Studies on Contamination of Heavy metals on Microbial Community Structure of Agroecosystem in the Vicinity of Lucknow (India) and Associated Environmental Hazards

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BABASAHEB
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Dipti

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Under the Supervision of

Prof. Rana Pratap Singh

DEPARTMENT OF ENVIRONMENTAL SCIENCE
SCHOOL FOR ENVIRONMENTAL SCIENCES
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
A CENTRAL UNIVERSITY) (NAAC A++ ACCREDITED)
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Summary

Globally, soil contamination is worrisome issue affecting the sustainable development and food safety. Soil is a dynamic natural resource that is necessary for the existence of life of plants, microbes and animals, and also considered as the major receiver of the relentless contaminants like heavy metals. In environment, these metal contaminants are majorly introduced through anthropogenic activities and some natural sources such as volcanic eruptions, erosion, weathering of rocks, fossil fuel combustion, organic manures, pesticides and fertilizer, disposal of industrial and urban wastes, metallurgical industries, mining, atmospheric depositions through aerosols and dust etc. In developing nations, rapid industrialization, urbanization, excessive usage of synthetic chemicals and metals in the terrestrial environs coupled with the partial or deficient environmental management practices have triggered large-scale contamination in soil, sediments and water. India is one of the developing nations, where the contamination of HMs in agricultural soils has comprehensively researched due to intense expansion in agricultural sector. However, these investigations mostly emphasized on the identification of HMs contaminant and its level in soil environ, ecological and health risk assessment, translocation and accumulation in food crops.

Particularly in Uttar Pradesh region, the distribution of these heavy metals in agricultural soil, surface soil near industrial areas, municipal waste dumping sites, roadside, sewage disposal sites and brick industries were reported from different districts and/or cities. HMs contamination in the soil not only alters the physicochemistry of soil, but also changes the structure as well as composition of microbial communities, thus causing loss of soil fertility. Soil microorganisms are regarded as a microbiological indicator of soil health because they are susceptible to

changes imposed by soil properties like pH, available nutrients, organic matter, and pollutants. Notwithstanding, the negative impacts of HMs on the soil microbes, there are very meagre investigations involved to explore their influence on the residing soil microbial communities and soil fertility. To my best knowledge, there is no previous study exploring about the indigenous microbial community of the HMs contaminated agricultural soil of studied area i.e Lucknow.

Our question was to assess the microbial community structure and impact of varying levels of HM contamination in agricultural soil of Lucknow vicinity areas, and also about indigenous fungal populations possessing potential application in the soil remediation process. We hypothesized that HMs contamination in agroecosystem imposes distinct effect on the micro-environment in which microbes resides and the alteration in the microbial communities structure (especially bacteria and fungi) would be linked with the changes in the physiochemical properties of the agricultural soil. The main objective of this study was to determine the impact of HMs contamination in agricultural soil on the bacterial and fungal abundance and diversity using metagenomic approaches. Moreover, these contaminated soils are important source of HM tolerant microbes and considering the important of metal tolerant microbes isolation and selection of HM tolerant fungal isolates was done to explore the possibility of their utilization in the bioremediation purposes.

In present study, total 30 soil samples were collected from various peri-urban agriculture areas of the vicinity of Lucknow (Uttar Pradesh). The first site was Gaughat (coded as GAF) that lies on the margins of Gomti River and the farmer in this area utilizes contaminated river water for irrigation, the second site Mohanlalganj (MAF) that also lies on the margins of Sai River and utilizes its water for irrigation, the third site lies near Sitapur municipal waste dumping sites (SAF), the fourth site was Bijnaur

(BAF) near brick kiln incinerators, the fifth selected site was Devaroad (DAF) near roadside and the last site was Barabanki (BbAF). At least 5 soil samples were collected from each individual site, which itself was a composite mixture of 5-7 sub-soils. For determination of physico-chemical properties, concentration of HMs, identification of microbial (bacterial and fungal) communities in soil and heavy metal tolerance in fungal isolates standard protocols were followed.

