Summary and overview

Soil microbes are vital for soil quality and soil ecosystem processes due to their involvement in organic matter dynamics, nutrient cycling, decomposition of organo-pollutants and soil formation by rock weathering. Characterizing microbial diversity and activity in soils, would enable a better management of the soil ecosystem functions, because the ability of an ecosystem to withstand serious disturbances may be reflected in its microbial component. How soil microbial activities and communities are affected by anthropogenic and environmental perturbations in semiarid alluvial regions of Indian subcontinent, which occupy about 37 % of the total geographic area of the country, is not well understood.

The present study investigated the effect of anthropogenic and environmental parameters on soil microbial activity and diversity in semiarid alluvial soils (developed on River sediment) around Mahi River region, western India. The soils along the region studied are well characterized geomorphologically and presented several unique features that are of interest in studying from a microbiological point of view. First, the alluvial soils have been dated and can be classified as relatively recent and older (last 30 ka to 45 ka); secondly, they have been mapped as agriculturally fertile or badlands, some of them of same chronological age and of common sediment origin; third, there are certain spots along the estuarine part of the river where treated industrial effluent is discharged in to the water bodies; fourth, some regions along the river margin have succumbed to seasonal floods and suffer regular inundation; lastly several exposed sections of the sediments up to several meters in depth are accessible which have been extensively characterized geochemically and geomorphologically and stratigraphy correlated with chronological dating of the sedimentation events is available. These exposed sections allowed access to deeper and older sediments many of which are buried soils or palaeosols. Several of the features of the region under study have been exploited in this work to compare microbiological activity and diversity and provided an opportunity to investigate different geomorphologic settings under a single climatic zone (semiarid). Further, the study was extended to coastal soils developed on carbonated rocks under same climatic zone wherein the microbial weathering of parental rock “miliolite” was studied.
The approach used for the different studies here was polyphasic, involving cultivation-dependent as well as molecular studies and *in-situ* activity measurements. Qualitative and quantitative components of microbial community structure have been assessed, which made it possible to understand to some extent the relationship between structure of functional microbial guilds and the soils parameters in the ecosystem. Following section provides a brief summary of the thesis, chapterwise, for the five Chapters (Chapters 3-7) which present the research findings of this work.

**Chapter 3. Establishing a link between soil characteristics and microbial activity processes in semiarid Mahi River basin by polyphasic approach**

Estuarine and alluvial soils were compared for microbial activity (as an index of soil enzymes i.e. dehydrogenase and protease) in relation with soil properties such as soil organic matter (SOM), soil moisture content (SMC), soil texture (sand, silt and clay) and soil pH has been addressed. The study was conducted at 7 sites spread over 3 locations (2 were from alluvial zone and 1 was from estuarine zone), each sampled at interval of 25 cm from the three exposed sections along a 30 km long stretch of the Mahi River in western India. High microbial activity was noticed in estuarine soils than in alluvial soils. Dehydrogenase activity in both alluvial and estuarine soils indicated positive correlations with SOM, SMC and a moderate correlation with clay content. On the contrary, the protease activity showed poor correlation with SOM, SMC and clay content of alluvial soils, however significant positive correlations were noticed in estuarine soils. No correlation was observed between these two enzymes. A negative correlation existed between soil depth and both the enzymes at *P* level < 0.001. The findings demonstrate that SOM and SMC, clay content and soil depth are the important determinants for dehydrogenase activity (indicative of organic matter transformation) in both alluvial and estuarine soils, whereas the soil depth is the lone determinant for protease in alluvial soils and its correlation with other properties in estuarine soils is site specific.

