Microsphere (2025) 3(1): 302-311
https://doi.org/10.59118/IAZE6012

Research Article

A Microcosm Study to evaluate the impact of Yamuna River water on the Soil Microbial Community and Enzyme Activity

Ranju Sharma[1] · **Ngangbam Sarat Singh**[2]*

Received: 19 July 2025 / Accepted: 20 August 2025 / Published online: 30 September 2025

Abstract

The Yamuna River, one of the most polluted rivers in the world, has been found to contain various contaminants. Agricultural fields near the river in Delhi-NCR region are irrigated with the river water itself, thus posing a great threat to human health as well as adverse effects on the soil microbial community. The present study evaluated the impact of this irrigation process on enzyme activity and microbial community of soil. Three sets of five pots each were treated with river water sampled from six different sites (two upstream, two middle stream, and two lower stream) within the stretch of Yamuna river in Delhi-NCR region, while the soil sample treated with tap water was taken as control. The result indicated that the soil sample treated with river water from lower stream had much higher level of organic pollution as shown by higher value of TOC (total organic carbon) as well as dehydrogenase and nitrate reductase enzyme activities. ARISA-ADAPT analysis showed overall, *Firmicutes* (50.84%) were the most abundant bacterial group followed by *Proteobacteria* (33.32%) and *Actinobacteria* (12.46%). They displayed a higher percentage of variability in relation to the contamination of river water. Furthermore, specific bacterial groups such as *Acidobacteria*, *Verrucomicrobia*, and *Aquificae* were exclusively found in soil samples that had been treated with river water. The study concludes that increase in the organic pollutants in the river water significantly alter the composition of microbial population as well as the soil enzyme activity. These microbial communities could be used in identifying the level of pollution in river water so that improved management techniques can be adopted.

Keywords: Yamuna river, Enzyme activity, Microbial diversity, ARISA-ADAPT, water pollution.

Introduction

The problem of environmental pollution is growing daily, posing threats to human health, causing climate change, reducing soil fertility, and making drinking water scarce. Various microcosm studies were used to understand the toxic effects of heavy metals, pesticides and other harmful chemicals on the environment [1,2]. Microcosm is a common tool in microbiological study, act as ecological models that encircle and then study a specific area of the natural environment. Soil and water microcosm studies enable us to observe the effects of selective pressures, such as xenobiotic occurrence on natural microbial communities under controlled conditions. These microcosms are kept in controlled environments with near-natural temperature, light, humidity, and other parameters. Since microbial communities are crucial to comprehending the effects of anthropogenic and environmental factors on ecosystems, such study helps to more clearly establish the causal relationship between a toxicant and its effects on these communities [2].

Yamuna River is one of the most polluted rivers in the world. Various harmful chemicals, pesticides and other organic and inorganic pollutants are either directly or indirectly discharged into the river [3]. Despite the fact that, the stretch of Yamuna River in Delhi (approx. 22 km) is only 2% of the entire river, the 22 major drains in Delhi and NCR regions which directly merge with the river at different points are mainly responsible for the 70% of the total pollution load of the river. Water quality parameters studied by CPCB during the study period showed that its water is unfit for living organisms (Table 1) [4].

[1]Soil Microbial Ecology and Environmental Toxicology Laboratory, Department of Zoology, University of Delhi, Delhi-110007, India.
[2]Environmental Toxicology Laboratory, Department of Zoology, University of Allahabad, Prayagraj, U.P 211002, India
***Corresponding author:** Dr. Ngangbam Sarat Singh[2]; sarat4sarat@gmail.com

Table 1. Water quality analysis of Yamuna River (CPCB report-2014)

	Up stream	Middle stream	Lower stream	Reference
DO (mg/l)	4	1.2	0	4
BOD (mg/l)	3	2.4	17.3	
Coliform	10003	12010833	23816667	
pH	7.5	7.9	8.9	
DDT (ng/l)	197.01± 61.31	506.74 ±427.56	1114.51±383.26	5
HCH (ng/l)	76.75±38.60	191.34±157.14	310.25±191.57	

*DO (Dissolved Oxygen), BOD (Biological Oxygen Demand), Coliform and pH from CPCB, 2014 [4] and pesticide data from Kaushik et al. 2008 [5].

Out of 22 major and minor drains in Delhi-NCR, two major drains i.e. Nazafgarh and Shahdara drain are responsible for 78% of the total pollution load. This unabated discharge of drain water directly in river is responsible for its deterioration. Presence of sewage waste reduce the dissolved oxygen and increased the biological oxygen demand. This indicates that how much polluted the water is. Since this polluted river water is used in various domestic and agricultural purposes, it is critical to understand the effect of polluted water on soil microbial ecology.

Numerous studies on the microbiology of the Yamuna River and its level of pollution have been conducted up to this point. A rise in pollution in the Yamuna River affects not just the structure of the surrounding soil microbial community but also the health of the soil in agricultural fields that receive irrigation from the river [6-8]. Studies have looked into the presence of certain heavy metals and pesticides in the Yamuna River's water and the soil in the vicinity [3, 9-10].

