CHAPTER 25

MARINE MICROBIAL BIODIVERSITY: PRESENT STATUS AND ADVANCED STATISTICAL PARADIGMS

By

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ABSTRACT

Microorganisms occur nearly everywhere in nature and occupy an important place in human view of life. The world ocean is the largest ecosystem on earth, and has been used for a variety of purposes by man for millennia. However, our knowledge regarding the marine microbial diversity is limited and based on the cultured fraction of the microorganisms. Difficulties in sampling microorganisms from the sea also limit their utilization. This paper discusses the present status of marine microbial diversity and the advanced statistical tools to estimate the marine microbial biodiversity. Microbial diversity can be predicted using statistical approaches that are routinely used in ecological studies. A case study based on the culture dependent method from Bay of Bengal (India) is presented here to imply different statistical tools. The richness is estimated by a univariate method which includes indices such as Simpson Index, reciprocal of Simpson's Index, rarefaction index and Margalef index. To estimate the species diversity and evenness, Shannon-Wiener index and Pielou's evenness are used respectively. The K-dominance curve can also be used as a graphical method of diversity estimation.

Keywords: biodiversity, case study, Bay of Bengal, Marine microbes, status, statistics tools.

INTRODUCTION

'Biological diversity' means the variability among living organisms from all sources and the ecological complexes of which they are part and it includes diversity within species or between species and of eco-systems. Diversity generally means "variation" and "differentiation", and "diversification", in contrast to "uniformity". It may also be understood as something static: "heterogeneity" then denotes "irregularities", "variety" and "differences". When assessing biological systems, diversity may also be seen as "richness" (Kratochwil 1999).

The highest overall marine diversity occurs in the tropical Indo-Western Pacific area, a region that includes waters off the coast of Asia, Southeast Africa, Northern Australia and the Pacific Islands. The higher levels of diversity appear directly related to ecosystem stress (Hoffman & Parsons 1991). However, biodiversity on planet Earth is under an alarming rate of extinction (Wilson 1992). Despite the catastrophic impacts on the entire biosphere we are still very ignorant about the number of existing

species on earth (e.g. May 1988, 1995; Hammond 1995). Therefore, an approach based on ecological and evolutionary processes should proceed to mapping and quantifying marine biodiversity at all structural levels (Cognetti & Maltagliati 2004).

Marine systems differ from terrestrial systems in so many ways that paradigms concerning patterns of biodiversity in terrestrial systems may not be applicable to marine situations (May 1994; Gray 1997). The fundamental difference between the terrestrial and marine ecosystems is that the former can relate its three-dimensional space to permanent or semi-permanent physical structures (Raghukumar & Anil 2003). The diversity of microorganisms associated with water, soil and flora and fauna is also incredibly rich. The world ocean has a coast line of 312,000 km and a volume of 1.46×10^9 km^3 with an average depth of 4000m (Rumney 1968), making it the largest ecosystem on earth. It has been used for a variety of purposes by humans for millennia but most studies of biological diversity relate to terrestrial systems and our knowledge of marine biodiversity lags far behind that on land (Ellingsen 2001, 2002; Ellingsen & Gray 2002).

Based on rRNA trees, the main extent of earth's biodiversity is microbial (Hugenholtz *et al.* 1998) but our knowledge of the extent and character of microbial diversity is very limited. Microorganisms are ubiquitous in the marine environment and are truly the 'unseen majority'. It has been estimated that more than 10^{29} microbes are in the world ocean (Whitman *et al.* 1998), with a mass of $0.6-1.9 \times 10^{15}$ g C (Karl & Dobbs 1998). Assessing microbial biodiversity is a difficult task and is a topic of considerable importance and interest to conserve and protect the microbial wealth (Das *et al.* 2006). Although microbiologists have applied laboratory based culture techniques to marine isolates for over a decade, we still lack a comprehensive view of the ecology of microorganisms in the sea.

PRESENT STATUS OF MICROBIAL BIODIVERSITY

Modern microorganisms have a long evolutionary history (on the order of 3.5 billion years), which has been played out largely in marine environments. Initially the living world was divided into two very distinct types of organisms, eukaryotes which have a nuclear membrane and prokaryotes that lack nuclear membranes; the latter include bacteria, which are the 'first and simplest division of living beings'. However, taxonomists of the twentieth century emphasized the 'Five Kingdoms' of life: animals, plants, fungi, protists (Protozoa) and monera (Bacteria) (Whittaker and Margulis 1978). More recently, Woese *et al.* (1990) proposed three primary lines of evolutionary descent, termed "urkingdoms" or "domains": Bacteria (eubacteria), Archaea (archaebacteria) and Eucarya (eukaryotes) (Fig. 1). These domains are identified by genetic distance in the composition of the 16S or 18S rRNA (Woese *et al.* 1990). In the sea, these three domains have overlapping size spectra, physiological characteristics, metabolic strategies and ecological functions. The bacteria and archaea are divisions of prokaryotes, organisms with, usually, a rigid cell wall and DNA loosely organized in a region of the cell termed as nucleoid (Woese 1994). Woese's discoveries and interpretations were widely acclaimed and accepted although there was opposition by Mayr (1998). The division-level diversity of the bacterial domain as inferred from 16S rRNA gene sequences showed 36 divisions (Fig. 2) but the number of bacterial divisions may be well over 40 (Hugenholtz *et al.* 1998). Several of the described divisions are well represented by cultivated strains and were the first to be characterized phylogenetically (Woese 1987). In Figure 2, 13 of 36 divisions are characterized only by environmental sequences and so are termed "candidate divisions". One of these candidate divisions is Marine group A, which represents the marine bacteria by environmental sequences.
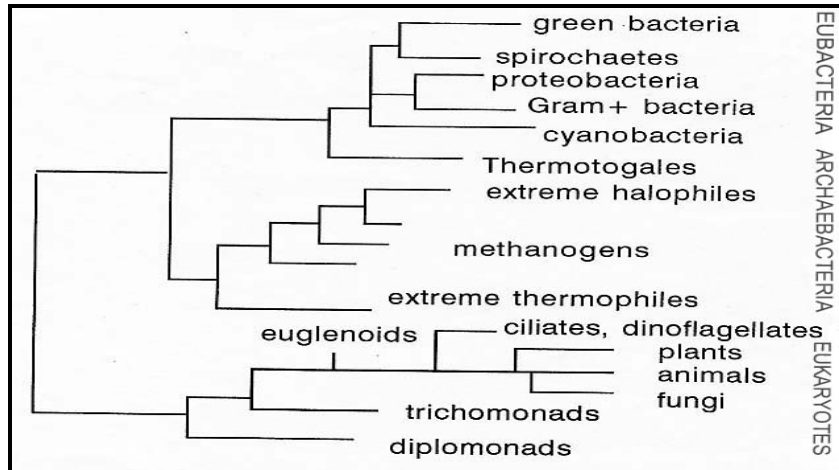
Figure 1. The "universal tree" based on rRNA sequenceing showing the three domains of life on earth (Fenchel 2001).
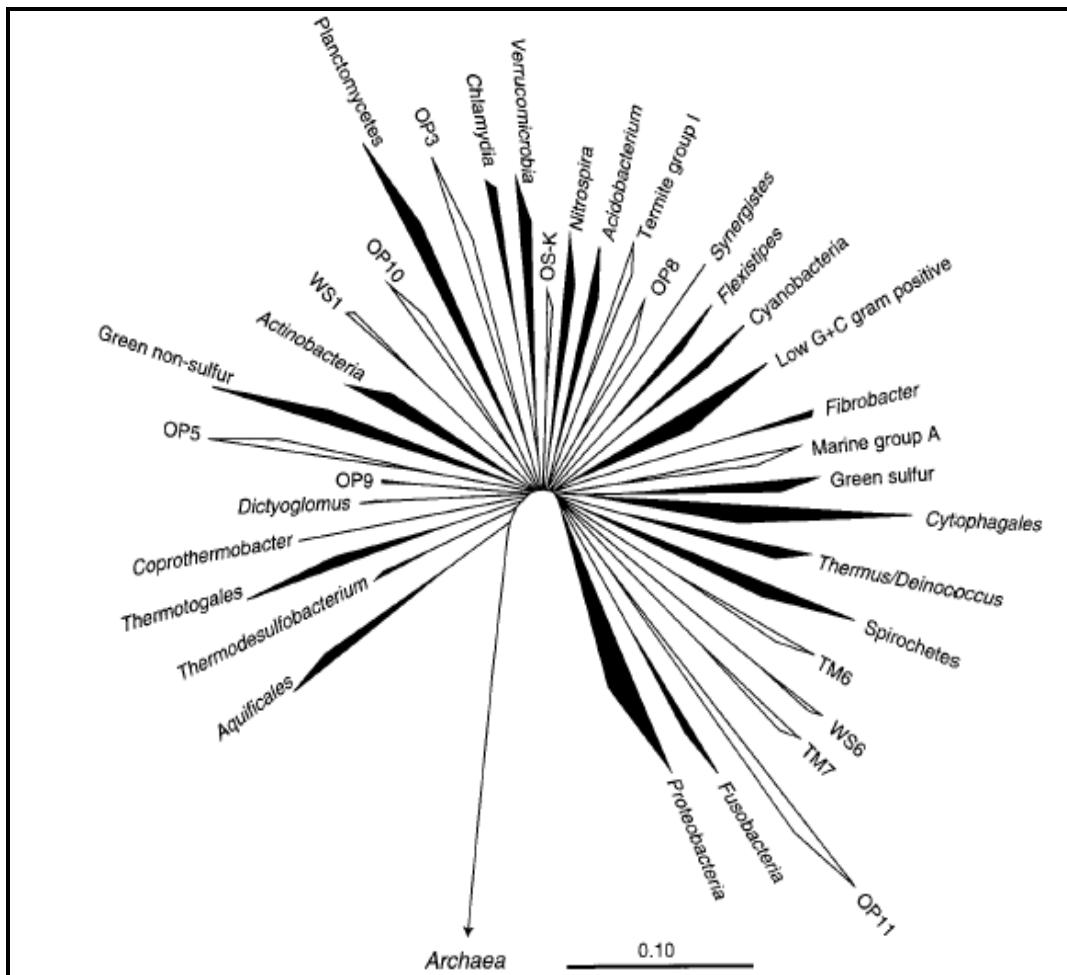

Figure 2. Evolutionary distance tree of the bacterial domain showing currently recognized divisions and putative divisions (Hugenholtz *et al.* 1998). The scale bar indicates 0.1 changes per nucleotide.

APPROACHES TO STUDY THE MARINE MICROORGANISMS

Communities can be analyzed and characterized in many different ways. One of the most common methods is by looking at community diversity. This is based on the relationship between the diversity of a community and its stability - the more diverse and complex the community, the more stable it is. Biodiversity and community structure are now recognized to be important determinants of ecosystem functioning and this ecosystem functioning is dictated to a large degree by biodiversity and the community structure that results from factors such as the richness and evenness of the diversity. Diversity at all levels, including infra-specific or genetic diversity that characterize populations of a species, species diversity that characterize communities, and in turn the community diversity that characterizes an ecosystem, all play a major role in understanding the biodiversity and ecosystem functioning (Raghukumar & Anil 2003).

