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# Isolation and Characterization of Heavy Metals Resistant Bacteria in Water Samples from Mambilla Artisanal Mining Site, Nguroje, Taraba State

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## ABSTRACT

The present study aimed to characterize and assess the resistance of specific bacterial strains obtained from water samples collected around the Nguroje area of the Mambilla Plateau in Sarduana Local Government. The focus was on their ability to tolerate various heavy metal ions and their potential applicability in remediating environments contaminated with heavy metals. Various culture media and lead, copper, and mercury salts were employed in the investigation. Water samples were gathered from diverse sources, including wells, streams, and an abandoned pit near the mining site. The isolated bacteria strains underwent characterization through both physical and chemical methods, encompassing Gram staining, coagulase, oxidase, catalase, indole, citrate, and utilization of glucose, galactose, and sucrose. The bacteria species identified were Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter aerogenes, and Streptococcus sp. Notably, Escherichia coli was the predominant bacterium across all three sampled sources, while Enterobacter aerogenes exhibited the lowest occurrence. Among the five bacterial isolates, namely Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa, three were subjected to metal resistance testing. Staphylococcus aureus demonstrated susceptibility at lead (Pb) concentrations of 0.20g/100mL, mercury (Hg) concentrations of 0.30g/100mL, and copper (Cu) concentrations of 0.10g/100mL. For Escherichia coli, the minimum inhibitory concentrations (MICs) were 0.15g/100mL for lead, 0.25g/100mL for mercury, and 0.20g/100mL for copper. Similarly, Pseudomonas aeruginosa exhibited MICs of 0.15g/100mL for lead, 0.20g/100mL for mercury, and 0.10g/100mL for copper. Interestingly, the resistant bacteria strains displayed a comparable resistance pattern towards antibiotics. Staphylococcus aureus showcased resistance to six out of ten tested antibiotics. Escherichia coli displayed the highest level of resistance (seven out of ten), while Pseudomonas aeruginosa exhibited resistance to five antibiotics. In conclusion, the isolated bacteria strains exhibit significant potential for employment as agents in bioremediation processes within environments contaminated by heavy metals. This study, underscores their promising role in addressing heavy metal pollution.

**Keywords:** *Heavy Metals; Bacteria; Water and Artisanal mining.*

## INTRODUCTION

Artisanal mining is a prevalent practice in many developing countries, providing livelihoods for millions of people [1]. However, it also poses significant environmental and health risks, particularly due to the release of heavy metals into water sources [2]. Heavy metal pollution is a serious environmental problem that can have detrimental effects on ecosystems and human health. Artisanal mining activities, which are often unregulated and informal, can contribute to the release of heavy metals into water sources. For example, the Mambilla artisanal mining site in Nguroje, Taraba State, Nigeria, has been extracting blue sapphire for over two decades, potentially leading to heavy metal contamination [3].

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Heavy metals, including lead, cadmium, mercury, and arsenic, are naturally occurring substances that can be released into the environment through various human activities, including mining [4]. Mining activities have been identified as one of the main sources of heavy metal contamination in water bodies, posing risks to ecosystems and human health [5]. Numerous studies have highlighted the alarming levels of heavy metal contamination in mining areas, particularly in water sources, emphasizing the need for effective remediation strategies [5]

Microorganisms, especially heavy metal-resistant bacteria, have emerged as potential agents in addressing heavy metal pollution. These bacteria possess specialized mechanisms that enable them to tolerate and thrive in environments rich in heavy metals [6]. Researchers have explored the use of these microorganisms in bioremediation efforts to restore contaminated environments and mitigate the far-reaching consequences of heavy metal pollution [3]. Bacteria have evolved diverse survival mechanisms, such as the expulsion of metal ions from cells and the conversion of these ions into less harmful forms, to thrive in metal-induced stress environments [2]. These resistance genes are typically harbored on chromosomes or plasmids and can be inducible [7]. Microorganisms can also engage in redox reactions with heavy metal ions, utilizing them for energy and growth [6]. Researchers have investigated the use of microbes capable of oxidizing or reducing metals to ameliorate sites contaminated by metal pollution [6].

In response to growing environmental awareness, regulatory actions have been taken to rectify past transgressions and protect against future environmental degradation and exploitation. These measures aim to preserve ecosystems and safeguard human well-being. Open-pit mining has multifaceted environmental ramifications, as the extraction and processing of ore yield substantial volumes of waste materials (Côte). The extraction process involves the removal of overburden and the generation of waste during ore extraction and separation.

