International Journal of
AGRICULTURE AND BIOSCIENCES
www.ijagbio.com P-ISSN: 2305-6622 E-ISSN: 2306-3599 editor@ijagbio.com

RESEARCH ARTICLE

Susceptibility to Heavy Metals and Hydrocarbonclastic Attributes of Soil Microbiota

Jaboro AG[1]*, Akortha EE[2] and Obayagbona ON[3]

[1]Precious Life Medical Center, Games Village, Kaura District, Abuja, Nigeria; [2]Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria; [3]Microbiology Laboratory, Edo Environmental Consults and Laboratory, Palm House Annex, Sapele Road, Benin City, Edo State, Nigeria

ARTICLE INFO

Received: June 13, 2013
Revised: August 08, 2013
Accepted: August 30, 2013

Key words:
Heavy metals
Heterotrophic
Hydrocarbonclastic
Microbial
Soils

***Corresponding Address:**
Jaboro AG
Jaborograndy@gmail.com

ABSTRACT

The culturable heterotrophic microbial and hydrocarbonclastic bacterial bio load of a hydrocarbon polluted soil and control soil was evaluated using pour plate method. The physicochemical qualities of the soils were determined using routine procedures. The identified isolates were screened for their potential hydrocarbonclastic activity. The tolerance of the bacterial isolates exposed varying concentrations of Pb^{2+}, Cu^{2+}, Hg^{2+}, Cd^{2+} and Ni^{2+} was ascertained using agar diffusion method. The mean heterotrophic bacterial counts of the hydrocarbon contaminated and control soils were 5.6×10^3 cfu/g and 1.4×10^4 cfu/g. Microbial isolates identified were; *Klebsiella oxytoca*, *Bacillus subtilis, Streptococcus* sp., *Pseudomonas aeruginosa*, *Bacillus megaterium*, *Staphylococcus epidermidis*, *Enterobacter aerogenes*, *Escherichia coli*, *Arthrobacter* sp., *Nocardia* sp. *Corynebacterium* sp., *Aspergillus versicolor*, *Aspergillus niger*, *Mucor mucedo*, *Penicillium chrysogenum* and *Penicillium* sp. *Nocardia* sp. (83%) had the highest percentage frequency of occurrence. The hydrocarbon polluted soil had higher hydrocarbon content in comparison to the control soil. *Aspergillus niger* exhibited the highest potential for hydrocarbon utilization as sole carbon source. There were variations in the susceptibility of the bacterial cultures to the concentrations of the heavy metals. There are possibilities in utilizing soil microbes as biosensors in the biomonitoring of environmental heavy metal pollution.

Cite This Article as: Jaboro AG, EE Akortha and ON Obayagbona, 2013. Suspectibility to heavy metals and hydrocarbonclastic attributes of soil microbiota. Inter J Agri Biosci, 2(5): 206-212. www.ijagbio.com

INTRODUCTION

Heavy metals are often defined as a group of metals whose atomic density is greater than 5 g/cm^3 (Gadd, 1992). Metals play a vital role in the metabolic processes of the biota. Some of the heavy metals are essential and are required by the organisms as micro nutrients (cobalt, chromium, nickel, iron manganese and zinc) and are known as 'trace elements' (Bruins *et al*., 2000). Nies (1999) and Hussein *et al.*, (2005), reported that heavy metals are involved in redox processes, act as catalysts in enzymatic reactions, and also regulate the osmotic balance. While some heavy metals are required in trace amounts as nutrients, they become strongly inhibitory for microorganisms at relatively low concentrations (Atlas and Bartha, 1993). Toxicity occurs through the displacement of essential metals from their native binding sites or through ligand interactions (Sevgi *et al.*, 2010). Nonessential metals bind with greater affinity to thiol containing groups and oxygen sites than do essential metals. Toxicity results from alterations in the conformational structure of nucleic acids [30]and proteins and interference with oxidative phosphorylation and osmotic balance (Said and Lewis, 1991). Heavy metals concentrations in soil are associated with biological and geochemical cycles and are influenced by anthropogenic activities such as agricultural practices, industrial activities and waste disposal methods (Eja *et al.,* 2003; Zauyah *et al.,* 2004). Contamination and subsequent pollution of the environment by toxic heavy metals have become an issue of global concern due to their sources, widespread distribution and multiple effects on the ecosystem (Nriagu, 1990).

Motor vehicle servicing centers popularly known as mechanic workshops are sources of automobile wastes in the Benin City metropolis. In these locations, fossil fuel products are used leading to excess accumulation of various forms of heavy metals. These accumulations deteriorate nearby farms and causes non-point source pollution (Aiyesanmi, 2005). Wastes from automobile

workshop activities include solvent, paints, spent heat, transfer fluids, hydraulic fluids, spent lubricants and stripped oil sludge (Osu and Okereke, 2010). Most of these wastes are dumped on our agricultural soils. Microbial survival in polluted soils depends on intrinsic biochemical and structural properties, physiological, and/or genetic adaptation including morphological changes of cells, as well as environmental modifications of metal speciation (Wuertz and Mergeay, 1997). Rough *et al.*, (1995) categorized mechanisms with which soil bacteria are able to detoxify heavy metals. The groups include; 1) intracellular sequestration, 2) export, 3) reduced permeability 4) extracellular sequestration and 5) extracellular detoxification. Almost all known bacterial resistance mechanisms are encoded on plasmids and transposons (Silver and Walderhaug, 1992) and it is probably by gene transfer or spontaneous mutation that bacteria acquire their resistance to heavy metals (Osborn *et al.*, 1997).

