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Heavy Metal Tolerance Profile among Bacteria from Auto-mechanic Workshop and Pristine Soil

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Authors' contributions

This work was carried out in collaboration between all authors. Author DRT designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed the literature searches. Authors BEA and IUB managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The study was aimed at investigating the heavy metal tolerance profile among bacteria from auto-mechanic workshop and pristine soil samples. Auto-mechanic workshop and pristine soil environments were randomly sampled within Calabar Metropolis. The research was undertaken within a period of six months. Standard microbiological methods were used to isolate, characterize and identify bacteria isolates from the collected soil samples, while heavy metal tolerance test of the bacteria isolates was carried out using agar dilution method. Bacterial isolates from auto-mechanic workshop soil samples showed a marginally higher percentage tolerability to Pb, Ni, Cr, Cd, Co and V than their pristine soil counterparts. *Serratia spp* (AMM_2, $AMME_2$), *Klebsiella sp* (AMM_3), *Corynebacterium* (AME_1), *Yesinia sp* (AME_3), *Pseudomonas* sp (AMT) and *Bacillus sp* (AME_4) tolerated high heavy metal concentration (300 µg/ml) of chromium, copper and lead either actively (bioaccumulation) or passively (adsorption) as compared to other bacteria isolates from both auto-mechanic workshop and pristine soil samples. Thus, these bacteria isolates could have applicability in bioremediation of heavy metal polluted environments and in the production of biosensors which can be utilized for both background and anthropogenic environmental heavy metal pollution.

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Keywords: Heavy metal; pristine; auto-mechanic workshop; remediation; ecosystem; tolerability.

1. INTRODUCTION

The introduction of heavy metals in the environment has increased rapidly since industrial revolution [1]. Heavy metals which are transitional trace metals and comprise the blocks of metals belonging to group 3 to 16 of the periodic table in periods of 4 or greater are found abundantly in almost all habitats [2]. Natural and anthropogenic activities such as mining and smelting discharges [3,4], are the main sources of heavy metals which are toxic, carcinogenic, mutagenic, teratogenic, allergic and immune – suppressive when above certain concentration living cells [5]. The presence of this toxic heavy metals in the environment imposes several hazards including; physiological and biochemical abnormalities in aquatic organisms [6], pulmonary oedema and rental effects in human and animal due to long term exposure to cadmium [7], stomach upsets, ulcer, kidney, liver damage and even death due to breathing and ingestion of large amount of chromium [8], Hallucinations, forget fullness, nerve damage, Parkinson's diseases, respiratory problems and sexual dysfunction caused by exposure to high levels of manganese [9], Asthma, pneumonia, sneezing, nausea, vomiting and serious effects on the heart caused by exposure to high levels of cobalt from either food intake or drink containing very high concentration of cobalt [10]. Several conventional chemical methods have been put into place for the treatment of these heavy metal contaminants in the environment but this approach has further resulted to the production of sludge and disposal problems, thereby paving way for biological methods and development which has emerged as an eco-friendly economic option [5]. Within the last decade, biomass metal absorption has been demonstrated to be an efficient process for mineralization and concentration of heavy metals from industrial residues [11]. Fungi and bacteria constitute the main components of the soil microbial biomass, however, bacteria possess some attributes that present them as good potential agents for metals bioremediation and some of these potentials include ability to withstand adverse environmental condition, low pH, low moisture content, low nutrient requirement and the production of extracellular enzymes like lipase [12]. Therefore, this study was designed to investigate the heavy metal tolerance profile among bacteria from auto-mechanic and pristine soil samples collected within Calabar Metropolis.

2. MATERIALS AND METHODS

2.1 Study Site and Sampling

Different auto-mechanic workshop and pristine soil environments in Calabar Metropolis were sampled randomly (Fig. 1). The auto-mechanic workshop sampled include; Auto-mechanic workshop located in Atekong, Ettaabgor, Mbukpa and Inyang while pristine soils sampled were; Staff Quarter-UNICAl, Cultural Centre Calabar, Botanical Garden-UNICAL and Inyang street.

2.2 Samples Collection

2.2.1 Soil samples

Soil samples were collected without bias, 20 g each of both the auto-mechanic workshop soils and pristine soils were collected from the top, 0 cm to 15 cm of the soils using sterile spoon and then placed separately in an oven sterilized aluminum foil (at 160℃ per hour). The soil samples were wrapped and placed in sterile 100 ml universal container.

2.2.2 Heavy metal samples

Heavy metal salts of vanadium, chromium, nickel, lead, cobalt, cadmium and copper were obtained from Vikham scientific research laboratories, located at U, J, Essuene stadium Calabar. The salts were used to prepare heavy metal stock solution of concentration – 1000 ppm.

