Bacterial diversity of Bhitarkanika mangrove ecosystem, Odisha, India

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Abstract

Bhitarkanika estuary is a highly diverse tropical mangrove ecosystem situated along the river delta of Brahmani and Baitarani before meeting the Bay of Bengal. Large number of heterotrophic bacteria present within the sediment and water are involved in different ecological functions and are capable of interfering in nutrient cycling thereby biological productivity. Heterotrophic bacterial diversity with respect to seasonal variation of several ecological parameters was studied from the five transects namely Dangmal, Ekakula, Gupti, Habalikathi and Kalibhanjadiha, within this ecosystem. During monsoon, highest heterotrophic bacterial population was recorded at Dangmal ($39.10\pm3.60\times10^3$ cfu/ml and $43.93\pm5.28\times10^5$ cfu/g) and lowest total heterotrophic bacterial population was recorded from Ekakula ($7.95\pm2.05\times10^3$ cfu/ml and $6.8\pm2.17\times10^5$ cfu/g) from both the water and sediment samples respectively. During summer, highest heterotrophic bacterial population was recorded at Habalikathi sediment sample ($165.58\pm41.76\times10^4$ cfu/g) and Kalibhanjadiha water sample ($10.287\pm11.82\times10^3$ cfu/ml) whereas lowest was recorded from Gupti sediment sample ($3.835\pm3.41\times10^4$cfu/g) and Ekakula water sample ($1.843\pm1.52\times10^3$ cfu/ml). Physico-chemical parameters such as pH, Organic carbon content, Organic matter content, Conductivity, CEC, Temperature, TDS, Ammonia content, Phosphate content, Nitrate content, Chloride content, Salinity, Dissolved Oxygen were studied to understand the impact of seasonal variation on heterotrophic biodiversity.

Culture independent bacterial diversity from sediment and water samples were studied by performing illumina sequencing of V3-V4 region of 16S rRNA which showed that Dangmal sediment sample contain the highest number of bacterial species (21,207 species) whereas Ekakula sediment sample contain the lowest (5,077 species). In case of water sample, highest no of bacterial species was recorded in Habalikhati (34,601 species) and lowest was recorded in Gupti (24,846 species). Species richness and chao 1 index was also carried out using alpha-diversity estimation followed by refraction curve and rank abundance plot for the sediment and

water samples. Comparison between microbial communities were done using Jaccard and Bray-Curtis index. This study revealed that unculturable fraction of bacteria varies greatly with respect to the culturable method. Predominant genera are *Acidovorax, Acinetobacter, Bacillus, Bergeriella, Burkholderia, Clostridium, Corynebacterium, Enterobacter, Proteobacteria, Escherichia, Moraxella, Neisseria, Pseudomonas, Serratia, Staphylococcus* and *Yersinia* from monsoon samples and *Clostridium, Acidovorax, Burkholderia, Pseudomonas, Streptococcus, Neisseria* and *Bacillus* from summer samples. The distribution and diversity of the heterotrophic bacterial population has not been well documented for Bhitarkanika mangrove ecosystem. Therefore, this study will form the database for bacterial genera available in Bhitarkanika Mangrove ecosystem.

Keywords: heterotrophic bacteria, Bhitarkanika mangrove, physico-chemical parameters, bacterial diversity.

Bacterial diversity of Bhitarkanika mangrove ecosystem, Odisha, India

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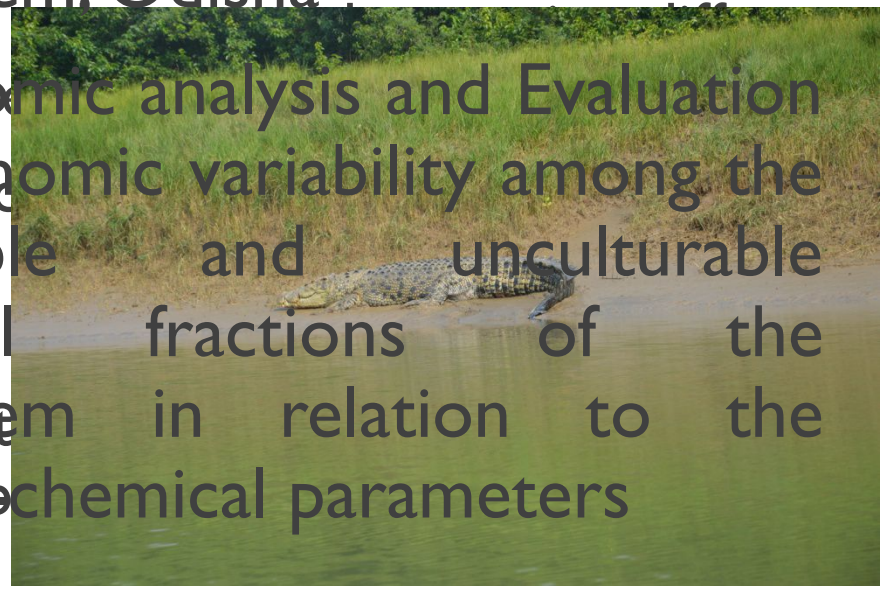
Background and Objectives

Background

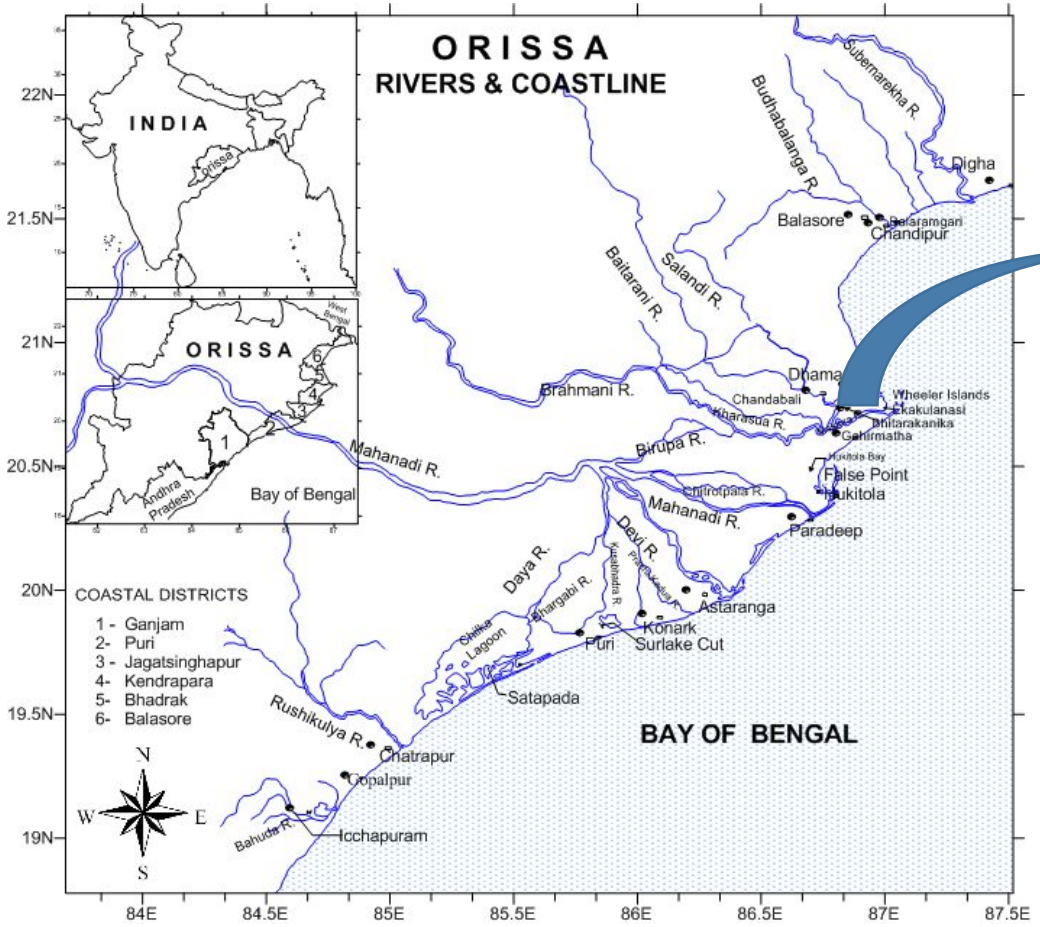
- Mangrove ecosystem of Bhitarkanika is highly rich in biological diversity as well as significant habitat for wildlife.
- Its brackish water environment is highly rich in organic matter due to **microbial enzymatic and metabolic activities**.
- It has been documented that microbial population in Bhitarkanika varies from **10^5 to 10^7 CFU/g** of soil whereas **10^4 to 10^7 CFU/ml** incase of water sample and **Gram negative bacteria** are more in comparison to Gram positive bacteria (Dash and Das., 2014; Thatoi *et al*., 2012)
- Population diversity have been found to shift seasonally, however role and function of these microbes in the ecosystem and effect of seasonal variation is not known.
- Vast microbial flora of this ecosystem is still untapped because of un-culturable nature of the microbes.

