

Isolation, Characterization and Molecular Phylogeny of Multiple Metal Tolerant and Antibiotics Resistant Bacteria Isolates From River Ganga

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Abstract – The current examination pointed toward distinguishing weighty metal tolerant bacteria in soil tests got from three distinct locales. The examples included mechanical effluents from assembling unit of batteries and invertors and homegrown waste tainted site. From every area, 8, 8 and 6 soil tests separately were gathered. The current investigation was centered around the isolation of multiple metal tolerant and antibiotics resistant bacterial strains from water tests of five unique Ghats of River Ganga, Varanasi, India. These strains were biochemically portrayed and their phylogenetic relatedness was accepted utilizing enhanced ribosomal DNA limitation investigation, fingerprinting and 16S ribosomal quality sequencing. The presence of heterogeneous gatherings of bacteria having a place with alpha, beta, gamma proteobacteria, and bacilli was taken note. A portion of the bacterial strains like *Pseudomonas*, *Serratia*, *Enterobacter*, and *Proteus vulgaris* were for the most part found at the Dashashwamedh Ghat and the Assi Ghat showing least inhibitory focus 200–300 mg/L for copper, nickel, lead, and chromium. The treatment of such bacteria would be actually quite troublesome. The examinations might additionally be stretched out to decide the relationship between's hefty metal and anti-microbial resistance in the tolerant segregates. The investigation is of importance in that such bacteria can be used for bioremediation of hefty metals in the climate especially in the treatment of sewage water and mechanical effluents.

Keyword – Isolation, Characterization, Molecular, Phylogeny, Multiple, Metal, Tolerant

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INTRODUCTION

Evaluation of tolerance power of such bacteria against heavy metals

Ecosystem gives essential requirements like sanctuary, food and business. All in all, these requirements might be viewed as biological administrations. The framework is answerable for giving variety (sea-going, earthbound) among living creatures. One of the fundamental parts of ecosystem is its capacity to clean, reuse contamination pollution in air, soil and water subsequently, keeping an environmental equilibrium. Soil is one of the significant ecosystems which give such conditions to microorganisms, creatures and people. Practically a wide range of microorganisms for example bacteria, organisms and infection and so on are found on the dirt which is quite possibly the main conditions for microorganisms which is effectively presented to numerous toxins. The assessment of the impacts of contaminations in the microbial populace thusly very important. Since the resistance of bacteria depends on their temperament

and climate as well as on actual factors, for example, the sort and grouping of substances as the microorganisms with critical resistance can uncover the state of their current circumstance.

In any case, with expansion in the populace levels generally speaking industrialization and different exercises a large portion of the ecosystems are going under huge danger and overburdened by contamination, corruption and obliteration. Natural debasement is a worldwide issue, fundamentally in agricultural nations with enormous populace in little region just as industrialization. People are answerable for such debasement in a few different ways statement of waste material, utilization of pesticides, mechanical waste and sewage ooze and so forth in climate. Throughout the long term, with the improvement of the enterprises, hefty metals which are either utilized or delivered as a result turns into a genuine danger to climate just as in abodes of living creatures. As of late, the release of mechanical wastewater from various assets containing weighty metals has brought about expansion in the number of inhabitants in resistant

bacteria such bacteria are especially more normal in modern regions in which the release of wastewater containing hefty metals into the streams has made the substantial metals be sorbed by soil particles and their exchange to far good ways from the mark of release.

Isolation and identification of heavy metal tolerant (Pb, Cd, Zn and Co) bacteria from industrial effluents, metal dumping sites and waste water

In the new years, the ecological contamination by weighty metals has turns into a huge issue all through the world. Weighty metals establish the gatherings of metals and metalloids which have nuclear thickness more noteworthy than 4000 kg m⁻³. Hefty metals, for example, Ni, Cr, Cu, Zn, Pb are available in the biota. These metals are otherwise called minor components which assume a vital part in different metabolic cycles of plant, creature and microorganisms. In the cutting edge occasional table hefty metals are remembered for d-block component, otherwise called change components. Zn, Hg, Cu, Pb, Cr, Ni, Cd, Mn, Co are some of them (Samanta et al, 2012 and Hookoom et al, 2013).

