11	Greyish	Greyish	Wrinkled	+++	++	+++	+++	PG	+
S402	896	<i>Streptomyces</i> sp. ABF8	Greyish pink	Pink	Smooth	+++	+	++	+++	+	++
S403	881	<i>Streptomyces</i> sp. ABF12	Dark brown	Dark brown	Flexible	+++	+	+++	+	++	+++
S501	893	<i>Streptomyces griseorubens</i> strain ABF13	Greyish white	White	Smooth	++	++	+	++	+++	+
S502	914	<i>Streptomyces carpaticus</i> strain ABF14	Dark brown	Dark brown	Smooth	+++	+	+++	PG	++	+
S503	888	<i>Streptomyces</i> sp. ABF15	Pink	Pink	Wrinkled	+++	++	+++	+	+	++
S504	893	<i>Streptomyces</i> sp. ABF9	Pink	Pink	Wrinkled	+++	+	++	++	++	+
S505	889	<i>Streptomyces</i> sp. ABF10	White centre gray	Gray	Straight	+++	+	++	+++	+	+
S601	917	<i>Streptomyces</i> sp. ABF786	Whitish	Whitish	Wrinkled	+++	++	+++	+	++	++

AM-aerial mass, SM-spore mass, and SA-surface area

(+) Positive; (++) Strong positive; (+++) Excellent; (-) Negative; (PG) Poor growth



Table 3. Biochemical and physiological characteristics of actinomycetes isolates from Patalganga estuary.

Name of the test	S101	S201	S202	S203	S204	S301	S302	S303	S304	S401	S402	S403	S501	S502	S503	S504	S505	S601
<b>Biochemical Characteristics</b>																		
Casein hydrolysis	++	+	-	-	+	+	+	-	+	-	+	+	+	+	+	+	+	-
Starch hydrolysis	++	+	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
H <sub>2</sub> O <sub>2</sub>	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	+	+	+
Nitrate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
H <sub>2</sub> S production	-	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-	+	+
Urease	+	-	+	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+
Lipase	+	-	-	-	+	-	-	+	-	+	-	-	+	-	+	+	-	-
Phosphate hydrolysis	-	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	+	-
Indole production	-	+	+	-	-	-	+	+	-	+	+	-	+	-	+	-	-	-
Methyl red	+	+	+	-	+	+	+	+	+	-	-	+	+	+	-	+	+	+
Vogesproskauers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Citrate utilization	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+
<b>Carbon source utilization</b>																		
Glucose	++	+	++	++	++	++	+++	+	++	++	++	++	++	+	++	++	++	+
Xylose	+	++	+	+	+	+	+	++	++	+	++	-	+	+	++	+	++	+
Adonitol	-	+	-	-	-	++	-	-	+	++	-	+	-	-	-	+	-	-
Rhamnose	+	++	+	-	-	+	+	++	+	++	-	-	-	+	-	++	+	+
Cellbiose	+	-	-	++	+	+	-	++	+	+	++	+	+	+	-	-	+	+
Melibiose	-	-	-	+	+	-	+	++	+	-	+	-	+	-	-	+	+	++
Saccharose	-	++	+	+	++	+	+	+	+	+	+	-	+	++	+	+	-	-
Raffinose	+	-	-	-	+	-	-	+	+	-	-	-	+	++	+	-	+	+
Trehalose	-	-	-	+	-	+	++	-	-	-	-	++	+	+	+	+	-	-
Arabinose	++	+	+	+	-	++	-	-	+	+	++	-	-	-	+	-	+	++
<b>Nitrogen source utilization</b>																		
ONPG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lysine	+	-	+	-	-	+	-	-	+	+	+	-	+	-	-	+	-	-
Ornithine utilization	+	-	-	-	+	+	-	+	+	+	-	+	-	-	-	+	+	+
Cysteine	+	-	-	-	-	+	-	+	-	+	-	-	+	-	+	-	+	-
Phenylalanine Determination (TDA)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>Growth at Temperature</b>																		
4°C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15°C	-	-	-	-	+	-	+	-	-	+	-	-	-	-	+	-	-	-
25°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
30°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
37°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
45°C	-	-	-	+	+	-	-	PG	+	-	+	-	-	+	-	PG	-	+
60°C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>Growth at pH range</b>																		
5.0	-	-	-	-	-	-	-	-	PG	-	PG	-	-	-	-	-	-	-
6.0	+	+	+	+	+	+	+	+	+	+	PG	+	+	+	+	+	+	+
7.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	PG	-	-	+
9.0	+	+	+	+	+	+	+	+	+	+	+	+	PG	+	+	+	+	+
10.0	PG	PG	PG	-	-	PG	PG	-	-	PG	-	-	-	-	+	-	-	-
<b>Growth in presence of</b>																		
1% NaCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2% NaCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5% NaCl	+	+	+	-	-	PG	-	-	+	+	+	+	-	PG	-	-	+	+
7% NaCl	-	-	-	-	-	-	-	PG	-	-	-	-	-	-	-	-	-	PG
10% NaCl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(+) Positive; (++) Strong positive; (+++) Excellent; (-) Negative; (PG) Poor growth

Table 4. Pearson correlation co-efficient analysis of physico-chemical properties of soil and total actinomycetes population.