Rathnayake *et al.*, (2010) stated that many approaches have been used to assess the risk posed by the contaminating metals in soil and water bodies. Currently, the tolerance of soil bacteria to heavy metals has been proposed as an indicator of the potential toxicity of heavy metals to other forms of biota (Olson and Thronton, 1982; Hassen *et al.*, 1998). Consequently, there is a dramatic increase in the interest on studying the interactions of heavy metals with microorganisms. The objectives of this study were; to isolate and identify the microbial flora of the soil samples. Determination of the susceptibilities of the bacterial isolates to varying concentrations of the metals; lead, copper, mercury, cadmium and nickel. Screening of the microbial isolates for hydrocarbonclastic activity and evaluating the physicochemical properties of the soils.

MATERIALS AND METHODS

Collection of soil samples

One hundred (100) g of top soil samples recently polluted with spent oil and other hydrocarbon fractions were collected from different locations at an auto-mechanic workshop located in Uwelu Spare Parts Market, Benin City, Edo State, Nigeria (Latitude: 6^o 22' 40.7274''; Longitude 5^o 35' 28.788'') using a soil auger at depths varying from 0 to 15cm. The soil samples were mixed together and dispensed in a labeled clean polythene bag (PS). About 100g of top soil sample was bored from a fallow farmland located near the Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria (Latitude: 6^o 23' 50.7474''; Longitude: 5^o 36' 54.1434'') at depths ranging from 0-15 cm. The soil was placed in a labeled clean polythene bag (CS). Sampling was conducted during the month of September, 2012. The soil samples were immediately transported to the laboratory for microbiological and physico-chemical analyses.

Enumeration and isolation of both heterotrophic microbial and hydrocarbonclastic bacterial counts using general purpose media and modified mineral salts medium

One (1) g of the respective fresh soil samples (PS and CS) were weighed and dissolved into 99 ml of sterile prepared peptone water diluent under aseptic conditions (Sharma, 2009). Serial fold dilutions were then made up to 10^{-5} and aliquots (0.1ml) of each dilution were cultured on plates of Nutrient Agar (NA) Potato Dextrose Agar (PDA) using pour plate method as described by Sharma (2009). Plating was done in duplicates. The nutrient agar plates were incubated aerobically at 35^{o}C for 48 hr. The potato dextrose agar plates were incubated at room temperature (28^{o}C±5^{o}C) for 5 days (Sharma, 2009). A petroleum based medium; Modified Mineral Salts Agar incorporated with 15 g of agar, 1% crude oil (Carbon source) (Amund *et al*., 1987; Okpokwasili and Okorie, 1988), was also used for the preliminary isolation of petroleum utilizing bacterial cultures from the polluted soil (PS). About 1ml of 10 mg/l Nystatin was added to the plates to discourage the growth of fungi (Rice and Hemmingsen, 1997). The culture plates were then incubated at 35^{o}C for 7 days (Kãstner *et al*., 1995). The resultant microbial colonies were enumerated and recorded as colony forming units (cfu) per gram of the soil sample (Sharma, 2009).

Characterization of the soil microbiota

Unique representative bacterial and fungal colonies were sub-cultured on freshly prepared nutrient agar and potato dextrose agar plates. These plates were incubated at 35°C for 24 hr and room temperature (28±2° C) for 4 days for bacterial and fungal cultures respectively (Jaboro, 2013). The colonial characteristics of the sub-cultured bacterial colonies were recorded. The bacterial isolates were further identified by the identification schemes of Holt *et al.* (1994) and Cullimore (2000). Isolated bacteria were also cultured on nutrient agar slants and stored at 2 ^{0}C. The sub cultured fungal isolates were identified on the basis of their morphological and microscopic features. Their microscopic attributes were examined using the wet mount technique (Sharma, 2009). Both lactophenol cotton blue and distilled water were used respectively as mountants. The microscopic structures observed were recorded and compared to those stated by Barnett and Hunter (1972). The fungal isolates were also transferred to potato dextrose agar slants and stored in aerated sterile cabinets which served as stock cultures.

