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Original Research Article

Isolation and Identification of Bacteria from Scrap Metal Dump Site Soil in Gada Biyu Area Gusau Metropolis

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Abstract: The increasing deposition of scrap metals in urban environments has contributed significantly to microbial corrosion and heavy metal contamination of soils, posing ecological and public health risks. The study aimed to determine the total mesophilic count, isolate and identify bacterial species present in the contaminated soils, quantify the concentration of selected heavy metals (Zinc, Cadmium, Copper, Lead, and Chromium), and assess the correlation between bacterial occurrence and heavy metal contamination levels. Soil samples were collected from three designated points (A, B, and C) across the dump site and the mesophilic bacterial count bacterial isolates were obtained using serial dilution and spread plate techniques on nutrient agar, followed by morphological and biochemical characterization. Heavy metal concentrations were determined through atomic absorption spectrophotometry after acid digestion of soil samples. The highest Mesophilic bacterial count ($5.5x10^6$) was observed in samples from the most heavily polluted areas (B4) while the least ($1.2x10^7$) was obtained from A2. The results revealed significant microbial diversity, with fifteen bacterial species identified, including Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumoniae, and Micrococcus luteus. These isolates displayed varied biochemical characteristics. Heavy metal analysis showed that Sample C had the highest concentrations of all tested metals particularly Zinc (0.34±0.04 ml/kg), Cadmium (0.20±0.05 ml/kg), and Lead (0.34±0.03 ml/kg) while Sample A recorded the lowest levels. It is concluded that the microbial diversity and heavy metal concentration in soil from a scrap metal revealed both high contamination level and the present of metal tolerant bacteria.

Keywords: Isolation, Identification, Metal Scrap, Dump Site Soil, Gada Biyu, Gusau.

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INTRODUCTION

Metal rusting, also referred to as corrosion, is the degradation of material properties, especially metals, due to chemical or electrochemical reactions in the environment (Zhenglin, 2020). It is a process that involves the reaction between iron or steel, atmospheric oxygen, and moisture, leading to the formation of iron oxide (rust). Bacteria play a significant role in the formation and progression of metal rusting (Yang *et al.*, 2020). Microbially induced corrosion (MIC) refers to the process of corrosion influenced by the presence or activity (or both) of microorganisms residing in biofilms on the surface of the corroded material (Mand *et al.*, 2019). MIC has been documented as a serious issue responsible for huge economic losses in various industries (Xiaowie *et al.*, 2018), contributing approximately 20% of annual loss in metals and non-metals (Kong *et al.*, 2018). Various microbial groups with different metabolisms, such as methane production, sulfate reduction, and iron oxidation, have been reported to enhance corrosion in the environment (Sachan and Singh, 2020).

Metal corrosion is influenced by several environmental factors, including humidity, temperature, and the presence of corrosive agents such as chloride ions and sulfur compounds. The microbial communities involved in MIC are known to accelerate corrosion through metabolic activities that generate acidic

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byproducts, biofilms, and direct electron transfer mechanisms. Iron-oxidizing bacteria such as Gallionella and Leptothrix contribute to iron corrosion by oxidizing ferrous ions to ferric ions, forming rust deposits that further deteriorate metal surfaces. Sulfate-reducing bacteria (SRB), including Desulfovibrio species, play a crucial role in the anaerobic corrosion of iron by producing hydrogen sulfide, which reacts with metal surfaces, leading to pitting corrosion and structural damage.

Heavy metals are non-biodegradable and are recognized as significant environmental contaminants due to their potential toxicity. Some metals, such as Nickel, Iron, Copper, and Zinc, play essential roles in metabolic reactions and are required as trace elements by organisms. However, other metals like Mercury, Silver, and Cadmium have no biological function in organisms and are toxic even at low concentrations (Nwagwu *et al*., 2017).

