Synopsis of the thesis on

GEOMICROBIOLOGY OF SUBSURFACE SEDIMENT CORES FROM THE ALLUVIAL MAHI RIVER BASIN, WESTERN INDIA

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1. Introduction

Microorganisms are referred to as the 'Janitors of the Earth' as they play important roles in soil/sediment geochemistry, hydrology, and ecology (Uroz et al., 2015). In soils and the sub-surface environment microorganisms are important drivers of biogeochemical processes. Link between microbial and geochemical parameters may help us unravel the factors that affect the activity and function of microbes in processes ranging from local to global scales (Druschel and Kappler, 2015).

1.1 Microbial activity in subsurface and its importance

The physical and chemical characteristics of deep soil environments are very different from the surface. Microbial life in deep subsurface soils is dependent upon chemical energy sources that have been buried in the sediment or enter as dissolved components organic matter and other reduced compounds (Mn(II), Fe(II), ammonia, sulfide) that can be oxidized with the release of energy. The factors affecting microbial physiology in the subsurface are availability of water, carbon, energy sources, terminal electron acceptors, and other environmental factors such as pH and temperature (Reith, 2011). Because microbes are highly responsive to their environments, examining the environmental factors that are characteristic of the deep soil provides a context for understanding the distribution and functioning of deep soil microbes.

Subsurface microorganisms are responsible for catalyzing the oxidation of organic matter coupled to a variety of electron acceptors just as microorganisms do in surface sediments, but at much slower rates. Glucose mineralization, N mineralization, $^{32}PO_4$ incorporation, ^{3}H-acetate incorporation has been found to decline with depth (Holden and Fierer, 2005).Taylor et al., 2002 reported that dehydrogenase activity, acidic phosphatase activity, alkaline phosphatase activity, β-D-glucosidase activity decrease significantly with depth.

Microorganisms also modulate the terrestrial and marine cycles of C, N, S, and P and some other elements. Microbial metagenomics study reveals that subsurface microbes play imperative role in climate-relevant subsurface biogeochemical processes (Long et al., 2016). Microbes can degrade contaminants that limit flux of contaminant (pollutants) to groundwater.

1.2 Subsurface microbial diversity

Diversity and gene quantification describe the abundance of specific bacteria or genes that are important in microbial ecology and assist to understand the roles and contributions of particular microbial and functional groups within ecosystem functioning (Smith and Osborn,

2009). Gram-positive bacteria and actinomycetes tend to increase in proportional abundance with increasing soil depth, while the abundances of Gram-negative bacteria, fungi, and protozoa were highest at the soil surface and substantially lower in the subsurface (Fierer et al., 2003).Lin et al., 2012 reported vertical stratification of subsurface microbial community composition across geological formations i.e. microbial community structure and richness varied substantially across the different geological strata recovered from depths of 9–52 m.

Organic matter content in deep subsurface strata can be extremely variable e.g. sandy aquifer sediments have lower organic carbon in compared to clayey confining beds. Organic acids produced in clayey confining beds strata due to microbial activities diffuse into the sandy aquifer sediments, where they are consumed by other respiratory microorganisms that are inherent within sandy strata which oxidize the organic acids to carbon dioxide (Lovley and Chapelle, 1995). Gradients of organic acids between confining beds and sandy aquifer sediments sufficient to support microbial metabolism. This way availability of different nutrients within different strata affects the numbers and diversity of microorganisms within stratigraphic sequence (Lovley and Chapelle, 1995).

1.3 Correlation of soil physicochemical properties with microbial activity and diversity

Several environmental factors, such as carbon and energy sources, mineral nutrients, growth factors, ionic composition, available water, temperature, pressure, air composition, electromagnetic radiation, pH, oxidation reduction potential, and interaction between microorganisms, can affect the ecology, activity and population dynamics of microorganisms in soil. Soil dehydrogenase activity, phosphatase activity, β-glucosidase activity positively correlates with soil organic matter and silt content while negatively correlate with sand and clay content of soil which indicate that soil physicochemical properties have profound effect on enzyme activity. Anthropogenic activities also affect soil enzyme activity such as dehydrogenase activity, β-glucosidase activity decrease in heavy metal contaminated soil (Oliveira and Pampulha, 2006) while municipal waste compost increase soil enzyme activity (Mora et al., 2005). Wang et al., 2016 demonstrate that bacterial community composition is significantly correlated with pH, water content, ammonium nitrogen, silicate silicon, nitrite nitrogen, organic carbon and organic nitrogen content.