Preparation of standard cultures for axenic bacterial isolates

Standard cultures were prepared for the isolates adapting the methods of Gerhardt *et al*. (1994) and Seely and Van-Denmark (1981). One hundred (100) ml of mineral salt broth was dispensed into each of three different conical flasks and inoculated with each purified isolate from each stock culture and incubated at 28°C for 24 hr. After incubation, the cultures were serially diluted up to 10^{-2} and 0.1 ml of each was added into sterile plates. Cool molten nutrient agar was added to the inoculated plates and incubated at 37°C for 24 hr. The plate counts were recorded and the values obtained were expressed as standard number of cells present in 0.1ml of the broth. This was used as the standardized culture.

Physicochemical analyses of the soil samples

Several physical and chemical properties of the various soil samples were determined. With the exception

of moisture content analysis, bulk density and water holding capacity analysis, the respective soil samples were placed on large wooden trays and air-dried for 72 hr. Lumps of moist soil samples were broken by hand prior to air drying of the samples. The air dried samples were also sieved using a 2mm mesh. Parameters which included electrical conductivity, pH, particle size distribution and Cation Exchange Capacity (CEC) were determined according to methods described by Radojevic and Bashkin, (1999). The Total Organic Carbon (TOC), total Nitrogen and Total Hydrocarbon Content (THC) of the soil samples were also evaluated in accordance with procedures stated by Onyeonwu (2000) and Bremmer and Mulvaney (1982). The moisture content, bulk density, water holding capacity were determined using procedures described by Kalra and Maynard (1991). The trace metal content (Pb, Cu, Cd and Ni) of both soils were determined using method described by Radojevic and Bashkin (1999) using a Digester (Gerhardt digester, UK) and Atomic Absorbance Spectrophotometer (AAS) (Buck Scientific model 210 VGP USA).

Determination of the heavy metal susceptibility of the bacterial isolates

Minimum inhibitory concentrations of (MICs) of the metals were determined by the Agar diffusion methods (Hassan *et al*., 2008) as described by Velusamy *et al*., (2011). The metals Pb^{2+}, Cu^{2+}, Hg^{2+}, Cd^{2+} and Ni^{2+} were used as $PbCl_2$, $CuSO_4$, $HgCl_2$, $CdCl_2$ and $NiCl_2$ salts respectively. Stock solution of the metals (100µg/ml) were prepared by weighing 40mg ($PbCl_2$), 75mg ($CuSO_4$), 120mg ($HgCl_2$), 170mg ($CdCl_2$) and 68mg ($NiCl_2$) and dissolved in 10ml of sterile distilled water. The solution was mixed thoroughly and strengths of 50 µg/ml and 25 µg/ml were made by double dilution method. These three different concentrations (100 µg/ml, 50 µg/ml and 25 µg/ml) of the respective metals were tested on the bacterial isolates and zones of inhibitions (mm) measured after 24 hr of incubation using a meter rule.

Screening for the ability to utilize Forcados blended crude oil as sole carbon source by the microbial isolates

The microbial isolates were screened for the ability to utilize Forcados blended crude oil as sole carbon source using mineral salt medium as described by Mills *et al*. (1978). The method employed was adapted from Okpokwasili and Okorie (1988). The medium (9.0 ml) was dispensed into 17 test tubes. Into each of the test tubes, 1.0ml of the blended crude oil was added. After capping, all the test tubes were tubes were sterilized at 121^{o}C for 15 min and allowed to cool. On cooling, the first set of test tubes (11 test tubes) were inoculated with 0.1 ml of standardized bacterial cell suspension of the respective bacterial isolates. The second set of test tubes (5 test tubes) were inoculated with one agar plug of the respective purified fungal isolates (collected with the aid of a sterile 4mm cork borer and forceps) (George-Okafor *et al*., 2009). One of the tubes which served as control was un-inoculated. All the tubes were incubated at room temperature for 14 days after which each tube was scored for optical density which indicated the utilization of petroleum hydrocarbon.

RESULTS

The results shown in Table 1 revealed that the mean heterotrophic bacterial, fungi and hydrocarbon utilizing bacterial counts of soil samples recently contaminated with petroleum products (PS) from the auto mechanic workshop and that of soil which had no recent contamination (CS). The mean heterotrophic bacterial counts were 5.6×10^3 cfu/g and 1.4×10^4 cfu/g for the PC and CS soils respectively. Mean heterotrophic fungi counts were 9.2×10^4 cfu/g and 5.1×10^4 cfu/g for PS and CS soil respectively. Mean hydrocarbon utilizing bacterial count for the PS soil was 2.8×10^2 cfu/g with nil hydrocarbon utilizing bacteria isolated from the CS soil after the period of incubation.

Bacterial isolates identified from the soil samples were: *Klebsiella oxytoca*, *Bacillus subtilis, Streptococcus* sp., *Pseudomonas aeruginosa*, *Bacillus megaterium*, *Staphylococcus epidermidis*, *Enterobacter aerogenes*, *Escherichia coli*, *Arthrobacter* sp., *Nocardia* sp. and *Corynebacterium* sp. (Fig. 1). *Nocardia* sp. (83%) had the highest percentage frequency of occurrence amongst the identified bacterial isolates while *Streptococcus* sp. (2%) had the least percentage occurrence (Fig. 1). Five (5) fungal isolates were also isolated and identified from the soil samples, namely; *Aspergillus versicolor*, *Aspergillus niger*, *Mucor mucedo*, *Penicillium chrysogenum* and *Penicillium* sp. (Fig. 2). *Penicillium* sp. (47%) had the highest percentage frequency of occurrence amongst the fungal isolates while *P. chrysogenum* was the least occurring fungal isolate (34%) (Fig. 2).

