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Isolation and Assessment of Metal Tolarance Bacteria for Heavy Metal Removal

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ABSTRACT
Bioremediation is the application microorganisms to reduce pollutant concentration of which is a natural process and its importance is increasingly considered for clean-up of metal contaminated and polluted ecosystem. Soil samples collected at three different locations namely UnguwaUku Mechanic Shop, KwakwacinDawanau Car Park Garage and School of Technology Botanical Garden were analyzed in order to determine the level of heavy metal contamination and bacteria diversity with the view to assess the bioremediation potentials of the isolated bacteria. Four heavy metal tolerant bacteria were isolated from the areas. These were identified by morphological and biochemical techniques as Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Klebsiellapneumoniae. Heterotrophic bacterial counts of the soil samples obtained from SOT site after 24, 48and 72hours of incubation were 2.08 x 10^5cfu/g, 2.24 x 10^5cfu/g and 2.56 x 10^5cfu/g respectively. These values were comparatively higher than those of soil samples obtained from the remaining two locations.The soil samples obtained from KofarRuwa and that of KwakwacinDawanau were found to be more neutral (pH 7.4 and pH 7.6) respectively. The moisture content and organic matter of both soil samples were found to be lesser than the SOT site (2.4%). The temperature of the SOT site (32.6˚C) is lesser compared to that of Kofarruwa (33.0˚C) and KwakwacinDawanau (38.8˚C).The concentration of Lead, Chromium, Zinc, Copper and Iron were determined with the help of Atomic Absorption Spectroscopy. Screening for heavy metal tolerant bacteria by plate diffusion method was conducted and the speciesS1, S2, S4 and S7 were identified as efficient species that were resistant toPb, Cr, Zn, Fe, and Cu respectively. Pseudomonas aeruginosa showed maximum tolerance to Pb (10 mM) while E. coli showed maximum tolerance to Fe (10mM). No Significance difference was observed between the heavy metals tolerant test of all the four bacterial isolates at 5% level of significance (P>0.05).

Key words: Mechanic workshop, heavy metals, bacteria isolates, bioremediation, Metal-tolerant

I. INTRODUCTION

Background to the study

Heavy metal is a general term used to describe a collection of metals and metalloids with an atomic density greater than 5.0 g/cm^3. These elements occur naturally in soils and rocks at different concentrations; they are also found in ground, surface water bodies and sediments(Duffus, 2002). Unchecked industrial and human activities have contributed significantly to pollution levels of these metals, in surface and subsurface soils when compared to those contributed from geogenic or natural processes (Dasaramet al., 2010).Soil being one of the repositories for anthropogenic waste, biochemical processes can mobilize the chemical substances contained in it to pollute water supplies and impact food chains thereby causing great harm to man. The high toxic and persistent natures of heavy metals in the environment have made them priority pollutants (Abechiet. al., 2010).

The sources and mechanism of discharge of heavy metals into the soil and water resource include; engine and lubricating oil, engine and gear box recycling, battery charging, welding and

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soldering, automobile body work and spraying painting and combustion processes (Adewole and Uchegbu, 2010). Wastes originating from such activities include spent lubricants, hydraulic fluids, worn-out parts, packaging materials, metal scraps, used batteries, discarded cans and stripped oil sludge. The heavy metals most frequently encountered in these wastes include copper, lead, cadmium, zinc, manganese and nickel, all of which pose risks for human health and the environment especially soil fertility levels. Conventional methods to remediate heavy metals contaminated site are excavation and solidification, these technologies are suitable to control contamination but not permanently remove heavy metals (Bahiet al., 2012). However, they have some disadvantages; among them are cost-effectiveness limitations, generation of hazardous by-products or inefficiency. On the other hand, biological methods potentially solve these drawbacks since they are easy to operate, do not produce secondary pollution (Su, 2014). Heavy metals having relatively high density are toxic at low concentration (Iramet al., 2013).

Microorganisms and plants are usually used for the removal of heavy metals. Application of microorganisms to reduce pollutant concentration is known as bioremediation which is a natural process and its importance (above or below the ground) is increasingly considered for clean-up of metal contaminated and polluted ecosystem. All the metals are toxic, but some of these are useful in low concentration (El-Shahawiet al., 2010).

