

Assessment of *Pseudomonas aeruginosa* Presence in Metal-Rust-Associated Soil from Scrap Dump Sites in Damba, Gusau, Zamfara State

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ABSTRACT

Metal corrosion, commonly referred to as rusting, poses significant public health risks by contaminating soil and drinking water systems, particularly along metallic pipelines. Previous studies have identified *Pseudomonas aeruginosa* as a major contributor to metal deterioration. This study aimed to detect and characterize *P. aeruginosa* in soil samples collected from a scrap metal dump site in Damba, Gusau, Nigeria. A comprehensive analysis was conducted to evaluate microbial diversity, heavy metal concentrations, and physicochemical parameters such as soil pH and temperature. Bacterial colony enumeration was performed using MacConkey agar, followed by Gram staining, biochemical testing, and molecular characterization for the identification of *Pseudomonas* species. Heavy metal concentrations were determined using Atomic Absorption Spectrophotometry (AAS). The colony-forming units ranged from 8.3×10^7 to 1.78×10^4 CFU/g. A total of 25 *Pseudomonas* spp. were identified biochemically, of which 5 isolates were further confirmed as *P. aeruginosa* through molecular techniques. Zinc, cadmium, copper, lead, and chromium were detected in all soil samples, with zinc showing the highest concentration (0.34 ± 0.04 mg/kg) and cadmium the lowest (0.01 ± 0.001 mg/kg). The results indicate that *P. aeruginosa* plays a significant role in metal corrosion processes. Although heavy metal levels were within acceptable limits of United State Environmental Protection Agency (USEPA), continuous monitoring and implementation of bioremediation strategies using metal-resistant bacteria are recommended to mitigate environmental and public health risks associated with scrap metal dump sites.

Keywords: *Pseudomonas aeruginosa*, metal corrosion, occurrence

1.0 INTRODUCTION

Corrosion is a natural process in which metals and their alloys undergo degradation, reverting to a more stable thermodynamic state through a series of chemical reactions or interactions with environmental elements (Li *et al.*, 2021). It is a global issue affecting critical sectors such as construction, oil refining, maritime operations, sewage, and water systems. The corrosion of materials like iron, steel, concrete, and stone compromises infrastructure and poses economic challenges. A specific type of corrosion, known as microbiologically influenced corrosion (MIC), is driven or exacerbated by microorganisms, often present as biofilms (Lou *et al.*, 2021). These microbes not only accelerate corrosion but, in some cases, inhibit it, underscoring the dual role of microbial activity in corrosion dynamics (Odeyemi and Alaba, 2024). The microbial influence on corrosion is primarily biochemical. Microorganisms can stimulate anodic or cathodic reactions through enzymatic activity, metabolite secretion, or hydrogen consumption (Victoria, 2021). In environments rich in heavy metals, microbial community structure and function are significantly affected. Heavy metals can impair microbial metabolism, alter enzyme specificity, damage cell membranes, and denature proteins and DNA (Renu *et al.*, 2021). Despite

this, certain microorganisms adapt to harsh, metal-laden environments through mechanisms such as metal sorption, intracellular accumulation, enzymatic reduction, and efflux systems (Kalaimurugan *et al.*, 2020; Pal *et al.*, 2022). Microorganisms are ubiquitous and possess remarkable metabolic flexibility, allowing them to survive and thrive in extreme conditions (Wu *et al.*, 2021). In heavy metal-polluted environments. Some bacteria exhibit tolerance by deploying efflux pumps, enzymatic detoxification, plasmid-mediated resistance, and other protective mechanisms (Mathivanan *et al.*, 2021). These adaptive traits make such bacteria excellent candidates for biotechnological applications, including bioremediation of heavy metal contaminated ecosystems (Biswas *et al.*, 2021). Given the growing threat of environmental pollution from industrial waste, scrap metals, and leachates, it is imperative to investigate these contaminated zones for resilient microbial strains with potential environmental applications.