Heavy metal contamination in agricultural soil of Lucknow and its ecological risk assessment

The levels of HMs at various locations were obtained in the range: Cr (2.956 -12.948 mg/kg), Cd (0.104 -12.700 mg/kg), Cu (5.947 - 30.545 mg/kg), Ni (6.725-19.633 mg/kg), Fe (2153.118 - 10873.894 mg/kg), Zn (17.638 - 92.581 mg/kg), Mn (96.386 - 301.797 mg/kg), Co (2.841- 7.493 mg/kg), As (0.409- 2.321 mg/kg), Se (0.143-1.557 mg/kg), Mo (0.050 – 1.009 mg/kg) and Pb (2.652 – 16.932 mg/kg). The mean concentration of Cr, Mn, Ni, Cu, Zn, Fe and Cd in the agricultural soils largely surpassed (several folds higher) the background values (BGV) of Lucknow. A comparison of obtained HMs levels in agricultural soil to various global standards showed that majority of HMs fell within the permissible limit, except Cu, Zn, Cd, Pb and Fe. The level of Cu and Pb in the Gaughat (GAF) site slightly exceeded USEPA guideline, whereas Zn content exceeded WHO guidelines. The level of Cd in GAF was much greater than the permissible limit for agricultural soil and this is a matter of concern for the public health safety, based on the CCME (2007), Indian and European standards limit. Secondly, Sitapur (SAF) site also contained higher Cu and Cd content as per WHO guideline. The toxic situation of these agricultural soils is evident from the Potential ecological risk index value of two sites, GAF and SAF falling in the “very high risk” and “considerable risk” category, respectively. Pollution load index also signifies “very high” and “moderately to high” pollution in GAF and SAF sites, respectively.

The probable reason for maximum HMs contents obtained in Gaughat area which lies on the margin of river Gomti is that farmer’s in this area utilized contaminated water of river directly for the irrigation purposes. This river is widely researched in the past few years due to increasing concentration of HMs and other organic pollutants, receiving it in the form of wastewater from heterogeneous pollution sources like electroplating industries, agrochemicals, sugar mills, tanneries, direct municipal sewage discharge through drains, direct dumping of wastes, discharge of household water from the slums residing on the banks of Gomti river etc. Whereas, the HM contamination observed in Sitapur (SAF) site might be due to presence of cities biggest municipal solid waste dumping (MSW) site lying close to agricultural areas of this region. The soil collected from the periphery MSW contained Cr (62.327 mg/kg), Mn (290.609 mg/kg), Cu (225.188 mg/kg), Zn (296.496 mg/kg), Pb (136.862 mg/kg) etc. During rainy season, these contaminants reach agricultural areas lying at lower altitude along with rain water via runoff.

Bacterial community structure of HM contaminated agricultural soil

The two highly contaminated agricultural soils i.e GAF and SAF obtained from the soil heavy metals monitoring study were selected for the metagenomics study in order to explore what type of microbial community (bacteria and fungi) is residing in these contaminated agricultural soils and the impact of metal contaminants on their community structure and diversity. A control soil (CON) sample was also collected from non-contaminated area for comparative analysis. At phylum level, the bacterial community was mainly predominated by Proteobacteria, Bacteroidetes, Acidobacteria and Actinobacteria phyla in HMs contaminated soils. At genus level, the bacterial communities were commonly dominated by *Sphingomonas, Lysobacter*, *Flavisolibacter*, *Prevotella*, *Fusobacterium, Stenotrophobacter*, *Leptotrichia* and *Capnocytophaga* among the studied soils. Alpha-diversity of microbes reflects that moderately contaminated agricultural soil harbour more diversity in comparison to highly contaminated and control soil. An analysis of metabolic functions performed by bacterial communities in soil demonstrated total 14 dominant pathways (with relative abundance >1%) such as ammonia oxidation, degradation of aromatic hydrocarbons, dehalgenation, nitrogen fixation, xylan degradation, chitin degradation etc. among the soil samples. Spearman correlation and Canonical correspondence analysis study between bacterial community and environmental factors (i.e physico-chemical parameters and heavy metals concentration) demonstrated that pH, OC, AP, Cd, Cu and Zn had a significant effect on bacterial community structure. Additionally, genus *Sphingomonas*, *Flavisolibacter, Terrimonas* and *Streptomyces* have a potential for bioremediation of HMs pollution.

