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Comparative Analysis of Bacterial Genera in Seasonally Inundated and Permanently Submerged Sediments of Sultanpur Lake – Ramsar Site, Haryana, India

Sandhya Bhat and Pamposh

University School of Environment Management, GGSIP University, Sector-16C,
Dwarka, New Delhi 110 078, India

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ABSTRACT

Sultanpur Lake is a shallow waterbody, within Sultanpur National Park, a Ramsar Site in Gurugram, Haryana. This area is home to a wide variety of plants and animals, and it is a crucial eating, resting, and nesting ground for local and migratory birds. The lake is under severe threat from rising pollution and summertime water shortage. The current study was undertaken to characterize the shifts in bacterial genera in the sediments of Sultanpur Lake brought on by intermittent drying and wetting. Next-generation sequencing of 16S rRNA amplicon was used to characterize the bacterial communities in Dry and Wet sediments of this lake and analyze the changes in them. There was a change in the community structure at the phylum as well as the genus level. The top bacterial genera in Wet sediments were *Clostridium sensus-stricto* (23%), *Sunxiuqinia* (17%), *Clostridium_XIVa* (12%), *Geobacter* (12%), *Bifidobacterium* (6%), *Caldilinea* (6%), *Dechloromonas* (6%), *Carboxylicivirga* (6%), *Desulfomonile* (6%) and *Bacillus* (6%) whereas the top ten genera in Dry sediments were *Clostridium sensus-stricto* (19%), *GPI* (17%), *Parcubacteria genera increate* (14%), *Lactobacillus* (12%), *Sunxiuqinia* (9%), *Alkaliflexus* (7%), *Methanobacterium* (7%), *Bacillariophyta* (5%), *Clostridium_III* (5%) and *Methanothrix* (5%). This compositional shift in the sediment bacterial communities in the Dry plots is towards communities that do not play a major role in the biogeochemical processes.

Key words : 16S rRNA amplicon, Next-generation sequencing, Bacterial community, Bacterial ecology, and biogeochemical processes

Introduction

Sultanpur National Park (28°28'N 76°53' E) is a famous National Park in the Gurugram district of Haryana. It is home to around 90 species of migratory birds from all over the world that come here to feed and spend the winter. It also supports a great variety of resident indigenous water birds and animals (Stewart, 2001; Rahmani *et al.*, 2016). The potential of this wetland is great as it sustains many

migratory and resident bird species and harbors great biodiversity. This place is a bird watchers paradise and a scenic getaway in a highly urbanized area. Sultanpur National Park was given the status of a bird sanctuary in 1969 and then declared a National Park in 1991 (Kothari, 1994). In August 2021 the Ministry for Environment, Forest and Climate Change declared it a Ramsar site. Ramsar sites are wetlands that are designated to be of international importance under the Ramsar Convention for na-

tional action and international cooperation regarding the conservation and sustainable use of their resources.

Monitoring of waterbodies is essential to safeguard the health of organisms that live in them or depend on them for their survival. It is also important for assessing the environmental health of the water body. Both physicochemical parameter monitoring, as well as biological analysis of microorganisms, is important to know about the health of wetlands. Monitoring measures like these provide insights into ways to devise strategies for the restoration and sustainability of water bodies. The health of a wetland is affected by the biogeochemical processes that take place in it. Bacterial communities present in the water bodies are the drivers of these processes. Sediments are the seats of these processes and sediment bacteria are the ultimate drivers of these processes. Bacteria are very sensitive to environmental changes and especially hydrology and any change in the sediment bacteria will have an effect on the biogeochemical processes (Oni *et al.*, 2015). So, maintaining good sediment bacterial diversity is very important for the sustainability of water bodies. Sediment bacterial communities were characterized in this study to understand the changes in the sediment bacterial communities in Sultanpur Lake due to seasonal inundation and dewatering using next-generation sequencing of 16S rRNA bacterial phylogenetic marker.