2.2.3 Media

The media used in the study were; nutrient agar (Hardy diagnostics, USA), Muller-Hinton agar and Tryptic soy broth (Hardy diagnostics, USA). The media were prepared according to manufacturer's instruction.

2.3 Sample Preparation

This was carried out according to the method described by [13]. 10 g of soil samples was aseptically weighed into 90 ml of sterile distilled water in a 100 ml conical flask. The samples were vortexed to homogenize and allowed to stand for 10 minutes. From the initial dilution, 10-fold serial dilutions were carried out in clean sterile test tubes containing 9 ml of sterile distilled water.

2.3.1 Plating procedures

This was carried out according to the method described by [5] with slight modification. 0.1 ml of desired dilutions 10^{-3}–10^{-5} were spread and plated in triplicate into nutrient agar supplemented with 50 µg/ml of nystatin to inhibit the growth of fungi. Plates were incubated at 35℃ and bacterial counts recorded after 24 h of incubation.

2.3.2 Purification of isolates

Following enumeration of total heterotrophic bacteria, colonies were picked at random and sub-cultured repeatedly into nutrient agar for purification. Purified isolates were stocked in nutrient agar slants for further studies.

2.3.3 Identification and characteristics of isolates

Purified isolates were characterized by gram morphology and biochemical test using the scheme in Bergey's manual of determinative bacteriology [14,15].

2.3.4 Heavy metal tolerance test

Agar dilution method as described by [16] was adopted. A loopful of 12-16 h bacteria culture in Tryptic soy broth was inoculated by streaking in duplicate on Muller-Hinton Agar plates supplemented with increasing concentrations (20 µg/ml, 100 µg/ml, 200 µg/ml, 250 µg/ml and 300 µg/ml) of the different heavy metal (chromium, vanadium, nickel, cobalt, copper, cadmium and lead). Plates were incubated for 24 h at 37℃. Following incubation, plates were examined visually for the presence or absence of growth. The presence of growth was recorded as resistance while absence of growth was recorded as sensible.

3. RESULTS

3.1 Heavy Metal Tolerance Test of Bacterial Isolates from Auto-mechanic Workshop Soil Samples

Table 2 shows the result of the heavy metal tolerance test of bacteria isolates from auto-mechanic workshop samples to 20 µg/ml, 100 µg/ml, 200 µg/ml, 250 µg/ml and 300 µg/ml of the different heavy metals employed. At 20 µg/ml of chromium, vanadium, nickel, cobalt, cadmium, copper and lead, all the bacteria isolates from the auto-mechanic workshop soil samples showed resistance to all the heavy metals used. At heavy metal concentration of 100 µg/ml, 19 out of the 21 isolates showed resistance to chromium, 15 of the isolates showed resistance to vanadium and nickel, 11 of the isolates showed resistance to cobalt, 18 of the isolates showed resistance to cadmium, 20 of the isolates showed resistance to copper, while all the isolates showed resistance to lead. At heavy metal concentration of 200 µg/ml, 13 out of the 21 isolates showed resistance to chromium, 11 of the isolates showed resistance to nickel, 11 of the isolates showed resistance to cobalt, 20 of the isolates showed resistance to copper, 21 of the isolates showed resistance to lead while all the bacteria isolates were sensitive to vanadium and cadmium at this same concentration. At heavy metal concentration of 250 µg/ml, 9 out of the 21 isolates showed resistance to chromium, 15 of the isolates showed resistance to both copper, 16 of the isolates showed resistance to lead while all the isolates were sensitive to nickel and cobalt. At heavy metal concentration of 300 µg/ml, 7 out of the 21 isolates showed resistance to chromium, 11 of the isolates showed resistance to copper while 14 of the isolates showed resistance to lead.

3.2 Heavy Metal Tolerance Test of Bacteria Isolates from Pristine Soil Samples

Table 3 presents the result of heavy tolerance test of bacteria isolates from pristine soil samples to 20 µg/ml, 100 µg/ml, 200 µg/ml, 250 µg/ml and 300 µg/ml of the different heavy metals used. At heavy metal concentration of 20 µg/ml, 13 out of the 14 isolates showed resistance to chromium and vanadium while all the isolates showed resistance to nickel, cobalt, cadmium, copper and lead. At heavy metal concentration of 100 µg/ml, 9 of the isolates showed resistance to chromium, nickel and cadmium, 10 of the isolates showed resistance to vanadium, 7 of the isolates showed resistance to cobalt while 11 of the isolates showed resistance to copper and lead. At heavy metal concentration of 200 µg/ml, 4 out of the 14 isolates showed resistance to chromium and cadmium, 2 of the isolates showed resistance to nickel, 7 of the isolates showed resistance to copper, 6 of the isolates showed resistance to lead while all the isolates were sensitive to vanadium and cobalt. At heavy metal concentration of 250 µg/ml, 3 out of the 14 isolates showed resistance to chromium, 1 of the isolate showed resistance to cadmium, 4 of the isolates showed resistance to copper and lead while all the isolates were sensitive to nickel. At heavy metal concentration of 300 µg/ml, 3 of the isolates resistance to copper, 3 of the isolates showed resistance to lead while all the isolates were sensitive to chromium and cadmium.

