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Isolation, identification, and characterization of heavy metal-resistant bacteria from soil samples collected at a cement company in Nigeria

AYANTSE LUBEM MARTINS[1], TATAH VERWIYEH SILAS[1,2], MOSES ADONDUA ABAH[2,♥], AROWORA KAYODE ADEBISI[2], ABU MICHAEL SUNDAY[2], ROY YOHANNA EMOCHONNE[2], CHINEDU CHRISTIAN IHEANACHO[3]

[1]Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria. Nsukka, P.M.B 923, Enugu State, Nigeria
[2]Department of Biochemistry, Faculty of Pure and Applied Sciences, Federal University Wukari. PMB 1020, Taraba State, Nigeria.
Tel.: +23-480-000-0000, ♥email: M.abah@fuwukari.edu.ng
[3]Department of Microbiology, Faculty of Pure and Applied Sciences, Federal University Wukari. PMB 1020, Taraba State, Nigeria

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Abstract. *Martins AL, Silas TV, Abah MA, Adebisi AK, Sunday AM, Emochonne RY, Iheanacho CC. 2024. Isolation, identification, and characterization of heavy metal-resistant bacteria from soil samples collected at a cement company in Nigeria. Asian J Trop Biotechnol 21: 26-32.* Many heavy metals, including cadmium, chromium, copper, lead, and zinc, are produced during cement-making. Even in low quantities, most of the heavy metals released are known to be harmful to plants and animals. The objective of this study was to isolate, identify and characterize heavy metal-resistant bacteria from soil samples collected at Benue Cement Company, Nigeria. Soil samples taken near the Gboko facility of Nigerian cement manufacturer Benue Cement Plc were tested for heavy metal-resistant bacteria. Results from the study revealed that compared to other metals (Pb, Cr, and Cd), the concentration of copper and zinc was consistently quite high across all sites. The levels of all components examined were determined to be higher than the limits allowed by the World Health Organization In this study, Cd, Zn, Cr, Cu, and Pb were found in soil samples taken from the Benue cement industry, according to the analysis of heavy metals. The results obtained from this study further revealed that out of 20 isolates only five (5) bacterial isolates, namely *Staphylococcus aureus, Escherichia coli, Proteus* sp., *Bacillus cereus,* and *Lactobacillus* which showed high levels of heavy metal resistance were selected for further studies in secondary screening. Based on the biochemical tests, *S. aureus* reacted positively to catalase and coagulase test. They were also seen to ferment lactose, sucrose and glucose. The *E. coli* reacted negatively to citrate, catalase, coagulase tests, and did not ferment sucrose. However, the bacteria fermented lactose and glucose. *Lactobacillus* reacted positively to only citrate but fermented the three sugars. *Proteus* sp. fermented glucose and sucrose and also reacted positively to citrate, catalase and urea tests. The *B. cereus* fermented glucose and sucrose. The bacteria also reacted positively to only citrate and catalase tests. Results of showed that *S. aureus* had MIC values between 12 and 16 mg/L, *E. coli* between 20 and 50 mg/L, *Proteus* species between 15 and 64 mg/L, and *B. cereus* between 10 and 18 mg/L, all against various metals. The *B. cereus* showed the lowest resistance to several heavy metals, while *E. coli* showed the highest resistance. Additionally, *E. coli* demonstrated a significant resistance level to all ten antibiotics examined. Antibiotic resistance was highest in *E. coli* and lowest in *Proteus* species. The findings of this study revealed that the four isolates that showed high tolerance to heavy metals could be used as inoculants to bioremediate cement sites that polluted by heavy metals.