Investigations on palaeosols intercalated within the late Quaternary continental sequences of Mahi River basin indicates that two palaeosols P1 and P2 have been dated back to ~ 45 and 30 ka, respectively. The two palaeosols were compared for their soil and microbiological characteristics. High microbial biomass carbon, soil respiration, microbial quotient, metabolic quotient (q-CO₂) and
dehydrogenase activity revealed that the organic matter decomposition and carbon flow to the atmosphere were much higher in P1 palaeosol. Soil in-situ enzymes, specific enzymatic quotients (SEQ) and specific enzyme indices (SPI) revealed that P1 has been more productive than P2. The order of biogeochemical cycling in P1 was N > P ≥ C whereas P2 showed N > P > C. 16S rRNA gene profiling using denaturing gradient gel electrophoresis (DGGE) indicated greater heterotrophic bacterial diversity and higher carrying capacity of P1 compared to P2. The contrasting microbial activities and diversity in these palaeosols point to different environments during palaeosol formation and are in general agreement to the climatic inferences drawn earlier workers.

A study involving the microbiological profiling of an entire exposed section (approximately 30 m in height) was carried out to explore critical physiochemical determinants that govern microbial activity in the different strata of exposed subsurface soils and sediments along the Mahi River bank. A total of 51 triplicate composite samples were collected at an interval of 25 cm from exposed soils and sediments at a subsurface section measuring about 28 m length near Rayka. Enzyme activities (dehydrogenase [DHA] and phosphatase [APA]), were varied particularly with the sampling depth and nature of the soil. Along the section, 5 microbial activity hot spots distributed at a depth of 2 to 2.5 m, 4.75 m, 6.5 m and 7.5 m were found. High microbial diversity as indicated by 16S rRNA-DGGE was noticed in sandy silt, silt with bedded calcrete, brown soil and surface top soil. DGGE based α-proteobacterial diversity studies revealed vertisol had higher alpha proteobacteria diversity which is an indicative of good soil health. Multivariate statistical analysis such as principle component analyses (PCA), canonical correspondence analysis (CCA), linear regression models and Pearson linear correlations were carried out to ascertain the critical geochemical, mineralogical and other soil parameters that may determine the microbial activity. Results indicated that organic carbon (OC), SMC, fungal spores (FS), black carbon (BC), structured carbon (SC), Al, Fe, Mg, Mn and Zr are the critical determinants for Rayka soil and sediment microbial activity at P <0.05. Further, microbial activity (as an index of enzymes and respiration) and their relation with various phosphorus fractions were deciphered. Significant positive relation (r = 0.8; P <0.001) between APA and organic phosphorous % (OP %) was observed. A weak negative correlation (r = 3.9) between inorganic phosphorous (IP) and APA also
noticed at $P < 0.05$. Basal soil respiration (BSR) was positively correlated with both IP ($r = 0.55$, $P < 0.05$) and OP ($r = 7.8$, $P < 0.01$). Although there was a significant ($P < 0.05$) relation between OP and DHA, the strength of the relation is ($r = 3.9$) weak. A negative correlation was observed between apatite inorganic phosphorous (AIP %) and BSR ($r = -0.58$).

**Chapter 4. Microbial activity and diversity profiles of agricultural, pristine, flood affected and unaffected soils of common sedimentary origin: Case studies from Rayka, Majpur and Pilludra Gujarat, western India.**

Agricultural (Agri) and pristine (Pri) soils (considered as badlands) studied in the present investigation were collected from two different fields situated near Rayka. Geologically both the sites were evolved simultaneously and fall under the same sedimentary origin. Significantly higher ($P < 0.05$, LSD) values of both $C_{mic}$ and BSR were found in agricultural soils. An increase in soil *in situ* enzymes was noticed with agricultural soils ($P < 0.05$, LSD, $N = 3$). Enzymes such as DHA, APA, GSA, and PSA were reduced to 29.1%, 43.4%, 18.9% and 25.78% respectively in pristine soils. Bacterial diversity of investigated with 16S rRNA based DGGE molecular fingerprinting indicated very complex but not very contrasting microbial diversity in both agricultural and pristine soils. Relatively high total 16S rRNA based microbial diversity was noticed in agricultural soils than in pristine soils.