The increasing contamination of soil by heavy metals has become a major concern, particularly due to its long-term ecological and health impacts. These pollutants, often stemming from industrial activities, scrap metal dumps, mining, and agriculture, contaminate soil and water,

affecting humans, animals, and plants (Masindi *et al.*, 2018). In Nigeria, the lack of accessible screening and treatment programs exacerbates this issue, leading to reduced agricultural productivity and compromised public health (Hossain *et al.*, 2020). Additionally, heavy metal toxicity disrupts microbial diversity and metabolic functions, weakening the natural resilience of the ecosystem (Shuaib *et al.*, 2021). The problem is further magnified in areas where scrap metal sites are situated near farmlands and water sources, threatening food and water safety. Despite increased awareness, most research on heavy metal pollution has focused on identifying the contaminants rather than exploring their biological impact or microbial responses. A critical gap exists in understanding how certain microorganisms, such as *Pseudomonas aeruginosa*, survive and potentially contribute to corrosion in such settings. Addressing this gap could reveal new insights into MIC and promote innovative bioremediation approaches. Given its adaptive capabilities, *P. aeruginosa* is a relevant model organism for studying microbial survival, resistance mechanisms, and interactions with heavy metals in contaminated environments.

1.1 Aim of the Study

The aim of the study was to assess the occurrence of *Pseudomonas aeruginosa* in metal-rust-associated soil, in Damba Area, Gusau metropolis.

1.2 Objectives

- i. To isolate *Pseudomonas aeruginosa* associated with metal rusting from the scrap metal dumping site.
- ii. To evaluate the concentration of heavy metals present in the scrap metal dumping site.
- iii. To genetically characterize *Pseudomonas aeruginosa* isolated from the scrap metal dump site using conventional PCR and Sanger sequencing methods.

2.0 MATERIALS AND METHODS

2.1 Study area

Damba a community in Gusau Local Government Area of Zamfara State of Nigeria. The Local Government Area is the State Capital of Zamfara State. It is situated at 12.1628° North latitude, 6.6745° East longitude. The capital city of Zamfara is Gusau, and its inhabitants are mainly Hausa and Fulani, with a population of over 5,833,494. Fig 3.1 the map of the study area Gusau, Zamfara State (Aliyu *et al.*, 2024).

2.2 Sample Collection

Samples were obtained from rust-associated soil found within local scrap yard environments. Three sampling points A (Bakin Kasuwa), B (Sabon Gari), and C (Bakin Tasha)—were selected. At each point, soil samples were

collected from the surface and approximately 20 cm depth using a soil auger, with three random locations sampled per site. Approximately 10 g of soil was collected per location and transferred into sterile polyethylene bags, stored in an icebox, and transported immediately to the laboratory for further analyses.

2.3 Isolation and Identification of Bacteria

2.3.1 Isolation of Bacteria

Ten grams of each soil sample were serially diluted from 10^{-1} to 10^{-6} dilutions. 10^{-2} and 10^{-6} dilutions were used for inoculation on MacConkey Agar. Using the spread plate method, 0.1 mL of diluted sample was plated and incubated at 37°C for 24 hours. Distinct colonies were subcultured on Nutrient Agar to obtain pure cultures (Chakma, 2022).

2.3.2 Bacterial Identification

Isolates were identified through Gram staining and biochemical characterization, following Bergey's Manual of Systematic Bacteriology as described by Issazadeh *et al.* (2013). Biochemical tests included IMViC, sugar fermentation, H₂S production, urease, catalase, and oxidase assays.

2.3.3 Gram Staining

Gram staining was performed on 24-hour cultures by heat-fixing smears, staining with crystal violet, iodine, and safranin, and decolorizing with 95% ethanol. Microscopic observation under oil immersion (100x) was used to differentiate Gram-positive (purple) and Gram-negative (pink) bacteria (Cowan *et al.*, 2003).

2.3.4 Motility Test

Motility medium was stabbed with isolates using a sterile wire loop and incubated at 35°C for 24 hours. Diffused cloudiness indicated motility, while growth restricted to the stab line indicated non-motile organisms (Cowan *et al.*, 2003).

2.3.5 Citrate Utilization Test

Isolates were inoculated on Simmons' citrate agar and incubated at 35°C for 48–72 hours. A blue color change indicated a positive result (Cowan *et al.*, 2003).

2.3.6 Indole Test

Cultures in 1% peptone water were incubated at 37°C for 24 hours. Addition of Kovac's reagent resulted in red coloration for indole-positive isolates (Cowan *et al.*, 2003).

2.3.7 Methyl Red and Voges-Proskauer (MR-VP) Tests

After incubation of isolates in MR-VP broth at 35°C for 48–72 hours, methyl red was used to detect acid

production (red = positive), and α -naphthol and KOH were added to detect acetoin production for VP (red = positive) (Cowan *et al.*, 2003).

