Summary
Rivers have always been the most important freshwater resources, along the banks of which our ancient civilizations have flourished, and most developmental activities are still dependent upon them. River water finds multiple uses in every sector of development like agriculture, industry, transportation, aquaculture, and public water supply etc. however, since old times, rivers have also been used for cleaning and disposal purposes. Huge loads of waste from industries, domestic sewage and agricultural practices find their way into rivers, resulting in large-scale deterioration of the water quality.

The concentration of metal pollutants in the environment is usually low excepting in specific areas, which are polluted by various hospitals and industrial wastes. Although some heavy metals are essential trace elements, most can be, at high concentrations, toxic to all branches of life including microbes by forming complex compounds within the cell. Because heavy metals are increasingly found in microbial habitats due to natural and industrial processes, microbes have evolved several mechanisms to tolerate the presence of heavy metals. Metal resistance is often associated with resistance to single or multiple drugs, phenolic compounds and pesticides.

Several hundred pesticides of different chemical nature are currently used for agricultural purposes all over the world. Because of their widespread use, they are detected in various environmental matrices, such as soil, water and air. Pesticides are divided into many classes, of which the most important are organochlorine and organophosphorus compounds. Organochlorine pesticides are known to resist biodegradation and therefore, they can be concentrated through food chains and produce a significant magnification of the original concentration at the end of the food chain.

The demonstration that wastewater can induce genotoxic effects in aquatic and terrestrial species has stimulated interest in this area. Progress in this field is in large part due to the development and widespread use of short-term genetic bioassays. Their simplicity, relative speed and low cost in experimentation, and small amount of samples required for analysis have permitted research in genetic toxicology to flourish. Results from genetic bioassays are relevant to human health because the toxicological target is DNA which exists in all forms of life.

In this study, efforts were made to evaluate the genotoxicity of the Yamuna River water and contaminated soil to highlight the putative hazards of industrial and domestic wastewater in the test system and also to investigate the patterns of resistance developed by certain Gram negative bacteria against heavy metals and antibiotics.
Significant findings along with their explanations are summarized as under:

I. Quantitative determination of heavy metals and pesticides

- Atomic absorption spectrophotometric analysis of water and soil samples revealed the presence of a variety of heavy metals viz. Fe, Zn, Cu, Cr, Pb, Cd and Ni. Test water samples (Mathura and Okhla) contained high levels of these metals as compared to that of control site (Sarsawa).
- Soil irrigated with wastewater contained elevated concentration of several heavy metals as compared to that of ground water irrigated soil and a significant difference was observed in the levels of these metals in two different soils.
- GC analysis of the water samples showed the presence of pesticides i.e. BHC, aldrin, endosulfan, dieldrin, 2,4-D, DDT, dimethoate and methyl parathion.
- Soil samples were also analyzed for the presence of pesticides by gas chromatography and a significant difference was observed between soil irrigated with wastewater and ground water irrigated soil.
- Some unidentified peaks in the gas chromatograms of the test water and soil samples were also observed, which showed the presence of other contaminants in the test samples.

These findings clearly indicated that test water and soil samples contained several organochlorine and organophosphorus pesticides. Several heavy metals were also present in the test samples. Wastewater irrigated soil contained elevated level of heavy metals as compared to that of ground water irrigated soil. This might be due to the strategic position of the area of our study namely the outskirts of Aligarh City which houses many lock manufacturing, steel and electroplating industries and their effluents would contain quite a large amount of these metals.

II. Heavy metal and antibiotic resistance in Gram negative bacteria isolated from the river water and soil

Certain Gram-negative bacteria (Pseudomonas spp., E. coli, and Azotobacter chroococcum) were isolated from the Yamuna water and soil, and identified on the basis of morphological, cultural and biochemical characteristics.

- All the bacterial isolates were tested for their resistance against certain heavy metals i.e. Hg\(^{2+}\), Cd\(^{2+}\), Cu\(^{2+}\), Cr\(^{3+}\), Cr\(^{6+}\), Zn\(^{2+}\), Ni\(^{2+}\) and Pb\(^{2+}\).
A high level of metal resistance was observed in bacteria isolated from water and soil irrigated with wastewater as compared to that of ground water irrigated soil. Most of the isolates showed MIC of more than 200 µg/ml to these metals. Further, MIC values upto 3200 µg/ml were recorded. Majority of the isolates from water and contaminated soil were resistant to multiple metal ions.

All the bacterial isolates were also tested for their sensitivity against 17 commonly used antibiotics/drugs and most of the isolates were resistant to multiple antibiotics.

Some of the multiple metal and antibiotic resistant strains were further screened for the presence of plasmid DNA and characterized by agarose gel electrophoresis. The molecular weight of the plasmids were calculated using photocapt software (Vilber Lourmat, France). The molecular weight of Pseudomonas plasmids were in the range of 49.9-56.8 kb, while that of E. coli were in the range of 51.0-53.5 kb.

Resistance markers could be transferred from multiple metal resistant strains to recipient E. coli K-12 AB2200 strain by conjugation.

Bacterial cells seem to have cured of plasmid DNA on treatment with ethidium bromide and acridine orange.