A large discrepancy in total viable count of diazotrophs was noticed. The counts were $2.5 \times 10^6$ (in agri soil) to $4.5 \times 10^4$ (in pri soil). Amplified ribosomal DNA restriction analysis (ARDRA) indicated that agricultural soil diazotrophic bacterial isolates fall in 12 different phylogenetic groups where as pristine soil isolates fall in 9 different groups at a similarity coefficient of 0.90. These results tentatively indicate that there was relatively higher diversity in culturable diazotrophs of agricultural soil than in non-agricultural soil. Most of the agricultural soil isolates are represented under phyla *Proteobacteria*, *Actinobacteria* and group *Firmicutes* whereas in pristine soil, most of the isolates were affiliated to *Firmicutes*. 16S rRNA based diazotrophic community was established with culture dependent -DGGE (CD-DGGE) and activity was determined based on their *in-situ* respiration activity. Higher diazotrophs diversity was noticed with agricultural soil than pristine soil by CD-DGGE. Relatively high amount of BSR activity ($88.24 \pm 9.3 \, \mu g \, CO_2-C \, ml \, broth^{-1}$)
was noticed with agriculture soil consortium than in pristine soil based consortium (52.84± 9.3 µg CO₂-C ml broth⁻¹).

Effect of annual floods (environmental impact) on soil microbial diversity and activity in agricultural soils has been studied. Three soil samples named as S1 (0-10 cm), S2 (10-20cm) and S3 (20-30cm) were collected with an interval of 10 cm from an agriculture field near Mujpur which was inundated with fluvial sediment. Control reference soil named S4 was collected from nearby unaffected agricultural field from Pilludra. Relatively high amount of heavy metals were detected in the flood affected soils. Inundated fluvial sediment significantly decreased ($P <0.05$) the soil biochemical properties ($C_{mic}$, BSR and SIR) in this very particular region. Microbial metabolic potential (q-CO₂) was higher in S1 and S2 samples. The detrimental effect of fluvial sediment on soil enzymes (DHA, GSA, APA and PSA) was noticed. Significant lower amount of total cultivable bacteria was observed in flood affected soils. Most probable number (MPN) indicates that high amount of the nitrogen fixers, denitrifiers, phosphate solubilising bacteria, cellulose degrading bacteria were observed in unaffected agricultural soil (S4). High amount of H₂S producers were indentified only with flood effected S1. Majority of the dominant bacterial phyla in S4 samples were Proteobacteria, Actinobacteria and Firmicutes; wile dominant phyla in S1 samples are found to be Firmicutes, Actinobacteria and Acidobacteria. Bacterial genera found in S4 sample were Bacillus (Family Bacillaceae), Naxibacter (Family Oxalobacteraceae), Arthrobacter (Family Micrococcaceae) and Beijerinckia (Family Beijerinckiaeae). Bacterial isolates affiliated to genera Bacillus, Promicromonospora. (Family Promicromonosporaceae) and Granulicella (Family Acidobacteriaceae) were found in S1 sample. The 16SrRNA gene based bacterial diversity as detected by DGGE was higher in S4 as compared to flood affected samples. Statistical analysis indicated an apparent negative impact of sand and heavy metals on microbial activity parameters. A strong positive correlation among SMC, TOC, clay and soil microbial activity parameters was noticed in this particular environment.

**Chapter 5. Microbial activity and diversity profiling in pristine and long term industrial effluent contaminated semiarid landscapes**
Soil pollution by elevated heavy metals is a major concern in alluvial plains. In the present study microbial activity, diversity and microbial ammonia oxidative functions in treated industrial waste effluent (IWE) polluted soils were investigated by polyphasic approach. Soil samples were chosen from three different locations under semiarid climate. Sample R1 is a natural pristine soil without any contamination, collected from a site nearby Ryaka. Samples R2 and R3 were collected from 2 different sites situated along the contaminated Mini River basin. These sites have been receiving mixed bag of contamination for several years by episodic streams of Mini River containing industrial waste water. R4 is a sediment sample collected from IWE settling lagoon near Sarod.