Microbial communities play an important role in the functioning of ecosystems and are key factors in various biological and chemical processes. As they respond to large-scale changes in the soil ecosystem in response to different environmental conditions, significant research has been focused to study the role of microorganisms in different environment [3,6,11]. The microbial populations are significantly impacted by various anthropogenic activities [3,6]. Therefore, it is crucial to characterize microbial communities and identify different microorganisms linked to particular habitat and their ecological roles.

Soil enzymes can respond quickly to changes in the ecosystem, and since they are easily quantifiable, are widely used as indicators of soil health [12]. Soil microbes such as bacteria, fungi, and archaea are the major sources of soil enzymes. As such, any changes in the soil environment which affect soil microbes have direct impact on soil's enzyme activities. Different soil enzyme activities indicate particular soil conditions. The activities of the urease and nitrate reductase enzymes are directly related to the quantity of nitrogenous compounds present in the soil. On the other hand, dehydrogenase is an intracellular respiratory enzyme found in all the living cells and its activity is corelated with the population of microbes in the soil [13]. The use of enzymes for environmental objectives has grown throughout time. They have also been employed in the detection and measurement of environmental conditions before to, during, and following the cleanup process.

In the present work, a microcosm study was done to observe the effect of Yamuna River water on soil microbial communities and enzyme activity. ARISA (Automated Ribosomal Intergenic Spacer Analysis) of 16S rRNA gene sequence was performed to observe the effect of river water from different streams on soil bacterial communities. The phylogenetic analysis was done using ARISA-ADAPT software. It is a culture independent technique and significantly more effective than T-RFLP in analysis the richness and community composition of bacterial species [6].

2. Material and Methods

2.1 Experimental set up

For microcosm study, soil sampling was done from Kamala Nehru Park (ridge area) near Delhi University. Soils from 1-15 cm depth were collected randomly and mixed evenly. Twenty different pots were taken and filled with 500 g of soil. Of these twenty pots, three sets of five pots each were treated with Yamuna River water sampled from upstream [Palla village (28°51'01.4"N 77°12'34.7"E) and Jagatpur village (28°44'52.6"N 77°13'30.6"E)],

Middle stream [Wazirabad (28°42'32.2"N 77°13'58.4"E) and IP Power station (28°37'34.7"N 77°15'27.7"E)], lower stream [Noida bridge (28°33'55.3"N 77°17'45.3"E) and Madanpur khadar (28°31'40.6"N 77°20'02.1"E)], respectively (Image 1). The remaining sets of five pots were treated with tap water and taken as control. Experiments were carried out for four months and soil sampling was done after every 15 days. Freshly sampled soil was used to observe the variation in soil enzyme activity while the rest of the soil was dried, sieved and kept at -20ºC for microbial diversity analysis.

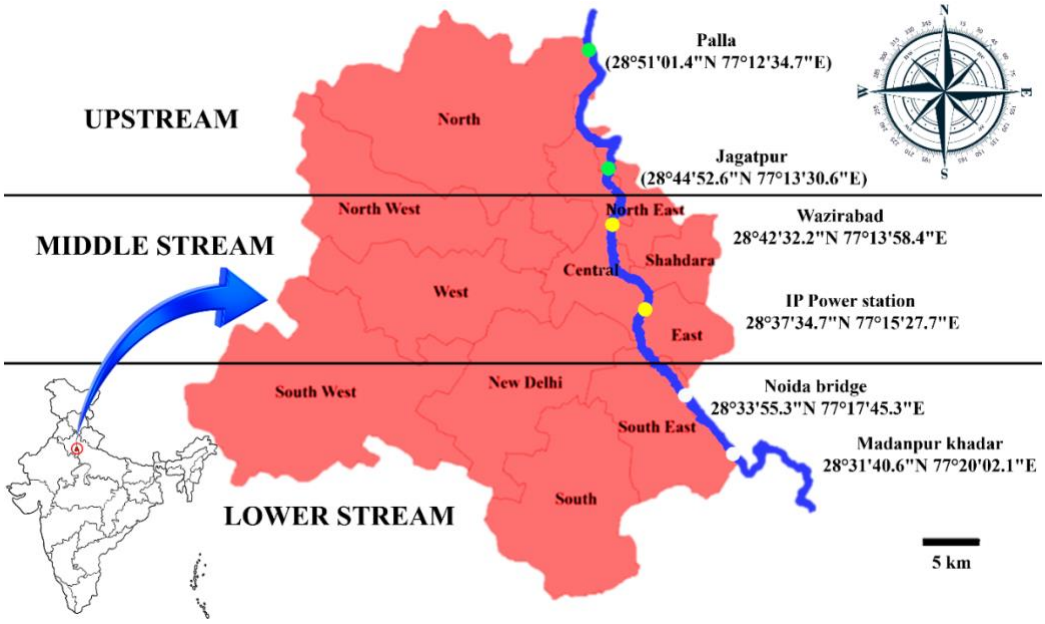


Figure 1: Water sampling points along the Yamuna River in Delhi -NCR region selected for the microcosm study.

2.2 Physico-chemical properties

Soil pH was measured using two different buffers having pH 4.0 and pH 7.0 using Corning pH meter [14]. Total organic carbon (TOC) of soil was determined using modified Walkley-Black method 1934 [15]. Soil enzyme activity i.e. Dehydrogenase enzyme activity has been determined using the method described by Thalmann (1968), Urease by Kandler (1988), and Nitrate reductase by Alef et al., (1995) [14, 16-17].