This study aims to unravel the intricate patterns of heavy metal resistance in bacteria isolated from the artisanal mining site on the Mambilla Plateau in Taraba State. Additionally, it seeks to evaluate the potential application of these bacteria in bioremediation strategies.

## MATERIALS AND METHODS

### Study Area

Nguroje, situated on the Mambilla Plateau within the Sardauna Local Government Area of Taraba State, Nigeria, serves as the focal point of this research. Its geographical coordinates correspond to approximately 6°57'0" North latitude and 11°7'0" East longitude.


**Figure** 1: Local map (modified from Google Earth) of the Mambilla Plateau Area

### Materials

A comprehensive array of materials encompassing culture media, equipment, reagents, chemicals, and utensils were employed.

**Sample Collection**

Water samples from diverse sources, namely Wells, Streams, and an Abandoned Pit in the vicinity of the Mambilla artisanal mining site at Nguroje, were meticulously collected. Sterile glass sample collection bottles were utilized, with a rope secured around the bottle neck to facilitate dipping and filling from strategic points within the water bodies. The samples were filled to approximately three-quarters (3/4) capacity, securely sealed, meticulously labeled, and then promptly transported to the laboratory within ice boxes to maintain their integrity.

**Preparation of Heavy Metal Treatments**

To achieve heavy metal treatments, a methodology akin to that outlined by [8], was adopted. Specifically, 0.05g, 0.10g, 0.15g, 0.20g, 0.25g, 0.30g, and 0.35g of Lead acetate, Mercury chloride, and Copper sulfate were precisely weighed and individually dissolved in 100mL of distilled water, within 250mL conical flasks.

**Sample Culturing**

The process of serial dilution and culturing hewed closely to the approach delineated by [9, 10].

**Isolation and Identification of Bacteria Isolates**

Employing the quadrant streaking method as illustrated by [10], bacteria isolation was executed. Subsequent identification of these isolates was based on their cell wall characteristics, involving the application of the Gram's staining technique. Further characterization ensued through conventional biochemical tests encompassing indole, catalase, oxidase, coagulase, glucose fermentation, fructose fermentation, and galactose fermentation tests. The protocols for these biochemical assessments adhered to those elucidated by Okereke and [9, 10].

**Heavy Metal Tolerance Testing**

Characterization of bacteria isolates was augmented by assessing their resistance and susceptibility profiles in the face of varied concentrations of distinct metallic compounds. Treatment solutions were prepared in accordance with the methodology outlined by [8]. This testing methodology entailed the use of the agar dilution technique. Specifically, 200µl portions of the pre-prepared heavy metal salt treatments (Lead - Ld, Copper - Cu, and Mercury - Hg) were incorporated into 20mL Nutrient agar within petri dishes, followed by thorough mixing and subsequent solidification. Utilizing a sterile wire loop, pure isolates were transferred to the surface of the agar media infused with heavy metals. Incubation of the petri dishes occurred at 37 ºC for a span of 24 hours. Evaluation post-incubation focused on the growth of bacteria colonies, with the Minimum Inhibitory Concentration (MIC) established as the concentration at which bacteria growth was impeded following incubation.

**Antibiotic Sensitivity Test (Disc-Diffusion Method)**

Leveraging the principle of radial diffusion, antibiotic-impregnated discs were positioned on agar plates previously inoculated with the test bacteria. Over time, the antibiotics diffused outward, creating a concentration gradient that hindered bacterial growth. Clear zones or rings formed around the antibiotic discs post-incubation, indicating growth inhibition. The methodology underlying this test aligned with established protocols and principles, as defined by the scientific community.

**RESULT**

**Overall Characteristics of Bacteria Isolated**

Table 1 presents the outcomes of distinct staining and biochemical assessments conducted on bacteria derived from diverse sample sources.