Materials and Methods

Study Site

Sultanpur Lake is a seasonal freshwater wetland in

the center of Sultanpur National Park (Fig. 1). This lake has erratic edges and greatly variable water levels due to its shallow nature and low precipitation during summers in that area (Singh *et al.*, 2017). In summer only a few small pools remain which serve as feeding grounds for its resident birds. Each year groundwater and irrigation canals are used to provide water in order to maintain the habitat for ducks and stop the loss of floral and animal variety (Singh *et al.*, 2021). This study was conducted to determine how the alternating cycles of drying and wetting affected the diversity of sediment-dwelling bacteria in this Ramsar site.

Sampling Strategy

Sampling was done in April 2018 from three different locations in Sultanpur Lake namely SUL1, SUL2, and SUL3. From each location, three sites were chosen for sampling SUL1a, SUL1b, and SUL1c. Each site was divided into two plots, Dry and Wet (Fig 2). Wet represents plots that are always submerged. Dry represents plots that were dry from April to August and then inundated. Water and sediment samples were collected in triplicates from these plots. Water samples were collected in 1L sterile bottles and water parameters were checked in the field. A sediment corer was used for sediment collection. The top 15cm of sediment was sieved through a 2mm mesh and put in sterile bags. From each site, the sediment samples collected from plots designated Dry and Wet plots were pooled to form six sets of composite samples namely SUL_1_Dry, SUL_1_Wet, SUL_2_Dry, SUL_2_Wet, SUL_3_Dry, and SUL_3_Wet. All the samples were kept on ice and transported to the laboratory and these samples were used for the extraction of metagenomic DNA.

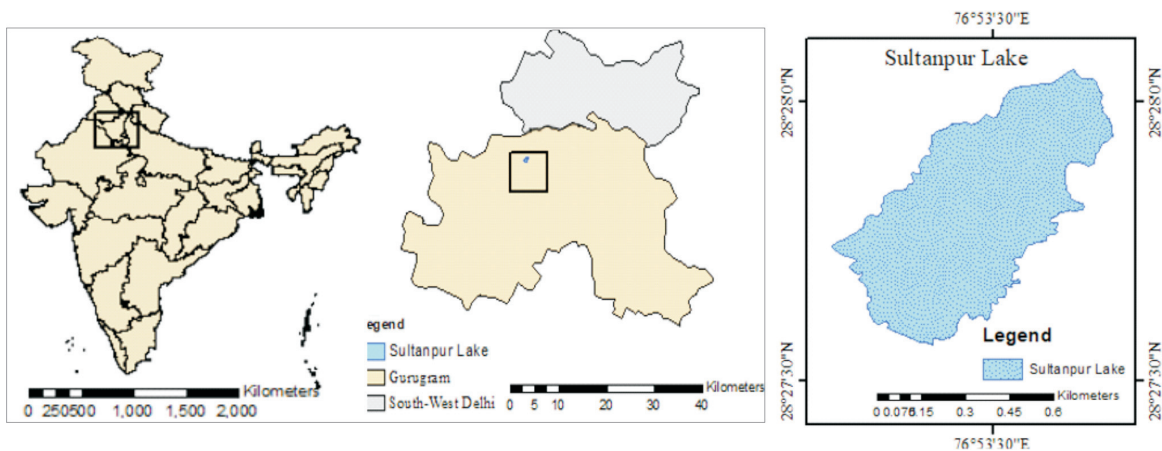


Fig. 1. Map of Sultanpur National Park and Sultanpur Lake



Fig. 2. Illustration of post-monsoon and pre-monsoon look of Sultanpur Lake and the sampling sites

DNA Extraction

Sediment samples were subjected to DNA extraction. Metagenomic DNA was extracted using the Mo Bio Power Soil® DNA Isolation Kit following the instructions given by the manufacturer. The DNA was eluted in 100 µl elution buffer and the DNA concentration of each extract was measured using the NanoDrop-1000 spectrophotometer (Thermo-Fisher Scientific). The DNA quality was checked using a 1% Agarose TAE gel. DNA purity was checked by using the 280/260 ratio as well 260/230 ratio.

16S rRNA Amplicon Generation

25ng of soil metagenomic DNA was used for 16S rRNA amplicon generation. This region of bacterial DNA serves as a phylogenetic marker and is highly conserved within species. A single amplicon of roughly 460 base pairs was produced by amplifying the 16S rRNA hypervariable region V3-V4 using universal primers (Berry *et al.*, 2011). This 16S rRNA amplicon was further amplified to generate libraries. Quantification of these amplicon libraries was done using the Qubit DNA HS quantification assay and the Bioanalyzer 2100.