Fungal community structure of HM contaminated agricultural soil

At phylum level, the fungal community was mainly predominated by Ascomycota and Mucoromycota across the studied sites. At genus level, the three most abundant OTUs corresponds to *Mortierella* (42.171%), *Chaetomium* (7.196%) and *Curvularia* (6.588%) in highly contaminated soil (GAF) highlighting their tolerance and adaptation to highly metal stressed condition; *Emericellopsis* (17.852%), *Curvularia* (12.401%) and *Parengyodontium* (6.975%) in less contaminated SAF soil and *Humicola* (36.654%), *Mortierella* (32.891%) and *Parengyodontium* (5.580%) in non-contaminated soil samples (CON). Alpha- diversity index showed that both the contaminated agricultural soil (GAF and SAF) harbour nearly similar diversity, which was less diverse as compared to the CON soil. Spearman correlation between the fungal community and environmental factors demonstrated that pH, TN, Cr, Fe, Zn, Cu, Cd and Pb had a significant major influence on the fungal community structure. Moreover, *Mortierella, Chaetomium, Fusarium* and *Talaromyces* have a potential for the bioremediation of HMs.

Isolation of Heavy metal tolerant fungi from HMs contaminated agricultural soil for assessing its HM absorption capacity

Since, GAF and SAF sites depicted higher contamination of Cd in the soil, therefore Cd tolerant fungi were isolated on 100 mg/L of cadmium nitrate amended PDA plate in order to assess their potential for remediation purposes. At this concentration, ten morphologically distinct microbes were obtained which were further purified on PDA plate. Firstly, the isolated fungal strains were screened for their tolerance against different concentrations of Cd. Among 10 isolates, only three isolates (coded as GF_2, GF_5 and SRF_4) of them have capability to grow on 1000 mg/L Cd amended plate. Hence, only three species were preferred for the further analysis. Further, the HM tolerance ability of three selected fungal isolates was tested against the concentration of certain other toxic metal contaminant and its was obtained that Isolate GF_2 has ability to tolerate Pb (1000 mg/L), Cu (500 mg/L), Co (250 mg/L), As (500 mg/L), Ni and Cr (100 mg/L); Isolate GF_5 can tolerate Pb (1000 mg/L), Cu (500 mg/L), Co (750 mg/L), Ni (500 mg/L), Cr (500 mg/L) while, showed no tolerance for As metal and Isolate SRF_4 was tolerant to Pb (1000 mg/L), Cu (500 mg/L), Co (500 mg/L), As (1000 mg/L), Ni and Cr (250 mg/L).

For the molecular identification of isolated microbes, ITS (internal transcribed spacer) region sequencing was performed. The identification of fungal strains species was done using Blast tool, NCBI database using the sequenced data. Isolate GF_2

depicted maximum resemblance with *Phanerochaete chrysosporium*; GF_5 showed maximum resemblance with *Diaporthe longicolla* and SRF_4 depicted maximum similarity with *Talaromyces* sp.