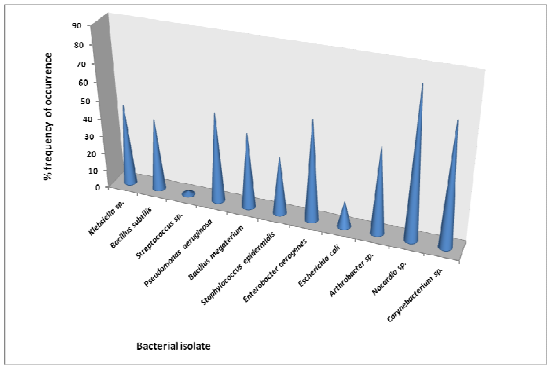

Fig. 1: Percentage frequency of occurrence of the bacterial isolates

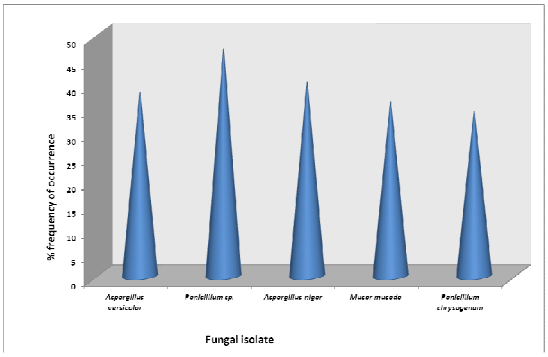

Fig. 2: Percentage frequency of occurrence of the fungal isolates

Table 1: Mean heterotrophic bacterial, fungal and hydrocarbon utilizing bacterial counts of soil contaminated with petroleum products and control Soil

Soil Sample	Mean Heterotrophic Bacterial Count (NA)	Mean Heterotrophic Fungi Count (PDA)	Hydrocarbon Utilizing Bacterial Count (MMSA)
Hydrocarbon Contaminated Soil (PS)	5.6×10^3	9.2×10^4	2.8×10^2
Uncontaminated Soil (CS)	1.4×10^4	5.1×10^4	Nil

Legend: NA: Nutrient Agar, PDA: Potato Dextrose Agar, MMSA: Modified Mineral Salt Agar

Table 2: Physicochemical properties of the soil samples

Parameters	PS	CS
pH	6.9	7.1
Conductivity (MHOS/cm)	20.7	11.9
Total Hydrocarbon Content (mg/Kg)	5600	600
Moisture Content (%)	76.1	88.9
Particle Size (%)		
Sand	69.40	82.04
Clay	26.34	17.94
Slit	4.26	0.04
Total Organic Carbon (%)	2.73	0.59
Nitrogen (mg/kg)	0.08	0.17
Phosphorus (mg/kg)	0.55	0.01
Cation Exchange Capacity (CEC)	3. 09	2.25
Water Holding Capacity (%)	76.67	55.00
Bulk Density (g/cm^3)	0.99	0.98

Legend: PS: Hydrocarbon Contaminated Soil, CS: Uncontaminated Soil

Table 3: Heavy metal content of the soil samples

Metals	Values (mg/kg)	
	PS	CS
Cu	1.80	0.10
Ni	ND	ND
Pb	0.22	0.10
Cd	0.10	ND

Legend: ND: below detectable level, PS: Hydrocarbon Contaminated Soil, CS: Uncontaminated Soil

Table 4: Forcados blended crude oil utilizing abilities of the microbial isolates

Microbial Isolates	Absorbance at 600nm
Bacterial	
Bacillus megaterium	0. 324 (+++)
Staphylococcus epidermidis	0.211 (+++)
Enterobacter aerogenes	0.103 (++)
Arthobacter sp.	0.114 (++)
Pseudomonas aeruginosa	0. 230 (+++)
Klebsiella oxytoca	0.035 (+)
Corynebacterium sp.	0.097 (++)
Klebsiella sp.	0.086 (++)
Bacillus subtilis	0.048 (+)
Streptococcus sp.	0.075 (++)
Nocardia sp.	0(-)
Fungal	
Aspergillus versicolor	0.016 (+)
Aspergillus niger	0.682 (++++)
Mucor mucedo	0.061(+)
Penicillium chrysogenum	0 (-)
Penicillium sp.	0.009 (+)
Control	0 (-)

Legend: +/-: score for the OD 600 nm reading.