Table 1. Description of sampling locations in Calabar metropolis- Cross River State

Location code	Name	Latitude	Longitude	Elevation	Sample
SQU	Staff Quarters UNICAL	4°56'04.51"N	8°20'69.40"E	114.3,A12.3	Soil
CCC	Cultural Center Calabar	4°57'05.97"N	8°19'04.80"E	165.5,A22.7	Soil
AMT	Auto-Mechanic Shop-Atekong	4°58'45.24"N	8°19'59.40"E	178.3,A13.3	Soil
AME	Auto-Mechanic Shop-Ettagbor	4°57'04.43"N	8°20'04.92"E	198.1,A16.2	Soil
AMM	Auto-Mechanic shop-Mbukpa	4°56'01.81"N	8°19'14.50"E	125.5,A17.5	Soil
AMI	Auto-Mechanic shop-Inyang	4°56'02.55"N	8°19'21.40"E	135,A17.7	Soil
BGU	Botanical Garden-UNICAL	4°57'08.64"N	8°20'34.20"E	195.7,A19.6	Soil
IS	Inyang Street	4°56'02.65"N	8°19'01.95"E	125.4A14.3	Soil

UNICAL- University of Calabar, Calabar

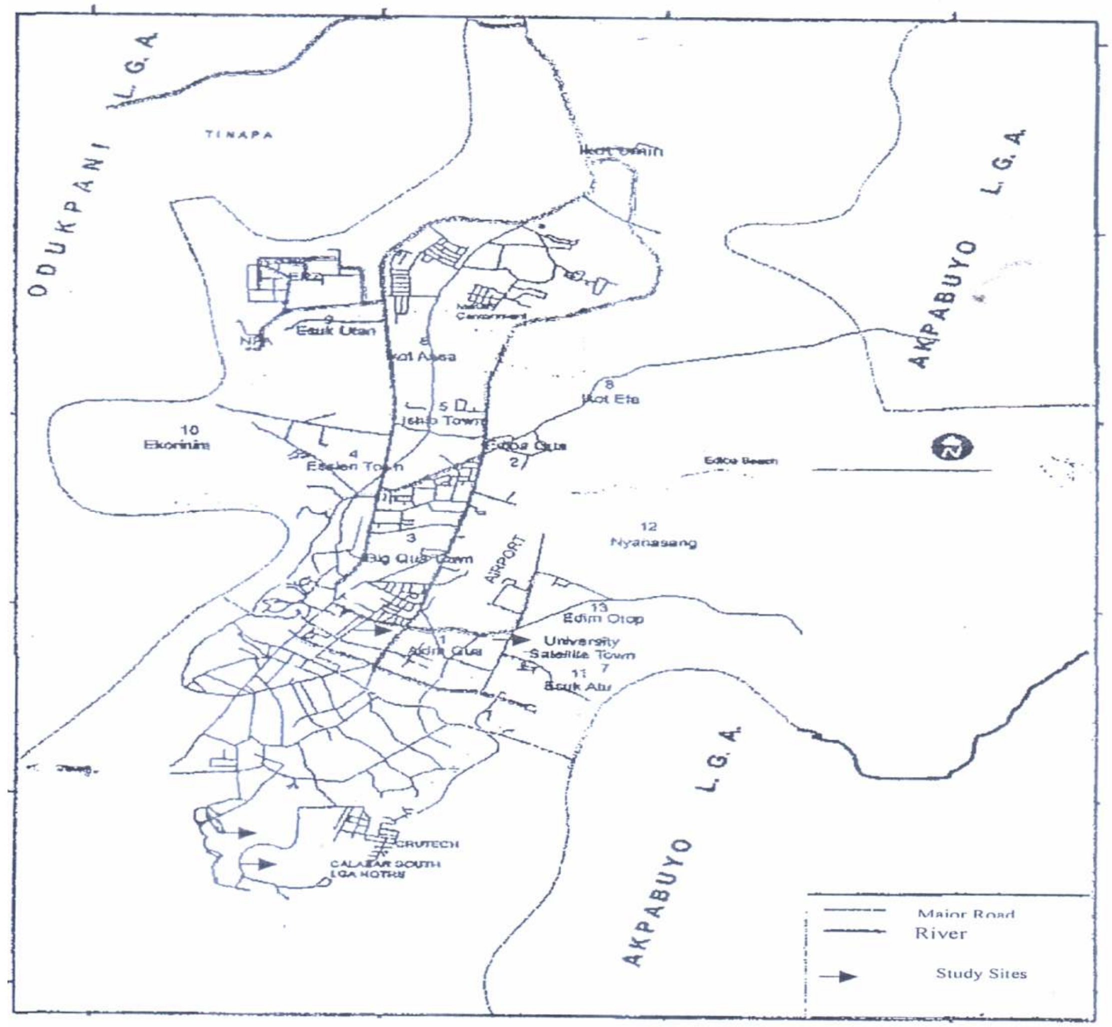

Fig. 1. Map of Calabar, showing the study area
Source: GIS Unit, University of Calabar, 2012

Table 2. Heavy metal tolerance profile of bacteria isolates from auto-mechanic workshop soils at varying concentration of different heavy metal

Isolates	Cr Concentration (µg/ml)					V Concentration (µg/ml)					Ni Concentration (µg/ml)					Co Concentration (µg/ml)					Cd concentration (µg/ml)					Cu concentration (µg/ml)					Pb Concentration (µg/ml)					Probable organism
	20	100	200	250	300	20	100	200	250	300	20	100	200	250	300	20	100	200	250	300	20	100	200	250	300	20	100	200	250	300	20	100	200	250	300	
AMT_1	R	R	R	R	S	R	R	S	S	S	R	R	R	S	S	R	S	S	S	S	R	S	S	S	S	R	S	S	S	R	R	R	R	R	S	*Bacillus sp*
AMT_2	R	S	S	S	S	R	R	S	S	S	R	S	S	SS	S	R	R	R	S	S	R	R	S	S	S	R	R	R	S	R	R	R	R	R	S	*Bacillus sp*
AMT_3	R	R	R	S	S	R	R	S	S	S	R	R	R	S	S	R	S	S	S	S	R	R	S	S	S	R	R	R	S	S	R	R	R	R	R	*Escherichia coli*