Blending of sewage alongside modern effluents into the Ganga stream annoy water quality. Dirtied water turned into a natural surroundings of different pathogenic bacteria having double properties in delivering multiple substantial metals tolerant limit just as antibiotics resistance. The current examination was centered around the isolation of multiple metal tolerant and antibiotics resistant bacterial strains from water tests of five distinct Ghats of River Ganga, Varanasi, India. These strains were biochemically described and their phylogenetic relatedness was accepted utilizing intensified ribosomal DNA limitation examination fingerprinting and 16S ribosomal quality sequencing. Coordinated effort of physiological, biochemical, and molecular methods could give total data about bacterial ID and portrayal. Presence of hurtful bacteria in the stream Ganga welcomes genuine consideration according to human wellbeing point of view since Ganga water is likewise utilized for drinking, sacred washing and water system purposes.

Varanasi, India (25°20' N, 83°00' E) lies 8.77 m above ocean level in the eastern Gangetic fields of India has somewhere around 84 Ghats. Ghats are riverfront steps prompting the banks of River Ganga. The blessed waterway Ganges and its Ghats are a home for a large number of locals other than being a spot visited by 300 individuals consistently in the mornings, and on celebration days 2,500 individuals show up each hour. As per World Health Organization (WHO) reports, in agricultural nations like India, practically 80% of the absolute water is being dirtied by release of homegrown sewage and untreated mechanical effluents (Sangu and Sharma, 1987). The WHO revealed (World Health

Organization [WHO], 2002) that irresistible illness like loose bowels, caused because of the admission of hazardous and amazingly dirtied water, is answerable for about 1.7 million passings every year around the world. As of late Times of India New Delhi September 2014 announced that the nature of Ganga water of two sacred spots Varanasi and Allahabad where millions take a plunge ordinary are generally unsuitable for washing reason.

Water of waterway Ganga at Varanasi district is essentially dirtied by modern effluents, homegrown sewage, and removal of dead bodies (Mishra and Tripathi, 2007; Pandey, Shubhashish, and Pandey, 2010). Around 1,503 enterprises (Chemical businesses, metal handling ventures, cowhide, and material enterprises) are situated in and around Varanasi (Anonymous, 2006; Industrial Directory, 1981; Rai and Tripathi, 2008). Heightening anthropogenic exercises, unpredictable urbanization, and sped up industrialization brought forth the stream water contamination especially weighty metals and microbial contamination (Rai, Mishra, and Tripathi, 2010). Either straightforwardly or in a roundabout way metals have been known to assume a significant part in practically all metabolic cycles, development, and advancement of the microorganisms (Bergey and Breed, 1994; Beveridge and Doyle, 1989). Nonetheless, these organic entities are compelled to adjust the expanding convergences of metals past their typical resistance level. Thusly, microorganisms have created different organic components like complexation, metal efflux frameworks, use or decrease of the metal during anaerobic breath to endure substantial metal pressure (Ehrlich and Brierley, 1990). Metal resistant bacteria can develop under high centralizations of metals and can assume an amazing part in the bioremediation of those metals (Bolan et al., 2014).

Presentation of antimicrobial specialists like antibiotics into the waterway and streams from modern contamination as metals, arisen as a steadily expanding wellbeing peril because of spread of anti-toxin resistant life form in water climate (Baquero, Martínez, and Cantón, 2008; Levy, 1997). Capacity of various types of bacteria in procuring multidrug resistance (MDR) has prompted extreme difficulty for people (Tenover and McGowan, 1996). A huge extent of MDR was found to contain plasmid borne portable resistance qualities that can communicate through bacterial populaces (Kumarasamy et al., 2010).