The study of marine microbial diversity is important in order to understand the community structure and the pattern of distribution in the different niches of the marine environment. But microbial diversity is one of the difficult areas of biodiversity research (Watve *et al.* 1999) and India's microbial diversity is perhaps one of the most significant in the world (Budhiraja *et al.* 2002). Although marine microbes have been studied for several decades (e.g Zobell & Upham 1944; Zobell 1946; Velankar 1957; Wood 1959; Iyer & Pillai 1976), only limited and scattered information is available and the number of species known to scientists is only the tip of an iceberg (Das *et al.* 2006).

a) Marine Bacteria: The phylogenetic groups of the eubacteria contain heterotrophic members, which indicate the genetic diversity of heterotrophic bacteria. This genetic diversity mirrors the functional diversity of heterotrophic bacteria (Staley 1996). To assess the diversity of heterotrophic bacteria in natural communities two separate approaches have been used. The traditional way of assessing the number of living bacteria is based on their ability to grow in culture media so they can be characterized phenotypically and genotypically. A large discrepancy between total and viable counts is a normal occurrence in these measurements. This discrepancy could be a consequence of the variety of environmental requirements and physiological adaptations of marine bacteria (Roszak & Colwell 1987) or of the difficulty in setting up nonselective culture media (Fry 1990). To better understand the physiology and ecology of bacterial species, their isolation in pure culture remains an essential step in microbial ecology. But for the marine environment, colony forming units (CFU) provide an inadequate description of the relative abundance of bacteria, because traditional cultivation methods do not mimic the real environmental conditions under which natural populations flourish (Ward *et al.* 1990) and also due to the unculturability of the organisms which account for 99% of the biota (Fuhrman & Campbell 1998). More recent molecular approaches do not require the bacteria to be cultivated; instead, the community diversity is assessed by an examination of its extracted nucleic acids and several other methods (Fig. 3). The gene sequences of the small subunit RNA as a molecular marker for identification of microorganisms has changed the perception of the diversity of microbial communities. The commonest gene, 16S ribosomal RNA gene, is used because ribosomal RNA is involved in protein synthesis so it evolves very, very slowly, as most mutations in the gene level are lethal. The genes encoding small subunit rRNA also reflect the evolutionary relationship of microorganisms (Woese 1987) and the sequences of these genes allow one to group and identify microorganisms. But the application of cloning and sequencing of 16S RNA genes are too laborious and time consuming to analyze a large number of samples, so genetic fingerprinting techniques have been developed, among which PCR-DGGE (Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis) fingerprinting is the most common tool used for monitoring variations in microbial genetic diversity, providing a minimum estimate of the richness of predominant community members. It has been used to investigate the diversity of microbial communities to determine the spatial and temporal variability of bacterial populations and to monitor community behaviour (Schafer & Muyzer 2001).
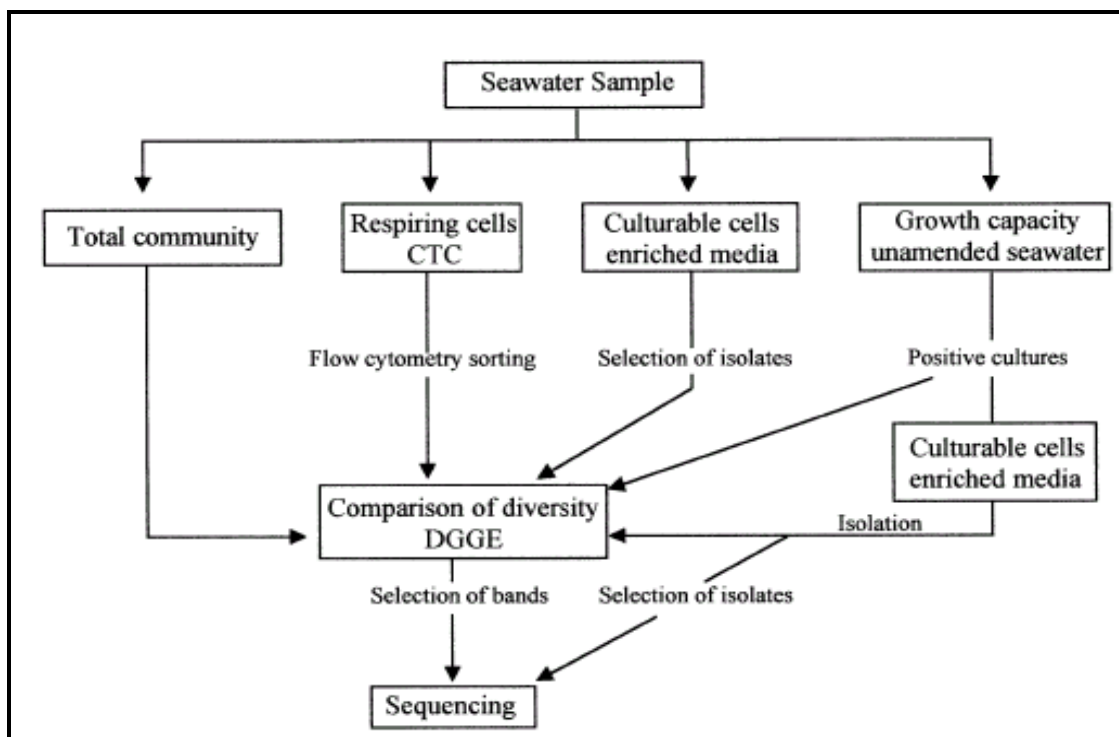
Figure 3. Strategy for comparing the genetic diversity of marine microbial communities (Bernard *et al.* 2000).

The principal advantage of using the classical cultivation approach is that organisms are isolated and therefore available for further study. But the culturability of bacterial cells is a species-dependent characteristic. Many marine bacterial species have unknown growth requirements and have not yet been cultured. Several media with different compositions have been proposed for isolating new species (Martin & MacLeod 1984; Gonzalez & Moran 1997) and a dilution culture technique has been developed to isolate oligotrophic species, which do not grow on nutrient-rich media (Button *et al.* 1993). In contrast, molecular approaches do not require the bacteria to be isolated. However, some disadvantages of the molecular approaches include the difficulty in capturing all bacteria from natural communities, and the presence of DNA from phages and higher organisms in the community. Furthermore, it is often not possible to determine the physiological type or species from its 16S rDNA sequence by comparing it directly with sequences in the NCBI database using BLAST (Basic Local Alignment Search Tool) as well as with the sequences available from the Ribosomal Database Project (RDP). For these reasons, it is impossible to determine diversity indices and species diversity of heterotrophic bacteria accurately in most communities using either cultivation or molecular approaches (Staley 1996).

b) Marine actinomycetes: Identification and classification are the difficult parts in traditional actinomycetes research. Several biochemical tests are performed and with the description given by Tresner *et al.* (1961), Shirling & Gottlieb (1966, 1968a, 1968b, 1969a, 1969b) and Nonomura (1974), the identification can be done. But the colony isolation is often the most frustrating and time-consuming task as it involves the examination of morphological characters. Hence, besides the traditional methods, the advanced method for the identification of actinomycetes through computer software Actinobase helps in the genus level identification where image files are stored using descriptions of International Streptomycetes Project (ISP) and other sources. Apart from these, analysis of 16S rRNA helps to determine phylogenetic relationships and makes possible the recognition up to species level using sequence signatures followed by BLAST search.

c) Marine fungi: With most of the methods available for fungal biomass and identification keys based on the vegetative hyphae or propagules (ascospores, basidiospores, conidia) (Kohlmeyer & Kohlmeyer 1991), fungi cannot always be identified (Nikolcheva *et al.* 2003). The obvious shortcoming to available protocols is the absence of propagules which might be due to the absence of species or to the presence of nonsporulating mycelium (e.g. Raghukumar *et al.* 2004, who isolated numerous fungi from the core samples of the Chagos Trench, Indian Ocean but most of which were non-sporulating and, therefore not identifiable). In the initial phases of fungal colonization, between the landing of propagules and their growth into a sporulating colony, newly arrived species will escape detection by traditional microscope based techniques. Molecular approaches characterize nucleic acids that are present in all stages of the fungal life cycle, and could circumvent the problems associated with the microscopy-based techniques.

Two methods can be used for fungal ecology study: terminal restriction fragment length polymorphism (T-RFLP) analysis (Liu *et al.* 1997) and denaturing gradient gel electrophoresis (DGGE) (Muyzer *et al.* 1993). Although both techniques require expensive equipments, many samples can be processed in a short time and allow profiling of the fungal community richness and evenness (Nikolcheva *et al.* 2003).

APPLICATION OF STATISTICAL TOOLS: A CASE STUDY

Although microbial diversity is one of the difficult areas of biodiversity research (Watve *et al.* 1999), the estimation of microbial diversity is required for understanding the biogeography, community assembly and ecological processes (Curtis *et al.* 2002). The number of species has been the traditional measure of biodiversity in ecology and conservation, but the biodiversity of an area is much more than the 'species richness' (Harper & Hawksworth 1994). Diversity can be predicted using statistical paradigms that estimate species number from relatively small sample sizes (Stach *et al.* 2003). But it has already been questioned by O'Donnell *et al.* (1996) whether indices used to quantify diversity in macroorganisms can be applied to microorganisms. Stach *et al.* (2003) found that the diversity indices which are used in ecological studies can also be used to study the microbial diversity. Hughes *et al.* (2001) found that both rarefaction and richness estimators can be used to microbial data sets, and highlighted the utility of nonparametric estimators in predicting and comparing bacterial species number. But Ravenschlag *et al.* (1999) relied on only the two diversity indices, species richness and species evenness, to study the bacterial diversity from permanently cold sediment of Arctic Ocean. Rarefaction and richness estimators rely on a species or operational taxonomic unit (OTU) definition. The limitations of this method is that OTUs are counted as equivalent despite the fact that some may be highly divergent and phylogenetically unique, where as others may be closely related and phylogenetically redundant (Martin 2002).

There are several arguments on the species concept in both bacterial and fungal systematics (O'Donnell *et al.* 1996; Watve & Gangal 1996). Ignoring all these controversies recent statistical analyses borrowed from population genetics and systematics have been employed and reviewed for use with microbial data sets to estimate species richness and phylogenetic diversity (Martin 2002). O'Donnell *et al.* (1996) advocated that in assessing the biodiversity of a site, instead of relying solely on estimates of species richness, information about the extent to which the species differ taxonomically should also be taken into consideration. This is 'Taxonomic Diversity' and is considered to be more indicative of the biodiversity of a habitat than species richness. At higher level of taxonomic rank such as phyla, diversity in marine habitats is greater than that in terrestrial environments even though the number of species may be lower (Laserre 1992). Estimation of both species richness and higher levels of taxonomic diversity in an ecosystem assume that all species or taxa have equal value and as such should be given equal weight in the quantification of diversity for conservation purposes (Williams *et al.* 1991). But 'ecosystem diversity' accounts all kinds of diversity within an ecosystem, so in addition to estimates of species richness and abundance, the habitat classification and structural measurement are important. Ecosystem diversity comprises: α-diversity, the diversity of species (genera, families) within a community or habitat (i.e. species richness) which defines the richness of a potentially interactive assemblage of organisms; β-

diversity, a measure of the rate and extent of change in species along a gradient between habitats (between-area) and is expressed as a similarity index; and γ-diversity, the richness in species over a range of habitats (within-area) in a geographical region or a biome (Bull & Stach 2004). Owing to difficulties in estimating the α-diversity of a microbial assemblage, it is difficult to see how estimates of ecosystem diversity which take full account of the microbial diversity of a habitat can be derived unless 'species richness' is replaced with an alternative measure or index of microbial diversity which does not rely on the classification and identification of microorganisms (O'Donnell *et al.* 1996).

