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RESEARCH ARTICLE

MICROBIOLOGICAL AND PHYSICOCHEMICAL CHARACTERISTICS OF SOIL CONTAMINATED WITH USED GENERATOR OIL

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ABSTRACT

The microbiological and physicochemical characteristics of soil contaminated with used generator oil were carried out. The media used were nutrient agar, Sabouraud dextrose agar mineral salt agar and mineral salt agar with antibiotics for the isolation of total heterotrophic bacteria total and fungi, hydrocarbon- utilizing bacteria and fungi respectively. The pour plate technique was employed. The total heterotrophic bacterial count ranged from 3.7 x 10^6cfu/g to 9.7 x 10^6cfu/g while the total heterotrophic fungal count ranged from 3.0 x 10^5cfu/g to 6.7 x 10^5cfu/g. The hydrocarbon-utilizing bacterial count ranged from 2.5 x 10^4cfu/g to 3.7 x 10^4cfu/g. The hydrocarbon-utilizing fungal count ranged from 1.5 x 10^3cfu/g to 2.4 x 10^3cfu/g. The microorganisms isolated were *Bacillus* species, *Klebsiella* species, *Micrococcus* species, *Pseudomonas* species, *Aspergillus* species, *Candida* species, *Mucor* species and *Penicillium* species The result of the physiochemical parameters ranged as follows pH, 3.9 – 4.7; conductivity, 118µs/cm to 159 µs/cm; total organic carbon, 19.85% to 26.10%; oil and grease, 420mg/kg to 1700mg/kg; total petroleum hydrocarbon, 386mg/kg to 1625 mg/kg; phosphate 2.41mg/kg to3.54mg/kg; sulphate, 12.80mg/kg to 19.40mg/kg; nitatre,1.53mg/kg to 2.91mg/kg; lead 0.63mg/kg to 1.62mg/kg; total iron, 36.7mg/kg to 63.1mg/kg; copper 1.52mg/kg to 3.04mg/kg; nickel, 0.081mg/kg to 0.083mg/kg; cadmium, 0.031mg/kg to 0.099mg/kg; manganese, 6.87mg/kg to 10.14mg/kg; barium, 0.03mg/kg to 0.102mg/kg; sodium, 15.8mg/kg to 23.8mg/kg; manganese, 1.80mg/kg t0 4.70mg/kg; potassium, 3.12mg/kg to 7.05mg/kg and calcium, 129mg/kg to 182mg/kg. It has been observed that the used generator oil had impacted negatively on the soils within the vicinity of the generating plants.

Key words: Microbiological, physicochemical, contaminated soil, characteristics, generator, used oil

INTRODUCTION

The release of toxic substance or energy into the environment that leads to the disruption of the normal ecosystem and also damage to human health or human resource is called pollution. Pollution is caused by the release of pollutants into the environment such that they cause measurable effect on aquatic, atmospheric and terrestrial environments. Scientific studies that can generate a better management of the environment can be used to fight pollution (Odu, 1992). Generator, which is an alternative source of power, is a major source of pollution in the environment. Pollutants from an electrical generator range from the smoke that is exhausted as a result of engine combustion. These smokes are known to contain high level of carbon monoxide which is very toxic to human health. Generator cause noise pollution as a result of the sound evolved during mechanical activities of the generator. They also contribute immensely to land pollution as a result of the wrong channeling of used oil that are dispersed during their operation. These used oils if improperly channeled to a collecting container may find their way into surrounding soil environment thereby causing pollution on land. This form of land pollution has an adverse effect on the soil surrounding the generator and its house. Most human activities contribute large quantity of pollutants in the environment. Activities from oil industries, mechanic workshops automobile also play a role in land pollution by oil. The biodegradation of these pollutant by microorganism present in the soil as a result of their metabolic diversity occurs when there is pollution in areas where microorganisms are present (Leahy and Colwell, 1990).