The pH of the PS soil is slightly acidic when compared to that of the CS soil which is slightly alkaline. The PS soil had a higher conductivity and total hydrocarbon content when compared to the CS soil (Table 2). However, the CS soil had high moisture content than the PS soil. The particle size of both soil samples revealed that the samples are sandy-loamy (Table 2). The total organic carbon and phosphorus content of the PS soil were higher than those of the CS soil; however, the CS soil had higher nitrogen content than the PS soil. The magnesium and calcium content of the PS soils were higher than those of the CS soil, with the PS soil having a higher cation exchange capacity than the CS soil. The PS soil had higher water holding capacity and bulk density than the CS soil although there was no marked difference between the bulk densities of both soil samples (Table 2).

Table 3 revealed the results of heavy metal concentrations in both the PS and CS soils. The heavy metals assayed for were Copper (Cu), Lead (Pb), Cadmium (Cd) and Nickel (Ni) (Table 3). It was discovered that the PS soil had varying amounts of Cu, Pb Cd and Ni (Table 3). The CS soils only showed only trace amounts of Cu and Pb only (Table 3). All the bacterial isolates with the exception of *Nocardia* sp. could utilize crude oil as energy source (Table 4). *Bacillus megaterium, Staphylococcus epidermidis* and *Pseudomonas aeruginosa* scored the highest utilization rates. Amongst the fungal isolates, *Aspergillus niger* exhibited the highest potential for hydrocarbon utilization while *Pencillium chrysogenum* failed to utilize crude oil (Table 4). The bacterial isolates were screened for their ability to tolerate mercury, lead, nickel, copper and cadmium at three (3) different concentrations of 100 µg/ml, 50 µg/ml and 25 µg/ml respectively (Table 5a and 5b). All the bacterial isolates exhibited varying degrees of susceptibility towards mercury at 100µg/ml and 50µg/ml (Table 5a and 5b). *S. epidermidis*, *Klebsiella oxytoca*, *E. aerogenes, P. aeruginosa*, *B. megaterium, Streptococcus* sp. and *Nocardia* sp. were resistant to mercury at a concentration of 25 µ/ml (Table 5a and 5b). *Corynebacterium* sp., *Klebsiella sp.* and *B. subtilis* displayed resistance towards lead at 100 µg/ml, 50 µg/ml and 25 µg/ml respectively (Table 5a and 5b). The bacterial isolates exhibited varied sensitivity against copper at various concentrations; 100 µg/ml, 50 µg/ml and 25 µg/ml respectively (Table 5a and 5b). The highest zone of inhibition (27 mm) was displayed by *B. subtilis* against copper (Table 5a). All the bacterial isolates were sensitive against Cadmium (100 µg/ml, 50 µg/ml and 25 µg/ml) and Nickel at a concentration of 100 µg/ml (Table 5a and 5b).

DISCUSSION

The microbial counts recovered from the contaminated soils were higher than those observed in respect of the pristine soil (Table 1). This observation was similar to a report by Okereke *et al.* (2007) which recorded higher microbial counts from crude oil polluted soils in comparison to non crude oil polluted soils collected in Egbema oil field, Ohaji/Egbema Local Government Area, Imo State. The microbial bio load recorded for hydrocarbon polluted soils obtained within the vicinity of the auto mobile workshops could be

Table 5a: Susceptibility patterns of the bacterial isolates to varying concentrations of heavy metals (Hg^{2+}, Pb^{2+}, Cu^{2+}, Cd^{2+} and Ni^{2+})

Elements	Conc. (µ/ml)	Zone of inhibition (mm)					
		Bacillus subtilis	*Staphylococcus epidermidis*	*Enterobacter aerogenes*	*Klebsiella oxytoca*	*Pseudomonas aeruginosa*	*Arthobacter* sp.
Mercury (Hg^{2+})	100	25	16	16	8	17	22
	50	17	12	8	2	6	16
	25	-	7	-	-	-	12
Lead (Pb^{2+})	100	-	8	4	11	10	7
	50	-	-	-	7	6	2
	25	-	-	-	-	-	-
Copper (Cu^{2+})	100	27	20	16	12	13	16
	50	20	16	11	8	11	16
	25	10	12	10	7	9	6
Cadmium (Cd^{2+})	100	36	32	29	26	24	26
	50	31	33	26	20	18	21
	25	27	25	17	16	13	21
Nickel (Ni^{2+})	100	20	24	15	16	19	21
	50	17	19	11	13	16	17
	25	16	16	6	10	6	16

Table 5b: Susceptibility patterns of the bacterial isolates to varying concentrations of heavy metals (Hg^{2+}, Pb^{2+}, Cu^{2+}, Cd^{2+} and Ni^{2+})