Objectives

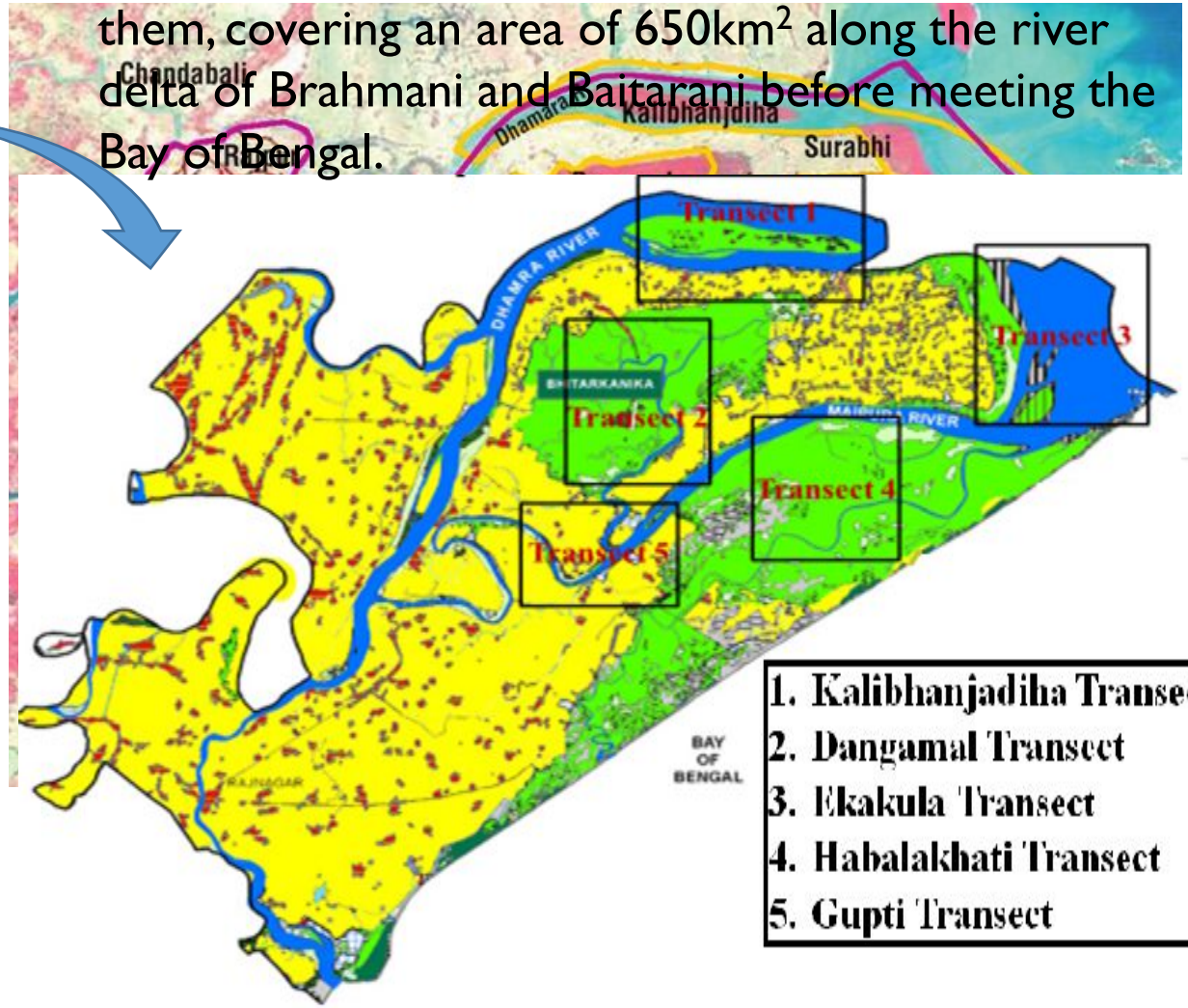
- Isolation and enumeration of culture dependent and culture-independent total heterotrophic bacteria from sediment and water of Bhitarkanika Mangrove ecosystem, Odisha
- Taxonomic analysis and Evaluation of taxonomic variability among the culturable and unculturable bacterial fractions of the ecosystem in relation to the Physico-chemical parameters



Study area



Bhitarkanika estuary of Odisha, India is one of them, covering an area of 650km^2 along the river delta of Brahmani and Baitarani before meeting the Bay of Bengal.



Methodology

Study site and collection of sample

- Five transects include **Kalibhanjadian, Dangmal, Ekakula, Habalikhati and Gupti**. Water (100 ml) and sediment samples (100 g) were collected in duplicates **twice a year i.e. Monsoon** (August 2016) and **Summer** (Post-monsoon) (March 2017) and on site analysis of physico-chemical parameters were conducted.

Analysis of physicochemical parameters

- pH, temperature and salinity (On site)
- Dissolved oxygen, Total Dissolved Solid, conductivity, chloride content, nitrate, phosphate, magnesium and calcium content and its total hardness will be tested in the laboratory (APHA, 1992).

Culture-dependent heterotrophic bacterial count

- Sediment and water samples were subjected to serial dilution followed by spread plating on Sea Water Nutrient agar (SWNA) and Zobell's Marine agar (ZMA) plates.
- Incubation at temperature as per the temperature of the samples for 24-48h.
- The total heterotrophic bacterial load was estimated for the respective samples using the standard formula: CFU/ml or g = No. of colonies x inverse of dilution factor/ volume taken.

Culture-independent bacterial population

- Culture-independent bacterial population of water and sediment samples were estimated by metagenomic analysis of the V3-V4 (Product size ~459bp) region of 16S rRNA at the Illumina platform.

Results

TOTAL HETEROTROPHIC BACTERIAL COUNT

Serial dilution of 1ml of water/1g of soil sample was spreaded on Zobell Marine Agar plates

Incubation at 37˚C for 48hrs

Total number of bacterial Colony Forming Units(CFUs) were counted

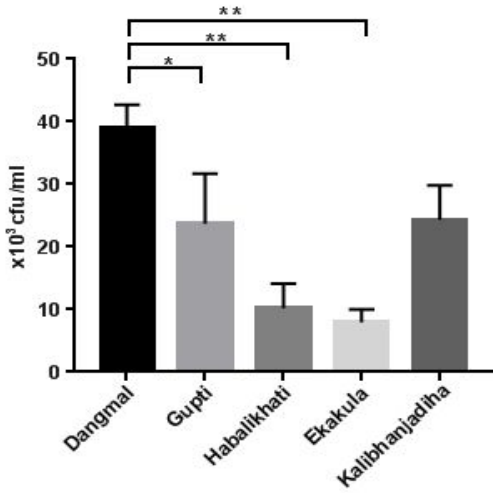
Isolation

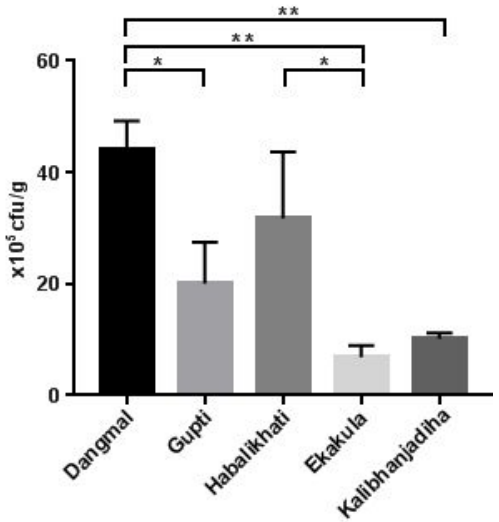
Different colonies were isolated according to their colony morphology

Water sample

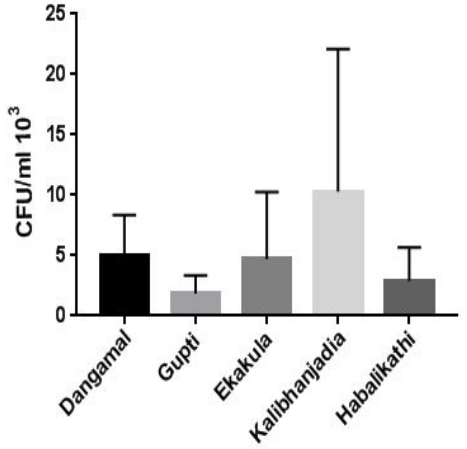
Sediment sample

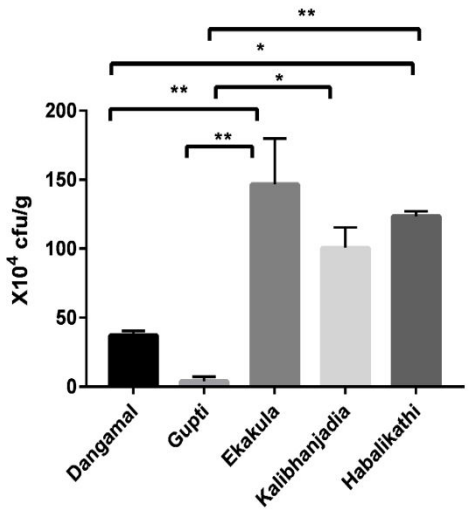
Monsoon





Summer

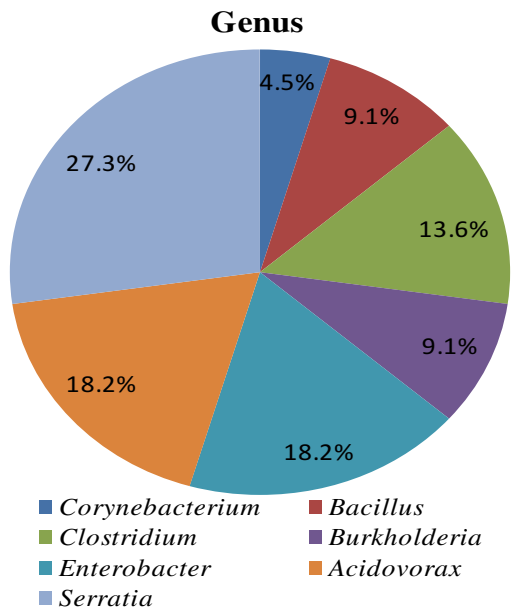




Relative abundance of bacterial diversity at Genus level (Dangmal)

