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Heavy Metals Tolerance and Antibiotic Susceptibility of Bacteria Isolated from Ashaka Solid Waste Disposal Site and Adjacent Source of Water

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Environmental pollution, particularly involving heavy metals (HMs) and pathogenic bacteria, is a global issue, with the cement industry contributing significantly. This study investigated heavy metal concentrations in soil and water samples from the Ashaka cement dump site in Gombe State, Nigeria, and isolated, identified, and characterized heavy metal-tolerant bacteria from these samples. Using Atomic Absorption Spectrophotometry after nitric acid digestion, the study found significant heavy metal concentrations exceeding recommended limits, indicating potential environmental pollution. Notable levels were found for zinc, mercury, lead, manganese, and chromium. Bacteria such as Escherichia coli, Klebsiella pneumoniae, Enterobacter sp., Bacillus sp., and Pseudomonas sp. were identified. These bacteria exhibited varied heavy metal tolerance and antibiotic resistance patterns. The results highlight the Ashaka cement dump site as a significant source of environmental contamination with heavy metals, emphasizing the need for proper waste management to mitigate health and ecological risks.

Keywords: Heavy metals, Pathogenic bacteria, Environmental pollution, Cement industry, Waste management.

1. Introduction

Cement production, primarily utilizing raw materials such as limestone, chalk, shale, clay, and sand, involves a series of processes including quarrying, crushing, fine grinding, and blending to achieve the correct chemical composition (Chatterjee, 2018). The cement industry significantly contributes to global living standards by generating direct employment and stimulating related economic sectors. However, the industry also poses considerable challenges to surrounding communities due to the generation of industrial solid wastes (Soliman and Moustafa, 2020). Technological advancements have enabled increased cement production volumes, consequently leading to higher waste generation with potential environmental impacts (Habert *et al.,* 2020).

Industrial activities today release substantial quantities of waste and pollutants into the environment, with cement manufacturing producing solid wastes such as clinker production residues, kiln dust, and fly ash from power plants. Maintenance activities also generate waste like used oil and metal scrap, often disposed of in open landfills, causing respiratory issues (Siddiqua *et al.,* 2022). These wastes, predominantly inorganic and heavy metals, pose ecological risks due to their toxic nature, affecting humans, animals, microorganisms, and soil respiration (Demkova *et al*., 2017).

Heavy metal pollution is a significant global problem, with the cement industry contributing to the release of large quantities of solid waste (Joshua *et al*., 2020). The soil environment, harboring high concentrations of metallic pollutants, suffers adverse effects on its microflora and fauna diversity (Nair, 2023). While some pollutants are negligible, heavy metals are particularly toxic even at low concentrations. Although metals naturally occur in the environment and play beneficial roles, excessive concentrations result in pollution, detrimentally impacting microbial communities (Naz *et al*., 2022).

Pollutant distribution varies, with certain contaminants more hazardous than others. Heavy metals, whether naturally occurring or introduced through industrial activities, accumulate in the environment, affecting soil and water ecosystems (Zhang and Wang, 2020). Studies show a correlation between heavy metal tolerance and

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antibiotic resistance in bacteria, facilitated by genetic exchanges and vertical gene transfer, complicating infectious disease treatments (Halema *et al.,* 2024).

Bioremediation of heavy metal pollution is a significant challenge in environmental biotechnology and microbiology. Industrial processes releasing heavy metals into aquatic systems pose severe risks to plants, animals, humans, and aquatic life due to their toxic concentrations (Uddin *et al*., 2021). Microorganisms, due to their large surface area and high metabolic activity, are crucial in introducing heavy metals into the food chain, necessitating immediate removal or detoxification (Chen *et al*., 2022).

Research highlights the threat posed by the indiscriminate disposal of heavy metal-containing solid wastes from industries like the Ashaka cement industry, impacting soil and water ecosystems. Prolonged exposure to such wastes can lead to environments conducive to the development of heavy metal-resistant bacteria, potentially acquiring genes for both antibiotic and heavy metal resistance, posing severe health risks (Gupta *et al*., 2022).

