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Hydrocarbons Utilizing Coliform from Polluted Soil (Sandy, Clay and Loamy) in Jega, Nigeria

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Abstract – Environmental pollution with spilled oil and waste hydrocarbons has become a problem of major concern on the global scale. The study determined the biodegradation potentials of coliform from soil treated with petrol in Jega metropolis. A total of nine (9) soil samples were collected and analyzed using Physicochemical and Microbiological standard techniques. The mean values of the physicochemical parameters tested ranged; pH (6.8a ± 0.01-7.8a ± 0.013), Temperature (30.3*b* ± 1.2–*32.6b*± 0.02), Electrical conductivity (77.3a ± 1.53-95.7d ± 1.53), Dissolved oxygen (2.4*e* ± 0.2-8.9*d* ± 0.3), Biological oxygen demand (2.2b ± 0.3 - 9.6a ± 0.3), Chemical oxygen demand (2.57*b* ± 0.2 − 11.3c ± 0.35), Nitrogen content (1.3*e* ±0.2 -5.3g ± 0.2) and Phosphorous contents (2.9*a* ±0.5- 7.4.c ± 3.4). The spectrophotometric biodegradation potential of the soil samples reveals the turbidity range of 2.3-7.5, after which the bacterial isolates as scoliform were obtained, the isolates were further biochemically characterized, their percentage of occurrence show; *Pseudomonas aeruginosa* with 5 (29.4%), *Escherichia coli* with 3 (17.6%), *Proteus vulgaris* with 3 (17.6%), *Citrobacter koseri* with 2 (11.8%), *Citrobacter freundii* with 2 (11.8%), while *Proteus mirabilis* with 2 (11.8%). The bacteria isolated fit for utilizing hydrocarbons as energy source. Therefore, microbes can be considered as a key component in the cleanup strategy for petroleum hydrocarbons remediation.

Keywords – Coliform, Pollution, Hydrocarbons, Biodegradation, Spectrophotometric

I. INTRODUCTION

Environmental pollution is a world problem, as every form of pollution especially that of oil, into the atmosphere in any part of the world will contribute to the global pollution and further aggravate the problem of climate change [1, 2]. Oil Pollution in Nigeria is as common as the air we breathe and several researches have also brought this awareness to the world [3]. In Nigeria, oil spillage is one of the greatest environmental problem currently battling with especially in the Niger Delta zone [4].

Crude oil pollution is currently considered to be a great threat to the health of living things in the environment including humans [5]. Nigeria records an average of 300 oil spills in the oil producing States annually, making the Niger Delta regions the most polluted part of Nigeria, affecting the air, soil and water bodies [6]. Apart from the Niger Delta regions, other places that serve as depots for petroleum products such as diesel, premium motor spirit, and kerosene among others have in one way or the other encountered oil spills during transportation or storage either accidentally or due to human error contributing to soil and water pollution [7, 8].

Coliform bacteria belong to the family *Enterobacteriaceae*, which comprises facultative anaerobic bacteria naturally inhabiting digestive tracts of warm-blooded animals including man. Therefore, such microorganisms are continuously discharged with feces, and *E. coli*, for example, makes up about 10% of the domestic sewage micro flora worldwide [9]. Bioremediation is a process that involves the use of living microorganisms or their enzymes in detoxifying and degrading environmental pollutants, thus, restoring a polluted environment [5]. The microbial population and activities in the affected environment such as microbial counts, respiration, biomass diversity, and enzyme activities can be used to evaluate the extent of bioremediation [10].

Microbial biodegradation of petroleum contaminants is cheap and environmentally friendly and can be enhanced for better cleanup operations [11]. Petroleum utilizing microorganisms are more in number in oil polluted environment than pristine environment. However, microorganisms that are indigenous to a petroleum contaminated site have been reported to best remediate the environment from oil spills and other pollutants than the non-indigenous microorganisms to that site [12]. Therefore, the study determined biodegradation potentials of coliform in soil (sandy, clay and loamy) which has been mixed with petroleum product in Jega, Kebbi State.