2. Rationale

Correlation of soil/sediments physicochemical properties with microbial activity, microbial diversity and abundance of functional genes reveal their geo-ecological functions in subsurface which is prime importance for human health, ecosystem functions, agriculture, and environmental management. It would be interesting to understand how the subsurface core samples of different stratigraphy differ in the geomicrobiological characteristics. The present work addresses the question how microbial activity and diversity vary with subsurface sediment characteristics and geological material age.

In the Indian subcontinent interaction between microbial parameters and sediments indigenous properties within subsurface sediments of river is not well established till date. Present study on Mahi river basin with respect to microbial parameters and sediments indigenous properties will help to understand river ecosystem and health of the river environment within Indian subcontinent.

3. Objectives

1) To understand the correlation of physicochemical characteristics of the sedimentary sequences and microbial activities in subsurface sediment cores
2) Study of microbial diversity in the stratigraphic sequence of sediment cores by culture dependent and culture independent methods
3) Determination of relative abundance of genes for selected enzymes from different biogeochemical cycles and establishing functional diversity in the stratigraphic sequence of sediment cores

4. Work done to fulfill above objectives

Site description and sample collection:

Sediment samples were collected from three distinct locations (**Chokari, Rampura (near Nandesari) and Rayka)** of the Mahi River basin, western India, which falls under semi-arid region with mean annual rainfall about 600-650 mm. **Chokari** (22°13’49"N, 72°55’41"E) lies within estuarine zone of Mahi river located downstream of Rayka and made up of sediments of middle to late Holocene forming a younger terraced surface. While, the alluvial zone of the Mahi River basin at **Rayka** (22°26'55"N, 73°05'27"E) comprises distinct geomorphic units and alluvial deposits of late Pleistocene age. The sediment and soil sequences along these cliffs have been dated back to 125ka (Juyal et al., 2000). **Rampura** (22°23'0.3"N, 73°05'15"E) sediment also

consists of alluvial deposits of late Pleistocene age and comprise similar stratigraphic sequence as Rayka but Rampura site is located on the left bank of Mini River (a tributary of Mahi river) basin near Nandesari industrial estate.Therefore, Rampura sediment is contaminated with different pollutants.

Samples from all three sites were collected by core drilling method. In drilling method plastic core liner was used. Tight fitting plastic end caps were placed onto the ends of the core liner to minimize contamination. Cutting of plastic core barrel open lengthwise using a small circular saw was done. Geological formations included within the core was identified and depth intervals were decided for subsampling. The outer third to two-thirds portion of each core was aseptically pared away and only the center most portion of the core was used for microbiological investigations. Autoclaved polythene bags were used for collecting the subsamples which were stored at -20^{o}C for DNA extraction and at 4^{o}C for other microbial analysis.

Sediment core samples were collected up to 17m, 28m and 25m depth respectively from Chokari, Rayka, and Rampura. In this study correlation of microbial parameters with geological properties of sediment core samples were done.

The research component of the thesis is divided in to **three chapters as follows**

1) Microbial characteristics and their association with sediment geochemical properties in 17 m deep estuarine core at Chokari, Mahi River Basin
2) Linkage of sediment indigenous properties with microbial parameters in two laterally deposited Late Quaternary sediment cores of the Mahi River basin: A case study from Rayka and Rampura
3) Evaluation of bacterial strains isolated from subsurface sediment samples at Rayka and Rampura for heavy metal tolerance and Cr(VI) removal ability

Chapter 1: Microbial characteristics and their association with sediment geochemical properties in 17 m deep estuarine core at Chokari, Mahi River Basin

To determine microbial activities of Chokari sediment core section microbial enzyme activities such as β – D-glucosidase, alkaline phosphatase, dehydrogenase, arylsulphatase, protease, fluorescein diacetate (FDA) activities were investigated directly using sediment samples as crude enzyme source. Notable phosphatase activities was observed throughout core profiles indicating P stress in CRD core. Interestingly within Chokari (CRD) sediment profile higher fluorescein diacetate (FDA) activities were observed at some of the deep samples. β–D-glucosidase, protease, dehydrogenase and arylsulphatase activities decrease significantly at higher depth within CRD sediment profile indicating that microbial activities involved in C, N, and S cycles are reduced at higher depth within vertical stratification of estuarine region of Mahi river basin. Dehydrogenase activities are reported to decline with increasing depth in subsoil profiles (Taylor et al., 2002).

Physicochemical characteristics of sediment samples such as organic C, inorganic C, moisture content, pH, salinity, TDS, conductivity, particle size analysis, major oxide, trace element, and rare earth element content has been obtained with collaboration from Dr. Anupam Sharma, Birbal Sahni Institute of Palaeosciences, Lucknow, India.