Table 1: Overall Characteristics of Bacteria Isolated

| *S/N* | *Gram Stain* | *CO* | *OX* | *CA* | *IN* | *CI* | *GL* | *GA* | *SU* | *G p* | *Bacteria Isolated* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | _ (Rod) | _ | _ | + | + | _ | + | + | + | + | *Escherichia Coli* |
| 2 | + (Cocci in clusters) | + | _ | + | _ | + | + | + | + | _ | *Staphyloccus aureus* |
| 3 | _ (Rod) | _ | + | + | _ | + | _ | _ | _ | _ | *Pseudomonas aeruginosa* |
| 4 | _ (Rod) | _ | _ | + | _ | + | + | + | + | + | *Enterobacter aerogenes* |
| 5 | + (Cocci in chains) | _ | _ | _ | _ | + | + | + | + | _ | *Streptococcs Sp.* |

**Key:**

*CO: Coagulase*
*OX: Oxidase*
*CA: Catalase*
*IN: Indole*
*CI: Citrate*
*GL: Glucose*
*GA: Galactose*
*SU: Sucrose*
*GP: Gas production*

**Occurrence of bacteria from different sources.**

Figure 1 illustrates the distribution and prevalence of bacteria in relation to their respective origins. The identification of these organisms hinged on a combination of colonial, morphological, and biochemical traits. Notably, the findings highlight that Escherichia coli exhibited the highest frequency, accounting for 22 instances (30.1%). Staphylococcus aureus and Streptococcus sp. followed closely with 14 occurrences each (19.2%), while Pseudomonas aeruginosa constituted 13 cases (17.8%). Comparatively, Enterobacter aerogenes presented the lowest incidence, with 10 instances (13.7%), across all three water sources.

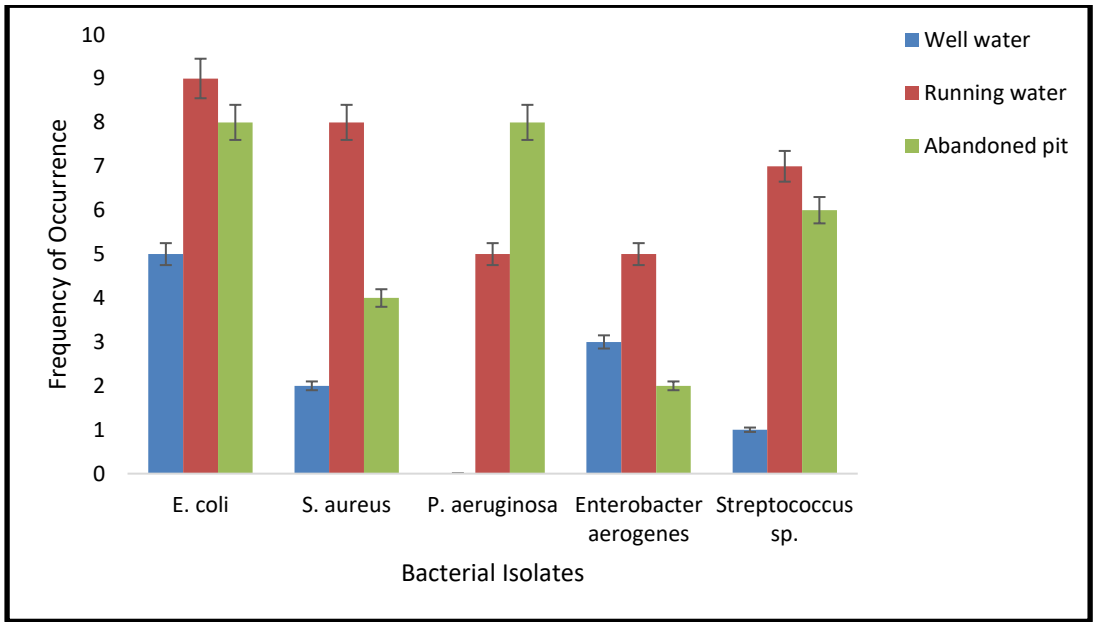


**Figure 1: Occurrence of bacteria from different sources.**

**Growth profile of *Staphylococcus aureus* at different metals concentrations**

Figure 2 depicts the growth dynamics of Staphylococcus aureus across varying concentrations of different metals. Notably, the bacterium exhibited its most robust growth within media infused with mercury, displaying a progressive decline in growth culminating at the 0.25g/100mL concentration, followed by inhibition at 0.3g/100mL. Lead, at a concentration of 0.20g/100mL, inhibited the growth of Staphylococcus aureus. Conversely, the bacterium demonstrated the least resistance to copper, with growth inhibition manifesting at the modest concentration of 0.1g/100mL.

