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Evaluation Of Heavy Metal Bioremediating Bacteria From Heavy Metal Polluted And Semi Pristine Environments

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Abstract:
This study compared the evaluation of heavy metal in semi-pristine and heavy metal-polluted environments. Samples of soil contaminated with heavy metals was obtained from Challawa industrial dump site Kano while semi-pristine soil samples were obtained from Bayero University Kano's Botanical and Ecological Gardens, and Jardin Botanique Geneva in Switzerland. Microbiological methods were used to isolate and identify the bacteria in the samples. The isolates were further screened for their ability to tolerate Lead (Pb) and Chromium (Cr) salts separately at various concentrations of 50, 100, 150, 200, 250, 300, 350, and 400 mg/l of each heavy metal after which their Minimum Inhibitory Concentration (MIC) was determined. Isolates that tolerated this heavy metal were further subjected for bioremediation assay. To provide the ideal circumstances for heavy metal removal, the bacteria that produced the greatest results while bioremediating these heavy metals independently were further improved. Seven bacteria were isolated; 2 from the semi-pristine sites and 5 from the polluted site; 1 Acinetobacter spp., 3 Psuedomonas spp., 2 Bacillus spp., and 1 Staphylococcus spp. Of all the isolates, Pseudomonas spp. from polluted soil tolerated more heavy metals with Minimum Inhibitory Concentration 280mg/l for Cr and 420mg/l for Pb. It also had the highest heavy metal removal of 58.28% for Cr and 72.97% for Pb while Pseudomonas spp. from Geneva had the least Cr removal of 25.79% and Bacillus spp. from the ecological garden had the lowest removal efficiency for Pb with 18.53%. The most efficient isolate for removing heavy metals was Pseudomonas spp. from the Challawa industrial dump site. The isolate showed the greatest reduction at an ideal pH of 7; the isolate removed heavy metals most effectively when incubated for 48 hours; the same isolate removed Pb and Cr efficiently at an ideal temperature of 37°C. Using bacteria in the bioremediation of heavy metals does not always require them to be able to withstand the heavy metals. Therefore, relevant regulatory organizations should regulate the careless dumping of untreated wastes by industries to improve the environment.

Keywords: *Sem pristine, Heavy metal, Polluted environments, Isolate, Dump site.*

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I. Background Of The Study

Heavy metals and other substances that are resistance-promoting agents in the environment can have a serious negative influence on public health. Notably among these heavy metal driving agents are Chromium (Cr) and Lead (Pb) which are usually discharged from tannery industries in Nigeria. Industrialisation as well as advancement in technology have increased the burden on the environment by releasing large quantities of dangerous waste, heavy metals (such as Cd, Cr, and Pb) and metalloids (elements with intermediate properties between those of typical metals and non-metals, such as arsenic and antimony), and organic contaminants that have imposed and caused serious damage on the ecosystem (Gaur *et al.*, 2014; Ayangbenro and Babalola, 2017). The build-up of heavy metals and metalloids in soils and waters continues to create great global health concerns because these metals and metalloids cannot be degraded into non-toxic forms, but persist in the ecosystem (Dixit *et al.*, 2015; Ayangbenro and Babalola, 2017). Contamination of the environment with heavy metals has increased beyond the recommended limit and is detrimental to all forms of life (Tak *et al.*,2013; Ayangbenro and Babalola, 2017). The maximum permissible concentration of some heavy metals in water as stated by the Comprehensive Environmental Response Compensation and Liability Act (CERCLA), USA, is 0.01, 0.05, 0.01, 0 .015, 0.002, and 0.05 mg/L for Ar, Cd, Cr, Pb, Hg, and Ag respectively (Chaturvedi *et al.*, 2015). The standard for soil, as established by the Indian standards for heavy metals, is 3–6, 135–270, 75–150, 250–500, and 300–600 mg/kg for Cd, Cu, Ni, Pb, and Zn respectively (Nagajyoti *et al.*, 2010).

Statement of the Problem

Excessive discharge of large mass of heavy metal containing waste been dumped at Challawa waste disposing site in Kano is a major source of Public Health concern. It can also affect natural bioremediation

process. Heavy metals are a group of pollutants (usually from industrial, agricultural and domestic activities) of major concern in the aquatic environment due to their toxicity (Sundaramoorthi *et al.,* 2011). The relevant toxic metal pollutants like Cr, Cd, Ni and Pb access the water bodies through industrial wastewater treatment plants Jabbari *et al*., (2010). Some of these heavy metals (Pb, Cd and Cr) are nonessential and general toxicant; they are multimedia pollutants which cause pollution of soil, water and atmosphere (Ghorbani *et al.,* 2008). Lead and chromium enter into the environment and human food chain in the process of usage as in lead-based gasoline, paints, gunshot, batteries and alloys. Lead is classified as a 2B carcinogen by the International Agency for Research on Cancer (IARC). As a result of very toxic effects, lead measurement for exposure monitoring is very important (Memon, *et al.,* 2005; Das and Kumari, 2016).