Keywords: Antibiotic resistance, bacteria, cement, heavy metals, soil

INTRODUCTION

In pursuing better living conditions, man has increasingly contributed to environmental challenges through industrialization and urbanization (Adekola et al. 2012). Animals and people alike suffer due to the disruption of nature's natural balance that he brings about through his exploitation of it (Udiba et al. 2012). Like many other developing nations, Nigeria is experiencing rapid urbanization and industrialization. With the increase in manufacturing and other service sectors, the country is realizing the importance of diversifying its economic base to secure its future (Afolabi et al. 2012). Cement manufacturing is a major contributor to the emissions of heavy metals such as Cd, Cr, Cu, Pb, and Zn (Al-Khashman and Shawabkeh 2006; Aman et al. 2008). The wind speed and particle size determine the distance that these heavy metals are carried in the soil by cement dust and stack fumes (Udiba et al. 2012; Tatah et al. 2016). Most of the heavy metals in cement dust come from the ingredients themselves. Approximately half of the total Cd, Cu, and Zn loads in cement are introduced via raw materials, with typical raw materials containing 25 mg/kg of Cr, 21 mg/kg of Cu, 20 mg/kg of Pb, and 53 mg/kg of Zn (Achternbosch et al. 2003). Even at low concentrations, most emitted heavy metals are toxic to plants and humans (Sherene 2010). The health effects of soil and water contamination can vary greatly depending on the type of pollutant, the exposure route, and the susceptibility of the exposed population (e.g., children, adults, or elderly) (Adekola et al. 2012). Anthropogenic heavy metal deposition can occur via several processes, including cement manufacture, although the main sinks for these metals are soil and water (Al-Khashman and Shawabkeh 2016; Tatah et al. 2017; Otitoju et al. 2022). Contamination of soil with heavy metals poses multiple problems due to their inability to break down biologically, a constant source of environmental anxiety to ecological preservation (Emmanuel et al. 2009; Tatah et al. 2020; Otitoju et al. 2022). Animals and humans are at risk when they consume plants, fruits, and vegetables with bioaccumulated harmful metals from polluted soil and water (Turner 2009; Udiba et al. 2012; Abah et al. 2021; Olawale et al. 2023).

In most cases, heavy metals kill microbes by blocking vital functional groups, displacing vital metal ions, or changing the active conformation of biological components. Nevertheless, microbes rely on certain metals at low concentrations as co-factors for enzymes and metalloproteins (Olawale et al. 2023). Microbes that are able to withstand the effects of heavy metals have begun to proliferate in the soil and water of industrialized areas (Emmanuel et al. 2009). The ability to produce new microbial strains with potent heavy metal detoxification activities is often determined by plasmids (Abimbola et al. 2019). Plasmid and transposon genes encode metal resistance in bacteria; this resistance can be transmitted between genera and even between different types of bacteria and in situ and indigenous microflora (Zhema et al. 2022). There is a strong correlation between metal and antibiotic resistance (Ansari and Malik 2007); heavy metal resistance is often far more prevalent than antibiotic resistance. Heavy metal-polluted settings often have bacteria resistant to antibiotics and heavy metals, as studies have shown that these genes tend to be located on the same plasmid (Malik and Jaiswal 2000). The organic compounds included in heavy metal contaminants can be broken down by bacteria and other microbes employed in bioremediation, leading to the harmless release of carbon dioxide and water (Rashed 2010). Therefore, bacteria have evolved multiple coping methods to withstand heavy metal uptake in settings polluted with metals. Consequently, these organisms can be bio-indicators to detect environmental heavy metal pollution (Das et al. 2016). The survival of these microorganisms relies on their inherent biochemical, structural, and physiological characteristics and genetic adaptations (Ozer et al. 2013). Therefore, the present research aimed to identify, isolate, and characterize heavy metal-resistant bacteria from soil samples at and around the Benue Cement Company in Nigeria. These bacteria could be useful in bioremediation efforts in areas where metals have been polluted.

MATERIALS AND METHODS

Study area

Gboko is home to Benue Cement Company Plc. (now Dangote Cement Company Plc.) in Nigeria's north-central area, specifically in the Gboko Local Government Area (LGA) of Benue State. Approximately 532 feet above mean sea level, it can be found with these coordinates: 7°24 42.45N, 8°58 31.28E (Figure 1). The research site was in a tropical subhumid zone where the average yearly temperature ranges from 23 to 34°C.

Collection of soil samples

Soil samples were collected from five separate farmlands within a five-kilometer radius of the cement industry, down to a 10 cm depth. The cement factory's surrounding water sources were also sampled using sterile glass bottles: stagnant water, flowing water, abandoned pits, stream water, and underground water. For analysis, the soil and water samples were brought to the laboratory in an ice box.

Metal analysis of the collected soil samples

The concentrations of metals in all samples were determined according to the AOAC (2010) using an Atomic Absorption Spectrometer (Varian SpectrAA 220 USA).

