https://doi.org/10.33472/AFJBS.6.Si3.2024.3182-3193

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

# Isolation and Identification of Heavy Metal Tolerant (Cr, Cu, Pb) Bacteria from Municipal Waste Dumping Site and Assessment of their MIC and Antibiotic Susceptibility

**Satyakam Agarwala[1&3*], Arijit Bora[2*], Pranab Jyoti Koch[3], Ankita Kalita[3]**

[1]Dept. of Bioengineering and Technology, GUIST, Gauhati University Gopinath Bordoloi Nagar, Jalukbari, Guwahati, Pin 781014
[2]Deputy Secretary, University Classes, Gauhati Univeristy, Gopinath Bordoloi Nagar, Jalukbari, Guwahati, Pin 781014
[3]Dept. of Applied Biology, School of Biological Sciences, University of Science and Technology Meghalaya, Ribhoi, Bariduwa, Pin – 793101, India

Corresponding author – arijitbora@gauhati.ac.in
Co-corresponding author – satya.agar2020@gmail.com

**Article Info**

Volume 6, Issue Si3, July 2024

Received: 21 May 2024

Accepted: 27 June 2024

Published: 13 July 2024

*doi: 10.33472/AFJBS.6.Si3.2024.3182-3193*

**ABSTRACT:**

With rapid urbanization and development, a huge amount of solid/liquid Municipal wastes are produced each day and are dumped at Municipal dumping grounds leading to environmental pollution with toxic, non-biodegradable, persistent heavy metals on-site and off-site. Current investigation emphasizes the isolation and identification of bacterial isolates capable of tolerating heavy metal concentrations (Copper, Chromium, and Lead). Five isolates from sludge were collected from the dumping site at Boragaon, Guwahati and based on their biochemical and morphological properties and 16S rDNA analysis, identification was done till the genus level. They were found to be *Bacillus cereus*, *Bacillus paramycoides*, *Bacillus velezensis* and *Lysinibacillus fusiformis*. All the isolates showed different MIC against Cu, Cr, and Pb at different concentrations. *Lysinibacillus fusiformis* showed maximum resistance against Cr at 900 ppm and the least was shown by *Bacillus cereus* at 500 ppm. Co resistance of heavy metals and antibiotics was also investigated, which showed many of the isolates were not only resistant towards Cu, Cr, and Pb but also were resistant towards Penicillin G and Streptomycin. *Bacillus* sp and *Lysinibacillus* sp showed varying susceptibility against other antibiotics namely Tetracycline, Ampicillin, Chloramphenicol, Ciprofloxacin, Azithromycin, and Norfloxacin. This has led to the conclusion of non-ethical and non-scientific disposal of wastes leading to the release and accumulation of toxic heavy metals to the ecosystem.

**Keywords:** heavy metals, municipal waste, antibiotic susceptibility, MIC, phylogenetic analysis

© 2024 Satyakam Agarwala, This is an open access article under the CC BY license (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you giveappropriate credit to the original author(s) and the source, provide a link to the Creative Creative Commons license, and indicate if changes were made

## 1. Introduction

"Heavy metals" in a broader sense indicates a group of metals with an atomic density higher than 4.0 g/cm$^3$, or something higher than water (Paul et al., 2015). Non-biodegradability of heavy metals is one of the prime sources of environmental pollution and a threat leading to its accumulation in different parts of the food chain, endangering animal and human health (Upadhyay et al., 2022, Ashraf et al., 2007). Metal contamination of anthropogenic origin can be due to metal mining and smelting, industries, atmospheric deposition, agricultural activities, and disposal of wastes (Haferburg., 2007, Ezzouhri et al., 2009). Many reports have confirmed that heavy metals like Cr, Cd, Pb, Hg, and Cu are immensely toxic for almost all organisms. Metal contamination has become a major concern for people living near Municipal solid waste dumping grounds, contaminating not only food but also water (Sanga et al., 2023, Verma et al., 2023, Balali-Mood et al., 2021). Flora and fauna are experiencing increased pressure due to the hazards caused by the discharge of heavy metals containing industrial effluents affecting the health of animals, plants, humans, and aquatic organisms (Robin et al., 2012). A wide method of evaluation of soil pollution is the determination of heavy metals concentration. Existence of metal in the soil due of atmospheric deposition and mineral weathering of anthropogenic origin and of natural sources are the major reason of its deposition in the soil (Hookoom et al., 2013). Heavy metals, including copper, iron, and zinc, are necessary trace elements for cells at low concentrations, but if found at higher concentrations in the contaminated soil, they can be hazardous (Pandey et al., 2012, Bánfalvi et al., 2011). Soil environments containing major contaminants like Pb and Cd, which are extremely poisonous can lead to cell membrane disruptions, enzyme activity alteration, and destruction of the assembly of DNA. Cadmium being most toxic pollutant of the soil, is released into the environment by mining, smelting, burning of plastics, batteries and fossil fuels, dumping of sewage sludge (Tang et al., 2006). Pb a major pollutant of soil, water, and air is extremely toxic not only to humans, as well as to animals, plants, and microorganisms (Raj et al., 2023, Low et al., 2000). High concentrations of both forms of chromium in the environment viz hexavalent (Cr (VI)) and trivalent (Cr (III)), are harmful (Chung et al., 2014). Leather tanning effluents, chromium electroplating, and nuclear wastes are listed as the major sources of Cr pollution (Marzan et al., 2017).