Heavy metal pollution is at present a fundamental environmental problem because metal ions persist in the environment because they are non-degradable in nature. Unlike organic contaminants, heavy metals cannot be broken down by chemical or biological processes. Therefore, they can only be transformed into less toxic species (Ayangbenro and Babalola, 2017). Toxic metals could accumulate in agricultural soils and get into the food chain, thereby becoming a major threat to food security. Heavy metals are also cytotoxic at low concentrations and could cause cancer in humans (Dixit *et al*., 2015).

Cr is a pollutant which exists in nature as the soluble highly toxic Cr (VI) anion and the less soluble, less toxic Cr (III) species (James, 2002). Cr VI is widely used in tanning, metal finishing, petroleum refining, iron and steel industries, inorganic chemical production, and textile processing as well as pulp production (Meriah and Tebo, 2002; Srinath *et al.,* 2002). Tanneries are the basic source of chromium contamination which releases Cr (VI) ranging from 40 – 25,000 mg/l of waste water; while the maximum tolerance of total Cr for public water supply has been fixed at 0.05 mg/l as per Indian standards (Benazir *et al*., 2010).

As a result of its carcinogenicity and mutagenicity, the United States Environment Protection Agency (USEPA) has designated Cr as a "Priority pollutant" also referred to as "Class A" pollutant (Srinath *et al.,* 2002). At high levels, heavy metals like chromium have the capacity to damage cell membranes, alter enzyme specificity; disrupt cellular functions and damage DNA structure (Bruins *et al.,* 2000). Cr (VI) activates p53 by Reactive Oxygen Species (ROS) mediated free radical reactions which occur during the oxidative-reduction of hexavalent Cr within the cell. Oxidative damage is regarded as an important mechanism in the genotoxicity of Cr (VI). Therefore, the need arises to remediate chromium before being discharged in the ecosystem (Benazir *et al.,* 2010).

Against these backdrops, this study seeks to provide answers to the following research questions: what heavy metal tolerant bacteria can be isolated, identified, and screened from semi pristine and polluted environments; do the isolates have the ability to bioremediate heavy metals (Cr and Pb) in polluted soil; what are the optimum physical conditions for bioremediation such as pH, temperature and incubation time using one factor at a time?

The aim of this study is to evaluate the ability of isolates to bioremediate heavy metals and assess the optimum conditions for bioremediation in semi pristine and polluted environments. The specific objectives are: to isolate, identify and screen heavy metal tolerant bacteria from semi pristine and polluted environments; to screen the isolates for the ability to bioremediate heavy metals (Cr and Pb) in polluted soil; to optimize physical conditions for bioremediation such as pH, temperature and incubation time using one factor at a time.

Standard approaches commonly applied to remove heavy metals from waste water and contaminated soil is: chemical (precipitation, neutralization) or physical (ion exchange, membrane separation, electro dialysis and activated carbon adsorption) methods (Das and Kumari, 2016). These processes may be non–viable at low concentrations. These processes are however expensive and not eco-friendly (Das and Kumari, 2016). Bioremediation technology has provided an alternative to regular techniques for remediating the metal-polluted soils (Khan, 2009). In the process of bioremediation microorganisms or their enzymes are used to return an environment which was earlier altered by contaminants to its original condition (Vinay *et al*., 2013). In relation to biosorption mechanisms, the implication of complex structure of microorganisms is that there are many ways for the metal to be taken up by the microbial cell. Biosorption mechanisms vary; and they may be classified with respect to a number of criteria. With respect to the dependence on the cell's metabolism, biosorption mechanisms can be divided into metabolism dependent, and non-metabolism dependent.

Method of Data Analysis

In order to isolate, identify, and screen heavy metal tolerant bacteria from semi-pristine and polluted environment, the following step-wise procedure were taken:

Identification and analysis of heavy metal concentrations in soil sample

Two heavy metals (Cr and Pb) in the soil sample were determined. 1g of the soil sample was weighed and prepared for digestion. Digestion was carried out in triplicates of 5ml of batches of the sample in a mixture of nitric acid and hydrochloric acid followed by heating at 100^{o}C for 45minutes to 1hour to almost dryness and

the volume made up to 60ml with distilled water. The digest was filtered to remove insoluble material that could clod the atomizer as was done by Begum *et al.,* (2009). The filtrates were then analyzed using Atomic Adsorption Spectrophotometry (Shimazu AAs, model AA-6800, Shimazu corporation) at the central laboratory of Bayero University Kano.

