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Isolation and Characterization of Carbonoclastic Bacteria Diversity in Oil-contaminated Soil in Cape Coast Metropolis, Ghana

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Authors' contributions

This work was carried out in collaboration among all authors. Author HDN conceived the study, collected the samples, analyzed data and drafted the manuscript. Author GCO participated in the study design and assisted in the preparation of manuscript. Author OFJ assisted in editing the manuscript. Author IAKG assisted in laboratory analysis, interpretation of data and drafting of manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The study aimed at the quantification, isolation and characterization of hydrocarbon degrading bacteria in oil-contaminated and pristine soils.
Methodology: Soil samples from petroleum hydrocarbon polluted sites at auto-mechanic workshops, a mechanic village, as well as pristine (control) soils, comprising of 14 sampling locations within Cape Coast Metropolis in the Central Region of Ghana were collected using standard sampling techniques. Collected soil samples were treated and cultured while enumerations, isolations and characterization of carbonoclastic bacteria associated were evaluated.

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Results: Bacterial populations isolated from hydrocarbon-polluted sites had higher aerobic counts ranging from 7.24-8.02 log_{10} cfu/g of soil when compared with the pristine sites (from 6.79-7.61 log_{10} cfu/g of soil). Also, soil samples from the mechanic village (8.76 to 7.48 log_{10} cfu/g of soil) recorded more bacterial counts than those from the mechanic garages (8.02 to 7.24 log_{10} cfu/g of soil). The calculated percentage profiles of all the hydrocarbon utilizing bacteria in the total culturable heterotrophic bacteria were low throughout the study, even though the percentage scores were all above 50%. A total of 19 hydrocarbon degraders were isolated. The isolates identified belong to the genera *Pseudomonas, Proteus, Bacillus* and *Enterobacter*.
Conclusion: The outcome of the study based on the bacteria populations, identification profiles, coupled with their survival and multiplications in designated medium amended with crude oil as the carbon and energy sources, suggest their petroleum hydrocarbon degrading capabilities, hence may be used in bioremediation applications.

Keywords: Bacteria diversity; polluted soil; mechanic workshop; carbonoclastic bacteria; petroleum hydrocarbon; used engine oil.

1. INTRODUCTION

Since the beginning of the Anthropocene, man has continuously explored and utilized the natural environment to his benefits through numerous activities such as agriculture, industrialization, urbanization among others. The overexploitation of natural resources and the lack of proper management of the environment have led to serious ecological consequences to flora and fauna, with humans being the worst affected. These anthropogenic activities have contributed to the upsurge of environmental pollution worldwide, with the release of petroleum hydrocarbons being no exception. Through industrialization and urbanization [1], mining pursuit [2], agronomy [3,4], manufacturing and conveying [5,6], significant amount of waste is created that contributes immensely to contaminants in the environment. Additionally, spent oils from automobile vehicles, wash away from metal wares and used batteries, organic and inorganic compounds [7] and heavy metals, via food chain and the environment, have recorded elevated amounts in humans by ingestion, skin absorption, inhalation of the volatile compounds, runoffs among others [8]. A looming picture of the devastating nature of the environments manifests in countries where petroleum is produced. Operations in the petrochemical industries, span from petroleum explorations, unintentional spills of petroleum products, leakages via distribution outlets and storage tanks, and effluents from the production platforms into the immediate environments and beyond have been a major concern [9,10]. Oil spill through accidental or deliberate means impacts negatively on microbial metabolism, which to an extent may cause destruction of viable microbial populations, affect nutrient cycles and arguably compromise soil fertility, and the environment as a whole [11].

Hydrocarbon contaminants can filter through the soil profile to pollute aquifers and other aquatic environments, and arable fields, which ultimately affects trophic levels of the ecosystem [12]. Petroleum products that contain benzene, toluene, ethylbenzene, xylene (BTEX), and naphthalene are deleterious to all life forms [13]. Oil spill also makes the soil deficient of oxygen, affecting roots of plants [14,15] and rendering large parcels of arable lands for staple crops production useless [16].

A wide range of microorganisms such as bacteria, fungi, algae, and viruses thrive in terrestrial habitats [17]. These microbes are instrumental in nutrient formation and transfers, as well as the growth of flora and fauna, to ensure ecological balance and cleanups in the environment [11]. The composition, structure, and performance of the soil is also attributed to presence of microbial populations [18]. Although the presence of diverse microbial populations in oil-contaminated environments gives a clue to effective degradation, not much is reported on algae and protozoa [19]. Microbial metabolism converts the toxic components of crude oil or petroleum products to ecologically harmless products of carbon dioxide and water [20]. The cleanup process hinges on the physicochemical properties of hydrocarbon contaminants, conditions of the environment, the presence of the microbes and their biodegradation potentials [21] and that the breakdown of the hydrocarbon pollutants are commandeered by resident microbes [22]. More so, out of the numerous microorganisms in the oil-polluted soils, only a small proportion acclimatizes to the

contaminated environment that predominates with the others recording low frequencies of occurrence. The molecular approach, however, becomes applicable to sites where a normal culture-dependent approach will not yield desired results [23].