Sequencing

The libraries were sequenced on the Illumina Mi Seq platform and read data generated. This data was in the form of FASTQ files. These files were processed using the bioinformatics software "Quantitative Insights into Microbial Ecology" (QIIME v1.9.1) (Caporaso *et al.*, 2010). The reads were clustered and OTU's picked using the default UCLUST algorithm

using 97% identity. These OTUs were assigned taxonomy by comparing with the default GreenGene reference database (Version gg_13_5) and annotated up to species level and an OTU table was generated.

Diversity Profiling

Diversity was assessed at two levels alpha and beta diversity. Alpha diversity is the analysis of species diversity in a single site and takes into consideration the richness and evenness of the different species in the site. It was calculated with measures like Chao1, Shannon, and the Simpson indices. Simpson index measures the richness, Chao 1 index measures the evenness, and the Shannon index takes into consideration both the species' evenness as well as the richness. Beta Diversity Analysis measures the dissimilarity of bacterial communities between different sites and is defined as the ratio between regional and local diversities. The metrics used were Euclidean distance among all groups.

Bioinformatics and statistical analysis

To summarize the inter-sample relationships between the wetlands the data were used to generate ordination plots. The goal of ordination plots is the visualization of beta diversity for the identification of possible data structures and the comparison of sites (Ramette, 2007). Principal Coordinate Analysis (PCoA) was used to compare dry and wet samples based on phylogenetic-based distance metrics (Euclidean). Genus-level Phylogenetic tree and heatmap were generated using MEGAN6. MEGAN is a microbiome analysis tool that performs interactive taxonomic analysis of large microbial community datasets (Huson *et al.*, 2016).

Data accessibility

Sequence data can be found at NCBI Sequence Read Archive (SRA) with the accession numbers SRX6085350 to SRX6085355.

Results and Discussion

At the time of sampling, the average temperature of the lake was 30°C, pH was around 8, and specific conductance was 289 μ S. The Dissolved Oxygen was in the range of 5 -6 mg/l.

With the help of sequencing analysis, 93 different bacterial species were identified in Dry sediments, and only 44 different bacterial species were identified in the Wet samples. Alpha diversity analysis revealed both the samples differed in diversity with Dry sediments having higher Shannon (3.24-3.52) and Chao1(79-90) indices than Wet samples Shannon (2.5-2.84) Chao1(34-40). Higher Shannon and Chao1 indices indicate higher bacterial community diversity not only in terms of the number of species but also in how the abundance of the species is distributed among all the species in the bacterial community. Lower diversity in Wet sediments may be due to more anaerobic conditions in these plots as these are always submerged in water.

At the phylum level, the bacterial community Structure of Dry and Wet plots showed *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* accounting for 63% of total abundance in Dry sediments and around 80% abundance in Wet sediments as the top three phyla in both the plots (Fig. 3). Bacterial species belonging to, phyla *Parcubacteria*, *Choloroflexi*, and

Actinobacteria are present in both the sites. The rest of the phyla contributing to bacterial community structure in both types of sediments were different. This difference may be due to the drying and rewetting of the sediments as all the other factors are the same in the sediment samples. The sediments of seasonally inundated plots have a high percentage of *Cyanobacteria* and these are totally absent in sediments that are always submerged in water, as these bacteria are aerobic and need sunlight. The Dry plots are near the periphery and have more oxic conditions than the wet plots explaining the abundance of these bacteria in Dry plots.

To analyze the change in the bacterial communities at the genus level in Wet and Dry sediments of Sultanpur Lake phylogenetic trees were generated. The phylogenetic tree (Fig. 4) makes the visualization of evolutionary relationships between different bacterial genera in the Dry and Wet sediments of Sultanpur easier. This also helped in analyzing which species in these two different plots evolved from a series of common ancestors. The comparative analysis of the data was done by employing non-parametric statistical analysis. As the species data is huge visualization plots were generated. Principal Coordinate Analysis Plot Figure 5(a) shows that Wet and Dry sediments are separated from each other denoting variation in the bacterial communities between the sites. From figure 5(b) it can be seen that 30% of the variation in the bacterial communities between the sites is explained by the PC1 and 27% by PC2. Figure 5(c) is a heat map depicting the relative abundance of the top 30 bacterial genera in Wet and Dry sediments.