The soil sample from the semi pristine environment and soil sample from the polluted area were serially diluted respectively to about 10^{-8} dilutions, in saline solution and 1ml each from the different samples from 10^{-5} to 10^{-8} was pour plated in nutrient agar plates. The plates were incubated at 37ºC for 24 hours in an inverted position (Velusamy, *et al.,* 2011). Bacteria colonies were then picked and sub cultured on a nutrient agar plate supplemented with Pb (NO_3) and Cr (VI) at concentration (10mg/l and 20mg/l) respectively. The inoculated plates were incubated for 48 hours at 37ºC. The well grown cultures on the nutrient agar plates supplemented with heavy metal were further inoculated on a nutrient agar slant in their pure form for further studies.

Bioremediation Activity Assay using Bacteria

The bioremediation potential of the isolates for removing heavy metal was assessed by batch experiment process. The bacteria were cultured in Nutrient Broth (NB) supplemented with the heavy metal salts at concentration of 10mg/l and kept under agitation in a rotatory shaker at 180rpm for 72 hours at 37ºC (Pathak *et al.*,2022). The samples were then centrifuged and further analyzed by AAS for the final heavy metal concentration (Pathak *et al.,* 2022).

The percentage (%) removal of the heavy metal was calculated for each as

% Removal = Ci – Ct/ Ct × 100

Where Ci is initial the concentration of heavy metal in the solution

Ct is the final concentration of heavy metal in the solution.

Optimization for Heavy Metal Removal

Temperature, pH and incubation time are the major factors which affects the adsorption process. Particularly, pH on bioremediation experiments was investigated by optimization process. The bacterial isolates were inoculated into Nutrient Broth (NB) medium amended with the heavy metals at varying conditions. To find out the optimum temperature for maximum adsorption of this toxic heavy metal, strains were incubated with a wide range of temperature of 30, 35, 37, 40, 45℃. The pH was varied from 5, 6, 7, 8, 9 while the incubation time was varied from 24, 36, 48, 60 and 72 hours. All the tests were performed in triplicate in the order of Babapoor *et al.,* (2022).

Isolation, Identification and Screening Heavy Metal Tolerant Bacteria from Semi Pristine and Polluted Environments

Table 1 reveals the preliminary concentration of the heavy metals (Cr and Pb) before the experiment began. It was shown from the result obtained from the analysis of the polluted soil that samples collected from the industrial dump site were more contaminated with Cr compared to soil samples collected along the canal with the highest concentration of 22.10 ± 0.113. When compared with the environmental protection regulatory limits the dump site results (D1) were found to exceed the standard. Furthermore, the major concentration results obtained were compared with the environmental protection regulatory limits as Abd *et al.,* (2013); and all were found to exceed the acceptable standard with highest concentration at the dumpsite (D1) 3.189 ± 0.240.

It was observed from the result in Table 2 that heavy metals (Cr and Pb) concentrations were all below the pollution regulatory limits in all the soil samples gotten from the semi pristine environments when compared to Nath *et al.,* (2012).

Table 3 presents a total of 7 bacteria which were isolated and identified as *Bacillus spp.* (2), *Pseudomonas spp.* (3), *Acinetobacter spp.* (1), and *Staphylococcus spp.* (1) after biochemical tests (Gram Stanning, Catalase test, Coagulase test, Citrate test, Indole test, Methyl Red test, Oxidase test, Hydrogen Sulphide test, Vogas Proskauer test, Urase test, Spore formation test, Tripple Sugar Iron test, Gelatin Hydrolysis test, and Gas Production test) were conducted on the bacteria isolates from the polluted and semi pristine environments, this finding is consistent with previous work by Benazir *et al*., (2010); Abbas *et al.,* (2014) .

Presented in Tables 4 and 5 are the tolerance levels of the isolates to Cr and Pb; these levels were used to screen the organisms after 48 hours Chihomvu *et al*., (2014); Syed and Chinthala, (2015); Hossain and Anwar, (2016) did. In both the Cr and Pb heavy metal concentrations, the first two (2) bacteria organisms were obtained from polluted environment while the other organisms were obtained from semi pristine environments. At 50mg/l and 100mg/l of the heavy metal concentrations all the bacteria isolates were observed to have grown after 48 hours of exposure in Cr and Pb concentrations, this finding was similar to that of Chihomvu *et al*., (2014). It was however noticed that while all the bacteria isolate in Lead concentration grew after 48 hours, only three isolates (*Bacillus spp., Pseudomonas spp.* (D2), *and Pseudomonas spp*. (E2)) grew in Cr concentration