Molecular methods have started another time of microbiology and may end up being useful in deciding the occupants of freshwater microbial networks (Mukherjee, Kumar, Kumar, and Chakraborty, 2013). Groupings dependent on 16S ribosomal quality uncover various refined just as crude microorganisms in different conditions as 16S ribosomal RNA (16S rRNA) particles contain both profoundly preserved just as factor districts (Woese,

1987). In the new couple of years, enhanced ribosomal DNA limitation investigation (ARDRA) has become a helpful instrument for appraisal of microbial local area structure changes as it is a basic, quick and solid strategy for assessment of bacterial variety in various conditions (Martínez-Murcia, Acinas, and Rodríguez-Valera, 1995).

In the current correspondence, the water nature of River Ganga has been investigated utilizing physicochemical properties. Isolation and biochemical portrayal of multiple metal tolerant and antibiotics resistant bacteria have been researched. Molecular portrayal of disconnects dependent on 16S rDNA quality successions and ARDRA fingerprints were performed. The meaning of the proposed study has been discovered by applying factual boundaries, for example, pearson relationship/relapse and head part examination (PCA) investigation.

The systems includes in anti-microbial resistance are regular determination, plasmid move and transformation. The utilization of antibiotics gives a particular benefit to resistant bacteria and after some time the populace is made out of totally resistant strains, the treatment of which becomes inadequate (Laxminarayan and Brown, 2001). Anti-infection resistance qualities are typically carried on chromosomes, plasmids or conjugative transposons. Resistance qualities are obtained through formation from anti-microbial makers (Chapman, 2003).

Plasmids are self-ruling hereditary components that repeat freely of the principle chromosome. Plasmids convey qualities that administer their replication, isolation and duplicate number just as extra phenotypic capacities like anti-infection resistance, metal resistance, union of bacteriocins and capacity to intercede cell to cell formation among others (Silver, 1992).

OBJECTIVES OF THE STUDY

1. Isolation and identification of heavy metal tolerant (Pb, Cd, Zn and Co) bacteria from industrial effluents, metal dumping sites and waste water
2. Evaluation of tolerance power of such bacteria against heavy metals

REVIEW OF LITERATURE

Thacker et al, (2016) Significant wellsprings of chromium contamination include: effluents from cowhide tanning, chromium electroplating, wood safeguarding, amalgam readiness and atomic squanders because of its utilization as a consumption inhibitor in thermal energy stations.

Kiyono and Hou, (2017) Mercury is perhaps the most poisonous metals in the climate. It is delivered

into climate in generous amounts through regular occasions and anthropogenic exercises

Vallee and Ulmer, (2017) The natural contamination by hefty metals comes from anthropogenic sources. These metals become portable in soils relying upon pH of the dirt and their speciation. A small portion of the absolute mass of the weighty metals can filter to spring or can become bioavailable to living organic entities, which require diverse measure of substantial metals however unnecessary level can be harming to them. Hg, Pb and plutonium are harmful metals as their collection in the body can cause genuine disease. Substantial metal poisonousness can bring about harmed or diminished mental and focal anxious capacity, lower energy level, and harm to blood structure, lungs, kidney, liver, and other imperative organs

Kjaergaard et al, (2018). Zn is a significant follow metal which is fundamental for the appropriate advancement of sex organ and bone development, especially in the youngsters and unborn embryos. At high fixation, it becomes destructive to wellbeing.

Benoff et al, (2018). Lead (Pb) and Chromium (Cr) are risky substantial metals found in the climate now days these metals influence diverse organic frameworks in the human body

Needleman et al, (2018). Indeed, even at low dosages lead might cause formative issue in hatchlings, babies and the youthful just as mind harm, social changes, respiratory issues, inebriation of focal sensory system and creation of unexplained male barrenness.