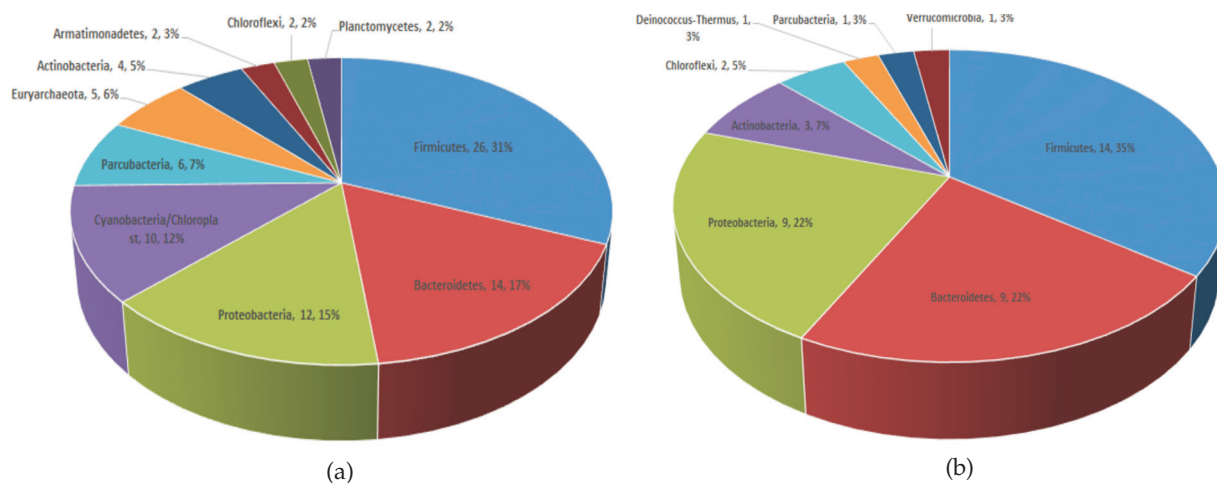


Fig. 3. Top ten bacterial phyla in the sediments of Sultanpur Lake (a) Dry and (b) Wet



Fig. 4. Phylogenetic tree depicting bacterial communities at the species level in sediments of Sultanpur (a) dry and (b) wet

The top ten bacterial genera in Wet sediments are *Clostridium sensu stricto* (23%), *Sunxiuqinia* (17%), *Clostridium_XIVa* (12%), *Geobacter* (12%), *Bifidobacterium* (6%), *Caldilinea* (6%), *Dechloromonas* (6%), *Carboxylicivirga* (6%), *Desulfomonile* (6%) and *Bacillus* (6%). The top ten genera in Dry sediments are *Clostridium sensu stricto* (19%), *GPI* (17%), *Parcubacteria genera increate* (14%), *Lactobacillus* (12%), *Sunxiuqinia* (9%), *Alkaliflexus* (7%), *Methanobacterium* (7%), *Bacillariophyta* (5%), *Clostridium_III* (5%) and *Methanotherix* (5%). At the genera level, it is observed that only three genera are common in both types of sediments *Clostridium sensu stricto*, *Sunxiuqinia* and *Bacillus*. The rest of

the genera are different. The dominance of *Clostridium* in sediments is indicative of an anaerobic environment in the Sultanpur sediments (Sun *et al.*, 2021). The genus *Clostridium sensu stricto* was the dominant genus in both Wet and Dry sediments. These bacteria are known to play a role in biomass decomposition (Zhao *et al.*, 2017). This genus is widely distributed in human and animal intestines, as this site is a bird sanctuary full of excreta from birds and animals explaining its abundance in the site. *Parcubacteria*, which are present in Dry sediments and absent in Wet sediments are present in a broad range of anoxic environments and are thought to be ectosymbionts or parasites of other

organisms (Nelson and Stegen, 2015).