1.1. Related Work

Most of the oil companies in Nigeria claim that acts of sabotage contribute the most to the release of petroleum into the environment relative to operational failure. This is corroborates to some spill data put in the public domain by the shell petroleum Development Company, Port Harcourt, Nigeria, the data show that oil spill incidents traceable to operational failure range from 7 to 35%; inferring that acts of sabotage are responsible for 65-93% of oil spill in Nigerian environment [13]. Several studies have described the ability of mixed bacterial consortia to degrade 28-51% of saturates and 0-18% of aromatics present in oil [14]. *Pseudomonas* species have been reported by [15] to represent one of the most versatile groups of organisms involved in the degradation of hydrocarbons, the species have also been reported to have specificity for a range of hydrocarbon compounds including biphenyl, PAHs and petroleum products commonly used in the Nigerian environment [16].

II. Methodology

2.1. Study sites

The study sites were GRA (sandy samples), Gindi area (loamy samples) and S/fada area (clay samples) in Jega area, Kebbi State Nigeria. The area is located at the latitude 12.3667°N and longitude 4.6333°E, with total area of 891 km2 and a population of 193,352 as of 2006 Nigeria census estimate. The inhabitant are predominantly Gimbanawa, with minority groups of Kambari, Zamfarawa and Zabarmawa.

2.2. Sampling

A total of nine (9) polluted soil (Sandy, Clay and Loamy) samples were collected from three (3) distinct sites labeled as S1-S3 (Sandy), C1-C3 (Clay) and L1-L3 (Loamy). This was aseptically performed using sterile polythene bags as described by [17].

2.3. Determination of Physicochemical Parameters

Parameters; pH, Temperature, Electrical Conductivity, Dissolved Oxygen, Biological Oxygen Demand, Chemical oxygen demand, Nitrogen and Phosphorous contents of the soil (Sandy, Clay, Loamy) samples were determined in accordance with [18,19].

2.4. Determination of Biodegradation Potentials of Isolates

The polluted soil samples were air dried and sieved using a 2mm mesh, then five grams of each sample was suspended in 250ml flask containing a mixture of 50ml of mineral salt broth. The flasks were incubated on a rotary shaker at 250rpm for 7days at 30^0C under agitation, after which, the content of biodegradation potential of the mixtures were measured using spectrophotometer in accordance with [20,21].

2.5. Isolation and Identification of Coliform

The fermentative solutions were diluted serially, aliquots from suitable factors were subjected to presumptive test (lactose broth) to assess their ability to ferment lactose by producing gas after 24 hours of incubation, gas produced from the tubes were streak plated on the surface of Eosin Blue Agar (confirm test) to further confirm the presence of coliform through their morphological features, the colonies from confirm test were sub-cultured onto an agar slant (completed test), where the resulting colonies were biochemically identified as described by [22,23].

III. Statistical Analysis

The analysis was performed using SPSS Software (version 20.0). The data obtained were expressed as mean+ standard deviation, and least significant difference between means and standard deviation was determined.

IV. Results And Discussion

Crude oil pollution is currently considered to be a great threat to the health of living things in the environment including humans. Nigeria records an average of 300 oil spills in the oil producing States annually, making the Niger Delta regions the most polluted part of Nigeria, affecting the air, soil and water bodies [24]. Early reports estimated that about 0.08 to 0.4% of the internationally produced crude oil is spilled into the environment [25]. Probably this value has currently increased because of the ever increasing activities related to oil extraction from reservoirs, transportation, processing, use as an energy source, and accidents and military conflicts. Spilled oil may be removed using physiochemical or microbiological means [26].