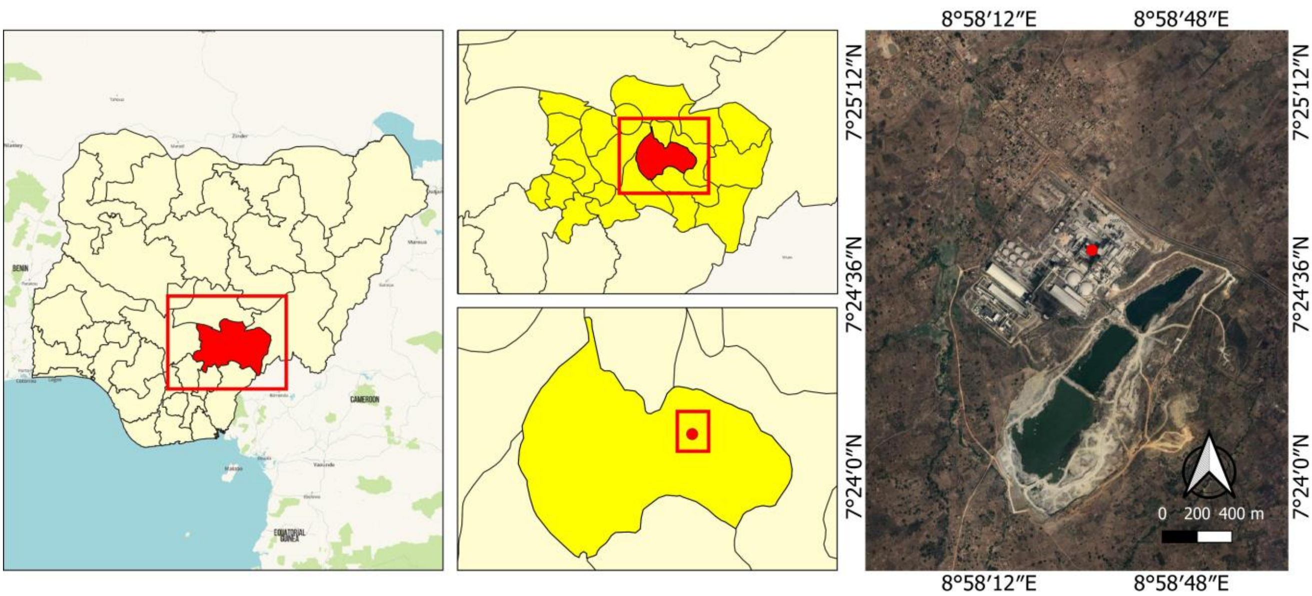

Figure 1. The location of sampling site in Gboko Local Government Area (LGA) of Benue State, Nigeria (Source: www.google.com)

Bacterial isolation from soil samples

To isolate bacterial isolates from the obtained soil samples, enrichment technique was used via added 5 g of soil to 500 mL of mineral salts media in sterilized Erlenmeyer flasks. Sole carbon source was applied as 2000 µL of crude oil and incubated with shaking for 7 days at room temperature. Then, ten-fold serial dilutions were performed for enrichment sample suspension and one mL of each dilution was poured into oil agar plates to isolate bacterial isolates and incubated at 30°C for 3-7 days. Sub-culturing repeated for selected pure colonies on agar plates and transferring on Nutrient agar slants for morphological, biochemical and molecular identification.

Morphological characterization

Morphological characteristics, include color and shape of colony, cell size, and motility (Cheesbrough 1991). Microscopic features were recorded for all isolates via Gram stain protocol.

Biochemical characterization

Biochemical tests were carried out according to the method of Cheesbrough (1991).

Catalase test

Gas bubbles detecting within 10 s after added purified bacterial culture to 5 mL of hydrogen peroxide solution, considered as a positive catalase test.

Urea test

Slanted two mL of urea medium which placed in bijou bottles applied for the incubated bacterial colony at room temperature. Red-pink colour in the medium was considered as a positive test for urease induction.

Indole test

Appearance of bright red and yellow color which composed after added 0.5 mL of Kovac's reagent to incubated bacterial culture at 35°C for 24 h on SIM media indicated a positive and negative results respectively.

Citrate test

Simmons citrate test was performed via inculcate Simmons Citrate Agar plates (TSBA, Himedia) surface with bacterial cultures then, incubated at 37°C up to 48 h. changing media colour from green to bright blue indicate positive reaction.

Carbohydrate fermentations

Some of the isolates showed a color change from pink to yellow, indicating the production of fermented sugars such as glucose, sucrose and lactose of a gas bubble in the Durham's tube.

Coagulase test

A drop of sterile distilled water was placed on each end of a sterile slide. A colony of test organism was emulsified on each spot to make thick suspensions. A loopful of plasma was added to one of the suspension and mixed gently. The slide was examined for clumping or clotting of the organism within 10 seconds. Plasma was not added to the second suspension which serves as control.