Unlike organic pollutants, metals cannot be degraded to harmless products, such as carbon dioxide, but rather persist indefinitely in the environment, complicating their remediation. Once heavy metals enter the environment, biological systems including microorganisms can accumulate and introduce the metals into food webs. Metals can exert toxic effects on microorganisms. However, metal exposure leads to the establishment of tolerant microbial populations, which are often represented by several Gram-positives belonging to Bacillus, Arthrobacter and Corynebacterium, as well as Gram-negatives such as Pseudomonas, Alcaligenes, Ralstonia, and Burkholderia (Ellis et al., 2003). In order to make the environment healthier for human beings, contaminated water bodies and land need to be rectified to make them free from heavy metals and trace elements. Metal-tolerant bacteria surviving in these habitats could be isolated, identified, and selected for their potential application in bioremediation of contaminated sites.

Statement of Research Problem

World Health Organization (WHO) estimates that about a quarter of the diseases facing mankind today occur due to prolonged exposure to environmental pollution (Adams et al., 2014). A number of technologies have been investigated to remedy the situation. Treatment processes have incorporated chemical, physical and biological methods or a combination of them. Treatment option include excavation and landfill disposal, incineration, surfactant application and a host of others that are expensive, environmentally unfriendly and only transfer the contaminants from one place to another. Heavy metal contamination is very possible in Kano metropolis considering the rampant activities and improper disposal of heavy metal-containing material handled in mechanic workshop clusters. These workshops are widely located in areas such as KofarRuwa market, UnguwaUku motor park, and Gadan Kaya/Tal'udu among several others. These places are for repairs and servicing of motor vehicles and other machineries.

Justification

Environmental contamination by heavy metals is consequently the most critical environmental problem as it poses significant impacts to the health of humans as well as the numerous ecosystems that support it. The contaminants infiltrate deep into underground, pollute ground water and surface water. There is a probability of entering the human food web through plants and aquatic animals that bioaccumulate them, consequently transferring them from one food chain to another in a process known as biomagnification. Bioremediation is inexpensive, naturally and socially acceptable and environmentally friendly. The use of heavy-tolerant bacteria as a bioremoval under study will help minimize the prohibitive cost associated with soil remediation, prevent significant soil texture alteration, prevent the transfer of pollutants from one medium to another and ensure a healthier technique for remediating heavy metals.

Aim and objectives

Aim

The aim of this study was to isolate metal tolerant bacteria and assess their potential in removing heavy metals from soil samples of two different mechanic sites (battery charging, welding and soldering)

Objectives

1. To determine the physico-chemical characteristics of the contaminated workshops soil

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2. Toconduct Heterotrophic bacterial counts of the soil samples obtained from mechanic workshops.
3. To conduct Biochemical characterization of the isolated bacteria
4. To Screen for heavy metal tolerant bacteria by plate diffusion method
5. To evaluate Maximum Tolerable Concentration (MTC) of heavy metals by bacterial isolates
6. To determine heavy metal removal by bacteria using atomic absorption spectrophotometer (AAS).

II. MATERIALS AND METHODS

Sample Site

Three sampling sites were selected for this study, consisting of two different workshops; one located at KofarRuwa Market (12.0272°N, 8.4974°E) where car battery charging is the main activities, and the other one located at Kwakwacindawanau Motor park (11.966°N, 8.5639°E) which are involved in welding and soldering, and also a control site at Ecological Study Area of School of Technology, Kano (11.9818°N, 8.4801°E) (this area is free from any human activities). All the sampling locations were be within Kano metropolis

Sample Collection

Soil samples were collected randomly using quadrant sampling technique and homogenized from five different spots. The soil samples were collected at a depth of 0-10 cm below the soil surface using soil auger and put into sterile Polyethene bags and transported to the Microbiology Research Laboratory, SOT Kano, Nigeria. The soil samples were stored in a refrigerator at 4^0C until they were analyzed

Physico-chemical Analysis of the Soil Samples

Determination of Hydrogen-ion Concentration (pH)

Twenty (20) grams of soil sample were air dried after sieving through 2-mm mesh size and transferred into a 50ml-beaker to which 20ml of distilled water were added. The soil suspension were stirred several times for 30 minutes using a glass rod. The suspension were allowed to stand for 30 minutes. The electrode of the pH meter (Jenway 3051 model) was then inserted into the partly settled suspension and measured the pH (Omotayoet al., 2012).