Scrap metal dumping has had a significant impact on microbial biota, vegetation, and human health due to the toxic effects of heavy metals found in the scraps. Scrap metals are valuable substances often collected by informal urban street collectors and sold to recycling companies. However, scrap metal dump sites, such as the Gada Biyu site in Gusau Metropolis, serve as major contributors to heavy metal contamination, impacting microbial diversity and the surrounding environment (Haladu *et al*., 2024). Elevated concentrations of heavy metals have been documented in such sites across various regions. The accumulation of heavy metals in these environments exacerbates microbial corrosion, further intensifying the degradation of metallic materials and leading to structural failures and environmental hazards.

Metal rusting, also referred to as corrosion, is the degradation of material properties, especially metals, due to chemical or electrochemical reactions in the environment (Zhenglin, 2020). It's a process that involves the reaction between iron or steel, atmospheric oxygen, and moisture, which leads to the formation of iron oxide (rust). Bacteria play a significant role in the formation and progression of metal rusting (Yang *et al*., 2020). Microbially induced corrosion (MIC) refers to the process of corrosion influenced by the presence of activity (or both) of microorganisms residing in biofilms on the surface of the corroded material (Mand *et al*., 2019). Microbial corrosion has been documented as a serious issue responsible for huge economic losses in various industries (Xiaowie *et al*., 2018). Approximately 20% of annual loss is contributed by microbially influenced corrosion (MIC) of metals and non-metals (Kong *et al*., 2018). A number of microbes of different groups with different metabolisms such as methane production, sulfate reduction, and iron oxidation have been reported to impart MIC that leads to enhanced corrosion in the environment (Sachan and Singh, 2020).

The study aimed to determine the total mesophilic count, isolate and identify bacterial species present in the contaminated soils, quantify the concentration of selected heavy metals.

MATERIALS AND METHOD

Study Area

This study was carried out in Gusau, the capital of Zamfara State in Northwestern Nigeria. The area is known to face significant environmental challenges, including pollution from industrial and anthropogenic sources. The Gada Biyu scrap metal dump site, which is a major contributor to local environmental degradation, served as the sampling location for this research.

Sample Collection

Soil samples were collected from three designated points (labeled A, B, and C) within the scrap metal dump site in Gada Biyu, Gusau Metropolis. Each sampling point included surface and sub-surface soil (up to a depth of approximately 20 cm), collected using a sterile soil auger. Three random spots were selected at each point to enhance representativeness. The samples were sieved, homogenized, and packed into clean, labeled polyethylene bags. They were preserved at 4°C and immediately transported to the Postgraduate Microbiology Laboratory, Federal University Gusau, for microbiological and physicochemical analysis.

Isolation and Morphological Characterization of Bacteria

Serial Dilution and Plating

The soil samples were serially diluted up to 10^{-6} dilution. From each dilution, 0.1 ml was aseptically inoculated onto nutrient agar plates using the spread plate technique. Plates were incubated at 37°C for 24 hours.

Mesophilic bacterial Count and Subculturing

After incubation, the number of bacterial colonies on each plate was counted. Colonies with distinctive appearances were sub-cultured into nutrient broth and re-streaked on fresh nutrient agar to obtain pure isolates.

Morphological Observation

Colony morphology including growth, elevation, shape, color, size, and margin were recorded. The colonial characteristics were used as preliminary indicators for bacterial classification (Neha *et al*., 2015).

Biochemical Identification of Bacterial Isolates

The bacterial isolates were subjected to series of biochemical tests including Gram staining, motility test, citrate utilization test, indole test, methyl red and Voges-Proskauer (MR-VP) tests, (Cowan and Steel, 2003) catalase test, coagulase test and urease test (Rainey *et al*., 2019) according to standard protocols described in Bergey's Manual of Systematic Bacteriology (Issazadeh *et al*., 2013).