2.3 Soil microbial community analysis

2.3.1 Soil DNA isolation

Soil DNA was isolated using Ultra Clean™ Soil DNA isolation kit, (Mo Bio Laboratories, CA, USA) according to the manufacturer's instructions with minor modifications. The quality of isolated DNA was evaluated on 0.8% w/v agarose gel (GeNei™, Banglore, India) and visualized in Molecular Imager (Gel Doc ™ XR + Biorad) and quantified at 260/280 nm by using NanoDrop (ND -1000, spectrophotometer, Thermo Fisher Scientific, DE, USA).

2.3.2 Amplification of 16S rRNA gene

PCR amplification from 50 ng of extracted soil DNA was conducted on PCR Thermocycler (BIO RAD, USA) with a total volume of 50 µl by using 1 µM concentrations of the universal primers ITS-F (5′-GTCGTAACAAGGTAGCCGTA-3′) and ITS-R (5′-GCCAAGGCATCCACC-3′) [18]. For ARISA forward primer was FAM labelled. Each 50 µl of PCR reaction mixture contains 200 mM of each dNTP, 2 Units of DNA polymerase and 1xPCR buffer and final volume make up was done with nuclease free water. Amplification was carried out under the following conditions: 5 min at 94°C, 30 cycles of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C, plus an additional 15 min cycle at 72°C. Amplified DNA was analysed by gel electrophoresis on 0.8% agarose gel with ethidium bromide. Bands were visualized in Gel Documentation system (Gel Doc™ XR+ BIO RAD, USA). Amplified samples were sequenced from SCI genome labs, Cochin, India**.** ARISA profile was visualized using Gene Mapper® software (version 4.0. Applied Biosystems) which can detect the fluorescently labelled fragments. Bands with different intensities belong to different bacterial groups and also reveal the difference in the number of particular groups [19].

2.3.3 Diversity Analysis

ARISA profile data consists of peak number, peak name, peak size (length) in base pair, peak area, peak height and data point. ADAPT software was used for ARISA data analysis. Input data file in ADAPT is in .csv format containing peak number, peak name, peak size, peak area and peak height. Output data helps to provide a table and graphical representation of the autotrophic/heterotrophic and the pathogenic/non-pathogenic average fraction for each sample.

2.4 Statistical tool

All samples were processed in triplicates. Level of significant difference in total organic carbon content and soil enzyme activity due to different treatment between all the experimental pots was assessed using Two Way Analysis of Variance (ANOVA) followed by the Tukey's test ($P < 0.001$, Sigma plot version 12.5).

Shannon Weiner Diversity Index was calculated using the equation,

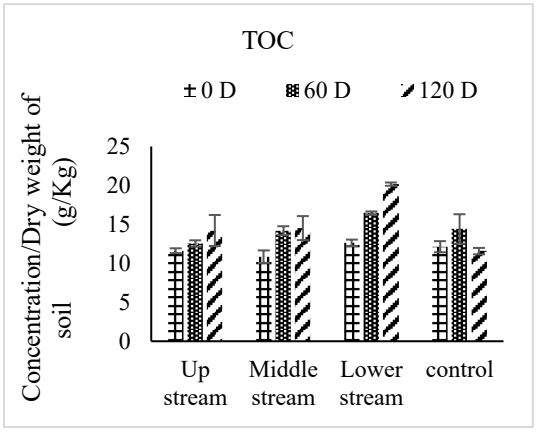
$$H' = - \sum (N_i/N) \times Ln (N_i/N)$$

Where, N_i = number of individuals of species "i" or % peak area of species "i" and N = total number of individuals of all species or total sum of the peak area of individual sample. It helps to compare the relative complexity among communities and to estimate the completeness of sampling [20]. Multivariate analysis provides an inexpensive and easy means of analysing data and was done using MVSP version 3.1. Complex data was analysed by PCA (Principal component analysis) using scattered plots. UPGMA (Unweighted Pair Group Method using Averages) dendrograms were prepared through cluster analysis using Jaccard coefficient [21]. This helps to determine the relationship between microbial OTUs (Operational Taxonomic Units) distribution and community composition as a function of seasons, geographic origin or habitat structure [22].