Determination of Total Organic Carbon (TOC)

The soil sample was grinned and passed through 0.5mm sieve and 1.00g soil was placed in a 250ml conical flask containing 10ml of 1N $K_2Cr_2O_7$ solution. The flask was swirled gently to dispense the soil. A quantity (20ml) of concentrated H_2SO_4 was added immediately and swirled gently until the soil and reagents were mixed. The contents were then swirled more vigorously for a minute. The flask was rotated again and allowed to stand on a sheet of asbestos for30 minutes after which 100ml of distilled water was added and allowed to cool. The suspension was filtered and followed by the addition of 5ml of O-phosphoric acid after which 3 drops of o-phenanthroline indicator was added and the contents titrated against 0.5N ferrous sulphate on a white background. As the end point approached, the solution turned to a greenish cast and then changed to dark green. At this point, the ferrous sulphate was added in drops until the color changed sharply from blue to red (maroon color) in reflected light against the white background. Blank determination in the same way was made but without soil to standardize the dichromate (Mclean, 1965). The result was calculated according to the following formula:

% Organic carbon (air-dried soil)=

$$\frac{\text{(Blank titer – Actual titer) x 0.3 x M x F}}{\text{Weight (g) of air dried soil}}$$

Where: M = Concentration of $FeSO_4$ and F=Correction factor = 1.33

Determination of Moisture Content

Five (5) grams of soil sample was transferred into pre-weighed can box with tight-fitting lid. The can box containing the moist soil sample was weighed immediately and placed with its lid off in the drying oven at a temperature of 105^oC (to dry the soil to constant weight) for 24hr after which the set up was removed from the oven and immediately covered with its lids, cooled in dessicator and weighed again (Luyaforet al., 1990). The moisture content of the soil sample was determined using the following formula:

Moisture content (%) =

$$\frac{W_2 - W_3\ (g) \times 100}{W3 - W1\ (g)}$$

Where:
W_1 = Weight of empty soil can box (g).
W_2 = Weight of can box + moist soil (g).
W_3 = Weight of can box + oven dry soil (g).

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Determination of heavy metal concentrations

About 3 g sieved soil samples were digested with a mixture of 10 ml concentrated hydrochloric acid (HCl) and 3.5 ml concentrated nitric acid (HNO_3). The mixtures were left overnight without heating under the switch-on fume cupboard and were heated for 2 hours to 140^{o}C on the next day. Distilled water was added to cool the digested sample and then filtered with filter paper and topped up to 100 ml with distilled water. The concentration of heavy metals Cu, Cr, Zn, Pb and Fe in the top soil samples were analyzed using an atomic absorption spectrometer (AAS) (Najibet al., 2012).

Microbiological Analysis

Total Heterotrophic Bacterial Count

Samples were enumerated by making ten-fold dilutions of the soil samples from 10^1 to 10^5. Aliquot 0.1ml of the 10^{-3} dilution was transferred and plated in nutrient agar amended with nystatin (0.5-1 µg/ml) for isolation of bacteria (Adams et al., 2014). The plates were prepared and inoculated in duplicates. The inoculated nutrient agar plates were then incubated at 37°C for 24 hours. After incubation, the colonies of the isolates were counted and expressed in CFU/g.; isolated colonies were then further purified by repeated sub-culturing

Identification of selected heavy metal-tolerant bacterial isolates

Bacterial isolates that exhibited the heavy metal-tolerant potential were then identified according to Bergey‘s manual of systemic bacteriology and other biochemical tests (Cheesebrough, 2013).

Gram’s staining

After the smear was prepared, the slide was flooded with crystal violet and allowed to stand for one minute. It was then washed off with tap water and then flooded with Gram‘s iodine (a mordant) and left for one minute after which the smear was washed off with tap water again, decolorized with alcohol (95%) until no more color washed off. This was the most critical step; care was taken so as not to over decolorize the smear as many Gram-positive organisms may lose the violet stain easily and thus appear to be Gram-negative after they are counterstained. The smear was washed off with tap water and safranin (counter stain) was applied and allowed to stand for 30 seconds. The smear was then washed off with tap water, drained and blotted gently with bibulous paper. The slide was air dried thoroughly before examined under the microscope. Gram-positive organisms retained the primary stain while Gram-negative ones took up the secondary (counter) stain (Cheseebrough, 2003).

