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Research Article

MICROBIAL COMMUNITY COMPOSITION IN CONTAMINATED SOIL SAMPLES OF THE YAMUNA RIVER

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Abstract- Yamuna is one of the important river of India and its stretch through Delhi is its most polluted part. Therefore it is important to study the effects of pollutants on microbial community structure to restorate it. For bioremediation, microbial community is needed to be investigated. 16S rDNA cloning technique was employed to study the microbial community composition. Results were analyzed by Canonical correspondence analysis (CCA) to understand the impact of environmental variables and coupled with Principle coordinate analysis (PCoA) to compare the diversity composition between the samples. Representative strains belonging to genera *Geobacter, Sphaerobacter, Exinguobacterium* and *Eubacterium* were dominant in the soil adjacent to contaminated river water. The presence of *Geobacter* indicated the phenolic compounds and organic compounds contamination in the river. On the other hand, the presence of *Sphaerobacter* indicated the sewage wastes in water while *Exinguobacterium* and *Eubacterium* showed the contamination of heavy metals. The indigenous bacterial communities were capable of decreasing the level of pollutants in the river water.

Keywords- Cloning, Microbes, Pollutants, 16S rDNA and Yamuna

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Introduction

Yamuna is one of the major rivers of India. In addition to its traditional and religious values, its water is also used for irrigation, domestic and industrial purposes. Due to anthropogenic activities and industrial effluents draining in Yamuna river, the river water got contaminated. Eighteen major drains including Najafgarh Drain and Shahadra Drain are increasing the contamination of Yamuna [1]. Central Pollution Control Board (CPCB), India in 2006 reported that Yamuna water is mainly polluted due to discharge of sewage (domestic and industrial) and agricultural wastes (agricultural residues, fertilizers, pesticides and excess salts), dumping of garbage and dead bodies. In 2007, high level of dissolved oxygen was recorded upstream and downstream, which was 8 mg/l and 10 mg/l respectively [2]. The average biological oxygen demand (BOD) level reported by the CPCB in 2008 was 2 mg/l upstream and 59 mg/l downstream. According to their report, lead, nickel and cadmium were present in very low concentrations whereas zinc and iron were in high amount. Among pesticides, Beta-Hexachlorocyclohexane was present in some areas of the river, whereas other pesticides e.g. endosulfan, aldrin, dieldrin and DDT were negligible [3]. Mishra in 2010 has reported that upstream of Yamuna river is less polluted compared to downstream.

In ecosystem soil microorganisms represents a large driving source and pool of nutrients. They play an important role in biogeochemical cycles, soil structure, and in regulating plant growth [4]. Soil microbial community compositions are sensitive to the disturbances which indirectly affects ecosystem functions [5]. A change in microbial diversity due to an environmental impact helps to take steps to remedy the situation. The microbial diversity analysis of soil adjacent to Yamuna water can be helpful to understand the soil quality and its bioremediation. Therefore, this study investigated the microbial community structure and enzyme activities in soil adjacent to Yamuna water in Delhi, India.

Materials and Method

Soil samples were collected in May 2010 from three locations along the Yamuna river stretch in Delhi, India [Fig-1]. Sample 1 (N28° 47'03.0" E077°12'07.2"), sample 2 (N28°37'44.9" E077°15'16.7) and sample 3 (N28°32'32.0" E077° 18'52.2"). Soil sampling was done randomly from 0-10cm soil depth. Each point contained replicates and each consists of three soil cores. Replicates of each sample were mixed thoroughly [6], sieved and stored at 4°C and -20°C.