Preparation of stock solutions for metals

All of the solutions utilized in the study were prepared using chemicals of a spectroscopic grade. In 1,000 mL of deionized water, sufficient quantities of $CdCl_2$, $PbCl_2$, $ZnCO_3$, $CrCl_3$, and $CuCl_2$ were dissolved to produce a synthetic stock solution of Cd (II), Pb(II), Zn (II), Cu (II), and Cr (II) effluent (1,000 mg/L). The stock solutions of the metal ions were serially diluted to prepare all other concentrations. Therefore, to balance the pH of the water-based solution, 0.1 M hydrochloric acid and 0.1 M sodium hydroxide were utilized.

Determination of Minimum Inhibitory Concentrations (MICs) of heavy metals

The plate dilution method was used to calculate MIC of the metals for each isolate as described by Bauer et al. (1966). Minimum Inhibitory Concentrations (MICs) of heavy metals were given as the concentrations at which microbiological growth might be inhibited.

Determination of antibiotic resistance

The disc diffusion method was employed according to the method of Bauer et al. (1966), to identify the antibiotic resistance pattern in various bacterial isolates. Strained strains were defined as those with a diameter of 12 mm or greater.

Data analysis

The results were analyzed statistically using One-Way Analysis of Variance (ANOVA), followed by Duncan multiple comparisonsusing Statistical Package for Social Science (SPSS) version 21. Significance between means was determined at a p-value of less than 0.05 ($p < 0.05$). The results for each treatment were presented as mean ± standard deviation.

RESULTS AND DISCUSSION

Heavy metal analysis of soil samples

Results of heavy metals analysis in and around the Benue Cement Factory is summarized in Table 1. Compared to other metals (Pb, Cr, and Cd), the concentration of copper and zinc was consistently quite high across all sites. The levels of all components examined were determined to be higher than the limits allowed by the World Health Organization (WHO 2010). In this study, Cd, Zn, Cr, Cu, and Pb were found in soil samples taken from the Benue cement industry, according to the analysis of heavy metals.

Isolation and identification of heavy metal-resistant bacteria

Results showed that total 20 isolates were isolated from the samples. Out of 20 isolates only five (5) bacterial isolates, namely *Staphylococcus aureus, Escherichia coli, Proteus* sp., *Bacillus cereus,* and *Lactobacillus* which showed high levels of heavy metal resistance were selected for further studies in secondary screening (Table 2).

Based on biochemical assays (Table 3) and morphological examination, *S. aureus* showed positive result to catalase and coagulase test. They were also seen to ferment lactose, sucrose and glucose. The *E. coli* reacted negatively to citrate, catalase, and coagulase test. It fermented lactose and glucose but did not ferment sucrose. *Lactobacillus* reacted positively to only citrate but fermented the three sugars. *Proteus* sp. fermented glucose and sucrose and also reacted positively to citrate, catalase and urea tests. The *B. cereus* fermented glucose and sucrose. The bacteria also reacted positively to only citrate and catalase tests.

Minimum Inhibitory Concentrations (MICs) of metals

The study investigated the resistance of four (4) of the five (5) isolates to the heavy metals Cd (II), Pb (II), Zn (II), Cu (II), and Cr (II). The *E. coli* and *Proteus mirabilis*, two of the most metal-resistant bacterial species, had the highest Minimum Inhibitory Concentration (MIC) values of all the isolates tested. Table 4 shows the results of separate determinations of Minimum Inhibitory Concentrations (MICs) of different metals. The *S. aureus* had MICs values between 10 and 16 mg/L, *E. coli* between 20 and 50 mg/L, *Proteus* species between 15 and 64 mg/L, and *B. cereus* between 10 and 18 mg/L against various metals.

Resistance to antibiotics

Table 5 shows the estimated antibiotic resistance of the most common ten tested antibiotics against the multiple metals resistant isolates (*E. coli* and *Proteus* species) to study the correlation between metals and antibiotic resistance in bacteria.

Table 1. Heavy metal concentration (mg/L) of soil

Samples	Pb	Cu	Zn	Cr	Cd
1	0.235 ± 0.00^a	4.670 ± 0.01^a	8.4600 ± 0.01^a	0.016 ± 0.00^a	0.6220 ± 0.00^a
2	0.131 ± 0.00^b	3.020 ± 0.00^b	9.5350 ± 0.02^b	0.021 ± 0.00^b	0.5210 ± 0.00^b
3	0.222 ± 0.00^c	3.775 ± 0.00^c	6.6400 ± 0.01^c	0.035 ± 0.00^c	0.4215 ± 0.00^c
4	0.145 ± 0.00^d	5.110 ± 0.01^d	6.5300 ± 0.01^d	0.025 ± 0.00^d	0.3160 ± 0.00^d
5	0.317 ± 0.00^e	4.850 ± 0.00^e	7.6150 ± 0.01^e	0.036 ± 0.00^c	0.5155 ± 0.00^e