Geobacter species are dominant in Wet sediments and are very important bacteria as they have a specialty to make electrical contacts with extracellular electron acceptors and other organisms explaining

their ubiquitous presence in anaerobic environments. These bacteria are very helpful in biogeochemical processes. They are very helpful in the bioremediation of aromatic hydrocarbons, uranium, and related contaminants in contaminated sites

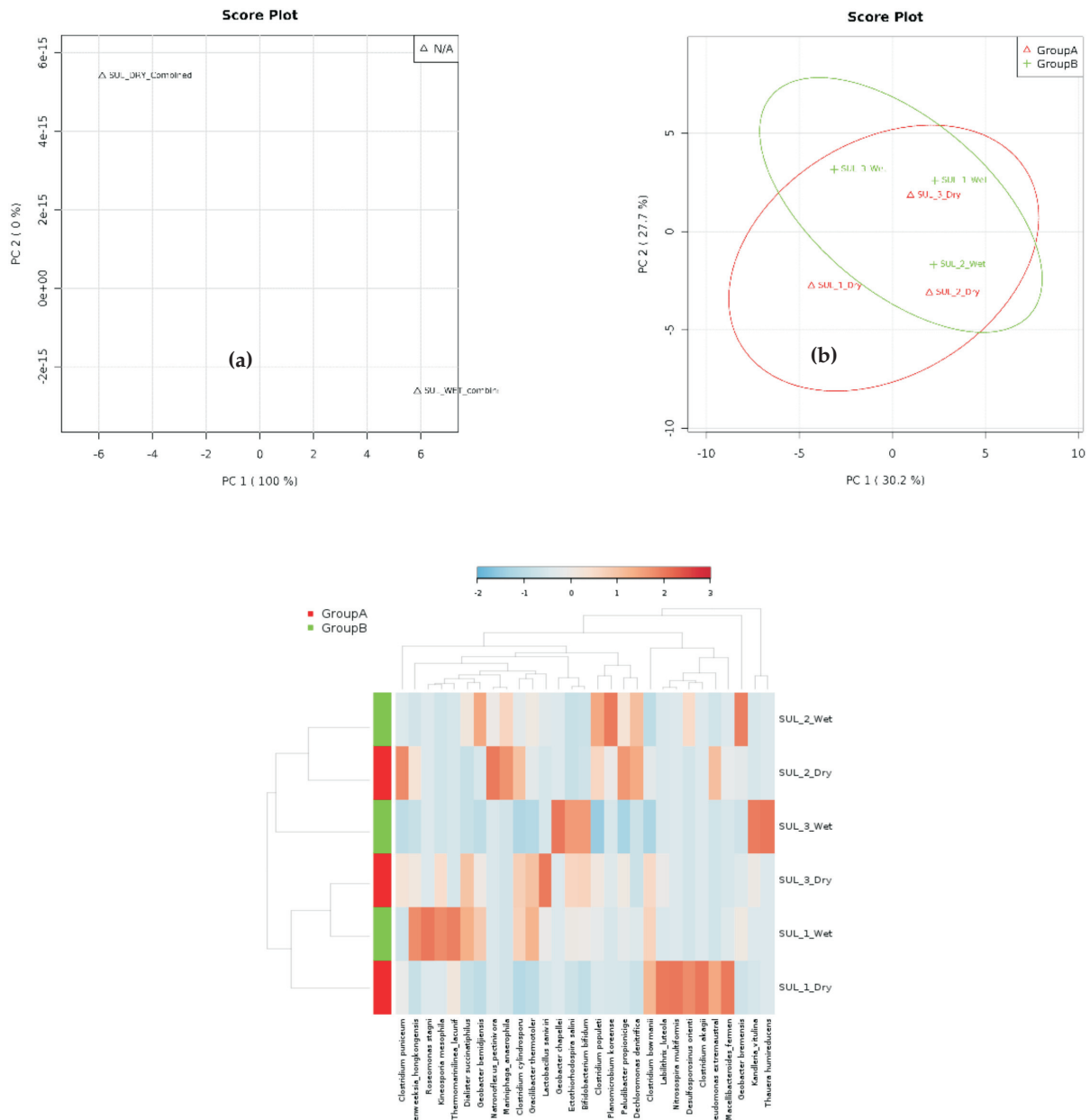


Fig. 5. (a) Principal coordinate analysis (PCoA) score plot based on sediment bacterial communities in all Wet and Dry sediment samples in Sultanpur lake. The two data points represent the combined bacterial species in wet and dry sediments.(b) PCoA score plot based on sediment bacterial communities in Wet and Dry sediment samples in Sultanpur lake. The different data points represent the bacterial species in wet and dry sediments at different sites.(c) Heatmap representing the relative abundance of bacteria at genus level in Wet and Dry sediment samples in Sultanpur lake.

