INTRODUCTION

Soil environment receiving upward micro-seepage of hydrocarbon was reported to directly stimulate the abundance, biodiversity and distribution of microbes in an environment [1]. The possibility to isolate large population of certain hydrocarbon utilizing bacteria from soil and sediments is generally taken as confirmation that those bacteria used hydrocarbon as their carbon source for their metabolism in that microenvironment [6]. Compelled by reservoir force, some of the gaseous constituents from oil and gas reservoirs can vertically infiltrate the concealment above and rise to the surface of the earth. Consequently, these gaseous and volatile constituents of hydrocarbon will lead to the mobilization of specific bacteria with the capacity to use such constituents to the near surface soil and they will colonize that particular...
habitats [9]. The fact that those bacteria utilize the gaseous compounds as growth substrates makes it possible for them to colonize those specific microenvironments where larger deposits of those carbonic constituents occur naturally [11], and the larger the population the denser the reservoirs may be [16].

The critical component of this kind of survey is that it does away with negative long wasted survey, where prospecting area will be prioritized according to the presence of large quantities of the hydrocarbon in particular reservoirs [11]. The ability to utilize hydrocarbon substrates as a carbon source is exhibited by a widespread and diverse microbes [8] widely distributed in oil reservoirs and sediments [8]. Using culture dependent isolation techniques, different bacterial genera have been characterized from these relevant environments [16]. The hydrocarbon utilizing bacteria isolated from the soil were species of *Bacillus, Enterobacter, Arthrobacter, Pseudomonas, Micrococcus, Seratia,* and *Corynebacterium* [2]. The present study has been conducted to detect and enumerate the indicator bacterial population with specific interest to those utilizing petroleum hydrocarbons as their source of carbon in their metabolism in the hydrocarbon exploration sites soil samples.

**MATERIALS AND METHODS**

**Study Area**
The study area is Kukawa, one of the 27 local government areas in Borno State, Nigeria. Kukawa is located in the northern part of Borno bordering four other local government areas including Abadam, Monguno, Guzamala and Marte. It also has international border with Chad with geographical coordinates 12°55′33″ North and 13°34′12″ East (Fig. 1). It has an average elevation/altitude of 277 meters. The majority populations are into farming and fishing with estimated population of over 25,000. Kukawa is considered one of the potential crude oil prospecting areas by the Nigerian National Petroleum Corporation (NNPC).

**Microbiological analysis of the soil samples**

**Enumeration of total aerobic heterotrophic and hydrocarbon utilizing bacteria**

Representative one-gram soil was sieved and weighted under aseptic condition and a tenfold serial dilution was performed as described by [4]. Nutrient agar was prepared according to manufacturer’s specification and used for the isolation of total aerobic heterotrophic bacteria while the Mineral salt medium supplemented with 5% per liter of diesel as oil agar (OA) was prepared and used for the isolation of hydrocarbon utilizing bacteria. Aliquot from the 10⁵ dilution was spread on the prepared plates and were incubated at room temperature (30 ± 2 °C) for 18 hours and 5 days for NA and OA, respectively. Bacterial colonies, which developed on the plates were counted and recorded as colony forming units per gram (CFU/g) of soil [5]. Pure cultures of the isolates were obtained by repeated sub-culturing on media used for primary isolation. The pure isolates were maintained on agar slants for further characterization and identification [4].

**Characterization and Identification of the hydrocarbon utilizing bacteria**

Bacterial isolates were characterized and identified after studying their Gram reaction as well as cell morphology. Bacterial biochemical identification was performed using the Bergey’s Manual of Determinative Bacteriology the tests were performed according to the methods described by [4].

**RESULTS AND DISCUSSION**
The abundance of hydrocarbon utilizing bacteria in soil samples was calculated by observing and counting the developed colonies. The hydrocarbon utilizing bacterial colonies and total heterotrophic bacterial colonies in 10cm observed on plates are shown in Fig. 2, with Kukawa (KW) indicating the least with 129×10⁵ CFU/g and Baga having the highest count of 269×10⁵ CFU/g for HUB. THB count was recorded highest at Baga (BG) 129×10⁵ CFU/g and least count was recorded at Bundur (BD) 106×10⁵ CFU/g.