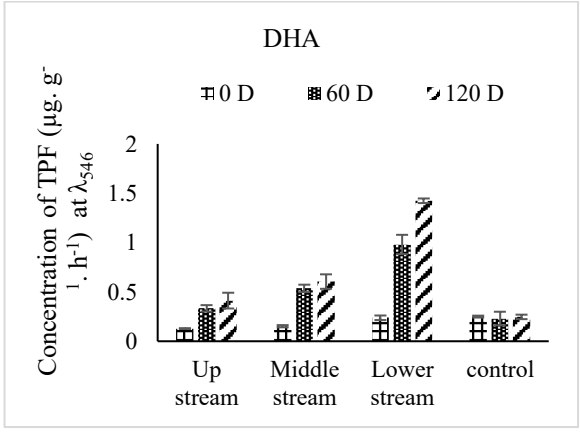
3. Results

3.1 Variation in physico-chemical properties

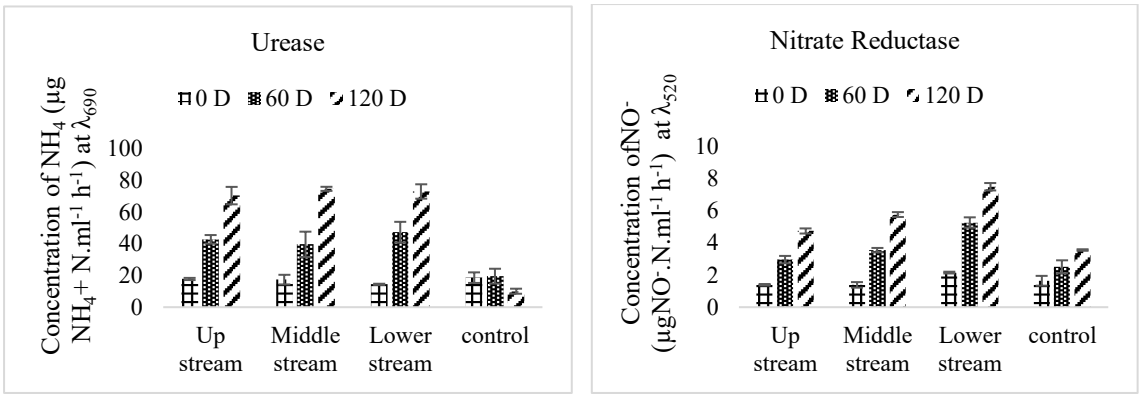
Microcosm study was done to observe the effect of Yamuna River water on soil enzyme activity and microbial diversity of control soil. The pH of all the studied soil sample ranged between 7 to 8. Total organic carbon, Dehydrogenase enzyme and Nitrate reductase enzyme activity were significantly higher ($P< 0.001$) in pots treated with river water from lower stream (Figure 1A, 1B and 1D). Urease enzyme did not show any significant variation among the entire soil sample treated with Yamuna River (Figure 1C). In all the fifteen pots treated with Yamuna River water, urease enzyme activity was significantly increasing ($P< 0.001$) from 60th day onward. Soil sample treated with tap water did not show any significant variation in total organic carbon content, Dehydrogenase enzyme activity, Urease enzyme activity and Nitrate reductase enzyme activity.



A.



B.



C. **D.**

Figure 1. Total organic carbon (A), Dehydrogenase enzyme (B), Urease enzyme (C) and Nitrate reductase enzyme (D) activity of experimental soil treated with Yamuna River water (upstream, middle stream and lower stream) and tap water (control). Result was shown as mean of five replicates for each stream.

3.2 Community shift in microbial population

Abundance and richness of soil microbial community study using ARISA technique and data analysis was done using ADAPT software. On the basis of ADAPT analysis heterogeneous distribution of ten different bacterial phyla were observed in soil treated with river water (Figure 2). Out of these, *Firmicutes* were the most abundant bacterial group with percentage relative abundance of 50.84% followed by *Proteobacteria* (33.32%) and *Actinobacteria* (12.46%). Other bacterial phyla viz. *Chlorbi* (1.31%), *Thermotoage* (0.85%), *Veerucomicrobia* (0.80%), *Bacteriodetes* (0.40%), *Nitrospirae* (0.35%), *Aquificae* (0.24%) and *Acidobacteria* (0.10%) were also present but in less relative abundance. Out of these bacterial phyla, control soil treated with tap water showed only seven bacterial phyla with *Firmicutes* (55.24%) as the most abundant group followed by *Proteobacteria* (30.52%), *Actinobacteria* (10.24%), *Chlorbi* (2.09%), *Thermotoage* (0.99%), *Bacteriodetes* (0.56%) and *Nitrospirae* (0.33%). *Aquificae, Acidobacteria* and *Verrucomicrobia* were not present in control soil samples treated with tap water.

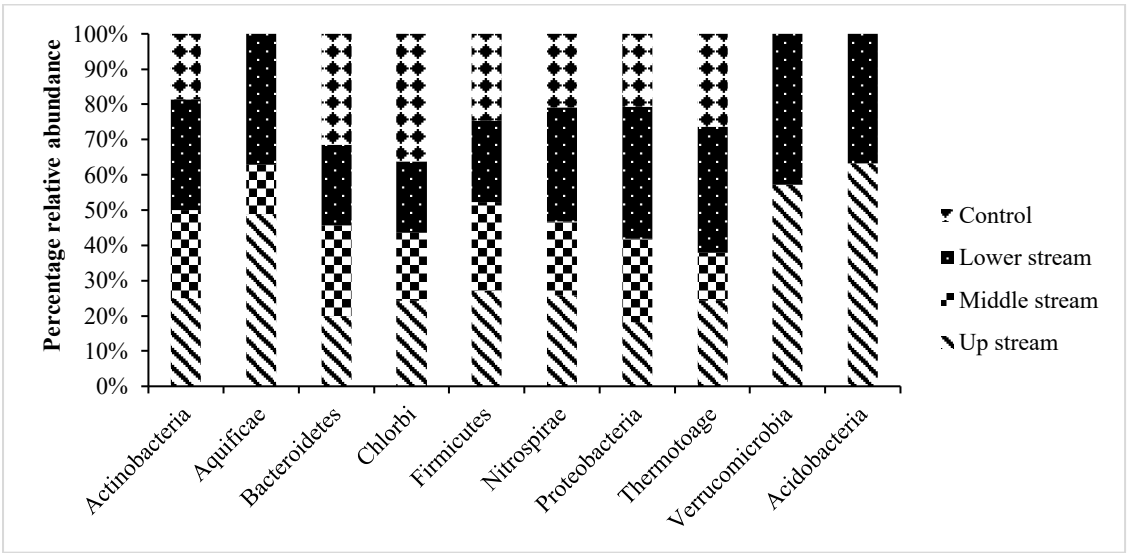


Figure 2. Percentage relative abundance of Bacterial phyla observed during the study period in soil treated with Yamuna River water (upstream, middle stream and lower stream) and tap water (control). Result was shown as mean of five replicates for each stream.