Various natural processes & urbanization have led to an accumulation of high amounts of heavy metals, which are mostly found in microbial habitats. Omnipresence of microbes is mostly involved in various biological processes of life, which has forced these organisms to adapt to high heavy metal conditions by adjusting themselves with different biological mechanisms (Habi et al., 2009). Genes related to tolerance mechanisms have been found both on chromosomes and plasmids. However, most studies have been paid a lot of emphasis on the mechanism by which metallic ions are sent out of the cell (Affan et al., 2009). Soil containing metal resistant microbes are dependent on a number of factors including intrinsic biochemical properties, genetic and physiological adaptation as well as cells morphological changes and ecological alterations in metal speciation process. These microbes have evolved several coping strategies to tolerate the increasing concentration of heavy metal ions under metal-stressed circumstances including the cellular accumulation of the metal ions, outflow of metal ions from the cell, and the reducing the concentration of the heavy metal ions (Edgar et al., 2004). Large numbers of studies have described and reported that indigenous microbes are important in the bioremediation or restoration of contaminated soil owing to their tolerance to high heavy metal concentrations. (Mia et al., 2019, Ge HW et al., 2009).

The prime goal of the current study is to characterize the isolates of heavy metal-resistant bacteria (Cu, Cr, and Pb) from a residential and industrial Municipal disposal site in Guwahati city. Additionally, it looks into how resistant they are to elevated levels of heavy metal contamination and measures how active they are against popular antibiotics, suggesting that

they may be resistant to other kinds of broad-range antibiotics as well. Lastly, MIC will assess their tolerance capacity. The taxonomic characterization will determine the types of bacteria that are present in the region.

## 2. Materials and methods

### Sample collection

A municipal garbage disposal site is a common dumping site where large amounts of wastes of anthropogenic origin is disposed every day. The collection site is located at Paschim Boragaon situated at the outskirts of Guwahati city, Kmarup (M) district, Assam (26.06.872° N latitude, 91.40.896° E longitude). For the present study sludge sample (50.0mL) was collected in sterile Falcon tubes and transferred to laboratory immediately. The tubes were sealed and kept under a low temperature of -20ºC for further analysis.

### Isolation and characterization of bacteria

The serial dilution method opted for the isolation of heavy metal-resistant bacteria (Cu, Cr, and Pb). 1mL of the sludge was added in 9.0mL of sterilized distilled water and was serially diluted from $10^{-1}$ to $10^{-9}$. Using the standard spread plate method, 0.1mL of the serial dilution sample was spread over a sterilized nutrient agar (HiMedia) medium containing 30ppm of each of Cupric sulphate ($CuSO_4$), Potassium dichromate ($K_2Cr_2O_7$) and Lead nitrate ($PbNO_3$) and incubated at 37ºC for 2 days. The bacterial isolates were observed and screened for microscopic, morphological, and biochemical characterization such as Methyl red, Vogues Proskauer, citrate utilization, indole production, oxidase, catalase, and starch hydrolysis. Identified isolates were cultures and further sub-cultured on the media incorporated with heavy metals to obtain pure cultures. (Pandit et al., 2013).

### Optimal growth conditions of the isolates

For optimum growth, pH was considered as a parameter for assessment. For determination, 5mL of Nutrient Broth (HiMedia) was taken in test tubes amended with 30ppm of the heavy metal, and pH was maintained at 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0 then autoclaved. The pH of the medium was adjusted using dilute HCl or NaOH. 0.1 mL of the overnight grown isolates were added in the test tubes and were incubated for 24 hours at a temperature 37ºC. The optimal growth of the isolates was noted using a spectrophotometer (Systronics UV-Vis double beam Spectrophotometer, 2206TS) at 600 nm.

### Antibiotic susceptibility test

Many reporters have reported the association of antibiotic resistance with metal resistance (Rani et al., 2010). Antibiotic sensitivity tests for the bacterial isolates were performed on Muller Hinton agar medium and were determined using the Kirby Bauer Disc diffusion method (Baccer et al., 1966). As many as ten antibiotic discs (HiMedia) of standard concentration were taken for the susceptibility tests and they are as follows: tetracycline (30µg), streptomycin (10µg), chloramphenicol (30µg), ampicillin (10µg), penicillin G (10µg), azithromycin (15µg), norfloxacin (10µg), and ciprofloxacin (5µg) respectively. Overnight grown bacterial culture was spread over the Muller Hinton media and incubated at 37ºC for 24 hours. Diameters of the zone of inhibition were measured, and the strains were classified as susceptible (*S*), intermediate (*I*), or resistant (*R*)or adhering to the standard antibiotic disc given by NCCLS guidelines (NCCLS., 2004).

**Minimum Inhibitory Concentration assay**

Overnight grown cultures of heavy metal resistant bacterial cells were harvested to their log phase of growth ($OD_{600}$ = 0.7) and adjusted to approx 2.8 x $10^9$ cells $mL^{-1}$ MIC for the heavy metal resistant isolates were then assessed using the protocol as described by Filali et al. (2000). Adjusted cell counts were grown on Nutrient Broth incorporated with each of increasing concentration of heavy metals namely, Cupric sulphate ($CuSO_4$), Potassium dichromate ($K_2Cr_2O_7$) and Lead nitrate ($PbNO_3$) until the isolates failed to grow or showed decreased growth. The initial heavy metal concentration was 100 ppm and it was increased till 1900 ppm. The culture was grown for 24 hours at 37ºC in a shaker incubator at 200 rpm. To assess the growth, absorbance was taken at 600nm using multiplate reader (GeNei Laboratories, Elisa Reader). MIC was taken as the concentration at which a particular bacterial strain could not grow (Filali et al., 2000, Rajbanshi et al., 2008).