AMT_4	R	R	R	R	R	R	R	S	S	S	R	R	R	S	S	R	R	R	S	S	R	R	S	S	S	R	R	R	R	R	R	R	R	R	R	*Pseudomonas sp*
AMT_5	R	S	S	S	S	R	S	S	S	S	R	S	S	S	S	R	R	R	S	S	R	R	S	S	S	R	R	R	R	S	R	R	R	R	R	*Klebsiella sp*
AMM_1	R	R	R	S	S	R	S	S	S	S	R	S	S	S	S	R	S	S	S	S	R	R	S	S	S	R	R	R	S	S	R	R	R	R	R	*Aeromonas sp*
AMM_2	R	R	R	R	R	R	R	S	S	S	R	R	R	S	S	R	R	R	S	S	R	R	S	S	S	R	R	R	R	R	R	R	R	R	R	*Serratia sp*
AMM_3	R	R	R	R	R	R	R	S	S	S	R	R	R	S	S	R	R	R	S	S	R	R	S	S	S	R	R	R	R	R	R	R	R	R	R	*Klebsiella sp*
AMM_4	R	R	R	S	S	R	S	S	S	S	R	R	S	S	S	R	S	S	S	S	R	R	S	S	S	R	R	R	R	S	R	R	R	R	S	*Enterobacter sp*
AMM_5	R	R	R	S	S	R	S	S	S	S	R	S	S	S	S	R	S	S	S	S	R	R	S	S	S	R	R	R	R	S	R	R	R	R	S	*Enterobacter sp*
AMM_6	R	R	S	S	S	R	S	S	S	S	R	S	S	S	S	R	S	S	S	S	R	R	S	S	S	R	R	R	R	S	R	R	R	R	R	*Aeromonas sp*
AME_1	R	R	R	R	R	R	R	S	S	S	R	R	R	S	S	R	R	R	S	S	R	R	S	S	S	R	R	R	R	R	R	R	R	R	R	*Corynebacterium sp*
AME_2	R	R	R	R	R	R	R	S	S	S	R	R	R	S	S	R	R	R	S	S	R	R	S	S	S	R	R	R	R	R	R	R	R	R	R	*Serratia sp*
AME_3	R	R	R	R	R	R	R	S	S	S	R	R	R	S	S	R	R	R	S	S	R	R	S	S	S	R	R	R	R	R	R	R	R	R	R	*Yesinia sp*
AME_4	R	R	R	R	R	R	R	S	S	S	R	R	R	S	S	R	R	R	S	S	R	R	S	S	S	R	R	R	R	R	R	R	R	R	R	*Bacillus sp*
AME_5	R	R	S	S	S	R	R	S	S	S	R	R	S	S	S	R	S	S	S	S	R	R	S	S	S	R	R	R	R	R	R	R	R	S	S	*Serratia sp*
IS_1	R	R	S	S	S	R	R	S	S	S	R	R	S	S	S	R	S	S	S	S	R	R	S	S	S	R	R	R	S	S	R	R	R	R	R	*Shigella sp*
IS_2	R	R	R	R	R	R	S	S	S	S	R	R	R	S	S	R	R	S	S	S	R	R	S	S	S	R	R	R	S	S	R	R	R	S	S	*Aeronomas sp*
IS_3	R	R	S	S	S	R	R	S	S	S	R	S	S	S	S	R	R	R	S	S	R	R	S	S	S	R	R	R	R	R	R	R	R	S	S	*Serratia sp*
IS_4	R	R	S	S	S	R	R	S	S	S	R	R	R	S	S	R	R	R	S	S	R	S	S	S	S	R	R	R	R	R	R	R	R	S	S	*Klebsiella sp*
IS_5	R	R	S	S	S	R	R	S	S	S	R	R	S	S	S	R	S	S	S	S	R	S	S	S	S	R	R	R	R	R	R	R	R	S	S	*Pseudomonas sp*