The above findings suggest that the Yamuna water and soil is heavily polluted with several types of pesticides, toxic metals as well as the potentially hazardous bacterial flora because of their capacity to resist one or the other well known chemotherapeutic agents. Moreover, the occurrence of resistant bacterial species can be detrimental for the ecosystem. Present study also indicated that not all species have evolved resistance mechanisms for metals and antibiotics. Many sensitive species might have been eliminated by the pollutants and their place is taken by the resistant species which have different ecological properties. The resistant microorganisms often failed to perform specific ecological functions. One step ahead of the above, we can envisage the alarming situation prevailing in our system and surrounding in the light of transmissible nature of R-plasmids.

III: Genotoxicity of the Yamuna River water

Test samples (Mathura and Okhla) displayed significant enhancement in the number of his+ revertant colonies as compared to that of control site (Sarsawa).

TA98, which are frame shift mutants were found to be the most sensitive strain. The number of revertants at the dose level of 10 µl/plate was 522 when tested with the
XAD concentrated water samples of Okhla as compared to that of Mathura at the same dose (464 his+ revertants).

- All the tester strains responded significantly even in the absence of S9 liver microsomal fraction.
- The mutagenic activity of XAD concentrated samples was further enhanced in the presence of S9 fraction.
- Liquid-liquid extracted water samples also exhibited a remarkable level of mutagenic activity. Strains TA98, TA100, TA97a displayed a linear dose response relationship with the test samples as long as the dose was within the tolerance limit of the strains. However, the presence of S9 fraction either slightly decreased the number of revertants or remains the same as compared with that in the absence of S9 fraction.
- Strains TA98 and TA97a exhibited the maximum mutagenic activity with 2411 and 1508 net revertants/liter of water respectively when treated with liquid-liquid extracts of Mathura in the absence of S9 fraction.
- XAD-concentrated water samples of Mathura resulted significant mutagenic response with TA97a (9825 revertants/liter) and TA98 (11738 revertants/liter) strains in the absence of S9 fraction.
- The number of net histidine revertants/l with strain TA98 in the absence of S9 fraction was 12918 when treated with XAD concentrated water samples of Okhla.
- The recA, lexA, and polA mutants of E. coli K-12 were highly sensitive in the presence of test samples (XAD-concentrated and liquid-liquid extracted) of Mathura and Okhla. All the mutants invariably exhibited a significant decline in their colony forming units (CFUs) as compared to their isogenic wild type counterparts. The lexA and polA mutants were found to be the most sensitive strains.
- The damage brought about in the cell in the presence of XAD-concentrates was found to be remarkably high as compared to liquid-liquid water extracts of the test sampling sites at the dose level of 20 μl/ml of culture.
- Extracellular treatment of bacteriophage λ with test samples resulted in significant loss of plaque forming units.
- The survival was 30% in lexA strain when the phage was treated with liquid-liquid extracted water sample after 6 h of treatment and it was only 17% in case of XAD-concentrated water samples of Mathura. The recA mutant also exhibited decline in their plaque forming units as compared to their isogenic wild-type counterparts.
The survival was 30.3% in \textit{lexA} strain when the phage was treated with liquid-liquid extracted water sample after 6 h of treatment and it was only 18.4% in case of XAD concentrated water samples of Okhla.

Mutagenicity assessment of the Yamuna River water demonstrated that it has been polluted by a mixture of genotoxins with both bacterial mutagenic and genotoxic effects. Our results are consistent with the idea that the test water samples would be remarkably genotoxic. Moreover, the water samples also initiate the SOS response and thus bring about the mutation in the bacterial DNA. The genotoxic effect of the test water samples would obviously pose a risk of neoplastic transformation in humans using untreated Yamuna River water for drinking.

IV: Genotoxicity of soil

- Agricultural soil irrigated with wastewater was found to be most responsive to the \textit{Salmonella} tester strains as compared to that of ground water irrigated soil.
- Extracts of soil with methanol were found to be more mutagenic in agricultural soil irrigated with wastewater than acetonitrile and acetone extracts. Extracted soil samples (methanolic) exhibited a significant degree of mutagenicity with TA98 strain (218 net revertants) at the dose level of 80 \textmu l/plate. The reversion property which displayed an increasing trend up to 80 \textmu l/plate declined sharply at 100 \textmu l/plate.
- Significantly enough the presence of S9 liver microsomal fraction exhibited an inhibitory effect on appearance of his* revertants.
- Extraction of soil with acetonitrile and acetone also displayed the maximum mutagenicity with TA98 strain both in the presence and absence of S9 fractions.
- Ames tester strains TA102 and TA104 were less sensitive to test samples.
- The \textit{recA}, \textit{lexA} and \textit{polA} mutants of \textit{E. coli} K-12 were sensitive to the test samples (soil extracts) as compared to their isogenic wildtype counterparts.
- \textit{polA} and \textit{lexA} mutants were the most sensitive strains when tested with soil extracts but the decline was more pronounced when they were treated with extracts of soil irrigated with wastewater than ground water extracted soil.
- The survival was 16.5%, 21.0% and 33.7% in \textit{polA} strain after 6 hours of treatment when tested with wastewater irrigated soil extracts of methanol, acetonitrile and acetone respectively.
Extracellular treatment of bacteriophage λ resulted in significant decline in plaque forming units in wastewater irrigated soil extracts as compared to that of groundwater irrigated soil.

These findings strongly suggest that the agricultural soil irrigated with wastewater contained certain genotoxic agents, which are capable of inducing mutations. In view of the common practice of application of untreated wastewaters to agricultural land in the neighboring area should be strictly prohibited as the pollutants might enter into the food chain and causing health hazards to humans.