Water sample

MONSOON



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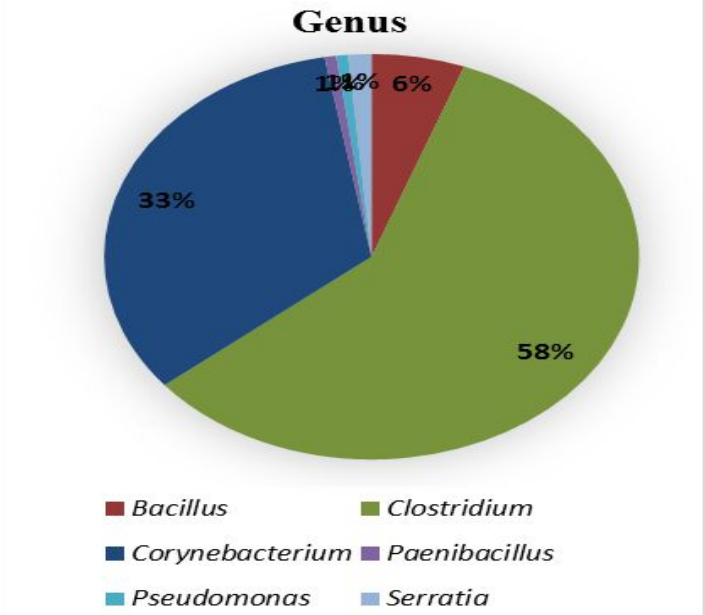
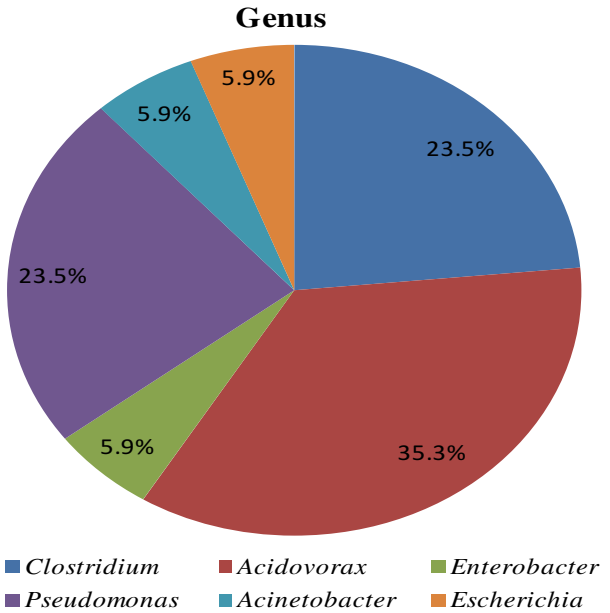


Sediment sample

MONSOON



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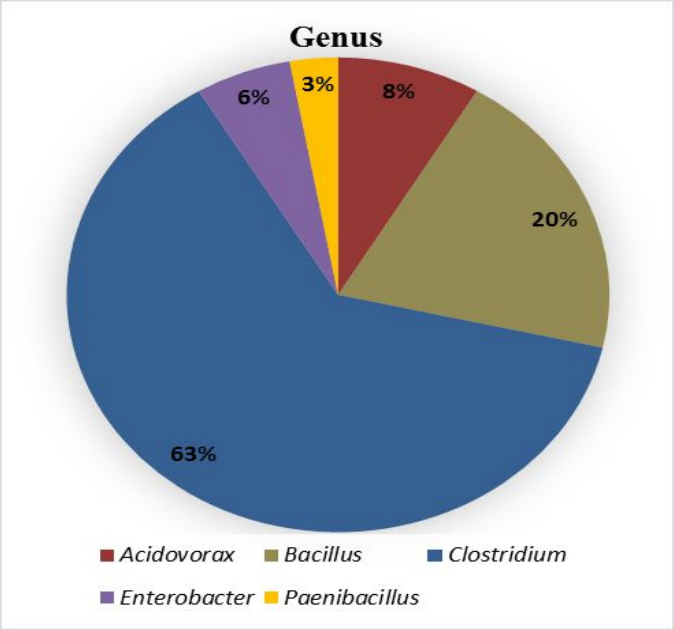

Relative abundance of bacterial diversity at Genus level (Gupti)

Water sample

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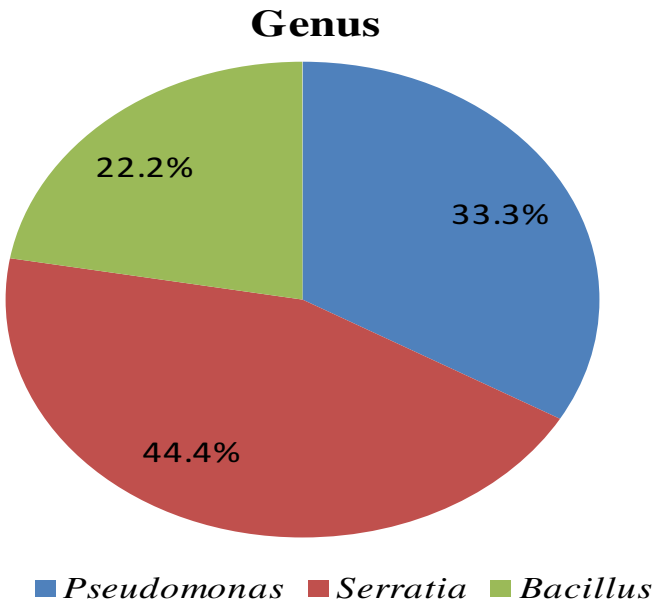

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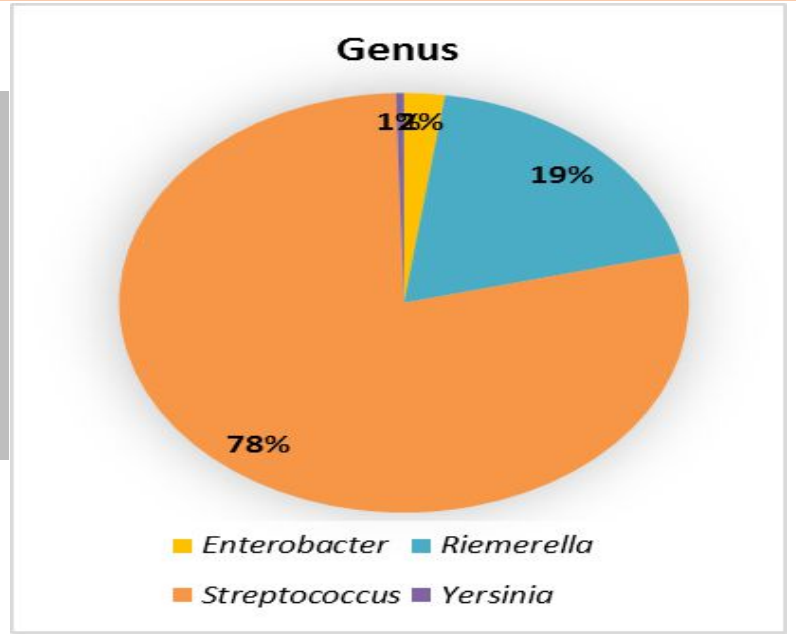

Sediment sample

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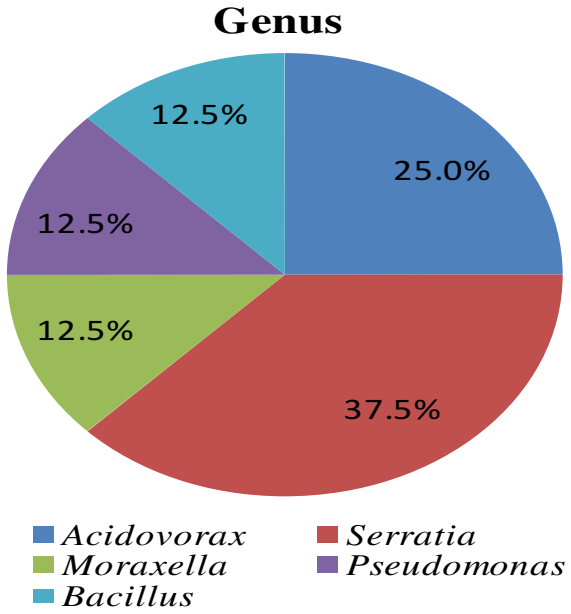