In Ghana, many towns and cities are bedeviled with haphazard siting of auto-mechanic workshops and garages, as new ones continuously spring up day-by-day. Spent lubricating oils, grease, and other related products are released indiscriminately during operations of mechanics, which seeps into the soil polluting the immediate environment and may subsequently end up in water bodies and aquifers. A cursory evaluation of many earmarked sites for investigation brought to fore, the deliberate spilling of spent oil on the immediate working areas with the pretense of moistening and preventing dusty environment. These routine practices amongst many of the auto-mechanics and their apprentices have a consequential effect on the environment. Although little or virtually no attention has been given to these worrisome acts, microorganisms associated with the hydrocarbon-contaminated environment have been reported as a source of biosurfactant production [24]. The present investigation, therefore, focused on the environmental analysis of indigenous bacterial strains associated with soil samples from auto-mechanic garages and the mechanic village in Cape Coast Metropolis of the Central Region of Ghana, with emphasis on the enumeration, isolation, morphological and biochemical characterizations of culture-dependent indigenous bacteria.

2. MATERIALS AND METHODS

2.1 Study Sites and Sampling

The investigation was carried out in auto-mechanic garages and a mechanic village with their history of operations ranging from 10-20 years and over 50 years respectively, both situated in the Cape Coast Metropolis of the Central Region of Ghana. A total of 14 sampling stations, comprising of 8 petroleum hydrocarbon-polluted sites, and 6 pristine sites designated as the controls to the test samples were used (Fig. 1). The geographical coordinates for the sampling sites are presented in Table 1. Composite soil samples from the sampling stations were collected with an auger at depth of 0-15 cm into well-labelled sterile, zip-locked polyethene bags and carefully packed in an ice chest and immediately transferred to the research laboratory of the Department of Laboratory Technology, University of Cape Coast, Ghana for microbiological analysis.

2.2 Chemical Reagents and Culture Media

All chemicals used for the investigation were of high analytical grade. The microbiological media, which included plate count agar (PCA) and nutrient agar (NA) were used for enumeration and isolation of total culturable heterotrophic bacteria (TCHB); Bushnell-Haas agar medium was used for counting, isolation, and purification of hydrocarbon-utilizing bacteria. All the culture media were prepared as per the manufacturer's specifications, and the glassware was cleaned with nitric acid and thoroughly rinsed with distilled water. The crude oil, which served as the carbon source and energy for microbial cultivation was obtained from the Petroleum Laboratory of the Ghana Standard Authority in the Greater Accra region of Ghana.

2.3 Enumeration of Total Culturable Heterotrophic Bacteria (TCHB) Counts

The TCHB counts of polluted and unpolluted soil samples from auto-mechanic garages and the mechanic village was determined using the pour plate method described by Chikere and Ekwuaba [25] with slight modification. From each sample, 1 g of the composite soil was added to 9 ml of sterile distilled water in Erlenmeyer flask and vortexed to ensure thorough mixing of suspension. Tenfold dilutions of the suspensions were made and 0.1 ml was aseptically dispensed into a dry, sterile Petri dish and proceeded by pouring 15 ml of sterile prepared, molten plate count agar (PCA) about 45ºC, swirled in a clockwise and counter-clockwise fashion and then incubated at 30ºC for 24 hours. Discrete colony-forming units were observed macroscopically after incubation and enumerated using the Stuart scientific colony counter.

2.4 Enumeration of Hydrocarbon-Utilizing Bacteria (HUB)

Hydrocarbon-utilizing bacteria were enumerated according to the vapor phase method with a slight alteration in the method described by Chikere and Azubuike [26]. Sterile Whatman (No 1) filter papers were saturated with crude oil and aseptically placed into the lids of inverted plates of each inoculated Bushnell-Haas agar (composition in g.L-1: $MgSO_4$ 0.2 g, $CaCl_2$ 0.02 g,

KH_2PO_4 1 g, K_2HPO_4 1 g, $FeCl_2$ 0.05 g, NH_4NO_3 1 g and 15 g agar-agar/L, pH 7.2±0.2) plates and in an inverted position were incubated at 30ºC for 14 days. After incubation, all plates with visible colonies of 30 to 300 were enumerated and expressed as colony-forming units per gram of soil (cfu/g). The percentage of hydrocarbon-utilizing bacteria (HUB) out of the total culturable heterotrophic bacteria (TCHB) was calculated using the formula:

$$\% \text{ HUB} = \text{HUB} / \text{TCHB} \times 100 \quad (1)$$

2.5 Characterization of Bacterial Isolates

The culturable bacterial colonies that showed different morphologies were isolated with an inoculating loop and repetitively sub-cultured by streaking on surfaces of nutrient agar plates to obtain pure cultures. Attempts to characterize the pure, fresh cultures of bacterial isolates were done by thorough macroscopic observations of the colonial characteristics, and the microscopic examination of cell morphological characteristics, as well as their biochemical assays. The colonial morphologies of the bacteria isolates, which included characteristics such as form, texture, color, margin, surface, opacity and motility were observed macroscopically. The cells were further observed microscopically for their Gram staining reactions and shapes. For the motility test, the sterile inoculating loop was used to pick 24 h old cultures and stabbed into a nutrient agar (semi-solid motility medium) in the test tube and incubated at 30ºC for 24 h. Non-motile bacteria demonstrated growth that was restricted to the stabbed openings with clear margins without extensions to the neighboring areas while motile bacteria showed spread growth from surface and beyond [27]. For gram staining techniques, a 24-hour old pure culture was used to prepare thin smear on a grease-free glass slide. The underside of the slide was gently passed about four times over a flame for fixation of the smear. Two drops of crystal violet solution were used to stain the heat-fixed smear and allowed to stand for 60 seconds, and thereafter rinsed with water. The rinsed smear was flooded with iodine solution for 30 seconds and subsequently rinsed with distilled water. Decolorization with 70% alcohol was done for 15 seconds and proceeded with another rinsing with distilled water. At this point, the smear was counterstained with 2 drops of Safranin for 60 seconds, and again rinsed with water for the last time, and thereafter air-dried. With the help of a microscope, the smear was observed under high power magnification (100x), using oil immersion. Gram-negative and Gram-positive cells visualized under the microscope as pink or red and purple colorations, respectively [28].

2.6 Biochemical Characterization of Isolates

To augment the motility and the staining tests of cultural characteristics to draw closer to the identification of bacterial isolates, the following biochemical assays such as methyl red test, Voges-Proskauer test, citrate test, urease test, indole test, oxidase test, catalase test, and triple sugar ions test were conducted.

Table 1. Geographical coordinates of sampling stations of auto-mechanic garages and mechanic village in Cape Coast Metropolis, Ghana

Sampling site	Sampling station	Latitude	Longitude
Auto-mechanic workshop	BYS	5º8’ 13’’ N	1º13’ 07’’ W
	GHS	5º7’ 41’’ N	1º13’ 23’’ W
	KS	5º8’ 34’’ N	1º17’ 05’’ W
	PS	5º7’ 14’’ N	1º16’ 22’’ W
Auto-mechanic workshop (control)	BYSC	5º8’ 13’’ N	1º13’ 08’’ W
	GHSC	5º7’ 40’’ N	1º13’ 22’’ W
	KSC	5º8’ 33’’ N	1º17’ 03’’ W
	PSC	5º7’ 03’’ N	1º16’ 34’’ W
Mechanic village	SS 1	5º6’ 49’’ N	1º15’ 36’’ W
	SS 2	5º6’ 46’’ N	1º15’ 35’’ W
	SS 3	5º6’ 44’’ N	1º15’ 35’’ W
	SS 4	5º6’ 42’’ N	1º15’ 33’’ W
Mechanic village (control)	SSC I	5º6’ 59’’ N	1º15’ 51’’ W
	SSC II	5º6’ 34’’ N	1º15’ 21’’ W

BYS-Brafoyaw Station, BYSC-Brafoyaw Station Control, GS-Greenhill Station, GSC- Greenhill Station Control, KS-Kakumdo Station, KSC-Kakumdo Station Control, PJS-Pedu Junction Station, PJSC- Pedu Junction Station Control, SS 1-Swidu Station 1, SS 2-Swidu Station 2, SS 3-Swidu Station 3, SS 4-Swidu Station 4, SSC I-Swidu Station Control I, SSC II-Swidu Station Control II