Key; Cr=chromium, V=vanadium, Ni=nickel, Co=cobalt, Cd=cadmium, Cu=copper, Pb=lead, R=Resistance, S=sensitive

Table 3. Heavy metal tolerance profile of bacteria isolates from pristine soil samples at varying concentrations of heavy metal

Isolates	Cr Concentration (µg/ml)					V Concentration (µg/ml)					Ni Concentration (µg/ml)					Co Concentration (µg/ml)					Cd concentration (µg/ml)					Cu concentration (µg/ml)					Pb Concentration (µg/ml)					Probable organism
	20	100	200	250	300	20	100	200	250	300	20	100	200	250	300	20	100	200	250	300	20	100	200	250	300	20	100	200	250	300	20	100	200	250	300	
SQU_1	R	S	S	S	S	S	S	S	S	S	R	R	S	S	S	R	R	S	S	S	R	R	R	R	S	R	R	R	R	R	R	R	R	S	S	*Citrobacter sp*
SQU_2	R	R	S	S	S	R	R	S	S	S	R	R	R	S	S	R	S	S	S	S	R	S	S	S	S	R	R	R	S	S	R	R	S	S	S	*Citrobacter sp*
SQU_3	S	S	S	S	S	R	R	S	S	S	R	R	S	S	S	R	S	S	S	S	R	R	S	S	S	R	S	S	S	S	R	R	R	R	R	*Yesinia sp*
SQU_4	R	R	R	R	S	R	R	S	S	S	R	S	S	S	S	R	R	S	S	S	R	R	S	S	S	R	R	S	S	S	R	S	S	S	S	*Serratia sp*
IS_1	R	R	S	S	S	R	R	S	S	S	R	S	S	S	S	R	R	S	S	S	R	S	S	S	S	R	R	R	R	S	R	R	R	S	S	*Yesinia sp*
IS_2	R	S	S	S	S	R	R	S	S	S	R	R	S	S	S	R	S	S	S	S	R	R	R	S	S	R	S	S	S	S	R	R	S	S	S	*Bacillus sp*
IS_3	R	S	S	S	S	R	R	S	S	S	R	S	S	S	S	R	R	S	S	S	R	S	R	S	S	R	R	S	S	S	R	R	R	S	S	*Serratia sp*
IS_4	R	R	R	R	S	R	S	S	S	S	R	R	S	S	S	R	S	S	S	S	R	R	S	S	S	R	S	S	S	S	R	R	R	R	R	*Citrobacter sp*
IS_5	R	R	S	S	S	R	R	S	S	S	R	S	S	S	S	R	R	S	S	S	R	S	S	S	S	R	R	R	R	R	R	R	S	S	S	*Bacillus sp*
BGU_1	R	R	S	S	S	R	R	S	S	S	R	R	R	S	S	R	R	S	S	S	R	R	R	R	S	R	R	S	S	S	R	R	R	R	R	*Bacillus sp*
BGU_2	R	R	R	R	S	R	R	S	S	S	R	S	S	S	S	R	R	S	S	S	R	S	S	S	S	R	R	S	S	S	R	S	S	S	S	*Bacillus sp*
BGU_3	R	R	S	S	S	R	R	S	S	S	R	R	S	S	S	R	S	S	S	S	R	R	S	S	S	R	R	R	S	S	R	R	S	S	S	*Serratia sp*
CC_1	R	S	S	S	S	R	R	S	S	S	R	R	S	S	S	R	S	S	S	S	R	S	S	S	S	R	S	R	S	S	R	R	S	S	S	*Serratia sp*
CC_2	R	R	R	S	S	R	R	S	S	S	R	S	S	S	S	R	S	S	S	S	R	R	S	S	S	R	R	R	R	R	R	S	S	S	S	*Yesinia sp*

