Microbial analysis and Heavy metal contamination in the soil of river (Yamuna) bank at Agra (U.P.)

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Abstract - The study was carried out on the samples of soil of the river bank ,Yamuna. Various tests were performed to isolate the bacteria including Indole test, Catalase test, MR-VP test, Urease test. The media used were nutrient agar, MacConkey agar, simmons citrate agar. The color of the soil is very important factor that describes the amount of organic content present in the sample. The pH of the soil were found to be alkaline in nature. The bacterial Populations are found to be pH, moisture and organic matter dependent. The soil samples were found to be coli form negative and positive. Micro organisms isolated were Klebsiella Pneumonae, Salmonella, Pseudomonas, Streptococcus, Enterobacter aerogenes, Morganella morganii, Proteus spp., E.coli, Shigella. The presence of *Escherichia* and *Shigella* indicated the sewage contamination in the river. Heavy metal accumulation in the soil also noticed.

Keywords: River bank soil, bacteria, soil properties, heavy metals, Atomic Absorption Spectrophotometer.

I. INTRODUCTION

The Yamuna is the longest and the second largest tributary river of the Ganges (Ganga) in northern India. Originating from the Yamunotri Glacier at a height of 6,387 metres on the south western slopes of Banderpooch peaks in the uppermost region of the Lower Himalayas in Uttarakhand, it travels a total length of 1,376 kilometers (855 mi) and has a drainage system of 366,223 square kilometres (141,399 sq mi), 40.2% of the entire Ganges Basin, before merging with the Ganges at Triveni Sangam, Allahabad (Joshi & Pandey, 2015). At present the condition of river Yamuna is very alarming not only water is polluted but also river bank soil is polluted by the disposal of industrial, agricultural waste. In the soil a number of metals are found that affects the microbial population of the soil (Oliveira and Pampulha, 2006). Soil provides a heterogeneous habitat for microbial life and is inhabited by a wide range of microorganisms whose numbers reach as high as million to billion g-1 of soil. There are six groups of organisms are found in soil. These include bacteria, fungi, protozoa, nematodes, arthropods and earthworms. Bacteria are the most abundant of the soil organisms and they existed in as many as a trillion species (Dykhuizen, 1998). Soil is a non-renewable, finite natural asset. Soils are systems of pores and aggregated mineral and organic particles in differing sizes. Within the pores microbes are continuously eating, respiring, reproducing, competing, cooperating, and responding to their environment. The soil environment consists of a variety of physical, biological and chemical factors that affect the abundance and diversity of microbes found in the soil. (Chau et al,