The Case Study

To apply statistical tools to microbial data sets for biodiversity estimation sediment samples were collected using Smith McIntyre Grab (coverage area $0.2m^2$) from the southeast continental slope of Bay of Bengal (India) in three different depth stations (i.e. ca. 200m, 500m and 1000m) along five transects (Fig. 4) and numbered consecutively from S48 to S62. The culture dependent method was followed to estimate the metabolically active total heterotrophic bacterial population. The samples were analyzed immediately on board onto Zobell's Marine Agar 2216e medium (HiMedia, India) in duplicate for bacteria by the spread plate method (Schneider & Rheinheimer 1988) after suitable dilutions. Colony forming units (CFU) were counted and expressed as CFU g^{-1} dry sediment weight. All the colonies were subsequently picked up, sub-cultured and maintained in slants for further studies. Standard bacteriological procedures were carried out to identify the isolates up to generic level following the schemes given by Baumann *et al.* (1972), Buchanan & Gibbons (1974), Starr *et al.* (1981), Oliver (1982) and Sneath (1986).
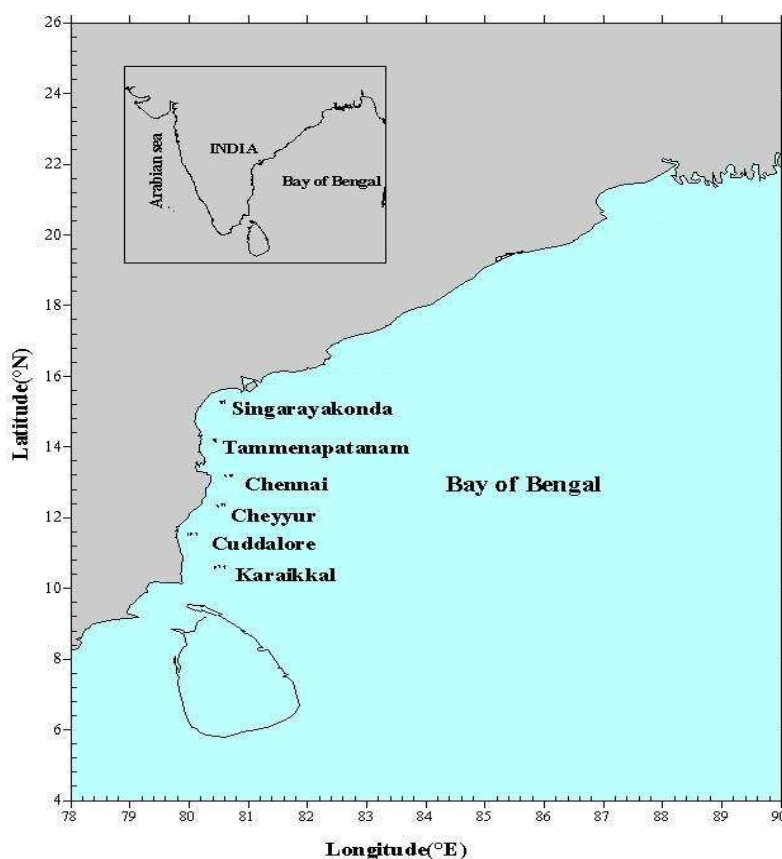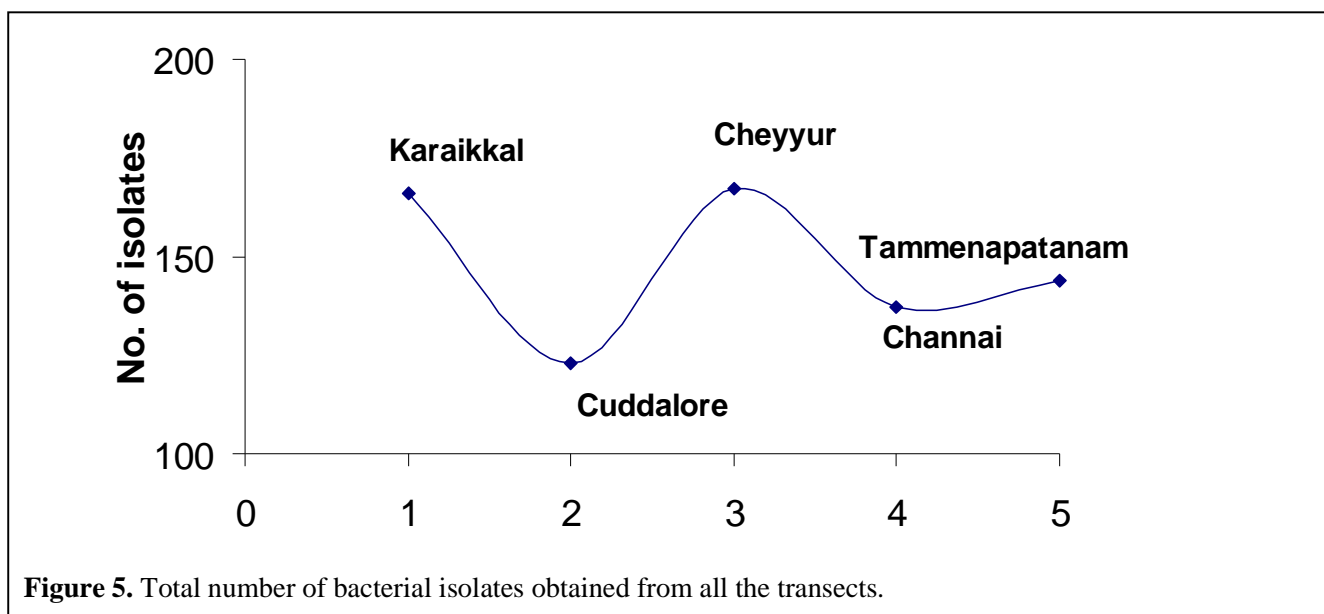

Figure 4. The study area- continental slope of the southeast coast of Bay of Bengal (India).

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Table 1. Numerical abundance of bacterial genera in the different stations.

Transects	St. no.	Location		*Corynebacterium*	*Arthrobacter*	*Micrococcus*	*Bacillus*	*Acinetobacter*	*Alteromonas*	*Cytophaga*	*Alcaligenes*	*Flavobacterium*	*Vibrio*	*Pseudomonas*
		Lat (N)	Long (E)											
Karaikkal	S48	10°34.96	80°26.70	4	2	5	8	3	3	2	4	3	9	11
	S49	10°36.64	80°30.67	1	2	5	6	2	4	1	1	4	11	8
	S50	10°36.16	80°36.23	5	4	7	14	1	5	3	5	5	10	21
Cuddalore	S51	11°31.82	79°59.01	3	9	6	12	2	2	1	4	6	11	13
	S52	11°31.44	80°02.16	4	5	5	16	2	4	1	2	3	14	19
	S53	11°32.03	80°07.55	5	2	4	14	4	1	3	4	1	15	18
Cheyyur	S54	12°18.81	80°29.35	2	1	6	14	1	3	2	8	3	12	11
	S55	12°21.68	80°33.49	5	2	7	18	4	3	3	4	1	14	20
	S56	12°21.34	80°36.10	3	5	6	14	2	5	2	3	2	12	10
Chennai	S57	13°09.78	80°36.66	2	2	4	11	1	5	1	4	5	9	8
	S58	13°09.95	80°41.99	1	2	3	20	2	3	4	2	2	15	17
	S59	13°10.21	80°43.18	2	4	6	7	5	2	1	1	2	9	5
Tammenapatanam	S60	14°10.65	80°24.97	3	2	8	10	2	3	4	2	3	11	9
	S61	14°10.99	80°26.37	7	5	5	9	2	3	4	1	2	11	10
	S62	14°10.23	80°27.04	2	4	5	12	6	4	1	3	1	9	11

Figure 5. Total number of bacterial isolates obtained from all the transects.

In total, eleven bacterial genera were identified (Table 1) and the transect-wise total number of isolates showed that highest value in Karaikkal transect followed by Cheyyur (Fig. 5).

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Statistical Treatment

Biodiversity indices measure the degree to which species or organisms in a sample are taxonomically or phylogenetically related to each other (Clarke & Warwick 1994). In molecular analysis of samples using DGGE (Denaturing Gradient Gel Electrophoresis), different samples are compared based on the number of DGGE bands detectable (i.e. genetic richness) and their relative staining intensity (i.e. evenness). Using the PCR-DGGE defined richness and evenness values, a Shannon-Wiener diversity index could be calculated (Schafer & Muyzer 2001). The diversity of fungi in leaf and woody litter of mangrove forests with the help of Simpson diversity and Shannon diversity indices was also described (Ananda & Sridhar 2004). The number of species is, however, not the only measure of diversity. The relative abundances of the different species are also important. An area in which the species are equally abundant would be regarded as more diverse than one where the same number of species are of disparate abundance. The above data were treated with the help of two computer programmes viz., PRIMER (Plymouth Routines in Multivariate Ecological Research ver. 5) (Clarke & Warwick, 2001) and BDPRO (Biodiversity Professional ver. 3).

A) Species richness indices: Species richness indices measure the total number of species and these indices have been used widely in the study of microbial diversity (Maria & Sridhar 2002; Stach *et al.* 2003; Grishkan *et al.* 2003; Bowman & McCuaig 2003; Gallagher *et al.* 2004; Ananda & Sridhar 2004). For example, for species richness (number of isolated species) Simpson index (D'), reciprocal of Simpson's Index (1/D), Margalef index (d), rarefaction index and 95% confidence intervals are in use.

i) Simpson index: Simpson elucidated the probability of any two individuals drawn at random from an infinitely large community belonging to different species.

$$\text{Simpson index, } D' = \frac{1}{\sum (Pi)2}$$

$$Pi = \frac{ni}{N},$$

where, ni = number of individuals of i, i^2 etc.
N= total number of individuals.

Simpson's index is heavily weighted towards the most abundant species in the sample and is less sensitive to species richness. It varies between 0 and 1 and moderately sensitive to sample size (Hosetti 2002). Our study showed (Figs. 6-9) the highest richness was in S58 (off Chennai) and the overall species richness was higher in the 500m depth stations. Species richness is a straightforward count of the number of species in a given area and increases with sample size (Magurran, 1988; Flach *et al.* 1999). Therefore, it may be estimated that the richness of species was higher in the 500m depth stations.

ii) Reciprocal of Simpson's index: Reciprocal of Simpson's Index (1/D) (Figs. 10-13) is used as a measure of diversity, since it has been widely used for ecological studies but also applied to microbial communities (Hill *et al.* 2003).
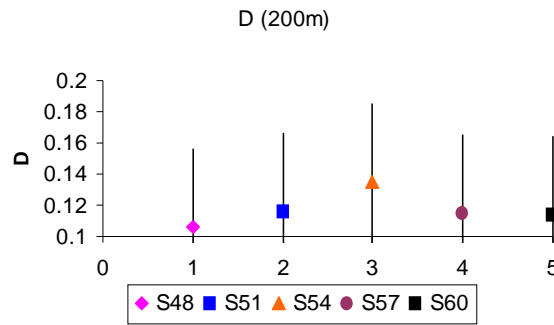
D (200m)

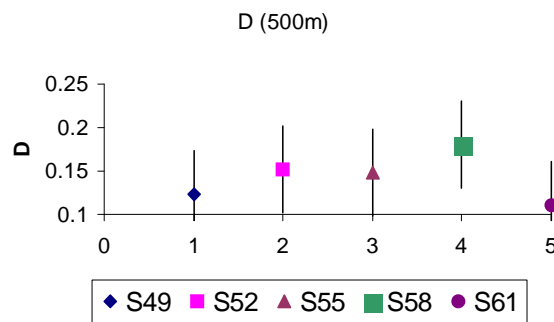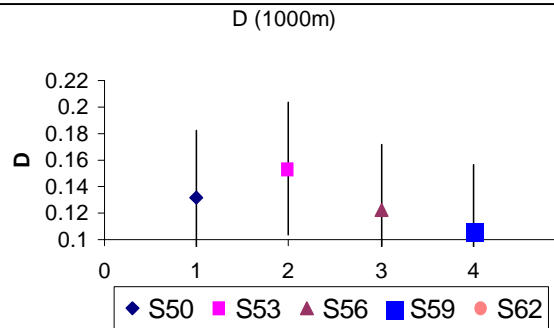**Figure 6.** Means and 95% confidence intervals of Simpson's index (D) for the 200m depth stations.

