Summary and Conclusions

SUMMARY

Although research on correlation between microbial and physicochemical parameters has made great progress in the past decade, their correlation within subsurface river floodplains in the Indian subcontinent is not well understood. River floodplains have global impact on biogeochemical cycling and have great economic importance as river landscapes can be used for various man-made activities therefore discovering microbial parameters and their correlation with surrounding geological parameters within vertically stratified floodplain ecosystems have immense importance. In this study, the geological complexities of subsurface stratified sediment sequences of the Mahi River basin were used as a model to understand the inter-relationship between microbial parameters with geological characteristics. Microbial characteristics and their correlation with sediment geochemical properties within three different cores from different locations in the Mahi river basin spanning up to ~28 m depth has been carried out. Evaluation of bacterial strains isolated from subsurface sediment samples for heavy metal tolerance and Cr(VI) removal ability from different consistency of soils was also carried out here to determined ability of bacterial isolates in reducing leaching of Cr(VI) through sediments of different consistency.

- Core samples were collected from Chokari (CRD - 22°13’49"N, 72°55’41"E), Rayka (RYD - 22°26'55"N, 73°05'27"E), and Rampura (RMD -22°23'0.3"N, 73°05'15"E) sediment profiles of the Mahi River basin by the rotary drilling method.
- CRD core was located at the river estuary and lies within a younger terrace of river basin while RYD and RMD cores lie within an older terrace of the river.
- Core drilling was carried out up to depth 17 m at CRD, 25 m at RMD and 28 m at RYD.
- Chokari (CRD) core is comprised of sandy silt, silt, silty sand, sand horizons of Holocene tidal flats estuarine deposits up to ~7.7 m depth and gravel, palaeosols, silty sand, sand, and silt horizons of Pleistocene fluvial deposits from 7.7 m to 17 m depth. Rayka (RYD) and Rampura (RMD) core comprised Late Pleistocene stratigraphic sequences that contain four distinct palaeosols among which three are brown color palaeosols and one is red color palaeosol. Further, the aeolian horizon was observed up to ~4.8 m depth in the RYD core while in the RMD core aeolian horizon was observed up to ~1 m depth.
- Among three cores, CRD core showed relatively higher dehydrogenase activity (DHA), β-glucosidase activity (β-GA), alkaline phosphatase activity (alkaline PA), protease enzyme

(PTA), and arylsulfatase activity (ASA) as compared to RYD and RMD cores indicating that estuarine core from a younger terrace surface had higher microbial activities as compared to fluvial cores from older terrace surfaces.

- Further, higher alkaline phosphatase (P cycle enzyme) and fluorescein diacetate hydrolysis activities were observed in all three cores as compared to dehydrogenase, β-glucosidase (C cycle enzymes), protease (N cycle enzyme), and arylsulfatase (S cycle enzyme) activities indicating P limitation in all three cores of Mahi River basin.
- Ecoenzymatic stoichiometry analysis also revealed P limitation in the CRD core, C and P limitation in the RYD core samples and C, N and P limitation in the RMD core samples.
- Plate counts on R2A agar indicated that heterotrophic bacteria ranging from 10^5 to 10^7 (per gram dry weight of sediment) were present in the CRD core throughout the different depths and in RYD and RMD cores heterotrophic bacteria were found in range of 10^3 to 10^5 per gram dry weight of sediment.
- The denitrifying bacterial population was observed within the range of 10^2 to 10^5 g^{-1} dry weight of sediment in the CRD core, 10^2 to 10^4 g^{-1} dry weight of sediment in RYD and RMD cores. Low counts of N_2 fixing bacteria were observed up to 4.5 m in RYD samples while they were found in relatively higher numbers up to ~11 m within the RMD core.
- Bacterial 16S rRNA gene copy numbers (determined by qPCR) ranged from 10^6 to 10^9 per gram dry weight of sediment in the CRD core; were in the range of 10^6 to 10^7 per gram dry weight of sediment within RYD core samples and in the range of 10^5 to 10^8 per gram dry weight of sediment within RMD core samples. Archaeal 16S rRNA gene copy numbers were observed within the range of 10^3 to 10^5 per gram dry weight of sediment in the RYD core and 10^4 to 10^5 per gram dry weight of sediment in the RMD core. qPCR based abundance of bacterial and archaeal 16S rRNA gene revealed that bacteria were more abundant in RYD and RMD cores as compare to archaea.
- *NirS* gene copy numbers were observed within the range of 10^4 to 10^7 per gram dry weight of sediment in the CRD core, within the range of 10^4 to 10^5 per gram dry weight of sediment in the RYD and 10^3 to 10^5 per gram dry weight of sediment in the RMD core.
- Relatively higher culturable counts, 16S rRNA gene copy numbers, denitrifiers counts and *nirS* gene abundance in the CRD core as compare to RYD and RMD cores suggesting presence of high denitrification rates in the estuarine region and also indicative of low

oxygen. In the fluvial cores also high denitrification suggests high nitrate, coupled with limited C and O_2 levels promoting loss of nitrates to gaseous nitrogen in these ecosystems.