2011). The soil environment consists of solid and porous fractions. In between these fractions, a variety of chemical and physical factors are affected by and and affect microbes (Nimmo, 2004).
Microbial processes directly affect their environments as well, contributing to the carbon, phosphorous, sulphur and nitrogen cycles (Kapoor 2006).
In microbial term, bacteria are highly ubiquitous and can be found in everywhere. The organisms are excellent materials for investigation by ecologist, physiologist, biochemist and biotechnologist. They possess a unique number of biological characteristic and they are considered to be one of the potential organisms which can be useful to mankind in various ways.
In the soil of river Yamuna at Agra different microbial species of bacteria are found, some of them are Klebsiella Pneumonae (Ravichitra et al 2014), Salmonella (Kunwar et al, 2013) , Pseudomonas (Dewaliya and Jasodani, 2013) , Streptococcus (Ryan and Ray, 2004), Enterobacter aerogenes (Mordi and Hugbo,2011), Morganella morganii (Trivedi et al,2015), Proteus spp.(Drzewiecka, 2016) ,E.coli (Gallagher et al,2012) , Shigella (Marler, 2015) . Soil micro organisms involved in nutrient transformation process, decomposition of resistant components of plant and animal tissue, Participate in humus formation, Predator to nematodes, Surface blooming reduces erosion losses, Improves soil structure, Maintenance of biological equilibrium. Microorganisms are involved in the production of oxygen, biomass control and 'cleaning' the Earth of remnants of dead organisms.
Nitrogen in its gaseous form (N2) makes up 78% of the atmosphere, but it cannot be absorbed and used as a nutrient by plants and animals. It must be converted by nitrifying (nitrosomonas) bacteria, so that it can enter food chains as a part of the nitrogen cycle (Ghaly and Ramakrishnan, 2015). Bacteria are the cause of some serious diseases, such as cholera, that plagued villages and towns centuries ago. Unfortunately, less developed countries that do not have effective sanitation systems are still affected by diseases caused by bacteria and viruses from sewage(Cabrales, 2010). Bacteria found in drinking water come from several sources. The most common source is the soil surrounding the water system. If e.coli is present in water, it means that harmful sewage contamination has occurred (Pandey et al, 2014). Bacteria can be described as either pathogenic or non-pathogenic, meaning whether or not they can cause disease. Pathogenic bacteria can overcome the body's natural defenses and invade healthy tissues. In addition, "opportunistic" or "secondary" pathogens are those that can cause an infection when an unusual opportunity, such as an open wound or suppressed immune system, presents itself. Very few types of bacteria are pathogenic.
Heavy metals like mercury, arsenic, copper, cadmium are affected not only fauna but also flora in the soil, thus affected the growth of vegetation and the population of microbes as microbes play a important role in ecological functions (Chibuike and Obiora, 2014). The reduction in microbial biomass from metal exposure was due to instantaneous death of microbial cells, disorder of important functions and change in population size and in viability or competitive ability of soil microorganisms (Boroń and Boroń ,2014). K. pneumoniae is an important cause of human infections. Diseases include urinary tract infections, pneumonia, septicemias, and soft tissue infections (Ajibade et al 2015). Most E. coli strains do not cause disease, but virulent strains can cause gastroenteritis, urinary tract infection and neonatal meningitis (Ademola et al, 2011).

II. MATERIALS AND METHODS

2.1 Sample Collection

Soil samples were collected from four sites of river Yamuna, Agra.
Site 1: Runukta (SU)
Site 2: Kailash Temple (M)
Site 3: Pohia Ghat (P)
Site 4: Water works (R)
Soil samples were collected during the month of April, 2016 from different locations of Agra City for isolation, identification and characterization of bacterial microflora and metals from Yamuna river soil. Approximately 0.5 Kg soil was collected at each sampling sites. The samples were collected in a sterile poly bags and immediately brought into the laboratory. The samples were assigned with numbers and collection date for record in the field note book before they are processed. The physico-chemical properties of soil like pH, elecrical conductivity, organic matter, NPK were determined. The pH of the soil was determined by making a soil solution and measured by digital meter.

2.2 Serial dilution

In serial dilution agar plate method, a known amount of material is suspended or agitated a known volume of sterile water blank to make a microbial suspension. 10 gm of soil samples were taken and dissolved in 100ml distilled

water in a beaker. Then 9 ml of distilled water was taken in selected each 5 test tubes. 1 ml of the soil suspension was mixed properly in first test tube containing 9 ml of distilled water. I ml of liquid suspension was taken out with the help of micropipette from the beaker and was added to the test tubes which marked as 10^{-1}. Again 1 ml of suspension was taken out from test tube 10^{-1} and mixed with the test tube marked as $10^{-2.}$ This process was repeated for other test tubes like 10^{-3}, 10^{-4}, 10^{-5}.The liquid suspension of each sample were incubated at 37°C for 24 hours.

2.3. Gram staining of different bacterial cultures:

Applying a primary stain (crystal violet) to a heat-fixed smear of a bacterial culture. The addition of iodide, which binds to crystal violet and traps it in the cell, Rapid decolorization with ethanol or acetone. Counterstaining with safranine. Blot the smear, air dry and observe. Examine under microscope.

2.4 Characterization of bacterial cultures

The pure cultures were characterized on the basis of colony morphology (Color, Shape,
Elevation and Optical Characteristics) and Gram's staining reaction.

2.5 Biochemical tests for soil of different sites for the isolation and purification of bacteria:

2.5.1 Indole test

Concentrated HCL 25 ml + 75 ml of amylalcohol 5g of paradimethylaminobenzaldehyde to the solution.