4.1. Physicochemical Parameters of the Soil Samples

The determination of physicochemical parameters of the soil samples (Table 1) reveals the pH mean range ($6.8a \pm 0.01$-$7.8a \pm 0.013$), with sample S3 had the highest mean of $7.8a \pm 0.013$, while sample C1 with the lowest mean of $6.8a \pm 0.01$. The values fall within the WHO standard (6.5–8.5). Optimum pH 7 has been attributed to affect many chemical and biological processes in soil permitting the survival of different organisms. According to [27], addition of nitrogen and sulfur fertilizer can lower soil pH over period of time. The increased of the pH may be caused by a loss of organic matter, removal of soil minerals when crops are harvested, erosion of the surface layer, and effect of nitrogen and sulfur fertilizer as reported by [28].

The Temperature ranged ($30.3\text{b} \pm 1.2$–$32.6\text{b}\pm 0.02$), with sample C1 had the highest mean of $32.6\text{b}\pm 0.02$, while sample L2 with the lowest mean of $30.3\text{b} \pm 1.2$. The values fall within the WHO guideline for soil temperature. [29] uphold that temperature greatly influences biological activity and growth; it determines the kind of organisms that live in the terrestrial regions. [30], reported that the decreased in temperature results in decreased in water and nutrient uptake in the soil. The Electric conductivity ranged ($77.3\text{a} \pm 1.53$-$95.7\text{d} \pm 1.53$), with sample L3 had the highest mean of $95.7\text{d} \pm 1.53$, while sample S1 with the lowest mean of $77.3\text{a} \pm 1.53$. The values obtained did not match with the WHO Guidelines for soil conductivity. This indicated the low level of nutrients in the oil. The findings disagree with [31] that the optimal soil Electrical Conductivity level ranges from 110-570 millisiemens per centimeter (mS/cm).

The Dissolved oxygen ranged ($2.4e \pm 0.2$-$8.9d \pm 0.3$), with sample S3 had the highest mean of $8.9d \pm 0.3$, while sample C1 with the lowest mean of $2.4e \pm 0.2$. The highest values obtained are greater than WHO standard for soil. DO is required for respiration and release of energy from food, thus it affects the growth, survival, distribution, behavior and physiology of some organisms on land. The dissolved oxygen content of soil is a single most important parameter that influences terrestrial biota life [32]. The Biological oxygen demand ranged ($2.2\text{b} \pm 0.3$ -$9.6\text{a} \pm 0.3$), with sample S1 had the highest mean of $9.6\text{a} \pm 0.3$, while sample C2 with the lowest mean of $2.2\text{b} \pm 0.3$. This agrees with WHO guidelines for soil BOD. As reported by [33], decreased or increase in BOD is as a result of certain environmental stresses (hot summer temperatures) and other human-induced factors (introduction of excess fertilizers to a water body) can lessen the amount of dissolved oxygen in a water body, resulting in stresses on local aquatic and terrestrial lives.

The chemical oxygen demand ranged ($2.57b \pm 0.2 - 11.3\text{c} \pm 0.35$), with sample C1 had the highest mean of $11.3\text{c} \pm 0.35$, while sample S2 with the lowest mean of $2.57b \pm 0.2$. The values are greater than the WHO guideline for soil COD. Increase in COD may be as a result of increased in the concentration of organic compounds materials, it also increases inorganic compounds susceptible to oxidation by the oxidant (typically dichromate) are present. [34], reported soil with high COD to have typically contains high level of decaying plant matter, human waste, or industrial effluents. The Nitrogen content ranged ($1.3e \pm 0.2$ - $5.3\text{g} \pm 0.2$), with sample C1 had the highest mean of $5.3\text{g} \pm 0.2$, while sample L3 with lowest of $1.3e \pm 0.2$. This did not agree with the WHO standard for soil nitrogen. [35], reported that nitrogen can be lost from agricultural lands through soil erosion and runoff, which do not normally account for a large portion of the soil nitrogen budget, but should be considered for surface soil quality issues. Incorporation or injection of manure and fertilizer can help to protect against nitrogen low through erosion or runoff. Where soils are highly erodible, conservation tillage can reduce soil erosion and runoff, resulting in less surface low of Nitrogen. Substantial amounts of Nitrogen is lost from the soil system through crop removal. Nitrogen can be lost in this way from manure and fertilizer products containing urea.