Catalase Test

This was carried out by introducing 2.0ml of hydrogen peroxide on a clean, grease-free glass tube. With the edge of a sterile glass rod, a colony of organism was picked and introduced onto the hydrogen peroxide on the slide. The presence of bubbles indicated a positive reaction while absence of bubble indicated a negative reaction (Bhattacharya et al., 2002).

Urease Test

Urea agar medium was inoculated with culture and incubated at 37ºC temperature for 48 hours. After incubation, change of colour from light orange to pink showed a positive result (Kummerer, 2004).

Oxidase Test

Few drops of 1% aqueous solution of tetramethyl-p-phenylenediamine hydrochloride was added to a loopful of bacterial culture on a clean slide. The slide was observed for development of purple colour within 5 seconds and result was taken (Cheesebrouhg, 2003).

Citrate Utilization Test

This was carried out by inoculating the test organism in a test tube containing Simon‘s citrate medium, was incubated for 24 hours. The development of deep-blue colour after the incubation period indicated a positive result (Udeaniet al., 2009).

Assessment of heavy metal resistance potential

Heavy metal resistance was determined by the plate diffusion method. To isolate metal resistant bacteria, 1.0 mM of metal solution (Pb, Zn,Cr, Cu and Fe) were prepared. Nutrient Agar media plates were swabbed with overnight culture of each bacterial isolate and in each plate wells were prepared with a sterile cork borer. Fifty microliter (50 µl) of appropriate heavy metal solution was poured in each well and plates were incubated at 37^{o}C for 24 hours. After incubation, the zone of inhibition was then measured. A zone size less than 1mm was then scored as heavy metal-tolerant strains (Hassenet al., 1998).

Statistical analysis

The data was analyzed by calculating mean ± SD analysis of variance (ANOVA) was performed by using Microsoft office Excel 2007. P value was calculate to observe the level of significance.

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Results showing P value less than 0.05 were considered as significant (P< 0.05).

III. RESULT AND DISCUSSION

Table 1 Physico-chemical properties of the soil samples obtained at KofarRuwa,KwakwacinDawanau Car Park Garage and SOT, Ecological garden.

Parameter	KofarRuwa	KwakwacinDawanau	Ecological garden, SOT
Ph	7.54	7.65	6.46
Temperature (˚C)	33.00	38.80	32.60
Organic Matter (%)	2.785	2.304	2.889
Moisture Content (%)	1.013	0.675	2.389
Chromium (mg/kg)	0.286	0.0853	0.0006
Lead (mg/kg)	1.12	1.19	1.01
Zinc (mg/kg)	11.5102	2.7757	1.3583
Copper (mg/kg)	0.139	0.079	0.047
Iron (mg/kg)	11.11	42.56	3.12

Table 1 present different Physico-chemical characteristics of the soil such as pH, temperature, organic matter, moisture content and heavy metal concentration were determined for both soil samples from three different locations (KofarRuwa,KwakwacinDawanau Car Park Garage and SOT Ecological garden). The soil samples obtained from KofarRuwa and that of KwakwacinDawanau were found to be more neutral (pH 7.4 and pH 7.6) respectively compared to the soil samples from SOT Ecological garden (pH 6.4). The moisture content and organic matter of both soil samples were found to be lesser than the SOT site (2.4%). The temperature of the SOT site (32.6˚C) is lesser compared to that of Kofarruwa (33.0˚C) and KwakwacinDawanau (38.8˚C). The concentration of Lead, Chromium, Zinc, Copper and Iron were determined with the help of Atomic Absorption Spectroscopy. The data reveals that the amount of Zinc is maximum with a concentration of 11.51 mg/kg in KofarRuwa, while Iron is both maximum from KwakwacinDawanau and SOT (42.56 mg/kg and 3.12 mg/kg respectively).