2.5.2 Methyl Red test

1-2 drops of Methyl red reagent was added, reagent remains red color if the test id positive.

2.5.3 Vogues Proskuer test

2-3 drops of Barrit reagent A & 1-2 drops of Barrit reagent B were added in the broth medium.

2.5.4 Catalase test:

Place a drop of 3% H2O2 on to the slide and mix. A positive result is the rapid evolution of oxygen (within 5-10 sec.) as evidenced by bubbling.

2.5.5 Urease test:

Streak the surface of a urea agar slant with a portion of a well-isolated colony or inoculate slant with 1 to 2 drops from an overnight brain-heart infusion broth culture. Leave the cap on loosely and incubate the tube at 35°-37°C in ambient air for 48 hours to 7 days.
Examine for the development of a pink color for as long as 7 days.

2.6 HEAVY METAL ANALYSIS OF THE SOIL SAMPLES:

The technique makes use of absorption spectrometry to assess the concentration of an analyze in a sample. It requires standards with known analytic content to establish the relation between the measured absorbance and the analytic concentration and relies therefore on the Beer-Lambert Law. Estimation of different heavy metal concentration in the soil sample was done by AAS. Sample was digested according to the standard procedure.

III. RESULTS AND DISCUSSION

3.1 Gram staining and colony morphology:

Table 1: Colony morphology and Gram staining of isolated bacterial cultures:

Culture name	Gram's stain	Shape (bacteria)	Color	Shape (colony)	Elevation	Optical Characteristics
RS1(1)	Gram Negative	Bacilli	yellowish	Circular	Convex	Opaque
RS1(2)	Gram Negative	Bacilli	White	Irregular	Convex	Opaque
RS1(3)	Gram Negative	Bacilli	White	Spherical	Convex	Translucent
RS1(4)	Gram Positive	Cocci	Creamy	Spherical, circular	Flat	Translucent
RS1(5)	Gram Negative	Bacilli	Pale white	Circular	Convex	Translucent
RS1(6)	Gram Negative	Bacilli	Yellow	Spherical	Flat	Opaque
RS1(7)	Gram Negative bacilli	Bacilli	Pale white	Spherical	Flat	Translucent
RS2(1)	Gram Negative	Bacilli	Pale white	Spherical	Convex	Opaque
RS2(2)	Gram Negative	Bacilli	creamy	Circular	Convex	Opaque
RS2(3)	Gram Negative	Bacilli	Yellowish	Circular	Convex	Translucent

RS2(4)	Gram Positive	Cocci	White	Irregular	Convex	Translucent
RS3(1)	Gram Negative	Bacilli	Pale white	Spherical	Flat	Translucent
RS3(2)	Gram Negative	Bacilli	White	Spherical	Flat	Translucent
RS3(3)	Gram Negative	Bacilli	White	Circular	Convex	Opaque
RS3(4)	Gram Negative	Bacilli	White	Circular	Flat	Opaque
RS3(5)	Gram Positive	Cocci	Creamy	Circular	Flat	Translucent
RS4(1)	Gram Positive	Cocci	Creamy	Spherical	Convex	Translucent
RS4(2)	Gram Negative	Bacilli	Pale white	Spherical	Convex	Opaque
RS4(3)	Gram Negative	Bacilli	Pale white	Spherical	Convex	Translucent

After examining of gram staining the isolates from the soil samples are found as gram +ve *Streptococcus* type bacterium and gram –ve type bacterium i.e. *E.coli, Pseudomonas spp., Enterobacter aerogenes, Klebsiella Pneumoniae, Salmonella, Proteus spp.,Morganella morganii*. Most of the culture show gram negative bacilli, while only few cultures show gram positive cocci colonies.

Fig. 1: Isolation of pure culture by streak plate method

3.2: Characterization of bacterial cultures

On the basis of colony morphology it was observed that the cultures from Yamuna soil were either white or pale white in color, creamy, yellow or yellowish. On the basis of the remaining parameters (shape, elevation and optical characteristics) the cultures were showing variable morphology.