ICP-AES analysis revealed the elevated levels of heavy metals in samples R2, R3 and R4. Particularly elevated heavy metals such as Cu, As, Cr and Pb were found in R3 and R4 samples. Significantly lesser ($P < 0.05$, LSD) values of soil $C_{\text{mic}}$, BSR and SIR and in-situ microbial enzymes such as DHA, APA, $\beta$-glucosidase (GSA), and protease (PSA) were found in metal polluted soils (R2, R3 and R4). Higher $q$-CO$_2$ values found in contaminated soils. Contrasting eubacterial community shift along the contamination gradient was noticed by 16S rRNA based DGGE molecular fingerprinting. No significant ($P < 0.05$) difference among the diversity indices $H'$ and $E^l$ was observed. However $E^l$ values were relatively high in R2 and R3 samples. A total of 26 bands were excised from the DGGE gel, reamplified, cloned and sequenced for phylogenetic analysis.

Major eubacterial phyla in R1 soil were affiliated to Poteobacteria and Actinobacteria, where as phyla Actinobacteria, Firmicutes and Acidobacteria were dominant in R2 and R3 soils. Phylum Spirochaeta was found only in R2 soil. Classes Alphaproteobacteria and Betaproteobacteria were found to be specific to R1 where as classes Epsilonproteobacteria and Gammaproteobacteria were specific to R2 and R3 samples. Major euabcterial phyla in contaminated sediment R4 were found to be Actinobacteria, Firmicutes and Acidobacteria. Dominance of unclassified bacteria was observed in contaminated soils (R2 and R3) and sediment (R4).

Potential nitrification rate (PNR) was found to be highly sensitive to soil contamination. Quantitative real time PCR (q-PCR) results indicate that relatively, high amount of bacterial 16S rRNA, archaeal 16S rRNA, ammonia monooxygenase
gene \(( amoA)\) of ammonia oxidizing archaea \(( AOA-amoA)\) and of ammonia oxidizing bacterial \(( AOB-amoA)\) copies (log transformed) were found in uncontaminated R1 soil than contaminated R2 and R3 soils. Both AOA and AOB-amoA gene copies significantly decreased in contaminated soils. In R1 the ratio of AOA-amoA to AOB-amoA gene was found to be high \((36.82 \pm 6.75)\), however the ratios significantly \((P <0.05, \text{LSD})\) decreased in R2 and R3 soils. DGGE analysis of \(amoA\) gene diversity indicated complex and contrasting AOA-amoA gene and AOB-amoA gene profiles along the contamination. Relatively, higher AOA-amoA and AOB-amoA gene diversity was noticed in pristine R1 soil than contaminated R2 and R3 soils. Phylogenetic analysis of the nucleotide sequences retrieved from DGGE bands revealed that thaumarchaeal “group 1.1b” was dominant AOA in these soils. Thaumarchaeal “group 1.1a” was found only in contaminated soils (R2 and R3) where as thaumarchaeal “group 1.1a associated” was found only in pristine soil R1. All the 10 AOB-amoA DGGE band clones were affiliated to \(\text{Nitrosospira}\)-like sequences and \(\text{Nitrosomonas}\)-like sequences. Nevertheless, \(\text{Nitrosospira}\)-like sequences were dominant ones, consisted of 70% of total sequences. \(\text{Nitrosomonas nitrosa}\) cluster-8 was found only in contaminated soils, whereas \(\text{Nitrosospira}\) cluster-11 was specific to pristine soil. \(\text{Nitrosospira}\) cluster-3a is common for all three soil samples. In addition to this, \(\text{Nitrosospira}\) cluster-1, 2, and 4 were also noticed among soil AOB-amoA genetic profile.