3.3 Statistical analysis

Diversity index of whole microbial community present in all the soil sample were also calculated. It was observed that initially the value of diversity index does not show any significant variation but with time significant increase was observed. During the whole study period, the average of diversity index was observed to be significantly higher in soil treated with river water from lower stream (H'=1.15±0.01) followed by middle stream (H'=1.14±0.04) and upstream (H'=1.09±0.03). Control soil treated with tap water showed very less diversity index *i.e.* H'=1.04±0.06.

Cluster analysis using Jaccard similarity index showed the similarity coefficient of microbial community among the four microcosms treated with water from three different streams and tap water (control). The microcosm treated with water from the lower stream had the lowest similarity coefficient index, 0.84, according to the results (Figure 3). When compared to the microcosm treated with tap water (control), the microcosms treated with water from the upstream and middle stream displayed similarity coefficient index values of 0.92 and 0.94, respectively. This shows that the water from upstream and middle stream are least polluted and is essentially the same as tap water. Conversely, the microbial community of soil is greatly impacted by the extremely polluted water from the lower streams. Similarly, PCA analysis showed high percentage of variance among the three streams. Lower stream on the right side of axis with 96.09% of variance along PC 1 while up and middle stream on the left with 3.80% of variance along PC2 (Figure 4).

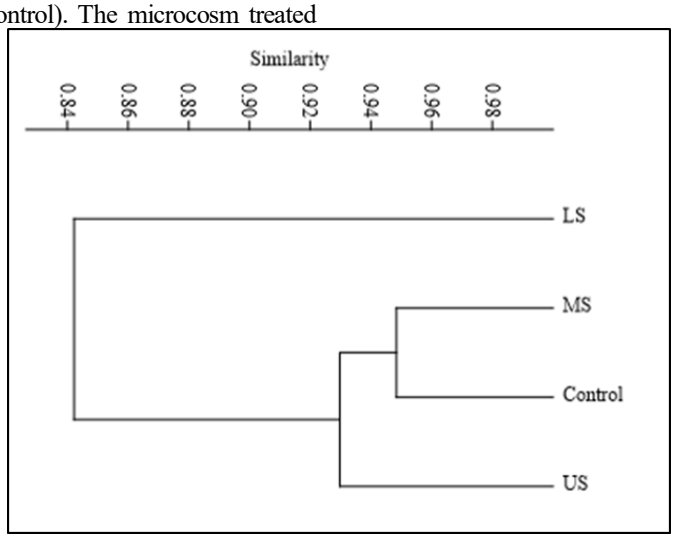


Figure 3. UPGMA dendrogram showing coefficient of similarity by using the Jaccard's index on the basis of ARISA-ADAPT profiles observed in soil treated with Yamuna River water from US (upstream), MS (middle stream), LS (lower stream) regions of Delhi-NCR.

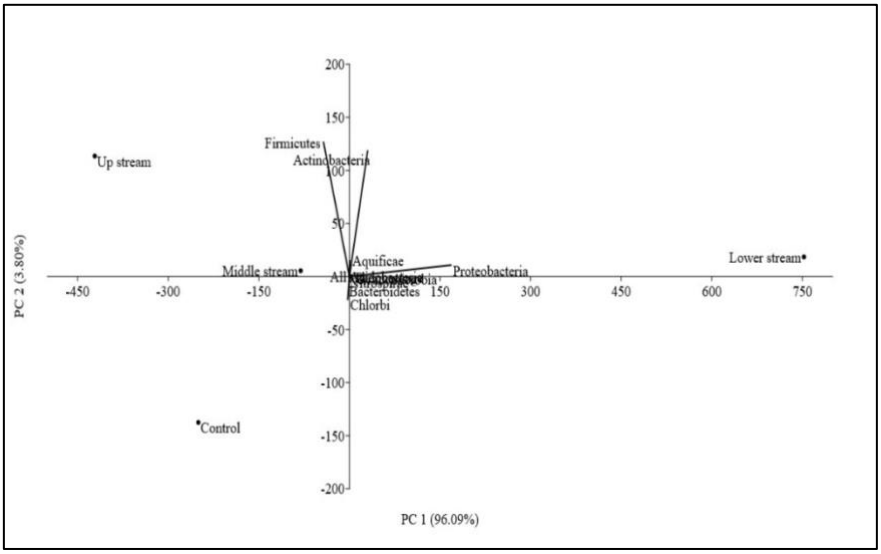


Figure 4. Principal component analysis graph showing percentage of variance among different Bacterial phyla on the basis of their relative abundance in Yamuna River (upstream, middle stream, lower stream). In the lower stream, *Proteobacteria* showed 96.09% of variance with respect to rest of the Bacterial phyla indicate *Proteobacteria* use contaminants as a source of energy and flourish in polluted water. while up stream and middle stream showed only 3.80% variance. *Firmicute* and *Actinobacteria* flourish in lower and upstream indicate that pollutant present in river water restrict the growth of these bacteria phyla.