**Taxonomic characterization and phylogenetic analysis**

A bacterial genomic DNA extraction kit (HiMedia – India) was used for the isolation of the genomic DNA of the bacterial isolates. By running the DNA on a 1.2% agarose gel and observing a single band with a higher molecular weight, the quality was assessed. Nanodrop (Biotech Instruments, USA) was used to determine the concentrations of each DNA sample. For later use, DNA was kept at -80ºC for storage. Using a Veriti® 96-well Thermal Cycler, a specific universal primer for 16S rRNA was employed to amplify the isolated DNA. A total volume of 25µl consisting of forward and reverse primers (27F–GAGTTTGATCATGGCTCAG and 149R–TACGGTTACCTTGTTACGACTT) of 10 pmol, 2.5 mM of $MgCl_2$, 200µM of deoxyribonucleotide triphosphates (dNTPs), Taq DNA polymerase of about 0.5 U, 1X of PCR buffer (Invitrogen Life Technologies, Brazil) and 50-100 ng of isolated genomic DNA of bacteria was used to perform PCR. Pre-denaturation of the DNA template was run at a temperature of 95 °C for a duration of 5 min followed by 39 cycles of denaturation of strands for a duration of 30 sec at a temperature of 95 °C. Followed by annealing for a duration of 45 seconds and then elongation step at 72°C for 1 minute. The final extension was done for about 7 min at 72°C. The resulting amplicons were then run and determined in 1.2% agarose gel using 0.5x TAE buffer. BDT v3.1 Genetic Analyzer was used along with a Cycle sequencing kit on ABI 3730xl for forward and reverse DNA sequencing reaction of PCR amplicon with forward primer and reverse primers. Using forward and reverse sequence data information, and with the help of aligner software, a consensus sequence of the rDNA gene was generated. The rDNA gene sequence was used to carry out BLAST with known database of NCBI Genbank with a view to determine the genus and species of the isolates. The first ten sequences were chosen and on the basis of maximum identity score, aligned using the ClaustalW multiple alignment software. Molecular Evolutionary Genetics Analysis Software XI was used for phylogenetic analysis and a tree was constructed using the neighbor-joining tree method (Saitou et al., 1987). Using the model given by Tamura-Nei (Tamura et al., 1993), genetic distance matrix was developed.

## 3. Results and Discussion

Municipal solid dumping sites contain varied domestic, economic, and industrial waste materials from different industries, which are the prime sources of heavy metal being dumped (Afolagboye et al., 2020, Imron et al., 2021). There is a negative impact of the heavy metals on the nature and quality of microbes and quantities of the soil (Hassen et al., 1998, Kormoker et al., 2019). The prime objective of the study is to investigate the tolerance potential of the bacterial isolates from municipal solid waste dumping site, considered as a major reserve of heavy metals (Ojiego et al., 2022b). This is in a view of better understanding of the properties

of the isolates, which can be a potential tool for reducing metal pollution by bacteria. In the current study, municipal dumping site at the outskirts of Guwahati at Paschim Boragaon was chosen (Fig-1). As many as 110 colonies were observed and were screened in a medium supplemented with heavy metals (Pb, Cr, Cu), and 5 colonies were selected based on their relative tolerance and resistance towards heavy metals for further studies (Fig-2). Based on their biochemical characterization, Cr (VI), Pb (II), and Cu (II) resistant bacterial isolates were caused to undergo identification using Bergey's Manual of Determinative Bacteriology (Holt et al., 1994). All 5 selected bacterial isolates shared almost similar morphology and biochemical characteristics: off-white colony, Gram-positive rods, and endospore-forming (Table 1). The isolates were grown on separate mediums containing heavy metals (Pb, Cr, Cu) and were identified as isolates resistance against specific heavy metals. They were named as : S1 (Copper), S2 and S3 (Chromium), S4 and S5 (Lead). On the basis of biochemical and morphological characteristics, *Bacillus* sp, and *Lysinibacillus* sp were identified. The gram staining results were quite correlated with the studies conducted earlier stating that most of the metal-tolerant bacteria were positive for Gram staining, especially Bacillus (Ndeddy et al., 2017). The isolates were then selected for further identification. Table 1 displays the results of morphological and biochemical findings. All the isolates S1, S2, S3, S4, and S5 showed optimum growth at 35ºC, which appeared to be the most suitable temperature. Optimum growth for S1, S4 and S5 was observed at pH 7.0, while optimum growth for S2 and S3 was noted at pH 7.5 and pH 7.0 respectively.

All the selected isolates were confirmed and authenticated based on the 16S rDNA sequence. Using BLAST-N analysis, the 16S rDNA sequence of the bacterial isolates was aligned and results revealed that the sequence query has 98-100% identity. Using 16S rDNA data of bacteria in GenBank it was used to reveal a 100% query coverage. The phylogenetic tree construct further concluded the extent of relatedness between the 16S rDNA sequence of the bacterial isolates and other related bacteria taken from the database (fig - 3). Based on the following information, result of the isolates showed that S1 is closely related to *Bacillus cereus* (100%), S2 to *Lysinibacillus fusiformis* (98.93%), S3 to *Bacillus paramycoides* (100%), S4 to *Lysinibacillus fusiformis* (99.93%) while S5 is closely related to *Bacillus velezensis* (99.74%) respectively. All the isolates have GeneBank accession numbers: MT507094.1, NR_042072.1, NR_157734.1, MK875175.1, and OQ600718.1 respectively. The genus *Bacillus* is one of the predominant bacterial genera found in soil, and several studies have reported their presence in various ecosystems (Saxena et al., 2019). Moreover, the genetic identifications of these isolates were in agreement with their biochemical reaction results.