after the same 48 hours period. While the two (2) bacteria isolates from the polluted environment (*Bacillus spp.* (D1), and *Pseudomonas spp.* (D2)) were observed to have grown at 200mg/l concentration of both Cr and Pb heavy metals after 48 hours, the other five (5) isolates from semi pristine environments (*Acinetobacter spp.*(P), *Pseudomonas spp.* (B1), *Bacillus spp.* (B2), *Staphylococcus spp.* (E1), and *Pseudomonas spp.* (E2)) did not grow upon exposure to the same concentration of both Cr and Pb heavy metals after 48 hours, this finding is also similar to Hossain and Anwar, (2016) previous submission. At a concentration of 250mg/l exposure to Cr only *Pseudomonas spp.* (D2) from the polluted environment grew after 48 hours and others did not grow, but the bacteria isolates were exposed to the same concentration of 250mg/l of Lead *Bacillus spp.* (D1), and *Pseudomonas spp.* (D2) grew after 48 hours.

Table 1: Preliminary Analysis of Heavy Metals Concentrations in Polluted Soil Samples

Samples	Chromium Concentration (mg/l)	Lead Concentration (mg/l)
C1	0.843±0.023	0.241±0.045
C2	0.397±0.018	0.147±0.009
D1	22.10±0.113	3.189±0.240
D2	8.863±0.016	0.256±0.046

Source: Experimental Analysis, (2023)
WHO/FEPA Regulatory Limit: Chromium 2.000mg/l; Lead 0.010mg/l

While bacteria isolate from both polluted and pristine environments did not grow when they were exposed to 300mg/l, 350mg/l and 400mg/l of Cr heavy metal respectively after 48 hours, upon exposure of the bacteria isolates to 300mg/l and 350mg/l concentration of Pb heavy metal, *Bacillus spp.* (D1), and *Pseudomonas spp.* (D2) which were both from the polluted environment grew after 48 hours but other isolates from the semi pristine environments did not grow within the period of time; in addition, when the isolates were exposed to 400mg/l of Pb concentration only *Pseudomonas spp.* (D2) grew after 48 hours but organisms did not grow this finding is consistent with submission by Syed and Chinthala, (2015).

Table 2: Preliminary Analysis of Heavy Metals Concentrations in Semi Pristine Environment

Sample	Chromium Concentration (mg/l)	Lead Concentration (mg/l)
Geneva (P)	0.056 ± 0.128	0.002 ± 0.516
BUK Botanical Garden (B)	1.050 ± 0.005	0.006 ± 0.117
BUK Ecological Garden (E)	1.782 ± 0.036	0.008 ± 0.048

Source: Experimental Analysis, (2023)
WHO/FEPA Regulatory Limit: Chromium 2.000mg/l; Lead 0.010mg/l

In order to determine the concentration at which the bacteria isolate from both the polluted environment and the semi pristine environment could no longer grow, the Minimum Inhibitory Concentration (MIC) test was conducted for Cr and Pb heavy metals in a similar manner as Dewi *et al.,* (2019). Result of the MIC test as presented in Table 6 reveals the MIC at which the bacteria isolates could tolerate before they could no longer tolerate the Cr and Pb heavy metals after 48 hours. It was observed that the MIC for *Bacillus spp.* D1 upon exposure to Cr could no longer grow after 220mg/l concentration but grew on at that concentration when it was exposed to Pb until the concentration became 385 mg/l and it could not grow after 48hours of exposure to the heavy metal. The MIC at which *Pseudomonas spp.* D2 could no longer grow upon exposure to Cr heavy metal was 280mg/l; it however required more concentration (420mg/l) of Lead to stop the organism from growing. There was no wide difference in the MIC of *Acinetobacter spp.* P and *Staphylococcus spp.* B2 which both had the same MIC values when exposed to Cr and Pb heavy metals as their MIC was found to be 120mg/l and 150mg/l respectively this is in tandem with Huet and Puchooa, (2017). The MIC of *Pseudomonas spp.* B1 revealed the same concentration (120mg/l) upon which they could no longer grow after 48 hours when exposed to both Cr and Pb heavy metals; the MIC of *Pseudomonas spp.* E2 also showed the same result in which the same concentration (150mg/l) was observed upon which the isolates could no longer grow after 48 hours of exposure to both Cr and Pb heavy metals this finding is consistent with previous studies by Marzan *et al.*, (2017); Soumitra *et al.*, (2019).