There is a knowledge gap regarding whether the development of resistance to heavy metals and antibiotics is solely due to environmental factors such as cement processing activities. Efforts by companies like Lafarge through initiatives like Geocycle aim to promote eco-friendly waste management (Lafarge.com). However, continuous discharge of untreated industrial solid waste necessitates identifying heavy metal-tolerant bacteria from waste disposal sites and adjacent water sources and investigating their antibiotic susceptibility patterns. This study aims to determine the concentration of heavy metals in soil near and from the dump site as well as in adjacent water sources and to Isolate, identify, and characterize heavy metal-tolerant bacteria from the soil at the dump site and from adjacent water sources.

2. Materials and Methods

2.1. Sampling site and sample collection

Ashaka Cement Factory, established in 1967, is situated in Funakaye Local Government Area, Gombe State, Northeastern Nigeria, and serves construction demands across the region. Located within the Northeastern region of Gombe, it lies between longitude 10°45' N and 11°00' N and latitude 11°15' E and 11°30' E.

Soil samples were collected from four distinct sites to evaluate contamination levels: the factory's primary dumping area (Site A), areas 2 meters (Site A2) and 4 meters (Site A3) from the dump site, and an adjacent water source (Site B) located near the quarry, which is situated several kilometers away from the main factory premises. To serve as a baseline for contamination levels, samples were also taken from the Bayero University Botanical Garden in Kano State (Site C), considered a control site due to its lower contamination relative to the industrially active Ashaka area. The choice of Kano State as a control, despite its own industrial activities, was made due to its relatively low pollution levels compared to the concentrated industrial discharge surrounding the Ashaka Cement Factory.

Sampling was conducted at a depth of approximately 20 cm from the soil surface using a soil auger and spatula. Samples were carefully placed in clean polyethylene bags to prevent external contamination, immediately transported to the laboratory, and stored at 4°C to maintain sample integrity until analysis. This handling method minimized the potential for contamination or alteration of the samples, ensuring reliable assessment of the environmental impact at each site (Neha et al., 2015).

2.2. Determination of Selected Heavy Metals Concentration

To determine heavy metals concentration in the dump site, the following steps were taken:

2.2.1. Digestion of water sample

This was carried out according to techniques as described by (Olafisoye *et al.*, 2013). Two point five (2.5 cm^3) of 1mol.dm^{-3} HNO_3 was added to 50 cm^3 of water sample and digested by heating on a plate until colourless solution was obtained. The digest was allowed to cool and then filtered into a 50 cm^3 volumetric flask. The **v**olume of the digested water sample was made to 50 cm^3 with distilled water

2.2.2. Digestion and analysis of soil samples

One gram (1.0g) of the soil sample was placed into 100 cm3 beaker and 15 cm^3 of aqua-regia

(HNO_3: HCl 3:1v/v) was then added. The mixture was boiled on a hot plate at 125^0C for 2hrs. Then, the digest was allowed to cool at room temperature and filtered through a whatman 541 filter paper in 100 cm^3 volumetric flask. The filtrate was made up to mark distilled water (Olafisoye *et al*., 2013). The filtrates were then analyzed using Atomic Absorption Spectrophotometry (Shimazu AAS, model AA-6800, Shimazu corporation) at the central laboratory of Bayero University Kano, which detects the elements in the liquid sample from a light source through the application of characteristics wavelengths 357.9 nm for chromium, 217 nm for lead, 213.9 nm for Zinc, 228.8 nm for Cadmium, 279.5 nm for Manganese and 253.7 nm for Mercury.