Table 2 Heterotrophic bacterial counts of the soil samples

Sample sites	**Incubation time (hours)**		
	24	**48**	**72**
KofarRuwa	152000±4000	162000±5291	184000±8718
Kwakwachi	148000±3606	160000±4583	180000±4359
Ecological garden, SOT	208000±4359	224000±5292	256000±7211

Values are means ± standard deviation

Heterotrophic bacterial counts (CFU/g) of the soil samples after 3 days of incubation at 30˚C
In this study, the heterotrophic bacterial counts of the soil samples obtained from SOT site after 24, 48and 72hrs of incubation were 2.08 x 10^5cfu/g, 2.24x 10^5cfu/g and 2.56 x 10^5cfu/g respectively. These values were comparatively higher than those of soil samples obtained from Kofarruwa (1.48 x 10^5cfu/g, 1.60 x10^5cfu/g and 1.80 x 10^5cfu/g) and KwakwacinDawanau (1.52 x 10^5cfu/g, 1.62 x10^5cfu/g and 1.84 x 10^5cfu/g) (Table 2). The standard deviation of the mean was also determined.

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Table 3 Biochemical characterization of the bacterial species

Test	Species S2	Species S7	Species S4	Species S1
Gram-staining	Gram negative, straight rods	Gram Negative, rod-Shaped	Gram positive, round-shaped	Gram Negative, rod-shaped
Catalase	+	+	+	+
Indole	+	-	-	-
Oxidase	-	-	-	-
Methyl red	+	-	-	+
Voges Proskauar	-	+	-	+
Citrate	-	+	-	+
Urease	-	-	-	+
TSI	-	-	-	+
Coagulase	-	-	+	-
Species	**E. coli**	**P. Aeruginosa**	**S. aureus**	**K. pneumonia**

Key: + Positive and – Negative result

The biochemical characterization of isolated tolerant bacteria was given in Table.3. The organisms identified include Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Klebsiellapneumoniae. The identified organisms were used for the removal of heavy metalsfrom contaminated soil.

Table 4 Screening for heavy metal tolerant bacteria by plate diffusion method

Test organism	Zone of inhibition of 1.0 mM of metal solution at concentration of 50 µl/ml				
	Pb,	Zn,	Cr,	Cu	Fe
Species 1	+	+	+	+	+
Species 2	+	+	+	+	+
Species 3	–	–	–	–	–
Species 4	+	+	+	+	+
Species 5	–	–	–	–	–
Species 6	–	–	–	–	–
Species 7	+	+	+	+	+

Key: + Growth not inhibited and – Growth inhibited

In plate diffusion method, results of zone formation signify the ability of the organisms as heavy metal-resistant or as sensitive strains to heavy metals. Heavy metal-resistant species show no inhibition of growth at a concentration of heavy metals, whereas heavy metal-sensitive species show inhibition of growth (S3, S5 and S6) at a concentration of heavy metals. Based on this concept, S1, S2, S4 and S7 were identified as efficient species that were resistant Pb, Cr, Zn, Fe, and Cu respectively (Table 4)

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Table 5Maximum Tolerable Concentration (MTC) of heavy metals by Bacterial isolates

Organism	Lead	Chromium	Zinc	Iron	Copper
Pseudomonas aeruginosa	10 Mm	5mM	2mM	2mM	5mM
E. coli	2Mm	5mM	5mM	10mM	5mM
S. aureus	1Mm	1mM	2mM	2mM	1mM
Klebsiellapneumoniea	1Mm	1mM	1mM	2mM	1mM

Heavy metal tolerance test by Pseudomonas aeroginosa showed maximum tolerance to Pb (10 mM) and minimum tolerance to Fe (2mM), Cu (5mM), Zn (2mM) and Cr (5mM) (Table 5 Figure 1). E. coli showed maximum tolerance to Fe (10mM) and minimum tolerance to Cr (5mM), Zn (5mM),Pb (2mM) and Cu (2mM) (Table 5 Figure 1).S. aureus showed maximum tolerance to Zn (2mM) and Fe (2mM) and minimum tolerance to Cr (1mM), Cu (1mM), and Pb (1mM) (Table 5 Figure 1). Klebsiellapneumoniae showed maximum tolerance to Fe (2mM) and minimum tolerance to Zn(1mM), Pb (1mM) and Cu (1mM) (Table 5 Figure 1). No Significance difference was observed between the heavy metals tolerant test of all the four bacterial isolates at 5% level of significance($P>0.05$), but it was evident from the results that there is significance difference ($P < 0.05$) in the rate of growth of the different bacterial isolates.