Key; Cr=chromium, V=vanadium, Ni=nickel, Co=cobalt, Cd=cadmium, Cu=copper, Pb=lead, R=Resistance, S=sensitive

3.3 Percentage Tolerability of Bacteria Isolates to Various Concentrations of Heavy Metal

Fig. 2 and Fig. 3 present the percentage tolerability of bacteria isolates from both auto-mechanic workshop and pristine soils respectively. The bacteria isolates from auto-mechanic workshop soil showed 100% tolerability to 20 µg/ml of chromium (Cr), vanadium (V), lead (Pb), nickel (Ni), cobalt (Co), cadmium (Cd) and copper (Cu). At heavy metal concentration of 100 µg/ml, the bacteria isolates showed 90% tolerability to chromium (Cr), 71% tolerability to vanadium (V) and nickel (Ni), 57% tolerability to cobalt, 86% tolerability to cadmium (Cd), 95% tolerability to copper (Cu) and 100% tolerability to lead (Pb). At heavy metal concentration of 200 µg/ml, the bacteria isolates showed 62% tolerability to chromium (Cr) 57% tolerability to nickel (Ni), 52% tolerability to cobalt (Co), 95% tolerability to copper (Cu) and 100% tolerability to lead (Pb). At metal concentration of 250 µg/ml the bacteria isolates showed 43% tolerability to chromium, 71% tolerability to copper (Cu) and 71% tolerability to lead (Pb). At heavy metal concentration of 300 µg/ml, the bacteria isolates showed 14%, 52% and 67% tolerability to chromium (Cr), copper (Cu) and lead (Pb). Almost the same pattern of tolerability although at a lesser extent was observed in bacteria isolates from pristine soil (Fig. 3). All the bacteria isolates from both auto-mechanic workshop and pristine soil could not tolerate 200 µg/ml of vanadium and 250 µg/ml of cobalt and nickel.

3.4 Comparability of Heavy Metal Tolerability by Bacteria Isolates from Auto-mechanic Workshop and Pristine Soils

Table 4 presents the results of comparability of heavy metal tolerability by bacteria isolates from both auto-mechanic workshop and pristine soil. It showed that *Serratia spp* (AMM_2, AME_2), *Klebsiella sp* (AMM_3), *Corynebacetrium sp* (AME_1) and *Yesinia sp* (AME_3) *Pseudomonas sp* (AMT_4) and *Bacillus sp* (AME_4) from auto-mechanic workshop soils tolerated 300 µg/ml of chromium (Cr), copper (Cu) and lead (Pb) while none of the isolates from the pristine soil samples were able to tolerate these three heavy metals at 300 µg/ml.

