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A Study of Bacteriological and Physicochemical Characteristics in Soils of Auto-mechanic and none Auto-mechanic Workshop Soils from Selected Areas in Calabar Metropolis

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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 literature searches. Authors BEA and SOI managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The research study was aimed at investigating physicochemical and bacteriological characteristics of auto-mechanic and none auto-mechanic workshop soil samples. Auto-mechanic and non auto-mechanic workshop environments were randomly sampled within Calabar Metropolis. The study was completed within a period of six months. Standard microbiological methods were used to isolate, characterize and identify bacteria from the collected soil samples. The mean heterotrophic bacteria counts were higher in the auto-mechanic workshop soils compared to their non auto-mechanic workshop soil counterpart. Bacteria isolates from the auto-mechanic mechanic workshop soil samples were identified as species of *Aeromonas, Serratia, Klebsiella, Corynebacterium, Yersinia, Shigella, Enterobacter* and *Escherichia coli,* while those isolated from the none auto-mechanic workshop soils were identified as *Citrobacter, Yersinia, Bacillus and Serratia*. Comparison of the mean physicochemical parameters of the auto-mechanic workshop and non

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auto-mechanic soil samples showed significantly lower values of pH and available phosphorus in the auto-mechanic workshop soils whereas C:N ratio, calcium, total hydrocarbon concentration effective carbon exchange capacity and base saturation percentage values were significantly ($P<0.05$) higher in the auto-mechanic workshop soil samples. The concentration of heavy metals varied widely among the location studied. The mean Zn, Cu, Fe, V, Ni, Cd, Pb, Co, Cr were higher in the auto-mechanic workshop soil samples than in the none auto-mechanic soil samples. However, the total hydrocarbon values for the studied auto-mechanic soil samples showed that the mean values of 5266.7 mg/kg, 4413.2 mg/kg, 3130 mg/kg and 4514.4 mg/kg (AMT, AMM AME, and AMI) were above the recognized biogenic value of 50 mg/kg reported by DPR (1991), as these is environmentally significant and indicative of gross contamination which could in turn stimulate a wide variety of environmental issues.

Keywords: Auto-mechanic workshop; physicochemical.

1. INTRODUCTION

With an ever increasing world's population, there is a constant increase in the demand for petroleum and petroleum products which apparently constitutes a source of environmental pollution [1], as large amounts of petroleum products handled on land every year contribute immensely to land pollution as a result of the wrong channeling of used oil such as spent engine oil, used hydraulic oil and brake pad oils [2]. These used oils is improperly channeled to a collecting container may find their way into surrounding soil environment thereby causing environmental hazard with adverse effect.

The toxicity of these crude oil or petroleum products varies widely depending on their composition, concentration, environmental factors and on the biological state of the organisms at the time of the contamination. Prolonged exposure to high waste oil concentration may cause the development of liver or kidney disease, possible damage to the bone marrow and an increased rate of cancer [3].

In addition, several researchers have shown that metals contained in these waste oil posses the following effects when present in the environment; malfunctioning of the nervous system, weakness in fingers, wrists or ankles, anemia and increase in blood pressure caused by high exposure of humans to lead either by breathing or swallowing [4]; Asthma, pneumonia, nausea, vomiting and sneezing caused by exposure to high levels of cobalt either from intake of food or drink [5]; Flu-like condition known as metal fever, liver and kidney damage, Wilson's disease and even death caused by long time exposure and infestation of copper [6]; damage of nervous system, cardiac and vascular diseases, skin rashes, nose bleeding, headaches, malaise and dizziness caused by high exposure to vanadium [7,8].

The biodegradation of these waste oil pollutant by microorganisms present in the soil as a result of their metabolic diversity occurs when there is pollution in areas where microorganisms are present [9]. Microbial degradation of hydrocarbon-contaminated site is performed with the help of a diverse group of microorganisms particularly the indigenous bacteria present in soil. A large number of pseudomonas strains capable of degrading poly aromatic hydrocarbons have been identified to include *Bacillus, Micrococcus, Alkaligenes spp, Flavobacterium, Corynebacterium, Serratia spp, Enterobacter spp* [10,11].

Therefore, the aim of this research work was to determine the bacteriological and physiochemical characteristics of auto-mechanic and none auto-mechanic workshop soils from selected areas in Calabar Metropolis, Cross River state.

2. MATERIALS AND METHODS

2.1 Study site and Sampling

Four different auto-mechanic and none auto-mechanic workshops were sampled randomly from different locations within Calabar-metropolis (Fig. 1). The auto-mechanic workshop sampled were collated from the top, 0 cm to 15 cm of the soil, using sterile spoon and then placed in an oven sterilized aluminum foil (at 160℃ per hour). The soil samples were then wrapped and placed in sterile 100 ml universal container.