Used oil is any oil that has been refined from crude oil or any synthetic oil that has been used and as a result of such use, is contaminated by physical and chemical impurities. Used oil can be as a result of the use of the following refine products such as used lubricants, synthetic oil, transmission and brake fluid, refrigerator oil, compressor oil, hydraulic fluids, heat transfer fluids and used motor engine oil. Used oil is typically contaminated or mixed with dirt, fine particle, water or chemicals, all of which affect the performance of the oil and eventually render it unstable. Used oil does not include product derived from vegetables or animal fats or petroleum distillates used as solvents. Used oil has been as solvents. Anti freezes, cleaning agents, gasoline jet fuels are not used oil. Used oil is not waste oil. Waste oil comes from oil wastes that have not been used, such as virgin fuel storage tank

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bottoms or virgin fuel spill clean up residual (Kobayashi and Rittman, 1992). Used oil is generated from many different sources. The most common sources are manufacturing companies, electric generator, mining /smelter operations, automotive maintenance facilities and air conditioning repair facilities (Kobayashi and Rittman, 1992). Used oil can be re-fined as lubricating oil, used as a clean fuel and reprocessed to create many petroleum based products. Used oil that is dumped or channeled to the ground or is put in storm drained can contaminate ground water which can be very difficult to clean up. Used oil in surface water has the potential to harm wild life by depleting the oxygen supply for fish and other aquatic life and by hindering the ability of birds to fly. When plants are grown in the soil or fed by water contaminated by used oil, they absorb high concentration of heavy metals. One of the indirect risk of such environmental dangers is the poisoning of the food chain which ultimately affects human health (Colombo *et al*., 1996). The aim of this research work is to determine the effect of used generating plant oil on the microbiological and physiochemical characteristics of the soil within Madonna University Elele campus.

MATERIALS AND METHODS

Collection of Samples

The soil samples were collected from seven different generating plants located at different points at Madonna University Elele Campus, Rivers State, Nigeria. The sampling points were Boys' hostel, Girls' hostel, Main school power house, Madonna University Teaching Hospital 1 and 2 and Ezeke. The samples were collected from different portions of each location with soil auger and put into sterile cellophane bags. The different portions were then mixed to get a composite sample. The samples were taken to the laboratory for analysis.

Chemical Reagents

The chemical reagents used in the study were of analytical grade and were products of BDH Chemicals, Poole's, England and Sigma Chemical Company, St. Louis Missouri, USA. The microbiological media used were products of Oxoid and Difco Laboratories, England. They included nutrient agar used for the estimation of total heterotrophic aerobic bacteria, purification of hydrocarbon utilizers and for pure culture; Sabouraud dextrose agar (SDA) used for the isolation of fungi. The modified mineral salt agar without and with antibiotic was used for the isolation of hydrocarbon-utilizing bacteria and fungi respectively.

Enumeration of Total Heterotrophic Bacteria and Fungi

Samples of the contaminated soil were serially diluted in ten folds. Total aerobic plate counts were determined using pour plate technique. Then the molten nutrient agar, MacConkey agar and Sabouraud dextrose agar at 45^0C were poured into the Petri dishes containing 1mL of the appropriate dilution for the isolation of the total aerobic bacteria and fungi and coliforms respectively. They were swirled to mix and colony counts were taken after incubating the plates at 30^oC for 48h for the bacterial count and 5days for the fungal count. The bacterial isolates were preserved by subculturing them into nutrient agar slants which were used for biochemical tests.

Enumeration of Hydrocarbon-Utilizing Bacteria and Fungi

The hydrocarbon-utilizing bacteria and fungi were determined. The mineral salt agar of Mills *et al*. (1978) as modified by Okpokwasili and Amanchukwu (1988) comprising per litre of distilled water NaCl, l0g; $MgSo_4.7H_20$, 0.42g; KCL, 0.29g; K_2HP0_4, Na_2HP0_4, 0.83; $NaN0_3$, 0.42g; agar,15g; pH 7.2 was used. To 990mL of the mineral salt medium in conical flasks was added 10mL of the lubricating oil which served as sources of carbon. However, for the hydrocarbon-utilizing fungi, the medium was supplemented with an antibiotic chloramphenicol. The hydrocarbon-utilizers were then enumerated after plating in duplicate using pour plate technique, 1mL of the appropriate dilutions of the samples on Petri dishes. The molten mineral salt agar medium without antibiotic and the one containing antibiotic at 45^0C were poured into the Petri dishes for the isolation of hydrocarbon-utilizing bacteria and fungi respectively. These were swirled to mix, allowed to solidify and incubated. Enumeration of the hydrocarbon-utilizers was performed after incubation at 30^oC for 7 days. Colonies of the hydrocarbon-utilizing bacteria growing on the agar plates were counted, isolated, purified by streaking on nutrient agar plates and kept on nutrient agar slants as stock cultures for characterization and identifications. In the case of hydrocarbon-utilizing fungi, the isolates were streaked to purify onto Sabouraud dextrose agar plates and kept on Sabouraud and agar slants as stock cultures for characterization and identification.