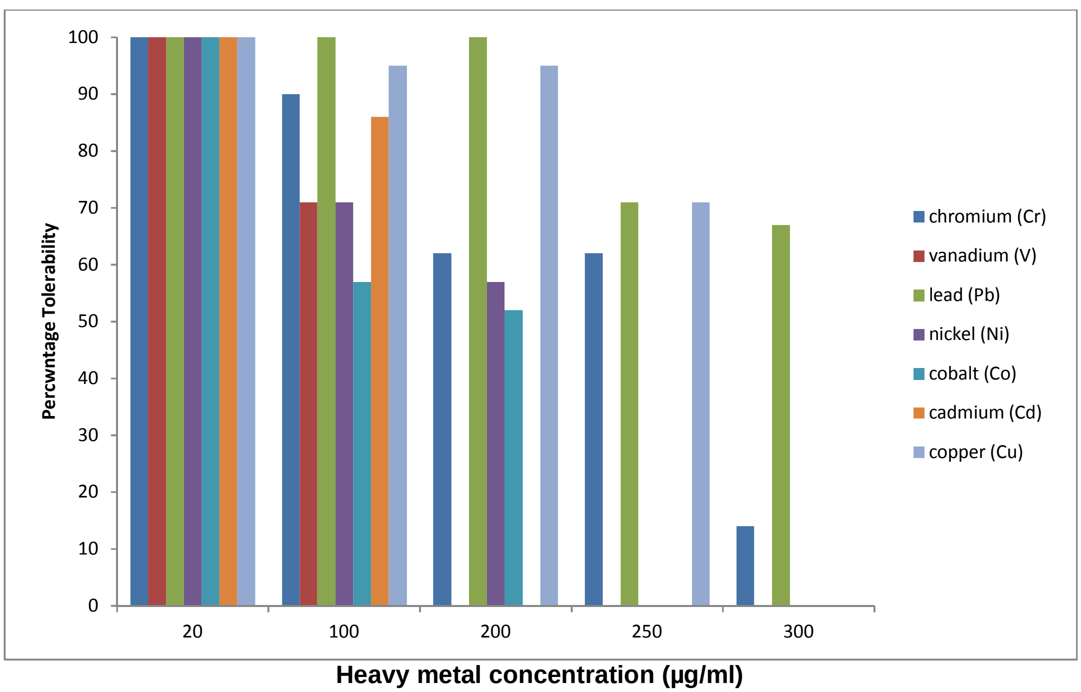

Fig. 2. Percentage tolerability of bacteria isolates from auto-mechanic workshop soil to increasing heavy metal concentration

(Cr=chromium, V=vanadium, Ni=nickel, Co=cobalt, Cd=cadmium, Cu=copper, Pb=lead, Number of isolates tested = 21)

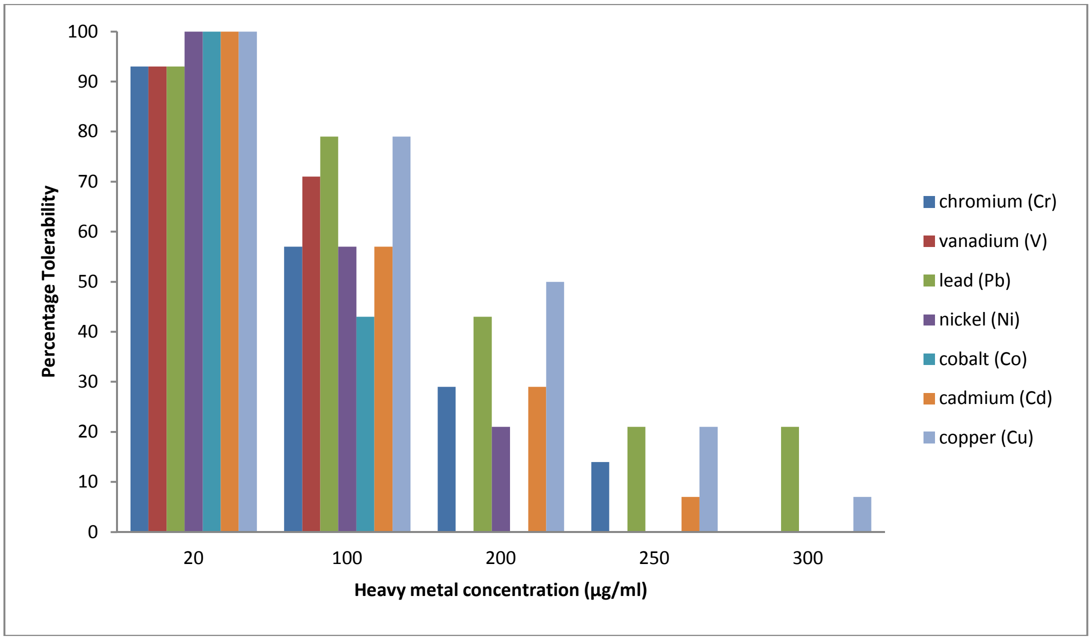

Fig. 3. percentage tolerability of bacteria isolates from pristine soil to increasing heavy metal concentration

(Cr-chromium, V=vanadium, Ni=nickel, Co=cobalt, Cd=cadmium, Cu=copper, Pb=lead, Number of isolates tested =14)

Table 4. Comparability of heavy metal tolerability by bacteria isolates from auto-mechanic workshop and pristine soil samples