Microcosm studies were conducted to investigate the initial response of AOB and AOA to heavy metals Cu and As. Two different acidic alfisols \(viz.\) RA and GZ spiked with different concentrations of As, Cu and As+Cu were incubated for 10 weeks. Significant reduction \((P <0.05)\) in copy numbers of archaeal 16S rRNA, bacterial 16S rRNA and AOB-amoA genes was noticed at elevated metal concentrations, while reduction in AOA-amoA gene copy numbers was minimal. AOA was found to be more abundant than AOB in all the treatments and the ratio of AOA to AOB ranged among 94-303 in RA soils and 21-39 in GZ soils. The soil PNR differed across different treatments, and significantly \((P <0.05)\) decreased with increasing As and Cu-concentrations. PNR was significantly correlated with both AOA and AOB, nevertheless the correlation was much stronger for AOA. DGGE of functional AOA-\( amoA\) gene revealed no apparent community shift for AOA even at higher concentrations of As and Cu. Phylogenetic analysis of AOA-amoA gene from
4 clone libraries indicated that all the AOA-amoA gene sequences (400) were placed within 3 distinct clusters from soil and sediment group 1.1b. Cluster-III of group 1.1b was found to be the most dominant one containing 51%-59% of total sequences, indicating their potential role in nitrification of acidic alfisols polluted with As and Cu.

**Chapter 6. Exploring multimetal resistant plant growth promoting bacteria (PGPB) for enhancing plant growth in heavy metal polluted soils.**

Metal resistant bacteria were isolated from sediment contaminated with industrial effluent R4. Isolates were identified and screened for their plant growth promoting (PGPR) traits. Gnotobiotic plant inoculation experiments were conducted with the most promising bacterial isolate identified as *Enterobacter* sp. (by 16S rRNA gene sequence phylogeny) to evaluate its plant growth promoting potential under elevated levels of Cr (VI) (up to 400 mg kg\(^{-1}\) soil) and Pb (II) (up to 400 mg kg\(^{-1}\) soil). *Enterobacter* sp. C1D was found to be multi-metal resistant in nature with multiple plant growth promoting traits. In order to know the mechanisms involved in plant growth promotion under elevated metal concentrations, PGPR traits were studied in presence of metal.

Cr (VI) toxicity was noticed in above and below ground biomass of *Vigna radiata* GM4 during plant experiments. However, plant inoculation studies with *Enterobacter* sp. C1D significantly increased (\(P = 0.05\)) root length, shoot length, shoot weight, root weight and chlorophyll content of the plants in a range of Cr (VI) treatments. Plant tolerance towards Cr (VI) measured as effective concentration (EC\(_{50}\)) showed higher values with *Enterobacter* sp. C1D treated plants as compared to the uninoculated plants at all concentrations of Cr (VI). High amount of acidic and alkaline periplasmic phosphatases, elevated IAA production and ACC deaminase activities probably enable *Enterobacter* sp. C1D to enhance plant growth in Cr (VI) amended soils.

**Chapter 7. Bacteriology of miliolite, a bioclastic lime stone: Bacterial diversity, activity and potential role of bacterial in weathering of miliolite**