2.3.8 Oxidase Test

Filter paper moistened with oxidase reagent was used to test colonies. Development of blue-purple coloration within 10 seconds was considered positive (Cowan *et al.*, 2003).

2.3.9 Catalase Test

A drop of 3% hydrogen peroxide was placed on a slide and mixed with an isolate. Immediate bubbling indicated catalase production (Barboza and Dias, 2024).

2.3.10 Coagulase Test

Colonies were emulsified with 10 μ L citrated human plasma on a slide. Agglutination within 10 seconds was interpreted as coagulase-positive (Barboza and Dias, 2024).

2.3.11 Urease Test

Isolates were inoculated in urease medium containing 40% urea solution. A color change from yellow to pink-red indicated a positive result (Rainey, 2019).

3.1 Heavy Metal Content Analysis

3.2.1 Sample Digestion

Two grams of dried soil sample were digested with 5 mL of H_2SO_4 -based digestion mixture in a digestion tube at 360°C for 2 hours until the solution became colorless. After cooling, 25 mL distilled water was added, shaken, and made up to 50 mL volume. The clear solution was decanted for elemental analysis (Li *et al.*, 2017).

3.2.2 Atomic Absorption Spectrometry (AAS)

Heavy metals (Zn, Cd, Cu, Pb, Cr) were quantified using PerkinElmer PinAAcle 900H AAS, following standard AAS protocols. The spectrometer (Brewer 171) was pre-heated for 20 minutes, and acetylene flame was ignited for 10 minutes for burner stabilization. Each element was measured using its respective wavelength, with distilled water used as a blank. Data were statistically analyzed using Relative Standard Deviation (RSD%) to compare metal concentrations across sites (Li *et al.*, 1997).

3.4 DNA Extraction and PCR Amplification

3.4.1 DNA Extraction

DNA from bacterial isolates was extracted using the Zymo Research ZR BashingBead™ Kit. About 50–100 mg of cells were lysed in 750 μ L lysis solution and bead-beaten. Supernatants were filtered through Zymo-Spin™ IV filters, followed by DNA binding with DNA buffer, washing, and elution with 100 μ L DNA elution buffer.

DNA was stored for downstream applications (Nwachukwu *et al.*, 2024).

3.4.2 PCR Amplification

PCR amplification was performed using universal bacterial primers:

- Forward (27F): AGAGTTTGATCCTGGCTCAG
- Reverse (1429R): GGTTACCTTGTTACGACTT

The 25 μ L reaction mixture contained: 2.5 μ L of 10x buffer, 1 μ L of $MgCl_2$ (25 mM), 1 μ L each of primers, 2 μ L of dNTP mix (2.5 mM), 0.1 μ L Taq polymerase, 1 μ L DMSO, 3 μ L of DNA template, and 13.4 μ L of nuclease-free water. Cycling conditions were: initial denaturation (94°C, 5 min), 36 cycles of denaturation (94°C, 30 sec), annealing (56°C, 30 sec), extension (72°C, 45 sec), and a final extension (72°C, 7 min). PCR products (~1500 bp) were visualized on 1.5% agarose gel stained with ethidium bromide using a 1 kb DNA ladder as marker (Hosseinzadeh and Sorkhou, 2024).

3.4.3 Sequencing

PCR products were purified via ethanol/EDTA precipitation, and sequencing reactions were conducted using standard Sanger sequencing protocols on a 96-well plate.

3.5 Data Analysis

Data obtained in this study were analyzed using IBM SPSS Statistics version 22. Descriptive statistics such as means and standard deviation were used to present results.

3.0 RESULTS AND DISCUSSION

3.1 Total Colony Count

The bacterial load varied significantly across soil samples and dilution levels. In Sample A, the bacterial concentration at the 10^{-2} dilution was too numerous to count (TNTC), indicating an extremely dense microbial presence. Even at higher dilutions, the microbial load remained substantial, with counts of 17×10^6 CFU/ml at 10^{-4} and 6.0×10^8 CFU/ml at 10^{-6} . In Sample B, the bacterial load was comparatively lower, recording 26×10^4 CFU/ml at 10^{-2} , 14.5×10^5 CFU/ml at 10^{-4} , and 8.3×10^7 CFU/ml at 10^{-6} . Sample C showed the lowest bacterial count among the three, with 17.8×10^3 CFU/ml at 10^{-2} , 9.8×10^5 CFU/ml at 10^{-4} , and 4.2×10^7 CFU/ml at 10^{-6} . These results suggest that Sample A had the highest microbial activity, followed by Sample B and Sample C.