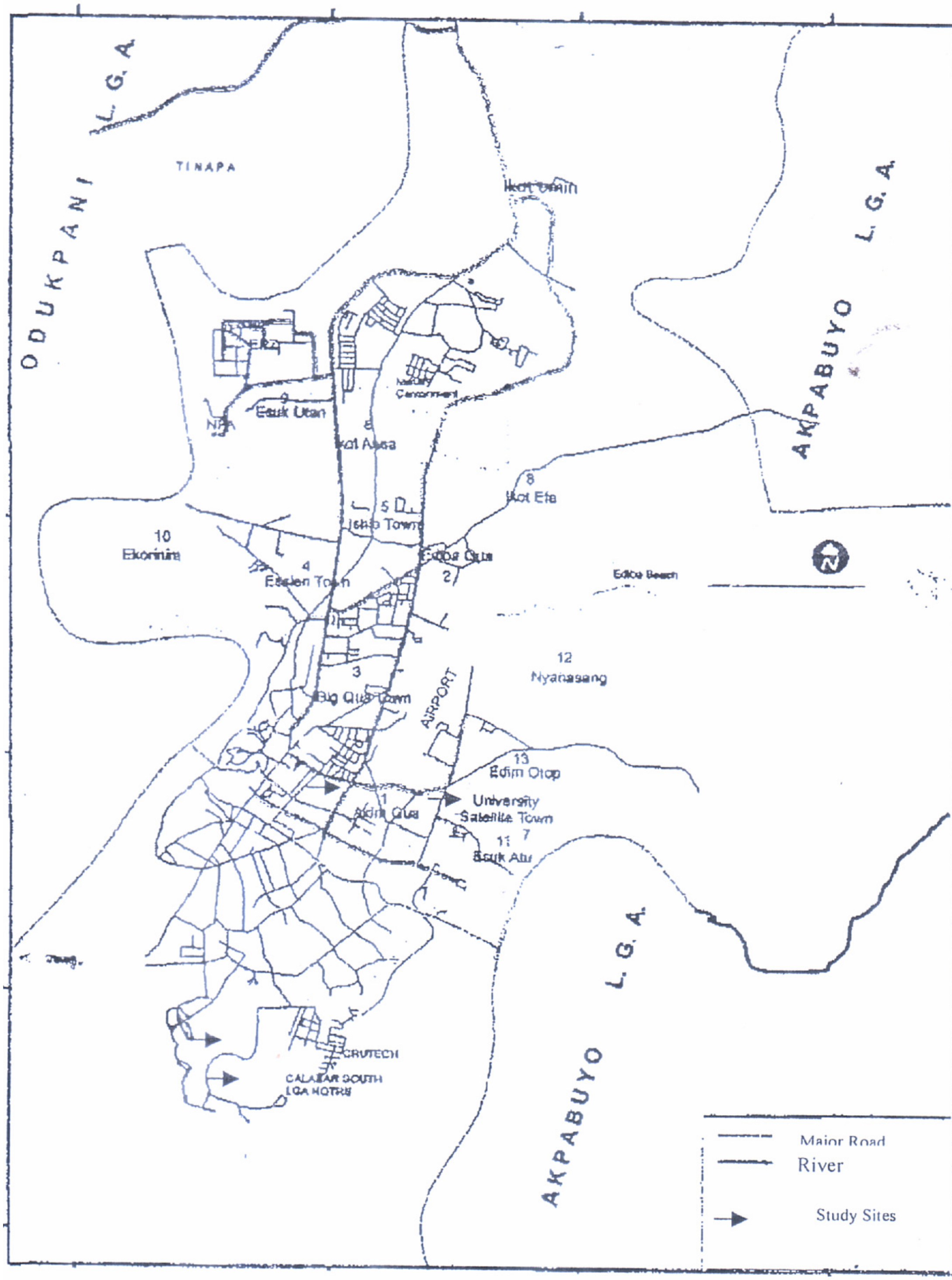

Fig. 1. Map of Calabar, showing the study area
Source: GIS Unit, University of Calabar, 2012

2.2 Media

The media used in the study were Nutrient agar, Motility Indole Ornithine (MIO) medium (Hardy diagnostics, USA), Simmon citrate medium (Acumedia, USA). These media were prepared according to manufacturer's instructions.

2.3 Chemicals and Reagent

Chemicals used in the study were of analytical grade. They include absolute alcohol, acetone, methanol (Sigma, USA), neutral red, methyl red indicators, phenol red indicator, urea (Titan Biotech, India). Reagents used were oxidase strips, indole kovacs and were products of Hardy diagnostics, USA.

2.4 Microbiological Analysis

2.4.1 Sample preparation

Ten (10) grams 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 this initial dilution, ten (10) fold serial dilutions were carried out in clean sterile test-tubes containing 9m/s of sterile distilled water.

2.4.2 Plating procedures

Zero-point-one (0.1) milliliter of desire dilutions, 10^{-3}-10^{-5} was spread plated in triplicate unto nutrient agar supplemented with 50 µg/ml of nystatin to inhibit the growth of fungi. Plates were incubated at 35°c and bacterial counts recorded after twenty-four (24) hours of incubation.

2.4.3 Purification and identification of isolates

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

2.4.4 Identification and characterization of isolates

Purified isolates were characterized by gram morphology and biochemical test using the scheme in Bergey's manual of determinative bacteriology [12,13].

2.5 Physicochemical Analysis

2.5.1 Processing of soil samples

Soil samples for physiochemical analysis were air dried, ground, sieved and homogenized; samples were then re-bagged in whirl-pak bags from which portions was used for physiochemical analysis.

2.5.2 Determination of pH

Soil pH was determined using soil solution ratio of 1:2 (10 g of soil in 20 ml of distilled water), [14]. The pH was read with pH meter.

2.5.3 Determination of organic-carbon

Organic carbon in soil was determined calorimetrically using modified Walkey-Balck method as described in methods in soil Microbiology and Biochemistry [15].

2.5.4 Total nitrogen estimation

Total nitrogen was estimated by the Macro-Kjeldahl Digestion method as outlined by [16]. Soil samples were digested with catalyst mixture and concentrated H_2SO_4. The digest were distilled with 10N NaoH into boric indictor (H_3BO_3). The distillate was titrated with $0.01NH_2SO_4$to a pink colour. Blank (without soil) was prepared and used percentage of total nitrogen in soil was calculated from the following equation:

$$\% \text{ total N} = \frac{M\ (T\text{-}B) \times 14 \times 100}{1000W}$$

Where

M = normality of H_2So_4, T=ml, burette reading for sample

B = ml, burette reading for blank, w=weight of sample

2.5.5 Available phosphorus

Soil available phosphorus was extracted with acid fluoride using the Bray P-II method. Phosphorus in the extract was determined calorimetrically [16].