Characterization and Identification of Hydrocarbon-utilizing Isolates

Bacterial isolates were characterized and identified after studying their Gram reaction as well as cell micro morphology. Other tests performed were spore formation, motility, and catalase production. Citrate utilization, oxidative/fermentative utilization of glucose, indole production, methyl red - Voges Proskauer reaction, urease and coagulase production, starch hydrolysis, production of H_2S from triple sugar iron (TSI) agar and sugar fermentation. The tests were carried according to the methods described by (Cheesborough, 2005; Adeoye, 2007; Agwung-Fobellah and Kemajou, 2007; Ochei and Kolhatkar, 2007). Microbial identification was performed using the keys provided in the *Bergey's Manual of Determinative Bacteriology* (1994). Fungal isolates were examined macroscopically and microscopically using the needle mounts technique. Their identification was performed according to the scheme of Barnett and Hunter (1972) and Larone (1986).

Determination of the Physiochemical Parameters

A number of physicochemical parameters of the contaminated soil samples were determined. They included pH, conductivity, nitrate, phosphate, sulphate. Others included oil and grease, total petroleum hydrocarbon, total organic carbon heavy metals and exchangeable cations. The pH was measured using Hach pH meter (Model EC1O);

conductivity was measured using Hach conductivity meter (Model CO150). Sulphate, nitrate and phosphate were determined using Barium chloride (Turbidimetric), Cadmium reduction and Ascorbic acid methods respectively. All analyses were in accordance with APHA (2005).

Heavy Metal Analysis

The heavy metals were determined using Unicom atomic absorption spectrophotometer (Model 969, Unicam) after digesting the samples with 10mL of water, 5mL of HCL (S.G. 1.19) and 1mL of HNO_3 (S.G. 1.42) in digestion containers. The digest was then analysed using the atomic absorption spectrophotometer. The method was adapted from ASTM (2003).

Determination of oil and grease and total petroleum hydrocarbon (TPH)

The method was adopted from ASTM (2003). The soil samples were air dried and sieved. Ten grams of the air dried sieved samples were weighed into 60ml glass bottles and 20ml of tetrachloroethylene was poured into the glass bottles. These bottles were placed into a shaker maintained at room temperature. The system was allowed to into a 20ml glass bottle using a glass funnel stuffed with cotton wool on which anhydrous sodium sulphate was placed. Analysis of the samples was done using Hach DR4000 spectrophotometer. The TPH was determined by treating the extracts with silica gel before analyzing with the spectrophotometer.

Determination of total organic carbon

The method used was adopted from ASTM (2003). One gram each of the air-dried samples was weighed out in duplicate and transferred to 250ml Erlenmeyer flask. Ten millimeters of 1N potassium dichromate solution and 20ml concentrated sulphuric acid was added and the flasks swirled until the soil and reagents were mixed. The flasks were allowed to stand on the sheet of asbestos for about 30minutes after 100ml of distilled water was added. Three drops of indicator was added and then titrated with 0.5N ferrous sulphate solution. The endpoint was observed when the colour changed sharply from blue to red (maroon colour) in reflected light against a white background.

Determination of Exchangeable Cations

The method for the determination was adopted from APHA (2005). The soil samples were first extracted using IN ammonium acetate solution. This was done by weighing 5g of sieved air dried samples and adding to 30ml of the extracting solution in a tube. This was shaken on a mechanical shaker for two hours. They were then centrifuged for five minutes and the supernatant carefully decanted into a 100ml volumetric flask. This was then made up to the mark with the extracting solution. The exchangeable cations (Na, K, Ca^{2+}, Mg^{2+}) of the extract were determined using Unicam Atomic Absorption Spectrophotometer, Model 969.