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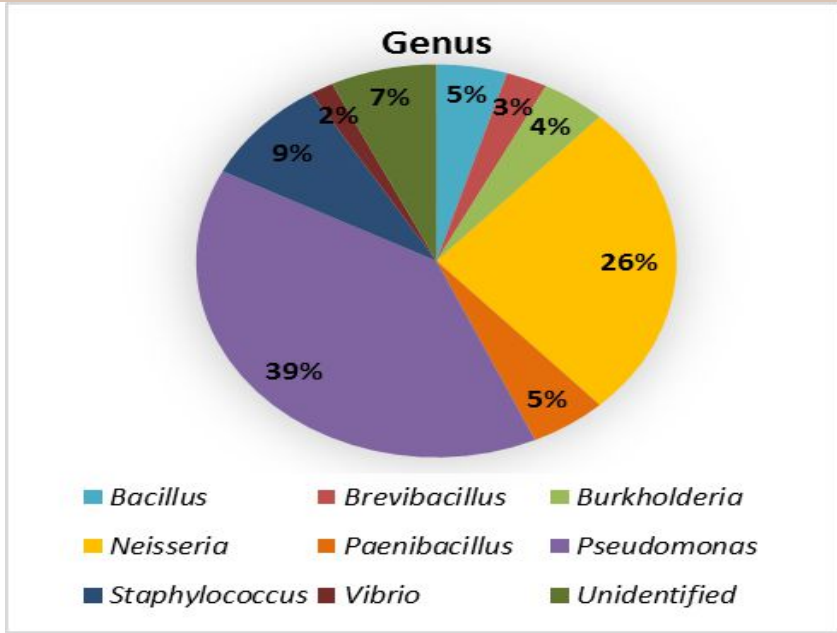

Relative abundance of bacterial diversity at Genus level (Habalikhati)

Water sample

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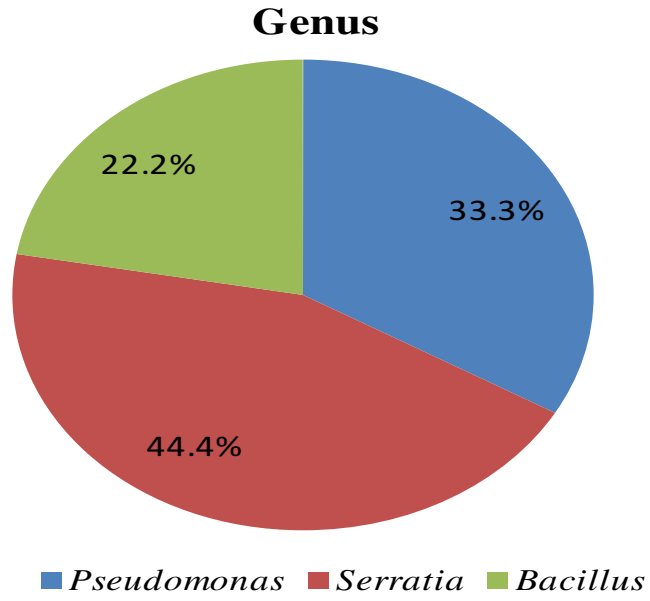

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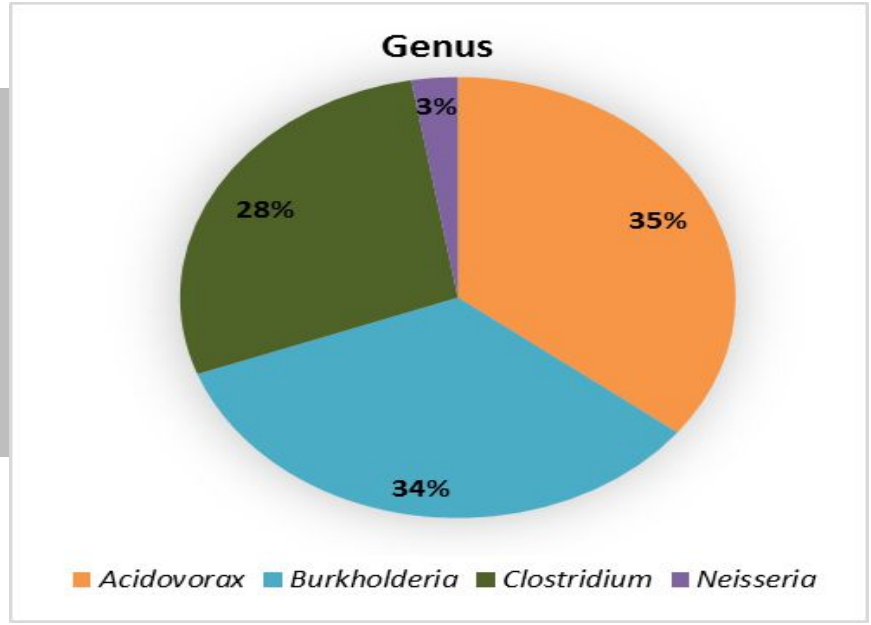

Sediment sample

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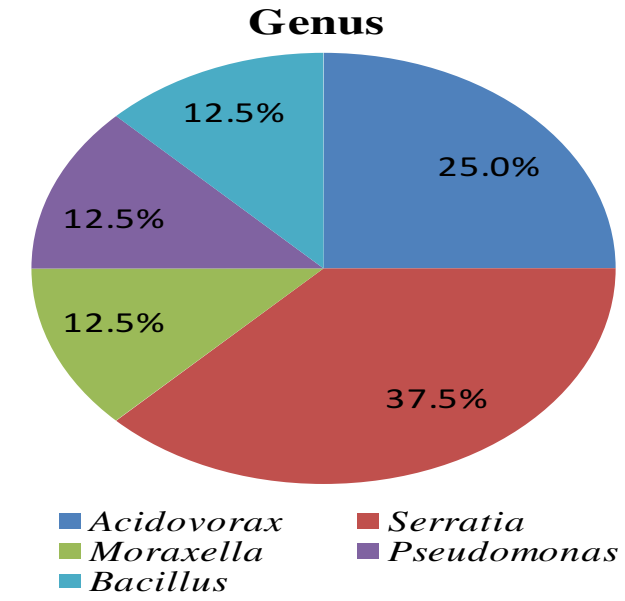

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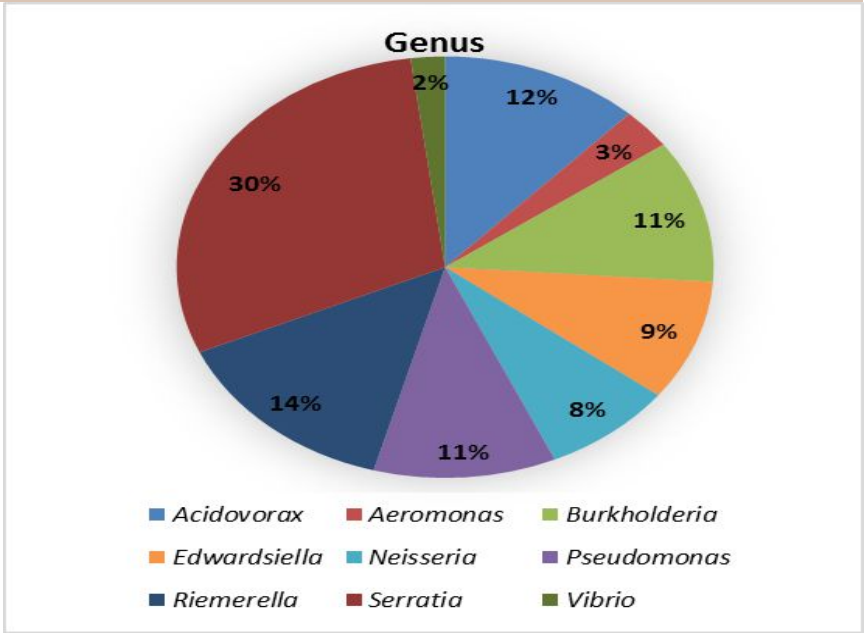
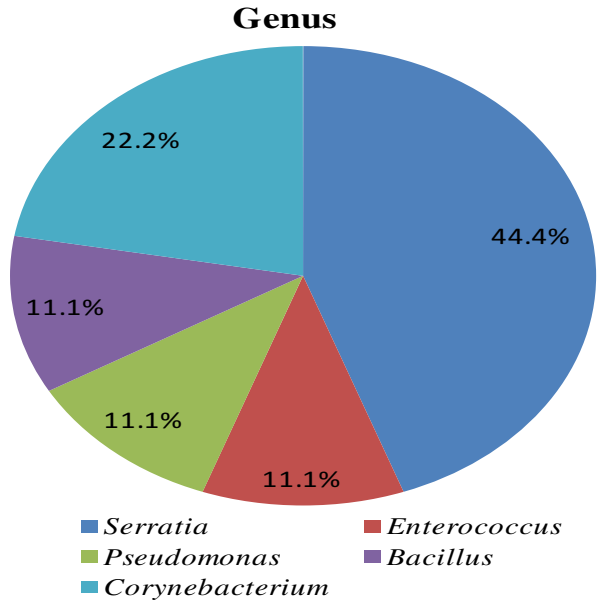