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2.2.3. Determination of Tolerance Level

Serial Dilution

One milliliter /one gram of both water and soil sample was transferred into sterile test tube containing one 9 mL of sterile distilled water to get 10^{-1} dilution. Then, 1 mL from 10^{-1} dilution was transferred into another 9 mL of sterile distil water to get 10^{-2} dilution, and the samples was further diluted to obtain various dilution of 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} respectively. 10^{-3} dilution was used for inoculation. (Oko *et al.2016)*

The serial diluents were aseptically inoculated onto different plates of Nutrient agar (NA) were incorporated with salts of lead (II) oxide, mercuric iodide, zinc chloride and chromic oxide (Pb^{2+}, Hg^{2+} Zn^{2+}, and Cr^{6+}) at concentration 50mg/l 100mg/l, 200mg/l, 300mg/l and 400mg/l. The media was used for the selective isolation of heavy metals tolerant bacteria. Nutrient agar (Oxford UK) was prepared according to the manufacturer's instructions, sterilized and cooled to 50°C.

2.3. Isolation of bacteria from samples

The serial dilutions were aseptically inoculated onto Nutrient Agar (NA) plates that were supplemented with salts of heavy metals, including lead (II) oxide, mercuric iodide, zinc chloride, and chromic oxide (Pb^{2+}, Hg^{2+}, Zn^{2+}, and Cr^{6+}) at concentrations of 50 mg/L, 100 mg/L, 200 mg/L, 300 mg/L, and 400 mg/L, following the methods described by Walley et al. (2008).

2.4. Identification of bacterial isolates from samples

The sample isolates were identified by comparing their morphological and biochemical characteristics with those of known taxa as described by Bergey's Manual for Determinative Bacteriology. (Brenner *et al*., 2005)

2.5. Gram Staining

Smears of young culture (18-24 hours old) of bacterial isolates were made on a clean glass slide. The smears were heat fixed and stained with crystal violet for 30-60 seconds. The dye was drained and then fixed with Lugol's iodine for 30 seconds. The slides were rinsed with tap water, decolourized with 95% ethanol for about 10 seconds and again washed with tap water. The slides were counter stained with Safranin for 30 seconds then rinsed, air dried and examined under the microscope using the oil immersion lens for gram reaction and cellular morphology (Cheesbrough, 2006).

2.6. Biochemical Test

Catalase test, Citrate Utilization Test, Indole Test and Urease Test was done as described by (Cheesbrough, 2006).

2.6.1. Methyl Red (MR) and Voges- Proskauer (VR) Test

MR test was carried out as described by Ochei and Kolhatkar (2000). A little portion of the isolates was inoculated into glucose phosphate peptone water medium and then incubated at 37 ^{o}C for 48hours. Few drops of methyl red will be added to the culture. MR positive form red colour at the surface of the medium while MR negative showed no colour change. MR test is usually done in conjunction with VR test. Retaining the initial colour in the medium during MR test indicate a positive VR test while change indicate VR negative.

2.6.2. Hydrogen Sulphide (H_2S) Production

This was done as describe by Cheesbrough (2006)

2.6.3. Fermentation of Sugar

Triple sugar iron (TSI) was prepared according to techniques as described by (Cheesbrough, 2006).

2.6.4. Molecular confirmation of the isolates identity

To confirm the identity of the isolates, PCR was used to amplify 16S rRNA gene in the bacteria

2.6.5. Genomic DNA Extraction

Genomic DNA extraction for amplification of 16S rRNA gene was performed using DNA extraction kit procedure (Quick-DNA™ Microprep Kit, Zymoresearch, USA).

2.6.6. PCR Amplification and Sequencing of 16S rRNA gene

The 16S rRNA gene of all the isolated strains were amplified by polymerase chain reaction using primers 27F (5'- AGAGTTTGATCMTGGCTCAG-3') and 1492R (5' ACCTTGTTACGACTT-3'). The PCR was carried out in a PCR Machine (Techne Techgene FTGENE5D, USA) under the following conditions: initial denaturation at 94ºC for 5 minutes; 30 cycles of denaturation at 94ºC for seconds, annealing at 5ºC for 30 seconds and extension at 72ºC for 30seconds.

The final extension was performed at 72ºC for 10 minutes. The amplified PCR products of 16S rRNA gene of bacterial strains were purified and sequenced using the primers 27F (5'- AGAGTTTGATCMTGGCTCAG-3') and 1492R (5' ACCTTGTTACGACTT-3').