Note: Mean values on the same column having different letters of the alphabet are statistically significant $p\leq 0.05$

Table 2. Morphological characteristics of isolated bacteria

Bacteria Isolates	*Staphylococcus aureus*	*Escherichia coli*	*Proteus* sp.	*Bacillus cereus*	*Lactobacillus*
Colony colour	Golden yellow	Pink	Cream	White	Yellow
Gram nature	Positive	Negative	Negative	Positive	Positive
Cell morphology	Cocci	Rod	Rod	Rod	Rod
Motility	Negative	Positive	Positive	Positive	Negative

Table 3. Biochemical analysis of isolated bacteria

Biochemical Parameters	*Staphylococcus aureus*	*Escherichia coli*	*Proteus* sp.	*Bacillus cereus*	*Lactobacillus*
Catalase	+	-	+	+	-
Citrate	-	-	+	+	+
Coagulase	+	-	-	-	-
Glucose	+	+	+	+	+
Indole	-	+	-	-	-
Lactose	+	+	-	-	+
Sucrose	+	-	+	+	+
Urea	-	-	+	-	-

Note: (+): Positive, and (-): Negative

Table 4. Minimum inhibitory concentration (MIC) of bacterial isolates to lead (Pb), copper (Cu), cadmium (Cd), zinc (Zn), and chromium (Cr)

Bacterial Isolates	MIC (mg/L)				
	Pb	Cu	Cd	Zn	Cr
Staphylococcus aureus	12.00	15.00	14.00	10.00	16.00
Escherichia coli	35.00	20.00	40.00	50.00	30.00
Proteus sp.	33.00	15.00	35.00	64.00	35.00
Bacillus cereus	15.00	18.00	10.00	15.00	12.00

Table 5. Antibiotic sensitivity of heavy metals resistant isolates

Antibiotics	Disc codes	Inhibition zone diameter (mm)		Resistance/sensitivity	
		Escherichia coli	*Proteus* sp.	*Escherichia coli*	*Proteus* sp.
Streptomycin	S	6	11	R	R
Gentamycin	Gen	9	28	R	S
Pefloxacin	PEF	34	7	S	R
Ciprofloxacin	CPX	5	30	R	S
Septrin	SXT	6	12	R	R
Chloramphenicol	C	8	19	R	S
Augmentin	AUG	12	22	R	S
Ofloxacin	OFX	30	17	S	S
Sparfloxacin	SP	10	8	R	R
Amoxicillin	AM	25	10	S	R

Note: S: Sensitive; R: Resistant

Discussion

In particular, the emissions of heavy metals such as Cd, Cr, Cu, Pb, and Zn from cement manufacture are a major cause of environmental contamination (Aman et al. 2008; Chattopadhyay and Grossart 2011). The primary source of these heavy metals in the soil is raw materials, but they can also be found in cement dust and stack fumes (Das et al. 2016). Because of their persistence in the environment and bioaccumulation in the food chain, heavy metals are hazardous to plants and people, even in low quantities (Ozer et al. 2013). Heavy metal contamination is a global concern; resolving it is important (Akhter et al. 2017).

How microbial communities react to heavy metals is conditional on the metals' concentration and accessibility (AOAC 2010). Heavy metals frequently inhibit microorganisms, essential for the biological remediation of heavy metal-polluted locations (Cheesbrough 2005). Therefore, finding microbes that can survive in environments with high concentrations of heavy metals is crucial for creating efficient bioremediation systems.

The results of present study showed that heavy metal pollutants, such as Cd, Zn, Cr, Cu, and Pb was found in soil samples from the Benue Cement Company in Nigeria. Bacteria resistant to heavy metals were isolated and identified. The *S. aureus*, *E. coli, B. cereus, Lactobacillus,* and *Proteus* species were the five strains chosen for additional analysis from 20 isolates. Three similar bacterial species, namely *E. coli, S. aureus,* and *Proteus* species have been identified from industrial effluents in Punjab, India (Summers and Silver 1972). Nath et al. (2012) also found *S. aureus* and *Proteus* species in sewage from Asam, India, garages, and petrol pumps. Because of their adaptability and favorable conditions, these bacterial species are likely be found in areas contaminated with heavy metals from cement dust.