Table 3: Biochemical test results for the four sites RS1, RS2, RS3, RS4:

Test/ Sites	**RS_1 (SU)**	**RS_2 (M)**	**RS_3 (P)**	**RS_4 (R)**
Gram staining	–	+	+	–
Indole test	–	–	–	–
Catalase test	–	–	–	–
Methyl Red test	–	+	–	+
Vogues Proskauer test	+	–	–	–
Urease test	+	+	+	–
Lactose sugar fermentation	–	+	–	–

From the above test results the bacterial genera that are found in the soil sample are Klebsiella Pneumoniae, Salmonella, Pseudomonas, Streptococcus, Enterobacter aerogenes, Morganella morganii, Proteus spp., E.coli, Shigella.

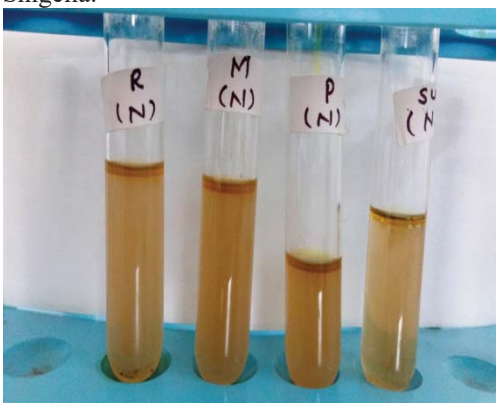

Fig. 2: Results of Indole Test

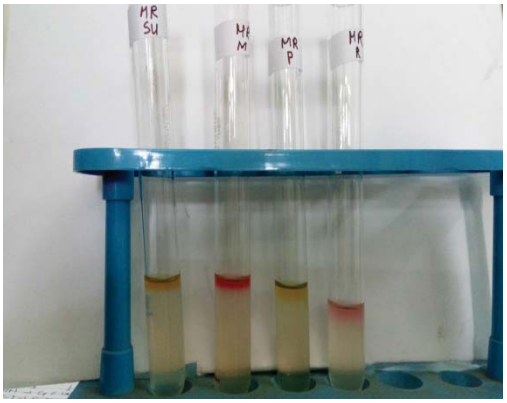

Fig.3: Results of Methyl Red (MR) Test

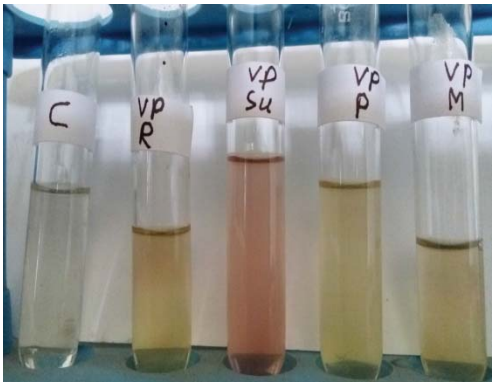

Fig. 4: Results of Vogues-Proskauer (VP) Test

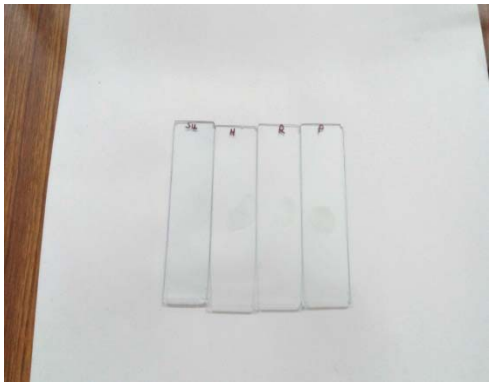

Fig. 5: Results For Catalase Test

Fig. 6: Results for Urease Test

Fig 7: Results for Lactose Fermentation Test

3.3: Results of Indole test:

Positive: Pink colored rink after addition of appropriate reagent

Negative: No color change even after the addition of appropriate reagent.
In our studies all samples were found Indole negative. Klebsiella Pneumoniae, Salmonella, Pseudomonas are present.