D (500m)

Figure 7. Means and 95% confidence intervals of Simpson's index (D) for the 500m depth stations.

D (1000m)

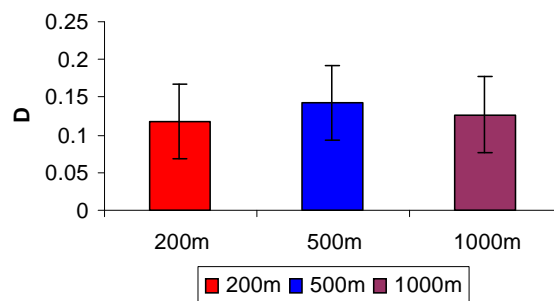**Figure 8.** Means and 95% confidence intervals of Simpson's index (D) for the 1000m depth stations.
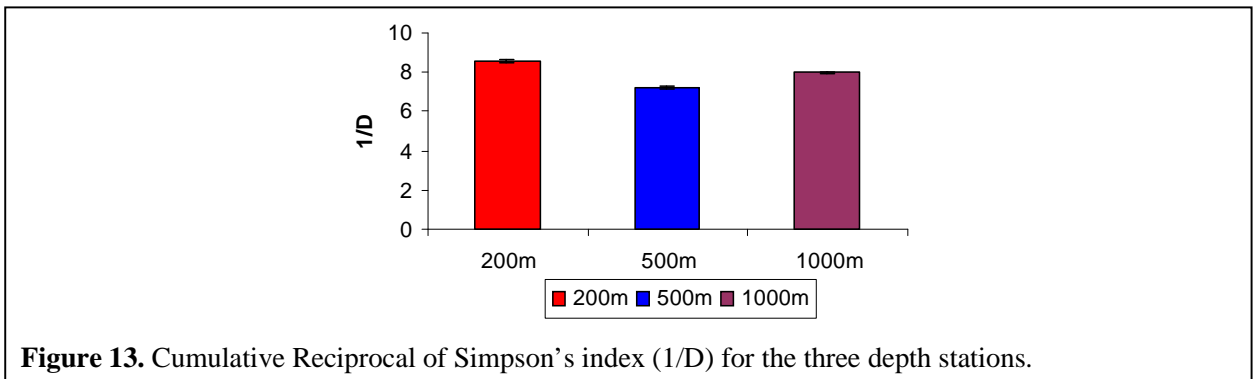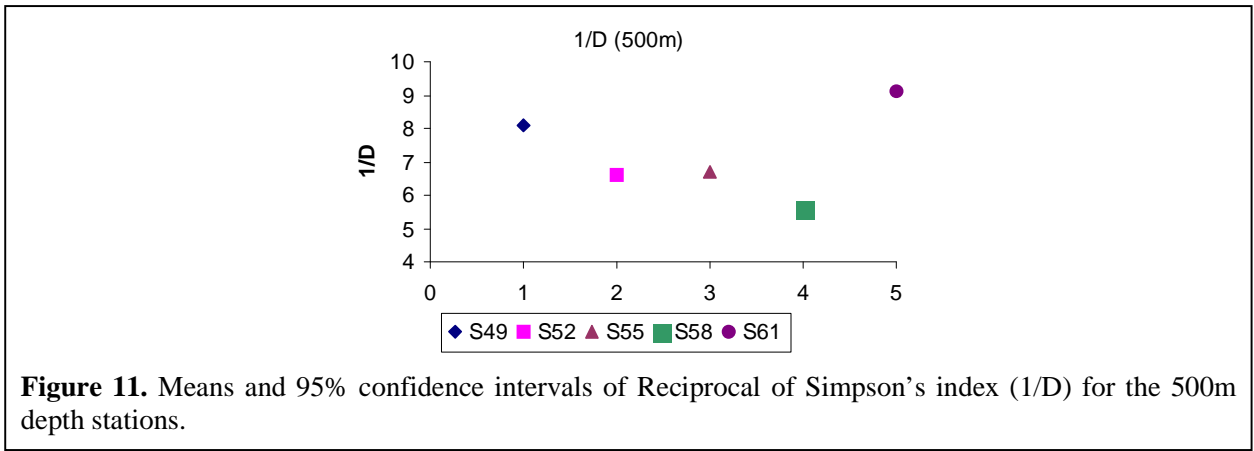

Figure 9. Cumulative Simpson's index (D) index for the three depth stations.

Figure 10. Means and 95% confidence intervals of Reciprocal of Simpson's index (1/D) for the 200m depth stations.

Figure 11. Means and 95% confidence intervals of Reciprocal of Simpson's index (1/D) for the 500m depth stations.

Figure 12. Means and 95% confidence intervals of Reciprocal of Simpson's index (1/D) for the 1000m depth stations.

Figure 13. Cumulative Reciprocal of Simpson's index (1/D) for the three depth stations.

iii) Margalef index: This index has a good discriminating ability and is weighted towards species richness. It is denoted by'd'. The advantage of this index over the Simpson index is that the values can be more than 1 unlike in the other index where the values will be varying from 0 to 1. This method of comparing species richness between different samples collected from various habitats is easy. The trend noticed in the diversity index is also observed in this index (Figs. 14-17) but the values were higher in the 200m and 1000m depth stations.

$$\text{Margalef index, } d = (S-1) / \log N$$
$$\text{where, } S = \text{Number of species}$$
$$N = \text{Total number of individual}$$
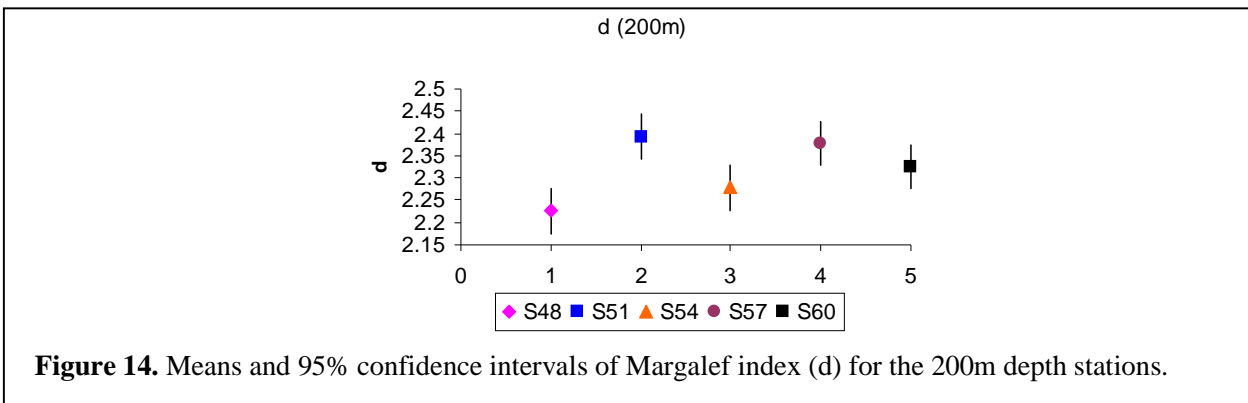
Figure 14. Means and 95% confidence intervals of Margalef index (d) for the 200m depth stations.
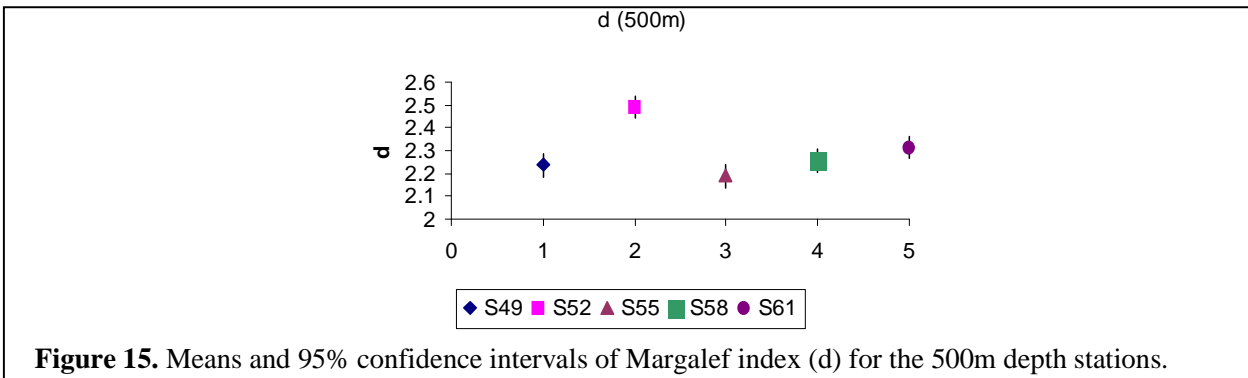
Figure 15. Means and 95% confidence intervals of Margalef index (d) for the 500m depth stations.
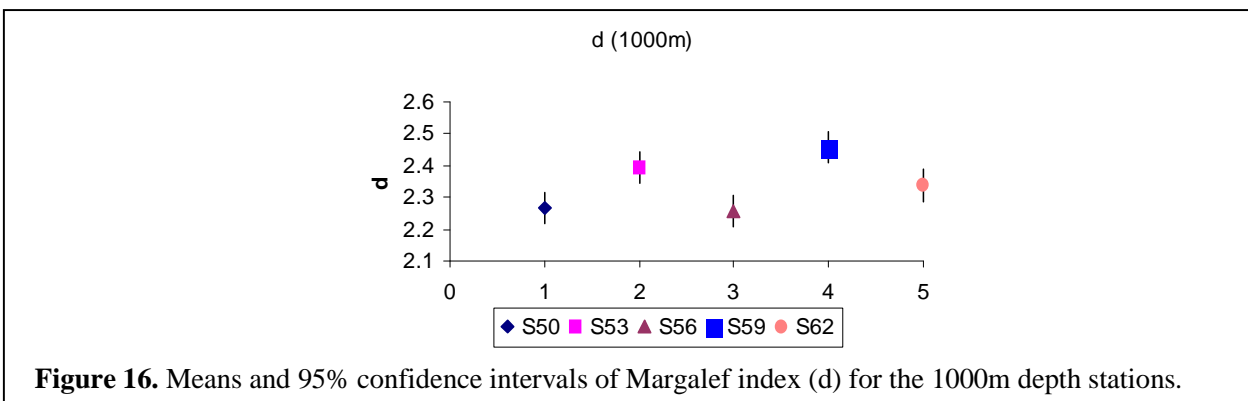
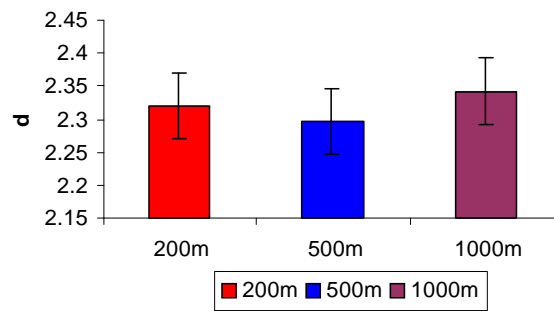**Figure 16.** Means and 95% confidence intervals of Margalef index (d) for the 1000m depth stations.