Miliolite and surface red soil sequences were collected from the miliolite-bearing natural section in Gopnath, Gujarat, India. Calcium was found to be the most
abundant element in miliolite and its proportion was found to be $75 \pm 6.71\%$ as revealed by X-ray fluorescence XRF. Potential carbonate dissolving bacteria were isolated, characterized and screened their ability to disintegrate miliolite. A total of 14 bacterial isolates showed significant Cz/Cs ratio (zone of clearance/colony size) on DB medium containing CaCO$_3$ and miliolite agar. Further, these isolates were chosen for organic acid profiling by HPLC. Lactic acid and acetic acid are found to be major organic acids, while pyruvic acid and malic acids are minor ones in assisting miliolite dissolution by these organisms. Phylogenetic diversity of carbonate dissolving bacteria was deciphered by ARDRA and 16S rRNA sequencing analysis. *Firmicutes* (Families *Bacillaceae* and *Staphylococcaceae*) and *Actinobacteria* (Family *Promicromonosporaceae*) represent two keystone culturable carbonate-dissolving heterotrophic bacterial phyla involved in carbonate dissolution and attendant miliolite weathering. Carbon sources had contrasting effects on bacteria mediated Ca$^{2+}$ release miliolite. Short-term microcosm experiments were conducted with isolates *Staphylococcus* sp. M16, *Bacillus* sp. M12 and *Xylanimonas* sp. RS25 for demonstration of bacterial mediated miliolite weathering. Results clearly indicated the bacterial metabolic activity in dissolution of calcite. Bacterial colonization, erosion and pitting of calcite crystals was well documented by scanning electron microscopy SEM. Calcite dissolution was further confirmed by XRD pattern and XRF analysis. The order of bacterial ability in dissolution of miliolite was found in the following order: *Staphylococcus* sp. M16 > *Bacillus* sp. M12 > *Xylanimonas* sp. RS25. SEM images indicate that bacterial activity increased the surface area of calcite, so that the process of chemical and biological weathering would be further accelerated. Calcite mineral surfaces were aggressively coated with a thick complex microbial biofilm by *Staphylococcus* sp. M16. The present study improves our conceptual understanding of microbial communities as important players in carbonate weathering, which has a wide range of implications; from the elucidation of biogeochemical cycles to the potential impact of atmospheric CO$_2$ sinks.

**Salient research findings of the work**

Table S1 provides a summary of the different microbial activity and diversity parameters analyzed in various settings. The key findings that have emerged from this work are emphasized below.
Relatively estuarine zone showed high microbial activity as compared to alluvial zone in the semiarid Mahi River region. Two soil enzymes dehydrogenase and protease showed different relationships with soil properties depending upon the alluvial and estuarine zones. SOM, SMC, clay content and soil depth are the main determinants for soil dehydrogenase activity and subsequent organic matter transformation in both zones. Soil protease activity and its critical determinates were found to be site specific.

The order of microbial activity in biogeochemical cycling in chronologically older (45 ka) P1 palaeosol was found to be N > P ≥ C, whereas in P2, relatively younger (30 ka) palaeosol it was N > P > C. P1 showed high heterotrophic bacterial diversity and broader carrying capacity than P2. Contrasting microbial activity and diversity in these Mahi River palaeosols indicate two different palaeo-environments and are in agreement with differences in climatic inferences drawn by earlier workers.

Microbial activity and diversity are affected by contrasting soil or sediment physicochemical gradient in the exposed section of nearly 30 m in the semiarid region. Multivariate statistical analysis indicated that OC, OP, SMC, FS, BC, SC, Al, Fe, Mg, Mn and Zr are the critical determinants for microbial activity in exposed shallow subsurface soils and sediments of Mahi River basin.

Soil management practices influenced soil physical and chemical characteristics and brought about changes in the soil microbial activity, community structure and function. Semiarid soil quality has been improved by sustainable agricultural practices. Higher bacterial diversity of eubacteria in agricultural soil as compared to pristine soil. Agriculture practice enhanced the diazotrophic bacterial population size. Floods have led to distinct alterations of the soil ecosystem functioning. Microbial mediated organic matter dynamics, nutrient cycling and decomposition processes in agriculture fields are decreased by fluvial sediment inundation. The soil health in the flood affected field is in endangered which suggest the importance of modifying management for sustainable agriculture. Enzymes, q-CO$_2$ values, bacterial phyla *Firmicutes*, *Actinobacteria* and *Acidobacteria* can be used as sensitive biological measure to assess or detect the soil disturbances lead by environmental perturbations in semiarid soils.
Contamination by industrial waste effluent (IWE) had significant negative impacts on bacterial abundance, activity and community structure in semiarid soil/sediments. Microbial parameters such as $C_{\text{mic}}$ and extracellular enzymes are found to be good responsive measures for monitoring the toxicity of IWE deposited in the environment. It can be inferred that dehydrogenase, $q$-$\text{CO}_2$ and bacterial phyla *Firmicutes*, *Actinobacteria* and *Acidobacteria* could be used as soil indicative parameters for soil contamination by mixed bag of pollutants.