Table 3: Isolation and Identification of Bacteria from the Soil Samples from Polluted and Semi Pristine Environments

Isolate code	Gram Stain	Shape	Catalase	Coagulase	Citrate	Indole	Methyl red	Oxidase	H_2S	VP	Urase	Spore formation	TSI	Gelatin hydrolysis	Gas	Inference
1	+	Rod	+	+	+	-	+	-	+	-	-	+	Y/Y	+	-	*Bacillus spp.*
2	-	Rod	+	-	+	-	-	+	-	-	-	-	R/R	+	+	*Pseudomonas spp.*
3	-	Cocco bacilli	+	-	+	-	-	-	-	-	-	+	Nd	-	-	*Acinetobacter spp.*
4	-	Rod	+	-	+	-	-	+	-	-	-	-	R/R	+	+	*Pseudomonas spp.*
5	+	Rod	+	+	+	-	+	-	+	-	-	+	Y/Y	+	-	*Bacillus spp.*
6	+	Rod	+	-	+	-	-	+	-	-	-	-	Nd	+	+	*Staphylococcus spp.*
7	-	Rod	+	-	-	-	-	+	-	-	-	-	R/R	+	+	*Pseudomonas spp.*

Source: Experimental Analysis, (2023)

Key:

+ = Positive
- = Negative
VP = Vogas Proskauer test
TSI = Tripple Sugar Iron test
H_2S = Hydrogen Sulphide test
Isolates 1 and 2 = Dumpsite
Isolates 3 and 4 = Semi pristine;
Isolates 5 and 6 = Botanical Garden
Isolates 7 = Ecological Garden
Nd = not done

Table 4: Tolerance Level of the Isolates to Chromium (Cr) after 48 hours

Samples	50 mg/l	100 mg/l	150 mg/l	200 mg/l	250 mg/l	300 mg/l	350mg/l	400 mg/l	Isolate
D1	+	+	+	+	-	-	-	-	*Bacillus spp.*
D2	+	+	+	+	+	-	-	-	*Pseudomonas spp.*
P	+	+	-	-	-	-	-	-	*Acinetobacter spp.*
B1	+	+	-	-	-	-	-	-	*Pseudomonas spp.*
B2	+	+	-	-	-	-	-	-	*Bacillus spp.*
E1	+	+	-	-	-	-	-	-	*Staphylococcus spp.*
E2	+	+	+	-	-	-	-	-	*Pseudomonas spp.*

Source: Experimental Analysis, (2023)

Key:

+ = Growth
- = No Growth
D1 and D2 = dump site soil sample E1 and E2 = Ecological Garden soil
P = Geneva soil sample
B1 and B2 = Botanical Garden soil sample

According to Srivastava and Thakur, (2007) *Acinetobacter spp.* isolated from pulp paper mill effluent could tolerate Cr (VI) concentration up to 500 mg/l. The MIC of *Bacillus spp.* E1 when exposed to Cr heavy metal revealed a concentration of 160mg/l upon which it could no longer grow after 48 hours but the isolate had earlier stopped to grow after 48 hours of exposure to Pb heavy metal when the concentration was 150mg/l.

Table 5: Tolerance Level of the Isolates to Lead (Pb) after 48 hours

Isolates	50mg/l	100mg/l	150mg/l	200mg/l	250mg/l	300mg/l	350mg/l	400mg/l
Bacillus spp. D1	+	+	+	+	+	+	+	-
Pseudomonas spp. D2	+	+	+	+	+	+	+	+
Acinetobacter spp. P	+	+	+	-	-	-	-	-
Pseudomonas spp. B1	+	+	+	-	-	-	-	-
Bacillus spp. B2	+	+	+	-	-	-	-	-
Staphylococcus spp. E1	+	+	+	-	-	-	-	-
Pseudomonas spp. E2	+	+	+	-	-	-	-	-

Source: Experimental Analysis, (2023)

Key:

+ = Growth
- = No Growth
D1 and D2 = dump site soil sample; E1 and E2 = Ecological Garden soil sample
B1 and B2 = Botanical Garden soil sample; P = Geneva soil sample

Table 6: MIC of the Isolates to Chromium and Lead

Isolates	Cr MIC (mg/l)	Pb MIC (mg/l)
Bacillus spp. D1	220	385
Pseudomonas spp. D2	280	420

Acinetobacter spp. P	120	150
Pseudomonas spp. B1	120	120
Staphylococcus spp. B2	120	150
Bacillus spp. E1	160	150
Pseudomonas spp. E2	150	150

Source: Experimental Analysis, (2023)

Screening of Isolates for the Ability to Bioremediate Heavy Metal (Chromium and Lead)