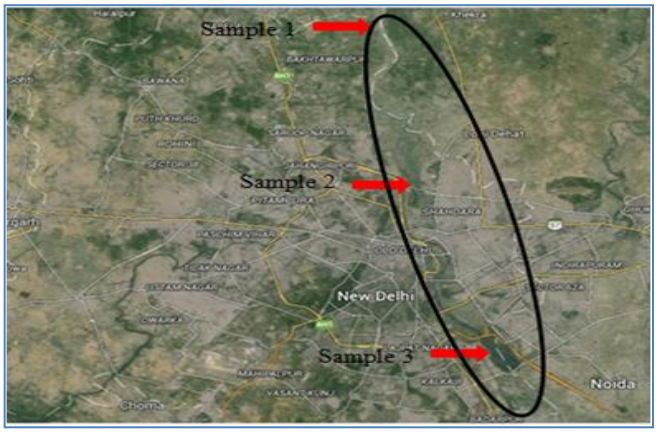

Fig-1 Sampling points along Yamuna River in Delhi, India. Water flows from first point towards third point. Yamuna River is important for the replenishment of Delhi city of their water supply

Soil pH was determined by J. Forster's method [7]. Modified Walkley-Black Method was used to determine total organic carbon [8]. Soil nitrogen analyzed by solid sample dry combustion using Elementar Vario El Cube CHNS Analyzer (Germany). Dehydrogenase activity (DHA) by R. Ohlinger's method [9] while

urease and nitrate reductase activity was determined by Kandeler's method [10, 11].

Soil DNA extraction was performed on 0.5g of soil in triplicates using MOBIO soil DNA kit. The quality of DNA was checked by 0.8% on agarose gel and quantified spectrophotometerically. 16S rDNA was amplified using universal primers 8F (5' AGAGTTTGATCCTGGCTCAG 3') and 928R (5'CCCTCAATTCCTTTGAGTT 3') [12]. PCR conditions as follows: 1 cycle at 95°C for 5 min (initial denaturation and activation of Taq polymerase), 30 cycles at 95°C for 1min, 55°C for 1min, and 72°C for 2 min, followed by final extension for 15min at 72°C in Applied Biosystem 2720 Thermal Cycler. Amplified product was checked on 0.8% agarose gel and images were captured. PCR amplicons at desired length were excised and eluted using a gel extraction kit (Promega, USA) and ligated into a pGEMT vector (Promega, USA). Ligated vector was transformed into *Esherichia coli* DH5alpha. The clone inserts were checked by using vector specific M13 primers. Positive clones per site were picked and digested with *Eco*RI. On the basis of banding patterns, different phylotypes were selected [13]. Plasmids were extracted and sent for sequencing to Xcelris Labs, Ahmedabad, India. The partial sequencing was done with M13 forward primer. Sequences were submitted to Gene Bank, JN418886 to JN418925. Data was evaluated by ANOVA using Sigma Plot version 12.0 and $p<0.05$ were treated significant. Calculation of diversity indexes, canonical correspondence, principle co-ordinate analysis and rarefaction curve was drawn by using Past3 software.

Results

Relationship between environmental factors, pollutants and bacterial communities in soil adjacent Yamuna has not been well studied. River is highly influenced by the anthropogenic activities. In this work soil microbial community composition and enzyme activities were studied. Soil physico-chemical characteristics are the parameters to investigate of soil health. First soil samples were analyzed for their physico-chemical properties [Table-1]. pH of soil sample 2 and 3 were more alkaline as compared to sample 1 [Table-1]. pH values of all samples were significantly different ($p<0.05$). Total organic carbon of most contaminated soil samples (2 and 3) was significantly high ($p<0.05$) as compared to sample 1.

Table-1 *Physico-chemical properties of soil samples*

Soil Physico-chemical properties	Sample 1	Sample 2	Sample 3
pH	$7.22^{a} \pm 0.03$	$7.5^{b} \pm 0.02$	$8.55^{c} \pm 0.02$
Total Nitrogen (%)	0.009	0.076	0.036
Organic Carbon (%)	0.65	3.7	1.7
Soil texture	41.4% sand,	66.78% sand	84.4% sand,
	47% silt,	23.98% silt,	8% silt,
	11.6% clay	8.76% clay	7.6% clay

Different superscripts (a, b and c) represents significant difference (Tukey multiple test, $p < 0.05$) and same superscripts represents no significantly. (±) are standard deviation of three replicates.