An expanding number of sicknesses are opposing treatment because of the spread of medication resistance inside bacterial populaces. This represents a genuine danger to effective treatment of numerous microbial diseases (Bhattacharjee et al, 1988). There is expanding worry over the utilization of bactericides, sanitizers and cleaning agents locally and medical care offices because of their capability to actuate the outflow of antimicrobial resistance qualities which encode multi-drug efflux siphons and their controllers. This component quite often makes resistance a wide assortment of antibiotics particularly in *E.coli*, *Salmonella*, *Pseudomonas* spp. what's more, other bacterial species (Moken et al, 1997). The pace of resistance improvement has required the WHO to pronounce the rise of anti-infection resistance as an unpredictable test driven by interconnected elements (WHO, 2012) henceforth the need to dissect and comprehend different elements that possibly add to advancement and spread of resistance like hefty metals. A few investigations have exhibited that dirt and water as beneficiary and repository conditions for anti-toxin and metal resilience qualities (Wright, 2010). Bacterial people group in these environs are presented to both hefty

metals and anti-infection stress between anti-toxin resistance and weighty metal resilience hence require concentrates to decide connection between's the anti-microbial resistance and substantial metal resilience.

RESEARCH METHODOLOGY

MATERIALS AND METHODS

Sample collection and physicochemical analysis

The regions chose for the current examination depend on the human use and contamination. Water tests were gathered from the bank of five Ghats (5 km region) viz. Dhashashwamedh ghat, Harishchandra ghat, Samne ghat, Rajendra Prasad Ghat and Assi Ghat. Water tests (1 L) were gathered month to month during the primary seven day stretch of every month from January 2014 to December 2014 from 15 cm profundity at 8.00–10.00 am close to bank of every Ghat in three recreate in 1 L corrosive washed glass bottles. Temperature, pH, and conductivity were estimated on the spot utilizing a thermometer, a pH-meter and a conductivity meter. Tests were then moved to the research facility under sterile condition for isolation of bacteria and examination of broke down oxygen (DO), biochemical oxygen interest (BOD), complete hardness (TH), all out alkalinity (TA), calcium (Ca²⁺), magnesium (Mg²⁺), sulfate (SO₄⁻), absolute disintegrated solids (TDS), all out nitrogen (TN) and all out phosphorus (TP), the last assessed utilizing the American Public Health Association [APHA] (1998) strategy. Assessment of TN content was performed by following the miniature Kjeldahl procedure utilizing a Gerhardt programmed analyser (model KB8S Kjeldatherm, Germany). Titrimetric techniques were for trying DO, BOD and TA while a gravimetric strategy was utilized for TDS (Vogel, 1978). Turbidometric technique was utilized for assessment of SO₄⁻² (Vogel, 1978). An ethylenediaminetetraacetic corrosive titrimetric technique was utilized to gauge Ca⁺², Mg⁺², and TH (Vogel, 1978). A nuclear ingestion spectrophotometer (Model 2380, Perkin Elmer, Inc. Norwalk, CT, USA), was utilized for assurance of Cr, Cd, Ni, Pb, Zn, and Cu. A Pearson relationship framework was built by utilizing SPSS programming rendition 16.0 to consider connection among physicochemical boundaries at five Ghats of River Ganga Varanasi, India.

Isolation and maintenance of bacterial isolates

To get a wide range of bacterial strains, water tests from every Ghats were gathered through centrifugation at 10,000 rpm for 15 min. Pellet was broken up in 100 µL disinfected refined water and spread on supplement agar (NA) media containing 0.5% peptone, 0.3% meat extricate/yeast separate, 1.5% agar, 0.5% NaCl, and brooded at 37°C. Various provinces were gotten on NA plates. The

single bacterial provinces showing special morphology were separated and subcultured something like multiple times to determine virtue of the confines. The immaculateness of the confines was observed regularly by minuscule examination. Purged bacterial confines were exposed to the biochemical and molecular distinguishing proof tests.