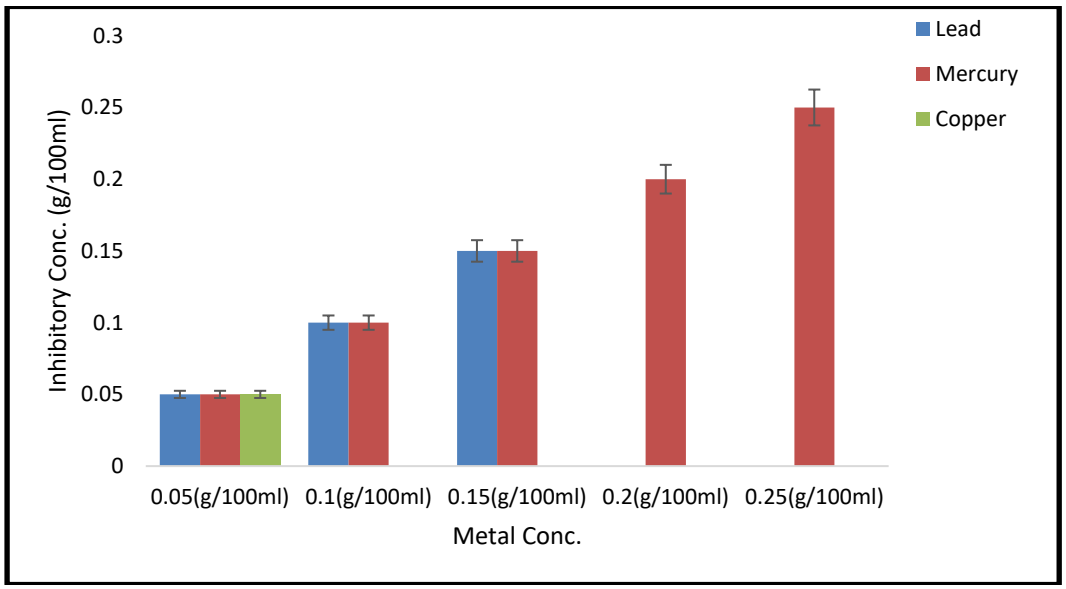


**Figure 2: Growth profile of *S. aureus* at different metals concentrations.**

**Growth profile of *E. coli* at different metals concentrations.**

Figure 3 portrays the growth behavior, revealing that *Escherichia coli* exhibited the utmost degree of resilience towards copper, as evidenced by its inhibition at the concentration of 0.25g/100mL. Comparatively, the bacterium displayed lesser resistance to mercury, with growth inhibition arising at the concentration of 0.20g/100mL. Notably, *Escherichia coli* evinced the least restraint against lead, as its growth was curbed at the modest concentration of 0.15g/100mL.