Elements	Conc. (µ/ml)	Zone of inhibition (mm)				
		Corynebacterium sp.	*Bacillus megaterium*	*Streptococcus* sp.	*Klebsiella sp.*	*Nocardia* sp.
Mercury (Hg^{2+})	100	16	10	19	26	18
	50	14	6	11	21	9
	25	8	-	-	16	-
Lead (Pb^{2+})	100	-	3	13	-	23
	50	-	-	4	-	12
	25	-	-	-	-	7
Copper (Cu^{2+})	100	16	13	16	12	16
	50	9	11	12	11	10
	25	6	9	7	8	5
Cadmium (Cd^{2+})	100	27	31	24	28	22
	50	22	20	19	16	18
	25	16	16	18	10	11
Nickel (Ni^{2+})	100	23	18	20	23	16
	50	18	10	12	18	-
	25	11	8	-	14	-

suggestive of the ability of this microflora to thrive and proliferate in these environments in spite of the deliberate exposure of these soils to varying doses of petroleum or its refined products. The dominance of *Nocardia* sp. amongst the identified microbial isolates (Fig. 1) is in tandem with a report by Cullimore (2000) which indicated that *Nocardia spp.* and *Arthrobacter spp.* are widely distributed in soil. He also observed that *Arthrobacter spp.* is known to form the largest non-mycelia component in many soils. The microbial isolates identified from the examined soils (Fig. 1 and 2) contrasted with a report by Sarma and Sarma (2010) who isolated *Staphylococcus sp.*, *Acinetobacter iwoffii* and *Enterobacter agglomerans* from contaminated soils obtained from Joipur oil field in South western Assam, India.

In contrast to the neutral pH recorded for the control soil, the contaminated soil sourced from the vicinity of the automobile workshop was acidic (Table 2). This observation was similar to a report by Ipeaiyeda *et al*., (2007) which stated pH values which ranged from 6.1 to 7.2 for top soil samples obtained from five auto mechanic workshops located at Iwo town, Osun state. IIembayo and Kolade, (2008) stated that high pH might reduce the mobility of some metal species down the soil strata while low pH values usually enhance metal distribution and transport in the soil. The total hydrocarbon content of the petroleum polluted soil was higher than that of the control soil (Table 2). This could be symptomatic of the intermittent anthropogenic discharges of petroleum or its refined products on these soils. This trend is similar to a report by Liu *et al*. (2009) which stated a higher concentration of petroleum hydrocarbons in crude oil contaminated soil obtained from Jidong oil field, Southern China in comparison to pristine soil collected from a fallow farmland in Southern China.). The organic carbon and phosphorus content of the hydrocarbon polluted soil was comparatively higher than the value recorded in respect of the pristine soil sample (Table 2). This trend was in disagreement with a report by Liu *et al*. (2009) who reported higher values for these macro elements in pristine soil in comparison to values obtained for crude oil polluted soil sample However, the nitrogen content of the control soil was comparatively higher that of the hydrocarbon contaminated soil (Table 2). The relatively low concentration of these macro elements in the analyzed soil samples would discourage the direct usage of these soils for farming purposes. The heavy metal concentration of the soil samples was very low (Table 3). However, the concentration of the respective heavy metals in the uncontaminated soil sample was lower in comparison to values recorded for the hydrocarbon polluted soil (Table 3). This observation is similar to an earlier report by Hashem (1995) who reported that the concentration of several heavy metals such as lead, iron, cadmium, copper,

iron and zinc were comparatively higher in hydrocarbon contaminated soil than levels detected in pristine control soil collected from Eastern Saudi Arabia. Ipeaiyeda *et al*. (2007) reported that anthropogenic activities such as disposal of waste lubricant oil and auto exhaust emission are most likely to be a significant source of heavy metal contamination of top soils in the vicinities of auto repair workshops.

Amongst the microbial isolates, *A. niger* displayed the highest biodegradation potentials (Table 4). *A. niger* was able to maximally utilize the inoculated crude oil as sole source of energy and carbon (Table 4). The hydrocarbonclastic activity of *A. niger* strains isolated from soils has been reported by George-Okafor *et al*., (2009) and Sebiomo *et al*. (2011).

All the bacterial isolates with the exception of *Nocardia* sp. showed resistance against lead (Pb^{2+}) at various concentrations; 100 µ/ml, 50 µ/ml and 25µ/ml respectively (Table 5a and 5b). This was not surprising, given that the bacterial flora of the contaminated soil might have been exposed to various types of petroleum products, some of which contain lead at various concentrations, which are disposed onto these soils within the vicinity of auto mobile workshops. Cadmium (Cd^{2+}), Copper (Cu^{2+}) and Nickel (Ni^{2+}) were toxic to the bacterial isolates at varying levels; 100 µ/ml, 50 µ/ml and 25µ/ml respectively (Table 5a and 5b). The sensitivity exhibited by the bacterial isolates was however proportional to the concentration of the heavy metal utilized (Table 5a and 5b). This trend was similar to a report of Rathnayake *et al.* (2010) which investigated the tolerance of trace metals such as Cd^{2+}, Cu^{2+} and Zn^{2+} by *Paenibacillus sp*. and *Bacillus thuringeinsis* isolated from a pristine soil. The sensitivity of *B. megaterium* to cadmium (Cd^{2+}) and copper (Cu^{2+}) was in disagreement with a report by Velusamy *et al*. (2011) who reported the resistance of this bacterium isolated from a petroleum polluted soil in Korea to concentrations of 50 and 10 mg/l^{-1}. *Nocardia* sp. had the least zone of inhibition against cadmium at 100 µ/ml concentration in comparison to the other bacterial isolates (Table 5a and 5b). It has been recognized since decades that soil "actinomycetes" (*viz. Nocardia*, *Micromonospora* and *Streptomyces*) were more tolerant to this metal than were the "eubacteria" and that gram-negative "eubacteria" (*viz. Alcaligenes*, *Agrobacterium*, *Chromobacterium*, *Enterobacter*, *Proteus*, *Rhizobium*) were more tolerant than were gram-positive bacteria (*viz. Bacillus*, *Brevibacterium*, *Corynebacterium* and *Micrococcus*) (Babich and Stotzky, 1977).