Relative abundance of bacterial diversity at Genus level (Ekakula)

Water sample

MONSOON



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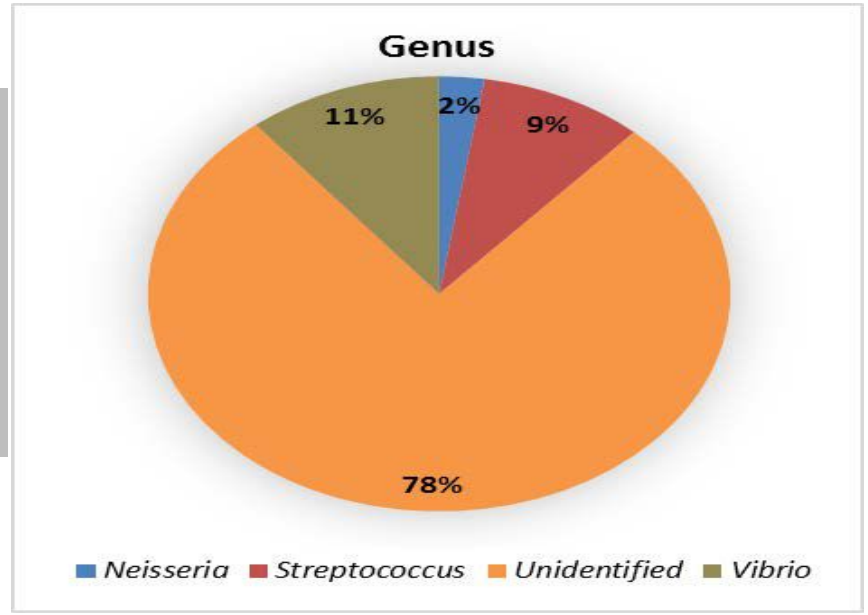

Sediment sample

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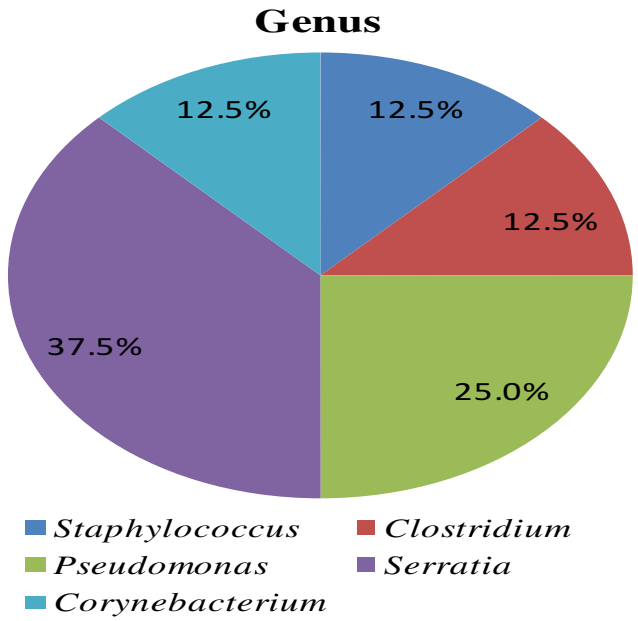

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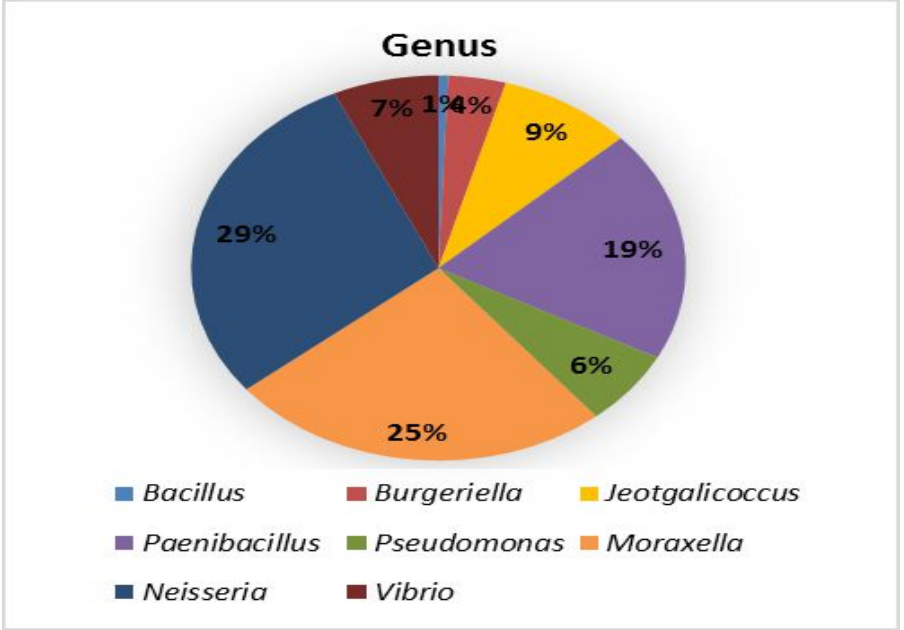

Relative abundance of bacterial diversity at Genus level (Kalibhanjadiha)

Water sample

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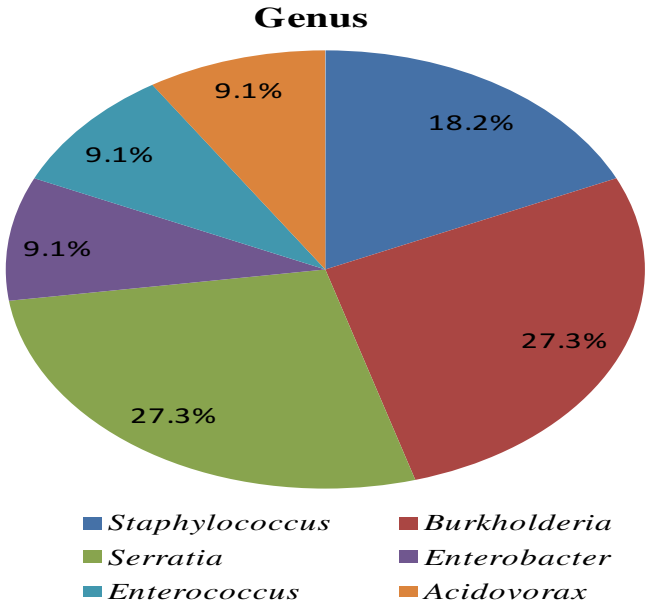

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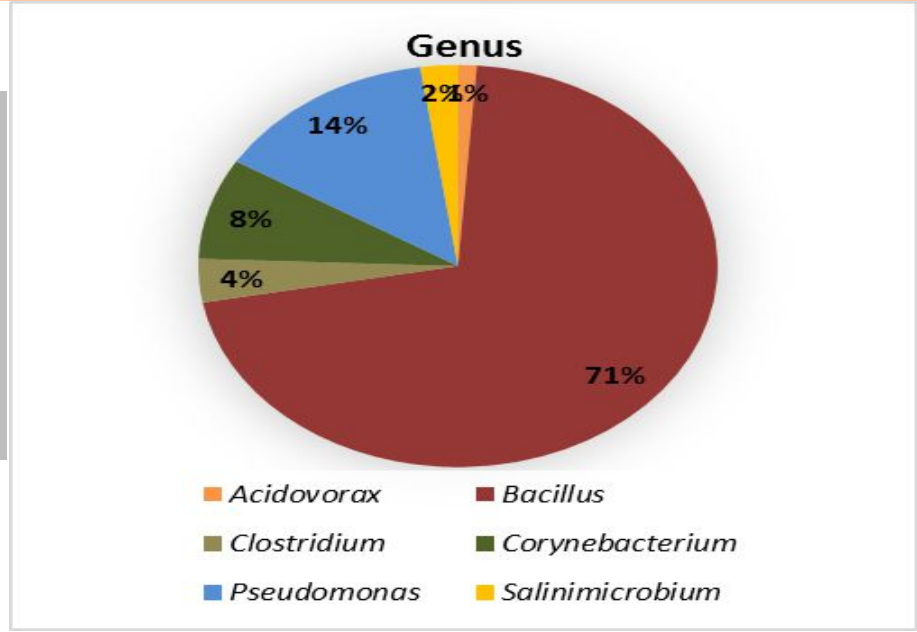

Sediment sample

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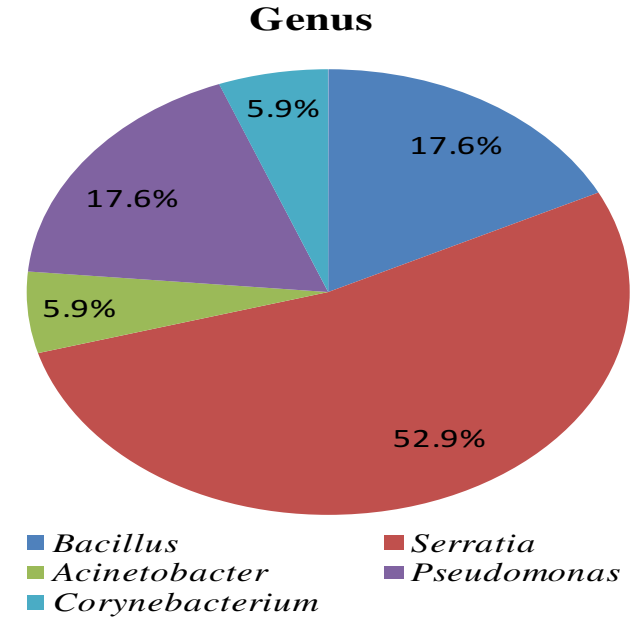