Metal tolerance assays

Minimum inhibitory concentration of metals

The detached unadulterated bacterial societies were checked for their particular least inhibitory focuses (MICs) towards every one of the metal particles. Various centralizations of metals particles like Zn, Cd, Cu, Ni, Pb, and Cr (0, 25, 50, and 100 mg/L) were added independently against each bacterial separates brooded in strong NA medium and afterward saved for 24–48 h at 37°C in hatchery. Those bacterial separates, in which development was apparent up to 100 mg/L were additionally developed at higher metal focus going from 150, 200, 250, 300, 350 to 400 mg/L. At that metal focus where bacterial detaches neglected to develop on plates even following 7 days of brooding were considered as the MIC for that specific bacterial strain (Narasimhulu, Rao, and Venu Vinod, 2010).

Antibiotics resistance assay

Bacterial strains were checked for antibiotics resistance design against ampicillin (Amp), chloramphenicol (Chl), nalidixic corrosive (Nal), tetracyclin (Tet), vancomycin (Van), ciprofloxacin (Cip), imipenem (Imi), erythromycin (Ery), streptomycin (Str), and polymixin (Pol) (Himedia, India) as per Kirby-Bauer plate dispersion strategy (Bauer, Kirby, Sherris, and Turck, 1966). A volume of 5 mL of supplement stock was ready for immunizing bacterial strains which were hatched for the time being at 37°C. The unadulterated stock societies were then cleaned over the sanitized 20 mL Mueller Hinton pre-made agar plates. Circles of 6 mm width were ready from sanitized Whatman channel paper. The 10 antibiotics at groupings of 30 mg/L were conveyed in 10 circles which were then apportioned on every one of the cleaned plates at fitting good ways from one another. Plates were then hatched at 37°C for 24 h. Zones of hindrance were estimated in breadth across the focal point of each zone in millimeters.

Biochemical assay

Citrate test

A volume of 100 mL Simmon citrate agar medium (15 g/L agar, 0.2 g/L ammonium dihydrogen phosphate, 0.08 g/L bromothymol blue, 0.8 g/L disodium ammonium phosphate, 0.2 g/L magnesium sulfate heptahydrate, 5 g/L sodium chloride, 2 g/L trisodium citrate and 15 g/L agar) pH 7.0 was ready

and afterward autoclaved. The disinfected Simmon citrate agar medium was poured in various glass tubes and set at a slant to get ready inclinations. From that point forward, the inclinations were streaked with various bacterial disconnects and saved for 48 h in the hatchery. The adjustment of shading from dull green to blue was seen after 48 h (Claus, 1989; Jawetz, 1989).

Catalase test

A little inoculum of bacterial disengages was moved to a spotless dry glass slide and barely any drops of hydrogen peroxide arrangement (3%) were added. Fast evolvement of oxygen bubbles gave the positive outcome for catalase (Facklam and Elliott, 1995).

DATA ANALYSIS

Results and discussion

In the current investigation, endeavors have been made to disengage distinctive metal tolerant just as anti-toxin resistant bacterial strains from the banks of Ghats of waterway Ganga and describe them utilizing different biochemical and present day molecular procedures. To break down water quality, physico-substance attributes of Ganga water close to Ghats was performed and observed to be higher than most extreme allowable cutoff set by Indian Standard Specification for Drinking Water (IS: 10500) consistently (Table 1). Out of five chosen Ghats, Assi ghat and Dashashwamedh ghat were observed to be most contaminated Ghats. Water nature of the Assi ghat has radically disintegrated in the new past as Assi Nala (Sewage) is straightforwardly associated with the Assi Ghat which is the blending point of untreated waste water, homegrown water and modern contaminations with the stream water. However, in Dashashwamedh ghat, outrageous degree of human impedance is potentially the significant explanation of expanded water contamination. Level of supplements viz. absolute nitrogen and complete phosphorus were observed to be higher in the Harishchandra ghat which is basically because of the ignition of human bodies on this specific ghat. Different variables like all out alkalinity and all out disintegrated solids were likewise high in a similar ghat since washing of garments is additionally extremely successive. The degree of weighty metals was discovered much past as far as possible at all the Ghats of waterway Ganga. As indicated by Indian Standard Specifications for Drinking Water IS: 10500 safe cutoff (Indian Standard Drinking Water Specification (Second Revision) IS 10500, 2012), Cd focuses past 0.01 mg/L becomes poisonous, Cr above constraint of 0.05 becomes cancer-causing and Pb past 0.1 could cause blood disease in human. Climatic affidavit of metals produced from vehicles, direct anthropogenic exercises and release of untreated modern effluents just as homegrown sewages into the stream could be the conceivable explanation of

metal burden in waterway Ganga (Pandey et al., 2010). The more elevated level of Cu in water may be because of consumption of lines, fittings, and utensils.