Figure

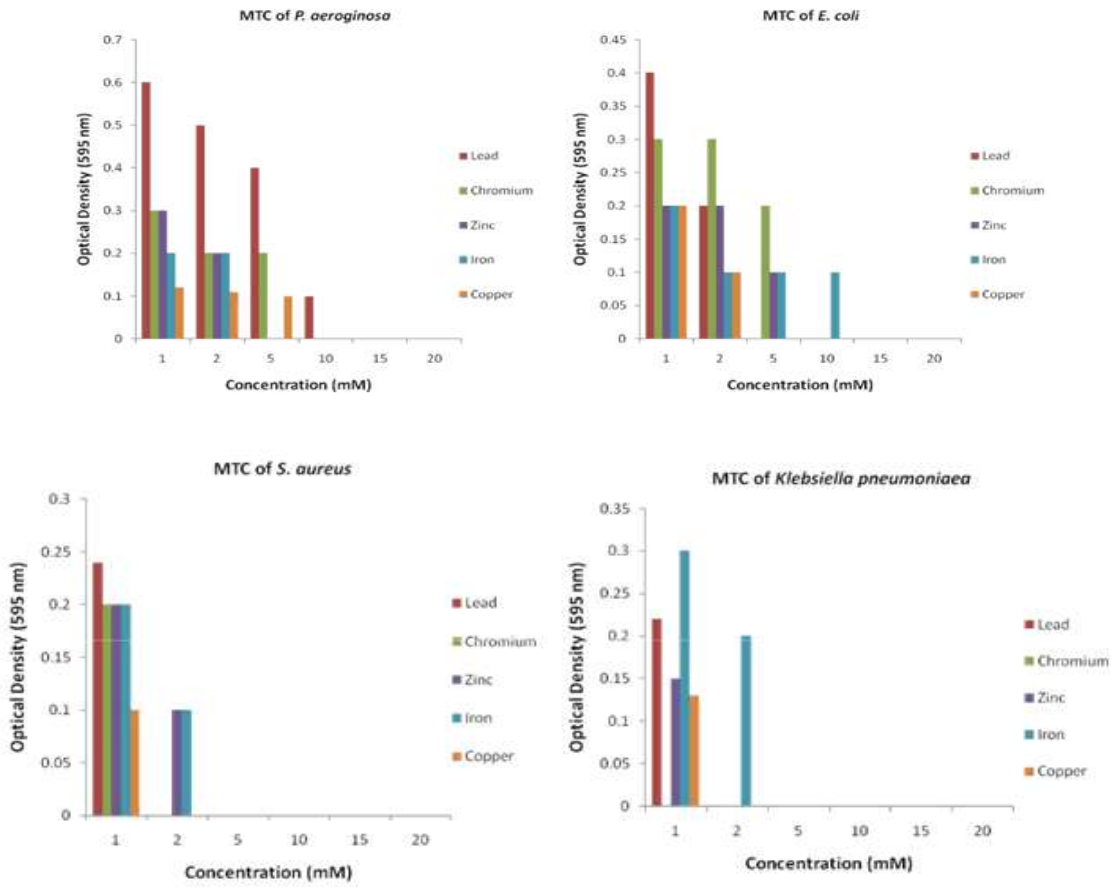

Fig 1: Maximum Tolerable Concentration (MTC) of heavy metals by Bacterial isolate

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Table 6: Heavy metals Removal by bacteria using (AAS) Machine.