Antibiotic resistance and decreased antibiotic susceptibility are known to be co regulated by heavy metal ions (Zhai et al., 2016). After an incubation of 24 hours, the plates were studied for the zone of inhibition to various antibiotic discs (Table 2). The study showed that S1 *Bacillus cereus*, S2 *Lysinibacillus fusiformis*, S3 *Bacillus paramycoides,* and S4 *Lysinibacillus fusiformis* are sensitive to antibiotics Chloramphenicol, Ciprofloxacin, Azithromycin and Norfloxacin showing maximum zone of inhibition. All the given strains either showed resistance towards penicillin G or intermediate sensitivity towards it. *Bacillus cereus* is known to be extremely resistant to b-lactam antibiotics (Yim et al., 2015), namely penicillin, ampicillin, carbenicillin, amoxicillin, oxacillin, cefazolin, and ceftriaxone. The association of b-lactamase activity with antibiotic resistance is thought to be the reason. This resistance may be associated with b-lactamase production (Park et al., 2009). S2 *Lysinibacillus fusiformis* and S3 *Bacillus paramycoides* showed sensitivity towards most of the antibiotics. S1 *Bacillus cereus* and S5 *Bacillus velezensis* showed maximum resistance towards antibiotics such as Ampicillin and Penicillin G. Similar is the case with S4 *Lysinibacillus fusiformis* which showed resistance towards tetracycline and penicillin G. The major triggering factor of heavy metal co-selection and increasing level of tolerance to certain antibiotics is always environmental

pollution in view of regulation of resistance (Nath et al., 2019). There is a possibility that antibiotic resistance and heavy metal resistance in *B. cereus* are co-expressed, more than a random occurrence, but rather the outcome of selection by environmental heavy metals as reported in some earlier works (Shammi et al., 2016, Dhanarani et al., 2016, Nath et al., 2018). Soil-dwelling microbes are always under selective pressure from the toxic substances released into the environment leading to the development resistance of the bacteria. (Gu Y et al., 2017, Cabral., 2016). This has led to the evolution of various resistance and detoxification mechanisms of metal-tolerant bacteria, which are mostly plasmid-mediated. The level of toxicity in the soil is mostly dependent on the geographical location, concentration of metal, and bacterial diversity (Rajkumar et al., 2010). The study revealed different metal tolerance by S1 *Bacillus cereus*, S2 *Lysinibacillus fusiformis*, S3 *Bacillus paramycoides*, S4 *Lysinibacillus fusiformis* and S5 *Bacillus velezensis* (Fig-4). MIC of the isolates for each heavy metal was determined at concentrations ranging from 100 ppm to 1900 ppm. Investigation led to the finding that *Lysinibacillus fusiformis* S2 showed higher threshold of tolerance towards Cr(II) at 900 ppm (0.247±0.018) followed by S4 *Lysinibacillus fusiformis* (0.231±0.037) and S3 *Bacillus paramycoides* (0.283±0.015), which showed resistance at 700 ppm towards Pb(II) and Cr(II) respectively. The strain of S1 *Bacillus cereus* (0.229±0.033), and S5 *Bacillus velezensis* (0.237±0.037) however, showed resistance towards Cu(II) and Pb(II) at 500 ppm. It's been reported that bioaccumulation of heavy metals inside the cells generally exceeds the rate of extracellular loss (Oladipo et al., 2018). Although cell wall makes up, as well as synthesis of metalloproteins controls varying quantities of metal accumulation by various isolates (Mosa et al., 2016).

Table 1: Table for Morphological and Biochemical characterization of isolates

| Isolates | S1 | S2 | S3 | S4 | S5 |
|---|---|---|---|---|---|
| Growth Conditions and Biochemical Tests | | | | | |
| pH | 6.0-7.0 | 6.0-7.5 | 7.0 | 6.0-7.0 | 6.0-7.0 |
| Temperature | 35 | 35 | 35 | 35 | 35 |
| Gram | + | + | + | + | + |
| Shape | Rods | Rods | Rods | Rods | Rods |
| MR | - | - | + | - | - |
| VP | + | + | - | + | + |
| Indole | - | - | _ | - | - |
| Citrate utilization | + | + | + | + | - |
| Catalase | - | + | + | + | + |
| Oxidase | + | + | + | + | + |
| Starch hydrolysis | + | + | + | + | + |
| Carbohydrate Fermentation | | | | | |
| Glucose | - | + | + | + | + |
| Sucrose | + | - | + | - | + |
| Fructose | + | + | + | + | + |

Table 2: Pattern of antibiotic sensitivity and resistance of the isolates (*S* sensitive, *I* intermediate, *R* resistance)

| Antibiotics | S1 *Bacillus cereus* | S2 *Lysinibacillus fusiformis* | S3 *Bacillus paramycoides* | S4 *Lysinibacillus fusiformis* | S5 *Bacillus velezensis* |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| Tetracycline | S | S | S | R | S |
| Ampicillin | R | I | I | I | R |
| Chloramphenicol | S | S | S | S | S |
| Penicillin G | R | I | I | R | R |
| Ciprofloxacin | S | S | S | S | S |
| Streptomycin | S | R | R | I | S |
| Azithromycin | I | S | S | S | I |
| Norfloxacin | S | S | I | S | S |