Heavy Metal Analysis of Soil Samples

Sample Digestion

Approximately 2 g of air-dried, sieved soil sample was weighed and transferred into a digestion tube. Five ml of a digestion mixture (sulfuric acid and catalyst) was added. The mixture was digested at 360°C for about 2 hours until the solution became clear. The digest was cooled, diluted with 25 ml of distilled water, shaken, and made up to 50 ml. It was allowed to settle, and the clear supernatant was decanted for metal analysis. The digested solution was filtered into a 100 ml volumetric flask and made up to the mark with distilled water for quantitative analysis (USEPA, 2018).

Metal Content Determination

The concentrations of heavy metals Zinc (Zn), Cadmium (Cd), Copper (Cu), Lead (Pb), and Chromium (Cr) were determined using Atomic Absorption Spectrophotometry (AAS) as described by Li *et al*., (2012). Three samples (A, B, and C) corresponding to different dump zones were analyzed to determine metal pollution gradients within the site.

RESULTS

Total Mesophilic Bacterial Count

The result for colony morphology of bacterial isolates obtained from the scrap metal dump site soil is presented in Table 1. It revealed a wide diversity in colony appearance, size, shape, and margins. All samples showed visible colony growth on nutrient agar, indicating the presence of viable bacteria. The morphology ranged from filamentous, punctiform, circular to irregular shapes. Most colonies appeared cream-colored, though some variations in white coloration were observed. The size ranged from very small to very large, and the margin characteristics included filamentous, undulate, lobate, and entire, reflecting heterogeneity in the bacterial population.

Table 1: Total Mesophilic Bacterial Count Obtained of the Scrap Metal Dump Site Soil

Sample Code	No. of colonies	Total Bacterial Count (CFU/g)
A1	208	2.08×10^7
A2	120	1.2×10^7
A3	144	1.44×10^7
A4	8	0.8×10^6
A5	248	2.48×10^7
B1	246	2.46×10^7
B2	242	2.42×10^6
B3	18	1.8×10^6
B4	55	5.5×10^6
B5	72	7.2×10^6
C1	32	3.2×10^6
C2	184	1.84×10^7
C3	184	1.84×10^7
C4	220	2.20×10^7

Biochemical Characteristics and Identification of Bacterial Isolates

A series of biochemical tests were conducted to identify the bacterial species isolated from the dump site samples. The results are presented in the Table 2. The tests include Gram reaction (GR), motility (MT), and metabolic tests such as indole (I), methyl red (MR), Voges-Proskauer (VP), citrate (CT), urease (UR), sugar fermentation (S, L, GL), hydrogen sulfide production (H_2S), and gas production (GS). Bacteria were identified based on their test reactions using standard bacteriological identification keys.

Table 2: Biochemical Characteristics of Bacterial Isolates Obtained from the Scrap Metal Dump Site Soil

Samples	GR	MT	I	MR	VP	CT	UR	S	L	GL	H_2S	GS	Isolates
1	-	-	-	+	-	-	+	-	+	+	-	-	*Escherichia coli*
2	-	-	-	-	+	-	+	-	+	+	-	+	*Klebsiella pneumoniae*
3	-	-	-	-	+	+	+	-	+	+	+	-	*Enterobacter aerogenes*
4	-	-	-	-	+	+	+	-	+	+	-	-	*Enterobacter spp*
5	-	-	-	-	+	+	-	+	+	-	-	-	*Pseudomonas spp*
6	-	-	+	-	+	+	+	-	-	+	-	-	*Proteus spp*
7	-	-	-	+	-	+	+	-	+	+	-	-	*Salmonella spp*
8	-	-	+	-	+	+	+	-	+	+	-	-	*Shigella spp.*
9	-	+	-	-	+	+	-	-	-	+	-	-	*Citrobacter spp*
10	-	-	+	-	+	+	+	-	-	+	-	-	*Serratia spp*
11	-	-	-	-	+	+	+	-	+	+	-	-	*Bacillus subtilis*