Organism	Heavy Metals	Initial Concentration (mg/L)	24 Hours (%)		48 Hours (%)		72 Hours (%)	96 Hours (%)
E. coli	Zinc	0.100	0.075	(29.5)	0.043	(54.7)	0.0390 (61.0)	0.0355 (64.5)
	Chromium	0.100	0.085	(17.5)	0.080	(18.0)	0.0809 (19.1)	0.0806 (19.4)
	Lead	0.100	0.073	(23.0)	0.045	(51.0)	0.0424 (57.0)	0.0342 (65.0)
	Iron	0.100	0.035	(62.5)	0.032	(63.8)	0.0360 (64.0)	0.0358 (64.2)
	Copper	0.100	0.021	(79.9)	0.014	(87.6)	0.0068 (93.2)	0.0062 (93.8)
P. aeruginosa	Zinc	0.100	0.076	(29.4)	0.048	(50.2)	0.0357 (64.3)	0.0278 (72.2)
	Chromium	0.100	0.074	(26.6)	0.066	(37.4)	0.0626 (37.4)	0.0498 (50.2)
	Lead	0.100	0.074	(24.6)	0.051	(49.9)	0.0341 (65.9)	0.0235 (76.5)
	Iron	0.100	0.029	(70.1)	0.022	(71.8)	0.0276 (72.4)	0.0274 (72.6)
	Copper	0.100	0.007	(90.3)	0.009	(92.1)	0.0075 (92.5)	0.0025 (97.5)
S. aureus	Zinc	0.100	0.087	(12.0)	0.077	(23.3)	0.0702 (29.8)	0.0631 (36.9)
	Chromium	0.100	0.084	(11.6)	0.082	(13.8)	0.0848 (15.2)	0.0827 (17.3)
	Lead	0.100	0.063	(33.7)	0.046	(51.4)	0.0435 (56.5)	0.0425 (57.5)
	Iron	0.100	0.082	(17.8)	0.086	(18.4)	0.0808 (19.2)	0.0801 (19.9)
	Copper	0.100	0.073	(29.7)	0.059	(40.1)	0.0506 (49.4)	0.0445 (55.5)
K. pneumonia	Zinc	0.100	0.098	(1.5)	0.098	(4.2)	0.0844 (15.6)	0.0729 (27.1)
	Chromium	0.100	0.085	(18.5)	0.075	(27.5)	0.0719 (28.1)	0.0651 (34.9)
	Lead	0.100	0.067	(37.3)	0.056	(45.4)	0.0505 (49.5)	0.0502 (49.8)
	Iron	0.100	0.052	(44.8)	0.054	(46.6)	0.0519 (48.1)	0.0507 (49.3)
	Copper	0.100	0.075	(20.5)	0.067	(35.3)	0.0427 (57.3)	0.0243 (75.7)

The residual heavy metal concentration was determined by the use of an atomic absorption spectrophotometer (AAS). Zinc concentration was observed reduced to 72.2% in the presence of Pseudomonas aeruginosa, Staphylococcus aureus, (36.9%), E. coli (64.5%) and Klebsiellapneumoniae (27.1%) from the initial concentration of 0.100 mg/ml after 96 hours of incubation period. Chromium concentration was reduced to 50.2% by Pseudomonas aeruginosa, Staphylococcus aureus (17.3%), E.coli (19.4%) and Klebsiella pneumonia (34.9%) from the initial concentration of 0.100 mg/ml after 96 hours of incubation period.Lead concentration was reduced by Pseudomonas aeruginosa to 76.5%, Staphylococcus aureus (57.5%), E. coli (65%) and Klebsiella pneumonia (49.8%) from the initial concentration of 0.100 mg/ml after 96 hours of incubation period. Copper concentration was reduced by Pseudomonas aeruginosa (97.5%), Staphylococcus aureus (55.5%), E. coli (93.8%) andKlebsiellapneumoniae (75.7%) from the initial concentration of 0.100 mg/ml after 96 hours of incubation period. Iron concentration was reduced by Pseudomonas aeruginosa to(72.6%), Staphylococcus aureus (19.9%), E. coli (64.2%) and Klebsiellapneumoniae by (49.3%) from the initial concentration of 0.100 mg/ml after 96 hours of incubation period. The percentages of removal by all the four bacterial isolates were shown in table 6. Significance

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difference was observed on metal removal by all the bacterial isolates ($P<0.05$).

IV. DISCUSSION, CONCLUSION AND RECOMMENDATIONS

Discussion

Presence of metals and other physicochemical parameters play an important role in developing metal tolerance in indigenous bacteria of particular site. It is emphasized that currently there is an increasing interest to isolate and identify some indigenous heavy metal tolerant bacteria and their possible use for the bioremediation of heavy metal contaminated areas (Abuzaret al., 2017).