Table 1. Physicochemical characteristics of 5 Ghats of River Ganga Varanasi

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Notes: \pm represents mean standard deviation with three replicates; note that the values are minimum and maximum; Site 1- Dashashwamedh Ghat, Site 2- Harishchandra Ghat, Site 3-Samne Ghat, Site 4- Rajendra Prasad Ghat, Site 5- Assi Ghat; EC-Electrical conductivity, BOD-Biological oxygen demand, DO-Dissolved oxygen, TN-Total nitrogen, TP-Total phosphorus, TA-Total alkalinity, TDS-Total dissolved solid, TH-Total hardness.

A stamped relationship between's physico-substance properties of water gathered from various Ghats was seen in the wake of playing out the measurable investigation utilizing Pearson's recipe of connection and relapse (Table 2). Metals like Zn, Fe, and Ni showed huge relationship with various abiotic factors. At higher focuses, metals apply poisonous impacts by shaping buildings with natural mixtures and harmful impacts of metals can change by changing metals structure (Das et al., 2013).

A sum of 115 culturable bacteria were separated based on their morphological and biochemical properties. Of these, 20 were chosen for additional investigations because of their capacity to endure harmfulness of six distinct metals (Zn, Cd, Cu, Ni, Pb, and Cr) and their resistance against 10 unique antibiotics. Bacterial segregates viz. *Enterobacter* sp., *Serratia* sp., *Pseudomonas fluorescens* and *Pseudomonas pseudoalcaligenes* had the option to develop at extremely high convergences of different weighty metals like Cu, Ni, Pb, and Cr. The base development inhibitory convergences of these

metals were in the middle of 300 and 400 mg/L (Table 3). *Comamonas* has recuperated fundamentally from Samne ghat and Rajendra Prasad ghat where the grouping of Pb was extensively high. Mechanical effluents are believed to be the significant wellspring of high Pb in these locales. The bounty of *Comamonas* in these very Pb contaminated destinations may be expected to their genomic transformation and resulting expanded resistance to Pb. Event and action of *Comamonas* strains have effectively been accounted for in Pb polluted soils (Konopka et al., 1999). Stoppel and Schlegel (1995) additionally announced metal tolerant strain of *Comamonas* sp. Albeit numerous *Comamonas* strains has been separated from clinical examples and viewed as entrepreneurial microbes (Willems et al., 1991).

The constant presence of *Serratia*, *Pseudomonas pseudoalcaligenes*, *Enterobacter* sp., *Proteus vulgaris* and *Shewanella* sp. in the water test gathered from the Assi ghat and Dashashwamedh ghat was noticed all through year. Alongside high MIC towards hefty metals, these detaches likewise showed MDR against Amp, Chl, Nal, Tet, Van, Imi, Cip, Ery, Pol, and Str (Table 4). Zone of hindrance of various antibiotics (Amp, Chl, Nal, Tet, Van, Imi, Cip, Ery, Pol, and Str) against touchy bacterial strains were found in the scope of 5.02 to 11.23 mm. But *Serratia*, *Pseudomonas pseudoalcaligenes*, *Comamonas*, and *Proteus vulgaris*, the wide range of various strains were touchy towards imipenem, a β -lactam carbapenem (Yong et al., 2002). Greatest zone of restraint delivered by imipenem in *Aeromonas hydrophila*, and *Ochrobactrum intermedium* viz. 11.03 and 11.23 mm, separately. Subsequently, the capacity of these bacterial strains to defeat the harmful impacts of various medications represents a significant issue in light of the fact that these medications are often utilized against bacterial contamination. Imipenem is a very good quality wide range drug basically used to battle nosocomial contaminations. In the Assi ghat, city civil waste and untreated sewage are the inescapable wellspring of water contamination and furthermore a reasonable climate for the development of human pathogenic bacteria.