2.7. Antibiotic susceptibility test

The antibiotic susceptibility pattern of the heavy metals tolerant bacteria isolated from soil and water

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samples was determined using the Kirby-Bauer disc diffusion technique as described by the Clinical Laboratory Standard Institute (CLSI, 2021)

2.8. Antibiotics resistance gene detection (blaNDM-1 and blaKPC)

2.8.1. Genomic DNA Extraction

Genomic DNA extraction for amplification of *blaNDM*-1 and *blaKPC* gene was performed using boiling method (see Table 1). A day-old culture was used for the DNA extraction. 50 ul of sterile distilled H_2O was pipetted into a micro centrifuge tube and few well isolated colonies were suspended into it. It was then vortexes for about 20 seconds then the cells were now heated for about 10 minutes in a water bath at 95°C then centrifuged at 15000rpm for 15 minutes. The DNA Content will be in the supernatant which was used as template DNA for the amplification of the resistant gene (Ayfan *et al.* 2021).

2.8.2. Gel Electrophoresis

1g of agarose was measured and the powder was mixed with 100ml 1XTAE in a microwavable flask for about 3 minutes until the agarose was completely dissolved. The agarose solution was allowed to cool down to about 50°C. Ethidium bromide (EtBr) was then added to final concentration of approximately 0.2-0.5 ug/ml. This EtBr binds to the DNA and allows one to visualized the DNA under ensuring that air bubbles are avoided allow it to sit at room temperature for 20 to 30 minutes until it has completely solidified.

2.8.3. Loading Samples and Running an Agarose Gel

Loading buffer was added to each of the DNA samples and allowed to solidify. The agarose gel was then place into the gel box (electrophoresis unit). The gel box was then filled with 1X TAE until the after which the samples were carefully loaded into the additional wells of the gel. The gel was then run at 100V until the dye line was approximately 75-80% of the way down the gel for about an hour. The power was then turned off, electrodes disconnected from the power source and the gel fully removed from the gel box. The DNA fragment was then viewed under UV light (Lee *et al*. 2021).

2.8.4. NDM-1 PCR Amplification protocol

The PCR was carried out using trans-gene master mix (Dream Taq-Inqaba). A total of 25ul reaction mix was prepared in a 0.2 ul nucleus free micro tube containing the following components. i. Master mix DreamTaq 12.5 ul, 0.5 ul of each of 10 uM NDM-1 forward and NDM-1 reverse (Table 3.1) ii. 5.5 ul of nucleous free water iii. 5ul of DNA template. The contents of the tube were mixed, capped and transferred into applied bio system 9700 thermocycler programmed with the following cycling conditions: Initial denaturation at 95°C for 5 minutes, followed by 40 cycles of denaturation at 94°C for 1-minute. Annealing then takes place at 51°C for 30 seconds, followed by extension at 72°C for minute, final extension at 72°C for 5 minutes finally holding at 4°C.

Table 1: Primers for the detection of blaNDM-1 and blaKPC used in the study

Primer name	Sequences	Annealing Temperature	Expected amplicon size	Reference
BlaNDM-1 (Fm)	5'-GGTTTGGCGATCTGGTTTTC-3'	56°C	621BP	Hou and yang,2015
BlaNDM-1 (Rm)	5'-CGGAATGGCTCACGATC-3'	58°C	621BP	Hou and yang,2015
BlaKPC (Fm)	5'- ATGTCACTGTATCGCCGTCT -3'	56°C	785BP	Yahaya *et al*.,2015
BlaKPC (Rm)	5'- TTACTGCCCGTTGACGCCC -3'	56°C	785BP	Yahaya *et al*.,2015

3. Results and Discussion

3.1 Results

Table 2 presents the analysis of heavy metal concentrations in soil samples from the polluted dump site, the adjacent water source located 100 meters from the dump site, and the B.U.K. Biological Garden soil sample, which serves as a control. When compared to environmental protection regulatory limits, all samples exceeded acceptable standards. The highest concentrations of heavy metals were found in samples A1 and A3 from the dump site, with manganese and chromium levels reaching 16.14 ± 0.0031 and 1.483 ± 0.0133, respectively. Although the control sample (C) had lower concentrations, it still exceeded permissible limits, with cadmium at 0.034 ± 0.001 and lead at 0.004 ± 0.002.