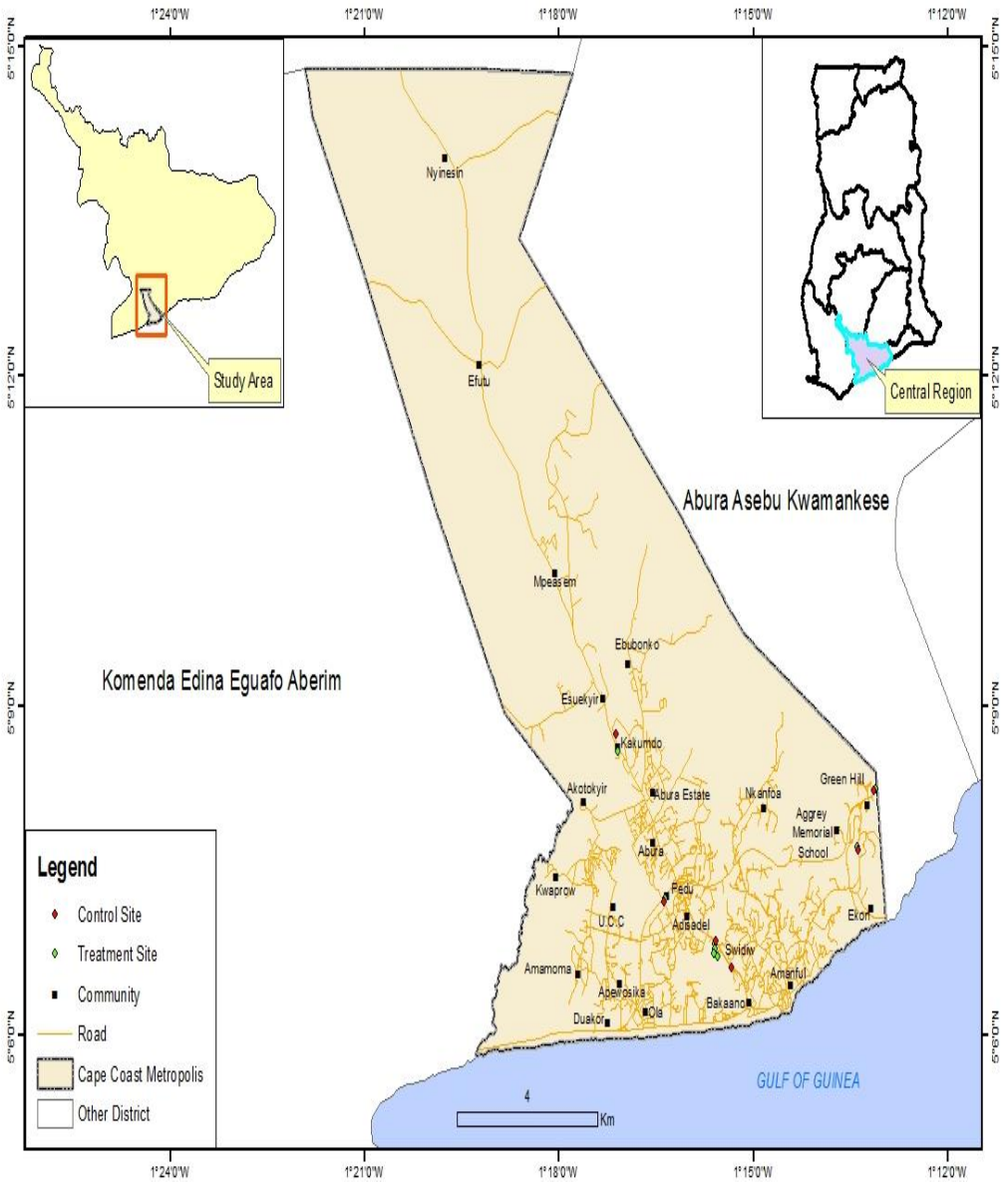

Fig. 1. Map of study area showing sampling locations in the Cape Coast Metropolis, Ghana

2.6.1 Methyl red test

One millimeter of a broth culture of the pure bacterial isolate under investigation was added to five millimeters of glucose phosphate broth (1 g glucose, 0.5% KH_2PO_4, 0.5% peptone and 100 mL distilled water) in a sterilized test tube and incubated at 30ºC for 48 hours. After incubation, two drops of methyl red solution was added to each test tube, shaken gently and observed for color change. Red coloration indicated a positive reaction [27].

2.6.2 Voges-Proskauer test

Again, one millimeter of a broth culture of the pure bacterial isolate was added to five millimeters of glucose phosphate broth (1 g glucose, 0.5% KH2PO4, 0.5% peptone and 100 mL distilled water) in a sterilized test tube and incubated at 30ºC for 48 hours. After incubation, 6% -naphthol and 6% sodium hydroxide were added to about 1 mL of the broth culture. A conspicuous red coloration after observation for 30 minutes suggested a positive reaction [27].

2.6.3 Catalase test

With the help of a sterile inoculating loop, a pure culture of bacteria isolate was put into a few drops of 3% hydrogen peroxide on a grease-free glass slide and observed immediately. Gas bubble detection was considered a positive test [29].

2.6.4 Citrate test

Ten milliliters of citrate medium prepared from 2.4 g citrate agar and 100 ml distilled water was dispensed into test tubes, and then sterilized and cooled to prepare slants. With the help of an inoculating loop, the bacterial isolate was inoculated by streaking the surface of the slants. After incubation, the utilization of citrate by a colour change from green to blue indicated a positive result [29].

2.6.5 Indole test

A loopful of the bacterial isolate was placed in a test tube containing 5 milliliters of Tryptone broth and incubated at 37°C for 48 hours. 0.5 milliliter of Kovac's reagent was added to the incubated bacterial culture, shaken gently and allowed to stand for 20 minutes to allow the rise of reagent. Observation of red color at the surface of the test tube indicated a positive result while yellow gave a negative result [29].

2.6.6 Oxidase test

A loopful of the bacterial isolate was smeared on the filter paper that had earlier been soaked with oxidase reagent. Color changes from blue to deep purple within 10 seconds if the organism is oxidase producing, which indicated a positive result [29].