Samples	GR	MT	I	MR	VP	CT	UR	S	L	GL	H_2S	GS	Isolates
12	-	+	-	-	+	+	+	-	+	+	-	-	*Micrococcus spp*
13	-	-	-	-	+	+	+	-	+	+	-	-	*Acinetobacter spp.*
14	-	-	-	-	+	+	+	-	+	+	-	-	*Staphylococcus aureus*
15	-	-	-	-	+	+	+	-	-	+	+	-	*Staphylococcus epidermidis*

Key: + = Positive; – = Negative; GR = Gram Reaction, MT = Motility, I = Indole, MR = Methyl Red, VP = Voges-Proskauer, CT = Citrate, UR = Urease, S = Sucrose, L = Lactose, GL = Glucose, H_2S = Hydrogen Sulfide, GS = Gas.

Concentration of Heavy Metals of Metal Scrap Dump Site Soil

The concentrations of heavy metals including zinc (Zn), cadmium (Cd), copper (Cu), lead (Pb), and chromium (Cr) were measured in soil samples from the dump site and the result is presented as Table 3. Sample A contains relatively low concentrations of all metals and represents the least contaminated site. Sample B exhibits moderate contamination, particularly with elevated lead and chromium levels. Sample C shows the highest levels of heavy metal contamination, especially zinc, cadmium, and lead, indicating a severe environmental impact likely due to prolonged accumulation from the dump site. The elevated levels in Sample C suggest a strong potential for microbial adaptation and/or resistance to heavy metals in this zone.

Table 3: Concentration of Heavy Metals of Metal Scrap Dump Site Soil

Sample	Zinc (ml/kg)	Cadmium (ml/kg)	Copper (ml/kg)	Lead (ml/kg)	Chromium (ml/kg)
A	0.18±0.02	0.06±0.02	0.17±0.01	0.01±0.001	0.01±0.001
B	0.16±0.03	0.07±0.01	0.18±0.02	0.16±0.03	0.18±0.05
C	0.34±0.04	0.20±0.05	0.29±0.06	0.34±0.03	0.16±0.07

DISCUSSION

The highest Mesophilic bacterial count ($5.5x10^6$) was observed in samples from the most heavily polluted areas (B4) while the least ($1.2x10^7$) was obtained from A2. The findings from this study revealed significant bacterial diversity and heavy metal contamination in soil samples collected from the Gada Biyu scrap metal dump site in Gusau Metropolis. These results align with the growing concern that improperly managed metal waste sites contribute to both microbial adaptation and environmental degradation (Alloway, 2019). The colony morphological characteristics observed across the fifteen samples demonstrated active bacterial proliferation in all soil samples analyzed. High colony counts such as those seen in Samples 4 (248 colonies), 3 (246 colonies), and 7 (242 colonies) suggest an abundant bacterial population in those zones, which may be influenced by nutrient availability and microbial adaptability to metal-rich environments. Variations in colony shapes including filamentous, punctiform, circular, and irregular along with differences in colony margins (e.g., entire, undulate, lobate) and size (very small to very large), reflect microbial heterogeneity and possibly the existence of different bacterial species cohabiting the dump site (Prescott *et al*., 2005).

The dominance of cream-colored colonies is consistent with previous studies indicating that many environmental soil bacteria, particularly Gram-negative enteric and Gram-positive bacilli, tend to form off-white to cream colonies under aerobic conditions (Neha *et al*., 2015). The flat colony elevation observed across all samples suggests non-mucoid, sediment-based bacterial growth typical of metal-exposed environments (Cowan and Steel, 2004).

Biochemical testing allowed the confirmation and tentative identification of fifteen bacterial isolates. These included members of *Enterobacteriaceae, Pseudomonadaceae, Bacillaceae*, and *Staphylococcaceae* families. Specific isolates such as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter aerogenes*, and *Pseudomonas aeruginosa* were identified based on distinct patterns in IMViC tests, sugar utilization, urease activity, H_2S production, and motility (Cheesbrough, 2006).