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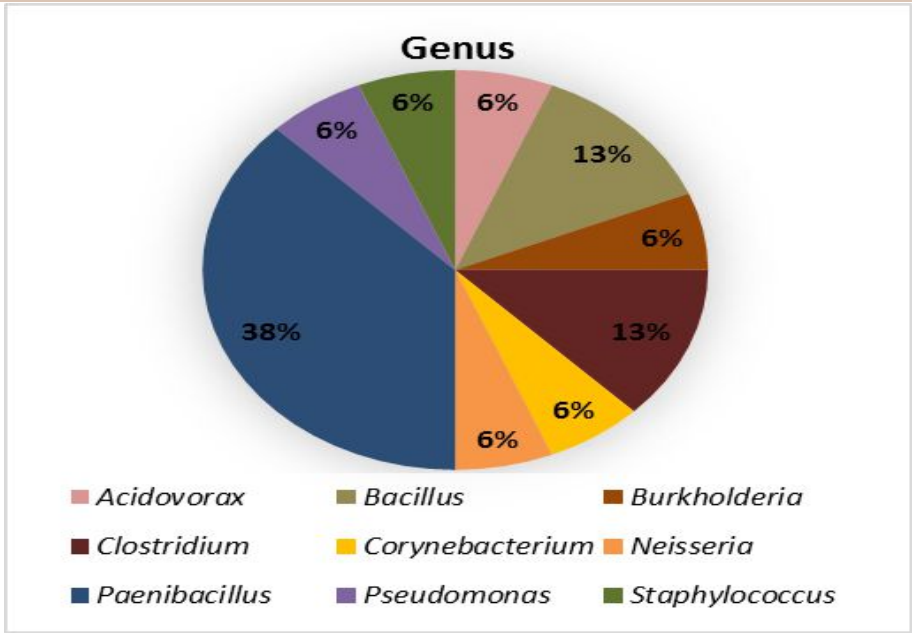
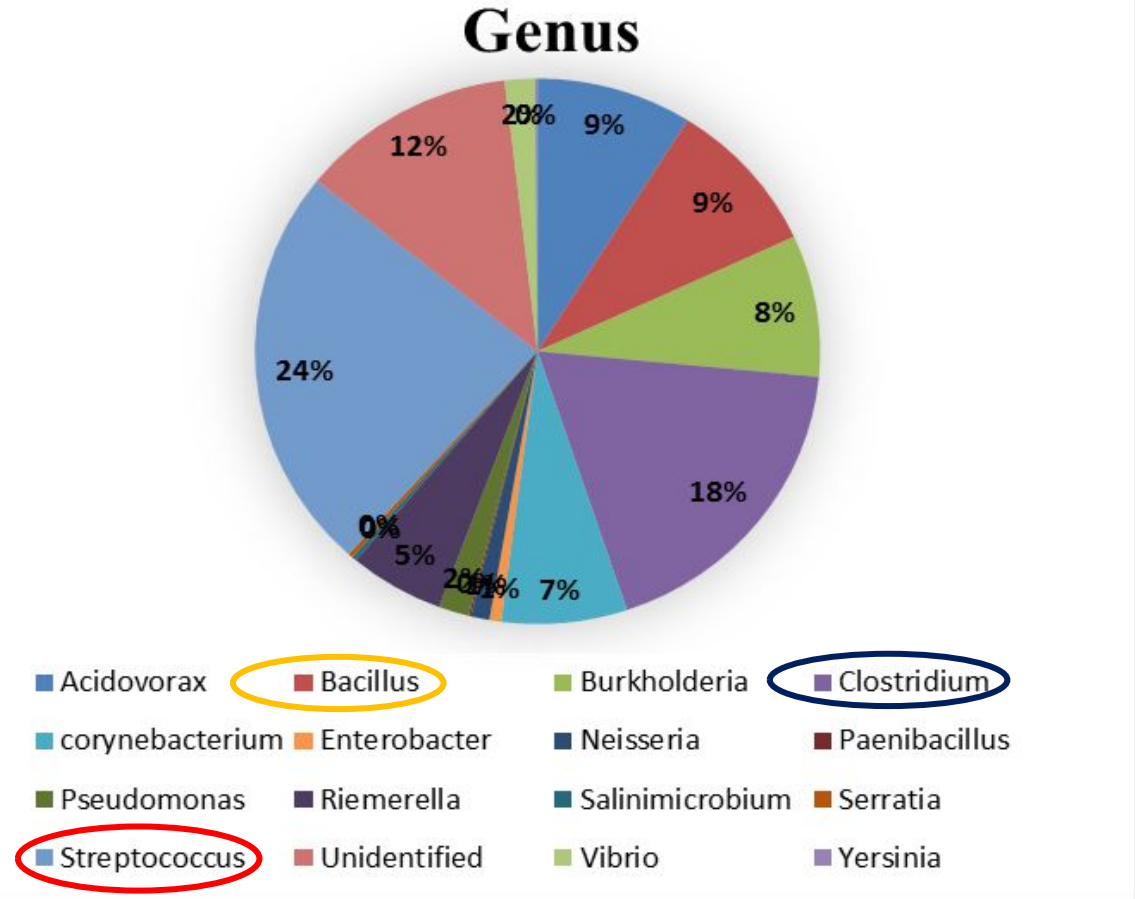
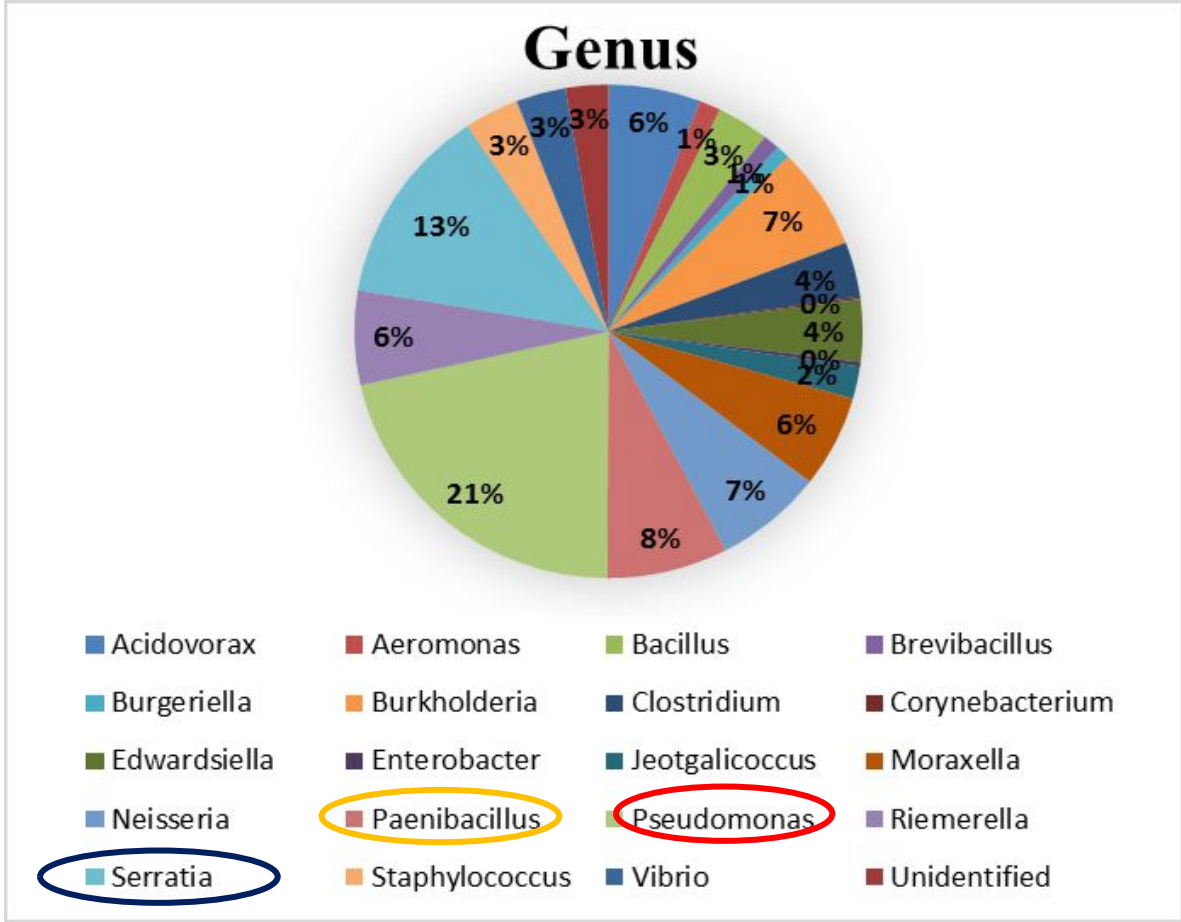