2.6.7 Triple sugar iron (TSI) test

This test is conducted to differentiate between microorganisms base on sugar (dextrose, lactose, and sucrose) fermentation and hydrogen sulfide production.as described by Manga and Oyeleke [30]. Slants of the medium were prepared and with the help of a sterile inoculating loop, each bacterial isolate was picked and stabbed into the butt with the entire surface streaked and incubated at 30ºC for 48 hours with a loose cap. After incubation, cracking of the surface of the medium indicated gas production while hydrogen sulfide formation was observed as blackening of the whole buffer or blackening at the slant butt junction. The yellowing of the butt indicated glucose fermentation, and the yellowing of both the butt and slant suggested the fermentation of lactose or sucrose or both.

2.7 Data Analysis

Data analysis was conducted using SPSS statistical package version 20.0 program for windows. Descriptive statistics such as mean and percentages were used while pie charts were generated with Microsoft Excel 2010 by Microsoft Inc.

3. RESULTS AND DISCUSSION

3.1 Total Culturable Heterotrophic Bacteria and Hydrocarbon-Utilizing Bacteria

In this present study, bacterial populations in different soil samples from sampling sites of auto-mechanic garages and a mechanic village were evaluated. The results of the total culturable heterotrophic bacteria (TCHB) count, the hydrocarbon-utilizing bacteria (HUB), and the estimated percentages of HUB in the TCHB counts of hydrocarbon-contaminated and pristine soils are presented in Table 2. The TCHB counts for soil samples from oil-contaminated sites (auto-mechanic garages, mechanic village), and their respective control sites ranged between 6.79 and 8.76 $\log_{10}$ cfu/g. Bacterial loads for test samples from auto-mechanic garages and mechanic villages were generally found to be higher than those from the pristine (control) sampling sites. The results further revealed higher bacterial counts from the mechanic village than the auto-mechanic sampling sites. This interesting revelation may be attributed to the over fifty years of continuous operations at the mechanic village where large volumes of spent oils released into the environment, which continuously served as the main source of energy coupled with other edaphic factors contribute immensely to the growth and multiplication of resident microorganisms as compared to the low bacterial counts in auto-mechanic garages base on the relatively few years of operation. The hydrocarbon contaminated soil sample (KS) recorded the highest count of 8.02 $\log_{10}$ cfu/g while the least count of 7.24 $\log_{10}$ cfu/g was present in GS. For the pristine (control) samples, BYSC recorded the highest culturable bacterial counts of 7.61 $\log_{10}$ cfu/g while KSC had least count of 6.79 $\log_{10}$ cfu/g. Soil sample SS3 from the mechanic village had the highest bacterial counts (8.76 $\log_{10}$ cfu/g) while SS1 had the least count (7.48 $\log_{10}$ cfu/g). Control samples (SSC I and SSC 2),

which had bacterial counts of 7.37 and 7.04 $\log_{10}$ cfu/g, respectively, were found to be lower than their hydrocarbon-polluted soil counterparts. Further observation revealed that both hydrocarbon-utilizing bacterial counts and their calculated percentages in the total culturable heterotrophic bacteria showed marked reductions as compared to the heterotrophic bacterial counts for all enumerations. The HUB and % HUB ranged from 4.07 to 5.53 $\log_{10}$ cfu/g, and 51.50 to 75.57%, respectively. The presence and high counts of hydrocarbon degraders in petroleum hydrocarbon-polluted and pristine (control) sites of the present study agree favorably with other researchers [31,32] who reported the availability of degrading microorganisms in many environments including unpolluted (pristine) sites. However, Aislabie, et al. [33] reported that the overall microbial diversity reduces in polluted sites. The enumeration results of the present study were similar to the works done by Ndibe, et al. [34] who enumerated the total viable bacterial counts of four different sampling sites from Kaduna in Nigeria. Ataikiru, et al. [35] also reported that total heterotrophic bacteria and hydrocarbon utilizing bacterial counts of polluted soils were higher than unpolluted soils. These findings, again corroborate with observations reported by several authors [25,36,37].

3.2 Bacterial Characterization

Bacteria isolated from oil-contaminated and pristine sites in the Cape Coast Metropolis were characterized based on their colonial and cellular morphological features (Table 3) and biochemical tests (Table 4). In all, 19 different bacterial colonies designated HDN 1 to HDN19 were isolated from nutrient agar supplemented with crude oil. Their demonstration of successful growth on nutrient media supplemented with crude oil suggests their ability to metabolize petroleum hydrocarbon. The number of bacteria isolated in the present study is consistent with the isolation of 19 single colonies from soil samples of Dhakar City in Bangladesh [38]. Similarly, bacterial isolated with varied populations were obtained on different culture media by other investigators. For instance [25] and [35] separately reported isolations of 47 and 45 pure bacterial cultures on Bushnell Haas agar and nutrient agar, respectfully. These variations in higher bacterial loads compared to the results of the present study may be attributed to many factors of which the source of soil sample and use of all-purpose media like the nutrient agar might have supported the growth of many varieties of microorganisms. Also, the use of selective media does promote growth of fastidious microbes, which may increase microbial growth of hydrocarbon utilizers in Bushnell Haas medium.