Fig – 1: Map view of the dumping site at Boragaon, Guwahati

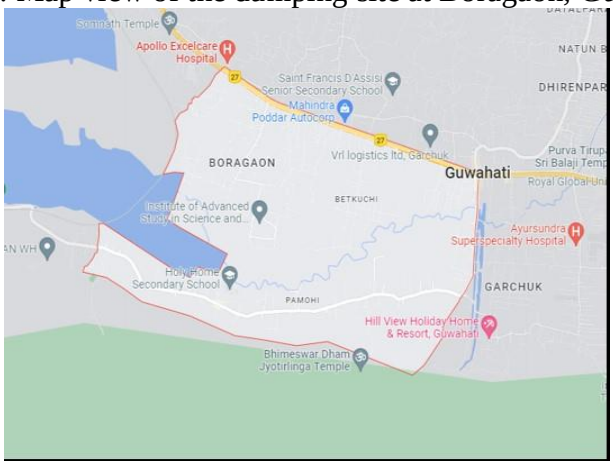



Fig – 2: Isolates growing on medium with heavy metals (a) Cu, (b) Cr, and (c) Pd (30 ppm)

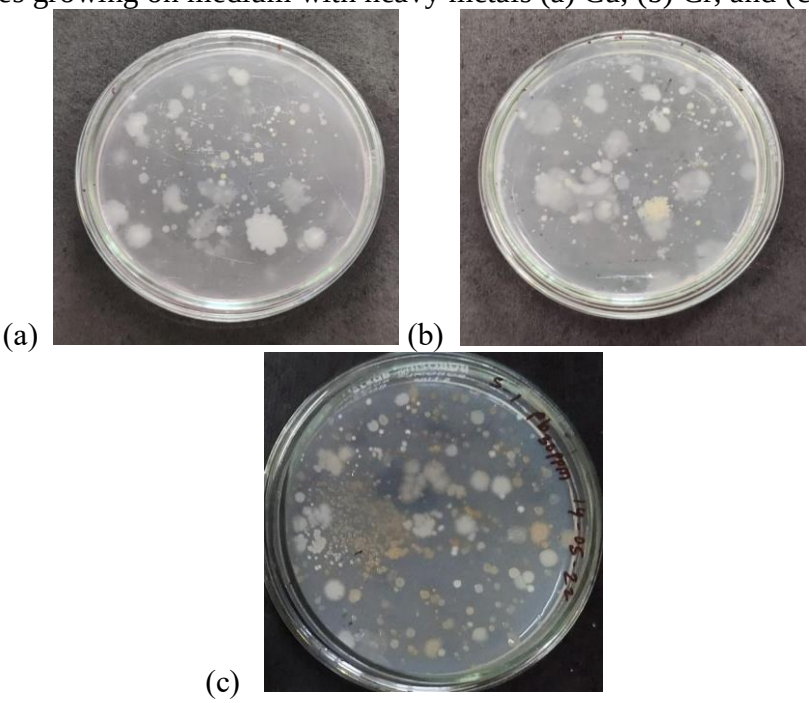
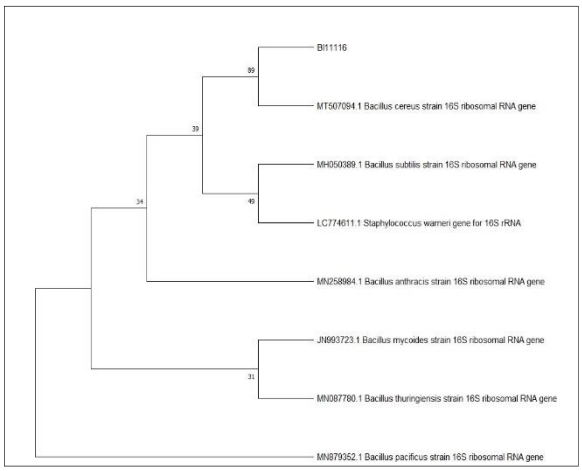
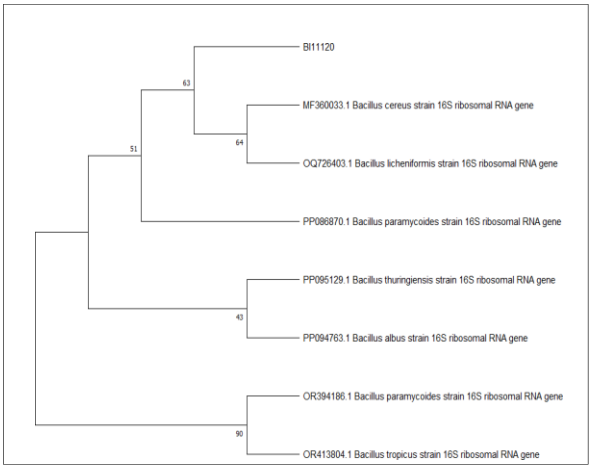


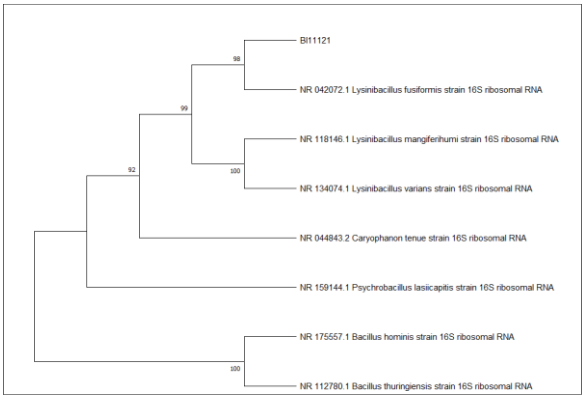

Fig-3: Phylogenetic relationship shown by Neighbour joining tree analysis, based on 16S rDNA sequences, between the reference bacteria draw out from GenBank Databases and the identified bacterial isolates. Numerical values at the branching points refer to Bootstrap values based on 1000 replicates.