The Phosphorous content ranged ($2.9a \pm 0.5$- $7.4.c \pm 3.4$), with sample L3 had the highest mean of $7.4.c \pm 3.4$, while sample S3 with $2.9a \pm 0.5$. Since this fraction of soil phosphorus derives from animals, microorganisms and plants, and their respective decomposition products, such compounds as phosphor proteins and sugar phosphates would predictably be present in soil. Their concentrations, however, may be so low as to escape detection [36].

Table 1: Physicochemical Parameters of the soil samples (Sandy, Clay and Loamy)

S	pH	Temp. (^{o}C)	EC (Us/cm)	DO (mg/kg)	BOD (mg/kg)	COD (mg/kg)	N (mg/kg)	P (mg/kg)
S1	$7.2a \pm 0.02$	$31.2b \pm 0.5$	$77.3a \pm 1.53$	$6.5c \pm 0.1$	$9.6a \pm 0.3$	$6.33a \pm 0.25$	$1.5e \pm 0.9$	$4.2a \pm 2.1$
S2	$7.5a \pm 0.05$	$31.2b \pm 0.3$	$77.7a \pm 1.16$	$8.9d \pm 0.3$	$2.6b \pm 0.1$	$2.57b \pm 0.2$	$1.3e \pm 0.2$	$7.2b \pm 0.1$
S1	$7.8a \pm 0.03$	$31.6b \pm 0.2$	$77.9a \pm 0.95$	$3.6e \pm 0.3$	$3.5b \pm 0.3$	$3.63b \pm 0.25$	$3.7f \pm 0.7$	$2.9a \pm 0.5$
C1	$6.8a \pm 0.02$	$32.6b \pm 0.2$	$85.7b \pm 1.53$	$2.4e \pm 0.2$	$5.2c \pm 0.2$	$11.3c \pm 0.35$	$5.3g \pm 0.2$	$5.1c \pm 1.9$
C2	$7.5a \pm 0.03$	$31.7b \pm 0.2$	$87.7b \pm 1.53$	$8.4d \pm 0.3$	$2.2b \pm 0.3$	$7.36a \pm 0.31$	$3.3f \pm 1.6$	$4.1ac \pm 1.7$
C3	$7.1a \pm 0.71$	$31.6b \pm 0.2$	$88.7b \pm 0.58$	$8.6d \pm 0.3$	$8.5a \pm 0.3$	$4.8b \pm 0.66$	$3.2f \pm 0.1$	$3.2a \pm 0.2$
L1	$6.8a \pm 0.01$	$31.3b \pm 0.5$	$89.4bc \pm 0.41$	$5.2c \pm 0.2$	$2.6b \pm 0.1$	$11.1c \pm 0.35$	$4.3fg \pm 0.6$	$4.2ac \pm 0.1$
L2	$7.4a \pm 0.03$	$30.3b \pm 1.2$	$90.7c \pm 1.53$	$2.7e \pm 0.3$	$6.4c \pm 0.3$	$5.34be \pm 0.21$	$5.0g \pm 0.5$	$6.1c \pm 0.1$
L3	$7.4a \pm 0.02$	$31.3b \pm 0.6$	$95.7d \pm 1.53$	$8.2d \pm 0.3$	$9.2a \pm 0.2$	$6.73e \pm 0.21$	$2.2ef \pm 0.1$	$7.4.c \pm 3.4$

Physicochemical parameters of soil samples are of means ± standard deviation, mean values with different alphabet appearing on the same column are significantly different otherwise they are the same.