Figure 17. Cumulative Margalef index (d) for the three depth stations.

iv) Rarefaction curve: This method gives an estimation of the decrease in apparent species richness of a community with unequal subsample size (Simberloff 1978) and was applied in the marine bacterial diversity (Ravenschlag *et al.* 1999), prokaryotic diversity studies of Antarctic continental shelf sediment (Bowman & McCuaig 2003) and in the soil bacterial diversity (Torsvik *et al.* 1990). A rarefied curve results from averaging randomization of the observed accumulation curve (Heck *et al.* 1975). Rarefaction has the feature that it allows the comparison of diversity from clone libraries of unequal sample size (Tiper 1979). It is calculated by using the rarefaction calculator of Sanders (1968) and Hurlbert (1971). It is a count-based measure and is independent of the total count or culturable viable count of microorganisms. Ananda & Sridhar (2004) used the rarefaction index to compare species richness among the marine fungal isolates during two seasons. It also can be downloaded from http://www.biology.ualberta.ca/jbrzusto/rarefact.php (Bowman & McCuaig 2003). It is also available with some software packages.

Rarefaction diversity plots he number of individuals on the X - axis against the number of species on the Y – axis (Sanders 1968). Rarefaction curves are plotted on a log scale in order to produce a unified display of stations with different total abundances. This allows the diversity to be visualised graphically in terms of the relationship between the species and individuals at each station. It can also be of considerable use in the comparison of investigations involving different sample sizes. Thus, the more diverse the community is, the steeper and more elevated is the rarefaction curve; coinciding with the richness, S58 (off Chennai) rarefaction was also steeper and hence, more diverse (Figs. 18-23).
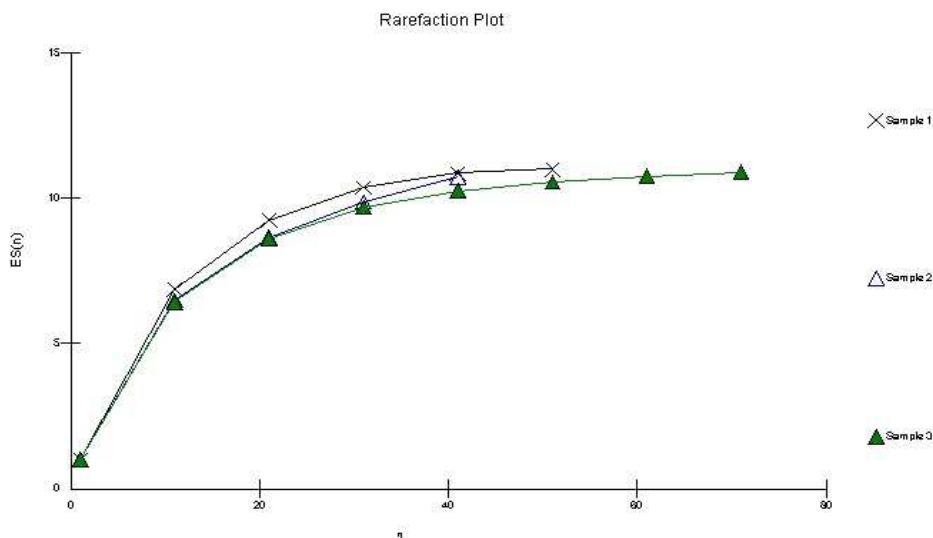

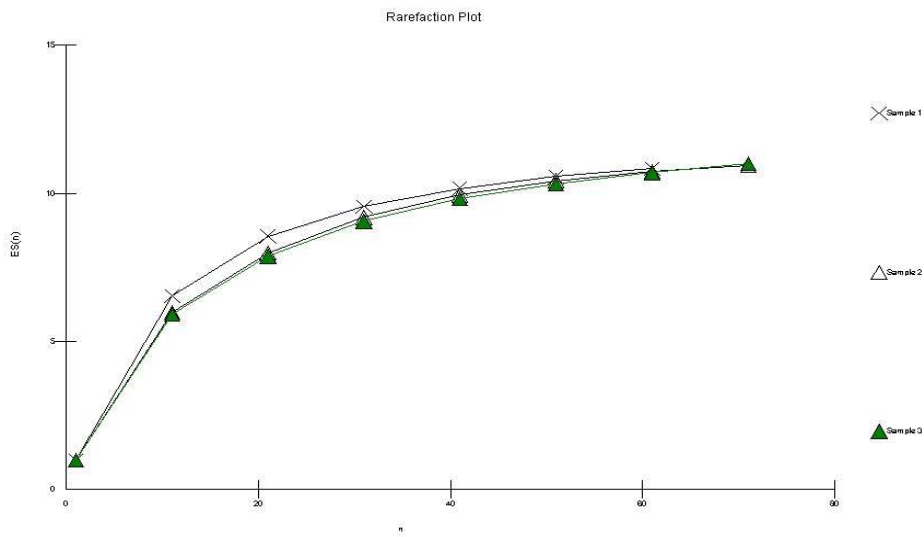**Figure 18.** Rarefaction curves for the three stations off Karaikkal.

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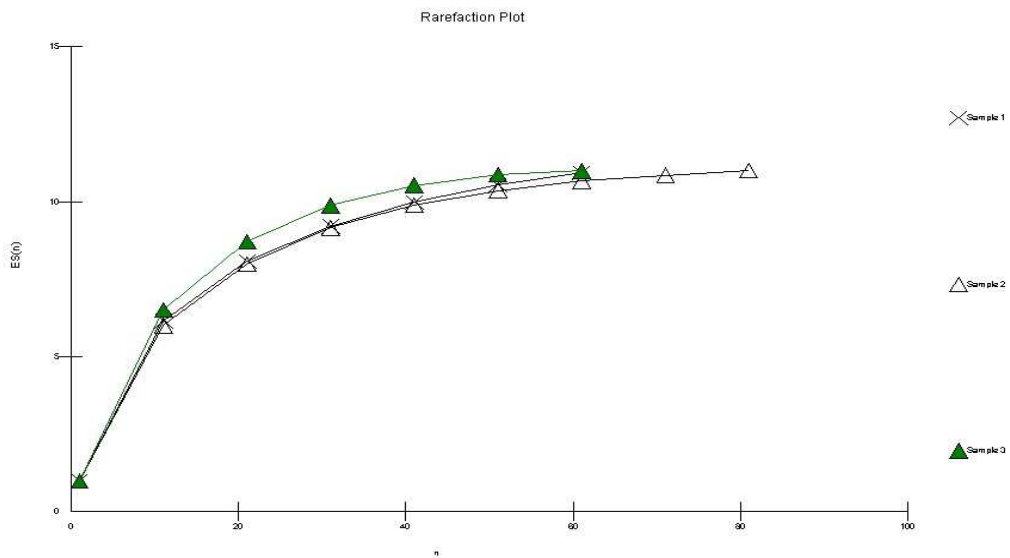**Figure 19.** Rarefaction curves for the three stations off Cuddalore.

Figure 20. Rarefaction curves for the three stations off Cheyyur.
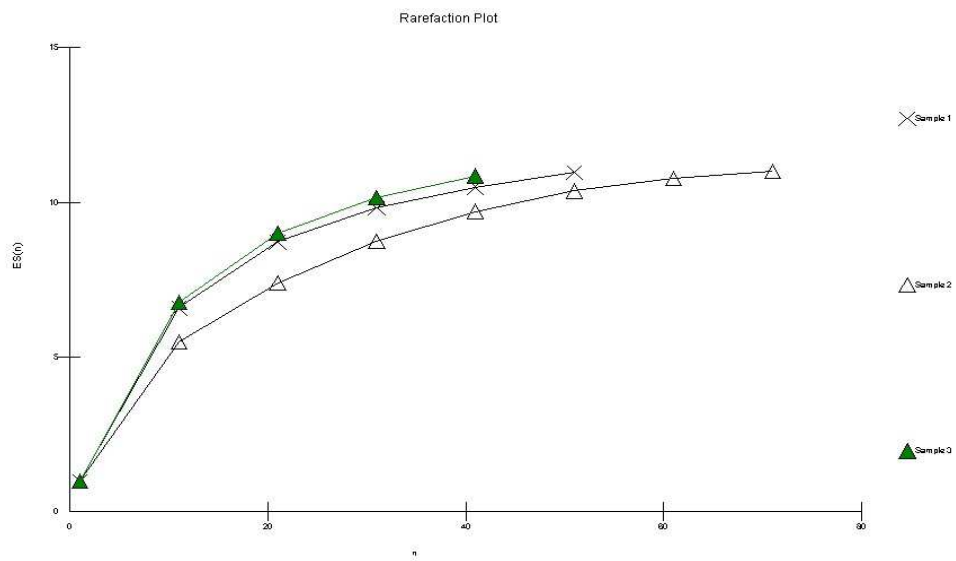
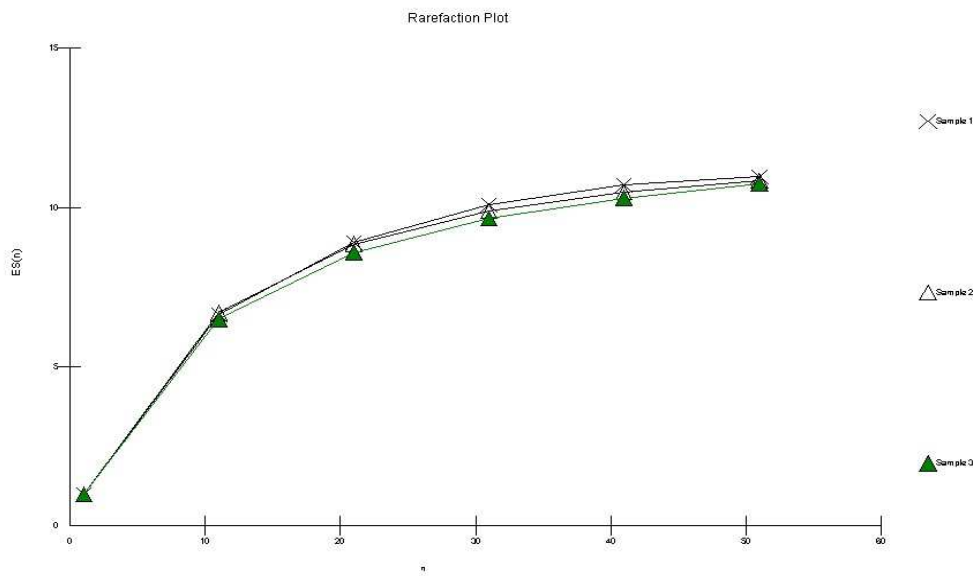**Figure 21.** Rarefaction curves for the three stations off Chennai.