2.5.6 Soil potassium and sodium determination

The method of [17] was adopted. Soil was extracted with IN NH_4OAC (pH 7.0) using 1:10

soil-solution ratio. Potassium and sodium extract was determined by flame photometry using flame photometer.

2.5.7 Nitrate, sulphate and phosphate determination

For extraction of nitrate and sulphate determination, 10 g each of the soil samples was weighed, transferred to 250 ml stopper conical flask and agitated with exactly 10 ml of distilled water (1:1 ratio) for 10 minute using a mechanical shaker. After agitation, samples was left for 30 minutes and filtered into Buchner funnels using Whatman No. 42 filter papers. Turbid filtrates were centrifuged at 3000 rpm for 5 minutes. The extraction for phosphate was carried out by weighing 10 g of soil into 10 ml of $NaHCO_3$ at pH 8.5, agitation and filtration was performed as described above. Nitrate, sulphate and phosphate were analyzed from the extracts.

2.5.8 Particle size analysis

Soil particle size distribution was determined using Bouyocos-type hydrometer method.

2.5.9 Estimation of total hydrocarbon (THC) and heavy metal concentration

THC was determined in the soil samples using the method described by [18]. 5 grams of each sample was extracted twice with 25 ml of toluene, filtered into 50 ml flack and made up to 50 ml with toluene. The absorbance of the filtrates was measured at 4200 m wavelength using spectrophotometer and THC concentrations calculated from the calibration graph.

Heavy metals (Pb, Cr, V, Ni, Co, Cd, Cu, Zn) concentrations in soil samples was determined following digestion in aqua regia (3:1HCL:HNO_3). 2 g of soil samples each was taken into 250 ml glass beaker and digested with 8 ml of aqua regia on a sand bath for two hours, following evaporation to near dryness, the samples were dissolved with 10 ml of 2% HNO_3, filtered and diluted into 50 ml with distilled water. Heavy metals in extract were analyzed using an atomic absorption spectrophotometer.

2.6 Data Collection and Analysis

Data on physiochemical parameters of the collected soil samples was subjected to analysis and significant means was determined using Duncan multiple range test ($p<0.05$) and T-test.

3. RESULTS

3.1 Heterotrophic Bacteria Counts

The mean heterotrophic bacteria count recorded from auto-mechanic and non auto-mechanic workshop soil samples are presented in Table 2. For the spent oil polluted soil samples, the mean heterotrophic bacteria counts ranged from 7.83×10^5 to 2.1×10^6 cfu/g and 1.07×10^5 to 9.10×10^5 cfu/g for the none auto-mechanic workshop soil samples respectively.

3.2 Morphological and Biochemical Characterization of Bacteria Isolates from Auto-mechanic Workshop

Table 3 presents the results of the morphological and biochemical characterization of bacteria isolates from auto-mechanic workshop soils.

Bacteria genera identified in the auto-mechanic workshop soil samples were species of *Bacillus, Pseudomonas, Aeromonas, Serratia, Klebsiella, Corynebacterium, Yersinia, Shigella, Enterobacter* and *Escherichia coli.* While bacteria isolates from the non auto-mechanic workshop were identified as species of *Citrobacter, Yersinia, Bacillus and Serratia* as shown in Table 5.

3.3 Physiochemical Properties of Soil Samples

The physiochemical properties of auto-mechanic workshop and none auto-mechanic soil samples are presented in Tables 5 and 6 respectively. The auto-mechanic soil samples were strongly acidic with pH values in the range of 4.36±0.01 to 5.67±0.20. The organic carbon content of the various auto-mechanic workshop soil samples was between 7.98±0.19% and 12.66±0.96% while total nitrogen content was between 0.35±0.02% and 0.66±0.03%. Consequently, the C:N ratio values ranged from 15.1±0.4 to 26.4±1.0. The auto-mechanic workshop soil levels of available phosphorus, calcium, magnesium, sodium and potassium vary from 11.82±0.22 to 20.87±0.19 mg/kg, 5.68±0.07 to 7.87±0.14 cmol/kg, 2.01±0.14 to 4.41±0.03 cmol/kg, 0.20±0.01 to 0.45±0.01 cmo/kg, and 0.12±0.01 to 0.21±0.02 cmol/kg respectively. The auto-mechanic workshop soil textural classes were loamy sand (AMM, AME and IS) and sandy loamy (AMA) with EA, ECEC and BS values ranging from 1.05±0.02 to

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
AMA	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

Table 2. Mean heterotrophic bacteria counts of auto-mechanic workshop and none auto-mechanic workshop soil samples

Soil sample	Location code	Mean heterotrophic bacteria count (cfu/g)
Auto-mechanic	AMA	1.37×10^6
	AME	9.10×10^5
	AMM	2.12×10^6
	AME	7.83×10^5
None Auto-mechanic	SQU	1.07×10^5
	CCC	1.41×10^5
	BGU	9.10×10^5
	IS	1.6×10^5

AMA = Auto-mechanic workshop soil – Atekong, AME = Auto-mechanic workshop soil – Etta abgor, AMM = Auto-mechanic workshop soil – Mbukpa, AMI = Auto-mechanic workshop soil-Inyang, SQU=Staff quarters-Unical, CCC=Cultural Centre Calabar, BGU=Botanical Garden Calabar, IS=Inyang street

1.12±0.02 cmol/kg, 10.07±0.18 to 13.4±0.23 cmol/kg and 88.4±0.16% to 90.7±0.21% respectively. All the physiochemical parameters from the different auto-mechanic workshop soil samples varied significantly (Duncan Multiple Range test, p<0.05).