Conclusion

Top soils collected from both the vicinities of the fallow farmland (control) and auto mobile workshop (hydrocarbon contaminated) contained varying numbers of culturable microflora. There were variations in the hydrocarbonclastic attributes and tolerance to differing concentrations of heavy metals by the individual micro organisms. This and future studies should provide some insight into heavy metal resistance in bacteria isolated from hydrocarbon contaminated soil ecosystems (auto mobile workshops) and could demonstrate the potentials of utilizing these microbes in the production of biosensors which can be utilized in biomonitoring of both background and anthropogenic environmental heavy metal pollution.

REFERENCES

Aiyesanmi AF, 2005. Assessment of heavy metal contamination of Robertkiri oil fields soil Science, 15: 42-46.

Amund OO, AA Adebowale and EO Ugoji, 1987. Occurrence and characterization of [277]hydrocarbon utilizing bacteria in Nigerian soil contaminated with spent motor oil. Indus J Microbiol, 27: 63-87.

Atlas RM and R Bartha, 1993. Microbial Ecology: Fundamentals and Applications. Addison Wesley Longman, Inc. Menlo Park, California, pp: 237-238.

Babich H and G Stotzky, 1977. Sensitivity of various bacteria, including actinomycetes, and fungi to cadmium and the influence of pH on sensitivity. Applied Environmental Microbiology, 33: 681-695.

Barnett HL and BB Hunter, 1972. Illustrated Genera of Imperfect Fungi. 3rd Edn. Burgess, New York.

Bremmer IM and CS Mulvaney, 1982. Total Nitrogen. In: American Society of Agronomy and Soil Science of America (editors), Methods of soil analysis. Agronomy Monograph, 9. Madison, Wisconsin, pp: 595-627.

Bruins MR, S Kapil and FW Oehme, 2000. Microbial resistance to metals in the environment. Ecotoxicol Environ Safety, 45: 198-207.

Cullimore DR, 2000. Practical Atlas for Bacterial Identification. CRC Press, Florida.

Eja ME, OR Ogri and OE Arikpo, 2003. Bioconcentration of heavy metals in surface sediments from the Great Kwa River Estuary, Calabar, Southeastern Nigeria. J Niger Environ Society, 1: 247-256.

Gadd GM, 1992. Metals and microorganisms: A problem definition. FEMS Microbiology Letters, 100: 197-204.

George-Okafor U, F Tasie and F Muotoe-Okafor, 2009. Hydrocarbon degradation potentials of indigenous fungal isolates from petroleum contaminated soils. J Physic Natur Sci, 3: 1-6.

Gerhardt P, R Murray, W Wood and N Kreig, 1994. Methods for General and Molecular Bacteriology. American Society for Microbiology, Washington DC.

Hashem AR, 1995. Influence of crude oil contamination on the chemical and microbiological aspects of Saudi Arabian soils. J King Saud Univ, 8: 11-18.

Hassan SHA, RNN Abskharon, SMF Gad El-Rab and AAM Shoreit, 2008. Isolation and characterization of heavy metal resistant strain of *Pseudomonas aeruginosa* isolated from polluted sites in Assiut, Egypt. J Basic Microbiol, 48: 168-176.

Hassen A, N Saidi, M Cherif and A Boudabous, 1998. Resistance of environmental bacteria to heavy metals. Bioresource Technol,, 64: 7-15.

Holt JG, NR Krieg and PHA Sneath, 1994. Bergey's Manual of Determinative Bacteriology, vol 4. Cambridge University Press, London.

Hussein H, S Farag, K Kandil and H Moawad, 2005. Tolerance and uptake of heavy metals by Pseudomonads. Process Biochem, 40: 955-961.

Ilembayo O and I Kolade, 2008. Profile of heavy metals from automobile workshops in Akure, Nigeria. J Envir Sci Tech, 1: 19-26.

Ipeaiyeda AR, M Dawodu, and Y Akande, 2007. Heavy metal contamination of top soil and dispersion in the vicinities of reclaimed auto repair workshops in Iwo, Nigeria. Res J Appl Sci, 2: 1106-1115.

Jaboro AG, 2013. Studies on antibiotic resistant plasmids isolated from bacteria in soil contaminated with petroleum products. Msc Thesis, Department of Microbiology, University of Benin.

