Poll Res. 41 (3) : 1062-1075 (2022)

ISSN 0257–8050
DOI No.: http://doi.org/10.53550/PR.2022.v41i03.044

EXPLORING THE BACTERIAL COMMUNITY IN THE SEDIMENTS OF AN ANTHROPICALLY POLLUTED URBAN CREEK LOCATED ON THE WEST COAST OF MUMBAI, INDIA

AASHNA MONGA[1,2], ABHAY B. FULKE[1*], ANIRUDH RAM[1] AND DEBJANI DASGUPTA[2]

[1]CSIR- National Institute of Oceanography (NIO), Regional Centre, Lokhandwala Road, Four Bungalows, Andheri (West), Mumbai 400 053, M.S., India
[2]School of Biotechnology and Bioinformatics, D.Y. Patil University, Navi Mumbai, India

(Received 16 February, 2022; Accepted 10 March, 2022)

ABSTRACT

Microbial communities with their key roles in nutrient cycling and degradation of pollutants are suitable bio-indicators in gauging the health of a marine ecosystem. However, insights into the structure and diversity of bacterial communities in sediments of Versova creek of Mumbai are lacking. Here we assessed the bacterial diversity and composition in sediments collected from two sampling stations for two months in Versova creek. The total sediment DNA was extracted and subjected to high throughput 16S rRNA sequencing technology which resulted in a total of 5467 unique OTUs (Operational Taxonomic Units) from four samples. 16S rRNA sequences revealed the dominance of phyla *Proteobacteria*, *Bacteriodetes*, *Tenericutes*, *Chloroflexi* and *Firmicutes*. Further taxonomic analysis revealed the surface sediments to be abundant in sulfate reducing taxa *Deltaproteobacteria (Desulfobacteraceae, Desulfobulbaceae* and *Desulfarculaceae)*. Organic matter loading and oxygen availability could be one of the prime factors affecting the bacterial community structure in the sediments of Versova creek. Additionally, except for Nickel and Copper, which surpassed the toxic thresholds set by international sediment quality standards, the concentration of most select heavy metals in the creek sediments revealed a moderate level of contamination. This study provides preliminary evidence of pollution in Versova creek due to release of contaminants beyond the assimilative capacity of the ecosystem. It provides a baseline assessment that can be used as a benchmark for future studies and policies that needs to be deployed in restoring the health of this system.

KEY WORDS: Heavy metal pollution, Microbial Diversity in marine sediments, 16S rRNA massively parallel sequencing, QIIME (Quantitative Insights Into Microbial Ecology), Alpha diversity, Beta diversity

INTRODUCTION

Tidal creeks and surrounding marshes support a large biodiversity of aquatic, marine and avian species that sustains complex food webs. Yet their ecological importance is under appreciated, as demonstrated by the lack of studies on such habitats relative to larger, better-known estuarine systems (Mallin and Lewitus, 2004). Versova Creek, located on the west coast of Mumbai, India, is one of the most polluted creeks in Mumbai. Emissions at Versova Creek derive from a variety of sources. It receives both primary and secondary treated wastewaters from the Malad and Versova treatment plants; wastewater releases from many small industrial units, such as steel, rubber, plastic, dye, and other miscellaneous industries, that have appeared in the neighbouring areas over the last decade. As a result of this partially processed and untreated waste being regularly released, the creek's environmental quality and aesthetics have deteriorated (Vijay *et al.,* 2010).

Nutrient cycling and organic matter remineralisation rely heavily on sedimentary

microbial communities (Cesare *et al.,* 2020). Marine sediments are not only hotspots for biogeochemical transformations, but they also act as a long-term reservoir for terrigenous and aquatic contaminants (Chen *et al.,* 2019). The response of microbial groups and sediments to increased organic carbon inputs has been well studied and is determined largely by physio-chemistry of the substrates, dissolved oxygen (DO) levels and microbial community composition in that ecosystem. The use of bacterial communities as bio indicators for aquatic ecosystems is based on the premise that bacterial diversity is a key factor affecting the biological quality of ecosystems due to its role in nutrient recycling, the degradation of pollutants, and the stability of ecosystems (de Paula *et al.,* 2021). Thus, changes in taxonomic diversity and the composition of bacterial communities is a suitable indicator of perturbations within an ecosystem (Liu *et al.,* 2018; Zhang *et al.,* 2019; de Paula *et al.,* 2021). Also because of their short life cycle and genetic organisation, sedimentary microbes can easily adapt and change their genetic makeup, which is preferred by contaminated environments, conferring them ability to survive (Cabral *et al.,* 2016). For instance, to withstand higher levels of heavy metals, bacteria have evolved metal resistance/tolerance mechanisms such as efflux pumps or sequestering heavy metals at intracellular or extracellular levels (Cesare *et al.,* 2020). Thus, improving our understanding of the diversity and dynamics of natural microbial assemblages in polluted ecosystems is crucial to suggest andoptimise bio stimulation approaches and, as a result, future bioremediation processes (Dell'Anno *et al.,* 2021).

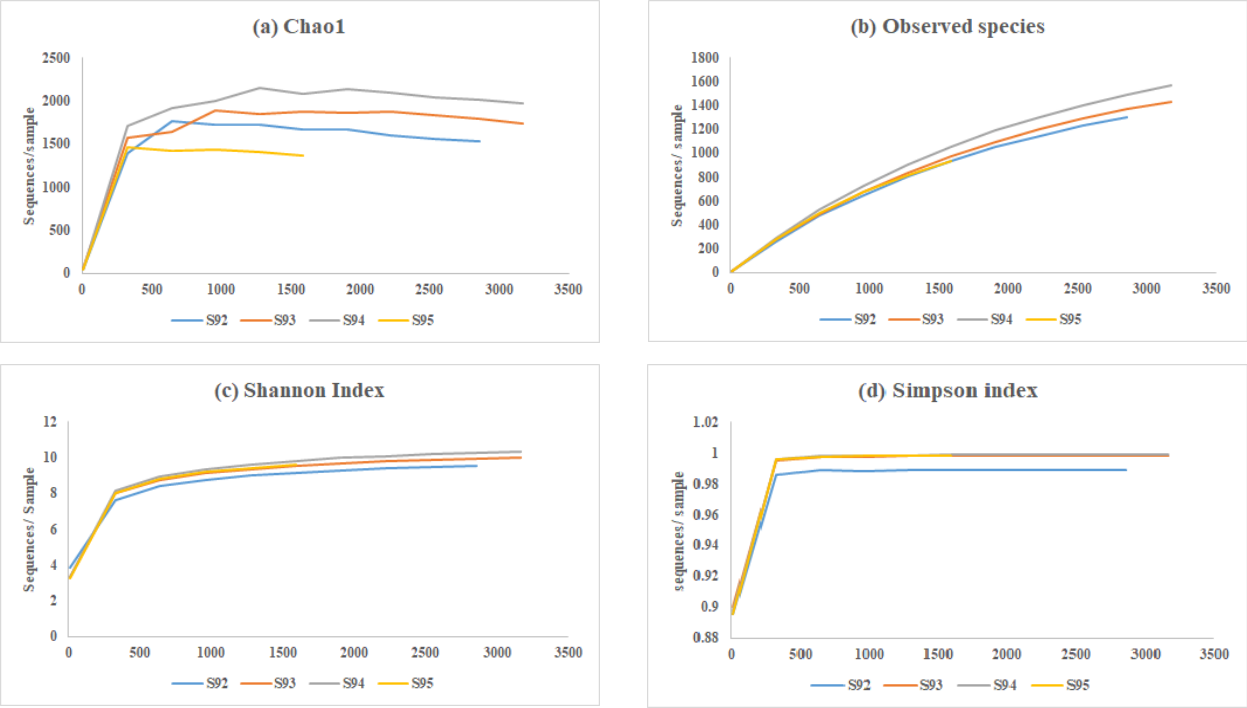
Multiple studies in microbial ecology have established numerous microbial groups with no axenic culture in a variety of habitats (uncultivable). But with the use of culture-independent approaches such as High-throughput sequencing technologies (Hiraoka *et al.,* 2016), it's now possible to detect "undiscovered" or "hidden" microbial biodiversity, extremophile presence, complex relationships between microbes and human pathogens, and nutrient cycling processes in symbiosis (Won *et al.,* 2017; Hiraoka *et al.,* 2016; Grossart *et al.,* 2020; de Paula *et al.,* 2021). The use of NGS techniques for phylogenetically informative marker genes like 16S ribosomal RNA (rRNA) has allowed researchers to conduct detailed investigations of the taxonomic diversity, and structure of marine microbial communities, suggesting that "marine metagenomics" is a novel and reliable tool for assessing and monitoring the health status of marine microbial communities (Won *et al.,* 2017; Hiraoka *et al.,* 2016; Sharma *et al.,* 2020).