Figure 22. Rarefaction curves for the three stations off Tammenapatanam.
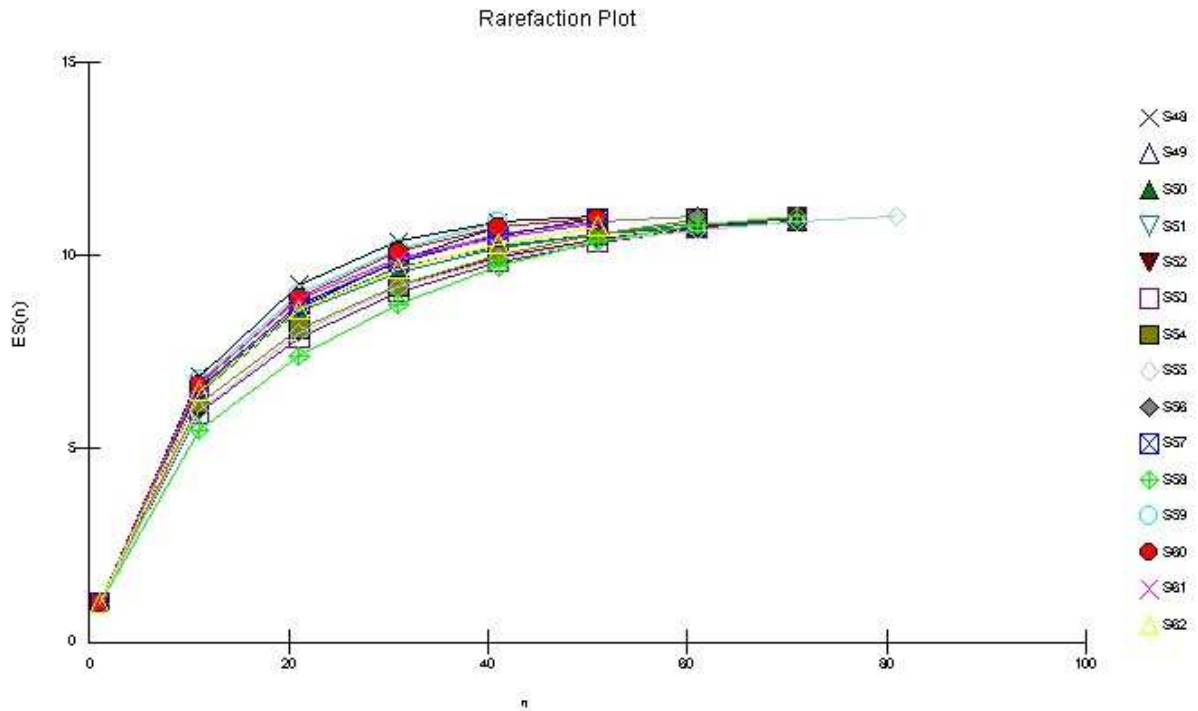
Figure 23. Rarefaction curves for all the stations off all the transects.

B) Species diversity index: More ecologically informative measures of biodiversity incorporate species diversity or abundance (Bull & Stach 2004). Shannon & Wiener (1949) derived a formula which is known as the Shannon index of diversity (H'). It is calculated to examine community changes (Valiela 1984). The values of Shannon diversity usually fall between 1.5 and 3.5 (Ajmal Khan 2004). This diversity is a very widely used index for comparing diversity between various habitats (Clarke & Warwick 1994). The more equally abundant the species are in an area, the more biologically diverse it is regarded.

$$\textbf{Shannon-Wiener Index}, H' = \sum_{i=1}^{s} Pi \log_2 P_1$$

Here P_i = Number of individuals in the ith species
S = Number of species.
Here *Pi* is the probability of finding each species *i* in a sampling plot.

The Shannon-Wiener index is moderately sensitive to sample size and places more weight on richness (Hosetti 2002). The Shannon-Wiener index showed (Figs. 24-27) that the 200m and 1000m stations were more or less equally diverse compared to 500m stations, where more diversity was found in S53 (off Cuddalore) and less was obtained from S58 (off Chennai).
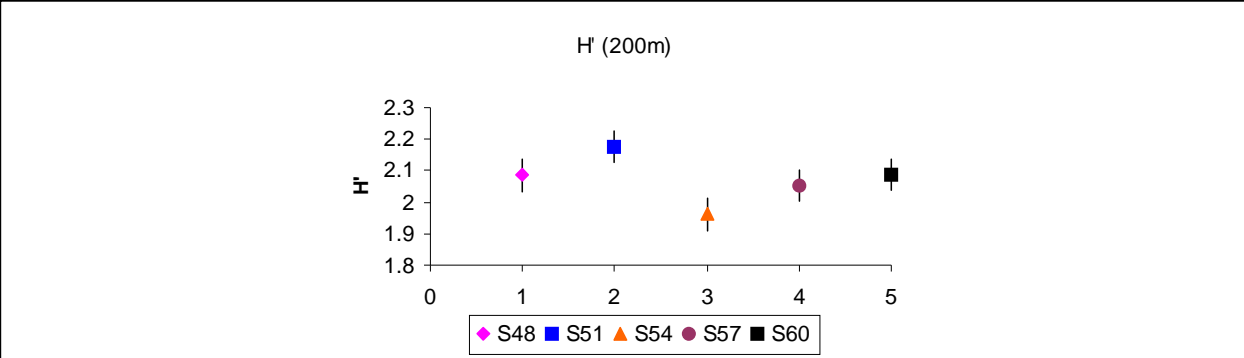
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Figure 24. Means and 95% confidence intervals of Shannon- Wiener (H') index for the 200m depth stations.
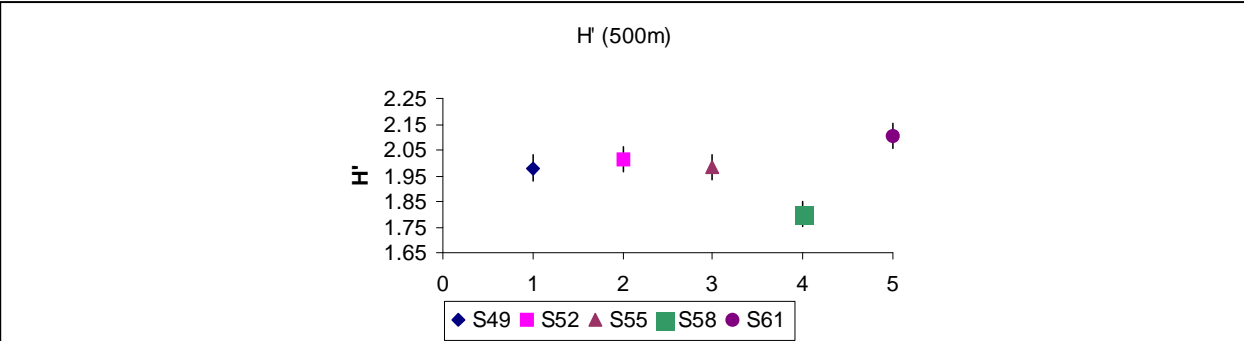

Figure 25. Means and 95% confidence intervals of Shannon- Wiener (H') index for the 500m depth stations.
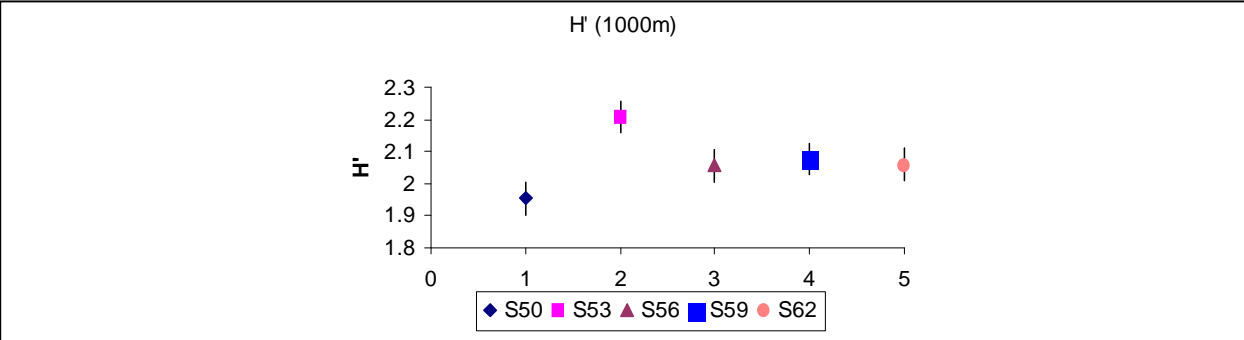

Figure 26. Means and 95% confidence intervals of Shannon-Wiener (H') index for the 1000m depth stations.
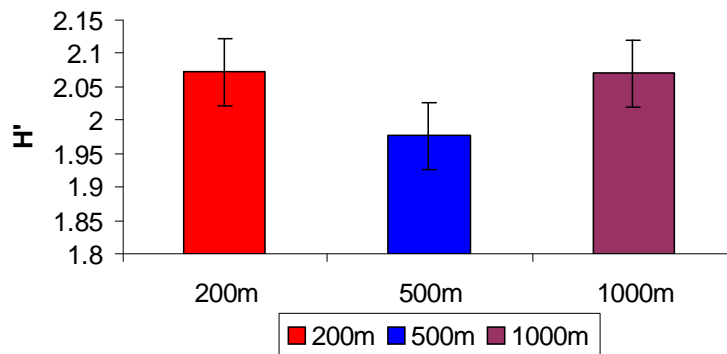
Figure 27. Cumulative Shannon- Wiener (H') index for the three depth stations.

C) Species evenness index: The evenness index is also an important component of the diversity indices. This expresses how evenly the individuals are distributed among the different species. Pielou's Evenness (Pielou 1966) is commonly expressed by *J'*. Maria & Sridhar (2002), Ananda & Sridhar (2004) used this in the diversity estimation of filamentous marine fungi and Ravenschlag *et al.* (1999) and Torsvik *et al.* (1990) used it in a bacterial diversity study. The evenness measure (J') in the present study (Figs. 28-31) largely followed the trend observed in the Shannon diversity index - the species in the 500m depth stations were less evenly distributed than at the 200m and 1000m depth stations. The overall diversity indices and their comparative analysis are outlined in Fig. 32.

$$\textbf{Pielou's evenness}, \text{J'} = \frac{H'}{H'\max}$$

Here H'= Shannon-Wiener diversity index
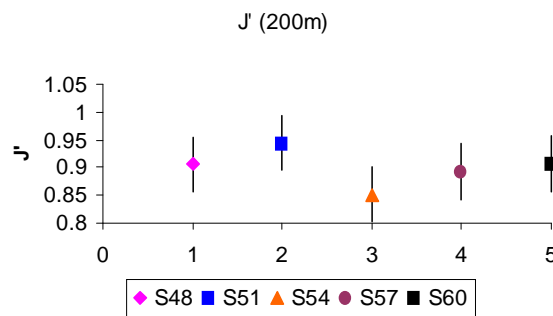H'_{max} = the maximum value of diversity for the number of species present (Pielou 1975).

Figure 28. Means and 95% confidence intervals of Pielou's evenness values (J') for the 200m depth stations.