For none auto-mechanic workshop soil samples as presented in Table 6, the pH values, organic carbon and nitrogen contents were in the range of 5.30±0.01 to 6.03±0.01, 9.83±0.25% to 11.83±0.59%, and 0.61±0.04% to 0.74±0.03% respectively. C:N ratio between 13.6±0.35 and 19.4±1.40 was obtained. Available phosphorus levels ranged from 31.29±0.71 to 43.91±0.40 mg/kg, while the values for Ca, Ma, Na and k were in the range of 1.40±0.02 to 0.34±0.01 cmol/kg and 0.13±0.11 to 0.23±0.02 cmol/kg respectively EA (1.07±0.01 to 1.15±0.01 cmol/kg), ECEC (6.27±0.05 to 10.21±0.04 cmol/kg and BS (80.8±0.14 to 90.5±0.21%) values were obtained. These parameters varied significantly among the none auto-mechanic workshop soil samples (Duncan Multiple Range test, p>0.05). The results of the particle size analysis showed that the none auto-mechanic workshop soil samples were loamy sand (SQU, IS) and sandy loam (BGU, CCC).

3.4 Comparison of Physicochemical Parameters of Auto-mechanic and None Auto-Mechanic Workshop Soil Samples

The mean physicochemical parameters of both the auto-mechanic and none auto-mechanic workshop soils are shown in Table 7. The observed mean values of pH, and available phosphorus were significantly lower in the auto-mechanic workshop soil than in the pristine soil, where as the C:N ratio, calcium, effective cation exchange capacity and base saturation percentage (Bs) values were significantly (p>0.05) higher in the auto-mechanic workshop soil than in the control soil. There was no significant difference in other parameters measured.

Table 3. Morphological and biochemical characterization of bacteria isolates from auto-mechanic workshop soil

Isolate	Oram RXN	Shape	Oxidase	Catalase	Motility	Indole	Ornithine	Methyl Red	Vogespr oskauer	Urease	Citoale	Starch hydrolysis	Acidfast	Slope	Butt	H2s	Gas	Probable organism
AMA_1	+	Rod	+	+	*	*	*	*	*	*	-	+	*	*	*	*	*	*Bacillus spp*
AMA_2	+	Rod	+	+	*	*	*	*	*	*	-	+	*	*	*	*	*	*Bacillus spp*
AMA_3	-	Rod	-	+	+	+	-	-	+	+	+	*	*	R	Y	+	AG	*Escherichia coli*
AMA_4	-	Rod	+	+	+	-	*	-	-	-	+	*	*	*	*	-	*	*Pseudomonas spp*
AMA_5	-	Rod	-	*	-	-	*	+	-	*	*	*	*	Y	Y	-	AG	*Klebsiellaspp*
AMM_1	-	Rod	+	*	-	*	*	*	-	*	*	*	*	R	Y	-	-	*Aeromonasspp*
AMM_2	-	Rod	-	*	+	-	-	+	-	-	*	*	*	R	Y	-	-	*Serratiaspp*
AMM_3	-	Rod	-	*	-	-	*	+	-	*	*	*	*	Y	Y	-	-	*Klebsiellaspp*
AMM_4	-	Rod	-	*	+	-	+	-	+	-	+	*	*	R	Y	+	AG	*Enterobacterspp*
AMM_5	-	Rod	-	*	+	-	+	-	+	-	+	*	*	R	Y	+	AG	*Enterobacterspp*
AMM_6	-	Rod	-	*	+	*	*	*	-	*	*	*	*	R	Y	-	-	*Aeromonasspp*
AME_1	+	Rod	-	+	-	*	*	+	*	*	*	-	-	-	*	*	*	*Corynebacteriumspp*
AME_2	-	Rod	-	*	+	-	-	*	-	-	*	*	*	Y	Y	-	AG	*Serratiaspp*
AME_3	-	Rod	-	*	-	-	-	*	*	-	*	*	*	R	Y	-	AG	*Yersinia spp*
AME_4	+	Rod	-	+	*	*	*	*	*	*	-	+	*	*	*	*	*	*Bacillus spp*
AME_5	-	Rod	-	*	+	-	-	*	-	-	*	*	*	Y	Y	-	AG	*Serratiaspp*
IS_1	-	Rod	-	*	-	-	+	*	*	-	*	*	*	R	Y	-	-	*Shigellaspp*
IS_2	-	Rod	+	*	+	*	*	*	-	*	*	*	*	R	Y	-	-	*Aeromonasspp*
IS_3	-	Rod	-	*	+	-	-	+	-	-	*	*	*	Y	Y	-	AG	*Serratiaspp*
IS_4	-	Rod	-	*	-	-	*	+	-	*	*	*	*	Y	Y	-	-	*Klebsiellaspp*
IS_5	-	Rod	+	+	+	-	*	-	-	-	+	*	*	*	*	-	*	*Pseudomonas spp*

*Key: R=Red, Y=Yellow, AG=Acid and gas, +=Positive, -=Negative, *=Not Determined*

Table 4. Morphological and biochemical characterization of bacteria isolates from pristine soil