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Table 2: Heavy metals content of soil obtained from dumping site and adjacent source of water of Ashaka cement dump site

PARAMETERS Mg/l	WHO, Limit	EPA, limit	SITEA1	SITEA2	SITEA3	SITEB	SITEC
Cadmium (Cd)	0.003	0.003	0.004±0.002	0.004±0.001	0.006±0.001	0.006±0.0007	0.002±0.001
Chromium (Cr)	0.05	2.00	0.685±0.0077*	1.066±0.0207*	1.483±0.0133*	0.106±0.030*	0.036±0.002
Manganese (Mn)	0.4	0.5	16.14±0.0031*	0.523±0.012	0.495±0.028	0.461±0.0009	0.1315±0.0434*
Lead (Pb)	0.01	0.010	0.207±0.0042*	0.215±0.005	0.627±0.015*	0.034±0.001	0.005±0.001
Zinc (Zn)	5.0	3.00	0.728±0.0049*	0.458±0.02	0.187±0.0062*	0.106±0.000*	0.087±0.0012
Mercury (Hg)	0.003	0.06	0.786±0.001*	0.746±0.156*	0.746±0.156*	1.167±0.07*	0.746±0.0567*

Values indicated by asterisk * indicate significant difference (p>0.05) across the group compared to the WHO MPL in Water using Duncan Multiple Range Test

SITE A1= Soil Sample from dump site
SITE A2 = Soil Sample, 2m away from A1
SITE A3 = Soil Sample, 4Mm away from A1
SITE B = Water Sample, 100m away from 100
WHO = World Health Organization
MPL = Maximum Permissible Limit
EPA = Environmental Protection Agency
SITE C = Soil Sample, BUK. Botanical Garden (Control)

Table 3: Biochemical Characterization of the isolated Tolerant Bacteria from the study

TEST	E. *coli*	K. *pneumonie*	*Enterobacter* sp.	*Bacillus* sp.	*Pseudomonas* sp.
Gram staining	Gram negative, rod shaped	Gram negative, rods	Gram negative, rod shaped	Gram positive rods	Gram negative rods
catalase	+	+	+	-	+
indole	+	-	-	-	-
oxidase	-	-	-	-	-
Methyl red	+	+	+	-	-
voges proskauar	-	+	+	-	+
citrate	-	+	+	-	+
urease	-	+	+	-	-
TSI	-	+	+	-	-
Coagulase	-	-	-	-	-

Key: + =Positive; - =Negative; VP = Vogas Proskauer test; TSI = Tripple Sugar Iron test; H_2S = Hydrogen Sulphide test

Table 4: Tolerance Level of the bacterial isolates to Zinc and Mercury after 48 hours during the study

	Heavy metal/conc.(mg/l)									
	Zinc					Mercury				
Isolates	50	100	200	300	400	50	100	200	300	400
E. *coli*	+	+	+	+	-	+	+	+	+	-
K. pneumonie	+	+	+	+	-	+	+	+	+	-
***Enterobacter* sp**	+	+	+		-	+	+	+	-	-
***Bacillus.* Sp**	+	+	+	+	-	+	+	+	+	-
***Pseudomonas* sp**	+	+	+	+	-	+	+	+	+	-

KEY
+ = Growth - = No Growth

Table 5: Tolerance Level of the Bacterial Isolates to Lead and Chromium After 48 hours of exposure

	Heavy metal /conc.(mg/l)									
	Lead					Chromium				
Isolates	50	100	200	300	400	50	100	200	300	400
E. *coli*	+	+	+		-	+	+	+	-	-
K. pneumonie	+	+	+		-	+	+	+	-	-
Enterobacter sp	+	+	+		-	+	+	+	-	-
Bacillus. Sp	+	+	+	+	-	+	+	+	+	-
Pseudomonas sp	+	+	+	+	-	+	+	+	+	-