The presence of *Escherichia coli* and *Klebsiella pneumoniae* indicates fecal contamination, possibly from human or animal waste runoff into the dump site. These bacteria are known opportunistic pathogens, and their presence in soil underscores the potential health risks to humans in the vicinity (Atlas and Bartha, 1998). The identification of *Pseudomonas aeruginosa* and *Proteus mirabilis* highlights the environmental adaptability of these species. *Pseudomonas* species are well-documented for their metal resistance and bioremediation potential. Their ability to thrive in metal-rich environments may be due to efflux systems, metal chelation, or biofilm formation which reduces metal toxicity (Gadd, 2010). Notably, the identification of *Bacillus subtilis* and *Micrococcus luteus* suggests the coexistence of Gram-positive spore-formers and environmental saprophytes. These organisms are known for their metabolic versatility and role in nutrient cycling, even under extreme environmental pressures (Madigan *et al*., 2018).

The analysis of heavy metal concentrations across Samples A, B, and C revealed varying degrees of contamination. Sample A had the lowest levels of heavy metals, with zinc, cadmium, and copper recorded at 0.18 ± 0.02 ml/kg, 0.06 ± 0.02 ml/kg, and 0.17 ± 0.01 ml/kg respectively. Lead and chromium levels in this sample were minimal (0.01 ± 0.001 ml/kg each), indicating relatively lower contamination at this site. Sample B showed moderate contamination, with slightly higher lead (0.16 ± 0.03 ml/kg) and chromium (0.18 ± 0.05 ml/kg) levels. Sample C, however, exhibited the highest concentrations of all heavy metals tested, particularly zinc (0.34 ± 0.04 ml/kg), cadmium (0.20 ± 0.05 ml/kg), copper (0.29 ± 0.06 ml/kg), and lead (0.34 ± 0.03 ml/kg). These findings suggest prolonged and intense deposition of metal-containing waste at Sample C, making it the most polluted zone of the scrap metal site (Adebayo *et al*., 2021). Elevated levels of cadmium and lead are of particular concern due to their non-biodegradable nature and high toxicity even at low concentrations. These metals can impair microbial functions, reduce biodiversity, and enter the food chain through plants or water contamination (Alloway, 2019; Li *et al*., 2012). Despite high concentrations of heavy metals especially in Sample C a diverse range of bacteria was still isolated. This indicates the presence of metal-tolerant or metal-resistant bacteria capable of surviving and proliferating under toxic conditions. The survival of such organisms can be attributed to their ability to detoxify or sequester metals, form protective biofilms, or express metal-specific resistance genes (Giller *et al*., 2009). This finding supports previous studies which demonstrated that bacteria isolated from contaminated sites often possess adaptive traits such as plasmid-encoded resistance mechanisms (Nies, 2003).

CONCLUSION

The highest Mesophilic bacterial count ($5.5x10^6$) was observed in samples from the most heavily polluted areas (B4) while the least ($1.2x10^7$) was obtained from A2. The results confirmed the presence of diverse bacterial species, with notable isolates including *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Micrococcus luteus*, and *Proteus mirabilis*. Furthermore, heavy metal analysis revealed variable contamination levels across the sampling sites. Sample C exhibited the highest concentrations of all tested heavy metals Zinc (0.34±0.04) Cadmium (0.20±0.05), Copper (0.29±0.06), Lead (0.34±0.03) and Chromium (0.16±0.07) while Sample A had the lowest levels Zinc (0.18±0.02), Cadmium (0.06±0.02), Copper (0.17±0.01), Lead (0.01±0.001) and Chromium (0.01±0.001). The elevated levels of cadmium, lead, and copper, particularly in Sample C, pose significant ecological and health risks, given their toxicity and non-biodegradable nature.

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