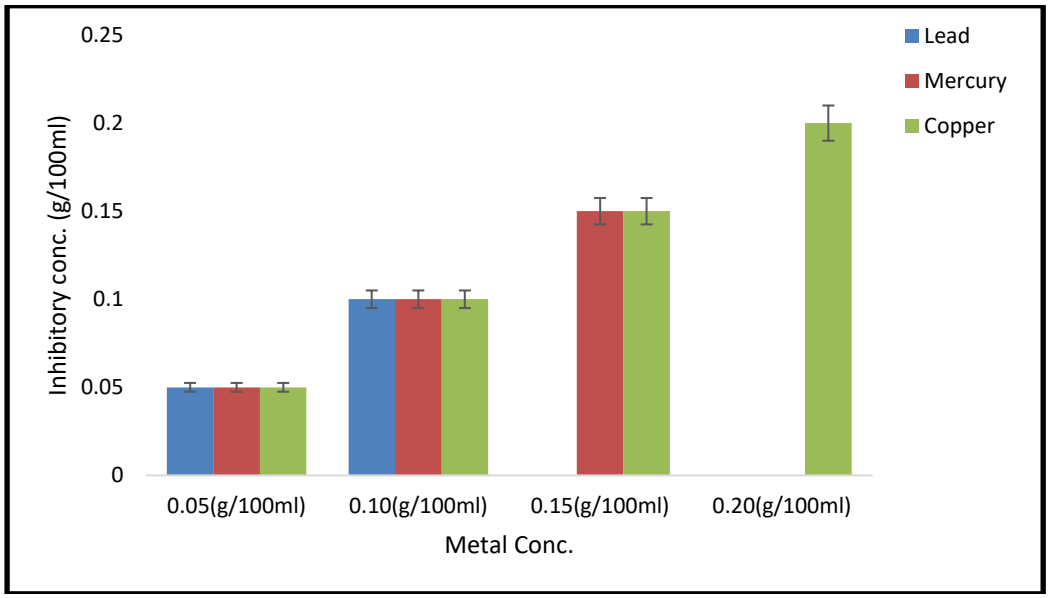


**Figure 3: Growth profile of *E. coli*at different metals concentrations.**

**Growth profile of *P. aeruginosa* at different metals concentrations.**

Figure 4 outlines the growth progression of Pseudomonas aeruginosa across varying concentrations of distinct metals. Notably, the bacterium demonstrated heightened resistance to mercury, with growth inhibition occurring remarkably early at a mere concentration of 0.20g/100mL. In comparison, lead also exerted an inhibitory effect, impeding the organism's growth at a concentration of 0.15g/100mL. Conversely, Pseudomonas aeruginosa displayed notable sensitivity to copper, as growth inhibition was observed at a minimal concentration of 0.10g/100mL.

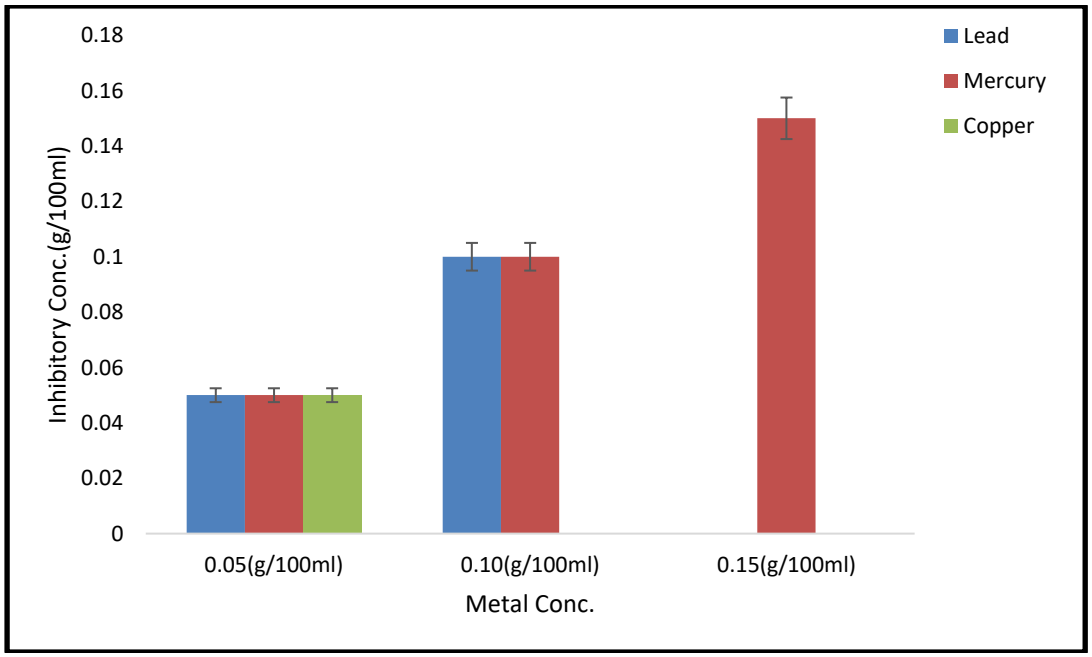


**Figure 4: Growth profile of *P. aeruginosa* at different metals concentrations.**

**Minimum Inhibitory Concentration (MIC) of Bacterial isolates**

Table 2 showcases the minimum inhibitory concentration (MIC) of the isolated organisms concerning Lead acetate, Mercury chloride, and Copper chloride. Notably, *Staphylococcus aureus* exhibited its highest level of resistance against Mercury, reflecting a MIC of 0.30g/100mL, trailed by Lead with an MIC of 0.20g/100mL. Comparatively, its resistance to copper was the lowest, with an MIC of 0.10g/100mL. *Escherichia coli* demonstrated its most formidable resistance against Mercury, registering an MIC of 0.25g/100mL, followed by Copper with an MIC of 0.20g/100mL, and displaying the least resistance towards Lead with an MIC of 0.15g/100mL. Likewise, *Pseudomonas aeruginosa* showcased its most pronounced resistance against Mercury, recording an MIC of 0.20g/100mL, followed by Lead with an MIC of 0.15g/100mL. In contrast, the organism's susceptibility to copper was the highest among the metals, revealing an MIC of 0.10g/100mL.