Isolate	Gram RXN	shape	Oxidase	Catalase	Motility	Indole	Ornithine	Methyl Red	Voges-proskauer	Urease	Citrate	Starch-hydrolysis	Acid-fast	Slope	Butt	H_2s	Gas	Probable organism
SQU_1	-	Rod	-	*	*	-	*	+	-	-	*	*	*	Y	Y	+	AG	*Citrobacter spp*
SQU_2	-	Rod	-	*	*	-	*	+	-	-	*	*	*	Y	Y	+	AG	*Citrobacter spp*
SQU_3	-	Rod	-	*	-	-	-	*	*	-	*	*	*	R	Y	-	AG	*Yersinia spp*
SQU_4	-	Rod	-	*	+	-	-	+	-	-	*	*	*	R	Y	-	-	*Serratia spp*
IS_1	-	Rod	-	*	-	-	-	*	*	-	*	*	*	R	Y	-	AG	*Yersinia spp*
IS_2	+	Rod	+	+	*	*	*	*	*	*	-	+	*	*	*	*	*	*Bacillus spp*
IS_3	-	Rod	-	*	+	-	-	+	-	-	*	*	*	R	Y	-	-	*Serratia spp*
IS_4	-	Rod	-	*	*	-	*	+	-	-	*	*	*	Y	Y	+	AG	*Citrobacter spp*
IS_5	+	Rod	+	+	*	*	*	*	*	*	-	+	*	*	*	*	*	*Bacillus spp*
BGU_1	+	Rod	+	+	*	*	*	*	*	*	-	+	*	*	*	*	*	*Bacillus spp*
BGU_2	+	Rod	+	+	*	*	*	*	*	*	-	+	*	*	*	*	*	*Bacillus spp*
BGU_3	-	Rod	-	*	+	-	-	+	-	-	*	*	*	R	Y	-	-	*Serratia spp*
CCC_1	-	Rod	-	*	+	-	-	+	-	-	*	*	*	R	Y	-	-	*Serratia spp*
CCC_2	-	Rod	-	*	-	-	-	*	*	-	*	*	*	R	Y	-	AG	*Yersinia spp*

*Key: R = Red, Y = Yellow, AG = Acid and Gas, + = Positive, - = Negative, * = Not Determined*

3.5 Total Hydrocarbon (THC) and Heavy Metal Analyses

The results of the Total Hydrocarbon (THC) and Heavy metal levels in auto-mechanic workshop soil samples are presented in Table 8 and 9. Total Hydrocarbon concentrations in auto-mechanic workshop soil samples ranged from 33130.27±27.392 to 5266.7±40.831 mg/kg while heavy metals concentrations were in the range of 0.012±0.0003 to 2863.1±43.028 mg/kg. The mean Total Hydrocarbon and heavy metal levels from the different locations varied significantly (p<0.05) (Table 7).

As shown in Table 9, the none auto-mechanic workshop soil samples had Total Hydrocarbon concentrations ranging from 9.893±0.315 to 26.543±0.505 mg/kg, with heavy metal levels ranging from 0.003±0.001 to 3735.20±60310 mg/kg.

4. DISCUSSION

In this study, the mean heterotrophic bacteria count was greater in the auto-mechanic workshop soil samples compared to the non auto-mechanic soil samples. This observation corroborates those of [19], who reported a higher heterotrophic bacteria count in soil contaminated with used petroleum products than in non-contaminated soil in Umuahia. Also study by [20] reported a higher total heterotrophic bacterial counts in oil contaminated soils major motor mechanic workshops than pristine soils in Benin-city metropolis, Edo state. This observation was also in line with similar study by [21], who reported higher microbial counts from crude oil polluted soils in comparison to pristine soils. The increased mean heterotrophic bacteria count which was observed in the auto-mechanic workshop soil samples could have been as a result of the increased hydrocarbon content and this is in agreement with the findings of [22]. Bacteria genera isolated from the auto-mechanic workshop soil were species of *Pseudomonas, Bacillus, Serratia, Klebsiella, Corynecbacetrium, Yersinia, Shigella, Enterobacter* and *Escherichia coli*, while bacteria species from the non auto-mechanic workshop soil samples as *Citrobacter, Yersinia, Bacillus* and *Serratia.* This observation is similar with that of study by [4], who identified species of *Acinetobacter, Micrococcus, Pseudomonas, Bacillus sp,* and *Serratia* from used lubricating oil contaminated soil. Also study by [23] reported the presence of *Klebsiella, Citrobacter, Micrococcus* and *Bacillus* species in petroleum contaminated soils. Similar study

Table 5. Physicochemical parameters of auto-mechanic workshop soil samples

Physicochemical parameters of sample	Location code			
	AMA	AMM	AME	IS
pH	5.67^c±0.20	4.37^a±0.01	5.40^b±0.21	4.36^a±0.01
Org.C (%)	8.04^a±0.21	12.66^c±0.91	7.98^a±0.91	10.65^b±0.38
Total Nitrogen (%)	0.35^a±0.02	0.48^b±0.01	0.53^b±0.02	0.66^c±0.03
C:N Ratio	22.90^c±0.5	26.4^b±1.0	15.1^a±0.4	16.1^a±0.7
Avail-P (mg/kg)	14.05^b±0.12	20.87^d±0.19	18.98^c±0.26	11.82^a±0.22
Ca (cmol/kg)	5.68^a±0.07	7.02^c±0.06	7.87^c±0.10	4.41^c±0.30
Mg (cmol/kg)	3.21^b±0.11	2.01^a±0.14	3.98^b±0.10	4.41^c±0.30
Na (cmol/kg)	0.20^a±0.01	0.38^b±0.01	0.28^a±0.02	0.45^c±0.01
K (cmol/kg)	0.12±0.01	0.21±0.02	0.16±0.01	0.20±0.02
EA (cmol/kg)	1.06^a±0.02	1.05^a±0.02	1.11^b±0.02	1.12^b±0.02
ECEC (cmol/kg)	10.07^a±0.18	10.67^a±0.20	13.4^b±0.23	13.2^b±0.20
BS (%)	88.9±0.20	90.5±0.13	88.4±0.16	90.7±0.21
Sand (%)	80.5	85.3	84.1	83.5
Silt (%)	6.6	10.5	4.9	6.6
Clay (%)	11.5	4.2	10.7	9.5
Soil texture	SL	LS	LS	LS