The primary aim of this study was to identify the diversity and composition of sediment bacterial communities in the Versova creek using 16S rRNA sequencing technology. For two months (August and October, 2017), we compared and evaluated community structures in the upper creek area, which received wastewater discharges from various point (WWTPs) and non-point sources, vs. the creek mouth, which is further away and receives the highest influx of seawater. This is a first systematic study surveying the dominant bacterial groups present under the erratic hypoxic/anoxic conditions to determine if oxygen availability is affecting the community structure and function.

MATERIALS AND METHODS

Study Area and Sample collection

Versova, our research area, is an important fishing village located on the west coast of Mumbai, India. Besides its socio-economic importance in serving the livelihood to most of the inhabitants of the village and around, we were keen on studying the Versova creek for two reasons: First, the water quality parameters of the creek have been previously monitored and recorded by the Maharashtra Pollution Control Board and this data (available since 2007) provided us with reliable parameters to identify the sampling locations with varied anthropogenic impact. Secondly, despite the fact that researchers have assessed the creek's hydrology, water and sediment quality (Vijay *et al.,* 2010; Vijay *et al.,* 2010), to our knowledge, no comprehensive studies have been undertaken yet to understand the microbial taxonomy, their abundance and community structures that are prevalent in the sediments of this particular anthropogenically impacted urban creek. This study selected the mouth of the creek (Stn. V2); and upper creek (Stn. V3) as sampling locations (Figure 1). The mouth of the creek represents a region with frequent hydrodynamic disturbances due to influx of seawater. The upper creek, on the other hand, represents a region with extreme anoxic environment and reduced salinity levels mostly due to wastewater discharges. Sediment samples (approx. 3 cm deep) were collected in triplicates from the two sampling stations for two months



Supplementray Material Fig. S1: Graphical representation of Alpha diversity indices.
Plots for alpha diversity indices across the sediment microbial communities showing levels of phylogenetic diversity in the four analysed samples, as illustrated by: (a) Chao1 diversity index, (b) Observed Species, (c) Shannon Index and (d) Simpson Index. The colour legend on the bottom centre depicts the respective samples.

(August and October 2017) using a Van Veen grab sampler. After collection, samples were placed in pre-sterilized bottles, stored in a portable ice box and transferred to a laboratory within 12 h.

Analysis of the water and sediment quality parameters and heavy metal content analysis

The sediment samples were analysed for their texture and total organic carbon (TOC) in the laboratory using standard methods. The bottommost layer of the overlying water over the sediment were measured for pH, temperature (T), dissolved oxygen (DO), biological oxygen demand (BOD), phosphate (PO_4^{3-}P), nitrate (NO_3^-N), nitrite (NO_2^-N) and ammonium (NH_4^+-N) using standard protocols. The select heavy metal content (Cu, Cr, Pb, Ni, Zn, Mn) in the sediment samples were calculated using USEPA Method 6020 (1994). Sediment samples were dried in electric oven and then powdered using a mortar pestle. For metal analysis, about 0.2 g of sediment was weighed and completely digested using acid cleaned Teflon beaker using 10 ml of Hydroflouric acid, 5 ml Perchloric acid, 5 ml Hydrochloric acid and 5 ml nitric acid on a hot plate heated to 180 °C. The digested sample was further diluted to 25 ml using Milli-Q water. Cr content was analysed using Inductive Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (DV 7300, Perkin Elmer). All acids employed were of superapur grade (Merck, Germany). The quality control of metal analysis in this work was evaluated using a reference standard of marine sediment SRM (MESS-4 of NRC, Canada). The certified and analytical values were found to be in good agreement.

eDNA Extraction, Polymerase Chain Reaction (PCR) and Massively Parallel sequencing

Complete genomic DNA was extracted in triplicate and purified from the sediment samples using the Power Soil DNA Isolation Kit (Mo BIO Laboratories, CA, USA) according to the manufacturer's protocol. A Nano Drop spectrophotometer was used to quantify the isolated DNA product. Post the concentration measurement, the integrity of the DNA isolation was confirmed by 0.8% agarose gel electrophoresis at 120V for 20 min. Purified DNA samples were held at -20°C. The DNA was then commissioned to *Bencos Research Solutions Pvt. Ltd*. Using unique primers, they amplified the region of approximately 469bp surrounding the V3-V4 hypervariable of the 16S rRNA gene was targeted

for bacterial biodiversity screening. Specific barcodes were applied to each of the PCR products before purification with the Invitrogen Pure Connection PCR purification kit for NGS library preparation. The Agilent Bioanalyzer was used to validate the libraries that had been prepared. Following that, the libraries were sequenced using a technique known as massively parallel sequencing using Illumina Miseq V3 600 cycles chemistry.

Optimization of Sequence Data and OTU Clustering and Taxonomy Assignment

The quality control parameters used to enrich the FASTQ files produced after the run were: (1) removal of reads with ambiguous nucleotides, (2) removal of reads 150bp, (3) removal of reads containing homo-polymers of >6bp. Trim-galore was used to de-replicate, consistency filter, and trim the raw sequences. PANDAseq was used to join the paired-end reads. Chimeric sequences, which are common PCR amplification artefacts, were then removed using the USEARCH process. Quantitative Insights into Microbial Ecology (QIIME v.1), a software application that analyses microbial community sequence data, was used to analyse the final reads (Kuczynski *et al.*, 2012). With UCLUST algorithm, sequences were deionized, clustered at 97% identification, and then assigned to operational taxonomic units based on their barcodes (OTUs). Taxonomy was assigned to representative sequences at 90% sequence similarity. This was done using against GreenGenes database using UCLUST algorithm. After the annotated OTUs were combined and translated to relative abundances and taxonomy, Chao1 (Chao, 1984), Observed Species, Shannon and Simpson Index were used to calculate alpha diversity. Beta diversity was calculated using Weighted UniFrac distances (Navas-Molina, 2013).

RESULTS

Environmental parameters and Heavy Metals Content

The sediment bed of Versova creek region is mainly composed of clayey silt. The results for the sediment and water quality parameters are presented in Supplementary Data Table S1. The temperature of the overlying water varied marginally between the samples. The pH was in the range of 6.81 to 7.10 with invariably low pH in the upper creek samples (S93 and S95) mostly due to sewage load. Salinity levels were invariably lower in the upper creek. The nutrients (listed in Table S1) were overall high in the samples especially during dry season. Perpetually low DO levels were recorded in the samples with values falling to zero at most instances along with high BOD (Biochemical Oxygen demand) levels. Sediments recorded high TOC (Total Organic

Supplementary Table 1. Physico-chemical parameters of the overlying water.
Physico-chemical analysis and Nutrient levels of the overlying water for all four analysed samples: Water Temperature (ºC); pH; DO (Dissolved Oxygen); BOD (Biological Oxygen Demand); Salinity; Phosphate; Nitrate; Nitrite and Ammonium. Measurement units are mentioned in the table.