3.4: Results of MR test:

Escherichia coli: MR test positive- appearance of red color after the addition of methyl red reagent.
Enterobacter aerogenes, Klebsiella,: MR test Negative- the lack of color change after the addition of methyl red.
3.5: Results of VP test: A positive test is represented by the development of a red color 15 minutes or more after the addition of the reagents indicating the presence of diacetyl, the oxidation product of acetoin. Klebsiella Pneumoniae is VP test positive and E.coli is VP negative bacteria.
3.6: Results of Catalase test:

Catalase Positive reactions: Evident by immediate effervescence (bubble formation)
Catalase Negative reaction: No bubble formation (no catalase enzyme to hydrolyze the hydrogen peroxide). Our samples are found to be Catalase negative i.e. Streptococcus is present.

3.7: Results of Urease test: Positive Reaction: Development of an intense magenta to bright pink color in 15 min to 24 h. Proteus spp. is present.

Negative Reaction: No color change.

E.coli, Shigella are present.

3.8: *Lactose Fermentation Test:*

An inoculum from a pure culture is transferred aseptically to a sterile tube of phenol red lactose broth. The inoculated tube is incubated at 35-37 C for 24 hours and the results are determined. A positive test consists of a color change from red to yellow, indicating a pH change to acidic.

Table 4: Physiochemical properties of soils collected from different places:

Site	pH	Electrical Conductivity Ds/m	Organic matter %	N (kg/ha)	K (kg/ha)	P (kg/ha)
RS_1	7.2	0.36	0.72	54.8	154	145
RS_2	6.9	0.45	1.62	76.2	243	134
RS_3	7.5	0.53	1.34	84.2	98	78
RS_4	7.8	0.42	1.45	72.3	149	106

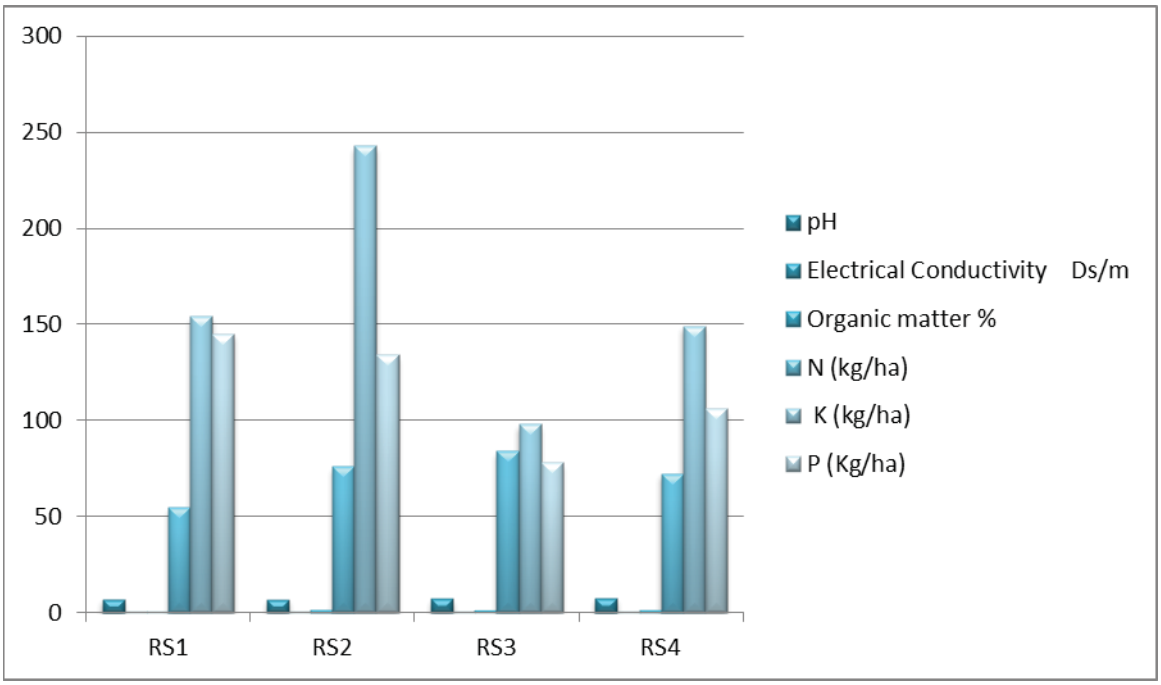

Fig 8: Physiochemical properties of soil.