The correlation analysis between physicochemical properties of sediment and microbial parameters in the CRD core revealed that dehydrogenase and β – D-glucosidase activity showed a significant positive correlation with org. C, pH, silt, clay, Fe_2O_3, P_2O_5, MgO, Cu, as well as Zn content. Alkaline phosphatase, protease, and arylsulphatase activity also showed a significant positive correlation with Fe_2O_3 and P_2O_5. All enzyme activities studied here exhibited a positive correlation with Cu, Zn, and pH. Sand content showed a significant negative correlation with dehydrogenase, β – D-glucosidase, protease, and arylsulphatase activities; sediment depth showed significant negative correlation with all the enzyme activities studied here while salinity showed a significant positive correlation with dehydrogenase and alkaline phosphatase.

Total viable heterotrophic bacteria of soils were enumerated on R2A agar (Reasonar's agar). Criteria for selection of distinct isolates for identification was based on their morphological characteristics and based on CFU/g dry wt of sediment. Plate count on R2A agar indicates that substantial number of heterotrophic microorganisms was present throughout complete sediment profile of CRD. Selected isolates were identified based on 16S *r*RNA gene

sequencing. Community DNA were extracted from sediment samples and the 16S rRNA gene amplification were carried out. Denaturing gradient gel electrophoresis (DGGE) was performed to determine microbial profiling. DGGE gel band pattern showed that high microbial diversity presents even at higher depth. These indicate that microbial diversity did not decrease with depth. Bacterial community composition assessment within CRD sediment profile also carried out by next-generation Illumina-based sequencing approach. Three sediment samples were selected from CRD vertical profile in which two samples belonged to estuarine strata and one sample belonged to Pleistocene strata. Beta diversity within these samples by Unweighted Pair Group Method with Arithmetic Mean (UPGMA) suggest that 50-70 cm (silt) and 106-108 cm (silt) depth samples lies within estuarine sediment sequence shows more closely related microbial community composition while 1020-1030 cm depth sample lies within fluvial sediment sequence shows distinct microbial community composition as compare to above two samples. Microbial diversity analysis carried out by culturable and uncluturable methods (DGGE and metabarcoding) revealed the dominance of bacterial phyla *Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, *Chloroflexi*, and *Acidobacteria* in the Mahi River estuarine core.

Denitrifying bacteria [enumerated by most probable number (MPN) method] were abundantly found in CRD subsurface sediment samples throughout complete sediment profiles. Further, abundance of different genes was determined by quantitative-PCR (qPCR). Quantification of eubacterial 16S rRNA genes by qPCR indicate that within CRD sediment profiles high number of bacteria were present even at higher depth and throughout complete sediment profiles. Additionally, here we reported the presence of 8.5×10^4 to 5.93×10^7 *nirS* gene copies of denitrifying bacteria (involved in N cycle) and not detectable to 5.93×10^7 gene copies of the *dsrB* gene of sulfate reducing bacteria (involved in S cycle) in the 17 m deep Chokari (CRD) estuarine core.

Within CRD sediment profile higher microbial activities were observed at upper estuarine region while lower microbial activities were observed at Pleistocene region lies beneath to estuarine region indicating that upper part of CRD sediment profile which comprise younger estuarine sediment is biogeochemically more active.

Chapter 2: Linkage of sediment indigenous properties with microbial parameters in two laterally deposited Late Quaternary sediment cores of the Mahi River basin: A case study from Rayka and Rampura

Within Rayka **(**RYD) and Rampura (RMD) sediment profile, high alkaline phosphatase activities were observed at some of the deep samples. Phosphatase activities were high throughout these sediment profiles core indicating P stress in both sediment profiles. Kobayashi et al., 2008 reported phosphatase activity consistently throughout the core sediments down to the deepest slurry sample from 342.5 m below seafloor. β–D-glucosidase, protease, dehydrogenase and arylsulphatase activities decrease at higher depth within RYD sediment profile indicate that microbial activities that involves in C, N, and S cycles are reduce at higher depth. Within RMD sediment profile protease, dehydrogenase and arylsulphatase activities decrease significantly at higher depth but higher β–D-glucosidase and FDA hydrolysis activities were observed at some of deep samples. Ecoenzymatic stoichiometry revealed C and P limitation in RYD core samples and C, N, and P limitation in RMD core samples.

Physicochemical and geochemical characteristics of sediment samples such as organic C, inorganic C, pH, salinity, TDS, conductivity, particle size analysis, major oxide, trace element, and rare earth element content has been obtained with collaboration from Dr. Anupam Sharma, Birbal Sahni Institute of Palaeosciences, Lucknow, India.

Total viable heterotrophic bacteria of soils were enumerated on R2A agar (Reasonar's agar). Plate count on R2A agar indicates that substantial number of heterotrophic microorganisms was present throughout complete sediment profile of RMD and RYD. Fliermans et al., 1989 suggest that despite the variations from one stratum to another, there was no obvious overall decrease in viable bacterial numbers with increasing depth and Das et al., 2013 also reported that depth does not affect heterotrophic bacterial population.