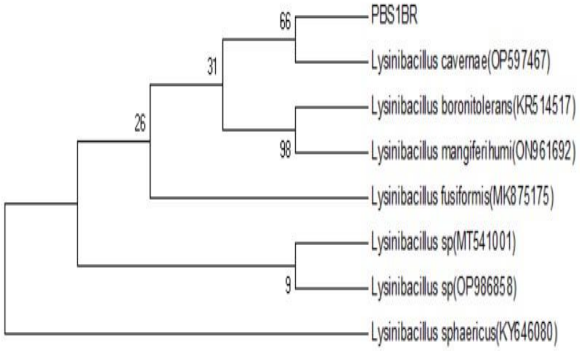

(a) S1 *Bacillus cereus*



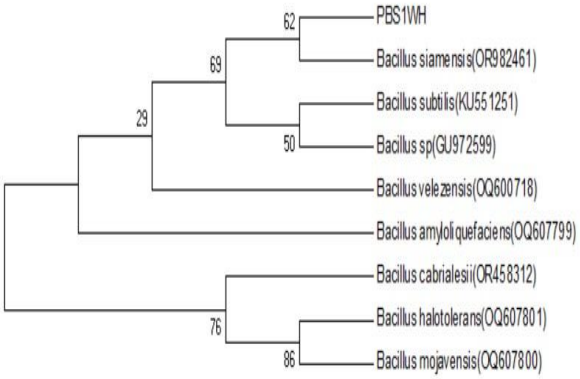

(b) *Lysinibacillus fusiformis*




(c) *Bacillus paramycoides*

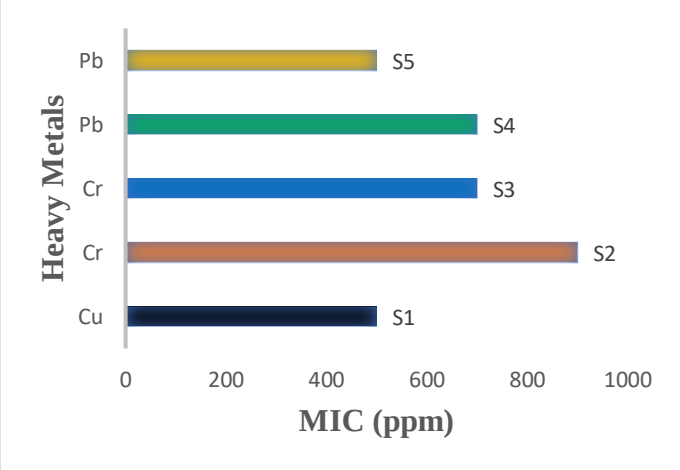



(d) *Lysinibacillus fusiformis*




(e) *Bacillus velezensis*

Fig-4: A visual representation of the MIC (ppm) for bacterial isolates resistant to heavy metals Copper, Chromium, and Lead.




## 4. Conclusion

A large number of wastes of anthropogenic origin, both domestic and industrial, are dumped at the solid waste dumping ground. This has led to the accumulation of waste not fit for the environment because most them contains heavy metals that are released directly into the ground. This has caused havoc not only at the site but also in the neighbouring area. The current study investigated the tolerance potentiality of the isolates against heavy metal concentration as well as their biochemical and molecular characterization. The study revealed metal tolerance by S1 *Bacillus cereus*, S2 *Lysinibacillus fusiformis*, S3 *Bacillus paramycoides*, S4 *Lysinibacillus fusiformis* and S5 *Bacillus velezensis*. Maximum tolerance was showed by S2 *Lysinibacillus fusiformis* against Cr at 900 ppm (0.247±0.018) followed by S3 *Bacillus paramycoides* against Cr at 700 ppm (0.283±0.015) and S4 *Lysinibacillus fusiformis* against Pb ppm (0.231±0.037). Antibiotic sensitivity of the isolates against antibiotics revealed co-regulation of heavy metal tolerance with antibiotic susceptibility. All the isolates showed efficient heavy tolerance at 35°C with a varying pH for all the isolates ranging from 6.0 to 7.5. Their unique metal tolerant abilities can be further used as a potential bioremediation agent to mitigate the level of environmentally unsafe heavy metals. This requires further studies on isolates isolated from Municipal dumping grounds to assess the heavy metal removal abilities.