Kalra YP and DG Maynard, 1991. Methods Manual for Forest soil and Plant Analysis. Minster of supply and services. Edmonton.

Kastner M, M Breuer-Jammali and B Mahro, 1995. Enumeration and characterization of the soil microflora from hydrocarbon-contaminated soil site able to mineralize polycyclic aromatic hydrocarbon (PAH). Appl Microbiol Biotechnol, 41: 267-273.

Liu R, Y Zhang, R Ding, D Li, Y Gao and M Yang, 2009. Comparison of archeal and bacterial community structures in heavily oil-contaminated and pristine soils. J Biosci Bioeng, 108: 400-407.

Mills AL, C Breuil, and RR Colwell, 1978. Enumeration of petroleum degrading marine and estuarine microorganisms by the most probable number method. Can J Microbiol, 24: 552-557.

Nies DH, 1999. Microbial heavy metal resistance. Appl Microbiol Biotechnol, 51:730-750.

Nriagu JO, 1990. Global metal pollution poisoning the atmosphere. Environment, 7: 87-93.

Okereke JN, SO Obiekezie, and KO Obasi, 2007. Microbial flora of oil-spilled sites in Egbema, Imo State, Nigeria. Afric J Biotechnol, 6: 991-993.

Okpokwasili GC and BB Okorie, 1988. Biodeterioration potentials of microorganisms isolated from car engine lubricating oil. Tribol Inter, 21: 215-220.

Olson BH and I Thronton, 1982. The resistance patterns to metals of bacterial populations in contaminated land. J Soil Sci, 33: 271-277.

Onyeonwu RO, 2000. Manual for Waste/Wastewater, Soil/ Sediment, Plant and Fish analysis. MacGill Environmental Research Laboratory Manual. Benin City.

Osborn A M, KD Bruce, P Strike and DA Ritchie, 1997. Distribution, diversity and evolution of the bacterial mercury resistance (mer) operon. *FEMS* Microbiol Rev, 19: 239-262. Osu CI and VC Okereke, 2010. Heavy metals contamination in relation to microbial counts in soils of automobile mechanic workshops, Port Harcourt metropolis, Rivers States, Nigeria. J Amer Sci, 6: 236-241.

Radojevic M and VN Bashkin, 1999. Practical Environmental Analysis. The Royal Society of Chemistry, Cambridge.

Rathnayake, IVN, M Megharaj, N Bolan and R Naidu, 2010. Tolerance of heavy metals by Gram positive soil bacteria. Inter J Civil Env Eng, 2: 191-195.

Rice LE and BB Hemmingsen, 1997. Enumeration of hydrocarbon-degrading bacteria. Methods Biotechnol, 2: 99-109.

Rough DA, BTO Lee and AP Morby, 1995. Understanding cellular responses to toxic agents: a model for mechanism-choice in bacterial metal resistance. J Indus Microbiol, 14: 132-141.

Said WA and DL Lewis, 1991. Quantitative assessment of the effects of metals on microbial degradation of organic chemicals. Appl Environ Microbiol, 57: 1498-1503.

Sarma A and H Sarma, 2010. Enhanced biodegradation of oil products by some microbial isolate supplemented with heavy metals. Inter J Bot, 6: 441-448.

Sebiomo A, AO Awosanya, and AD Awotodu, 2011. Utilization of crude oil and gasoline by ten bacterial and five fungal isolates. J Microbiol Antimicrob, 3: 55-63.

Seely HW and PJ Van-Denmark, 1981. A Laboratory Manual of Microbiology, 3rd edition. WH Freeman and Co, New York.

Sevgi E, G Coral, AM Gizir and MK Sangün, 2010. Investigation of heavy metal resistance in some bacterial strains isolated from industrial soils. Turkish J Biol, 34: 423-431.

Sharma K, 2009. Manual of Microbiology, tools and techniques. Ane books. Pvt Ltd, New Delhi.

Silver S and M Walderhaug, 1992. Gene-regulation of plasmid-determined and chromosome-determined inorganic ion transport in bacteria. Microbiol Rev, 56: 195-228.

Velusamy P, YM Awad, SAM Abd El-Azeem and YS Ok, 2011. Screening of heavy metal resistant bacteria isolated from hydrocarbon contaminated soil in Korea. J Agri Life Environ Sci, 23: 40-43.

Wuertz S and M Mergeay, 1997. The impact of heavy metals on soil microbial communities and their activities. In: Van Elsas JD, Trevors, JT and Wellington EMH (editors), Modern Soil Microbiology. Marcel Dekker, New York, pp: 607-639.

Zauyah S, B Juliana, R Noorhafizah, CI Fauzah and AB Rosenami, 2004. Concentration and speciation of heavy metals in some cultivated and uncultivated ultisols and inceptisols in Peninsular Malaysia. Proceedings of the Super-Soil 3rd Australian and New Zealand Soils Conference, University of Sydney Australia.