4. Discussion

Microcosm study is a very useful and reliable technique which helps us to observe the activity of a particular organism or an environmental factor in a controlled manner. It helps us to understand the whole process in a short period of time. Presently, Yamuna River is regarded as one of the most polluted rivers in world. Many big cities situated along the course of Yamuna River depend directly or indirectly on river water for its livelihood.Keeping in mind the deteriorating states of Yamuna River water due to increase in pollution, it becomes utmost important to know the effect of polluted river water on soil, the main life supporting medium. Soil is the mixture of minerals, organic matter, gases and countless micro- and macro- organisms that can support plant and animal life [23]. It takes part in various biogeochemical cycles.

In the present experiment, effects of Yamuna River water on soil enzyme activity and microbial diversity were studied. After 120 days, it was observed that river water from lower stream which is the most polluted stretches of the river has significant effect on physico-chemical properties, enzyme activity and microbial community structure of soil. Total organic carbon includes all the elements (hydrogen, oxygen, nitrogen, etc) that are components of organic compounds and not just carbon. Municipal solid waste is the rich energy source of carbon and increases the soil microbial population and its activity [24]. Total organic carbon content was highest in soil treated with river water from lower stream. This might be due to the presence of sewage waste of various drains (Najafgarh drain and Shahdara drain) which directly dumped their waste into the Yamuna River. These municipal wastes are the rich source of organic carbon and also act as a source of energy for soil microbes [24]. Of the 22 major and minor drains which dump their waste into the Yamuna River, Shahdara drain is the largest drain. It dumps its waste directly into Yamuna River at Noida Bridge (lower stream).

Soil enzymes are either intracellular or extracellular in nature. They are mostly microbial in origin, so variation in any environmental condition which affects the microbial population, influence the activity of soil enzymes. Sewage sludge is composed of highly oxidizable organic substrate and contains huge amount of biomass which results in increasing the dehydrogenase enzyme activity [3]. The higher dehydrogenase enzyme activity was observed in soil treated with river water from lower stream might be due to the higher pollution level of Yamuna River water at lower stream. Dehydrogenase enzyme is an intracellular enzyme responsible for oxidative phosphorylation. It is linked with microbial respiration; hence it is a good indicator of viable microbial system in soil. Municipal waste contains various organic and inorganic nutrients which act as a source of energy for the microbes and leads to increase in soil microbial biomass.

Chemical hydrolysis of urea within the soil is a very slow process therefore hydrolysis of urea in soil is mainly brought about by the action of urease enzyme [25]. In this microcosm study, urease enzyme showed higher activity in all the experimental pots treated with river water except control, treated with tap water. Urease enzyme activity was significantly increased from 60^{th} day onwards. Urease enzyme is closely related with nitrogenous waste present in the environment because it is the main composition of nitrogenous waste excreted by mammals. Addition of domestic waste and sewage waste directly into the river increases the concentration of urea in the river water. Most commonly used nitrogenous fertilizers such as urea also lead to an increase the concentration of urea in soil and also in water through leaching. Therefore, soil samples treated with Yamuna River water showed higher urease activity. Urease enzyme helps in mineralization of these waste products and release nitrogen as a source of energy for soil microbes [25].

Nitrate reductase also showed higher activity in pot soil treated with lower stream water. Nitrate reductase is a denitrifying enzyme which turns oxides back into nitrogen gas and nitrous oxides. Denitrification occurs only in the absence of oxygen. Organic pollutants create anaerobic conditions within the soil which enhance the activity of denitrifying enzyme [24]. In Delhi many untreated sewage wastes drain directly enter into the Yamuna River. One of the largest drains i.e. Shahdara drain directly dumps into the Yamuna River at Noida bridge. So, at Noida bridge Yamuna River water becomes most polluted. This may increase the activity of nitrate reductase enzyme present in soil from pot treated with river water from lower stream.

4.1 Effect of Yamuna water on microbial community structure

Since soil enzymes are mainly of microbial origin, hence variation in their activity indicates the variation in soil microbial activity. Various sewage drains which directly dump into the Yamuna River are the rich source of organic carbon and also act as a source of energy for soil microbes. They increase the activity and diversity of microbial community [3,24]. *Firmicutes, Proteobacteria* and *Actinobacteria* showed community shift with each other. *Firmicutes* also showed higher species richness and relative abundance than *Proteobacteria,* and *Actinobacteria* followed by other bacterial groups were found to be present in the soil. Among the different bacterial groups present in the soil, *Firmicutes*, *Proteobacteria* and *Actinobacteria* showed more percentage of variability against the water pollution. Relative abundance of *Firmucutes* decrease from upstream to lower stream while that of *Proteobacteria* and *Actinobacteria* increased. Presence of Pollutants in river water cause a community shift among the bacterial groups present at that place. Various studies have been done which show that sewage water and municipal waste cause a community shift in bacterial population [6,24, 26- 27].