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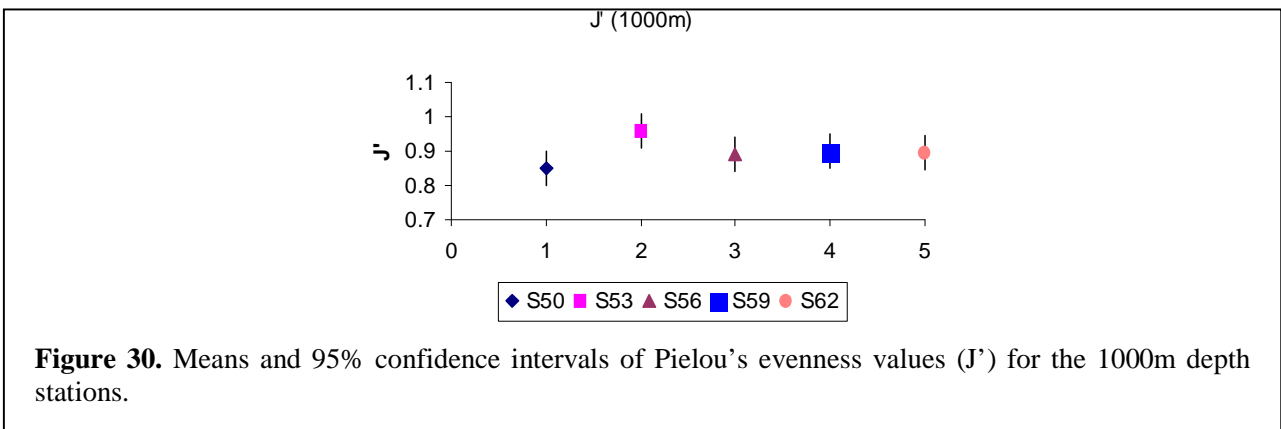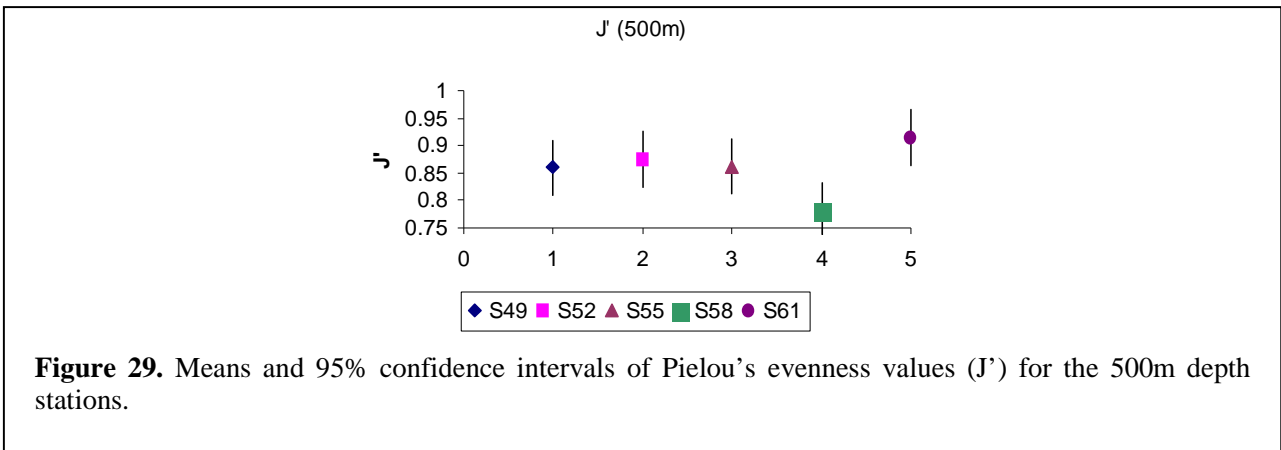**Figure 29.** Means and 95% confidence intervals of Pielou's evenness values (J') for the 500m depth stations.

Figure 30. Means and 95% confidence intervals of Pielou's evenness values (J') for the 1000m depth stations.

Figure 31. Cumulative Pielou's evenness index (J') for the three depth stations.

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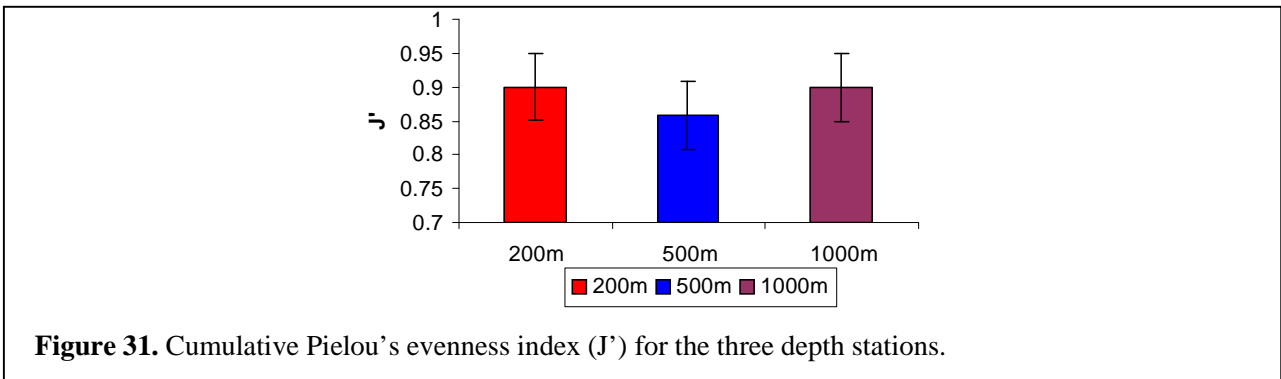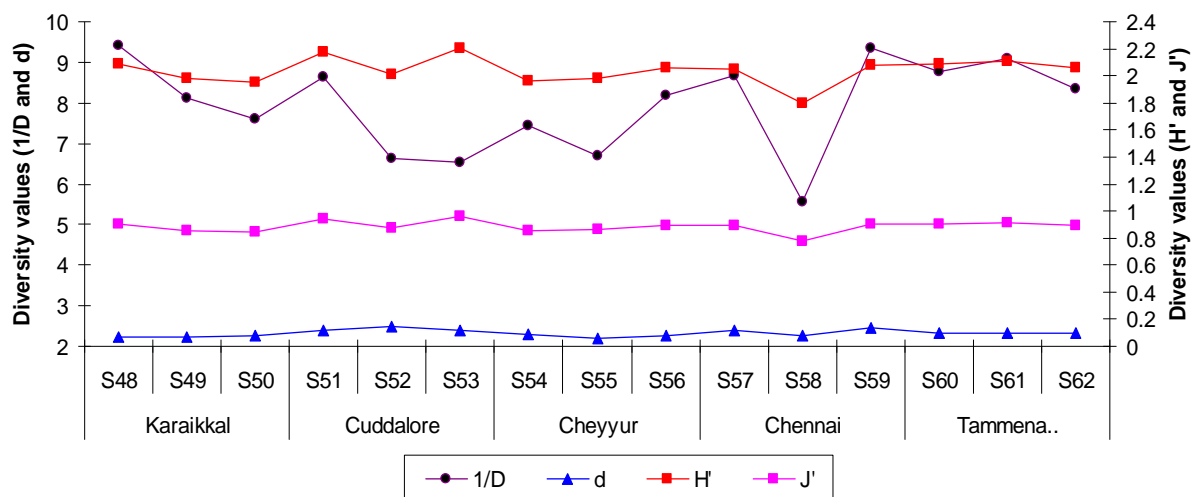
Figure 32. Comparative diversity indices for all the stations.

D) Rank abundance (Graphical representation): There are two commonly used methods for graphically presenting distribution of individuals among species (Clarke 1990):

i) log-normal distribution for species abundance classes against number of species and
ii) K- dominance curves (Lambshead *et al.* 1983), which rank the species in decreasing order of abundance (Figs. 33-38).

Figure 33. K- dominance curves for the three stations off Karaikkal.

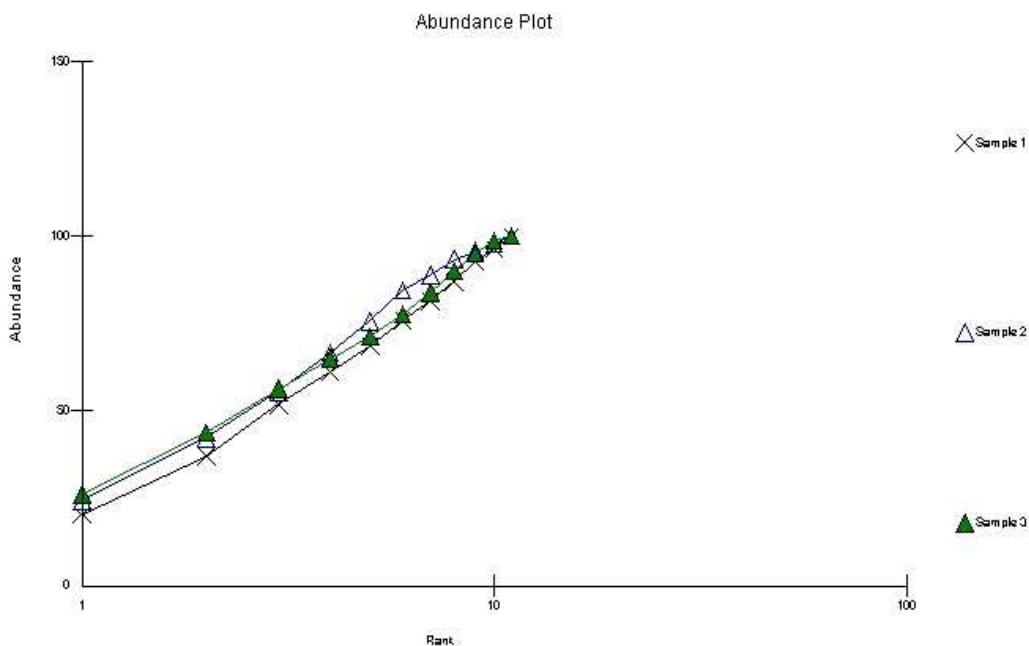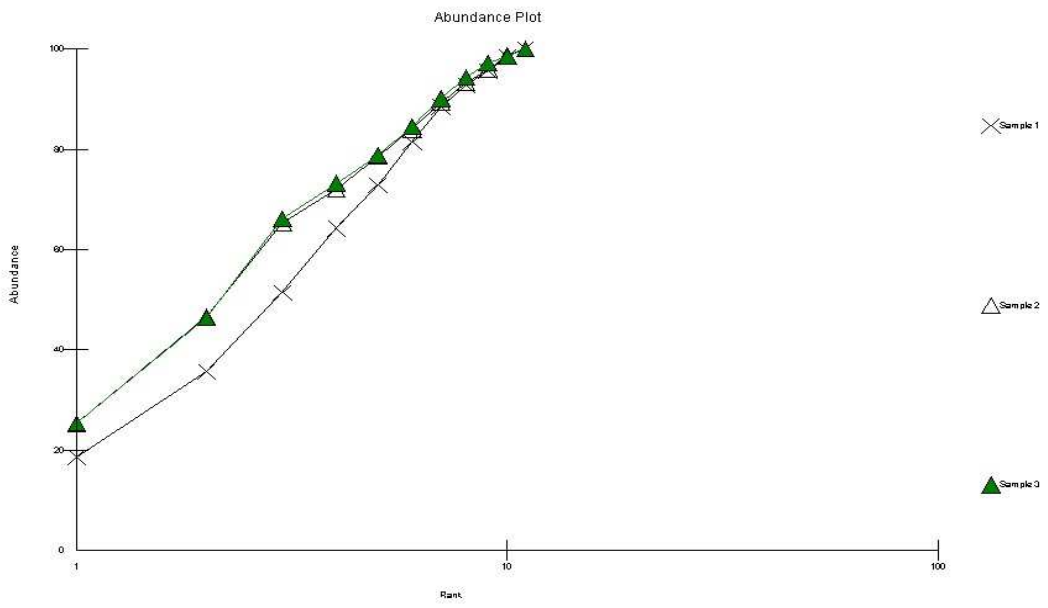**Figure 34.** K- dominance curves for the three stations off Cuddalore.
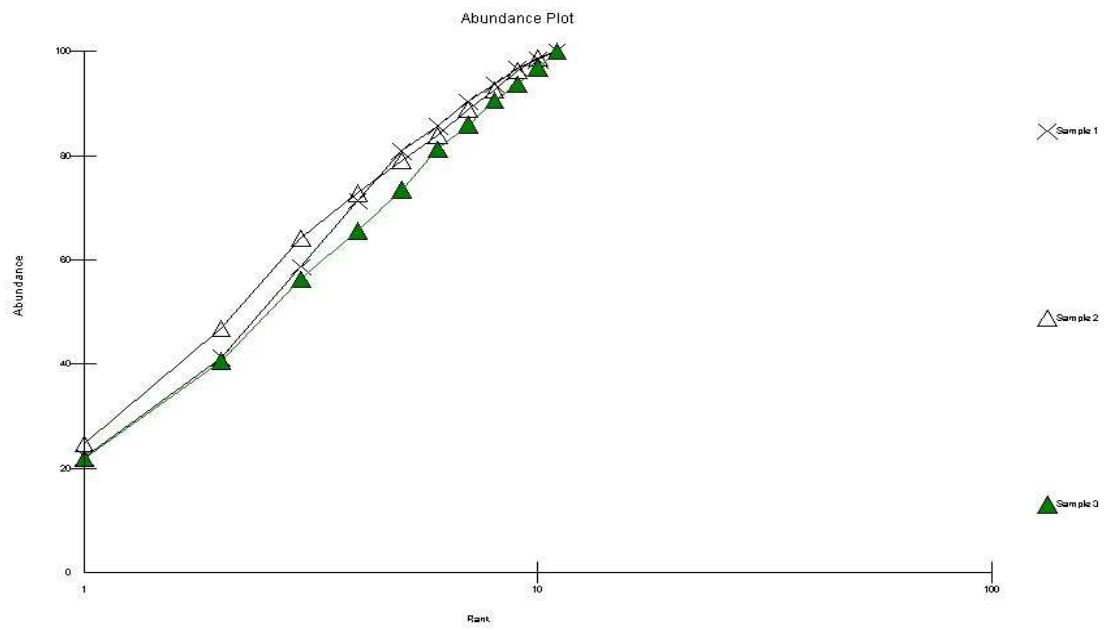