KEY + = Growth - = No Growth

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Table 6: Antibiotics Mean Zone of growth Inhibition (mm) against the Isolates

Antibiotics (ug)	*E. coli*	*K. pneumonie*	*Enterobacter sp.*	*Bacillus sp.*	*Pseudomonas sp.*	Percent (%)
IMP (10)	19.21±0.30(R)	21.83±3.36(S)	17.53±1.18(R)	19.95±1.08(I)	16.99±1.12(R)	60
DO (30)	8.31±0.19(R)	9.77±1.96(R)	17.91±1.96(S)	6.00±0.00 (R)	19.21±1.70 (S)	60
S (300)	17.63±0.22(S)	10.29±2.53(R)	15.97±1.19(S)	19.95±1.08(S)	19.95±1.08(S)	20
CRO (30)	10.12±0.65(R)	9.81±2.13(R)	13.98±1.22(R)	20.31±0.53(S)	9.77±1.96(R)	80
AMC (20/10)	6.00±0.00(R)	15.37±1.16(R)	6.00±0.00(R)	6.00±0.00(R)	7.99±1.10(R)	100
CIP (5)	20.15±0.50(R)	11.50±0.61(R)	13.98±1.22(R)	21.32±0.47(R)	19.95±1.08(R)	100
AMP (10)	6.00±0.00(R)	6.00±0.00(R)	6.00±0.00(R)	13.30±0.34(S)	11.5±0.61(R)	80
MER (10)	6.00±0.00(R)	6.00±0.00(R)	6.00±0.00(R)	9.77±1.96(S)	6.00±0.00(R)	80
SXT (25)	10.18±1.46(R)	6.00±0.00(R)	6.00±0.00(R)	6.00±0.00(R)	6.00±0.00(R)	100
C (30)	10.25±1.02(R)	17.57±0.82(I)	10.12±0.27(R)	6.00±0.00(R)	6.00±0.00(R)	100
Resistance Percent (%)	90	90	80	50	80	

CLSI, (2021). KEY: µg=microgram
IMP=Imipenem,AMP=Ampicillin,S=Streptomycin,AMC=Amoxicillin,C=Chloramphenicol,DO=Doxycycline,MER=Meropenem,CRO=Cefriaxon,CIP=CiprofloxacinSXT=Sulphamethoxazole/Trimethroprim R = Resistant I = Intermediate S = Susceptible

Table 7: Mean Zone of Inhibition (mm) of the Isolates Sourced from the Control Site CLSI, (2021)

	ISOLATES					
Antibiotics(µg)	*E.coli*	*K. pnuemonia*	*Enterobacter sp*	*Bacillus sp.*	*Pseudomonas sp.*	%Resistance
IMP (10)	24.41±0.95(S)	25.33±1.39(S)	21.53±1.41(S)	20.07±0.11(S)	24.39±1.14 (S)	0
DO (30)	12.38±0.49 (I)	15.91±0.56(S)	16.69±1.33(S)	17.65±.0.72(S)	22.711±1.53(S)	0
S (300)	18.74±1.10(S)	17.43±1.04(S)	18.58±1.24(S)	16.23±0.55(S)	21.33±1.07(S)	0
CRO (30)	24.53±1.05(S)	25.61±0.53(S)	26.03±0.98(S)	21.59±0.73(I)	21.65±1.50(I)	0
AMC (20/10)	15.46±0.48(I)	20.38±1.05(S)	6.00±0.00(R)	15.53±0.67(I)	19.10±0.57(S)	20
CIP (5)	26.61±0.66(S)	23.43±0.66(I)	26.34±1.11(R)	26.68±0.89(S)	26.07±0.75(S)	20
AMP (10)	15.53±0.66(S)	12.80±1.47(R)	12.67±1.12(S)	18.11±0.97(S)	18.38±1.05(S)	20
MER (10)	24.03±0.87(S)	18.23±0.28(R)	23.01±0.98(S)	24.45±0.97(S)	24.52±1.03(S)	20
SXT (25)	19.14±0.96(S)	9.64±0.87(R)	17.77±1.29(S)	20.24±0.39(S)	17.02±0.74(S)	20
C (30)	18.08±1.01(S)	27.33±1.26(S)	20.11±0.17(S)	21.68±1.33(S)	23.93±1.82(S)	0
%Resistance	0	30	40	0	90	