The results of heavey metal resistance revealed that *E. coli* and *Proteus* species had varying Minimum Inhibitory Concentrations (MICs) for heavy metals, such as Pb, Cu, Zn, and Cr. The resistance pattern exhibited by *E. coli* and *Proteus* species usually forms in reaction to environmental stresses caused by heavy metals (Gullberg et al. 2014). Research by Bauer et al. (1966) shows that certain bacteria, such as *E. coli* and *Proteus* species, can withstand and even eliminate heavy metals. Therefore, to mitigate heavy metal toxicity, other studies have investigated biosorption and microbial production of metal-binding compounds (Kabata-Pendias and Mukherjee 2007).

The varying degrees of toxicity that various metals have on bacterial cells may explain why their Minimum Inhibitory Concentrations (MICs) vary among different strains of bacteria. Zinc wasthe least poisonous metal, with a maximum concentration of 64 mg/L in one isolate. In contrast, copper was the most toxic, with no isolated organism having a MIC higher than 20 mg/L in *E. coli*.

Ansari and Malik (2007) measured several metals' Minimum Inhibitory Concentrations (MICs) against soil-isolated *E. coli* bacteria (Bauer et al. 1966). Maximum MICs for mercury (32 mg/L), cadmium (200 mg/L), copper (400 mg/L), nickel (800 mg/L), and lead (1600 mg/L) were observed. In addition, MICs for Cd (4-7 mM), Cr (0.7 mM), Ni (6.75-8.5 mM), Pb (6 mM), As (6.5-15 mM), and Hg (0.75 mM) were found in sewage multi-metal resistance isolates such *Proteus* species, *Acinetobacter radioresistens,* and *Pseudomonas aeruginosa* (Nath et al. 2012). Species of *Pseudomonas*, in contrast, showed remarkable resistance to copper, zinc, lead, arsenate, and mercury (Mohammad et al. 2015). Metal resistance was rather high among our bacterial isolates compared to other strains reported to be metal-resistant.

Bacteria have evolved various resistance mechanisms in response to environmental heavy metal stress. According to Das et al. (2016), when exposed to high concentrations of harmful metals, microbes create a wide variety of metal-binding compounds, some particular and others more general, which can reduce the impact of the metals and facilitate their uptake. Detoxification and heavy metal removal from polluted environments could use these processes (Raja et al. 2009). Therefore, considering the prevalence of heavy metal contamination at numerous sites, bioremediation using multiple metal-resistant bacteria holds great promise (Wei et al. 2009).

Therefore, Summers and Silver (1972) mention a correlation between metal resistance in microbes and antibiotic resistance. Since efflux pumps in bacterial membranes mediate both forms of resistance, microbial populations can develop antibiotic resistance when heavy metals and antibiotics are present in specific settings (Abskharon et al. 2010). The *P. mirabilis* and *E. coli* were resistant to most antibiotics tested in the present investigation. The *E. coli*, the most antibiotic-resistant strain, was also the most metal-resistant. These results point to typically high Minimum Heavy Metal Concentrations (MHCs) or the concentration needed to cause antibiotic resistance While both isolates exhibited some degree of antibiotic resistance, the resistance level was most pronounced in *E. coli* and lowest in *Proteus* species. Ciprofloxacin showed inhibitory zone of 5 mm against *E. coli* and 7 mm against *Proteus* specie when tested with perfloxacin. From the 10 antibiotics tested, *E. coli* showed no resistance to Perfloxacin (PEF), Ofloxacin (OFX), and Amoxacillin (AM) with 34, 30, and 25 mm zone diameters, respectively. On the other hand, *Proteus* species did not show resisance to GEN CPX, C, AUG, and OFX. According to Raja et al. (2009), along with resistance to ampicillin, tetracycline, chloramphenicol, kanamycin, erythromycin, streptomycin, and nalidixic acid, *P. aeruginosa*, *A. radioresistens*, and *Proteus* species, all have multiple metal resistances.

In conclusion, the results showed that the four characterized isolates, namely *B. cereus, S. aureus, E. coli*, and *Proteus* species exhibited an impressive tolerance to heavy metals. These findings exhibited that microorganisms can adapt to polluted heavy metal environments and be utilized in bioremediation initiatives. More importantly, environmental managers must address the correlation between heavy metal pollution and antibiotic resistance.

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REFERENCES

Abah MA, Okoli EC, Olawale O, Ozioma PE, David CB, Zephaniah HS. 2021. Determination of selected pesticide residues in leafy vegetables (*Amaranthus spinosus*) consumed in Donga, Taraba State. Intl J Biochem Bioinf Biotechnol Stud 6 (2): 9-16. DOI: 10.37745/ijbbbs.15.

Abimbola OA, Olawale OA, Anthony IO, Obasola EF. 2019. Metal-resistance encoding gene-fingerprints in some bacteria isolated from wastewaters of selected printeries in Ibadan, South-western Nigeria. J Taibah Univ Sci 13 (1): 266-273. DOI: 10.1080/16583655.2018.1561968.