From the above Values of pH it is clear that the soil of river Yamuna is almost alkaline in nature. At the RS1, RS3, RS4 site it is 7.2, 7.5, 7.8 and at site RS2 it is 6.9 basic in nature, for the growth of microorganisms this site is good as the microbes grow from basic to normal pH range. Electrical conductivity varies in an order RS1>RS4>RS2>RS3. At Runukta it is found higher than the other sites, Kailash Temple and Rambagh has approximately the same values while Pohia Ghat has the minimum. The soil is non- saline in nature that support the growth of microorganisms. Organic matter is regarded as the ultimate source of nutrients and microbial activity in the soil. All the three sites has the similar amount of organic matter i.e.,1.62%, 1.34%, 1.45% except RS1 which is

0.72%. The amount of NPK varies from one site to another, they are nutrients for the growth of plants. Nitrogen is found at an order of RS3>RS2>RS4>RS1.The amount of Potash is maximum at site RS2 while it is minimum at RS3. Phosphate is higher at RS1 site and has low value at RS3.
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Table 6: HEAVY METAL ANALYSIS RESULT

S.No.	Sampling Sites	Cd (mg/kg)	Cr (mg/kg)	Pb (mg/kg)	Cu (mg/kg)	As (mg/kg)	Zn (mg/kg)
1	Runukta	14.6	53.4	174	63.8	23.2	115
2	Kailash Temple	14.5	43.6	246	38.3	28.6	129
3	Pohia Ghat	18.2	68.7	198	52.4	17.8	223
4	Rambagh	20.3	71.4	220	45.4	24.8	136