Org.C=Organic Carbon, Avail-P=Available Phosphorus, AMA=Auto-mechanic workshop, Atekong, AMM=Auto=mechanic workshop soil-Mbukpa, AME=Auto-Mechanic workshop soil-Ettaagbor, AMI=Auto-mechanic workshop soil-Inyang, EA=Exchangeable Acidity, ECEC=Effective Cation Exchange Capacity, BS=Base Saturation, LS=Loamy Sand, SL=Sandy Loam; means in row with same superscript letter are not significantly different from each other (Duncan Multiple Range test, p>0.05)

Table 6. Physicochemical parameters from none auto-mechanic workshop soil samples

Physicochemical parameters of sample	Location code			
	SQU	**BGU**	**CCC**	**IS**
pH	5.30^{a}±0.01	5.51^{a}±0.02	6.03^{b}±0.01	5.73^{a}±0.02
Org.C (%)	11.78^{b}±0.41	9.83^{c}±0.59	11.8^{b}±0.59	10.18^{a}±0.45
Total Nitrogen (%)	0.74^{b}±0.03	0.72^{b}±0.05	0.61^{a}±0.04	0.73^{b}±0.09
C:N Ratio	15.9^{b}±7.30	13.6^{b}±0.35	19.4^{c}±1.40	13.9^{a}±0.91
Avail-P (mg/kg)	31.29±0.71	37.52^{b}±0.80	43.91^{c}±0.40	42.05^{d}±1.06
Ca (cmol/kg)	5.73^{c}±0.01	4.18^{b}±0.03	1.40^{a}±0.02	4.39^{b}±0.02
Mg (cmol/kg)	2.14^{a}±0.01	3.24^{b}±0.01	3.38^{c}±0.02	412^{d}±0.01
Na (cmol/kg)	0.14^{a}±0.01	0.22±0.02	0.23^{b}±0.01	0.34^{c}±0.01
K (cmol/kg)	0.13^{a}±0.11	0.23^{b}±0.02	0.14^{a}±0.03	0.22^{b}±0.02
EA (cmol/kg)	1.07^{a}±0.01	1.15^{b}±0.01	1.12^{b}±0.02	1.14^{b}±0.01
ECEC (cmol/kg)	9.21^{b}±0.03	9.02^{b}±0.04	6.27^{a}±0.05	10.21^{c}±0.04
BS (%)	87.86±0.11	87.08±0.14	84.27±0.33	90.5±0.21
Sand (%)	82.2	80.8	80.4	84.1
Silt (%)	7.8	8.2	7.5	7.3
Clay (%)	10	11	11.5	10
Soil texture	LS	SL	SL	LS

Org.C=Organic Carbon, Avail-Pc=Available Phosphorus, EA=Exchangeable Acidity, ECEC=Effective Cation Exchange Capacity, BS (Base Saturation, LS=Loamy Sand, SL=Sandy Loam, SQU-Staff Quarters-UNICAL, BGU=Botanical Garden CCC=Cultural Center Calabar, IS=Inyang street; same alphabets means the values are not significantly different from each other while different superscripts means they differ significantly

Table 7. Comparison of mean physicochemical parameters of auto-mechanic and none auto-mechanic workshop soil sample

Physicochemical parameters	Auto-mechanic workshop soil N=4	Pristine soil N=4	T-test	df	p-value
pH	4.95±0.11	5.60±0.02	2.60	6	2.45^{s}
Org.C(%)	9.83±0.44	10.96±0.43	1.71	6	2.45^{Ns}
Total Nitrogen (%)	0.51±0.02	0.70±0.05	1.02	6	2.45^{Ns}
C:N Ratio	20.13±0.65	15.70±0.99	4.88	6	2.45^{s}
Avail-P(mg/kg)	16.43±0.20	38.69±0.74	32.45	6	2.45^{s}
Ca (cmol/kg)	6.80±0.16	3.92±0.02	12.86	6	2.45^{s}
Mg (cmol/kg)	3.40±0.16	0.22±0.01	0.62	6	2.45^{Ns}
Na (cmol/kg)	0.33±0.01	0.23±0.02	0.82	6	2.45^{Ns}
K (cmol/kg)	0.17±0.02	0.18±0.05	0.05	6	2.45^{Ns}
EA (cmol/kg)	1.09±0.02	1.12±0.01	0.25	6	2.45^{Ns}
ECEC (cmol/kg)	11.84±0.20	8.68±0.13	7.78	6	2.45^{s}
BS(%)	89.62±0.18	87.43±0.20	5.02	6	2.45^{s}

EA=Exchangeable acidity. ECEC=Effective cation exchange capacity, BS = base Saturations, N^{S}= Not significant, S=significant

by [24] also reported to have isolated *Pseudomonas, Bacillus, Micrococcus, Flavabacterium* and *Klebsiella* from oil contaminated soil in Niger delta.

The physicochemical properties of any soil in the event of pollution with organic or chemical compounds are bound to alter. In this study, changes in some physicochemical parameters of auto-mechanic workshop soil were observed in relation to its non auto-mechanic soil counterpart. The auto-mechanic workshop soils in this study were characterized by significant low pH, available phosphorus and high C:N ratio, calcium, effective cation exchange capacity (ECEC) and percentage of base saturation in relation to the non auto-mechanic soil. This observation corroborates with that of [19], who reported a low pH, and high calcium, magnesium and C:N ratio in soil contaminated with used petroleum products in Umuahia, as compared with uncontaminated soils. Other physicochemical parameters showed no significant difference between the auto-mechanic workshop and non auto-mechanic soil. It is well known that in the event of petroleum product spillage on soil the activities of microbes that are able to degrade petroleum hydrocarbon alter the soil pH due to the accumulation of acidic metabolites.