(Lovley *et al.*, 2011). The abundance of *Lactobacillus* species, in Dry sediments, points to contamination by the feces of birds and animals (Volokhov *et al.*, 2012). *Alkaliflexus imshenetskii* an alkaliphilic anaerobic hydrolytic bacterium present in Dry sediments helps decompose oligosaccharides and monosaccharides (Detkova *et al.*, 2009). *Methanobacterium* is a *Euryarchaeota* that is strictly anaerobic and uses ammonia as a nitrogen source. This is methane-producing archaea present only in Dry sediments (Boone, 2015). Benzene is a very common contaminant of surface waters and it is very tough to degrade it. *Dechloromonas* species can completely mineralize benzene and other mono-aromatic compounds to CO₂ in the absence of O₂ with nitrate as the electron acceptor (Coates *et al.*, 2001). The abundance of this species in Wet sediments points to the fact that bacteria which are capable of biogeochemical processes are dominant in Wet sediments. *Desulfomonile* is an anaerobic sulfate-reducing, delta-proteobacteria that is usually present in sewage sludge bacterial consortium (DeWeerd *et al.*, 2015) and it has a role in the degradation of sludge. It is present in Wet sediments and absent in Dry sediments. Genus *Caldilineae* of the phylum *Chloroflexi* is also predominant in Wet sediments. These are filamentous and help in stabilizing flocs of activated sludge in a wide range of Waste Water Treatment Plants (Yoon *et al.*, 2010). In the Wet sediments of Sultanpur Lake, these must be fulfilling the same function. The community structure analysis of Wet and Dry sediments at the genus level has revealed that in the Wet sediments bacterial communities which are very important for biogeochemical processes are predominant.

Conclusion

This study found that there is a shift in the bacterial community structure between seasonally inundated and completely submerged sediments in Sultanpur Lake. This shift is at the phylum level as well as the genus level. Statistical analysis revealed that 30% of the variation in the bacterial community structure is explained by changes in the hydrological regime while the rest of the variation can be attributed to environmental parameters. This compositional shift is towards communities that are not very useful in biogeochemical processes. According to the findings, it can be safely said that water shortage is a major stressor of this wetland. It not only threatens the life of wildlife it also affects the bacterial ecology

of sediments. So, maintaining the water level in the lake throughout the year will help in sustaining and restoring this water body.

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Conflict of Interest

No conflict of Interest.

References

- Berry, D., Mahfoudh, K.B., Wagner, M. and Loy, A. 2011. Barcoded primers used in multiplex amplicon pyrosequencing bias amplification *Applied Environmental Microbiology*. 77(21) : 7846-7849.
- Boone, D. R. 2015. *Methanobacterium*. *Bergey's Manual of Systematics of Archaea and Bacteria*. 1-8.
- Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.L., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J. and Knight, R. 2010. QIIME allows the analysis of high-throughput community sequencing data. *Nature Methods*. 7(5) : 335-336. <https://doi.org/10.1038/nmeth.f.303>.
- Coates, J. D., Chakraborty, R., Lack, J. G., O'Connor, S. M., Cole, K. A., Bender, K.S. and Achenbach, L.A. 2001. Anaerobic benzene oxidation coupled to nitrate reduction in pure culture by two strains of *Dechloromonas*. *Nature*. 411 (6841) : 1039-1043.
- Detkova, E. N., Zaichikova, M. V. and Kevbrin, V. V. 2009. Physiological and biochemical properties of the alkaliphilic anaerobic hydrolytic bacterium *Alkaliflexus imshenetskii*. *Microbiology*. 78(3): 273-279.
- De Weerd, K. A., Todd Townsend, G. and Sufliata, J.M. 2015. *Desulfomonile*. *Bergey's Manual of Systematics of Archaea and Bacteria*. 1-5.
- Huson, D.H., Beier, S., Flade, I., Górska, A., El-Hadidi, M., Mitra, S., Ruscheweyh, H. J. and Tappu, R. 2016. MEGAN community edition-interactive exploration and analysis of large-scale microbiome sequencing data. *PLoS Computational Biology*. 12(6): e1004957.
- Kothari, A. 1994. *Conserving Life: Implications of the Biodiversity Convention for India*. Kalpavriksh, New Delhi