Keys: S= samples, Temp= Temperature, EC= Electrical conductivity, DO= Dissolved oxygen, BOD= Biological oxygen demand, COD= Chemical oxygen demand, N= Nitrogen and P= Phosphorous.

4.2. Spectrophotometry Biodegradation Potentials of the Isolates

The determination of biodegradation potential (Figure 1) ranged from 2.3-7.5, with clay soil had the maximal turbidity of 7.5, while Loamy soil with minimal turbidity of 2.3. The increase in biodegradation potential of organisms could be due to sufficient nutrients of the soil environment. The higher the water vapor permeability and water absorption, the faster is degradation [3,27]. Different factors influencing hydrocarbon degradation have been reported by [38], one of the important factors that limit biodegradation of oil pollutants in the environment is their limited availability of microorganisms.

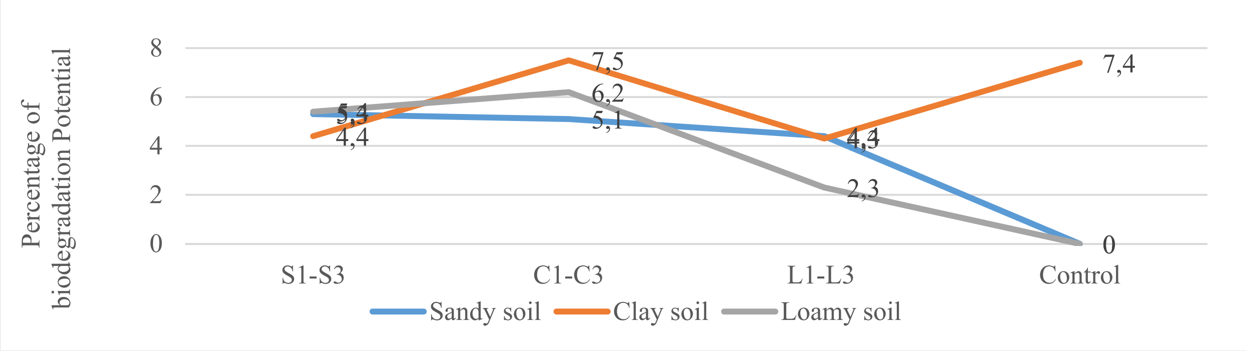

Figure 1: Percentage of biodegradation potential

4.3. Total Hydrocarbons Utilizing Coliform Count

The most probable number of coliform count (Figure 2) ranged (21-1100), with sample 3 had the lowest of 21, while sample 2 had the highest of 1100 MPN index per 100ml. This did not conform to WHO guideline for soil coliform. [8], stated that an MPN index number represents the MPN of bacteria in the original sample based upon the statistical probability of the coincidence of microorganisms in each sample replicate A 95% confidence interval represents a range of actual counts in a sample, whereby there is a 95% probability that any sample containing a number of microorganisms within that range would yield the same result by MPN techniques [39].