Tables 7 and 8 show the results of the percentage removal of heavy metals from the environments after the isolates were exposed to heavy metals from both the polluted and semi pristine environments. All the organisms were subjected to a concentration of 10mg/l of the heavy metals and the percentage removal was estimated with respect to the final concentration that was left after 72 hours that the isolates were subjected to the concentration heavy metals as was also done by Audu *et al.,* (2020). While upon exposing *Bacillus spp.* 1 to 10mg/l of Cr heavy metal, it was observed that the organism removed 49.81% of the heavy metal from the polluted soil sample; when the same isolate was exposed to 10mg/l of Lead heavy metal, the organism removed 63.32% of the heavy metal. When *Pseudomonas spp.* 2 isolate was exposed to 10mg/l of Cr heavy metal, the organism was found to have removed 58.28% of the heavy metal from the polluted soil sample; but when the same bacteria isolate was exposed to 10mg/l of Lead heavy metal, the organism was discovered to have removed 72.97% of the heavy metal from the polluted soil sample. *Acinetobacter spp.* 3 isolate was discovered to remove 32.17% of Cr heavy metal concentration from the semi pristine soil sample but when the isolates were exposed to Lead heavy metal, 49.17% of the Lead concentration was removed from the semi pristine soil sample. *Pseudomonas spp.* 4 bacteria isolate was found to remove 25.79% of Cr heavy metal concentration from the semi pristine soil sample; the same bacteria isolate when exposed to Lead heavy metal concentration removed 51.27% of the Pb heavy metal concentration from the semi pristine soil sample. While *Staphylococcus spp.* 5 isolates removed 26.82% of Cr heavy metal concentration in the semi pristine soil sample, the same bacteria isolate removed 36.37% of Pb heavy metal concentration in the semi pristine soil sample. It was also discovered that while *Bacillus spp.* 6 isolates removed 40.12% of the of Cr heavy metal concentration in the semi pristine soil sample, the same organism removed 18.53% of Pb heavy metal concentration in the semi pristine soil sample. While *Pseudomonas spp.* 7 isolates removed 39.86% of Cr heavy metal concentration in the semi pristine soil sample, the same bacteria isolates removed 21.69% of Pb heavy metal concentration in the semi pristine soil sample.

Table 7 Screening of the Isolates for Ability to Bioremediate Chromium

Isolate	Initial Concentration (mg/l)	Final Concentration (mg/l)	% Removal
Bacillus spp. 1	10.00	5.019 ± 0.110	49.81%
Pseudomonas spp. 2	10.00	4.172±0.09	58.28%
Acinetobacter spp. 3	10.00	6.783±0.043	32.17%
Pseudomonas spp. 4	10.00	7.421±0.178	25.79%
Staphylococcus spp. 5	10.00	7.318±0.025	26.82%
Bacillus spp. 6	10.00	5.988±0.222	40.12%
Pseudomonas spp. 7	10.00	6.014±0.018	39.86%
Control	10.00	8.886±0.045	11.14%

Source: Experimental Analysis, (2023)

Table 8 Screening of the Isolates for Ability to Bioremediate Lead

Isolate	Initial Concentration (mg/l)	Final Concentration (mg/l)	% Removal
Bacillus spp. 1	10.00	3.668± 0.021	63.32%
Pseudomonas spp. 2	10.00	2.703± 0.051	72.97%
Acinetobacter spp. 3	10.00	5.081±0.030	49.17%
Pseudomonas spp. 4	10.00	4.873±0.041	51.27%
Staphylococcus spp. 5	10.00	6.363±0.040	36.37%
Bacillus spp. 6	10.00	8.147±0.040	18.53%
Pseudomonas spp. 7	10.00	7.831±0.667	21.69%
Control	10.00	9.19±0.128	8.1%

Source: Experimental Analysis, (2023)

Pseudomonas spp. 2 from the polluted dump site was observed to have the highest percentage (58.28%) removal of Cr as the most efficient bioremediating agent of Cr and the least efficient bioremediating agent of Cr was *Pseudomonas spp.* 4 which was obtained from semi pristine environment with 25.79.0% removal of the heavy metal. On the other hand, *Pseudomonas spp.* 2 from the polluted dump site was also observed to have the highest percentage (72.97%) removal of Pb as the most efficient bioremediating agent of Lead this is in tandem with previous findings by Girma, (2015); Mihdir *et al*., (2016); Nath *et al.,* (2019) and

the least efficient bioremediating agent of Pb was *Bacillus spp.* 6 obtained from botanical garden with 21.69% removal of the heavy metal this finding is consistent with previous study by Benazir *et al*., (2010).