- Lovley, D.R., Ueki, T., Zhang, T., Malvankar, Nikhil, S., Shrestha, Pravin, M., Flanagan, Kelly, A., Muktak A., Jessica, E.B., Ludovic, G., Amelia, E.R., Holmes, D.E., Franks, A.E., Roberto, O., Risso, C. and Nevin, K. 2011. Geobacter: the microbe electric's physiology, ecology, and practical applications. *Advances in Microbial Physiology*. 59 : 1-100.
- Nelson, W. C. and tege, J.C. 2015. The reduced genomes of Parcubacteria (OD1) contain signatures of a symbiotic lifestyle. *Frontiers in Microbiology*. 6 : 713.
- Oni, O. E., Schmidt, F., Miyatake, T., Kasten, S., Witt, M., Hinrichs, K. U. and Friedrich, M. W. 2015. Microbial communities and organic matter composition in surface and subsurface sediments of the Helgoland mud area, North Sea. *Frontiers in Microbiology*. 6: 1290. <https://doi.org/10.3389/fmicb.2015.01290>
- Rahmani, A.R., Islam, M.Z. and Kasambe, R.M. 2016. *Important bird and biodiversity areas in India: priority sites for conservation* (revised and updated). Bombay Natural History Society, Indian Bird Conservation Network, Royal Society for the Protection of Birds and Bird Life International (UK), 1992.
- Ramette, A. 2007. Multivariate analyses in microbial ecology. *FEMS Microbiol Ecol*. 62 : 142–160. <https://doi.org/10.1111/j.1574-6941.2007.00375.x>
- Singh, P., Javed, S., Shashtri, S., Singh, R.P., Vishwakarma, C.A. and Mukherjee, S. 2017. Influence of changes in watershed land use pattern on the wetland of Sultanpur National Park, Haryana using remote sensing techniques and hydrochemical analysis. *Remote Sensing Applications: Society and Environment*. 7: 84-92.
- Singh, J., Hooda, S., Phogat, A. and Malik, V. 2021. Avian Diversity and Habitat Use of Sultanpur National Park, Haryana, India. *Asian Journal of Conservation Biology*. 10(1) : 124-13.
- Stewart, R.E. 2001. *Technical Aspects of Wetlands: Wetlands as Bird Habitat*. National Water Summary on Wetland Resources, United States Geological Survey 86.
- Sun, W., Yang, K., Li, R., Chen, T., Xia, L., Wang, Z. and Sun, X. 2021. The spatial distribution characteristics of typical pathogens and nitrogen and phosphorus in the sediments of Shahe Reservoir and their relationships. *Scientific Reports*. 11(1): 1-10.
- Volokhov, D. V., Amselle, M., Beck, B. J., Popham, D. L., Whittaker, P., Wang, H., Kerrigan, E. and Chizhikov, V.E. 2012. *Lactobacillus brantae* sp. nov., isolated from feces of Canada geese (*Branta canadensis*). *International Journal of Systematic and Evolutionary Microbiology*. 62 (Pt_9) : 2068-2076.
- Yoon, D.N., Park, S.J., Kim, S.J., Jeon, C.O., Chae, J.C. and Rhee, S.K. 2010. Isolation, characterization, and abundance of filamentous members of *Caldilineae* in activated sludge. *The Journal of Microbiology*. 48(3): 275-283.
- Zhao, D., Cao, X., Huang, R., Zeng, J. and Wu, Q. L. 2017. Variation of bacterial communities in water and sediments during the decomposition of Microcystis biomass. *PLoS One*. 12(4): e0176397.
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