Due to industrial and agricultural development in India, there is a rapid increase in the admissible limits of phenols, catechol and its derivatives in both soil and water which leads to severe toxicity of the indigenous biota of aquatic ecosystem including algae, protozoa, invertebrates and vertebrates. Microorganisms play a central role in the biodegradation of organic matter in the environment but only few species of micro-organisms are known to be potent degraders. The technical limitation to study natural diversity lies in the fact that only 0.1-1% of the micro-organisms are cultivable under standard laboratory condition. The predominant reason behind uncultivability of microorganisms is that they do not live segregated in the environment; they require co-operation of other species to establish biological and environmental relationships. Therefore, it is essential to study the microbial interactions and their associated metabolic potential in complex microbiomes. To fulfil this objective, we employed high throughput shotgun sequencing using Ion-torrent NGS platform to characterize and dissect the complete and detailed microbial community structure and their functional potential. Sewage sludge, river water and pesticide-contaminated soil samples were taken to fulfil our first objective. This study could help to deduce the possible metabolic pathways associated with each microbiome and role of microbial interactions to perform the degradation and other metabolic activities.

The study of pre- and post-treated sewage sludge provides comparative and detailed analysis of the taxonomic and functional potential of microbial consortia associated with sludge treatment process. Our analysis has shown the predominance of different microbes associated with each sludge stage and performing functions related to carbon mineralization and biodegradation of organic matter. Since sewage sludge represents complex microbial consortia associated with biodegradation processes, we have explored the functional pathways of biodegradation and community structure associated with it. Oxygenases are the fundamental enzymes involved in degradation of xenobiotics, therefore, it have studied the variation in oxygenases associated with each dataset. Our results showed the abundance of diverse oxygenases belonging to 17 different degradation pathways in sewage sludge revealing the high concentration of aromatic compounds in sewage. Taxonomy assignment of degradation pathways showed the abundance of *Betaproteobacteria* in chlorocyclohexane and chlorobenzene, caprolactam, atrazine and styrene degradation pathways, whereas ethylbenzene degradation pathway showed the abundance of both syntrophs and *Betaproteobacteria*, indicating their possible association for degradation of end products as an alternative source of organic carbon (Morris *et al.*, 2013). Microbial

communities involved in benzoate degradation pathway showed the abundance of *Beta-* and *Deltaproteobacteria* followed by methanogens, *Firmicutes* and *Chloroflexi*, whereas taxa assignment to naphthalene degradation pathway showed the abundance of *Gamma-* and *Deltaproteobacteria*. Taxonomy binning studies of raw sludge showed the genera of *Sulfurospirillum* to be highly active and abundant in all xenobiotic degradation pathways suggesting the ability of *Epsilonproteobacteria* in degradation of xenobiotic compounds.

As not much information is available regarding the fresh river water habitat and Sutlej river water has been under the effect of anthropogenic factors due to human activity during its course, therefore, we have selected it for the detailed investigation of taxonomy structure and functional role in terms of biodegradation of organic matter. Our results suggested the significant abundance of pathogenic bacteria *Acinetobacter* and *Stenotrophomonas* in river water showed the contamination of river water with hospital effluents. In addition, freshwater ecosystem practically plays an underestimated role in terms of biogeochemical cycles. Our study suggested that microbial communities present in river water ecosystem follows the net heterotrophic system. Carbon and energy flow is mediated by the presence of organic matter present in river water. Thus, this underestimated ecosystem, plays a surprisingly essential role in the storage, oxidation and release of carbon and energy. Our study has also suggested the dominating microbial communities present in freshwaters and their associated metabolic potentials in terms of biodegradation. It has been found that freshwater metagenome has a plenty of genes related to degradation of hydrocarbons and aromatic compounds. Peripheral pathways of aromatic compound degradation were found to be abundant, followed by genes related to metabolism of central intermediate aromatics. Phenylpropanoid dioxygenases were the most abundant oxygenases suggesting the active degradation of tannins, lignin and other plant cell components.

In case of pesticide-contaminated soil, we have chosen soil samples from village Balloh of Bathinda, Punjab, as its soil is known to be contaminated with lots of pesticides and thus, contributing to the enrichment of particular type of microbial community structure. The sequencing depth for balloh soil metagenome was relatively less than as compared to river water and sludge metagenomes, therefore, there may be the loss of certain rRNA and protein features. However, our data suggested the abundance of *Alphaproteobacteria* (order *Rhizobiales*) which may be performing the function of nitrogen-fixation for plants. The relative dominance of *Gammaproteobacteria* in balloh soil indirectly suggest the biodegradation of organic matter. Other microbes which are found to be dominating the

soil microbial communities are known to degrade catechol, phenol, propionate, phenylpropionate, chlorinated phenols and also for phosphate and uranium removal from the soil. Reads assigned to peripheral pathways for aromatic compound degradation were found to be abundant followed by pathways for central aromatic intermediates. A total of 646 reads were assigned to oxygenase genes, corresponds to 23 different types of oxygenases performing diverse metabolic and degradation functions in balloh soil.