KEY:IMP=Imipenem,AMP=Ampicillin, S=Streptomycin, AMC=Amoxicillin, C=Chloramphenicol,
DO=Doxycycline, MER=Meropenem, CRO=Cefriaxon, CIP=Ciprofloxacin,
SXT=Sulphamethoxazole/Trimethroprim R = Resistant I = Intermediate S = Susceptible µg=microgram

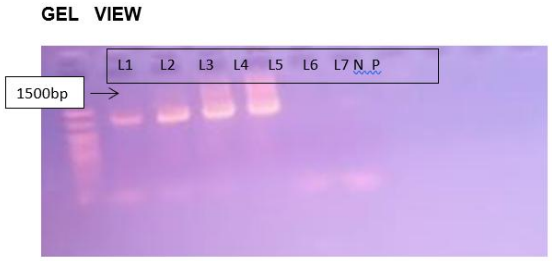


Figure 1: Amplified PCR Product of the test isolates

Key
Lane 1: Ladder
Lanes 2, 3, 4, and 5: Amplified PCR product of EU, PU, KU and BU
Lane 6: Negative control
Lane 7: Positive control

Table 3 shows results obtained from isolation and biochemical characteristics for the identification of bacteria from the polluted soil and water samples that were screened. The gram reactions test result showed that there were both gram –ve and gram +ve bacteria. All were rod shaped except one which was cocco bacilli. They all tested positive for catalase and negative to indole, Vogas Proskauer and urease test. A total of 5 bacteria were isolated and identified as E. *coli, Klebsiella pneumonie, Enterobacter sp , Bacillus sp* and *Pseudomonas sp,* The data presented in Table 2 indicates a significant presence of heavy metals in the soil and water samples collected from the Ashaka cement dump site. Cadmium concentrations were elevated in all dump site samples, with values of 0.004±0.002 mg/l, 0.004±0.001 mg/l, and 0.006±0.001 mg/l for Sites A1, A2, and A3, respectively. Chromium (Cr) levels in the soil samples (0.685±0.0077 mg/l, 1.066±0.0207 mg/l, and 1.483±0.0133 mg/l) also surpassed the EPA's MPL for soil (2.00 mg/l), indicating significant contamination

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Presented in Figure 1 are amplified PCR products of the isolates sourced from the study site that ware used molecular confirmation of their identity.

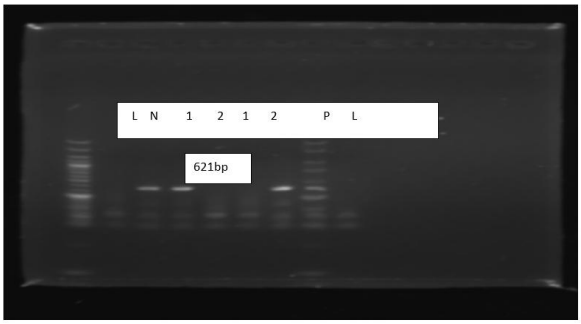


Figure 2: Agarose Gel electrophoresis of amplified *bla*NDM-1 and *bla*KPC Resistance Genes.

L: Ladder
N: Negative control for *bla*NDM-1
1 &2: Amplified PCR products (blaNDM-1 and blaKPC)
P: Positive control
DNA Marker: GeneRuler 1 kb DNA ladder (Thermo Fisher Scientific)

Presented in Table 4 is tolerance level of these isolates to zinc and mercury after 48hrs.all the isolates from the dump site had the highest tolerance level of around 300mg/l while *Enterobacter* sp from dump site tolerated net with 200mg/l.

Presented in Table 5 is tolerance level of these isolates to lead after 48hrs. *Pseudomonas* spp and *Baccillus.* spp from the dump site had the highest tolerance level of about 300mg/l tolerance level. All the other isolates tolerated up to 200mg/l after 48hrs.