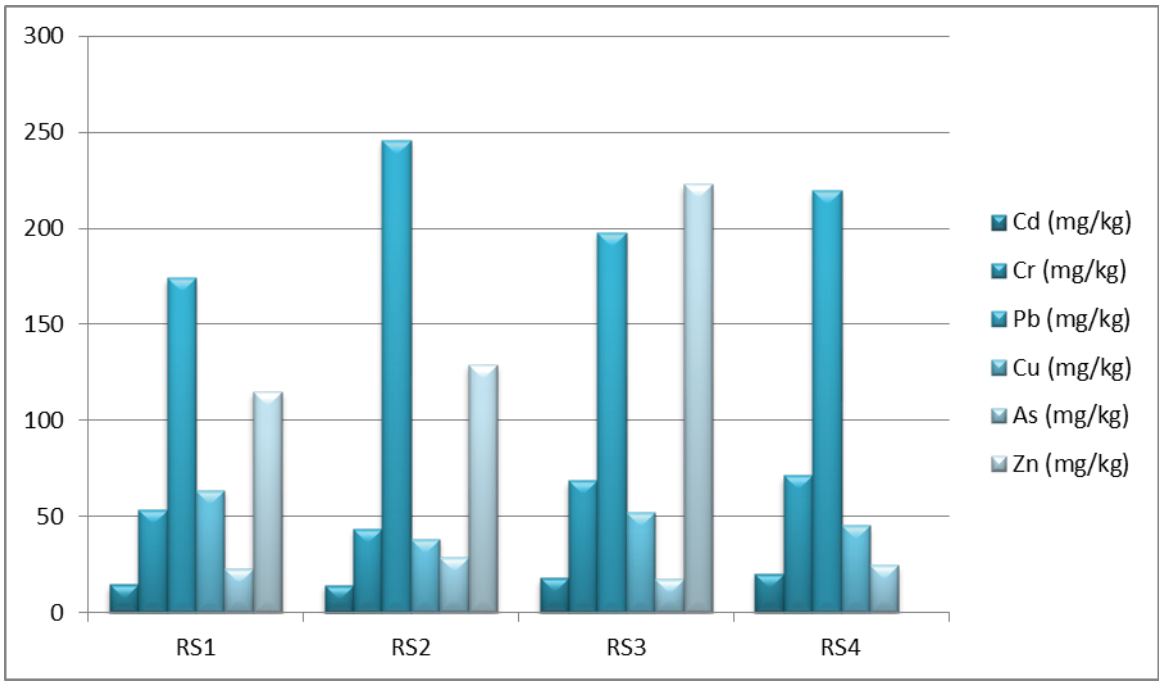

Fig.9: Heavy metal concentration at sampling sites of soil.

The above table the concentration of heavy metals are shown, which not only pollute the Yamuna river water but also affect the microbial life in soil that contributes in the ecological balance. The concentration of Cadmium is higher at Rambagh i.e. 20.3(mg/kg), Which is the last sampling site of the study area. Yamuna flows from Delhi to Agra,so Runukta is in first among all the sites the concentration of Cadmium is approximately same in the sample of soils of Runukta and Kailash Temple, where as 18.2 (mg/kg) at Pohia Ghat. Again Rambagh has the highest concentration of Chromium 71.4(mg/kg) the limits are higher than the permissible limits, it is minimum at site RS1.

The values of Lead is in the order of RS2>RS4>RS3>RS1.The percentage of Copper is max. at Runukta (63.8mg/kg) and minimum at Kailash Temple (38.3mg/kg) While 52.4 mg/kg at Pohia Ghat and 45.4 mg/kg at Rambagh. Arsenic was found in the order of RS2> RS4> RS1> RS3 and Zinc in a order of RS3> RS4> RS2> RS1. The values of heavy metals in the soil of river Yamuna were found much higher than the permissible limits, Which may be due to the disposal of industrial industrial waste, agricultural waste runoff of river Yamuna to the bank so contaminate the soil also.

IV. CONCLUSION

The isolated bacteria in the soil of river Yamuna are Klebsiella Pneumoniae, Escherichia coli, Salmonella, Streptococcus, Enterobacter aerogenes, Proteus spp., Pseudomonas . The presence of E.coli or Klebsiella Pneumoniae are the indicator of potential health risks, those faecal possess. The pH of the soil found alkaline in nature almost all the sites, favourable for the growth of microorganisms.The values of salinity, organic matter, N,P,K of soil samples were in the permissible limits.

Heavy metals (Cr, Cd, Pb, Cu, As, Zn) contamination is higher may be due to the industrial and municipal waste disposal in the water that not only pollute the water but also nearby soil of the river. The contamination through pollutants affect the microbial life in the soil also affect the nearby population. Efforts have been made to prevent these contaminations the waste should be treated at the point sources.

REFERENCES

[1] Joshi H.C., PandeyI. P. (2015) . Evolution of the Water Quality of River Yamuna. Using RS and GIS from Dakhpathar to Yamuna Nagar and Its Management, International Journal of Innovative Research in Science, Engineering and Technology, Vol. 4, Issue 10, 9670-9673.

[2] Oliveira A and Pampulha M.E., 2006. Effects of Long-Term Heavy Metal Contamination on Soil Microbial Characteristics, JOURNAL OF BIOSCIENCE AND BIOENGINEERING © 2006, The Society for Biotechnology, Japan Vol. 102, No. 3, 157–161. 2006 DOI: 10.1263/jbb.102.1,157-161.

[3] Dykhuizen, D. E., 1998. Santa Rosalia revisited: Why are there so many species of bacteria? Journal of Microbiology 73, 25-33.

[4] Chau, J.F., Bagtzoglou A.C., and Willig M.R.,2011. The Effect of Soil Texture on Richness and Diversity of Bacterial Communities, Environmental Forensics, 12:333–341, 2011 Copyright C Taylor & Francis Group, LLC ISSN: 1527–5922 print / 1527–5930 online DOI: 10.1080/15275922.2011.622348,333-341.