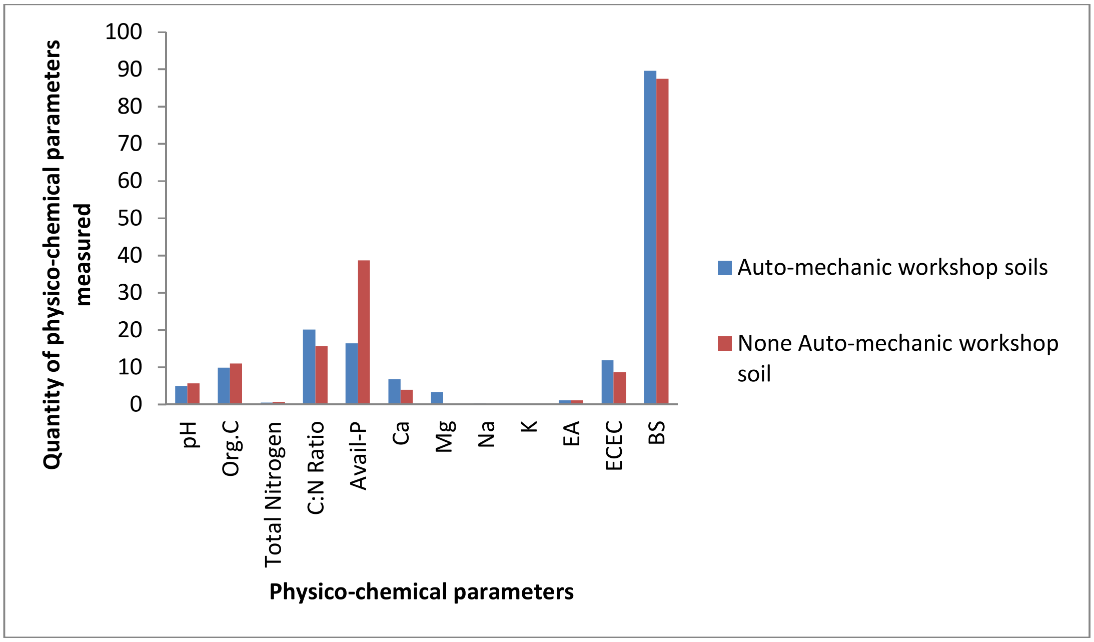

Fig. 2. Comparism of mean physico-chemical parameters of auto-mechanic and none auto-mechanic workshop soil samples

Key: Org.C – Organic carbon, C:N Ratio – Carbon Nitrogen ratio, Avail-P – Available Phosphorus, Ca – Calcium, Mg – Magnesium, Na – Sodium, K – Potassium, EA – Exchangeable Acidity, ECEC – Effective Cation Exchange Capacity, BS – Base Saturation

Table 8. Total hydrocarbon and heavy metal concentration of auto-mechanic workshop soil samples

Physicochemical parameters of sample	Location code			
	AMA	AMM	AME	IS
THC (mg/kg)	5266.7^{c}±40.83	4413.2^{b}±6.009	3130.27^{a}±27.392	4513.4^{b}±43.004
Zn (mg/kg)	322.00^{d}±0.428	167.16^{b}±0.756	43.673^{a}±0.781	219.60^{c}±5.256
Cu (mg/kg)	25.256^{c}±0.890	7.550^{a}±0.756	15.332^{b}±2.933	7.560^{a}±0.854
Pe (mg/kg)	2729.0^{c}±6.883	2863.1^{d}±43.028	2566.2^{b}±29.362	2355.1^{a}±34.56
V (mg/kg)	21.426^{d}±8.52	19.646^{c}±0.790	15.396^{b}0.007	5.840^{a}±0.317
Ni (mg/kg)	4.579^{b}±0.551	51.082^{d}±0.882	8.609^{c}±1.527	2.114^{a}±0.016
Cd (mg/kg)	0.84^{b}±0.012	0.073^{a}±0.017	1.085^{c}±0.111	0.075^{a}±0.016
Pb (mg/kg)	0.104^{a}±0.012	2.178^{b}±0.180	0.012^{a}±0.003	5.546^{c}±0.452
Co (mg/kg)	7.304^{d}±0.889	4.573^{c}±0.539	2.662^{a}±0.002	3.772^{b}±0.001
Cr (mg/kg)	78.78^{a}±2.846	18.374^{c}±1.829	24.58^{b}±1.090	16.543^{a}±1.772

THC=Total Hydrocarbon, AMA=Auto-mechanic workshop soil – Atekong, AMM = Auto-mechanic workshop soil-Mbukpa, AME-auto-mechanic workshop soil – Etta=agbor, AMI=Auto-mechanic workshop soil Inyang; same alphabets means the values are not significantly different from each other while different superscripts means they differ significantly

Table 9. Total hydrocarbon and heavy metal concentration of none auto-mechanic soil samples

Physicochemical parameters of sample	Location code			
	SQU	BGU	CCC	IS
THC	11.350[b]±0.350	26.543[c]±0.505	10.352[a]±0.542	9.893[a]±0.315
Zn (mg/kg)	9.12[a]±0.231	18.013[b]±0.062	36.394[d]±0.587	20.013[c]±0.081
Cu (mg/kg)	1.556[a]±0.158	9.517[c]±0.20	5.432[b]±0.205	1.762[a]±0.032
Fe (mg/kg)	3735.20[d]±60.310	32.16[a]±2.451	1425[b]±1.432	2715[c]±1.321
V (mg/kg)	0.335[b]±0.07	0.084[a]±0.004	0.283[b]±0.002	1.125[c]±0.002
Ni (mg/kg)	0.101[b]±0.005	0.052[a]±0.002	0.137[c]±0.002	0.325[c]±0.001
Cd (mg/kg)	0.095[a]±0.003	0.013[a]±0.002	0.046[b]±0.002	0.063[b]±0.003
Pb (mg/kg)	0.073[b]±0.002	0.058[a]±0.004	0.090[c]±0.003	0.062[a]±0.002
Co (mg/kg)	0.003[a]±0.001	0.013[a]±0.001	0.026[b]±0.001	0.084[c]±0.001
Cr (mg/kg)	0.035[b]±0.003	0.017[a]±0.004	0.056[b]±0.003	0.072[c]±0.002