Sewage and municipal wastes contain various organic and inorganic nutrients. These nutrients may act as a source of energy for microbial metabolic growth [24]. Increase in the relative abundance of *Proteobacteria* and *Actinobacteria* may be due to their role in degrading various organic and inorganic waste in river water [28-29]. Increase in their relative abundance explain their resistance toward increase in pollutant concentration. These are well-known decomposer of various organic and inorganic material present in soil. On the other hand, *Firmicutes* are obligate aerobes might be due to this reason their relative abundance decreases as the level of dissolved oxygen decrease from upstream to lower stream. Decrease in DO level indicates increase in pollutant concentration which create anaerobic conditions [4]. This might be responsible for decrease in the relative abundance of *Firmicutes*.

Aquificae, Acidobacteria and *Verrucomicrobia* groups was found to be absent in control soil. Among these three, *Aquificae* and *Verrucomicrobia* are fresh water bacterial groups and found in lakes, ponds and oceans. These two groups are generally found in anthropogenic wastes which is mostly found near river sides due to various anthropogenic activities. *Acidobacteria* group found in hot springs and metal contaminated environment [30]. This signifies that they may also act as a pollution indicator.

Cluster analysis and PCA were performed to have an overview of the association of bacterial communities present in soil treated with Yamuna River water from three different streams. In Cluster analysis soil samples treated with river water from lower stream showed very less coefficient of similarity with other soil samples and control also. This signifies that river water at upstream and middle stream is least polluted while from lower stream was highly polluted. Correspondingly in PCA, lower stream showed high percentage of variability. This signifies that lower stream had high percentage of variability among the bacterial groups than up and middle stream. Yamuna river water at lower stream is regarded as most polluted as the DO level at this site is very low [31]. Variations in the concentration of pollutants in Yamuna River water at the different streams cause a variability in the abundance of soil microbes.

5. Conclusion

This study shows that increase in pollution level of Yamuna River within Delhi-NCR regions has profound effects on soil physio-chemical properties, microbial diversity and enzyme activities. The percentage abundance of *Firmicute, Proteobacteria* and *Actinobacteria* were higher in the sample treated with river water from the most polluted site i.e lower stretch of Yamuna River in Delhi-NCR region. Furthermore, some bacterial groups such as *Acidobacteria*, *Verrucomicrobia*, and *Aquificae* were only found in the sample treated with river water. The results show that increased organic pollutant enhance microbial enzyme activity and favours the growth of pollution adapted bacterial groups while reducing diversity of sensitive species. These findings highlight the serious ecological risk caused by untreated sewage and industrial waste discharge into the river. Moreover, the specific pollutant adapted bacterial groups could be used as a biomarker to assess the river water quality.

Conflict of Interest

We have not any financial/commercial conflicts of interest.

Acknowledgements

I implore my profound gratitude to DST-DU Purse program of the University of Delhi, Delhi-110007 for providing us with all the necessary research facilities.

References

1. Ahmad, Z., Rehman, A.Ur., Anees, M., 2013 Microcosmic study of nickel stress towards soil bacteria and their biochemical characterization. *J. Bio-Mol. Sci.* 1,37-44.

2. Caracciolo, A.B., Bottoni, P., Grenni, P., 2012 Microcosm studies to evaluate microbial potential to degrade pollutants in soil and water ecosystems. *Microchem.* J, 1, 5.

3. Sharma, R., Singh, N.S. and Singh, D.K., 2020. Impact of heavy metal contamination and seasonal variations on enzyme's activity of Yamuna River soil in Delhi and NCR. *Appl Water Sci.* 10(3), p.83.

4. CPCB., 2014 Highlights of the Yamuna River water quality monitoring in Delhi stretch based on monthly study carried out during the year 2014 http://cpcb.nic.in/wqm/2014/RIVERWATER%20DATA%202014_6.htm.

5. Kaushik, C.P., Sharma, H.R., Jain, S., Dawra, J., Kaushik, A., 2008 Pesticide residues in river Yamuna and its canals in Haryana and Delhi, India. *Environ. Monitor. Assess*. 144: 329–340.

6. Sharma, R., Singh, N.S. and Singh, D.K., 2019a. Soil microbial diversity of peri-urban agricultural field and riverbank along Yamuna River in Delhi, India. *SN Appl Sci*. 1(1), p.22.

7. Sharma, R., Singh, N.S. and Singh, D.K., 2019b. Diversity of nitrogen-fixing bacteria in agricultural field soil along the Yamuna River, Delhi, India. *Agri. and Nat. Res.* 53(6), pp.566-572.

8. Sodhi, K. K., Kumar, M. and Singh, D. K., 2021. Assessing the bacterial diversity and functional profiles of the River Yamuna using Illumina MiSeq sequencing. *Arch Microbiol*, 203(1), 367-375.

9. Bhardwaj, R., Gupta, A. and Garg, J. K. 2017. Evaluation of heavy metal contamination using environmetrics and indexing approach for River Yamuna, Delhi stretch, India. *Water sci*, 31(1), 52-66.