To understand the changes in the microbial diversity composition 16S rDNA clone library was constructed. From the 16S rDNA clone libraries 41 OTUs were identified. Unidentified bacterial population was abundantly distributed throughout the samples. In particular firmicutes showed more relative abundance in [Sample-2 and 3]. In sample 1 population of firmicutes (*Exiguobacterium* sp., *Eryipelothrix* sp., *Salinicoccus roseus*) planctomycetes, chloroflexi (*Sphaerobacter* sp.,) and proteobacteria (*Geobacter* sp., *Aquimonas* sp., *Klebsiella pneumonia*) were present in same percentage [Fig-2]. To determine the relation between environmental variables and microbial community structure, CCA (Canonical correspondence analysis) was evaluated [Fig-3].

Rarefaction curve is a technique to calculate the species richness. In our samples rarefaction curve reaches plateau [Fig-4]. This indicates that the soil was sampled with good level of confidence. Distribution of bacterial population in ecosystems was calculated by diversity index. Simpson's diversity index and Shannon diversity index were used to calculate the richness of the bacterial population. Richness of the bacterial population in samples is estimated by diversity index calculation; high value of diversity indexes represents richness of the community. Chao1 values indicates that sample 1 and 3 were more rich in bacterial communities. Evenness (E) and equitability (J) values indicated the evenness of the population i.e. the samples were not dominated by any particular bacterial genera [Table-2]. Diversity indexes values indicating the high microbial diversity in the samples. Principle co-ordinate analysis (PCoA) was performed to determine the similarity between the samples [Fig-5]. All samples were placed at different coordinates in the PCoA plot which revealed distinctness in the composition of the bacterial communities. High percentage of *Geobacter*, *Sphaerobacter*, *Exinguobacterium* and *Eubacterium* was observed in the soil samples.

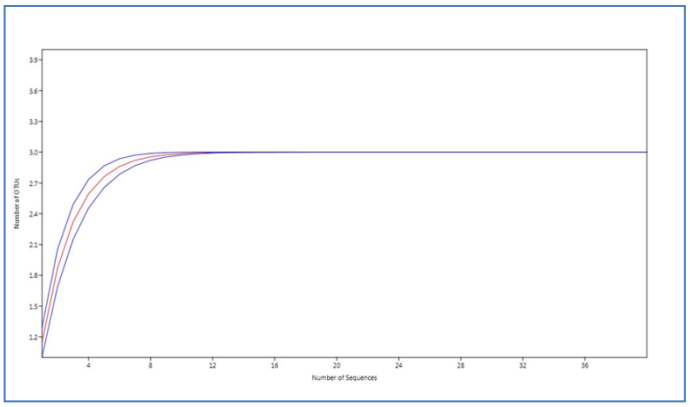

Fig-2 Relative abundance of sequences in the 16S rDNA clone libraries

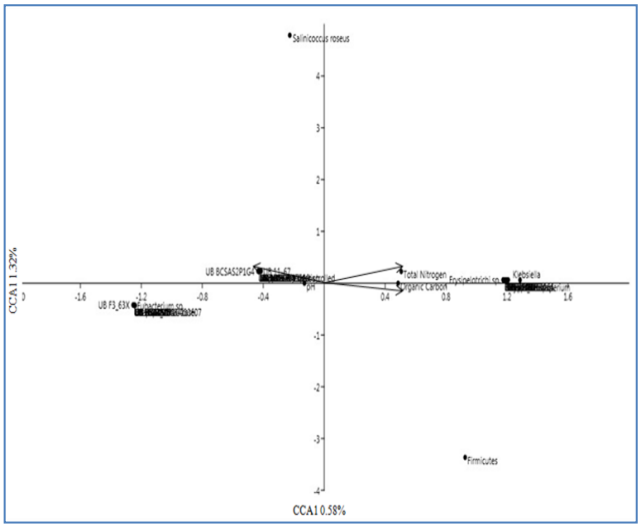

Fig-3 Canonical correspondence analysis (CCA) showing the relationship between the microbial community structure and environmental factors