Denitrifying bacteria and N_2 fixing bacteria were enumerated by most probable number (MPN) method. Denitrifiers are abundantly found in RMD and RYD subsurface sediment samples throughout complete sediment profiles. N_2 fixers were found up to certain depth after that N_2 fixer was not obtained within RMD and RYD sediment profile. Abundance of different genes was determined by qPCR. Community DNA were extracted from sediment samples and quantification of different gene were carried out. Quantification of 16S rRNA bacterial gene indicate that within RMD and RYD sediment profiles high number of bacteria were present even

at higher depth and throughout complete sediment profiles. Beck et al., 2011 reported 10^8 to 10^6 16S rRNA gene copies per cm^3 sediment from 0.5 to 20 m below seafloor within North Sea tidal-flat coastal quaternary sediments. Quantification of *nirS* gene of denitrifying bacteria (involved in N cycle) indicate that denitrifying bacteria were observed throughout both RMD and RYD complete sediment profiles. In this study, we also determined the abundance of *czcA* gene which encoded component of a Co/Zn/Cd efflux protein. *CzcA* gene abundance observed within ranged from 0 (not detected) to 659 copies per 10^5 16S rRNA gene copies within RYD core samples [total Zn content ranging from 35.9 to 81.4 ppm] and 0 (not detected) to 774 copies per 10^5 16S rRNA gene copies within RMD core samples [total Zn content ranging from 48.4 to 227 ppm].

Principal component analysis and Pearson's correlation analysis indicate significant linking of specific geochemical factors with microbial parameters in both the core profiles. However, the strength and direction (positive or negative) of correlation varied between the two sediment profiles. Notable among these were the strong positive relationship between P_2O_5 content and various enzyme activities and negative correlation of salinity and pH with enzyme activities in RYD core but not in RMD core. Likewise, a positive correlation between Ni and *czcA* gene abundance was observed in the RMD core but not in the RYD core. These results emphasize that sediment geochemical properties correlate differently with microbial parameters in the Late Quaternary subsurface sediment sequences. It would be interesting to study further the potential reasons for this distinguishable correlation pattern observed in both cores. Additionally, this work could assist future investigation on the alternation of microbial activity via deviating abiotic factors that link with microbial parameters to improve sediment ecosystem functioning of the region.

Chapter 3: Evaluation of bacterial strains isolated from subsurface sediment samples at Rayka and Rampura for heavy metal tolerance and Cr(VI) removal ability

Heavy metal contamination is major worldwide problem at industrial waste contaminated sites. Based on the metal bioavailability, metals in the environment can be classified into two categories. One is bioavailable (soluble, nonsorbed, and mobile) and another is non-bioavailable (precipitated, complexed, sorbed and nonmotile) (Kelley et al., 2002). Much of the research on metal bioavailability has been done in soil system because understanding the fate of metals in soil and sediments is crucial to determine the effects of metals on the biota. Soils/sediment usually exhibit higher concentrations of metals than water because metals are more likely to accumulate in the soil than being diluted and carried elsewhere in water.

Heavy metal resistance microbes could be potential agent for bioremediation of heavy metals polluted sediment (to reduce bioavailable metal) and it is cost effective. Bioremediation is defined as the "use of living organisms to clean up pollutants from soil, water, or wastewater" (EPA, 2016). Heavy metal is not completely degraded but oxidation states of heavy metals can be change by various microbes which reduce its bioavailability and therefore become less toxic for the biological systems. Microbial heavy metal tolerance in subsurface samples is indicative of long term ecotoxicological impact of metals and also could also reflect metal contamination of groundwater. However, the heavy metal tolerance characteristics of microbes isolated from subsurface river sediment profiles are still obscure.

In the present study, determination of heavy metal tolerance of bacterial strains isolated from two Late Quaternary sediment profiles (~28 m and ~25 m deep) located at the Mahi river basin was carried out. Identification of bacterial isolates by 16S rRNA gene sequencing revealed that bacterial isolates affiliated with phyla Actinobacteria, Proteobacteria, Firmicutes, and Bacteroidetes were dominant in both sediment profiles. Heavy metal tolerance of bacterial strains as determined by plate diffusion assay revealed order of metal tolerance as follows: Hg(II)<Cd(II)< Ni(II)<Cu(II)=Cr(VI). Chromate removal study in liquid medium suggested that bacterial strains procured from subsurface possessed the ability to remove Cr(VI) with varied magnitude. A packed bed column experiment indicated that bacterial strains obtained from subsurface have potential for Cr(VI) removal from different particle size consistency of the sediments.

5. References

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