	<b>Temp</b>	<b>pH</b>	<b>Al</b>	<b>Cr</b>	<b>Mn</b>	<b>Fe</b>	<b>Co</b>	<b>Ni</b>	<b>Cu</b>	<b>Zn</b>	<b>Hg</b>	<b>Corg</b>	<b>P</b>	<b>TAP</b>
<b>Temp</b>	1													
<b>pH</b>	0.7368	1												
<b>Al</b>	0.8300	0.2402	1											
<b>Cr</b>	-0.8211	-0.5604	-0.5581	1										
<b>Mn</b>	-0.3702	-0.0804	-0.9164	0.8099	1									
<b>Fe</b>	-0.9657	-0.6580	-0.3041	0.9365	0.5815	1								
<b>Co</b>	-0.6666	-0.5653	-0.3809	0.7927	0.5297	0.7610	1							
<b>Ni</b>	-0.9123	-0.7074	-0.2527	0.9356	0.5934	0.9600	0.6865	1						
<b>Cu</b>	-0.1452	-0.4791	0.4959	-0.0596	-0.4424	0.0631	0.4893	0.0168	1					
<b>Zn</b>	-0.8493	-0.9395	0.1534	0.7001	0.1821	0.8076	0.7367	0.8331	0.5221	1				
<b>Hg</b>	0.1506	-0.3027	0.6081	-0.3883	-0.6552	-0.2696	0.1823	-0.3169	0.9271	0.2469	1			
<b>C<sub>org</sub></b>	0.7228	0.4064	0.6276	-0.9079	-0.8491	-0.8277	-0.4849	-0.8451	0.4399	-0.4705	0.6835	1		
<b>P</b>	-0.3957	-0.4400	-0.00721	0.4259	0.1366	0.4432	0.8831	0.3528	0.8022	0.6103	0.5723	-0.0203	1	
<b>TAP</b>	0.5236	0.2734	0.7712	-0.8331*	-0.9273**	-0.6689	-0.4150	-0.6991	0.5331	-0.2968	0.7202	0.9611**	0.0505	1

Table 5. Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	7.751	55.367	55.367	7.751	55.367	55.367	5.739	40.994	40.994
2	4.358	31.128	86.495	4.358	31.128	86.495	4.743	33.881	74.875
3	1.442	10.301	96.796	1.442	10.301	96.796	3.069	21.922	96.796
4	.345	2.467	99.264						
5	.103	.736	100.000						
6	2.054E-15	1.467E-14	100.000						
7	5.370E-16	3.836E-15	100.000						
8	2.281E-16	1.629E-15	100.000						
9	1.523E-16	1.088E-15	100.000						
10	7.347E-17	5.248E-16	100.000						
11	-1.011E-16	-7.225E-16	100.000						
12	-2.371E-16	-1.694E-15	100.000						
13	-3.263E-16	-2.331E-15	100.000						
14	-5.375E-16	-3.839E-15	100.000						

Extraction Method: Principal Component Analysis

Table 6. Principal component matrix

	Component Matrix <sup>a</sup>			Rotated Matrix <sup>b</sup>		
	PC1	PC2	PC3	PC1	PC2	PC3
Temp	-.867	-.260	.314	-.935	.184	-.104
pH	-.630	-.589	.357	-.874	-.183	-.271
Al	-.495	.637	-.579	.108	<b>.986</b>	-.020
Cr	.994	-.053	.069	<b>.739</b>	-.651	.163
Mn	.760	-.527	.354	.253	-.957	-.015
Fe	.958	.131	-.135	<b>.874</b>	-.409	.148
Co	.788	.418	.448	.532	-.432	<b>.726</b>
Ni	.951	.097	-.234	<b>.908</b>	-.376	.055
Cu	-.026	.971	.235	.171	.500	<b>.848</b>
Zn	.750	.610	-.232	<b>.912</b>	.061	.390
Hg	-.347	.897	.195	-.088	<b>.662</b>	.719
C <sub>org</sub>	-.906	.395	.139	-.665	<b>.706</b>	.237
P	.423	.705	.558	.277	-.096	<b>.949</b>
TAP	-.816	.535	-.012	-.471	<b>.818</b>	.246

Extraction method: principal component analysis

Bold indicates correlation is significant at the 0.01 level (2-tailed)

<sup>a</sup> 3 components extracted

<sup>b</sup> Rotation method: varimax with Kaiser normalization