Nitrification activity was found to be significantly ($P < 0.05$) decreased by IWE in semiarid soils. Relatively, AOB-amo$A$ gene diversity is more decreased than AOA-amo$A$ diversity by IWE. Thaumarchaeal “group 1.1b” might be playing potential role in ammonia oxidation of pristine and IWE contaminated soils.

Soil potential nitrification rate (PNR) was significantly decreased by heavy metals As and Cu in acidic alfisols. AOA were found to be more abundant than AOB in As and Cu spiked acidic alfisols. Together these results may implicate the dominant role of AOA over AOB in ammonia oxidation observed in Cu and As amended acidic alfisols. AOA-community did not respond to As and Cu in acidic alfisols. Thaumarchaeal AOA group 1.1b might be playing potential role in ammonia oxidation of oligotrophic acidic soils. These results could support further the hypothesis that physiological differences between AOA and AOB may facilitate them to occupy distinct ecological niches. These results indicate that soil PNR could be used as soil quality indicator for heavy metal perturbations.

Heavy metals significantly reduced plant growth parameters of *Vigna radiata* GM4 (mung bean) plants. The bacterial strain *Enterobacter* sp. C1D exerted substantial growth promoting effects on the *Vigna radiata* var GM4 plants in Cr (VI), Pb (II) stress and unstressed conditions. Plant tolerance towards both Cr (VI) and Pb (II) has been significantly increased by *Enterobacter* sp. C1D. High amount of acidic and alkaline periplasmic phosphatases, elevated IAA production and sufficient ACC-deaminase activities probably enable *Enterobacter* sp. C1D to enhance plant growth in Cr (VI) and Pb (II) amended soils.
Bacterial phyla *Firmicutes* and *Actinobacteria* represent two keystone culturable carbonate-dissolving heterotrophic bacteria involved in *in-vitro* carbonate dissolution and attendant miliolite weathering. The order of bacterial ability in dissolution of miliolite was in the following order *Staphylococcus* sp. M16 > *Bacillus* sp. M12 > *Xylanimonas* sp. RS25. Lactate and acetate are two major organic acids in dissolving miliolite by the organisms. The efficacy of organic acids in dissolution of miliolite was found to be in following order: acetate > lactate > succinate > gluconate > pyruvate.

Other contributions in terms of tangibles obtained through this work are as follows:

- About 500 new nucleotide sequences have been added to the GenBank database. Out of these 80 are 16S rRNA gene sequences from cultivated microbes as well as clones. They represent bacteria present in hitherto unexplored environmental settings. Remaining 425 are sequences of AOA-*amoA* and AOB-*amoA* genes. These sequences represent potential ammonia oxidizing microbes in polluted soils.

- A collection of novel bacterial isolates have been obtained. They have interesting properties such as N fixation, multimetal resistance, miliolite (carbonate) dissolution. A particular multi-metal resistant bacterial isolate *Enterobacter* C1D, has excellent potential as a biofertiliser for plant growth promotion in metal polluted soils. Carbonate dissolving bacterial isolates obtained could be further utilized in applied biotechnological aspects (e.g. dissolution of carbonate clogs from irrigation pipes).
Table S1: Summary of the microbial activity and diversity parameters of discrete soil ecosystems of Mahi River basin. Rows with same colour are compared with each other.