## 5. Reference

1. Afolagboye, L. O., Ojo, A. A., Talabi, A. O., 2020. Evaluation of soil contamination status around a municipal waste dumpsite using contamination indices, soil- quality guidelines, and multivariate statistical analysis. SN Applied Sciences, 2:1864. https://doi.org/10.1007/s42452-020-03678-y
2. Baccer R, Kirby M, Sherris J, Turek M (1966) Antibiotic susceptibility testing by standard single disc diffusion method. Am J Clin Pathol 45:493–496
3. Balali-Mood, Mahdi, et al. "Toxic mechanisms of five heavy metals: mercury, lead, chromium, cadmium, and arsenic." *Frontiers in pharmacology* 12 (2021): 643972.
4. Bánfalvi G. Heavy metals, trace elements and their cellular effects. Cellular effects of heavy metals. 2011;3-28.
5. Cabral L, Júnior GVL, de Sousa STP, Dias ACF, Cadete LL, Andreote FD, Hess M, de Oliveira VM (2016) Anthropogenic impact on mangrove sediments triggers differential responses in the heavy metals and antibiotic resistomes of microbial communities. Environ Pollut 216:460–469

6. Chung DY, Kim HI, Chung YH, Lee MJ, Yoo SJ, Bokare AD, Sung YE. Inhibition of CO poisoning on Pt catalyst coupled with the reduction of toxic hexavalent chromium in a dual-functional fuel cell. Scientific reports. 2014;4(1):7450.
7. Dhanarani S, Viswanathan E, Piruthiviraj P, Arivalagan P, Kaliannan T (2016) Comparative study on the biosorption of aluminum by free and immobilized cells of *Bacillus safensis* KTSMBNL 26 isolated from explosive contaminated soil. J Taiwan
8. Edgar RC: *Nucleic Acids Res* **2004,**32(5):1792-1797.
9. Filali BK, Taoufik J, Zeroual YFAZ, Dzairi FZ, Talbi M, Blaghen M. Waste water bacterial isolates resistant to heavy metals and antibiotics. Current Microbiology. 2000;41:151-156.
10. G. Haferburg and E. Kothe, "Microbes and metals: interactions in the environment," *Journal of Basic Microbiology*, vol. 47, no. 6, pp. 453–467, 2007.
11. Ge HW, Lian MF, Wen FZ, Yun YF, Jian FY, Ming T. Isolation and characterization of the heavy metal resistant bacteria CCNWRS33-2 isolated from root nodule of *Lespedeza cuneata* in gold mine tailings in China. J Hazard Mater. 2009; 162:50-56.
12. Gu Y, Van Nostrand JD, Wu L, He Z, Qin Y, Zhao F-J, Zhou J (2017) Bacterial community and arsenic functional genes diversity in arsenic contaminated soils from different geographic locations. PLoS ONE 12(5): e0176696
13. Habi S, Daba H. Plasmid incidence, antibiotic and metal resistance among *entero bacteriacea* isolated from Algerian streams. Pak. J Biol. 2009; 12:1474-1482.
14. Hassen, A., Cherif, M., Say, S., Boudabous, A., 1998. Effect of heavy metals on Pseudomonas aeruginosa and Bacillusthuringiensis. Bioresource Technology, 68(1): 73 – 82.
15. Hookoom M, Puchooa D. Isolation and identification of heavy metals tolerant bacteria from industrial and agricultural areas in Mauritius. Current Res. in Micobiol. & Biotech. 2013; 1(3):119-123.
16. Imron, M. F., Kurniawan, S. B., Abdullah, S. R. S., 2021. Resistance of bacteria isolated from leachate to heavy metals and the removal of Hg by Pseudomonas aeruginosa strain FZ-2 at different salinity levels in a batch bio-sorption system. Sustainable Environment Research, 31:14. https://doi.org/10.1186/s42834-021-00088-6
17. Inst Chem Eng 69:61–67
18. J. G. Holt, N. R. Krieg, P. H. A. Sneath, J. T. Staley and S. T. Williams, *Bergey's Manual of Determinative Bacteriology*, 9th Ed. (Williams & Wilkins, Baltimore, 1994).
19. Kormoker, J. Proshad, R., Islam, M. S., Ahmed, S., Chandra, S., Uddin, M., Rahman, M., 2019. Toxic metals in agricultural soils near the industrial area of Bangladesh: ecological and human health risk assessment. Toxicity Review, https://doi.org/10.1080/15569543.2019.1650777.
20. L. Ezzouhri, E. Castro, M. Moya, F. Espinola, and K. Lairini, "Heavy metal tolerance of filamentous fungi isolated from polluted sites in Tangier, Morocco," *African Journal of Microbiology Research*, vol. 3, no. 2, pp. 35–48, 2009.
21. Low, K.S. Lee, C.K. and Liew, S.C. (2000): Sorption of cadmium and lead from aqueous solution by spent grain. *Process Biochemistry* **36**, 59-64.
22. Marzan, Lolo Wal, et al. "Isolation and biochemical characterization of heavy-metal resistant bacteria from tannery effluent in Chittagong city, Bangladesh: Bioremediation viewpoint." *The Egyptian Journal of Aquatic Research* 43.1 (2017): 65-74.
23. Mia, R., Selim, M., Shamim, A., Chowdhury, M., Sultana, S., Armin, M., Hossain, M., Akter, R., Dey, S., Naznin, H. (2019): Review on various types of pollution problem in textile dyeing & printing industries of Bangladesh and recommendation for mitigation. – Journal of Textile Engineering & Fashion Technology 5: 220-226.