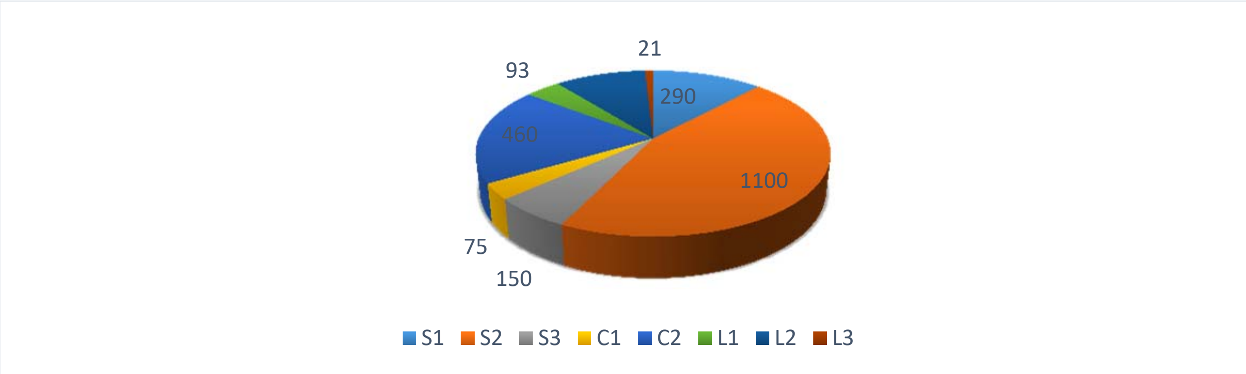

Figure 2: Total heterotrophic coliform count (MPN index/100ml) of the soil samples

4.4. Biochemical Characterization and Percentage of Coliform

The biochemical characterization and percentage of the isolates (Figure 3) revealed; *Pseudomonas aeruginosa* 5 (29.4%), *Escherichia coli* 3 (17. 6%), *Proteus vulgaris* 3 (17. 6%), *Citrobacter koseri* 2(11.8%), *Citrobacter freundii* 2 (11.8%), and *Proteus mirabilis* 2 (11.8%). The percentage of occurrence obtained disagrees with [9] who reported *Enterobacter hormaechei* subsp. *oharae* (56.6%), *Klebsiella* spp. (36%), and *Escherichia coli* (7.4%) in a study coliform bacteria for bioremediation of waste hydrocarbons in raw domestic sewage of Kuwait City. Similarly, the coliform genera obtained are similar with those reported by [21, 40] in their study Isolation and Selection of a highly tolerant microbial consortium with potential for PAH biodegradation from heavy crude oil-contaminated soils and microbial population of soil and water around petroleum depot Suleja, Nigeria, and their hydrocarbon utilization respectively.

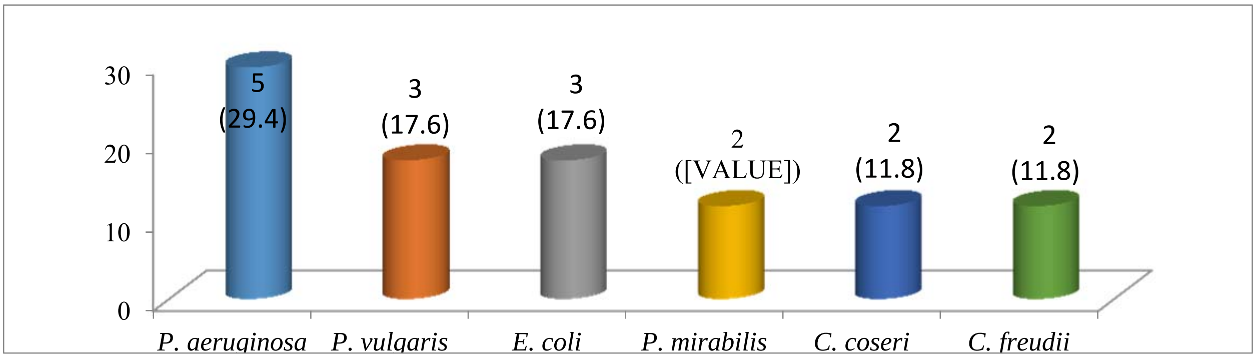

Figure 3: Frequency and percentage of the isolates

V. Conclusion And Future Scope

The bacteria demonstrated in this research reveal their tolerance to hydrocarbons as source of energy and cleanup strategy. As it was obvious that, petroleum polluted sites pose a threat to human life due to severe health problems caused by adverse health effects from exposure to oil-soil contamination. Therefore, microbial degradation can be considered as a key component in the cleanup strategy for petroleum hydrocarbon remediation. Therefore, once pollution is being detected; sampling, chemical analyses, evaluation of parameters and its effect must be done to avert the menace of hydrocarbons to our environment.

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