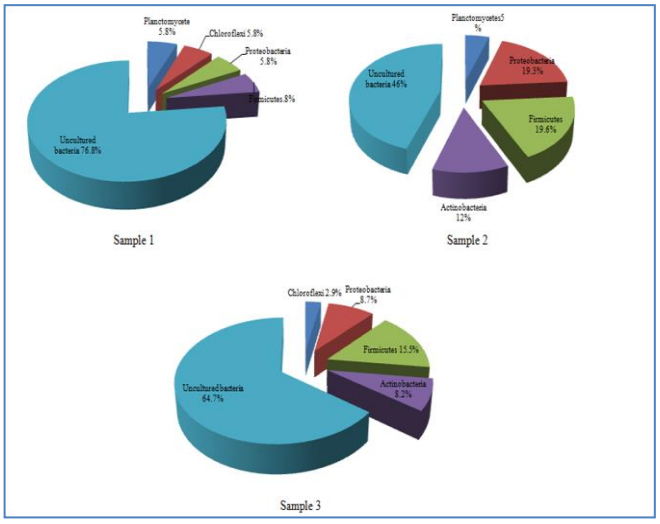

Fig-4 Rarefaction curve analysis for soil samples 1 (upper blue), sample 2 (red) and sample 3 (lower blue)

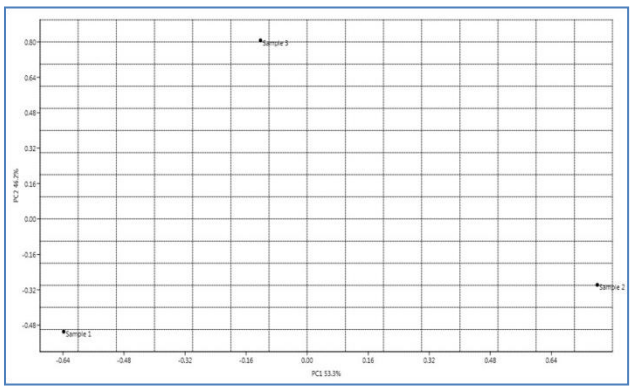

Fig-5 Principle coordinating analysis (PCoA) obtained on comparing the samples using Bray Curtis similarity coefficient

Table-2 *Diversity indexes calculated from 16S rDNA clone libraries*

	Sample 1	Sample 2	Sample 3
Simpson Index, 1-D	0.92	0.899	0.91
Shannon Index, H	2.95	2.58	2.89
Evenness, e^H/S	1.124	1.022	1.12
Equitability, J	1.041	1.008	1.04
Chao1	16	13	16

Soil enzymes are important indicators of soil environment quality. The level of dehydrogenase activity was ranged between 3±0.02 to 4.5±0.06 TPF/gm dry wt. of soil while the level of urease enzymes was ranged between 11.6±1.8 and 15.5±1.6 N/gm dry wt. soil. The highest activity of nitrate reductase was observed in sample 3 (13.2±0.2 N/gm dry wt. soil) compared to sample 1 and 2, 6.8 ± 0.16 and 9.6 ± 0.23 N/gm dry wt. soil respectively.

Discussion

The pH of the soil depends on the presence of pollutants. pH of soil samples ranged between neutral to alkaline. This might be due to the presence of soluble salts in industrial discharge [14]. Organic carbon content, which is another parameter to determine soil health, indicates the presence of nutrient sources in fewer amounts in the polluted soil samples [15].