Overall GENRIC distribution of bacteria in Bhitarkanika Mangrove ecosystem

Water sample



Sediment sample



Physico-chemical parameters

Table 1. Correlation matrix for the physico-chemical parameters of water samples

Parameters	THB	pH	Temp	Ammonia	Phosphate	Nitrate	Ca++	Mg++	Hardness	Chloride	Salinity	Conductivity	TDS	DO
THB	1.00													
pH	0.15	1.00												
Temp	0.04	0.87	1.00											
Ammonia	0.06	0.05	0.16	1.00										
Phosphate	0.05	0.08	0.24	0.99***	1.00									
Nitrate	0.62	-0.20	-0.46	0.44	0.36	1.00								
Ca++	-0.87*	-0.13	0.14	-0.29	-0.23	-0.90*	1.00							
Mg++	-0.22	0.41	0.59	-0.61	-0.52	-0.88*	0.60	1.00						
Hardness	-0.54	0.21	0.45	-0.54	-0.45	-0.99***	0.85	0.93*	1.00					
Chloride	-0.77	-0.13	0.11	-0.49	-0.42	-0.93*	0.98**	0.71	0.91*	1.00				
Salinity	-0.58	0.11	0.44	-0.35	-0.25	-0.98**	0.89*	0.85	0.97**	0.92*	1.00			
Conductivity	-0.82	-0.05	0.17	-0.45	-0.39	-0.94*	0.98**	0.70	0.91*	0.99***	0.91*	1.00		
TDS	-0.83	-0.06	0.14	-0.46	-0.40	-0.93*	0.98**	0.68	0.90*	0.99***	0.89*	1.00	1.00	
DO	-0.08	0.32	-0.15	-0.19	-0.28	0.31	-0.27	-0.27	-0.30	-0.27	-0.49	-0.20	-0.17	1.00

Values in the table indicate Pearson's r value. Level of significance ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$ at $n = 3$

Table 2. Physico-chemical properties and the total heterotrophic bacterial population of sediment samples of Bhitarkanika mangrove ecosystem.

Parameters	Dangmal	Gupti	Habalikhati	Ekakula	Kalibhanjadiha
THB ($X10^5$ cfu/g)	43.96±5.28	20.01±7.45	31.76±11.93	6.8±2.17	7.98±3.56
pH	7.34±0.19	7.48±0.42	7.73±0.41	7.27±0.04	7.92±0.26
Organic Carbon (%)	1.06±0.07	0.38±0.09	0.89±0.02	0.17±0.02	0.85±0.08
Organic Matter (%)	1.82±0.12	0.66±0.16	1.53±0.04	0.30±0.04	1.47±0.14
Sediment texture	Clay	Clay	Clay	Sandy Loam	Clay
Conductivity (mS/cm)	0.32±0.08	1.09±0.07	0.98±0.07	0.51±0.05	1.31±0.08

Culture-independent studies

Alpha diversity

Sediment

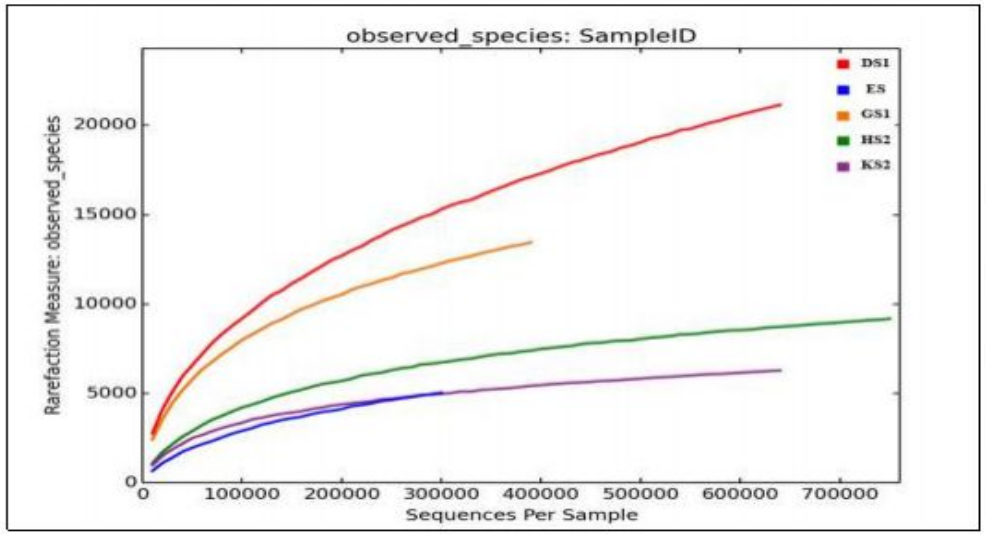
Stations	Observed species
Dangmal	21207.0
Ekakula	5077.0
Gupti	13517.0
Habalikhati	9193.0
Kalibhanjadiha	6299.0

Water

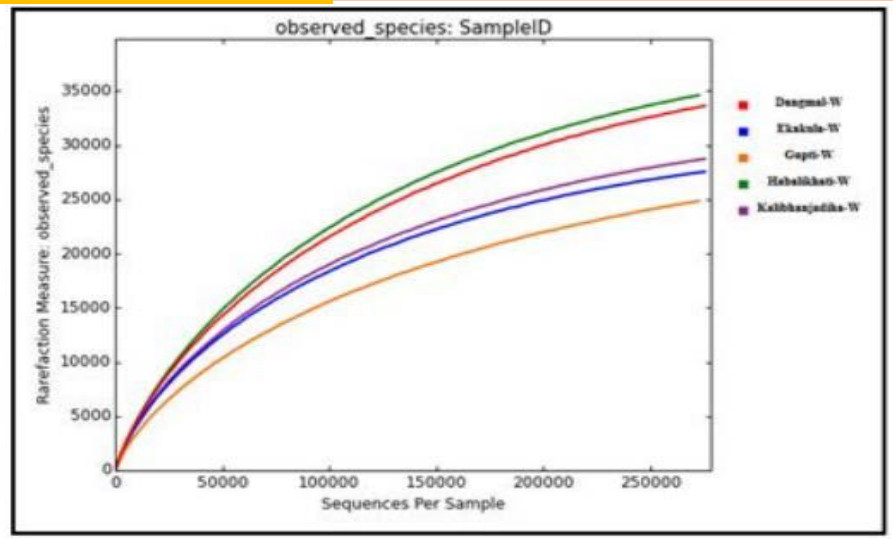
Stations	Observed species
Dangmal	33610.0
Ekakula	27555.5
Gupti	24846.5
Habalikhati	34601.3
Kalibhanjadiha	28729.1

Rarefaction curve

Sediment

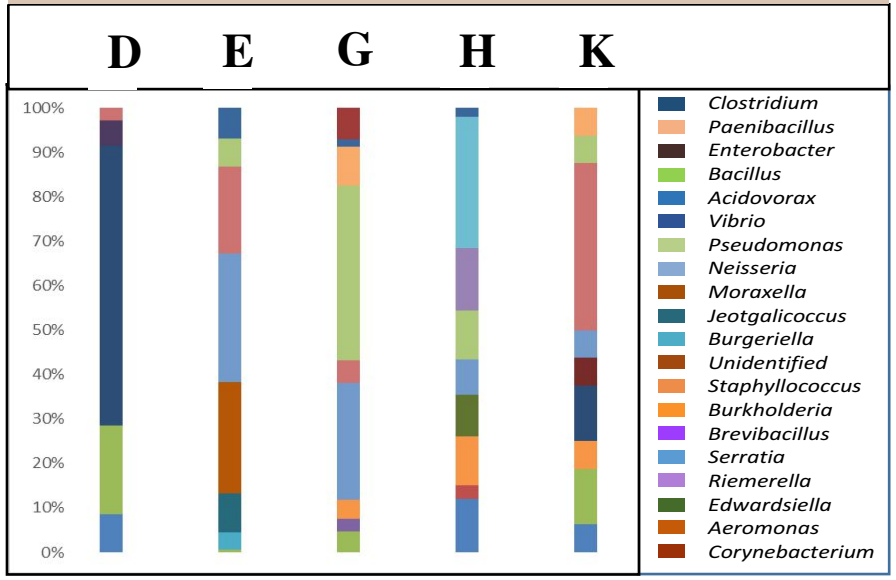


Water

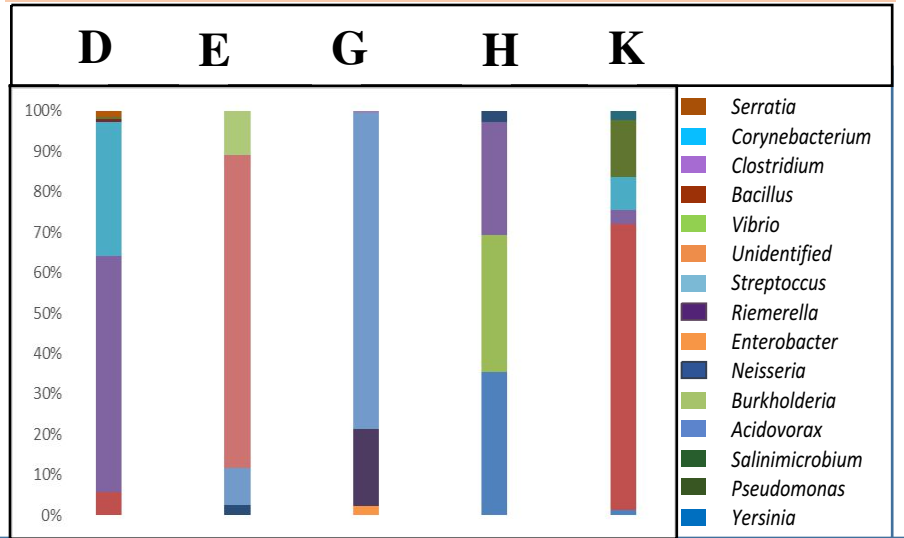


CULTURABLE vs UNCULTURABLE FRACTION

Sediment



Water



Genera (Cuture-dependent)