Sampling station/	Tide	WT (°C)	pH	DO (ml/l)	BOD (mg/l)	Salinity (pmol/mol)	PO_4^{3-}-P (µmol/l)	NO_3^--N (µmol/l)	NO_2^--N (µmol/l)	NH_4^+-N (µmol/l)
V2 (August) S92	**HT**	27.4	7.9	5.76	9.5± 0.0	18.13± 0.05	9.80± 0.04	21.80± 0.12	14.10± 0.01	56.62± 0.3
	LT	26.1	7.4	2.88	12.67± 0.0	10± 0.10	40.57± 0.04	0.15± 0.06	0.09± 0.01	96.75± 0.3
V3 (August) S93	**HT**	26.2	7.8	0.96	25.34± 0.0	13.67± 0.10	37.87± 0.04	24.03± 0.12	6.10± 0.02	75.12± 0.3
	LT	26.4	7.3	0.00	34.31± 0.0	3.91± 0.10	11.03± 0.06	0.25± 0.12	0.03± 0.02	31.62± 0.2
V2 (October) S94	**HT**	26.5	7.56	3.09	12.67± 0.18	36.49± 0.0	36.35± 0.09	0.0	0.2± 0.0	27.43± 1.55
	LT	26.2	7.10	0.00	17.42± 0.0	28.48± 0.10	39.11± 0.07	0.0	0.0	140.21± 1.61
V3 (October) S95	**HT**	27.9	7.0	0.00	38.01± 0.0	24.03± 0.0	36.89± 0.20	0.0	0.0	371.19± 1.86
	LT	26.3	6.81	0.00	47.52± 0.0	19.39± 0.10	31.67± 0.12	0.0	0.0	260.64± 2.08

Carbon) values atypical of marine systems receiving organic wastes.

Despite the fact that determining the heavy metal content is one of the first steps in determining the pollution status of the sediments, the calculated values do not explicitly represent the possible toxicity for the ecosystem's resident microbial population. So, following Cesare *et al.*, 2020's methodology, the data collected were analysed based on previously developed sediment quality guidelines (SQGs). ERL (Effects Range Low) and ERM (Effects Range Medium) are the most widely used thresholds for predicting the impact of metals on biota (Effects Range Median). ERL denotes the lower limit of metal concentrations above which harmful effects on the biota are unlikely to occur, while ERM denotes the upper limit of metal concentrations above which major deleterious effects on the resident biota are predicted (Burton, 2002).

Fig. 2 depicts the select metals studied in this report, along with their corresponding ERL and ERM (provided by the National Oceanic and Atmospheric Administration, NOAA). In all four samples, Ni concentrations surpassed the ERM values, with nearly 2-fold increases in S93 and S94, indicating a significant anthropogenic presence in the sediments due to Nickel (Fig. 2). Furthermore, Cu levels exceeded the ERM in one sample (S94), while Zn and Mn levels were significantly higher than the ERL values in all samples, indicating a moderate pollution due to these metals.

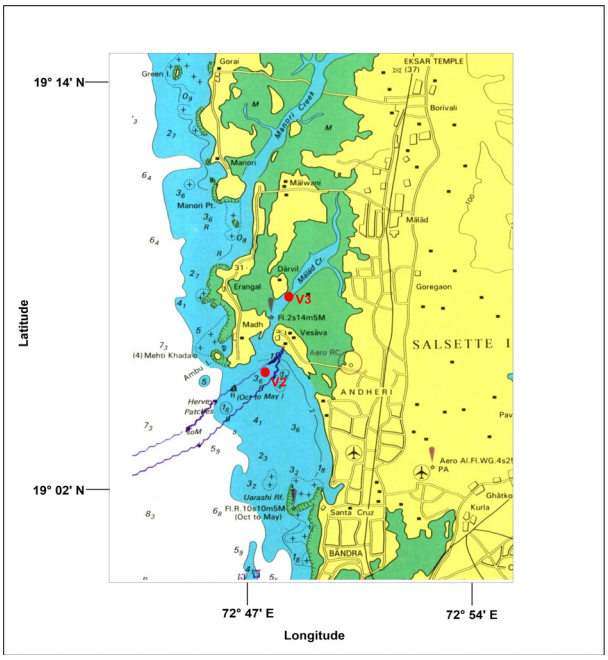


Fig. 1. Sampling Information.
Geographical map of Versova Creek, Mumbai shows the two sampling regions along the transect of the creek, V2- creek mouth and V3- upper creek. Samples were collected from the depth of 0-3 cm twice in 2017 (August and October 2017). Refer to Table 1 for sample ID's and GPS coordinates of the sampling locations.

Taxonomic richness (OTUs abundance) in the upper creek vs. creek mouth

Although the difference was only 1.2 times, the total number of OTUs for the sediment microbial communities present at the creek's mouth (1494) was higher than the upper creek communities (1240) (Table 1). The similarity in the taxonomic composition of the bacterial communities among the samples was also examined using Venn diagram (Fig. 3). We discovered that 33 OTUs were shared by all of the conditions S92 to S95, 139 were shared by S94 and S95, 180 were shared by S92 and S93, and 642 and 595 were exclusive to S92 and S93,

Table 1. Master Table.
Information on sample ID's (S92, S93, S94, S95); sampling months (August and October 2017); GPS coordinates of the sampling locations in Versova creek; Number of quality reads generated from 16S rRNA high throughput sequencing; average values for alpha diversity indices (Chao1, Observed_species, Shannon and Simpson index).

Sample ID	Sample station/ Month	GPS coordinates	No. of quality reads	No. of OTUs per sample	Chao1	Obs_ species	Shannon Index	Simpson Index
S92	V2 August	N 19.137792; E 72.800394	25,011	1315	1527	1296	9.5	0.989
S93	V3 August	N 19.148087; E 72.803977	24,084	1467	1733	1432	10.0	0.998
S94	V2 December	N 19.137839; E 72.800320	23,974	1672	1976	1566	10.2	0.999
S95	V3 December	N 19.148451; E 72.804363	23,043	1013	1372	937	9.6	0.998

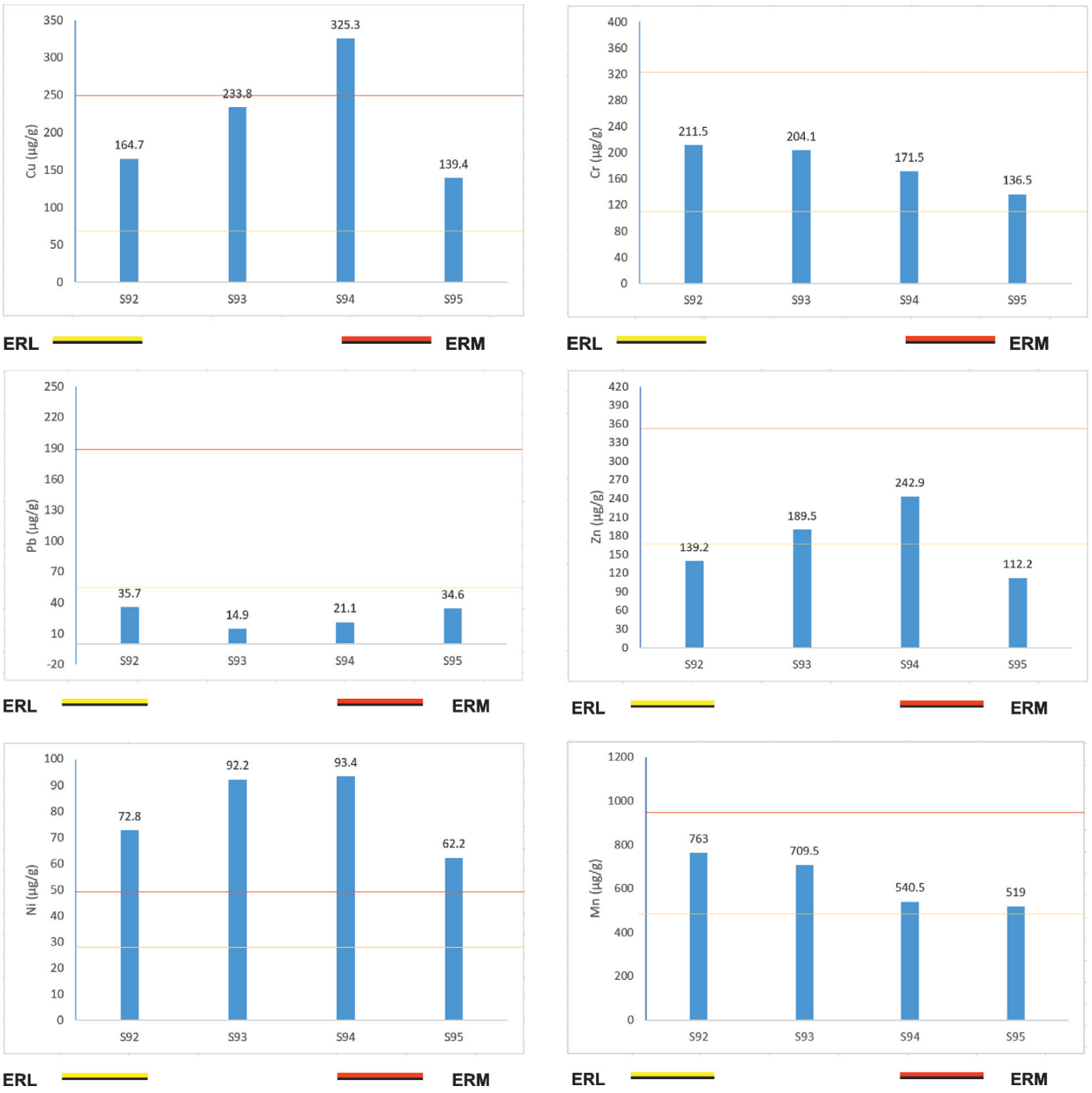


Fig. 2. Metal pollution analysis.
An illustration of metal content (Cu, Cr, Pb, Ni, Zn, Mn) across all collected sediment samples. The yellow line and red line depict the ERL (Effects-Range-Low) and ERM (Effects-Range-Median) concentration for each element respectively, as defined by NOAA Sediment Quality guidelines (Burton, 2002).