Table 6 shows the result of the antibiotic susceptibility revealed that all isolates were resistant to most of the antibiotics. E.*coli* was resistant to all antibiotics except streptomycin, *Klebsiella pneumonie* is also resistant to all antibiotics except Chloramphenicol and imipenem and also *Enterobacter* sp is also resistant to all antibiotics except Doxycycline and streptomycin .

Table 7 the result of the antibiotic susceptibility of the isolates from control site revealed that all isolates were susceptible to two or more antibiotics. Isolates were 0% resistant to most of the antibiotics used.

3.2. Discussion

Soil and water samples showed significant heavy metal contamination, particularly with cadmium, chromium, manganese, lead, and mercury, which exceeded both the World Health Organization (WHO) and Environmental Protection Agency (EPA) maximum permissible limits (MPL). These elevated levels corroborate previous studies linking industrial activities to heavy metal contamination in soils (Chibuike and Obiora, 2014; Singh et al., 2010). For example, cadmium concentrations were markedly higher than control sites, posing severe risks to human health. Similarly, chromium, manganese, and lead were present in concentrations consistent with other industrially impacted areas (Prasad *et al*., 2021), while mercury levels uniformly surpassed acceptable limits (Islam *et al.*, 2017).

The biochemical characterization of bacterial isolates from the dump site revealed the presence of various heavy metal-tolerant strains, including *Escherichia coli*, *Klebsiella pneumoniae*, and *Bacillus* sp. These isolates demonstrated tolerance to heavy metals such as zinc, lead, and mercury, suggesting potential utility in bioremediation efforts. Antibiotic susceptibility tests, however, revealed a concerning pattern of multidrug resistance (MDR). Strains of *K. pneumoniae* and *Enterobacter* sp. showed resistance to key antibiotics like amoxicillin and streptomycin. These findings suggest the production of beta-lactamases and carbapenemases as resistance mechanisms (Bush and Bradford, 2020). The detection of resistance genes, including blaNDM-1 and blaKPC, further confirms the presence of multidrug-resistant strains, aligning with global concerns about rising antibiotic resistance (Rodrigues *et al*., 2024).

A critical concern raised in the study is the co-selection mechanism between heavy metal tolerance and antibiotic resistance in bacterial populations. The findings support the theory that environmental contamination triggers the co-regulation of resistance genes, increasing both the ecological and public health risks posed by industrial pollution (Yuan *et al.*, 2020). Given the presence of metal-resistant bacteria, including *E. coli*, *K. pneumoniae*, and *Bacillus* sp., the potential for bioremediation strategies is promising. The study highlights the urgent need for stringent environmental policies and the development of new antimicrobial agents to address the growing threat of antibiotic resistance in contaminated regions (Ahmad *et al.,* 2021; Salam *et al.,* 2023).

4. Conclusion

This study investigates the concentrations of heavy metals and the presence of heavy metal-tolerant bacteria in soil and water samples from the Ashaka cement dump site in Gombe State, Nigeria. The cement industry significantly contributes to environmental pollution through the release of industrial solid wastes containing heavy metals. Samples from the dump site and adjacent water sources were analyzed using Atomic Absorption Spectrophotometry, revealing heavy metal concentrations exceeding recommended limits, indicating potential environmental pollution. High

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levels of zinc, mercury, lead, manganese, and chromium were found. Bacteria such as *Escherichia coli*, *Klebsiella pneumoniae, Enterobacter* sp., *Bacillus* sp., and Pseudomonas sp. were isolated and identified, showing varied heavy metal tolerance and antibiotic resistance patterns.

The study highlights the significant contamination of the Ashaka cement dump site with heavy metals, posing ecological and health risks. Proper waste management practices are necessary to mitigate these risks. The presence of heavy metal-tolerant bacteria and their antibiotic resistance patterns further emphasize the need for environmental monitoring and remediation efforts.

Authors' Contributions

Authors UAM and IY conceived the idea UAM conducted the experiment. AAI prepared the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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