Abskharon RNN, Hassan SHA, Kabir MH, Qadir SA, El-Rab SMFG, Wang MH. 2010. The role of antioxidants enzymes of *E. coli* ASU3, a tolerant strain to heavy metals toxicity, in combating oxidative stress of copper. World J Microbiol Biotechnol 26: 241-247. DOI: 10.1007/s11274-009-0166-4.

Achternbosch M, Bräutigam KR, Hartlieb N, Kupsch C, Richers U, Stemmermann P. 2003. Heavy Metals in Cement and Concrete Resulting from the Co-Incineration of Wastes in Cement Kilns with Regard to the Legitimacy of Waste Utilisation. Forschungszentrum Karlsruhe GmbH, Karlsruhe, Germany.

Adekola O, Whanda S, Ogwu F. 2012. Assessment of policies and legislation that affect management of wetlands in Nigeria. Wetlands 32: 665-677. DOI: 10.1007/s13157-012-0299-3.

Afolabi A, Francis AF, Adejompo, F. 2012. Assessment of health and environmental challenges of cement factory on Ewekoro Community Residents, Ogun State, Nigeria. Am J Hum Ecol 1 (2): 51-57. DOI: 10.11634/21679622150479.

Akhter K, Ghous T, Andleeb S, Nasim F.H, Ejaz S, Zain-ul-Abdin, Khan BA, Ahmed MN. 2017. Bioaccumulation of heavy metals by metal-resistant bacteria isolated from *Tagetes minuta* rhizosphere, growing in soil adjoining automobile workshops. Pak J Zool 49: 1841-1846. DOI: 10.17582/journal.pjz/2017.49.5.1841.1846.

Al-Khashman OA, Shawabkeh RA. 2006. Metals distribution in soils around the cement factory in southern Jordan. Environ Pollut 140: 387-394. DOI: 10.1016/j.envpol.2005.08.023.

Aman T, Kazi AA, Sabri MU, Bano Q. 2008. Potato peels as solid waste for the removal of heavy metal copper (II) from waste water/industrial effluent. Colloids Surf B: Biointerfaces 63: 116-121. DOI: 10.1016/j.colsurfb.2007.11.013.

Ansari MI, Malik A. 2007. Biosorption of nickel and cadmium by metal resistant bacterial isolates from agricultural soil irrigated with industrial wastewater. Bioresour Technol 98: 3149-3153. DOI: 10.1016/j.biortech.2006.10.008.

AOAC. 2010. The office Methods of Analysis of AOAC international. 16th Edn. the Association of Official Analytical Chemists, Arlington, USA.

Bauer AW, Kirby WMM, Sherris JC, Turck M. 1966. Antibiotic susceptibility testing by standardized single disc diffusion method. Am J Clin Pathol 45: 493-496. DOI: 10.1093/ajcp/45.4_ts.493.

Chattopadhyay MK, Grossart HP. 2011. Antibiotic and heavy metal resistance of bacterial isolates obtained from some lakes in northern Germany. NSHM J Pharm Healthc Manag 2: 74-75.

Cheesbrough M. 1991. Medical Laboratory Manual for Tropical Countries: Microbiology. ELBS Edition, Cambridge.

Cheesbrough M. 2005. District Laboratory Practice in Tropical Countries. Cambridge University Press, New York.

Das S, Dash HR, Chakraborty J. 2016. Genetic basis and importance of metal resistant genes in bacteria for bioremediation of contaminated environments with toxic metal pollutants. Appl Microbiol Biotechnol 100 (7): 2967-2984. DOI: 10.1007/s00253-016-7364-4.

Emmanuel E, Pierre M.G, Perrodin Y. 2009. Groundwater contamination bymicrobiological and chemical substances released from hospital waste water: Health risk assessment for drinking water consumers. Environ Intl 35: 718-726. DOI: 10.1016/j.envint.2009.01.011.

Gullberg E, Albrecht LM, Karlsson C, Sandegren L, Andersson DI. 2014. Selection of a multidrug resistance plasmid by sublethal levels of antibiotics and heavy metals. mBio 5 (5): e01918-14. DOI: 10.1128/mbio.01918-14.

Kabata-Pendias A, Mukherjee AB. 2007. Trace Elements from Soil to Human. Springer, Berlin. DOI: 10.1007/978-3-540-32714-1.

Malik A, Jaiswal R. 2000. Metal resistance in *Pseudomonas* strains isolated from soil treated with industrial wastewater. World J Microbiol Biotechnol 16: 177-182. DOI: 10.1023/A:1008905902282.