respectively. The largest number of OTUs and unique OTUs were identified in Sample S94.

Community compositionand relative abundance

Using 16S rRNA high throughput technology, the microbial communities in the sediment samples were profiled. A total of 5467 unique OTUs were classified based on a 97% sequence similarity. With the aid of the GreenGene Database, they were classified into taxonomic labels. Table 1 shows the number of OTUs that were generated from the samples. A total of 50 phyla were discovered, the majority of which were found in all samples. The detected OTUs at the phylum, and higher order levels (class, order/family and genus) were plotted graphically to visualise the abundance of microbial communities residing in our samples (Fig. 4a,b,c,and d) respectively. Our results from the sediments for

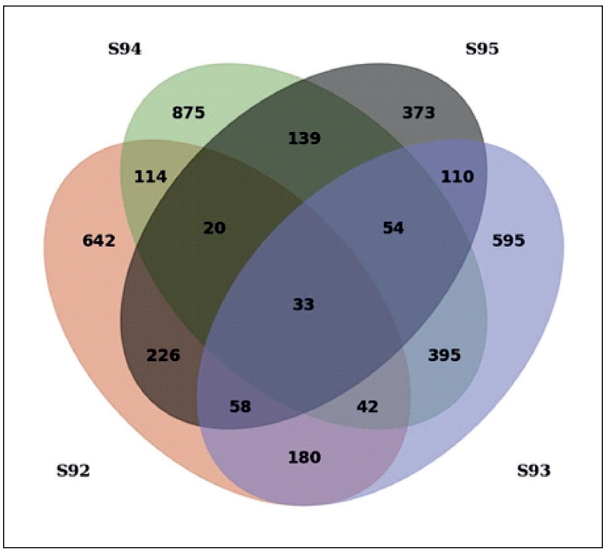


Fig. 3. Four-way Venn diagram.
A four-way Venn diagram depicting the number of shared and unique OTUs in microbial communities of the sediments of Versova creek. 33 OTUs were shared by all of the conditions S92 to S95, 139 were shared by S94 and S95, 180 were shared by S92 and S93, and 642 and 595 were exclusive to S92 and S93, respectively. The largest number of OTUs and unique OTUs were identified in Sample S94.

the August 2017 samples showed that sample S92 contained the group abundance in the order of the phyla. Proteobacteria (63%) are followed by Bacteroidetes (8%), Chloroflexi (5%), Caldithrix (2%), Spirochaetes (1.2%), Planctomycetes (1%), and Tenericutes (1%). While the composition found in sample site S93 was in the order Proteobacteria (45%), Bacteroidetes (18.3%), Tenericutes (10%), Chloroflexi (5%) and Firmicutes (3.5%) (Fig. 4a).At the sub-phylum level, microorganism population analysis for the S92 sample revealed a predominance of *Deltaproteobacteria* (*Desulfobacterales* and *Desulfarculales;* 15.2%), followed by *Epsilonproteobacteria* (*Camylobacterales*; 21.6%), *Gammaproteobacteria* (*Thiotrichales* and others; 20.6%), and *Alphaproteobacteria* (*Rhodobacterales* and others; 5.4%). In contrast, similar findings were observed in sample S93 for the group Proteobacteria, with the exception of *Gammaproteobacteria* (4.3%), which had a much lower abundance than S92 (Fig. 4b, c). In contrast to S92, however, there was a significant increase in the relative abundance of *Bacteroidia* (13.9%), *Mollicutes* (10.0%), and *Anaerolineae* (4.3%). *Desulfobacteraceae* (*Desulfococcus* and others; 2.7%) prevailed in S92, while *Helicobacteraceae* (*Sulfurimonas*; 13.1%) (Fig. 4c, d) made up most of the *Campylobacterales*. In addition, the majority of *Thiotrichales* in S92 were classified as *Piscirickettsiaceae* (2.7%) and *Thiotrichaeae* (3.3%). A similar pattern was observed among the bacterial groups at the family/genus sublevels in sample S93.

Tenericutes (25.1%), Proteobacteria (19.8%), Bacteroidetes (14.6%), Firmicutes (6.5%) and Chloroflexi (3%) were the most prevalent microbial classes in S94 samples collected in December. Proteobacteria (31.4%), Bacteroidetes (7.5%), Chloroflexi (6%), Tenericutes (3%), Cyanobacteria (2.7%), and Firmicutes (2.4%) were the most common classes in sample S95 (Fig. 4a). At the subphylum stage, *Mollicutes* (*Acholeplasmatales* and others; 25.1%) were the most abundant, followed by *Bacteroidia* (13.2%), *Deltaproteobacteria* (7.1%), *Clostridia* (*Clostridiales*; 6.5%), *Gammaproteobacteria* (5.8%), *Campylobacterales* (4.4%), and *Anaeroliniea* (2.7%) (Fig. 4b, c). In the upper creek sample (S95), *Deltaproteobacteria* (15.3%) was the most widespread subphylum, followed by *Gammaproteobacteria* (9.7%), *Bacteroidia* (6.2%), *Epsilonproteobacteria* (4.9%), *Mollicutes* (3%), *Clostridia* (2.4%), and *Anaerolineae* (2.3%). In addition, the *Epsilonproteobacteria* in sample S94 is dominated by *Campylobacteraceae* (*Arcobacter*; 4.4%) and *Helicobacteraceae* (*Sulfurimonas* and others; 4.4%) (Fig. 4c, d). The *Desulfococcus, Desulfobacter*, and others; 2.9% also dominated the community. Furthermore, *Alteromonadaceae* (*Alteromonadales*; 2.2%), *Oleiphilaceae* (*Oceanospirillales*; 2.4%), and *Piscirickettsiaceae* (0.6%) dominated the *Gammaproteobacteria* group (Fig. 4c, d).

Alpha and Beta Diversity Characteristics

We measured both alpha and beta diversity using various indices to estimate group diversity for each sample and differences in composition among them. The reported values are included in Table 1 as well. The four sediment samples did not vary significantly in terms of alpha diversity parameters (OTU richness and eveness). Sample S94 had the highest OTU richness and eveness in the sample composition, while S95 had the lowest richness and S92 had the lowest eveness. The results of the various alpha diversity estimators were plotted against the number of sequences per sample as rarefaction curves, with the line length representing OTU richness. The Chao1 plots for all four samples had a similar steep initial slope, suggesting that each library had a single dominant OTU that accounted