10. Asim, M. and Nageswara Rao, K. 2021. Assessment of heavy metal pollution in Yamuna River, Delhi-NCR, using heavy metal pollution index and GIS. *Environ Monit Assess,* 193(2), 103.

11. Sazykina, M. A., Minkina, T. M., Konstantinova, E. Y., Khmelevtsova, L. E., Azhogina, T. N., et al., 2022. Pollution impact on microbial communities' composition in natural and anthropogenically modified soils of Southern Russia. *Microbiol res*, *254*, 126913.

12. Sainju, U. M., Liptzin, D. and Dangi, S. M. 2022. Enzyme activities as soil health indicators in relation to soil characteristics and crop production. *Agrosys Geosci Environ,* 5(3), e20297.

13. Singh, P., Purakayastha, T. J., Mitra, S., Bhowmik, A., Tsang, D. C. 2020. River water irrigation with heavy metal load influences soil biological activities and risk factors. *J. Environ. Manage*., 270, 110517.

14. Alef, K., Nannipieri, P., 1995 Methods in Applied Soil Microbiology and Biochemistry. Third ed. Academic Press, London.

15. Walkley, A., Biack, I.A., 1934 An Examination of the Detjare Method for Determining Soil Organic Matter and a Proposed Modification of the Chromic Acid Titration*. J. Soil Sci.* 37,29-36.

16. Thalmann, A., 1968 Zur Methodik der Bestimmung der Dehydrogenaseaktivität im Boden mittels Triphenyltetrazoliumchlorid (TTC), Landwirtsch Forsch 21,249–258.

17. Kandeler, E., Gerber, H., 1988 Short+term assay of soil urease activity using colorimetric determination of ammonium. *Biol. Fert. Soils*. 6,68-72.

18. Slabbert, E., Van, Heerden, C.J., Jacobs, K., 2010 Optimisation of automated ribosomal intergenic spacer analysis for the estimation of microbial diversity in fynbos soil. *S Afr. J. Sci.* 106,1-4.

19. Fisher, M.M., Triplett, E.W., 1999 Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. *Appl. Environ. Microbiol*. 65,4630–4636.

20. Feng, B.W., Li, X.R., Wang, J.H., Hu, Z.Y., Meng, H., Xiang, L.Y., Quan, Z.X. 2009 Bacterial diversity of water and sediment in the Changjiang estuary and coastal area of the East China Sea. *FEMS Microbiol. Ecol.* 70,236–248.

21. Ramette, A., 2007 Multivariate analyses in microbial ecology. FEMS Microbiol Ecol 62,142–160.

22. Kent, A.D., Yannarell, A.C., Rusak, J.A., Triplett, E.W., McMahon, K.D., 2007 Synchrony in aquatic microbial community dynamics. *ISME J.* 1,38–47.

23. Al-Kaisi, M.M., Lal, R., Olson, K.R. and Lowery, B., 2017. Fundamentals and functions of soil environment. In Soil health and intensification of agroecosytems (pp. 1-23). Academic Press.

24. Garbini, G.L., Caracciolo, A.B., Rolando, L., Visca, A., Borello, D., Cosentini, C., Gagliardi, G., Ieropoulos, I. and Grenni, P., 2023. Effects of

25. municipal waste compost on microbial biodiversity and energy production in terrestrial microbial fuel cells. *New Biotechnology*, 78, pp.131-140.

26. Sigurdarson, J.J., Svane, S. and Karring, H., 2018. The molecular processes of urea hydrolysis in relation to ammonia emissions from agriculture. *Rev. Environ. Sci. Biotechnol*. 17(2), pp.241-258.

27. El-Khateeb, M., Hassan, G.K., El-Liethy, M.A., El-Khatib, K.M., Abdel-Shafy, H.I., Hu, A. and Gad, M., 2023. Sustainable municipal wastewater treatment using an innovative integrated compact unit: Microbial communities, parasite removal, and techno-economic analysis. *Ann. Microbiol.* 73(1), p.35.

28. Gangola, S., Joshi, S., Bhandari, G., Pant, G., Sharma, A., Perveen, K., Bukhari, N.A. and Rani, R., 2023. Exploring microbial diversity responses in agricultural fields: a comparative analysis under pesticide stress and non-stress conditions. *Front. Microbiol.* 14, p.1271129.

29. Okoye, A.U., Selvarajan, R., Chikere, C.B., Okpokwasili, G.C. and Mearns, K., 2024. Characterization and identification of long-chain hydrocarbon-degrading bacterial communities in long-term chronically polluted soil in Ogoniland: an integrated approach using culture-dependent and independent methods. *Environ Sci Pollut Res Int*, pp.1-19.

30. Thrash, J.C., Coates, J.D., 2015. Acidobacteria phyl. nov.. In Whitman WB (ed.). Bergey's Manual of Systematics of Archaea and Bacteria. John Wiley & Sons. pp. 1–5.

31. Halliday, E., McLellan, S.L., Amaral-Zettler, L.A., Sogin, M.L., Gast, R.J., 2014 Comparison of Bacterial Communities in Sands and Water at Beaches with Bacterial Water Quality Violations. *PLoS ONE*, 9: e90815.

32. Dhillon, M.K., George, M.P., Mishra, S., 2013 Water quality of River Yamuna – Delhi stretch. *Int. J. of Environ. Sci.* 3,1416-1423.