Table 2: Minimum Inhibitory Concentration (MIC) of Bacterial isolates to Lead (Pb), Copper (Cu), Mercury (Hg).

| ***S/N*** | ***Bacterial Isolates*** | ***MIC (g/100ml)*** | | |
|---|---|---|---|---|
| | | ***Pb*** | ***Cu*** | ***Hg*** |
| 1 | *Staphylococcus aureus* | 0.20 | 0.10 | 0.30 |
| 2 | *Escherichia coli* | 0.15 | 0.25 | 0.20 |
| 3 | *Pseudomonas aeruginosa* | 0.15 | 0.10 | 0.20 |

**Antibiotic activity on metal resistant isolates.**

Table 3 outlines the prevalence and distribution of antimicrobial effectiveness against distinct antibiotics concerning metal-resistant isolates. Among these isolates, Escherichia coli exhibited the highest degree of resistance, rendering it impervious to 7 out of the 10 antibiotics tested. Staphylococcus aureus followed suit, displaying resistance to 6 out of 10 antibiotics, while Pseudomonas aeruginosa showcased the lowest level of resistance, being impervious to 5 out of the 10 antibiotics assessed.

Table 3: Antibiotic Sensitivity of Lead, Mercury and Copper resistant Isolates

| ***S/N*** | ***Strain*** | ***Sensitive Antibiotics*** | ***Resistant Antibiotics*** |
|---|---|---|---|
| 1 | *Staphylococcus aureus* | Streptomycin, Zinnacef, Perfloxacin and Gentamycin. | Roceplin, Ciprofloxacin, Septrin, Erythromycin, Ampiclox and Amoxicillin. |
| 2 | *Escherichia coli* | Amoxicillin, Pefloxacinand Ofloxacin. | Augumentin, Gentamycin, Sparifloxacin, Streptomycin, Septrin, Chloramphenicol and Ciprofloxacin. |
| 3 | *Pseudomonas aeruginosa* | Chloramphenicol, Gentamycin, Augumentin, Ofloxacin and Ciprofloxacin. | Amoxicillin, Perfloxacin, Streptomycin, Septrin and Sparifloxacin. |

**DISCUSSION**

The current investigation, focused on the "Isolation and Characterization of Heavy Metals Resistant Bacteria in Water Samples," was conducted at the Mambilla artisanal mining site in Nguroje, Taraba State. The extracted bacteria encompassed Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter aerogenes, and Streptococcus sp., selected based on their prevalence in the sampled water sources. The identification and

characterization of these organisms were guided by established methods involving colonial, morphological, and biochemical criteria.

Notably, three bacterial species—Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa—align with those identified by [11] in Punjab, India, extracted from industrial effluents. Similarly, Staphylococcus aureus and Pseudomonas aeruginosa were reported by [12] in Assam, India, sourced from sewage, industrial effluents, garages, and petrol pumps. This concurrence underlines the adaptability of these bacteria to environments laden with industrial pollutants.

In the context of their occurrence across various water sources at the mining site—namely well water, running water, and abandoned pits—Escherichia coli dominated, particularly in running water, signifying its role as an indicator of potential fecal contamination. Staphylococcus aureus and Streptococcus sp. also exhibited notable prevalence. Interestingly, Enterobacter aerogenes displayed the least presence, possibly due to its inherent sensitivity to heavy metals, thus affecting its abundance in such contaminated settings.

Among the tested bacterial isolates, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa were examined for metal resistance, demonstrating distinctive responses across lead (Pb), mercury (Hg), and copper (Cu) concentrations.

Remarkably, Staphylococcus aureus displayed robust growth when subjected to 0.05g/100mL of lead, maintaining similar growth at 0.10g/100mL. This finding aligns with [13] report that this bacterium resisted 1000 µg/ml of lead. However, growth declined significantly at 0.15g/100mL and ceased entirely at 0.20g/100mL. This value is lower than the reported 5000 µg/ml by the same authors. The discontinuation of growth at 0.20g/100mL points to lead's cytotoxic effects, highlighting the balance between bacterial resistance mechanisms and heavy metal toxicity. Likewise, Staphylococcus aureus exhibited notable growth on plates with concentrations ranging from 0.05g/100mL to 0.15g/100mL of mercury, consistent with [14] finding that this bacterium resisted 150 µg/ml of mercury. Colony numbers decreased at 0.20g/100mL and continued diminishing until growth ceased at 0.30g/100mL of mercury. However, the decline at 0.20g/100mL is higher than the reported 50 µg/ml by the same authors. This strong resistance underscores Staphylococcus aureus' adaptation to mercury, potentially involving efflux pumps and metal-binding proteins. Conversely, Staphylococcus aureus' growth was limited in copper-containing medium, with sparse growth at 0.05g/100mL. This sensitivity could be attributed to copper's potent antimicrobial properties and its disruption of vital cellular processes.