CONCLUSION

Blending of sewage alongside mechanical effluents into the Ganga waterway irritate water quality. Dirtied water turned into a natural surroundings of different pathogenic bacteria having double properties in delivering multiple substantial metals tolerant limit just as antibiotics resistance. Cooperation of physiological, biochemical and molecular procedures could give total data about bacterial ID and portrayal. Presence of destructive bacteria in the waterway Ganga welcomes genuine consideration according to human wellbeing point of view since Ganga water is additionally utilized for drinking, blessed washing and water system purposes.

REFERENCES

- [1] Abbas SZ, Rafatullah M, Ismail N and Lalun J (2014). Isolation, identification, and characterization of cadmium resistant *Pseudomonas* sp. M3 from industrial wastewater. *Journal of Waste Management*; pp. 10-1155.
- [2] Abolude DS, Augustina DO, Zebedee B and Olajide OO (2013). Analyses of heavy metals in water and sediment of Bindare stream, Chikaji industrial area SabonGari. *International Journal of Scientific Research in Environmental Sciences (IJSRES)*; 1(6): pp. 115-121.
- [3] Abou-Shanab R. A. I., van Berkum, P. and Angle J.S. (2007). Heavy metal resistance and genotypic analysis of metal resistance genes in gram-positive and gram-negative bacteria present in Ni-rich serpentine soil and in the rhizosphere of *Alyssum murale*. *Chemosphere*; 68: pp. 360-367.
- [4] American Public Health Association [APHA]. (1998). Standard methods for the examination of water and waste water. Washington, DC: Author.
- [5] Baik, K. S., Park, S. C., Kim, E. M., Bae, K. S., Ann, J. H., Ka, J. O., ... Seong, C. N. (2008). Diversity of bacterial community in freshwater of Woopo wetland. *The Journal of Microbiology*, 46, 647–655.10.1007/s12275-008-0135-x
- [6] Baquero, F., Martínez, J. L., & Cantón, R. (2008). Antibiotics and antibiotic resistance in water environments. *Current Opinion in Biotechnology*, 19, 260–265.10.1016/j.copbio.2008.05.006
- [7] Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disc method. *American Journal of Clinical Pathology*, 45, 493–591.
- [8] Bergey, D. H., & Breed, R. S. (1994). Biochemical identification of enterobacteriaceae. *Bergey's Manual of Determinative Bacteriology*, 1, pp. 416–417.
- [9] Beveridge, T. J., & Doyle, R. (1989). Metal ions and bacteria. New York, NY: Wiley.
- [10] Felsenstein, J. (1985). Phylogenies and the comparative method. *The American Naturalist*, 125, pp. 1–15.10.1086/284325
- [11] Heyndrickx, M., Vauterin, L., Vandamme, P., Kersters, K., & De Vos, P. (1996).

Applicability of combined amplified ribosomal DNA restriction analysis (ARDRA) patterns in bacterial phylogeny and taxonomy. Journal of Microbiological Methods, 26, pp. 247–259.10.1016/0167-7012(96)00916-5

- [12] Indian Standard Drinking Water Specification (Second Revision) IS 10500. (2012). Retrieved from <http://www.cgwb.gov.in/Documents/WQ-standards.pdf>
- [13] Industrial Directory (1981). Government of India ministry of micro, small & medium enterprises, brief industrial profile of Varanasi District (updated) 1981. Retrieved from <http://dcmsme.gov.in/dips/DIP,%20Varanasi.pdf>
- [14] Isenberg, H. D., & Sundheim, L. H. (1958). Indole reactions in bacteria. Journal of Bacteriology Bacteriology, 75, pp. 682–690.

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