![Fig. 1. Map of Borno Showing Kukawa (Right) and Kukawa showing the 10 ward of AL (Alagarno), BG (Baga), BW (Barwati), BD (Bundur), DR (Doro), DG (Dogoshi), KU (Kauwa) KW (Kukawa), KK (Kekeno), and YY (Yoyo) (Left)](image)

![Fig. 2. Distribution of total heterotrophic bacteria (THB) and hydrocarbon utilizing bacteria (HUB) counts at the various sample sites from the depth of 10cm. Keys: AL (Alagarno), BG (Baga), BW (Barwati), BD (Bundur), DR (Doro), DG (Dogoshi), KU (Kauwa) KW (Kukawa), KK (Kekeno), and YY (Yoyo).](image)
The count of HUB at 20 cm depth in the various sample sites shows highest count at Baga (BG) \(282\times10^5\) CFU/g and least count at Kauwa \(128\times10^5\) CFU/g. The count of THB was highest at Bawarti (BW) \(220\times10^5\) CFU/g and least count was \(110\times10^5\) CFU/g at Alagarno (AL). Similarly HUB count at 30 cm depth in the various sampling sites shows highest count at Baga (BG) \(285\times10^5\) CFU/g and the least count at Kauwa (KW) \(128\times10^5\) CFU/g. The count of THB was highest at Kauwa (KU) \(147\times10^5\) CFU/g and the least count at Alagarno (AL) \(72\times10^5\) CFU/g.

The diversity of hydrocarbon utilizing bacteria in the sampling sites shows various genera and species, among the most occurring genus, Bacillus is dominating the sampling sites followed by Enterobacter, Actinomycetes.

The prospect of discovering hydrocarbon (Fossil fuel) reservoirs in soil and sediments by biological method is tinted by the fact that the hydrocarbon utilizing bacteria range between \(10^3\) and \(10^5\) CFU/g in soil/sediment receiving hydrocarbon micro-seepages depending on the ecological conditions [13]. The soil from relative higher depth indicates low bacterial count in view of oxygen deficient conditions at sea bed [14]. In the present study area, Baga shows higher population of hydrocarbon utilizing bacteria range between \(269\times10^5\) and \(285\times10^5\) CFU/g of soil sample, which is significant and proves the seepage of lighter hydrocarbon accumulations from oil and gas reservoirs. Other geologic and physical prospecting studies suggest that hydrocarbon seepage of subsurface origin is present in the Baga offshore study area and indicate that the area has encouraging prospects for petroleum exploration.
In most cases the main dominant genera bacteria in soils receiving upward seep of hydrocarbon are Bacillus, Pseudomonas, Actinomycetes, Micrococcus, Corynebacterium and Proteus [2,3]. In the present study, the most dominant occurring bacterial genus was Bacillus. Bacillus was reported to be the predominated genus, especially in the hydrocarbon polluted soils [1]. This may be because Bacillus species are tolerant bacteria to harsh conditions due to their ability to produce spores, which may defend them from the toxic effects of the hydrocarbons [10]. Although, hydrocarbon utilizing bacteria may be expected to be readily isolated from a petroleum-polluted environment, the same degree of expectation may be anticipated for a total unrelated environment [8,11]. The rate of hydrocarbon seepage is rapid as shown in the higher increase of hydrocarbon utilizing bacteria in the subsurface soil specifically 10 cm depth. This may be due to the fact that the microorganisms in the soil have efficient ability in utilizing the residual hydrocarbon as a source of carbon and energy. Indigenous and adapted microorganisms are more efficient for biodegradation of oil pollutant.

CONCLUSION

The results of this study revealed that hydrocarbon-utilizing bacteria are abundant and widely distributed in the soils Kukawa where crude oil exploration is underway; the diverse species of bacteria that are using hydrocarbon micro-seepage as their source of carbon in their metabolism are isolated in higher population indicating the positive prospect of the sites.

REFERENCES