For the observations of colonial morphological features of the total isolates presented in Table 2, 17(89.47%) of the isolates were predominantly circular with isolates HDN 5 and HDN 7 showing irregular forms. The bacterial isolates showed diverse colors, ranging from colourless, white, yellow, with creamy colour dominating. The margins of the colonies were mainly (89.47%)

Table 2. Total heterotrophic bacteria count, hydrocarbon-utilizing bacteria and percent of hydrocarbon-utilizers

Sampling site	Location ID	TCHB ($\log_{10}$ cfu/g)	HUB ($\log_{10}$ cfu/g)	% HUB out of TCHB
Auto-mechanic garage	BYS	7.91	5.50	69.53
	GS	7.24	4.18	57.74
	KS	8.02	4.13	51.50
	PJS	7.60	4.07	53.55
Mechanic village	SS1	7.48	5.03	67.25
	SS2	8.04	5.33	66.29
	SS3	8.76	5.45	62.22
	SS4	7.79	5.53	70.99
Auto-mechanic garage (control)	BYSC	7.61	5.27	69.25
	GSC	7.04	4.49	63.78
	KSC	6.79	4.24	62.45
	PJSC	6.91	4.23	61.22
Mechanic village (control)	SSC 1	7.37	5.53	75.03
	SSC 2	7.04	5.32	75.57

TCHB-Total Culturable Heterotrophic Bacteria, HUB-Hydrocarbon-utilizing Bacteria, BYS-Brafoyaw Station, BYSC Brafoyaw Station Control, GS-Greenhill Station, GSC- Greenhill Station Control, KS-Kakumdo Station, KSC-Kakumdo Station Control, PJS-Pedu Junction Station, PJSC- Pedu Junction Station Control, SS 1-Swidu Station 1, SS 2-Swidu Station 2, SS 3-Swidu Station 3, SS 4-Swidu Station 4, SSC 1-Swidu Station Control 1, SSC 2-Swidu Station Control 2

Table 3. Morphological (colonial and cellular) characteristics of hydrocarbon-utilizing bacteria of auto-mechanic workshops and mechanic village soils in the Cape Coast Metropolis, Ghana

Isolate code	Morphological characteristics							
	Form	Color	Margin	Elevation	Surface	Opacity	Size	Shape
HDN 1	Circular	Cream	Entire	Flat	Wrinkle	Opaque	Small	Short rod
HDN 2	Circular	Cream	Entire	Convex	Shinny	Translucent	Small	Short rod
HDN 3	Circular	Colorless	Entire	Raise	Shinny	Transparent	Tiny	Short rod
HDN 4	Irregular	Cream	Lobate	Raise	Smooth	Opaque	Large	Rods in chains
HDN 5	Circular	Cream	Entire	Raise	Shinny	Opaque	Medium	Short rod
HDN 6	Irregular	Cream	Lobate	Raise	Shinny	Translucent	Medium	Rod
HDN 7	Circular	Yellow	Entire	Raise	Shinny	Translucent	Small	Rod
HDN 8	Circular	White	Entire	Raise	Shinny	Transparent	Small	Short rod
HDN 9	Circular	White	Entire	Raise	Shinny	Translucent	Small	Coccus
HDN 10	Circular	Cream	Entire	Raise	Shinny	Opaque	Small	Coccus
HDN 11	Circular	White	Entire	Raise	Shinny	Transparent	Small	Rods in chains
HDN 12	Circular	White	Entire	Raise	Shinny	Transparent	Medium	Coccus
HDN 13	Circular	Cream	Entire	Convex	Shinny	Translucent	Small	Rod
HDN 14	Circular	White	Entire	Raise	Shinny	Transparent	Medium	Coccus
HDN 15	Circular	White	Entire	Raise	Shinny	Transparent	Medium	Short rod
HDN 16	Circular	White	Entire	Raise	Shinny	Transparent	Small	Short rod
HDN 17	Circular	Cream	Entire	Raise	Shinny	Translucent	Medium	Short rod
HDN 18	Circular	Colourless	Entire	Raise	Shinny	Transparent	Medium	Short rod
HDN 19	Circular	Cream	Entire	Raise	Shinny	Transparent	Medium	Short rod

HDN 1 = Bacillus, HDN 2=Acinetobacter , HND 3= Pseudomonas, HND 4 = Bacillus, HND 5 = Bacillus, HDN 6 = Pseudomonas, HDN 7 = Pseudomonas, HDN 8 = Pseudomonas, HDN 9 = Bacillus, HDN 10 = Acinetobacter, HDN 11 =Pseudomonas 12 = Pseudomonas, HDN 13 = Bacillus, HDN 14 = Streptococcus, HDN 15 = Proteus, HDN 16 = Enterobacter, HDN 17 = Proteus, HDN 18 = Alcaligenes, HDN 19 = Enterobacter