In the case of Escherichia coli, growth was notably inhibited in lead-containing medium, with minimal colonies at 0.05g/100mL and even fewer at 0.10g/100mL. Complete inhibition occurred at 0.15g/100mL. These findings contrast with [14] report that this bacterium resisted 250 µg/ml of lead. This sensitivity aligns with Escherichia coli's adaptability to lead-polluted environments, where higher resistance might result in lead accumulation within the cell.

Unlike lead's effect, Escherichia coli exhibited favorable growth in mercury-containing medium at 0.05g/100mL and 0.10g/100mL, with reduced growth at 0.15g/100mL. Growth suppression occurred at 0.20g/100mL. The significant resistance of Escherichia coli to copper was evident, with substantial growth at concentrations up to 0.15g/100mL, 0.10g/100mL and 0.15g/100mL. The growth however eventually reduced significantly at 0.20g/100mL and then seized at 0.25g/100mL. suggesting robust mechanisms for copper tolerance.

Pseudomonas aeruginosa exhibited sensitivity to the tested heavy metals. Growth was minimal in lead-containing medium at 0.05g/100mL and 0.10g/100mL, and absent at 0.15g/100mL. Similarly, moderate growth was observed at 0.05g/100mL of mercury, but higher concentrations suppressed growth, indicating sensitivity to mercury's toxicity. The observation that Pseudomonas aeruginosa showed moderate resistance to lead, mercury, and copper, with growth inhibition at 0.15g/100mL, 0.20g/100mL, and 0.15g/100mL, respectively, is consistent with [14] report that this bacterium resisted 200 µg/ml of lead, 200 µg/ml of mercury, and 150 µg/ml of copper. Importantly, copper exhibited the highest toxicity against Pseudomonas aeruginosa, with minimal growth observed at only 0.1g/100mL. This sensitivity underscores Pseudomonas aeruginosa's susceptibility to copper-induced oxidative stress and damage. Pseudomonas aeroginosa from this study was the least resistant isolate and will at least require genetic modification if its potential utilization in bioremediation becomes necessary.

Comparing the cytotoxic effects of the tested heavy metals, it was observed that Staphylococcus aureus exhibited inhibition at a concentration of 0.20g/100mL against lead, whereas both Escherichia coli and Pseudomonas aeruginosa were inhibited at 0.15g/100mL. This outcome highlights that among the Gram-positive bacteria isolates, particularly Staphylococcus aureus, demonstrated remarkable resistance to lead (Pb). Consequently, Staphylococcus aureus

emerges as a promising candidate for remediating lead-contaminated environments. Similar trends in metal resistance were discerned with mercury, as Staphylococcus aureus faced inhibition at 0.30g/100mL, while both Escherichia coli and Pseudomonas aeruginosa were inhibited at 0.20g/100mL. This underscores Staphylococcus aureus's potential in mercury-contaminated environment remediation.

The dynamics shifted significantly with copper, wherein the previously most resilient Staphylococcus aureus, along with Pseudomonas aeruginosa, exhibited reduced resistance, being inhibited at a concentration of 0.10g/100mL of copper. In contrast, Escherichia coli displayed a higher threshold for inhibition at 0.25g/100mL. Consequently, Escherichia coli emerges as a superior candidate among the three bacterial species for addressing copper pollution. This intriguing observation adds complexity to the comprehension of the exact mechanisms underpinning metal resistance in this study. Nevertheless, it is rational to acknowledge that even at low concentrations, heavy metals exhibit toxicity toward living cells.

In the conducted study, the minimum inhibitory concentration (MIC) of various bacteria isolates against heavy metals was examined. Notably, Staphylococcus aureus exhibited the highest resistance to lead, with an MIC of 0.20g/100mL. This value contrasted with findings by [11] from Punjab, where the average MIC for lead against three strains was 0.025g/100mL. Similarly, another study by [12] in Assam reported a lead MIC of 0.11g/100mL against Staphylococcus aureus. The elevated MIC values in the current research imply that these bacteria might have encountered lead exposure at the mining site, contributing to their resistance. Regarding mercury, the bacterial isolates demonstrated high MICs—0.30g/100mL for Staphylococcus aureus, 0.25g/100mL for Escherichia coli, and 0.20g/100mL for Pseudomonas aeruginosa. These values far exceeded the MIC of mercury against Escherichia coli reported by [15] in the mercury-polluted Yamuna River (0.000055g/100mL or less). This suggests that the bacterial strains from this study could be promising for bioremediation efforts in mercury-contaminated environments.