Our next objective was to prepare the functional libraries from all these samples and screen them for novel oxygenases. For this, we have constructed the functional libraries in fosmids and screened them using 1% (w/v) catechol. The positive clones were sequenced to study the oxygenases and the associated biodegradation pathways. Depending upon the sequence information, we have categorised the fosmid clones into six groups. Analysis of six fosmid groups indicated the presence of a total of nineteen oxygenases. Out of 19 genes, sixteen were annotated as dioxygenases and three were predicted as monooxygenases. Dioxygenases were further classified into ring-hydroxylating dioxygenases and ring-cleaving dioxygenases. Four genes encoding for RHDs were found whereas twelve were annotated as ring-cleaving dioxygenases. All classes of dioxygenases were found in our fosmids such as taurine dioxygenase (TauD) belongs to 2OG (oxoglutarate) dependent dioxygenase category; 2,3-dihydroxybiphenyl 1,2-dioxygenase (BphC)- a typical extradiol dioxygenase; class III DOPA 4,5-dioxygenase; iron-sulfur containing oxygenase from Rieske dioxygenase and gentisate 1,2-dioxygenase with characteristics similar to EDOs. Two EDOs belonging to class I family 1.2.C were found in group III and IV fosmids. The genes found in fosmid groups were successfully classified into cluster of orthologus genes (COG) categories. Most of the genes were assigned COG category Q that denotes secondary metabolites biosynthesis, transport and catabolism. Genes for replication, recombination and repair (COG category L) were found in group I, II, III and VI suggesting their origin from plasmids via transposons or insertion elements. The degradation genes and their organization was not found to be previously known suggesting that xenobiotic degradation in nature has been performed by combined action of many fragmental pathways. EDOs found were showing similarity with either metagenomic fragments or uncultured bacteria and most of them were not characterized yet. We have also annotated some of the hypothetical proteins as EDOs and successfully confirmed their dioxygenase nature by the activity assays. Thus, sequence analysis of the fosmid groups obtained by functional metagenomics is extremely useful in capturing unknown and unidentified EDOs

form yet uncultured bacteria. Therefore, we have chosen uncharacterized EDOs on the basis of their sequence similarity and proceed for the preparation of their recombinant versions in *E. coli* to determine the capability of uncultured bacteria for aromatic compound degradation.

For the preparation of recombinant versions of dioxygenases, we have selected 2,3-dihydroxybiphenyl 1,2-dioxygenase (BphC-SD3), Catechol 2,3-dioxygenase (C23O-RW1), Metapyrocatechase (MPC), DOPA 4,5-dioxygenase and Catechol 1,2-dioxygenase. We have completely characterized BphC-SD3 biochemically as well as structurally. Our results suggested that BphC-SD3 is efficient and specific in oxidizing 2,3-dihyroxybiphenyl (2,3-DHB) > catechol > 3-methylcatechol (3-MC). BphC-SD3 is moderately thermostable, retaining ~85% relative activity at 65°C. We have successfully solved the crystal structure of BphC-SD3 at 2.6Å. C23O-RW1 showed the substrate specificity in the order of catechol > 4-CC > 2,3-DHB > 3-MC whereas MPC showed the substrate specificity of 2,3-DHB > 3-MC > catechol. BphC-SD3 and C23O-RW1 were found to be halotolerant, capable of retaining the maximal activity even upto 4 M NaCl. The substrate-specificity and halotolerant ability of our enzymes would be utilized in the decontamination of saline industrial effluents. We have also performed preliminary experiments for the detection of catecholic compounds using BphC-SD3 by cyclic voltammetry techniques. Our results suggested that BphC-SD3 is successfully able to detect catechol on single-carbon electrode indicating its future use in developing the sensitive and reliable biosensor.

Taken together, this study would not only help in designing better bioremediation strategy for degrading xenobiotic compounds but also suggests the use of certain taxonomic groups to be incorporated in bioremediation agents. The efficiency and specificity of our novel oxygenases would help in developing new enzyme system for bioremediation. The stability of BphC-SD3 would help in constructing a specific, sensitive and reliable biosensor for the detection of catecholic compound in real samples.