Table 1: Total Bacterial Mesophilic Count Obtained from the Sample Site (cfu/ml)

Samples	CFU/ml (10 ⁻²)	CFU/ml (10 ⁻⁴)	CFU/ml (10 ⁻⁶)
A	TNTC	17 × 10 ⁶	6.0 × 10 ⁸
B	26 × 10 ⁴	14.5 × 10 ⁵	8.3 × 10 ⁷
C	17.8 × 10 ³	9.8 × 10 ⁵	4.2 × 10 ⁷

Keys: A = Bakin Kasuwa, B = Sabon Gari, C = Bakin Tasha; TNTC = Too Numerous to Count

3.2 Biochemical Identification of Bacterial Isolates

Biochemical characterization identified *Pseudomonas aeruginosa* in samples A1, B3, and C1. In all cases, the isolates were Gram-negative rods and tested positive for motility, citrate utilization, oxidase, and catalase. They tested negative for indole, methyl red, coagulase, and urease. The uniform biochemical responses across different samples affirm the presence of *P. aeruginosa* in all the three sampling locations sampled (Bakin kasuwa, Sabon gari and Bakin tasha).

Table .2: Biochemical Identification of Bacterial Isolates Obtained from the Samples

Sampl e	Organism	Gra									
		m Stai n	Mo t	Ci t	In d	M R	Ox i	Ca t	Co a	Ur e	U r
A1	<i>P. aeruginosa</i> - rod	+	+	-	-	+	+	-	-	-	-
B3	<i>P. aeruginosa</i> - rod	+	+	-	-	+	+	-	-	-	-
C1	<i>P. aeruginosa</i> - rod	+	+	-	-	+	+	-	-	-	-

Keys: A = Bakin Kasuwa, B = Sabon Gari, C = Bakin Tasha, + = Positive, - = Negative; Mot = Motility, Cit = Citrate, Ind = Indole, MR = Methyl Red, Oxi = Oxidase, Cat = Catalase, Coa = Coagulase, Ura = Urease

3.3 Number of Bacterial Isolates

The total number of bacterial isolates obtained from the three samples was 25. Sample A yielded 7 isolates, Sample B had 8, and Sample C recorded the highest with 10 isolates. The prevalence of *Pseudomonas aeruginosa* in all samples suggests its wide distribution across the dump site. The higher number of isolates in Sample C may indicate more favorable environmental conditions for bacterial proliferation.

The presence of *P. aeruginosa* in all the samples might be as a result of it been able to survive in harsh conditions, it develop resistance to heavy metals and antibiotics which

can be genetically transferred and it also contribute to mobilization or immobilization of metals through redox process or biosorption. Additionally, it could be sequel to the presence of other contaminants (like hydrocarbons-e.g., lubricants, and organic pollutants or antibiotics) not captured by heavy metal analysis.

As a means of control, Ibrahim *et al.* (2014) reported Bacillus, Pseudomonas, Staphylococcus, Micrococcus, Klebsiella, Escherichia as the predominant bacteria prevailing in normal soils of Zamfara. *P. aeruginosa* might have dominated the sampling sites assayed because of it's metal tolerance, environmental stress adaptation and pollution selection pressure.

Table 3: Number of Bacteria Isolated from the Soil Samples of the Metal Scrap Sites

Sample	Number of Isolates	Organism Isolated
A	7	<i>P. aeruginosa</i>
B	8	<i>P. aeruginosa</i>
C	10	<i>P. aeruginosa</i>
Total	25	

Keys: A = Bakin Kauwa, B = Sabon Gari, C = Bakin Tasha

3.4 pH and Temperature of Soil Samples

Environmental conditions such as pH and temperature can significantly influence microbial activity. Sample A had an acidic pH of 5.04 and a temperature of 36.1°C, conditions conducive to acidophilic microbial growth. Sample B showed a pH of 5.84 and a slightly higher temperature of 37.34°C, while Sample C had the most neutral pH (6.02) and the highest temperature (38.67°C). The relatively neutral pH in Sample C may reduce acid-driven corrosion, despite high microbial activity supported by elevated temperatures.