Mohammad MM, Mohammad RN, Mahmood A, Korosh K. 2015. *Lamium album* or *Urtica dioica*? Which is more effective in decreasing serum glucose, lipid and hepatic enzymes in streptozotocin induced diabetic rats: A comparative study. Afr J Tradit Complement Altern Med 12: 84-88. DOI: 10.4314/ajtcam.v12i5.13.

Nath S, Deb B, Sharma I. 2012. Isolation and characterization of cadmium and lead resistant bacteria. Glob Adv Res J Microbiol 1 (11): 194-198.

Olawale OF, Abah MA, Emmanuel OP, Otitoju GT, Abershi AL, Temitope DF, Andrew AE, Abdulkadir S, John A. 2023. Risk assessmen of heavy metal content in yam tubers locally produced in selected local government areas of Taraba State, Nigeria. Asian J Nat Prod Biochem 21: 6-12. DOI: 10.13057/biofar/f210102.

Otitoju O, Moses AA, Otitoju TG, Bilyaminu H, Emmanuel CO, Patience UO. 2022. Risk assessment of pesticide residues in water samples from river Gongola, Adamawa State, Nigeria. World J Adv Res Rev 13 (01): 424-432. DOI: 10.30574/wjarr.2022.13.1.0015.

Ozer G, Ergene A, Icgen B. 2013. Biochemical and molecular characterization of strontium-resistant environmental isolates of *Pseudomonas fluorescens* and *Sphingomonas paucimobilis*. Geomicrobiol J 30: 381-390. DOI: 10.1080/01490451.2012.694977.

Raja EC, Selvam SG, Omine KA. 2009. Isolation, Identification and Characterization of Heavy Metals Resistant Bacteria from Sewage. International Joint Symposium on Geodisaster Prevention & Geoenvironment in Asia, Fukuoka, 205-211.

Rashed MN. 2010. Monitoring of contaminated toxic and heavy metals, from mine tailings through age accumulation, in soil and some wild plants at Southeast Egypt. J Hazard Mater 178: 739-746. DOI: 10.1016/j.jhazmat.2010.01.147.

Sherene T. 2010. Mobility and transport of heavy metals in polluted soil environment. Biol Forum 2: 112-121.

Summers AO, Silver S. 1972. Mercury resistance in a plasmid bearing strain of *Escherichia coli.* J Bacteriol 112 (3): 1228-1236. DOI: 10.1128/jb.112.3.1228-1236.1972.

Tatah S, Ogodo A.C, Kaa LC, Agwaranze DI. 2016. The potential use of *Alternaria alternata* in bioremediation of wastewater contaminated by Hexavalent Chromium ion. Trends Sci Technol J 1 (1): 115-118.

Tatah VS, Ibrahim KLC, Ezeonu CS, Otitoju O. 2017. Biosorption kinetics of heavy metals from fertilizer industrial waste water using groundnut husk powder as an adsorbent. J Appl Biotechnol Bioeng 2 (6): 00049. DOI: 10.15406/jabb.2017.02.00049.

Tatah VS, Yakubu EO, Ayantse LM, Dearsley FU, Uba SA. 2020. Determination of heavy metals in soil and water samples from Mambilla Artisanal Mining Site and its environs. Trends Appl Sci Res 15: 125-132. DOI: 10.17311/tasr.2020.125.132.

Turner AH. 2009. Urban Agriculture and Soil Contamination: An Introduction to Urban Gardening, Environmental Finance Centre, EPA Region5, University of Louisville, Louisville.

Udiba UU, Ogabiela EE, Hammuel C, Ade-Ajayi AF, Odey MO, Yusuf U, Abdullahi M, Gauje B. 2012. Post remediation assessment of contaminants levels in soil, dareta village, Zamfara, Nigeria. Trends Adv Sci Eng 4 (1): 70-79.

Wei GH, Fan LM, Zhu WF, Fu YY, Yu JF, Tang M. 2009. 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 162: 50-56. DOI: 10.1016/j.jhazmat.2008.05.040.

WHO. 2010. Environmental Health Criteria 101. Methyl Mercury. World Health Organization, Geneva. https://wedocs.unep.org/20.500.11822/29413.

Zhema PA, Moses AA, Emochone RY, Emmanuel CO, Asemave SS, Bilyaminu H. 2022. Investigation of trace metal contamination in bread baked and sold in Wukari. Glob Sci J 10 (2): 2076-2082. DOI: 10.11216/gsj.2022.02.60325.