Cadmium removal efficiency and uptake capacity of *Phanerochaete chrysosporium, Diaporthe longicolla* and *Talaromyces* species

With the increasing concentration of Cd in the PDB media, the metal uptake capacity and removal efficiency of fungal isolates was significantly increased and a notable increased uptake capacity was noticed at the 100 mg/L concentration of Cd. The highest Cd uptake was observed in *D. longicolla* (16.038 mg/g DW), followed by *Talaromyces* (15.848 mg/g DW) and least was obtained in *P. chrysosporium* (15.743 mg/g DW) with a removal efficiency of 89.5 %, 86.3% and 84.8% in broth medium, respectively. The removal of HMs involves various mechanisms such as accumulation, precipitation, adsorption and reduction as investigated through SEM-EDS and FTIR analysis. SEM analysis reflected that morphology of the mycelia of *P. chrysosporium* was altered from smooth, enlarged and regular surface of the hyphae to dense, undistinguishable, distorted, compactly packed fungal hyphae, which might be a fungal detoxification mechanism. FTIR analysis of Cd treated fungal biomass inferred that functional groups such as –NH, –OH, –CH, C=O, S=O which exist on the surface mycelia of *P. chrysosporium* participated in Cd biosorption. Similarly, SEM analysis of mycelium of *Talaromyces sp*. was also altered from smooth, cylindrical and branched mycelia in control to tightly aggregrated, irregular mycelial surface in Cd treated biomass and such morphological changes are consequence due to toxic effect of HMs. Moreover, the FTIR analysis inferred functional groups such as –NH, –CH, C≡C, C=O, SO_2, COO^- and phosphate groups that exist on the surface mycelia of *Talaromyces sp.* participated in Cd biosorption. Contrarily, the SEM study of *D. longicolla* revealed no significant influence of Cd on the growth of this fungus in Cd treated biomass, except intertwining fungal hyphae and small irregular buds. This also showed that fungi can tolerate high concentration of toxic metal without any major toxicity responses. FTIR analysis revealed that functional groups like –OH, –NH, –CH, C≡C, C=O and COO^- lying on the cell wall surface of D. longicolla were might involve in the adsorption of Cd.

Key findings of the study

- Among all studied sites, the agricultural soil present on the margins of Gomti River in Gaughat area demonstrated Cd above Indian standards range of 3-6 mg/kg, Cu and Pb higher than USEPA guideline (30 and 10 mg/g, respectively); whereas, the agricultural soil near municipal waste dumping sites contained Cu and Cd slightly above WHO standard value.
- The concentration of Cr (1.1-5.95 folds), Mn (2.2-6.8 folds), Ni (2.2-6.5 folds), Cu (1.4-7.4 folds), Zn (1.2-6.1 folds), Fe (1.01-3.3 folds) and Cd (1.2-141.1 folds) in the agricultural soils largely surpassed the background values of Lucknow; whereas, Pb (1.46-2.98 folds) at some sites.
- The bacterial community of the contaminated agricultural soils was mainly dominated by members of Proteobacteria, Bacteroidetes, Acidobacteria and Actinobacteria phyla.
- Environmental factors such as pH, OC, AP, Cd, Cu and Zn were majorly influencing the bacterial community structure of the soil.
- The fungal community of the studied soil was dominated by members belonging to Ascomycota and Mucoromycota phyla.
- Environmental factors such as pH, TN, Cr, Fe, Zn, Cu, Cd and Pb were majorly influencing the fungal community structure of the investigated soil.

- Fungal strains namely *Phanerochaete chrysosporium*, *Diaporthe longicolla* and *Talaromyces sp.* isolated from the contaminated soil possess heavy metal tolerance against various HMs.
- Among all isolates, *D. longicolla* showed maximum Cd removal efficiency (89.5%) in PDB medium at 100 mg/L concentration of Cd.

Recommendations

A regular monitoring of the concentration and distribution of heavy metals in the agricultural soil, especially those lying close to any direct contaminated source is utmost important to prevent the environment from various toxicological impacts of HMs in terms of soil fertility and food produces. As, Rivers are important source of irrigational water and contaminated water is playing a huge role in creating soil pollution with various organic and inorganic pollutants including HMs, the government needs to take strict preventive steps to reduce it via some awareness campaign among farmers.