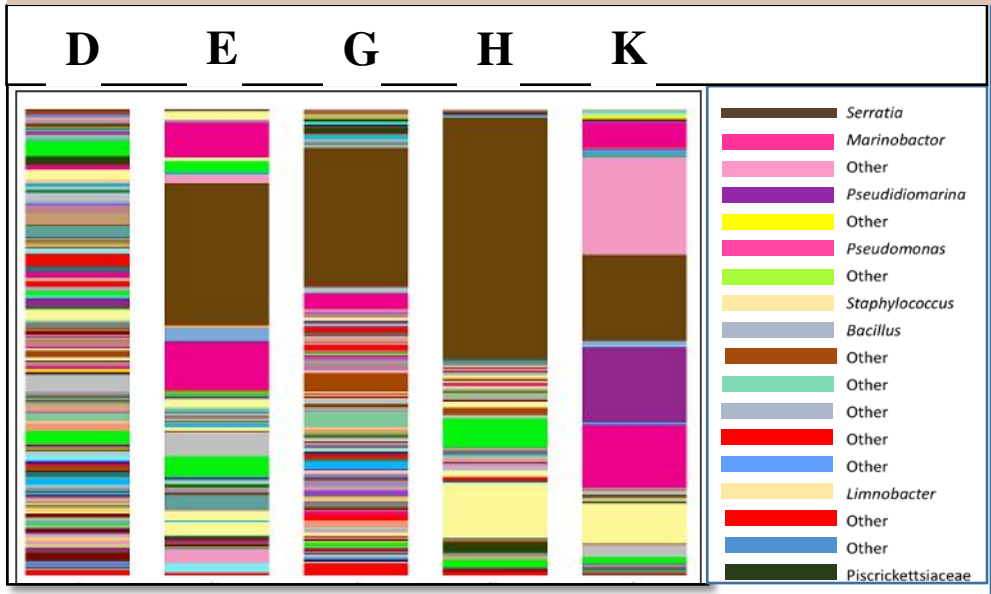
Streptococcus
Pseudomonas
Clostridium
Serratia
Bacillus
Acidovorax
Burkholderia
Corynebacterium
Moraxella

Genera (Culture independent)

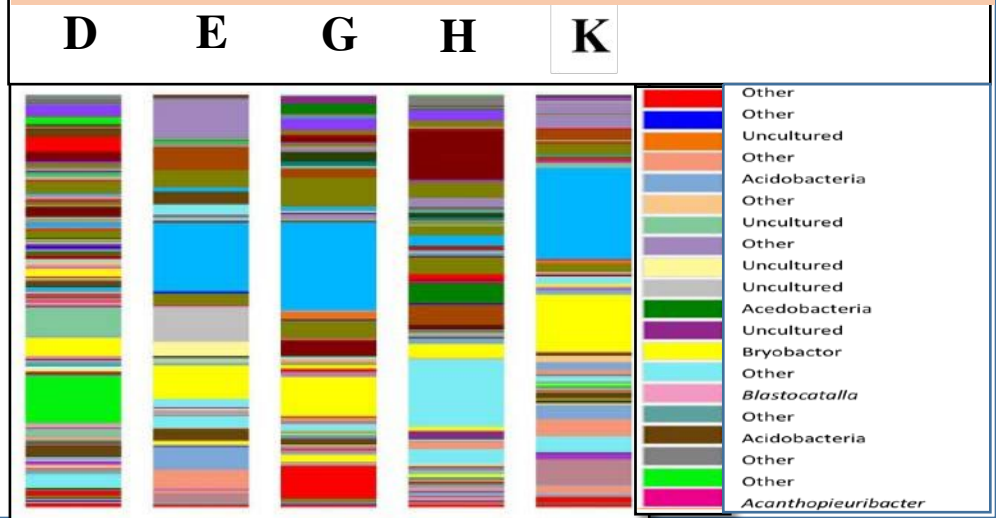
Marinobacter
Alcanivorax
Kordiimonas
Pseudospirillum
Sedimentibacter
Limnobacter
Streptomyces

0.921% (~1%) of total microbial population of Bhitarkanika Mangrove is culturable and remaining **~99%** is uncultured.

Sediment



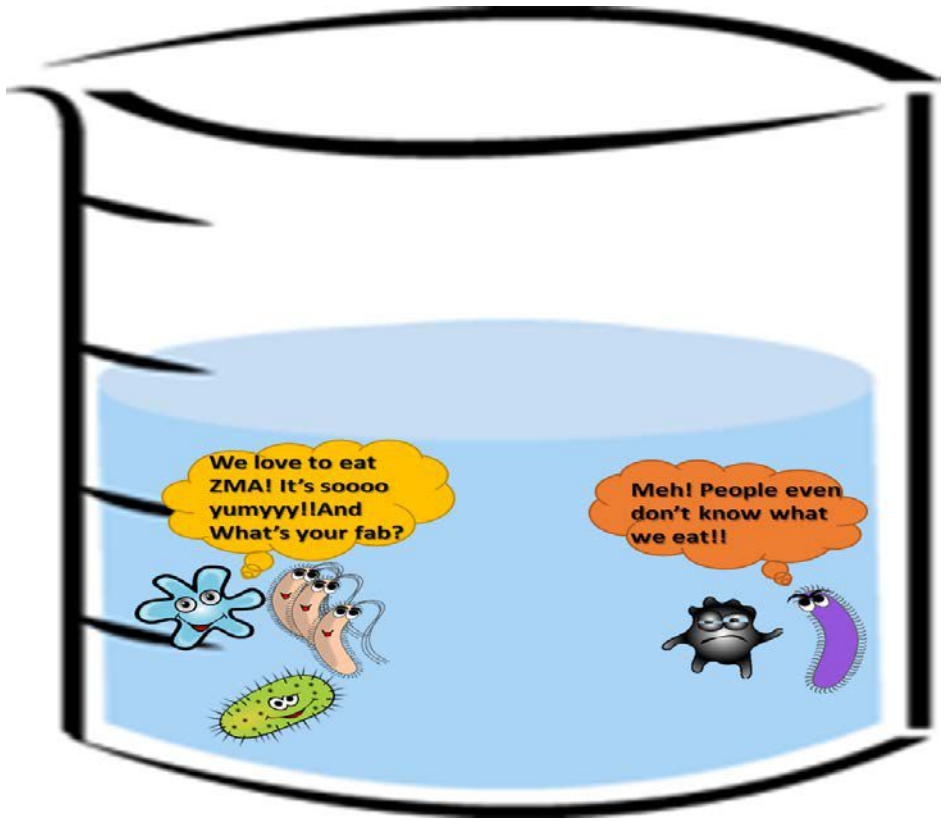
Water



Highlights

- Occurrence of pathogenic bacterial genera such as ***Streptococcus, Serratia, Staphylococcus, Neisseria, Clostridium*** showed that the sites such as Dangmal, Gupti and Kalibhanjadiha water and sediment are influenced by the anthropogenic activities.
- Mangrove ecosystem accounts for 25% of the earth's coastline and 75% of the tropical coastline. Bacteria, having role in mangroves is vital for biogeochemical cycles and transformations of most nutrients.
- **However, the question still exists: what are all they doing?**
- Bhitarkanika mangrove ecosystem is lagging behind to elucidate the question. A complete document on the microbial diversity is way far for this ecosystem.
- So a detailed analysis and further study is ongoing to elucidate the role of the microbial community in nutrient cycling and productivity in the mangrove ecosystem.

Conclusions



- As many as 21000 species from sediments and 30000 species from water have been recorded.

Total no. of OTU (sediment)	Total no of cultured bacteria (Sediment)	Total no. of OTU (Water)	Total no of cultured bacteria (Water)
40249	663	113269	751

- Cultivable fraction is 1.64% in sediment sample and 0.663% in water sample (of total microbial population).
- Overall, 0.921% of total microbial population of Bhitarkanika Mangrove is culturable and remaining ~99% is uncultured.
- Seasonal variation has been studied and found that during monsoon bacterial population and species diversity is more.
- Major physico-chemical factors regulating the bacterial population are nitrate and calcium in water and organic carbon in sediment.

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THANK YOU