In this study, a total of four heavy metal resistant bacteria were isolated from soil samples collected from KofarRuwa, KwakwacinDawanau and SOT Ecological garden site in Kano, Nigeria. The bacteria were selected in the presence of Zn^{2+}, Cr^{3+}, Pb^{2+}, Cu^{2+} and Fe^{3+}at concentrations of up to 2mM. The isolates were identified as Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus and Klebsiellapneumoniae on the basis of their cultural, morphological and biochemicalcharacteristics. Similar findings of occurrence of heavy metal resistant bacteria in contaminated soil samples have been reported by (Fagade and Adetutu, 1999). Likewise, this work conformed to Enimieet al. (2016) who isolated five bacterial isolates with maximum tolerance trend for cadmium, chromium, nickel and zinc which were identified as Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Proteus vulgaris and Klebsiellapneumoniae from petroleum refinery effluent.

The maximum tolerable concentration (MTC) of heavy metals was designated as the highest concentration of heavy metals that allowed growth after 24 hours (Schmiatt and Schlege, 1994). The MTC value for Pb (10mM) and Fe (10mM) in this study were higher than that obtained by Fagade and Adetutu (1999) which ranged between 2 and 2.5 mM, but similar with the findings of Owolabi and Hekeu (2014). However, the MTC values for Cr (5 mM), Cu (5mM) and Zn (5mM) are much lower than those reported by Oyetibo and others (2010) where the MTC for Cr was 17 mM. These studies propose variability in the potency of bacteria towards heavy metals to which they are resistant. This variation in response might be due to the difference in resistance mechanisms (Abouzeidet al., 2009). Toxicity testing in liquid medium facilitates a good evaluation of metal toxicity in polluted environments, such as industrial effluents and sewage sludge leachates (Hassenet al., 1998).

pH plays a very critical role in microbial metal resistance and uptake by influencing the metal speciation and solution chemistry as well as surface properties of bacterial cells. Results indicated that the optimum pH for all the bacterial strains was 7.0 using the controlled medium (no metal) whereas the optimum pH with Pb, Cr, Zn, Fe and Cu was around 6 and 7 (Table 1). These results were in agreement with those of Congeevaramet al. (2007), who found that optimal pH for growth and bioaccumulations of Cr^{6+} and Ni^{2+} by the heavy metal-resistant bacteria Micrococcus spp. was pH 7. The selected metal-resistant strain showed that their growth was only slightly affected with different pH values. Therefore, it is clear that growth of all the bacterial isolates is not inhibited with different pH values and this fact makes them a strong candidate for future application in metal bio removal.

The heavy metals reduction by each bacterial isolate revealed P. aeruginosa as the best heavy metal-tolerant bacteria followed by E. coli, K. pneumoniae and S. aureus (Fig. 1). Similar findings of heavy metals reduction by bacteria have been reported. It has the ability to degrade heavy metals present in industrial effluent (Enimieet al., 2016). In another research Pseudomonas putida showed removal rates of100% for Thalium, 96% for Lead, 83% for Vanadium, 71%for Cobalt, 57% for Nickel, 49% for Copper and 45% for protection strategy for facing contaminated environment (Duraisamy and Thatheyus, 2018).

Conclusion

In this study, $Cr^{3+,}$ $Pb^{2+}Zn^{2+}$ $Fe^{3+,}$ and Cu^{2+}resistant bacteria were isolated from three different sampling sites. The four isolates were identified as Pseudomonas aeruginosa, Escherichia coli, Klebsiellapneumoniae and Staphylococcus aureus and they all showed various level ofmaximum tolerance to different concentrations of lead, chromium, zinc, copper and iron. The efficacy of removal of the four heavy metals in decreasing order was P. aeruginosa>E.coli>K. pneumoniae>S. aureus. Overall, Pseudomonas aeruginosa had the highest percentage reductionof heavy metal analyzed by AAS in the study. Thus, Pseudomonas aeruginosa is more effective for bioremediation of heavy metals among the four heavy metals bacteria isolated.

Recommendations

It is recommended that:

1. All the four bacterial isolates should serve as potential candidates to be utilized in future to detoxify heavy metal contaminated soil within natural environments.

2. The studies assessing the potential ability of the selected isolates to remove heavy metals from contaminated soils and their plasmid profiles should be evaluated.
3. Further researches should be conducted to determine the mechanisms by which the bacterial isolates reduce heavy metals

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