Isolate	Auto-mechanic workshop soil				Pristine oil				
	Probable organism	Heavy mental conc. (300 µg/ml)			Isolate	Probable organism	Heavy metal con. (300 µg/ml)		
		Cr	Cu	Pb			Cr	Cu	Pb
AMT_1	*Bacillus sp*	S	R	S	SQU_1	*Citrobacter sp*	S	R	S
AMT_2	*Bacillus sp*	S	R	S	SQU_2	*Citrobacter sp*	S	S	S
AMT_3	*Escherichia coli*	S	S	R	SQU_3	*Yesinia sp*	S	S	R
AMT_4	*Pseudomonas sp****	R	R	R	SQU_4	*Serratia sp*	S	S	R
AMT_5	*Klebsiella sp*	S	S	R	IS_1	*Yesinia sp*	S	S	S
AMM_1	*Aeromonas sp*	S	S	R	IS_2	*Bacillus sp*	S	S	S
AMM_2	*Serratia sp****	R	R	R	IS_3	*Serratia sp*	S	S	S
AMM_3	*Klebsiella sp****	R	R	R	IS_4	*Citrobacter sp*	S	S	R
AMM_4	*Enterobacter sp*	S	S	S	IS_5	*Bacillus sp*	S	R	S
AMM_5	*Enterobacter sp*	S	S	S	BGU_1	*Bacillus sp*	S	S	R
AMM_6	*Aeromonas sp*	S	S	R	BGU_2	*Bacillus sp*	S	S	S
AME_1	*Corynebacterium sp****	R	R	R	BGU_3	*Serratia sp*	S	S	S
AME_2	*Serratia sp****	R	R	R	CC_1	*Serratia sp*	S	S	S
AME_3	*Yesinia sp****	R	R	R	CC_2	*Yesinia sp*	S	R	S
AME_4	*Bacillus sp****	R	R	R					
AME_5	*Serratia sp*	S	R	S					
IS_1	*Shigella sp*	S	S	R					
IS_2	*Aeromonas sp*	R	S	S					
IS_3	*Serratia sp*	S	R	S					
IS_4	*Klebsiella sp*	S	R	S					
IS_5	*Pseudomonas sp*	S	R	S					

*Key: Cr = chromium, Cu = copper, Pb = lead, S = sensitive, R = Resistance, *** = potential useful organisms for bioremediation*

4. DISCUSSION

Heavy metal tolerability among bacteria isolates from auto-mechanic workshop pristine soil was investigated. The microbial bioload recorded for the hydrocarbon polluted soils obtained within the vicinity of the auto-mechanic workshops could be as a suggestive ability of this micro flora to proliferate in these environments despite the deliberate exposure of these soils to varying doses of petroleum or its refined products [17]. The result of the study reviewed that the sensitivity exhibited by bacteria isolates from both the auto-mechanic workshop and pristine soils was proportional to the concentration of the heavy metals utilized. This trend was similar to report by [18], who investigated the tolerance of trace metals such as Cd^{2+}, Cu^{2+} and Zn^{2+} by *Paenibacillus spp* and *Bacillus thuringiensis* isolated from a pristine soil. A greater number and percentage of bacteria isolates from the auto-mechanic workshop soils in this study were able to tolerate to the seven heavy metals (Pb, Ni, Cr, Cd, Co, Cu and V) tested compared to those from the pristine soil. This was not surprising, as the marginal difference could have been due to the selective pressure from the metal content of their growth environment (auto-mechanic workshop soil), since such sample sites are constantly contaminated with petroleum products and metal fillings [19]. Researchers have shown that microorganisms have evolved several mechanisms to tolerate the uptake of metal ions in order to survive metal toxicity and these mechanisms have been proven to include surface binding or reduced uptake, increased efflux intracellular sequestration, enzyme detoxication and active transport [20,21]. In comparing heavy metal tolerability by bacteria isolates from the auto-mechanic workshop and pristine soils, *Serratia spp* (AMM_2, AME_2), *Klebsiella sp* (AMM_3), *Corynebacterium sp* (AME_1) and *Yesinia sp* (AME_3), *Pseudomonas sp* (AMT_4) and *Bacillus sp*(AME_4) tolerated high concentration (300 µg/ml) of chromium, copper and lead. This observation however, was not surprising as research by [22] on the bioremediation of effluents from magnetite and bauxite mines using *Bacillus spp* and *Pseudomonas aeruginosa* reported that both of the two organisms effectively absorbed Cd, Ca, Zn and Pb effectively. Similar study by [23] have also reported *Klebsiella oxytoca* to have metal binding potential. Hence the identified isolates from this study could be useful in the bioremediation of heavy metal contaminated ecosystem, as heavy metals does not easily decompose in the environment and cannot be easily eliminated in the environment by the use of other conventional chemical methods that further results to toxic deposits in the environments [24].

5. CONCLUSION

It is obvious that this study has provided some insight into heavy metal resistance in bacteria isolates from hydrocarbon contaminated soil ecosystem (auto-mechanic workshops), as *Serretia spp* (AMM_2, AME_2), *Klebsiella sp* (AMM_3), *Corynebacterium sp* (AME_1) and *Yesinia sp* (AME_3), *Pseudomonas sp* (AMT_4) and *Bacillus sp* (AME_4) were able to tolerate high concentration (300 µg/ml) of chromium, copper, and lead either actively (bioaccumulation) or passively (adsorption). Thus these bacteria isolates could have applicability in bioremediation of heavy metal polluted environments and in the production of biosensors which can be utilized in biomonitoring of both background and anthropogenic environmental heavy metal pollution.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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