Table 4: pH and Temperature of Soil Samples of the Metal Scrap Sites

Sample	pH	Temperature (°C)
A	5.04	36.1
B	5.84	37.34
C	6.02	38.67
Mean (X)	5.63	37.37
SD	±0.522	±1.29
CV%	9.27%	34.44%

Keys: SD = Standard Deviation, CV% = Coefficient of Variation

3.5 Heavy Metal Concentrations in Soil Samples

Heavy metal analysis revealed varying levels of contamination across the samples. Sample A had the lowest concentrations of all metals, including Zn (0.18 ml/kg), Cd (0.06 ml/kg), Cu (0.17 ml/kg), Pb (0.01 ml/kg), and Cr (0.01 ml/kg). Sample B showed moderate levels, particularly in Pb (0.16 ml/kg) and Cr (0.18 ml/kg). Sample C recorded the highest levels of all tested metals, with Zn (0.34 ml/kg), Cd (0.20 ml/kg), Cu (0.29 ml/kg), Pb (0.34 ml/kg), and Cr (0.16 ml/kg). This suggests that Sample C is the most heavily contaminated and may pose the highest ecological and corrosion-related risk.

Overall, for all the samples assayed and from the environmental and public health perspectives, the limits of heavy metals obtained could mean that there is a previous exposure or a biological warning sign due to soil's history or surrounding activities indicating the vulnerability of the soil. However, the *P. aeruginosa* recovered from the samples might be as a result of transfer to water systems via runoffs of agricultural, recreational or construction sites especially where humans and animals and/or their excreta interact with soil.

Table 5: Concentration of Heavy Metals in Soil Samples of the Metal Scrap Sites

Sample	Zn (ml/kg)	Cd (ml/kg)	Cu (ml/kg)	Pb (ml/kg)	Cr (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

Keys: A = *Bakin Kauwa*, B = *Sabon Gari*, C = *Bakin Tasha*

3.6 Molecular Identification

Molecular analysis was performed on five samples using 16S rRNA gene amplification. The primers 27F and 1429R successfully amplified the DNA of *P. aeruginosa* in all samples. Electrophoresis confirmed amplification with clear DNA bands (Figure 4.1). Phylogenetic analysis using the Neighbor-Joining method yielded a tree with a total branch length of 1.53118071, and evolutionary distances were calculated using the Maximum Composite Likelihood method. The analysis, conducted in MEGA6 software, involved 21 nucleotide sequences with 1274 positions, providing insights into the genetic relatedness of the isolates.