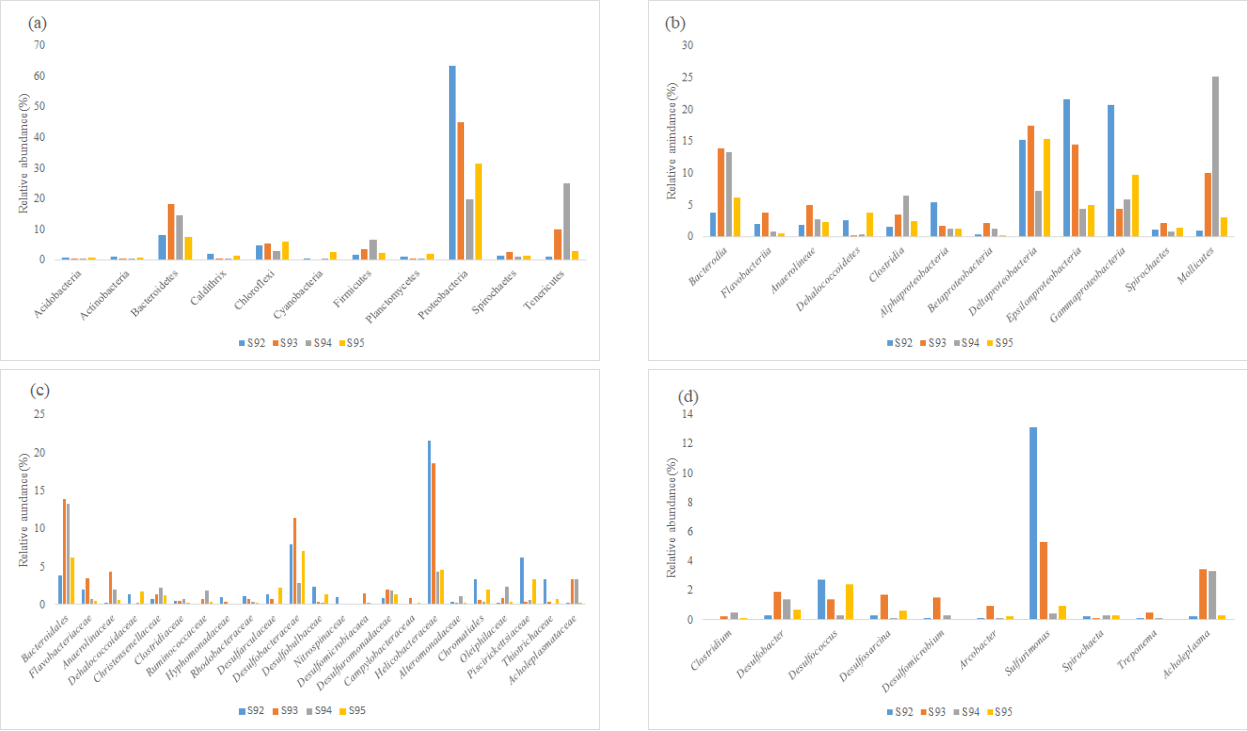


Fig. 4. Taxonomic classification and relative abundance (%) of microbial population.
Graphical representation of taxonomic classification and comparison (% relative abundance) of the bacterial reads of the metagenome in the sediment samples of Versova creek, based on (a) phylum level. (b) class level, (c) higher order/ family, (d) genus level, identified in the study. The legend appears at the bottom centre of each graphical representation

for more than half of the reads. The graphical plots for alpha diversity indices; Chao1, Observed Species, Shannon and Simpson Index are available as Supplementary Material (Fig. S1).

Beta diversity patterns of the sediment communities (among sample differences in OTU composition) were examined by PCA (Principal Component Analysis) ordination (Fig. 5). S93 and S94 were more similar to one another as explained by PC2 (31.96%) and PC3 (29.04%). S95 was separated from S93 and S94 with PC1 explaining 39% of the variance observed. S92 on the other hand was the least related to the rest of the samples.

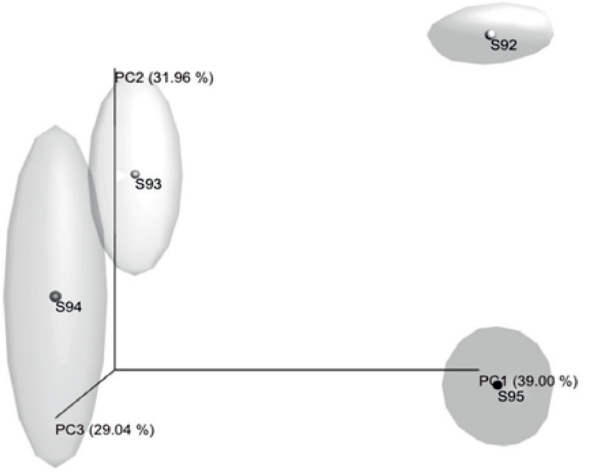


Fig. 5. Principal Component Analysis.
Diagram to illustrate the beta diversity patterns of the microbial communities present among the four sediment samples based on Weighted UniFrac distances associated with the segregation of communities along the PCoA axes.

DISCUSSION

This study deals with the culture-independent investigation of the bacterial community composition and diversity present in the sediments of a polluted urban creek, located on the west coast of Mumbai. Interestingly, 11.6% of the sequences obtained did not fit into any taxonomic hierarchy, hence they were grouped as 'others'(uncultured bacteria), which pointed to the yet-to-be-cultured bacteria present in marine sediments. The metal content in the sediments and basic physico-chemical

parameters of the overlying water were also assessed. The average DO levels were invariably low in the creek falling to zero during low tides with high TOClevels in the surface sediments. Null values of DO, salinity fluctuations and high pollution levels in the creek have been reported previously (Singh *et al.*, 2002). Overall, the concentrations of most of the heavy metals found in our samples revealed moderate levels of contamination except Ni, whose concentrations exceeded the ERM thresholds in all the samples tested, implying a significant anthropogenic effect in the sediments due to Ni. Sediment Ni levels have been linked to a variety of human activities, including mining, smelting, manufacturing, sewage sludge, and wastewater outfalls. Because of its toxicity, persistence, and bioaccumulation, WHO has listed metallic Ni as Group 1 (possible human carcinogen) (Eisler, 2008). Despite the fact that there are no high-quality nickel chronic toxicity data for estuarine or marine sediments (Gissi *et al.*, 2016), the presence of nickel in Versova Creek should not be overlooked. In S94, the Cu concentration was also higher than the ERM levels. Cu is typically found in coastal waters near industrialised bays and estuaries, and is one of the most toxic metals to a variety of marine life (Trannum *et al.*, 2004). Babich and Stotzky in 1983 addressed the synergy between Ni and Cu in their toxicity towards autotrophic microorganisms.

The dominance of Proteobacteria, Bacteroidetes, Tenericutes, Chloroflexi, and Firmicutes in our samples were in line from previous findings in the soil microbiota of polluted marine ecosystems (Zhang *et al.*, 2014, Choi *et al.*, 2016; Liu *et al.*, 2018). Bacterial diversity analysis in the metagenome of Arabian sea also identified the dominance of Proteobacteria that play important roles in nutrient cycling (Nair *et al.*, 2017). Based on Bray-Curtis dissimilarity, our samples formed three clusters (Fig. 6) that is shown along with the heatmap showing abundance based heirarchial clustering. S92 and S94 (Cluster I) were most similar with the dominance of bacterial groups *Bacterioidia, Anaerolinieae, Clostridia, Desulfobacterales, Campylobacetrales*, and *Mollicutes*. S95 (Cluster II) on the other hand depicted lower abundance of groups *Bacteriodia and Mollicutes* and higher abundance of *Desulfobacterales*. S92 (Cluster