Optimisation of Physical Conditions for Bioremediation

Figures 1 and 2 reveal the effect of pH on bioremediation of heavy metals (Pb and Cr) for the optimization of the best bioremediating isolate (*Pseudomonas spp.* 2) which was from the polluted dump site environment. The *Pseudomonas spp.* 2 isolate was found to be more effective as a bioremediating agent for both Pb and Cr heavy metals at a pH of 7 at which it bioremediated 73.22% and 79.93% of Pb and Cr from the dump site respectively.

Figures 3 and 4 show the optimal incubation time for the *Pseudomonas spp.* 2 which is the best bioremediating isolate to bioremediate Pb and Cr heavy metals from the polluted dump site environment respectively. The optimal incubation time for the heavy metals was at 48 hours where the isolate bioremediated 77.19% and 75.08% of Pb and Cr from the polluted soil environment respectively.

Furthermore, Figure 5 and 6 present the optimal temperature for the best bioremediating agent (*Pseudomonas spp.* 2) to bioremediate Pb and Cr heavy metals from the polluted dump site environment respectively. The optimal temperature for the heavy metals was 37ºC at which the *Pseudomonas spp.* 2 isolate bioremediated 89.81% and 75.11% of Pb and Cr from the polluted dump site environment respectively.

II. Conclusion And Recommendation

The study concludes that the development of resistance by environmental bacteria is not caused by chronic exposure to heavy metals or antibiotics as bacteria isolated from polluted and semi pristine environments exhibited similar antimicrobial pattern.

Bacteria do not necessarily need to tolerate heavy metals to be used in the bioremediation of heavy metals contaminated soil samples.

It is therefore recommended that indiscriminate untreated waste disposal by industries should be controlled by all the regulatory bodies concerned so as to have a better environment.

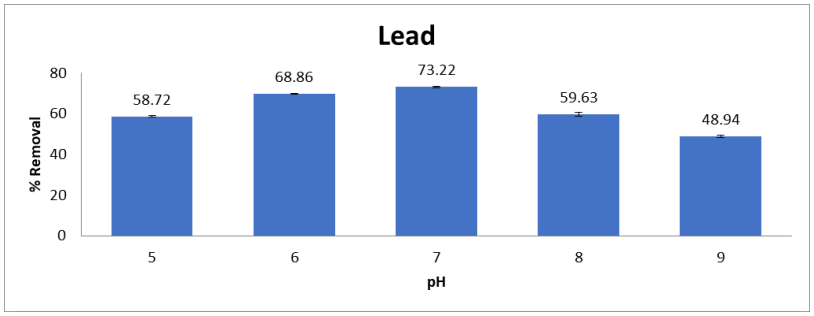

Figure 1: Optimal Ph In Bioremediating Lead From Polluted Dump Site Environment
Source: Experimental Analysis, (2023)

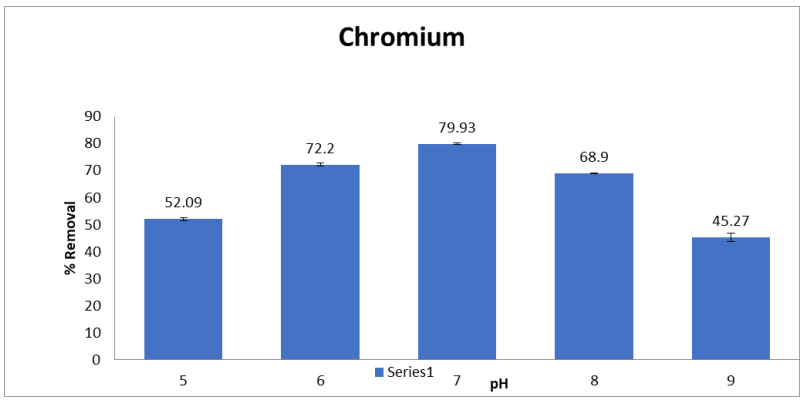

Figure 2: Optimal Ph In Bioremediating Chromium From Polluted Dump Site Environment
Source: Experimental Analysis, (2023)

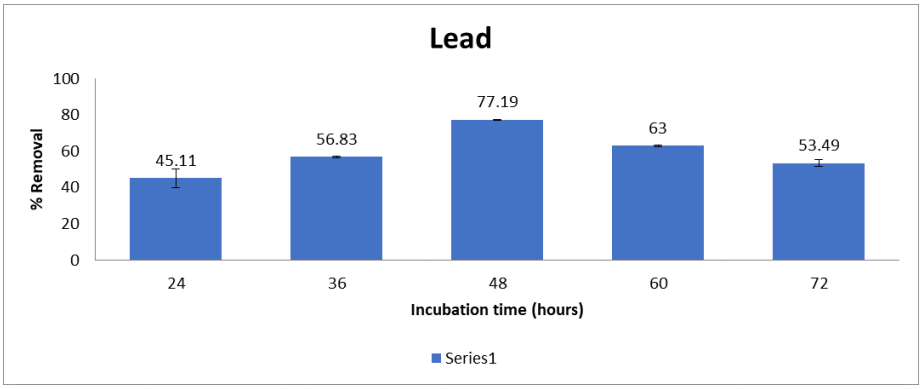

Figure 3: Optimal Incubation Time In Bioremediating Lead From Polluted Dump Site Environment
Source: Experimental Analysis, (2023)