entire, with isolates HDN 4 and HDN 6 showing lobate margins. 17(89.47%) of the bacterial isolates showed raised elevations, while HDN 1 was observed as flat, and HDN 2, HDN 13 were convex. Aside from HDN 1 and HDN 4 that showed wrinkled and smooth colonial surfaces respectively, the remaining 17 (89.47%) isolates had shinny (glistening) surfaces. Furthermore, there were marked variations in the opacity of the isolates; 9(47.37%) isolates were observed as transparent, 6(31.58%) were translucent and the remaining 4(21.05%) were opaque. When the cells from individual isolates were examined microscopically, 9(47.37%) isolates, and 8 (42.10%) isolates were observed as small and medium-sized respectively, while 1(5.26%) isolate was classified as tiny size and another 1 (5.26%) isolate was observed as large size. For the shapes and arrangement of the individual cells, 15(78.95%) isolates out of all the 19 isolates observed were found be rod-shaped, of which isolates HDN 4 and HDN 11 had rod-in-chains (staphylococci) arrangements. HDN 9, HDN 10, HDN12 and HDN 14 isolates, however, were observed as having cocci shape. The results of the motility, Gram reactions and the biochemical characteristics leading to the identification of the bacterial isolates are presented in Table 3. It was observed that the majority (84.21%) of the bacterial isolates were non-motile (Table 3 and Fig. 2). For Gram staining by microscopic identification of the 19 isolated bacteria, 11(57.89%) isolates were Gram-negative while 8(42.11%) isolates showed Gram-positive characteristics. The biochemical characterization of bacteria isolated revealed dominance for positive tests in catalase (84.21%), citrate (68.42%) and oxidase (63.16%) isolates. However, dominance for negative tests was recorded for methyl red (73.68%), Voges Proskauer (63.18%) and indole (94.74%) isolates. The TSI results revealed the presence of many non-fermenters of carbohydrate while others showed carbohydrate fermentations with acid accumulations. The aforestated conventional biochemical and Gram staining

results in conjunction with the colonial and cellular details helped in the preliminary identification of bacteria genera as *Pseudomonas, Bacillus, Proteus and Enterobacter*, which may suggest their active participation as indigenous petroleum hydrocarbon degraders of contaminated soil at auto-mechanic garages and the mechanic village in the Cape Coast Metropolis. These isolates among others have been reported as hydrocarbon utilizers by many investigators [39, 40,20]. An earlier study by Watanabe [41] revealed the isolation of *Micrococcus, Pseudomonas,* and *Bacillus* as hydrocarbons degraders at contaminated bioremediation site. Isolations from petroleum hydrocarbon contaminated soils in car parks, Port Harcourt, Nigeria revealed the presence of *Serratia, Pseudomonas, Proteus, Klebsiella, Micrococcus* and *Staphylococcus* species [42], whereas *Micrococcus, Bacillus, Corynebacterium, Vibro, Pseudomonas and Flavobacterium* were isolated from contaminated soils from Aluu and Mogho axis of Port Harcourt [36]. Again, Ataikiru, et al. [35] isolated *Acinetobacter, Bacillus, Enterobacter, Escherichia, Klebsiella, Micrococcus, Proteus, Pseudomonas, Staphylococcus* alongside other fungal species from hydrocarbon polluted soils in Effurum, Delta State, Nigeria. In a related study, Eze, et al. [43] reported *Bacillus, Pseudomonas, Escherichia, Staphylococcus, Klebsiella, Citrobacter, Micrococcus* species as hydrocarbon degraders from Umuahia, Abia State, Nigeria. Shaopeng, et al. [44] reported the presence of mainly *Bacillus* sp, *Arthrobacter* sp., *Microbacterium oxydans* and the fungus, *Rhizobium* spp from oil-contaminated soils in China. Uad, et al. [45] and Hassanshahian, et al. [46] have respectively isolated hydrocarbon-degrading bacteria from leaking gas station contaminating groundwater in Southern Amazon, Brazil and from oil-contaminated sites in the Persian Gulf, respectively.