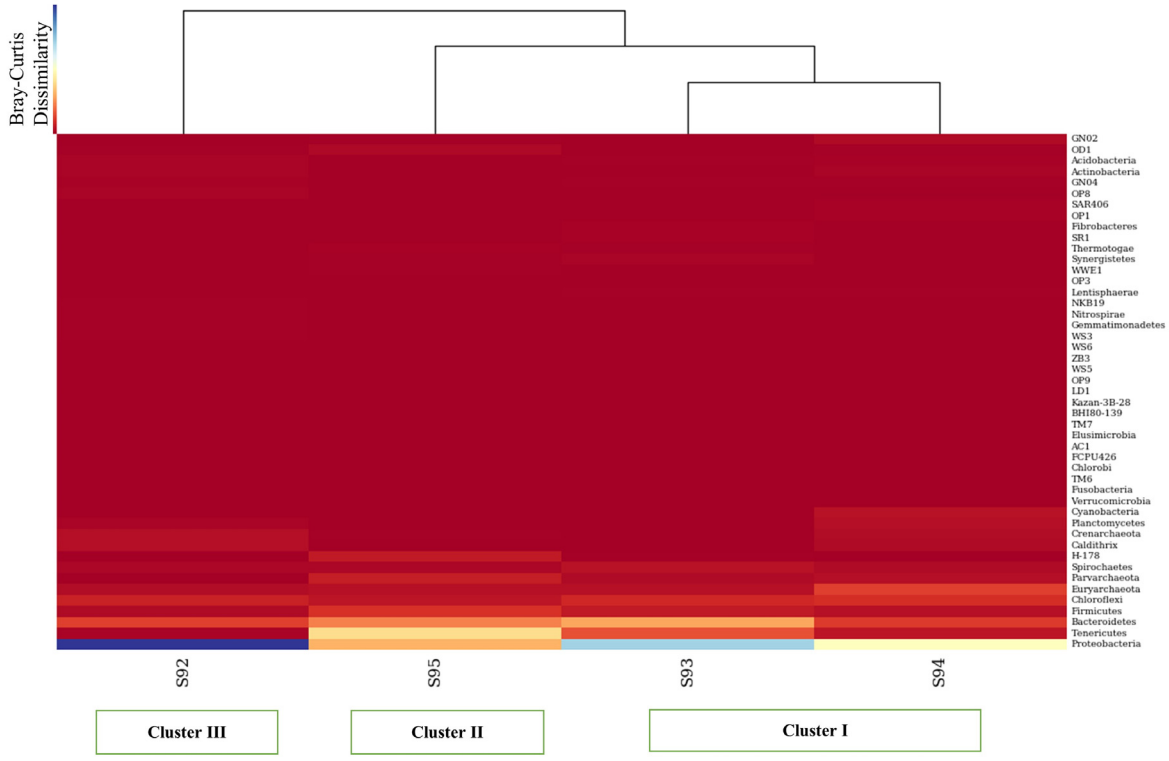


Fig. 6. A hierarchical bi-clustering of the samples.
Bi-clustering of all the samples performed using heirarchial clustering based on the abundances of different species at the level of the phylum. Based on Bray-Curtis Dissimilarity, three clusters formed- Cluster I (S93 and S94), Cluster II (S95) and Cluster III (S92).

III) was most atypical, with striking abundances of *Epsilonbacteria* (*Sulfurimonas)*, *Gammaproteobacteria*, and *Mollicutes*.

In normal marine sedimentary environments, sulfate reduction and aerobic respiration are responsible for most of the organic carbon oxidation processes (Henrichs and Reeburgh, 1987). Aerobic respiration predominantly takes place in the top layer of the sediments while in the layers below in hypoxic or anoxic environments, sulfate reducing bacteria reduce sulfate to sulphide primarily. Notably, in our case in the top 3 cm of the marine conditions seem to be very anoxic and there's quite a few anaerobes. The sulphate-reducing taxa (*Deltaproteo bacteria*) were abundant in the surface sediments of Versova creek (7.3%), where *Desulfobacteraceae* (7.3%), *Desulfobulbaceae* (1.1%) and *Desulfarculaceae* (1.1%) predominated the samples. This could be due to creek sediments receiving oxidizable organic waste in excess of their assimilative capacity. A study in Baltic Sea organic-rich surface sediment layer have reported the predominance of similar bacterial groups and linked that to role of sulfate reducers in organic matter mineralization under hypoxic/anoxic conditions (Sinkko *et al.,* 2011). Changes in oxygen availability in surface sediments could affect microbial community composition in benthic habitats (Nilsson *et al.,* 2000) and with elevated organic matter loading (respiratory oxygen uptake rates exceed the oxygen supply) (Sinkko *et al.,* 2011), anaerobic and sulfate reduction becomes the main metabolic pathway dominating over aerobic respiration. Several bacterial groups representing both oxic and anoxic metabolic processes are involved in sulfur cycling in marine sediments. *Sulfurimonas*, for instance, the one of the most abundant genus identified in our samples, was responsible for most of the chemoautotrophic activity in the Bohai Sea of China where this community and their activity were also positively associated with heavy metal contamination in the area (Lu *et al.,* 2019). Although, only two classes (*Anaerolineae*: 3% and *Dehalococcoidetes*: 1.7%) were identified in the phylum Chloroflexi (4.7%) in all the sediments. Though little is known about this phylum, they are mainly heterotrophic and dominate organic-rich marine sediments (Webster *et al.,* 2011).

Class *Alphaproteobacteria* was the least abundant Proteobacteria class in all of our samples. Though the sequences mainly belonged to *Rhodobacterales* and *Sphingomonadale*s. Various *Rhodobacterales* members are involved in the utilisation of various organic and inorganic compounds, sulphur oxidation, aerobic anoxygenic photosynthesis, carbon monoxide oxidation, and the development of secondary metabolites (Pohlner *et al.,* 2019), while *Sphingomonads* are capable of a wide range of metal and nutrient transformations, implying that they play roles of functional relevance to the maintenance of an healthy ecosystem (Wakelin *et al.,* 2008). Above findings indicate that organi carbon loadingand oxygen availability could be one of the factors that could atleast party drove the bacterial community composition in our samples from Versova creek, a hypothesis that has already been supported by a number of studies (Sinkko *et al,* 2011; Mahmoudi *et al.,* 2015; Chen *et al.,* 2019).

The prevalence of *Gammaproteobacteria, Desulfobacterota, Bacteriodia, and Campylobacterota* related sequences have been identified previously in heavy metal contaminated sediments of Pula bay (North Adriatic Sea) (Cesare *et al.,* 2020). Heavy metal contamination is known as one of the most significant hazards around the world, owing to its toxicity and persistence in the environment (Chen *et al.,* 2019). Microbes, on the other hand, have developed a number of HM-tolerant mechanisms, such as efflux pumps or extracellular or intracellular sequestration of HMs. *Sulfurimonas*, for example, encode for Type II SQRs (Sulfide: Quinone reductase), which play a role in heavy metal tolerance (Han and Perner, 2015). Hpn and Hpn-like proteins were discovered in a *Helicobacter* species that make them resistant to toxic Ni levels and play important roles in Ni detoxification and sequestration, according to another report (Seshadri, 2009). As for our study, culture based studies would be needed to firmly comment upon the microbial heavy metal resistance development in the creek sediments.

The impact of anthropogenic pollution in sediments resulting in reducing the specie eveness was rather evident when all the sample communities were enrichedby a single OTU, *Helicobacteraceae* (*Sulfurimonas*) and *Clostridia* (*Clostridium*). The apparent enrichment of *Helicobacteraceae* was consistent with a general trend for *Epsilonproteobacteria*, which are now being confirmed to be abundant in a wide range of anoxic environments afflicted by heavy metals, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), municipal and industrial wastes (Obi *et al.,* 2016, Quero *et al.,* 2015). *Clostridia*, on the

other hand, is well known to form a substantial part of the human gut microbiota (Lopetuso *et al.*, 2013). Furthermore, in all of the samples, gammaproteo bacteria (*Helicobacteraceaea* : 12%) formed the majority of sequences in all sediment samples in this study and particularly a large proportion in samples from month of August (S92: 21.5% and S93:18.6%). Within this the orders *Thiotrichales, Alteromonadales, Chromatiales, Oceanospirrales, and Methylococcales* were most commonly detected. McCarren et al. (2010) have previously discovered that adding high-molecular-weight (HMW) dissolved organic matter to seawater incubations stimulated many phylogenetic groups within *Alteromondales* and *Thiotrichales*, indicating that these groups may play a role in the breakdown of HMW-organic matter particularly in sediment bed where low oxygen levels are present. *Oceanospirillales*, on the other hand, have been found to breakdown simple aliphatic hydrocarbons aerobically in marine conditions and have been reported to carry genes coding for n-alkane and cycloalkane degradation, according metatranscriptomic analysis (Hazen *et al.*, 2010; Mason *et al.*, 2012).

Piscirickettsiaceae were also found in abundance in samples S92 and S95. While little is known about this genus, it does contain a single species, *Piscirickettsia salmonalis*, an intracellular fish pathogen that affects salmon and other fish species in the marine environment (Fryer and Hedrick, 2003). Members of *Anaerolinea* abundant in our samples are thermo- or mesophilic and are mostly present in activated sludge samples from waste water treatment plants (Sekiguchi *et al.*, 2003). Also present in the samples were various salt-tolerant bacteria such as *Marinobacter* (Li *et al.*, 2013) and Cyanobacteria such as *Stramenopiles* (Hayes and Sliwa, 2003) and *Chroococales* (Tindall *et al.*, 1978), though in small abundance.