Copper emerged as the most toxic heavy metal among those tested, displaying MICs of 0.10g/100mL against Staphylococcus aureus and Pseudomonas aeruginosa, and 0.20g/100mL against Escherichia coli. The pronounced toxicity of copper aligns with existing research [16], which highlights its harm to various organisms. Notably, the MICs observed in this study exceeded those from [11] where the MICs for copper against Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa were 0.034g/100mL, 0.038g/100mL, and 0.026g/100mL, respectively. This variance could be attributed to the bacteria strains adapted to the specific Nguroje area.

Examining antibiotic resistance among the metal-resistant isolates, all exhibited multidrug resistance, echoing findings by [11]. Escherichia coli displayed the highest antibiotic resistance, while Pseudomonas aeruginosa exhibited the least. The intricate relationship between heavy metals and antibiotics was evident, potentially contributing to the prevalence of antibiotic-resistant strains in the region. The co-existence of heavy metals and antibiotics could foster cross-resistance and impact antibiotic bioavailability.

Research findings have underscored that in certain microbial communities within specific environments, the simultaneous presence of heavy metals and antibiotics contributes to the emergence of microbial resistance to antibiotics, including multidrug resistance [17]. This premise guided the execution of antibiotic sensitivity testing in our study. For instance, the introduction of copper (Cu) into soils has been demonstrated to foster both copper resistance and resistance to antibiotics such as ampicillin, chloramphenicol, and tetracycline [18].

This phenomenon of heightened antibiotic resistance might be explained in a couple of ways. Firstly, the existence of heavy metals appears to enhance the proliferation of indigenous bacteria within the microbial community, which might already possess genes responsible for antibiotic resistance. Another plausible explanation is that the presence of heavy metals alongside antibiotics in the environment could potentially induce antibiotic resistance in bacteria that would otherwise be sensitive to antibiotics [19].

In our present study, we observed that the bacterial isolate displaying the least resistance to heavy metals exhibited significant resistance to antibiotics. This suggests that the minimum concentrations of heavy metals required to trigger antibiotic resistance are often quite low. However, [20] cautioned that even concentrations of heavy metals capable of inducing antibiotic resistance lie below the MIC of heavy metals. Consequently, even trace amounts of these metals within the body could undermine antibiotic therapies. This is particularly concerning for residents of Nguroje and the surrounding areas who rely on wells and other water sources near mining sites, as they are at a notable risk of developing antibiotic resistance due to heavy metal contamination.

Additionally, considering that antibiotics are secondary products of resident microbiota, it has been proposed that interactions between heavy metals and antibiotics can lead to cross-resistance. The effective concentrations of both

heavy metals and antibiotics could be influenced by the chelation of antibiotics with metals, thereby decreasing the bioavailability of each compound [21]. For instance, metallic cations like copper (Cu) can bind with antibiotics like tetracycline, forming complexes that lower the availability of the antibiotics to act against bacteria [22, 23], consequently diminishing their efficacy. Therefore, it is reasonable to posit that heavy metals exert direct impacts on antibiotic resistance by serving as catalysts and/or inducers of antibiotic resistance genes while simultaneously reducing the accessibility of antibiotics.

## CONCLUSION

The findings of this study indicate that the tested bacteria isolates, namely Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa, displayed a notable ability to withstand and resist the heavy metals examined. It's worth noting that successful bioremediation relies on factors like site-specific attributes such as nutrient availability, moisture levels, temperature, redox potential, and the concentration of contaminants. This bioremediation process can take the form of either bio stimulation, which enhances existing microbial populations, or augmentation, involving the introduction of additional microbial biomass.

Based on the outcomes of this investigation, there is a suggestion that these bacterial isolates could prove effective in bioremediation efforts within environments contaminated with heavy metals, given consistent maintenance of the relevant conditions. Nevertheless, it's imperative to conduct a thorough assessment of the pathogenic potential of these heavy metal-resistant isolates to ascertain their safety for the environment before progressing to field trials.

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