THC= Total Hydrocarbon, SQU=Staff Quarters – UNICAL, BGU=Botanical Garden-UNICAL, CCC= Cultural Center Calabar, IS=Inyang street. Same alphabets means the values are not significantly different from each other while different superscripts means they differ significantly

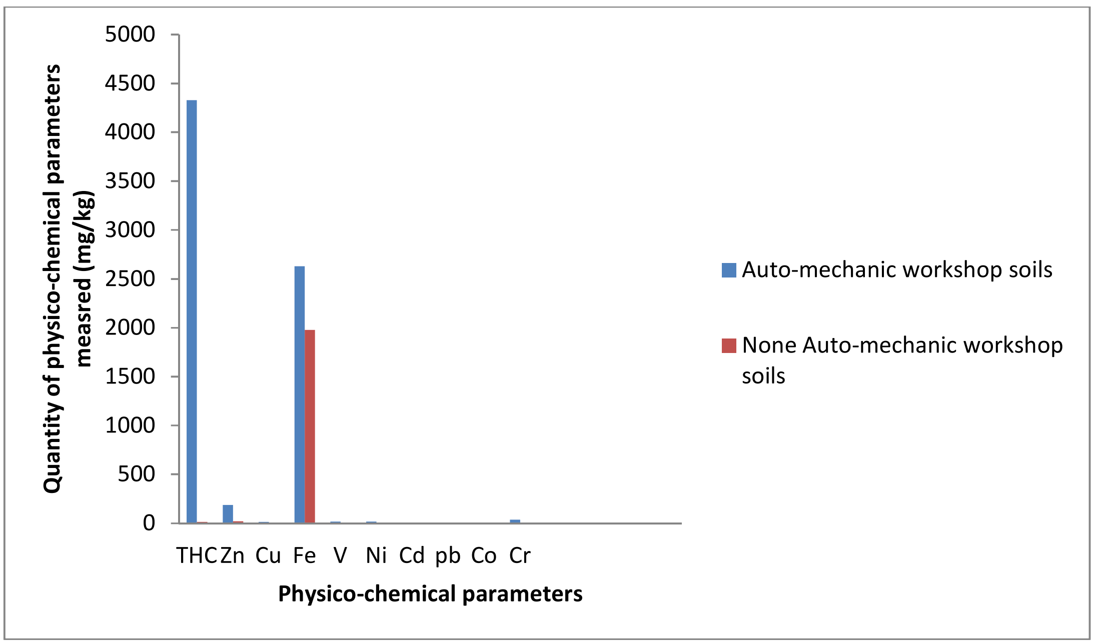

Fig. 3. Comparative analysis of mean physico-chemical parameters of auto-mechanic and none auto-mechanic workshop soil samples

Key: THC - Total hydrocarbon, Zn - Zinc, Cu – Copper, Fe- Iron, V – Vanadium, Ni – Nickel, Cd – Cadmium, Pb – Lead, Co – Cobalt, Cr - Chromium

The pH values of both the auto-mechanic workshop and non auto-mechanic soils were acidic. This observation is in agreement with the result of [25], who reported pH values of 4.5 and 4.7 for soils contaminated with spent engine oil. However, the increased acidic levels in the auto-mechanic workshop soil could be attributed to the microbial breakdown, of this pollutant to yield acidic metabolites. The auto-mechanic workshop soil showed a significantly low available phosphorus as compared to its non auto-mechanic soil sample counterpart. This observation corroborates with the findings of [26], who reported a lower available phosphorus in used motor oil contaminated soils compared to non-contaminated soils in Ota, Ogun state. The low available phosphorus may be due to the proliferation of heterotrophic bacteria in the auto-mechanic workshop soil samples. The auto-mechanic workshop soil samples in this study recorded considerably high values of total hydrocarbon. The high values could be as a result of indiscriminate disposal of petroleum and its products such as spent engine oil, used hydraulic oil, used crankcase oil, grease, diesel and spent lubricating oil that characterized the sampling sites. The THC values for the auto-mechanic workshop soil samples showed that the mean value of 5266.7 mg/kg, 4413.2 mg/kg, 3130 mg/kg and 4514.4 mg/kg for auto-mechanic workshop soil (AMA, AMM, AME and AMI) were above the recognized biogenic value of 50 mg/kg reported by [27] while that of the control-pristine soil, 11.358 mg/kg, 26.543 mg/kg, 10.352 mg/kg and 9.893 mg/kg (SQU, BGU, CCC, IS) was below the limit.

5. CONCLUSION

From this study, it is evident that the total hydrocarbon in auto-mechanic workshops in Calabar exceeds the recognized biogenic values as this is an indicative of gross contamination which could in turn stimulate a wide variety of environmental issues. Therefore, this calls for environmental consciousness to be instilled into auto-mechanic workshops to avoid indiscriminate disposal of spent oil. For remediation of the afore-mentioned consequences, the study has revealed the availability and survival of different bacteria species in these auto-mechanic workshop soil samples. Therefore, there is need for further research on the waste oil biodegradation potentials of these isolates, as they could serve as a more eco-friendly approach in cleaning and healing of such environments.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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