<table>
<thead>
<tr>
<th>Sample</th>
<th>DHA</th>
<th>APA</th>
<th>PSA</th>
<th>GSA</th>
<th>BSR</th>
<th>Cmic</th>
<th>SIR</th>
<th>q-CO₂</th>
<th>DGGE Diversity</th>
<th>Dominant bacterial phyla&lt;sup&gt;1&lt;/sup&gt;</th>
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</thead>
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<tr>
<td>Agricultural soils</td>
<td>High*</td>
<td>High*</td>
<td>High*</td>
<td>High*</td>
<td>High*</td>
<td>High*</td>
<td>N. S.</td>
<td>High*</td>
<td></td>
<td>Proteobacteria, Actinobacteria,Firmicutes</td>
</tr>
<tr>
<td>Pristine soils</td>
<td>low*</td>
<td>low*</td>
<td>low*</td>
<td>low*</td>
<td>low*</td>
<td>low*</td>
<td>N. S.</td>
<td>low*</td>
<td></td>
<td>Firmicutes</td>
</tr>
<tr>
<td>Industrial effluent</td>
<td>low*</td>
<td>low*</td>
<td>low*</td>
<td>low*</td>
<td>low*</td>
<td>low*</td>
<td>High*</td>
<td>low*</td>
<td></td>
<td>Acidobacteria, Actinobacteria, Firmicutes</td>
</tr>
<tr>
<td>Unaffected pristine soil</td>
<td>High*</td>
<td>High*</td>
<td>High*</td>
<td>High*</td>
<td>High*</td>
<td>High*</td>
<td>High*</td>
<td>low*</td>
<td></td>
<td>Bacterioidetes, Actinobacteria, Verrucomicrobia, Proteobacteria</td>
</tr>
<tr>
<td>Flood affected agricultural soils</td>
<td>low*</td>
<td>low*</td>
<td>low*</td>
<td>low*</td>
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<td>low*</td>
<td>High*</td>
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<td>Unaffected agricultural soils</td>
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<td>Proteobacteria, Actinobacteria, and Firmicutes</td>
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<td>Subsurface</td>
<td>High*</td>
<td>High*</td>
<td>-</td>
<td>High</td>
<td>High</td>
<td>-</td>
<td>-</td>
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<td></td>
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</tr>
</tbody>
</table>

<sup>1</sup>Dominant bacterial phyla: Proteobacteria, Actinobacteria, Firmicutes.
Vertisols | (60 to 65) | (250 to 500) | (3 to 5) | (6 to 8) |  
| Red soils | Low | low* | low* | low* |  
| | (40 to 60) | (120 to 300) | (0.82 to 2) | (5 to 6.25) |  
| Burried Palaeosol P1 | High* | High* | High* | High* | High* | High* | High* | -  
| | (81.5) | (180) | (502.3) | (166.5) | (3.05) | (282.6) | (7.3) | (0.25) |  
| Burreid Palaeosol P2 | High* | low* | low* | low* | low* | low* | low* | low* | -  
| | (47.6) | (129.9) | (358) | (68.1) | (1.78) | (221) | (3.26) | (0.19) |  
| Estuarine soils | High* | - | High* | - | - | - | - | - |  
| | (20-80) | - | (500 to 1800) | - | - | - | - | - |  
| Alluvial pristine soil | low* | low | - | - | - | - | - | - |  
| | (12 to 50) | (200 to 1200) | - | - | - | - | - | - |  

DHA: dehydrogenase; APA: phosphatase; PSA: protease; GSA: glucosidase; Cmic : Microbial biomass C; BSR: basal soil respiration; SIR: substrate induced respiration; q-CO₂: metabolic quotient; High and low indicate relative comparison where * indicates significant at \( P <0.05 \). ¥ deciphered by 16S rRNA sequencing. Actual values are provided (in parenthesis) for allover comparison. The units for the values are - DHA: µg of TPF g⁻¹ soil h⁻¹; APA: µg p-NP g⁻¹ soil h⁻¹; PSA: µg tyrosine g⁻¹ soil 2 h⁻¹; GSA: µg p-NP g⁻¹ soil h⁻¹; Cmic : mg kg⁻¹ soil; BSR: µg CO₂-C gm⁻¹ soil h⁻¹; SIR: µg CO₂-C gm⁻¹ soil h⁻¹; qCO₂: µg CO₂-C mg Cmic 24h⁻¹; N.S.: Not significant. ¥: Higher ammonia oxidizers (AOA and AOB) diversity.