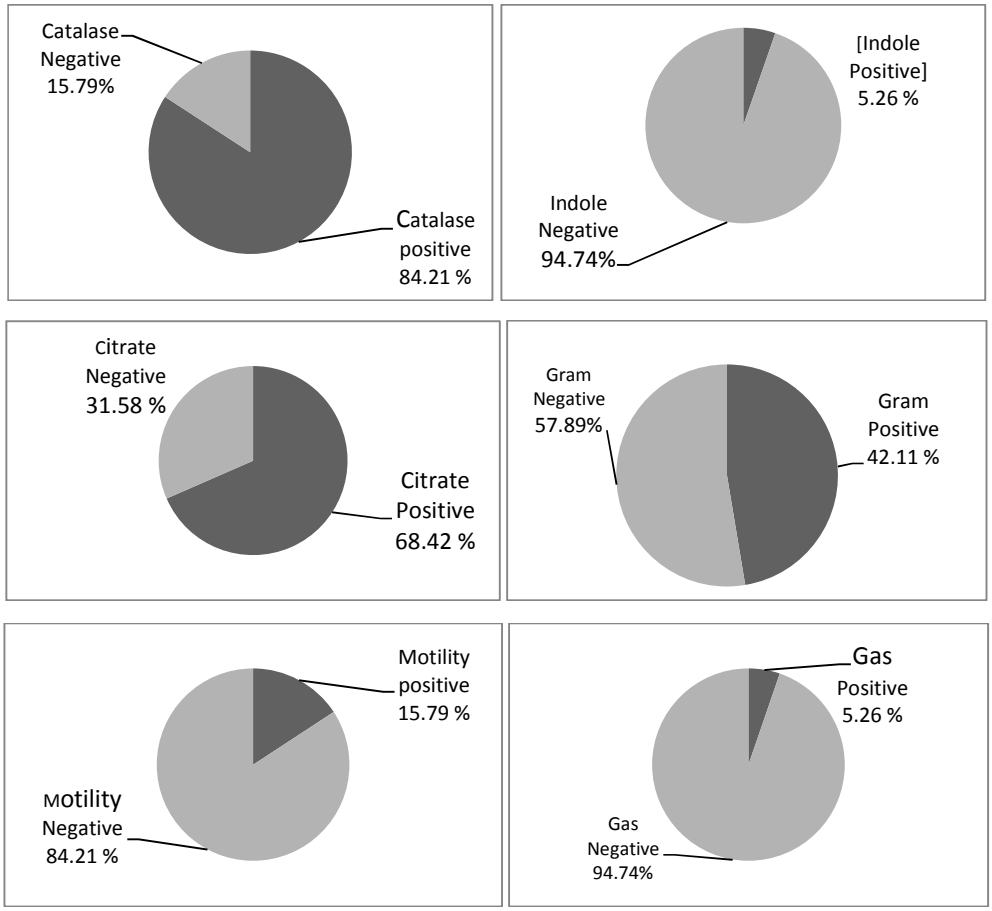

Fig. 2. Percentage profiles of some biochemical tests of bacterial strains from hydrocarbon-contaminated and pristine soils in the Cape Coast Metropolis

Table 4. Gram reactions and biochemical tests of hydrocarbon-utilizing bacteria of auto-mechanic workshops and mechanic village soils in the Cape Coast Metropolis, Ghana

Isolate code	Motility	Gram reaction	Biochemical tests						
			MR	VP	Catalase	Indole	Citrate	Oxidase	TSI
HDN 1	-	-	+	-	+	-	+	+	K/NC
HDN 2	-	-	-	-	-	-	+	-	A/AG
HDN 3	-	-	-	-	+	-	+	+	K/NC
HDN 4	+	+	-	+	-	-	+	+	K/A
HDN 5	+	+	-	+	+	-	+	+	K/A
HDN 6	-	+	-	-	+	-	+	+	K/NC
HDN 7	-	-	+	-	+	-	+	+	K/NC
HDN 8	-	-	+	+	+	-	-	-	NC/C
HDN 9	-	+	-	-	+	-	+	-	A/A
HDN 10	-	+	-	-	+	-	+	+	K/NC
HDN 11	-	-	+	+	+	-	-	+	K/NC
HDN 12	-	-	-	-	+	-	-	-	K/NC
HDN 13	-	+	+	+	+	-	-	-	A/A
HDN 14	-	+	-	-	-	-	-	+	K/NC
HDN 15	-	-	-	-	+	-	+	+	K/NC
HDN 16	+	-	-	-	+	-	+	-	A/A
HDN 17	-	-	-	+	+	-	+	+	K/NC
HDN 18	+	-	-	+	+	-	+	+	A/A
HDN 19	-	-	-	+	+	-	+	-	K/NC

MR-Methyl red, VP-Voges Proskauer, TSI-Triple Sugar. HDN 1 = Bacillus , HDN 2=Acinetobacter , HND 3= Pseudomonas, HND 4 = Bacillus, HND 5 = Bacillus, HDN 6 = Pseudomonas, HDN 7 = Pseudomonas, HDN 8 = Pseudomonas, HDN 9 = Bacillus, HDN 10 = Acinetobacter, HDN 11 =Pseudomonas 12 = Pseudomonas, HDN 13 = Bacillus, HDN 14 = Streptococcus, HDN 15 = Proteus, HDN 16 = Enterobacter, HDN 17 = Proteus, HDN 18 = Alcaligenes, HDN 19 = Enterobacter

4. CONCLUSION

This study has revealed that oil-contaminated and pristine soils from auto-mechanic workshops and mechanic village in Cape Coast Metropolis harbor large bacterial populations with higher loads associated with the oil-polluted sites. Similarly, soil samples from the mechanic village recorded higher bacterial counts than auto-mechanic. It further revealed the successful isolations of 19 bacterial hydrocarbon utilizers, which Pseudomonas predominated after characterization. The isolations of the hydrocarbon-degrading bacteria suggest that they can be useful in bioremediation of hydrocarbon polluted sites of the auto-mechanic and the mechanic village, and can as well combine with another bacterial consortium for replication in other oil-contaminated sites.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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