24. Mosa KA, Saadoun I, Kumar K, Helmy M, Dhankher OP (2016) Potential biotechnological strategies for the cleanup of heavy metals and metalloids. Front Plant Sci 7:303
25. Nath S, Deb B, Sharma I (2018) Isolation of toxic metal-tolerant bacteria from soil and examination of their bioaugmentation
26. Nath, Soumitra, et al. "Isolation and identification of metal-tolerant and antibiotic-resistant bacteria from soil samples of Cachar district of Assam, India." *SN Applied Sciences* 1.7 (2019): 727.
27. National Committee for Clinical Laboratory Standards (NCCLS), Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically: Approved, 2004
28. Ndeddy Aka, R.J., Babalola, O.O., 2017. Identification and characterization of Cr-, Cd-, and Ni-tolerant bacteria isolated from mine tailings. Biorem. J. 21 (1), 1–19. Nies, D.H., 2003. Efflux-mediated h
29. Ojiego, B. O., Ilo, O. P., Okolo, J. C., Igborgbor, J. C., Ishaku, T. , Abdullah, S. A., Gadzama, I. M. K., Bolorunduro, P., 2022b. Concentrations of heavy metals in soil samples from dumpsites located at Kuje and Kwali area councils, Abuja, Nigeria. Journal of Materials and Environmental Sciences, 13(9): 1037- 1046
30. Oladipo OG, Ezeokoli OT, Maboeta MS, Bezuidenhout JJ, Tiedt LR, Jordaan A, Bezuidenhout CC (2018) Tolerance and growth kinetics of bacteria isolated from gold and gemstone mining sites in response to heavy metal concentrations. J Environ Manage 212:357–366
31. Pandey G, Madhuri S. Heavy metals causing toxicity in animals and fishes. Research Journal of Animal, Veterinary and Fishery Sciences. 2014;2(2):17-23.
32. Pandit, R., Patel, B., Kunjadia, P., & Nagee, A. Isolation, characterization and molecular identification of heavy metal resistant bacteria from industrial effluents, Amala-khadi Ankleshwar, Gujarat. *Inter J Environ Sci*, (2013); *3*(5), 1689-1699.
33. Park, Y., J. B. Kim, S. B. Shin, J. C. Kim, S. H. Cho, B. K. Lee, J. Ahn, J. M. Kim, and D. H. Oh. 2009. Prevalence, genetic diversity, and antibiotic susceptibility of Bacillus cereus strains isolated from rice and cereals collected in Korea. J. Food. Prot. 72: 612–617.
34. Paul D.S., and Sinha S.N. (2015): Isolation and characterization of a phosphate solubilizing heavy metal tolerant bacterium from river Ganga, West Bengal, India, Songklanakarin. *J. Sci. Technol*; 37:651e657.
35. potentiality by pot studies in cadmium-and lead-contaminated soil. Int Microbiol 21:1–2
**36.** Q. Affan, E. Shoeb, U. Badar, J. Akhtar, *J. Basic. Appl. Sci*, **2009**., 5(2):55-60**.**
37. R. Ashraf., T. Adam ali, *Pak. J. Bot*., 2007., 39(2),629-36.
38. Raj, Kshyanaprava, and Alok Prasad Das. "Lead pollution: Impact on environment and human health and approach for a sustainable solution." *Environmental Chemistry and Ecotoxicology* 5 (2023): 79-85.
39. Rajbanshi A. Study on heavy metal resistant bacteria in guheswori sewage treatment plant. Our Nature. 2008;6(1).
40. Rajkumar M, Ae N, Prasad MNV, Freitas H (2010) Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol 28(3):142–149
41. Rani MJ, Hemambika B, Hemapriya J, Kannan VR (2010) Comparative assessment of heavy metal removal by immobilized and dead bacterial cells: a biosorption approach. Afr J Environ Sci Technol 4(2):077–083
42. Robin, R., Muduli, P., Vardhan, K., Ganguly, D., Abhilash, K., 2012. Heavy metal contamination and risk assessment in the marine environment of Arabian Sea, along the Southwest coast of India. Am. J. Chem. 2, 191–208.

43. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 1987; 4:406-25.
44. Sanga, Victor Fanuel, Christina Fabian, and Fikira Kimbokota. "Heavy metal pollution in leachates and its impacts on the quality of groundwater resources around Iringa municipal solid waste dumpsite." *Environmental Science and Pollution Research* 30.3 (2023): 8110-8122.
45. Saxena AK, Kumar M, Chakdar H, Anuroopa N, Bagyaraj D (2019) Bacillus species in soil as a natural resource for plant health and nutrition. J Appl Microbiol 128(6):1583–1594. https ://doi.org/10.1111/jam.14506
46. Shammi T, Ahmed S (2016) Heavy metal tolerance and antibiotic resistance of *Bacillus* spp isolated from two major rivers in Bangladesh. Bangladesh J Microbiol 30(1–2):17–22
47. Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 1993; 10:512-26.
48. Tang, X.Y. Zhu, Y.G. Cui, Y.S. Duan, J. and Tang, L. (2006): The effect of ageing on the bioaccessibility and fractionation of cadmium in some typical soils of China. *Environment International* **32**, 682-689.
49. Upadhyay, Richa. "Heavy metals in our ecosystem." *Heavy Metals in Plants*. CRC Press, 2022. 1-15.
50. Verma, Nitin, et al. "Assessment and impact of metal toxicity on wildlife and human health." *Metals in water*. Elsevier, 2023. 93-110.
51. Yim, J. H., K. Y. Kim, J. W. Chon, D. H. Kim, H. S. Kim, D. S. Choi, I. S. Choi, and K. H. Seo. 2015. Incidence, antibiotic susceptibility, and toxin profiles of Bacillus cereus sensu lato isolated from Korean fermented soybean products. J. Food. Sci. 80: 1266–1270.
52. Zhai Y, He Z, Kang Y, Yu H, Wang J, Du P, Zhang Z, Hu S, Gao Z (2016) Complete nucleotide sequence of pH11, an IncHI2 plasmid conferring multi-antibiotic resistance and multi-heavy metal resistance genes in a clinical *Klebsiella pneumoniae* isolate. Plasmid 86:26–31