[5] Nimmo J.R. 2004, Porosity and Pore Size Distribution, U.S. Geological Survey, Menlo Park, CA 94025, USA, 1-11.

[6] Dr. Kapoor. A., 2006, Department of Microbiology Swami Shraddhanand College Alipur, Delhi – 1100 36, 2-34.

[7] Ravichitra K.N. , Prakash P.H. , Subbarayudu S. and Rao U.S., 2014, Isolation and antibiotic sensitivity of Klebsiella pneumoniae from pus, sputum andurinesamples, Int.J.Curr.Microbiol.App.Sci (2014) 3(3): 115-119.

[8] Kunwar R, Singh M.H., Mangla M.V. , Hiremath M.R , 2013, Outbreak investigation: Salmonella food poisoning, medical journal armed forces india 69 (2013) 388 -391.

[9] Dewaliya V, Jasodani R, 2013, Isolation and Identification of soil isolates of Pseudomonas species via FAME analysis, IJSR - INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH, 360-361.

[10] Ryan KJ, Ray CG, eds. (2004). Sherris Medical Microbiology (4th ed.). McGraw Hill. pp. 293–4. *ISBN 0-8385-8529-9.*

[11] Mordi R.M, Hugbo P G,2011, Frequency of Isolation of Enterobacter Species from a Variety of Clinical Specimens in a Teaching Hospital in Nigeria, Tropical Journal of Pharmaceutical Research December 2011; 10 (6): 793-800

[12] Trivedi M K , Branton A , Trivedi D , Nayak G , Gangwar M and Jana S, 2015, Antibiogram and Genotypic Analysis using 16S rDNA after Biofield Treatment on Morganella morganii, Adv Tech Biol Med 2015, 3:3. doi: 10.4172/2379-1764.1000137, 1-8.

[13] Drzewiecka D, 2016, significance and Roles of *Proteus spp*. Bacteria in Natural Environments, Microb Ecol (2016). Doi: 10.1007/s00248-0720-6.

[14] Gallagher M A, Karthikeyan R , Mukhtar S, 2012, Growth Kinetics of Wildlife E. coli Isolates in Soil and Water, Journal of Environmental Protection, 2012, 3, 838-846.

[15] Marler B, 2015, Shigella is a Very, Very Nasty Bacteria, http://www.foodpoisonjournal.com/

[16] Ghaly AE, Ramakrishnan VV (2015) Nitrogen Sources and Cycling in the Ecosystem and its Role in Air, Water and Soil Pollution: A Critical Review. J Pollut Eff Cont 3: 136. doi:10.4172/2375-4397.100013

[17] Cabral P.S.J.,2010, Water Microbiology. Bacterial Pathogens and Water, Int J Environ Res Public Health. 2010 Oct; 7(10): 3657–3703.

[18] Pandey P.K, Kass P H, Soupir M L, Biswas S and Singh V P, 2014Contamination of water resources by pathogenic bacteria, *AMB Express*20144:51 DOI: 10.1186/s13568-014-0051-x

[19] Chibuike G.U.and S. C. Obiora S.C., 2014, Heavy Metal Polluted Soils: Effect on Plants and Bioremediation Methods, Applied and Environmental Soil Science Volume 2014 (2014),ArticleID 752708,12pages http://dx.doi.org/10.1155/2014/752708

[20] Boroń A L and Boroń P,2014, The Effect of Industrial Heavy Metal Pollution on Microbial Abundance and Diversity in Soils — A Review,759-770.

[21] Ajibade V. A, Oyebode, J. A. and Oyeyemi, B. F, 2015, Incidence and Susceptibility of Beta-Lactamase Producing Klebsiella pneumoniae to Extract from Phyllanthus niruri, International Journal of Scientific and Research Publications, Volume 5, Issue 7, July 2015 1 ISSN 2250-3153,1-3.

[22] Olaniran A.O, Kovashnee Naicker K, Pillay B ,2011, Toxigenic Escherichia coli and Vibrio cholerae: Classification, pathogenesis and virulence determinants, Biotechnology and Molecular Biology Review Vol. 6(4), pp. 94-100.