RESULTS

The results of the microbiological physicochemical characteristics are shown in Tables 1-4. Table 1 shows the microbial counts from the various samples. The total heterotrophic bacterial counts ranged from 3.7 x 10^6cfu/g to 9.1 x 10^6cfu/g. The girls hostel generating plant had the highest count of 9.7 x 10^6cfu/g while the least count of 3.7 x 10^6cfu/g was recorded in the Madonna university generating plant 1.The ANOVA, $P < 0.05$ showed that there was significant difference in the count among the locations. The total heterotrophic fungal count ranged from 3.0 x 10^5cfu/g to 6.7 x 10^4cfu/g. The highest count of 6.7 x 10^5cfu/g was from Madonna main generating plant 1 while the Madonna main generating plant had the least count of 3.0 x 10^5cfu/g. The ANOVA, $P < 0.05$ showed that there was significant difference in the counts among the stations. The hydrocarbon-utilizing bacterial count ranged from 2.5 x 10^4cfu/g to 3.7 x 10^4cfu/g while the hydrocarbon-utilizing fungal count ranged from 1.5 x 10^3cfu/g to 2.4 x 10^3cfu/g. The girls' hostel generating plant had the highest hydrocarbon utilizing bacterial count of 3.7x 10^4cfu/g while the least count of 2.5 x 10^4cfu/g was from Boys hostel and Madonna main generating plant respectively. The boys' hostel and Madonna university generating plants had the highest hydrocarbon-fungal count of 2.4 x 10^3cfu/g while Madonna University generating plant 1 had the least count of 1.5 x 10^3cfu/g.The ANOVA, $P > 0.05$ showed that there was no significant difference in the counts among the locations for the hydrocarbon-utilizing bacteria and fungi.

The microorganisms isolated and their percentage occurrences are shown in Table 2. *Bacillus* species had the highest occurrence of 38.89% while the *Micrococcus* species had the least occurrence of 11.11% for the bacterial isolates. For the fungal isolates, *Aspergillus* species had the highest occurrence of 42.86% while the *Mucor* species had the least occurrence of 10.71%. The result of the physiochemical parameters are shown in the Table 3. The result ranged as follows: pH, 3.9 – 4.7; conductivity, 118µs/cm to 159 µs/cm; total organic carbon, 19.85% to 26.10%; oil and grease, 420mg/kg to 1700mg/kg; total petroleum hydrocarbon, 386mg/kg to 1625 mg/kg; phosphate 2.41mg/kg to3.54mg/kg; sulphate, 12.80mg/kg to 19.40mg/kg; nitatre,1.53mg/kg to 2.91mg/kg; lead 0.63mg/kg to 1.62mg/kg; total iron, 36.7mg/kg to 63.1mg/kg; copper 1.52mg/kg to 3.04mg/kg; nickel, 0.081mg/kg to 0.083mg/kg; cadmium, 0.031mg/kg to 0.099mg/kg; manganese, 6.87mg/kg to 10.14mg/kg; barium, 0.03mg/kg to 0.102mg/kg; sodium, 15.8mg/kg to 23.8mg/kg; manganese, 1.80mg/kg to 4.70mg/kg; potassium, 3.12mg/kg to 7.05mg/kg and calcium, 129mg/kg to 182mg/kg. The ANOVA, $P < 0.05$ showed that there significant difference in the mean values of conductivity, oil and grease, total petroleum hydrocarbon, total organic carbon, sulphate, total iron, sodium, manganese, potassium and calcium among the different locations. The ANOVA, $P > 0.05$ showed that there is no significant difference in the values of other parameters among the locatons.

Table 1: The microbial counts of the various samples from the generating plants

Sample identity	THBC	HUBC	THFC	HUFC
BHG	8.9×10^6	2.5×10^4	6.2×10^5	2.4×10^3
GHG	9.7×10^6	3.7×0^4	6.3×10^5	2.4×10^3
MMG	7.4×10^6	2.5×10^4	3.0×10^5	2.0×10^3
JG	6.2×10^6	2.6×10^4	3.4×10^5	2.2×10^3
MUTHG	3.7×10^6	2.6×10^4	6.7×10^5	1.5×10^3
MUTH 2	6.7×10^6	2.8×10^4	4.5×10^5	2.4×10^3
EGP	4.9×10^6	3.2×10^4	4.1×10^5	1.8×10^3
Control	7.2×10^7	1.0×10^2	8.4×10^5	1.2×10^1

Legend: BHG =Boys Hostel generator; GHG =Girls Hostel generator; MMG = Madonna Main generator; JG =Jubilee generator; MUTH 1 = Madonna University Teaching Hospital generator; MUTH 2 = Madonna University Teaching Hospital 11; EG = Ezeke generator; THBC = Total heterotrophic bacterial count; THFC =Total heterotrophic count; HUFC = Hydrocarbon utilizing bacterial count and HUFC = Hydrocarbon –utilizing fungal count.