- Abundance of *dsrB* gene was observed within the range of 0 (not detectable) to 10^7 per gram dry weight of sediment in the CRD core. The abundance of the *czcA* gene was observed within the range of $<10^3$ (not detectable) to 10^4 per gram dry weight of sediment in both sediment cores, however more number of samples at different depths showed no detectable *czcA* gene copies in the RYD core as compared to the RMD core.
- Isolation and identification of culturable heterotrophic bacteria revealed that culturable bacteria belonging to *Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, and *Firmicutes* phyla were dominant within all three cores. Organisms belong to these phyla have been among the most abundantly sampled organisms due to the ease of their cultivation might be due to this dominance of these four phyla were observed in all three cores.
- Taxonomic affiliation of DGGE band sequences observed corresponded to organisms of the phylum *Proteobacteria* (87%), *Firmicutes* (7%), *Chloroflexi* (3%), and *Bacteroidetes* (3%) in the CRD core.
- Amplicon analysis of V3-V4 region of the 16S rRNA gene by Illumina sequencing (i.e. metabarcoding) was performed for three representative core samples includes 0.5-0.7 m depth sample (CRD 2), 1.6-1.8 m depth sample (CRD 6), and 10.2 to 10.3 m depth sample (CRD 27) revealed that *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, *Chloroflexi*, and *Acidobacteria* were most abundant top five phyla observed in these CRD core samples.
- DGGE analysis of microbial community composition revealed that core samples up to depth ~ 6 m clustered distinctly with respect to core samples that lie from ~ 6 m to 17 m depth. Likewise, the metabarcoding study also indicates that the upper part of CRD core samples (i.e. CRD 2 and CRD 6) had distinct microbial community composition than the lower part of the CRD core sample (i.e. CRD 27).
- Functional prediction from metabarcoding data of CRD core samples indicates that ammonium oxidizers, atrazine metabolizing bacteria, nitrite reducer (involved in denitrification process), nitrogen-fixing bacteria, sulfate reducer, sulfide oxidizer, xylan degrader, and bacteria that carried out dehalogenation are prominent within CRD core samples.

- Correlation between microbial parameters and physicochemical parameters determined by principal component analysis (PCA) and Pearson's correlation analysis indicated that bulk org. C, salinity, P_2O_5, Fe_2O_3, MgO, Cu, Ni, V, and Zn are correlated or associated significantly with microbial parameters in the CRD core. In RYD and RMD cores, bulk org. C is not correlated significantly with microbial characteristics but various trace elements, rare earth elements, K_2O, P_2O_5, EC, TDS, and salinity correlate or link significantly with microbial parameters. The direction and magnitude of the correlations observed between sediment microbial and physicochemical characteristics differ in all three core profiles might be due to different dynamics of *in situ* mineral-microbes interactions and/or discrepancy in bioavailability of elements.
- Among all bacterial strains obtained from Rayka and Rampura cores, ~7% bacterial strains showed the highest tolerance (i.e.10-20 ppm) for Hg(II), 13% showed tolerance capacity >50 ppm for Cd(II), ~25% of bacterial strains exhibit tolerance >100 ppm for Cr(VI), 5% bacterial strains had the highest (i.e. 300-400 ppm) tolerance capacity for Cu(II) and ~12% bacterial strains showed tolerance capacity between 200-300 ppm for Ni(II).
- The five bacterial strains showing the MIC greater than 40 ppm for Cr(VI) were selected to determine their Cr(VI) removal efficiency in R2A and SE+Peptone broth. Among these five isolates, *Bacillus* sp. RYD 15.1A exhibited higher Cr(VI) removal efficiency in R2A and SE+Peptone both while *Streptomyces* sp. RMD 42.2B exhibits the highest Cr(VI) removal efficiency among all five isolates in SE+Peptone broth hence these two bacterial strains were used further for microcosm study.
- *Bacillus* sp. RYD 15.1A was able to remove 87%, 88.6%, and 49% Cr (VI) from leachate of medium sand, fine sand, and silt and clay containing packed columns respectively [all columns have 5 ppm Cr(VI)]. Likewise, *Streptomyces* sp. RMD 42.2B was able to remove 91.6%, 93.7%, and 69% Cr (VI) from leachate of medium sand, fine sand, and silt and clay packed columns respectively [all columns have 5 ppm Cr(VI)].
- The significant percent reduction of Cr(VI) by *Bacillus* sp. RYD 15.1A and *Streptomyces* sp. RMD 42.2B in the leachate of the experimental columns as compared to the control columns indicate that both strains are capable of removing Cr(VI) from a different type of

soil materials and highlights the implication of subsurface microbes in bioremediation of metal pollutants.

CONCLUSION

Detectable microbial enzyme activities throughout depth in all three core profiles, abundance of heterotrophic bacteria (based on culturable counts and 16S rRNA gene abundance), denitrifying bacteria (based on MPN counts and *nirS* gene abundance) and sulfate-reducing bacteria (based on *dsrB* gene abundance) observed in the Mahi River cores suggesting role of the Mahi River floodplain sediments in C, N, P, and S biogeochemical cycling. Enzymatic stoichiometry analysis revealed P limitation within all three cores, C limitation in RYD and RMD core samples, and N limitation in RMD core samples. Furthermore, relatively greater microbial abundance and activities observed in the estuarine core that lies on younger terrace than fluvial cores of older terrace indicating that younger sediment deposits of Mahi River basin are microbiologically more active than older sediment deposits. This might be due to greater nutrition limitation in older sediment deposits as compare to younger sediment deposits as indicated by enzymatic stoichiometry. Microbial diversity study revealed that bacteria belong to phyla *Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, and *Firmicutes* were present in all three core profiles of Mahi River basin. Correlation between microbial and physicochemical parameters revealed key physicochemical parameters that linked with microbial characteristics such as org. C, P_2O_5, and salinity. These key physicochemical parameters could assist future investigation to altered microbial activities for improving sediment ecosystem functioning of the region. Moreover, chromate removal studies in liquid media and a packed bed column experiment revealed that bacterial strains obtained from the subsurface have the ability to remove Cr(VI) from liquid media as well as ability of preventing Cr(VI) leaching through sediments of different consistency. This finding may be exploited in further exploring the potential of subsurface microbes for Cr(VI) removal from soils/sediments of different textural characteristics for prevention of leaching in to the ground water.