These results could indicate that a number of factors are at play in forming the community structures in the contaminated creek's sediments. Despite the limited number of sampling points in our sample, the alpha diversity indices revealed that the upper creek locale had an apparent loss in microbial diversity (richness) in the post-monsoon season (S95). Although no consistent trend in seasonal variations in community structures could be found, sample S94 from station V2 (creek mouth) was unquestionably the most contaminated in terms of heavy metal concentrations. The only notable observation for community level shifts the scope of this analysis was the atypical community composition found in sample S92, with a striking higher relative abundance of *Sulfurimonas, Thiotrichales, Alteromonadales, and Chromatiales* in addition to a dramatic lower abundance of *Mollicutes*. While we did not describe the community structures of the overlying water in this sample, numerous studies have indicated that dispersal limitations between water and sediment populations may be to blame for the diverse community structures and dynamics (Leibold *et al.*, 2004). Since the exchange of particles and nutrients, as well as the movement of water, are only minimised by a thin sediment water interface (SWI) (Austen *et al.*, 2002), this may be a possible hypothesis for future research.

In our knowledge, our study is the first report assessment of sediment microbial communities in Versova creek, which could serve as a platform for future research into major microbial populations and their metabolic roles in the system. The presence and distribution of anaerobes in the top layer of the sediment was worth speculating on as oxygen availability could be driving much of the observed bacterial community structure in the sediments. Eutrophication and consequent deoxygenation of marine waters can impact the oxidation–reduction balance in sediments, as well as linked biogeochemical processes. Due to limited sample numbers we could identify any particular trend or seasonal shifts in the microbial communities along the transect of the creek. Further studies with multiple sampling points along the creek along with RNA or isotopic approaches may help us comment better on the bacterial groups and their roles in driving the nutrient cycling in this ecosystem as well as the fate of contaminants such a metals.

CONCLUSION

Environmental pollution has become a significant problem in Versova Creek due to unregulated discharge of untreated or partially treated sewage and wastewaters. To, our knowledge no systematic studies have been published about the function of even the most dominant sedimentary microbial groups in Versova creek. In our study, we have demonstrated with 16S rRNA high throughput sequencing, the dominance of sulfate reducing taxa indicating excessive organic inputs to be one of the major factors in shaping the communities in the anoxic creek sediments. Thus, in conclusion it would

be reasonable to say that though the sewage that was being treated in aerated lagoons before being released in Versova creek, from our results it was clear that either the volume released is much higher than the creek could assimilate or aerated lagoons are malfunctioning.

ACKNOWLEDGEMENT

Authors are grateful to Director, CSIR-National Institute of Oceanography and Scientist-In-charge, CSIR-National Institute of Oceanography, Regional Centre, Mumbai and Head at Department of Biotechnology and Bioinformatics, D. Y. Patil University for their encouragement and support throughout the period of this study.

REFERENCES

Austen, M.C., Lambshead, P.J.D., Hutchings, P.A., Boucher, G., Snelgrove, P.V., Heip, C., King, G., Koike, I. and Smith, C. 2002. Biodiversity links above and below the marine sediment-water interface that may influence community stability. *Biodivers Conserv.* 11: 113-136.

Babich, H. and Stotzky, G. 1983. Synergism between nickel and copper in their toxicity to microbes: mediation by pH. *Ecotoxicol Environ Saf.* 7: 576-587.

Burton Jr, G. A. 2002. Sediment quality criteria in use around the world. *Limnology.* 3: 65-76.

Cabral, L., Júnior, G.V.L., de Sousa, S.T.P., Dias, A.C.F., Cadete, L.L., Andreote, F.D., Hess, M. and de Oliveira, V.M. 2016. Anthropogenic impact on mangrove sediments triggers differential responses in the heavy metals and antibiotic resistomes of microbial communities. *Environ Pollut.* 216: 460-469.

Chao, A. 1984. Nonparametric estimation of the number of classes in a population. *Scand J Stat.* 265-270.

Chen, J., McIlroy, S.E., Archana, A., Baker, D.M. and Panagiotou, G. 2019. A pollution gradient contributes to the taxonomic, functional, and resistome diversity of microbial communities in marine sediments. *Microbiome.* 7: 1-12.

Choi, H., Koh, H. W., Kim, H., Chae, J. C. and Park, S. J. 2016. Microbial community composition in the marine sediments of Jeju Island: next-generation sequencing surveys. *J Microbiol Biotechnol.* 26(5): 883-890.

Dell'Anno, F., Rastelli, E., Tangherlini, M., Corinaldesi, C., Sansone, C., Brunet, C., Balzano, S., Ianora, A., Musco, L., Montereali, M.R. and Dell'Anno, A. 2021. Highly Contaminated Marine Sediments Can Host Rare Bacterial Taxa Potentially Useful for Bioremediation. *Front Microbiol.* 12: 326.

Di Cesare, A., Pjevac, P., Eckert, E., Curkov, N., Šparica, M.M., Corno, G. and Orliæ, S. 2020. The role of metal contamination in shaping microbial communities in heavily polluted marine sediments. *Environ Pollut.* 265: 114823.

Eisler, R. 1998. Nickel hazards to fish, wildlife, and invertebrates: a synoptic review. US Department of the Interior, US Geological Survey, Patuxent Wildlife Research Center.

Fryer, J.L. and Hedrick, R.P. 2003. *Piscirickettsia salmonis*: a Gramnegative intracellular bacterial pathogen of fish. *J Fish Diseases.* 26: 251-262.

Gissi, F., Stauber, J.L., Binet, M.T., Golding, L.A., Adams, M.S., Schlekat, C.E., Garman, E.R. and Jolley, D.F. 2016. A review of nickel toxicity to marine and estuarine tropical biota with particular reference to the South East Asian and Melanesian region. *Environ Pollut.* 218: 1308-1323.

Grossart, H. P., Massana, R., McMahon, K. D. and Walsh, D. A. 2020. Linking metagenomics to aquatic microbial ecology and biogeochemical cycles. *Limnol Oceanogr.* 65: S2-S20.

Han, Y. and Perner, M. 2015. The globally widespread genus *Sulfurimonas*: versatile energy metabolisms and adaptations to redox clines. *Front Microbiol.* 6: 989.

Hargrave, B. T., Holmer, M. and Newcombe, C. P. 2008. Towards a classification of organic enrichment in marine sediments based on biogeochemical indicators. *Mar Pollut Bull.* 56: 810-824.

Hayes, K. R. and Sliwa, C. 2003. Identifying potential marine pests–a deductive approach applied to Australia. *Mar Pollut Bull.* 46(1): 91-98.

Hazen, T. C., Dubinsky, E. A., DeSantis, T. Z., Andersen, G. L., Piceno, Y. M., Singh, N. and Mason, O. U. 2010. Deep-sea oil plume enriches indigenous oil-degrading bacteria. *Sci.* 330(6001): 204-208.

Henrichs, S. M. and Reeburgh, W. S. 1987. Anaerobic mineralization of marine sediment organic matter: rates and the role of anaerobic processes in the oceanic carbon economy. *Geomicrobiol J.* 5: 191-237.

Hiraoka, S., Yang, C.C. and Iwasaki, W. 2016. Metagenomics and bioinformatics in microbial ecology: current status and beyond. *Microbes Environ.* ME16024.

Kuczynski, J., Stombaugh, J., Walters, W.A., González, A., Caporaso, J.G. and Knight, R. 2012. Using QIIME to analyze 16S rRNA gene sequences from microbial communities. *Curr Protoc Microbiol.* 27: 1E-5.

Leibold, M.A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J.M., Hoopes, M.F., Holt, R.D., Shurin, J.B., Law, R., Tilman, D. and Loreau, M. 2004. The metacommunity concept: a framework for multiscale community ecology. *Ecol Lett.* 7: 601-613.

Li, R., Zi, X., Wang, X., Zhang, X., Gao, H. and Hu, N. 2013. Marinobacter *Hydro carbonoclasticus* NY-4, a novel denitrifying, moderately halophilic marine bacterium. *Springer Plus*. 2: 1-9.