Environmental factors play an important role in influencing bacterial population [16] and to determine this relation between environmental variables and microbial community structure, CCA (canonical correspondence analysis) was done. CCA analysis showed that bacterial communities that were influencing by total nitrogen, organic carbon content were different from those populations which were affected by pH. From this, it can be summated that the soil pH was strongly affecting the bacterial community composition. The rarefaction curve investigation of clone libraries revealed that the sampling was done at saturation level for bacterial diversity analysis, implying that most of the bacterial phylotypes were observed. Richness of bacterial population was calculated using Shannon and Simpson index. In our samples index values showed that contaminated soil samples were rich in bacterial communities. Microbial communities in contaminated soil samples of Yamuna River showed evenness. Evenness in the microbial community structure ensures that the population has much ability to consume pollutants with different metabolic pathways [17]. To determine the similarity between samples, PCoA was performed which showed that the samples were different from each other in bacterial population composition. This might be due to difference in the concentration of contaminants in the soil samples.

Many studies have shown that composition of microbial communities in environment is diverse and are sensitive to anthropogenic activities. It has been proposed that composition of bacterial community is an indicator of pollutants [18]. Like, the presence of *Geobacter* genus in our samples indicated the contamination of soil samples with phenolic compounds and organic compounds [19]. Similarly habitation of *Sphaerobacter* in samples indicated sewage waste contamination in Yamuna water [20]. This is rather relevant as river Yamuna is the dumping site where all the waste including industrial and domestic are dumped. Existence of bacterial genera like *Exinguobacterium* and *Eubacterium* revealed heavy metal contamination in the samples [21]. Phylum like Planctomycete was found to be present in soil sample 1 and 2 which have already been reported to remove nitrogen from nitrite and ammonium from waste waters [22]. Clones belonging to these genera showed that native bacterial population of the samples was capable of remediating the river naturally.

Enzymatic activities are regularly monitored for estimating the effect of contaminants on the soil microbial populations [23]. Dehydrogenase activity (DHA) is a standard method to check the metabolic activity of microorganisms in organically or inorganically contaminated samples [24]. Urease is another important enzyme used as an indicator of soil health. It carried out the conversion of organic nitrogen to inorganic nitrogen [25]. The activity of these enzymes in contaminated samples of Yamuna River indicates the ability of microorganisms for their production [26, 27]. The activity of nitrate reductase was high in sample 3 which was alkaline in nature. This was previously reported that nitrate reductase activity showed positive correlation with alkalinity of the soil samples [28]. Soil enzymatic activities are good indicators to evaluate impact of pollutants on soil health.

Microbial diversity analysis and enzymatic analysis support the richness and evenness at Yamuna site in spite of less concentration of organic carbon. This suggests that the bacterial communities were utilizing the pollutant as a carbon and energy source. The presence of bacterial genera like *Geobacter*, *Eubacterium* and *Eryipelothrix* having capability of tolerating heavy metal stress also increases the richness of the soil bacteria.

Conclusion

From this work, it can be concluded that the presence of diverse bacterial populations in the soil samples adjacent to contaminated river water. Bacterial activities and diversity may be linked with high anthropogenic activities including industrial waste. Microbes can be utilized to use as biomarker for the polluted soil and screened for bioremediation for the batter quality of the river water.

Acknowledgment

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Abbreviations

16S ribosomal Deoxyribonucleic acid (16S rDNA)
Canonical correspondence analysis (CCA)
Principle coordinate analysis (PCoA)
Central Pollution Control Board (CPCB)
Biological oxygen demand (BOD)

Author contributions

Sushma Sharma: Research work, analysis and manuscript.
Khushboo Singh: Research work, analysis and manuscript
Dileep Kumar Singh: Principal Investigator

Conflict of interest

All authors declare that they have no financial or commercial conflicts of interest.

Ethical approval

This article does not contain any studies with human participants or animal performed by any of the authors.

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