Figure 35. K- dominance curves for the three stations off Cheyyur.
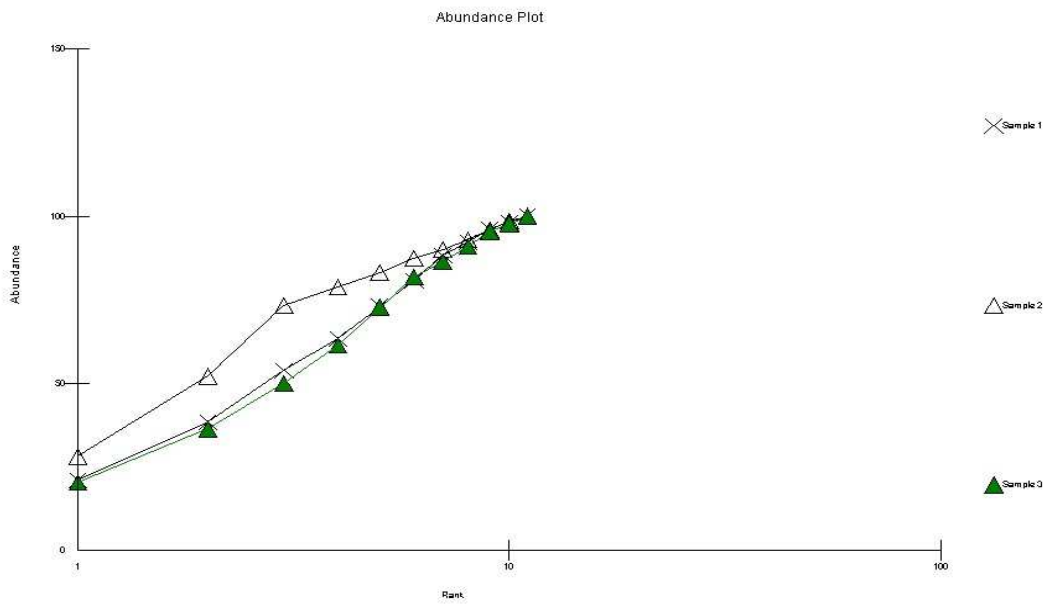
21

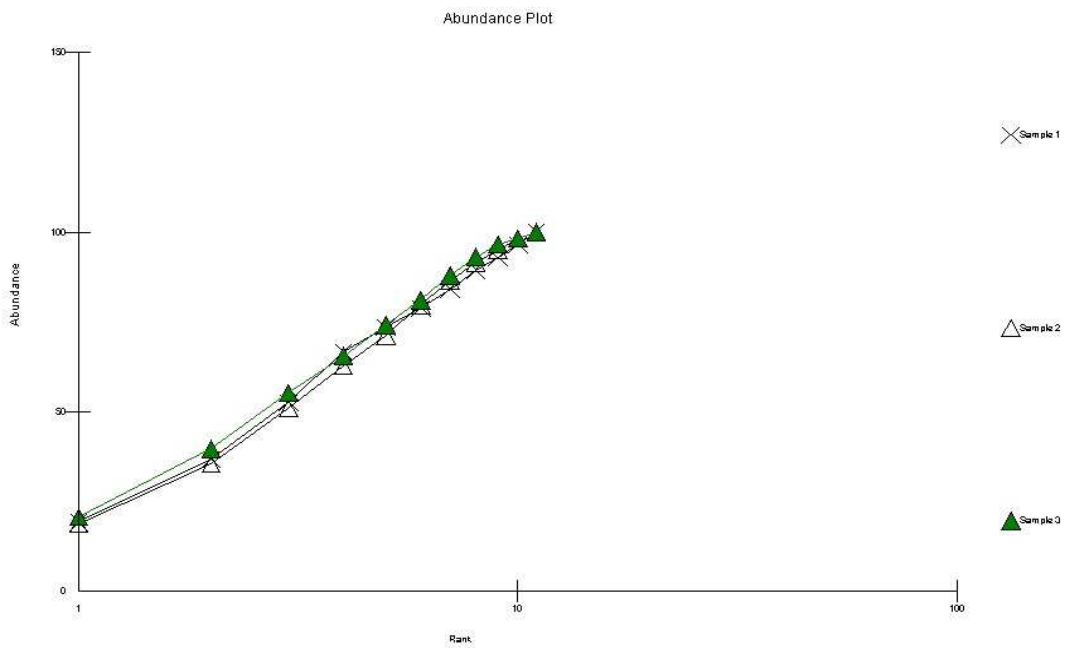**Figure 36.** K- dominance curves for the three stations off Chennai.

Figure 37. K- dominance curves for the three stations off Tammenapatanam.

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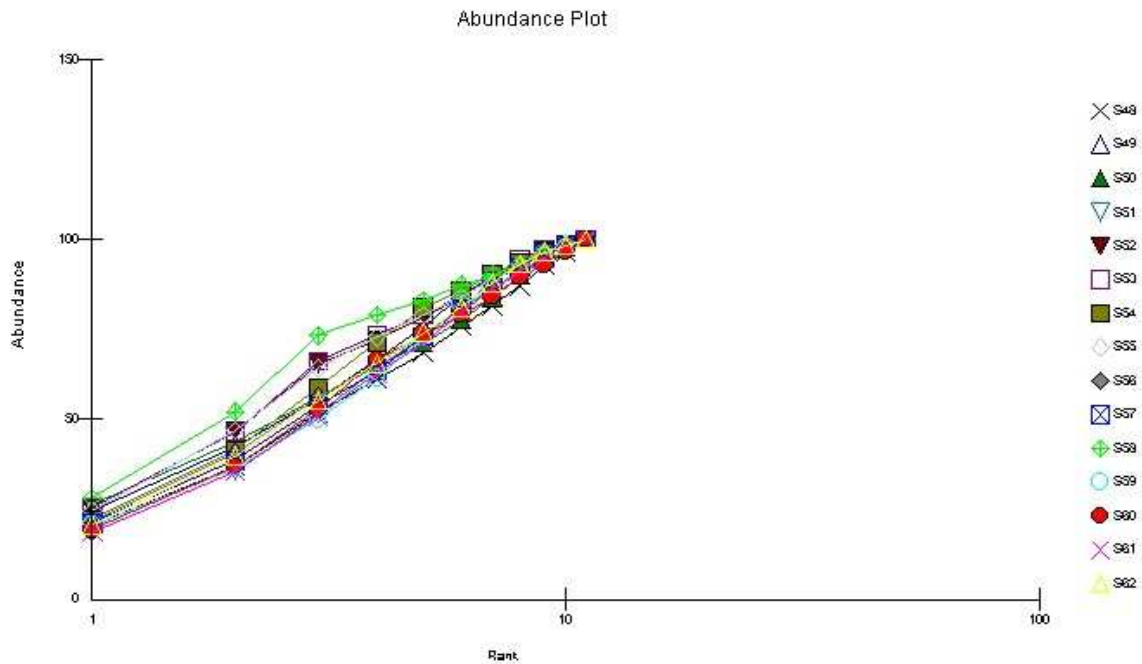
Figure 38. K-dominance curves for all the stations off all the transects.

E) Cluster analysis: Cluster analysis was done to assess the similarities between stations. The most commonly used clustering technique is the hierarchical agglomerative method. The results of this are represented by a tree diagram or dendrogram with the x- axis representing the full set of samples and the y-axis defining the similarity level at which the samples are groups or fused (Fig. 39). The Bray-Curtis coefficient (Bray & Curtis 1957) was used to produce the dendrogram (Biodiversity Professional V.3). This method classifies objects judged to be similar according to distance or similarity measures. Bray-Curtis similarity (single link) using Group-Average clustering appears to give a useful hierarchy of clusters. With the agreement of diversity index, S58 (off Chennai) also showed more abundant species. Bray-Curtis similarity coefficient has been shown to accurately reflect true similarity. The hierarchy of the dendrogram is determined by group average fusion. The dendrogram derived here did not show major difference in clustering the stations. The highest level similarity was found in S53 and S55. As S58 was more diverse, it was clustered separately. Different diversity values from the different stations are tabulated and presented in Table 2.

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Figure 39. Bray-Curtis similarity (Cluster analysis) for all the stations.

Table 2. Diversity values from the different stations.

Transects	St.No.	S	N	d	J'	H'	D	1/D
	S48	10	57	2.226	0.9057	2.085	0.106	9.414
Karaikkal	S49	10	56	2.236	0.8597	1.980	0.123	8.115
	S50	10	53	2.267	0.8480	1.953	0.132	7.596
	S51	10	43	2.393	0.9446	2.175	0.116	8.625
Cuddalore	S52	10	37	2.492	0.8752	2.015	0.151	6.623
	S53	10	43	2.393	0.9587	2.208	0.153	6.522
	S54	10	52	2.278	0.8515	1.961	0.135	7.426
Cheyyur	S55	10	61	2.189	0.8618	1.984	0.149	6.694
	S56	10	54	2.256	0.8931	2.056	0.122	8.195
	S57	10	44	2.378	0.8922	2.054	0.115	8.667
Chennai	S58	10	54	2.256	0.7816	1.800	0.180	5.559
	S59	10	39	2.457	0.9018	2.076	0.107	9.366
	S60	10	48	2.325	0.9065	2.087	0.114	8.769
Tammenapatanam	S61	10	49	2.313	0.9148	2.106	0.110	9.101
	S62	10	47	2.338	0.8944	2.059	0.120	8.348

CONCLUSION

Diversity is a measure of the complexity of the community structure and is increased or decreased by physical, chemical and biological factors. High diversity generally indicates a balanced, stable responsive community. Low diversity occurs in an area where the community is dominated by a few species. Among the large number of indices, it is often difficult to decide which is the best method of measuring diversity. A rather more scientific method of selecting a diversity index is on the basis of whether it fulfils certain criteria -- ability to discriminate between sites, dependence on sample size, what component of diversity is being measured and whether the index is widely used and understood. The relative abundances of the different species at each site were described by univariate methods (Shannon-Wiener index, Margalef index, Simpson index, Pielou's evenness index, Rarefaction curves), but in the graphical technique (K-

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dominance) the relative abundances of different species were plotted as a curve, which showed more information regarding the distribution than a single index. So, both the methods were applied to get the clear picture of the microbial biodiversity of Bay of Bengal (India).

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