Liu, J., Chen, X., Shu, H.Y., Lin, X.R., Zhou, Q.X., Bramryd, T., Shu, W.S. and Huang, L.N. 2018. Microbial community structure and function in sediments from e-waste contaminated rivers at Guiyu area of China. *Environ Pollut.* 235: 171-179.

Lopetuso, L.R., Scaldaferri, F., Petito, V. and Gasbarrini, A. 2013. Commensal Clostridia: leading players in the maintenance of gut homeostasis. *Gut Pathog.* 5: 1-8.

Lu, M., Luo, X., Jiao, J.J., Li, H., Wang, X., Gao, J., Zhang, X. and Xiao, K. 2019. Nutrients and heavy metals mediate the distribution of microbial community in the marine sediments of the Bohai Sea, China. *Environ Pollut*. 255: 113069.

Mahmoudi, N., Robeson, M. S., II, H. F. C., Fortney, J. L., Techtmann, S. M., Joyner, D. C. and Hazen, T. C. 2015. Microbial community composition and diversity in Caspian Sea sediments. *FEMS Microbiol Ecol.* 91(1): 1.

de Paula, M., Costa Silva, T. A., Araújo, A. S., & Lacorte, G. A. 2021. Assessments of Bacterial Community Shifts in Sediments along the Headwaters of São Francisco River, Brazil. *Conserv*. 1(2): 91-105.

Mallin, M.A. anḍ Lewitus, A.J. 2004. The importance of tidal creek ecosystems. *J Exp Mar Biol Ecol.* 2: 145-149.

Mason, O. U., Hazen, T. C., Borglin, S., Chain, P. S., Dubinsky, E. A., Fortney, J. L. and Jansson, J. K. 2012. Metagenome, metatranscriptome and single-cell sequencing reveal microbial response to Deepwater Horizon oil spill. *The ISME J*. 6(9): 1715-1727.

McCarren, J., Becker, J. W., Repeta, D. J., Shi, Y., Young, C. R., Malmstrom, R. R. and DeLong, E. F. 2010. Microbial community transcriptomes reveal microbes and metabolic pathways associated with dissolved organic matter turnover in the sea. *Proc Nat Acad Sci*. 107(38): 16420-16427.

Nair, H. P., Puthusseri, R. M., Vincent, H. and Bhat, S. G. 2017. 16S rDNA-based bacterial diversity analysis of Arabian Sea sediments: A metagenomic approach. *Ecolo Genet Genom*. 3: 47-51.

Navas-Molina, J.A., Peralta-Sánchez, J.M., González, A., McMurdie, P.J., Vázquez-Baeza, Y., Xu, Z., Ursell, L.K., Lauber, C., Zhou, H., Song, S.J. and Huntley, J. 2013. Advancing our understanding of the human microbiome using QIIME. *Method Enzymol*. 531: 371-444.

Nilsson, H. C. and Rosenberg, R. 2000. Succession in marine benthic habitats and fauna in response to oxygen deficiency: analysed by sediment profile-imaging and by grab samples. *Mar Ecol Prog Ser*. 197: 139-149.

Obi, C.C., Adebusoye, S.A., Ugoji, E.O., Ilori, M.O., Amund, O.O. and Hickey, W.J. 2016. Microbial communities in sediments of Lagos Lagoon, Nigeria: elucidation of community structure and potential impacts of contamination by municipal and industrial wastes. *Front Microbiol*. 7: 1213.

Pohlner, M., Dlugosch, L., Wemheuer, B., Mills, H., Engelen, B. and Reese, B.K. 2019. The majority of active *Rhodobacteraceae* in marine sediments belong to uncultured genera: A molecular approach to link their distribution to environmental conditions. *Front Microbiol*. 10: 659.

Quero, G.M., Cassin, D., Botter, M., Perini, L. and Luna, G.M. 2015. Patterns of benthic bacterial diversity in coastal areas contaminated by heavy metals, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). *Front Microbiol.* 6: 1053.

Sekiguchi, Y., Yamada, T., Hanada, S., Ohashi, A., Harada, H. and Kamagata, Y. 2003. *Anaerolinea thermophila* gen. nov., sp. nov. and *Caldilinea aerophila* gen. nov., sp. nov., novel filamentous thermophiles that represent a previously uncultured lineage of the domain Bacteria at the subphylum level. *Int J Syst Evolut Microbiol*. 53(6): 1843-1851.

Seshadri, S. 2009. *Roles of Nickel Binding Proteins in Helicobacter Species*. PhD Thesis, University of Georgia.

Sharma, N., Kumar, J., Abedin, M.M., Sahoo, D., Pandey, A., Rai, A.K. and Singh, S.P. 2020. Metagenomics revealing molecular profiling of community structure and metabolic pathways in natural hot springs of the Sikkim Himalaya. *BMC Microbiol.* 20: 1-17.

Singh, V. V., Joseph, L. and Sundaram, S. 2002. Environmental conditions off Mumbai with reference to marine fisheries. *Appl Fish Aquac*. 2(2): 49-53.

Sinkko, H., Lukkari, K., Jama, A. S., Sihvonen, L. M., Sivonen, K., Leivuori, M. and Lyra, C. 2011. Phosphorus chemistry and bacterial community composition interact in brackish sediments receiving agricultural discharges. *PLoS One*. 6(6): e21555.

Sushant, M. and Sujit, S. 2014. Studies on some aspects on the biology of green mussel *Perna viridis* (Linnaeus, 1758) from Versova creek, Mumbai, northwest coast of India. *International Research J Sci Eng*. 2: 47-50.

Tindall, D.R., Yopp, J.H., Miller, D.M. and Schmid, W.E. 1978. Physico-chemical parameters governing the growth of *Aphanothece halophytica* (Chroococcales) in hypersaline media. *Phycol.* 17(2): 179-185.

Trannum, H. C., Olsgard, F., Skei, J. M., Indrehus, J., Øverås, S., & Eriksen, J. 2004. Effects of copper, cadmium and contaminated harbour sediments on recolonisation of soft-bottom communities. *J Exp Mar Biol Ecol*, 310(1): 87-114.

USEPA Method 6020. 1994. Environmental protection Agency, Washington, DC.

Vijay, R., Sardar, V. K., Dhage, S. S., Kelkar, P. S. and Gupta, A. 2010. Hydrodynamic assessment of sewage impact on water quality of Malad Creek, Mumbai, India. *Environ Monit Assess.* 165: 559-571.

Vijay, R., Mardikar, T., Kushwaha, V. K., Kamble, S. and Wate, S. R. 2014. Assessment of sewage pollution in Malad creek, Mumnai, using high resolution image analysis India. ISRS Proceeding Papers of Sort Interactive Session ISPRS TC VIII International Symposium on "Operational Remote Sensing Applications: Opportunities, Progress and Challenges", Hyderabad, India, December 9 – 12.

Wakelin, S.A., Colloff, M.J. and Kookana, R.S. 2008. Effect of wastewater treatment plant effluent on microbial function and community structure in the sediment of a freshwater stream with variable seasonal flow. *Appl Environ Microbiol.* 74: 2659-2668.

Webster, G., Sass, H., Cragg, B. A., Gorra, R., Knab, N. J., Green, C. J. and Parkes, R. J. 2011. Enrichment and cultivation of prokaryotes associated with the sulphate-methane transition zone of diffusion-controlled sediments of Aarhus Bay, Denmark, under heterotrophic conditions. *FEMS Microbiol Ecol.* 77(2): 248-263.

Won, N.I., Kim, K.H., Kang, J.H., Park, S.R. and Lee, H.J. 2017. Exploring the impacts of anthropogenic disturbance on seawater and sediment microbial communities in Korean coastal waters using metagenomics analysis. *Int J Environ Res Publ Health.* 14: 130.

Zhang, L., Zhao, T., Wang, Q., Li, L., Shen, T. and Gao, G. 2019. Bacterial community composition in aquatic and sediment samples with spatiotemporal dynamics in large, shallow, eutrophic Lake Chaohu, China. *J Freshw Ecol.* 34(1): 575-589.

Zhang, W., Bougouffa, S., Wang, Y., Lee, O.O., Yang, J., Chan, C., Song, X. and Qian, P.Y. 2014. Toward understanding the dynamics of microbial communities in an estuarine system. *PloS one.* 9: e94449.