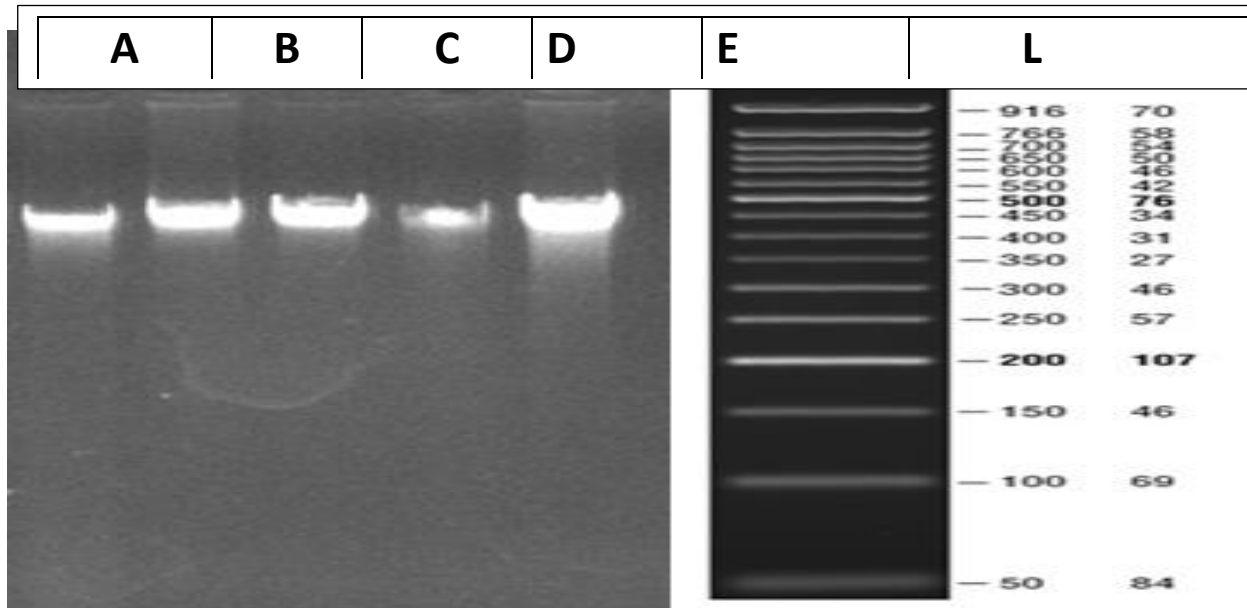


Figure 1: The image of electrophoresis showing the bands and ladder of the DNA that was amplified to be sample A – 450bp, B – E 500bp as expected.

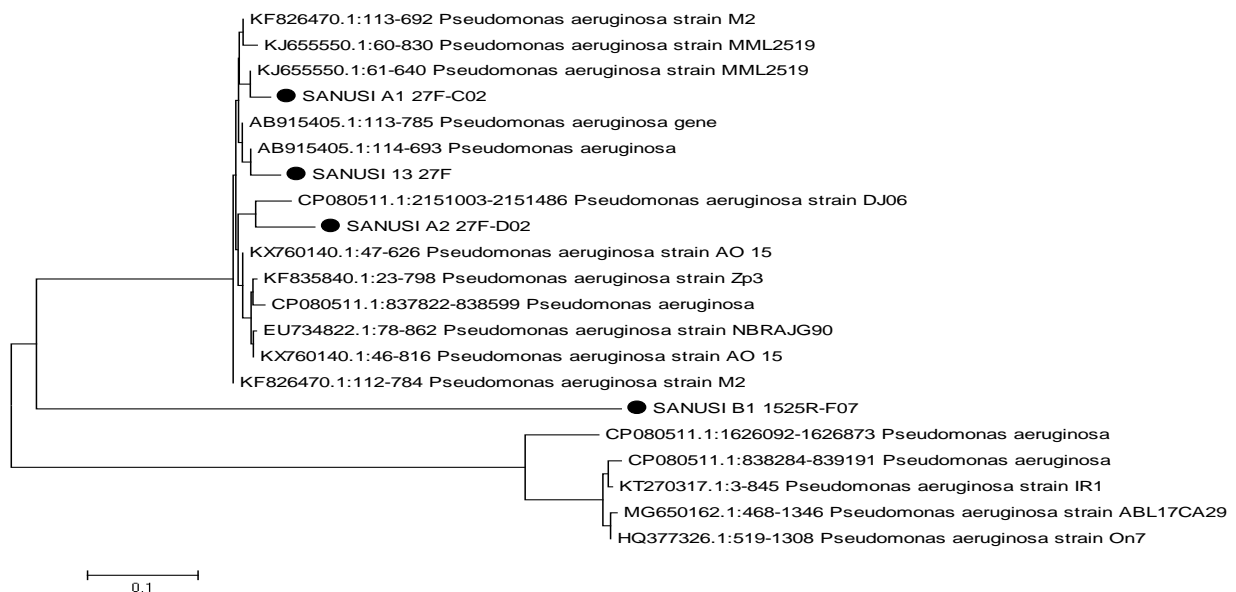


Figure 2: Evolutionary relationships of taxa obtained from the Metal Scrap Sites

The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 1.53118071 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analysis involved 21 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There were a total of 1274 positions in the final dataset. Evolutionary

analyses were conducted in MEGA6 (Saitou N. and Nei M. (1987; Tamura *et al.*, 2013).

4.0 Conclusion

This study successfully isolated *Pseudomonas aeruginosa* from soil at the scrap metal dump site, confirming its association with metal rusting. High concentrations of heavy metals were observed, influencing microbial activity and corrosion potential. Genetic characterization using PCR and Sanger sequencing validated the identity of *P. aeruginosa*. The findings highlight the bacterium's adaptability, biofilm formation, and role in Microbially Induced Corrosion



(MIC). Environmental factors such as pH, temperature, and metal concentration significantly impacted microbial diversity. Monitoring these variables is crucial for mitigating MIC and environmental hazards..

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