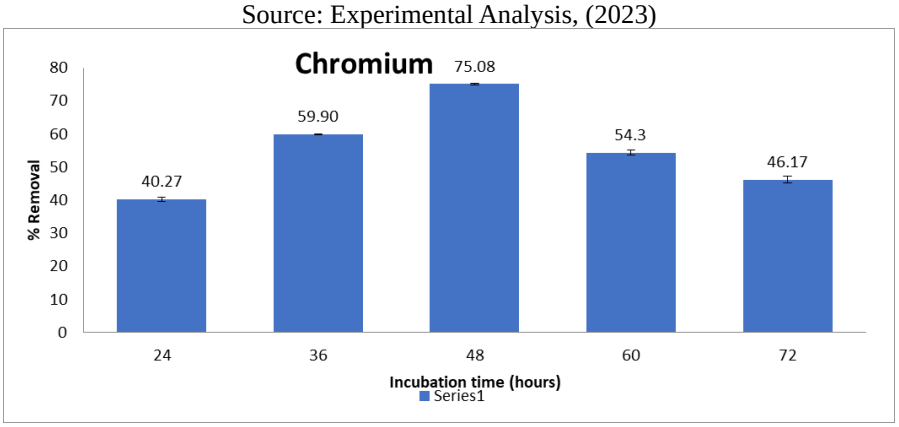

Figure 4: Optimal Incubation Time In Bioremediating Chromium From Polluted Dump Site Environment
Source: Experimental Analysis, (2023)

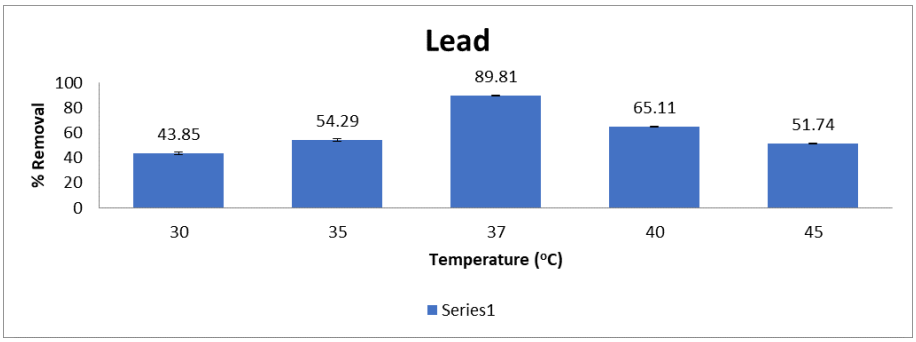

Figure 5: Optimal Temperature In Bioremediating Lead From Polluted Dump Site Environment
Source: Experimental Analysis, (2023)

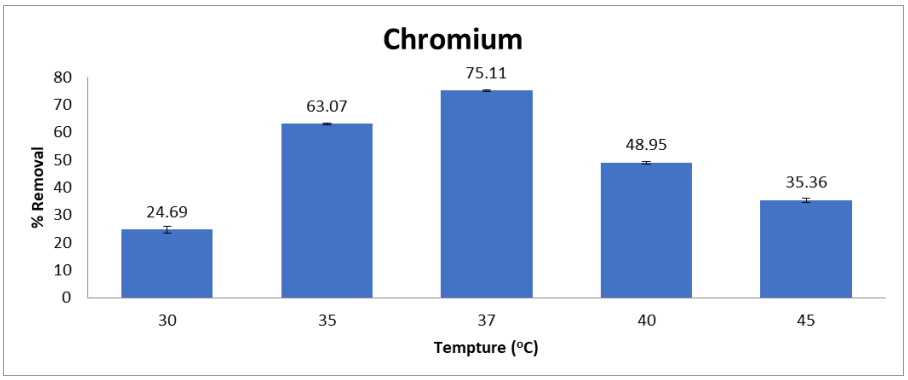

Figure 6: Optimal Temperature In Bioremediating Chromium From Polluted Dump Site Environment
Source: Experimental Analysis, (2023)

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