Table 2: Microorganisms isolated and their percentage occurrence

Bacteria	No of isolate	% occurrence
Bacillus species	7	38.89
Pseudomonas species	5	27.78
Klebsiella species	3	22.22
Micrococcus species	2	11.11
Fungi		
Aspergillum species	12	42.86
Penicillium species	7	25
Mucor species	3	10.71
Candida species	6	21.43

Table 3: The values of the physicochemical parameters of the soil samples

Parameter	BHG	GHG	MMG	JG	MUTHGI	MUTHG3	EG	Control
pH	4.6	4.1	4.4	3.9	4.0	4.7	4.5	6.8
Conductivity (µs/cm)	148	159	132	128	143	118	137	90
Total organic Carbon (%)	24.25	26.10	25.40	22.50	20.60	19.85	21.70	14.20
Oil and grease (mg/kg)	1,250	1,700	1,320	1,250	815	420	1,050	100
Total Petroleum Hydrocarbon (mg/kg)	1180	1652	1281	1020	735	386	985	80
Phosphate (mg/kg)	3.71	3.54	2.90	3.12	2.63	3.09	2.41	1.25
Sulphate (mg/kg)	18.20	19.40	16.50	17.10	13.40	12.80	13.30	8.50
Nitrate (mg/kg)	2.82	2.91	2.25	1.87	1.53	1.94	2.06	5.0
Lead (mg/kg)	1.24	1.62	0.97	1.05	0.87	0.63	0.73	0.4
Total Iron (mg/kg)	56.4	63.1	59.2	48.0	36.7	35.9	43.5	10
Copper (mg/kg)	2.88	3.04	2.72	2.29	1.76	1.52	2.03	0.54
Nickel (mg/kg)	0.061	0.083	0.05	0.064	0.037	0.018	0.029	0.010
Cadmium (Mg/kg)	0.085	0.099	50.06	0.058	0.046	0.031	0.054	0.012
Manganese (Mg/ kg)	9.28	10.14	38.82	7.93	7.63	6.87	8.15	2.65
Barium (mg/kg)	0.062	0.102	0.05	0.043	0.038	0.052	0.054	0.012
Vanadium (mg/kg)	0.121	0.104	90.12	0.102	0.129	0.213	0.084	0.05
Chromium (mg/kg)	0.032	0.058	40.041	0.124	0.102	0.129	0.213	0.083
Sodium (mg/kg)	20.6	23.8	18.8	16.2	17.1	15.8	18.5	3.86
Magnesium (mg /kg)	4.03	4.70	3.13	2.99	2.53	1.80	2.69	0.98
Potassium (mg/kg)	6.19	7.05	5.36	4.46	3.70	3.21	4.08	1.05
Calcium (mg/kg)	166	182	157	140	138	129	142	20

Legend: BHG =Boys Hostel generator; GHG =Girls Hostel generator; MMG = Madonna Main generator; JG =Jubilee generator; MUTH 1 = Madonna University Teaching Hospital generator; MUTH 2 = Madonna University Teaching Hospital 11; EG = Ezeke generator; THBC = Total heterotrophic bacterial count; THFC =Total heterotrophic count; HUFC = Hydrocarbon utility bacterial count and HUFC = Hydrocarbon –utilizing fungal count.

DISCUSSION

Environmental pollution from electric generating plant is on the increase especially in Nigeria where the normal source of power is scarce. Oil pollution from these generators on land is usually very difficult to clean up and it has steadily increased. Thus the mean counts of hydrocarbon- utilizing bacteria and fungi obtained from the contaminated were very high when compared with the control. This may be attributed to the fact that contaminated soils often harbour a vast array of microbial flora that is capable of utilizing the hydrocarbon as energy and carbon source. It has been observed that hydrocarbon discharge to the ecosystem may result in the the microbial population in the soil (Okpokwasili and Nnubia, 1995; Okpokwasili and Odokuma, 1996). Fresh oil spill contains substances that inhibit bacterial growth and microbes can suffer lethal effects after the accumulation of the oil pollutants. Environmental stresses brought about by the contamination could be adduced for the reduction in microbial diversity but increasing the population of few surviving species. Previous reports have proved extensive microbial diversity with population estimated between approximately 4×10^3 to 10^4 species per g of uncontaminated soil (Bornenman *et al*., 1996; Adesemoye *et al*., 2006). Some microorganisms are more abundant in areas of high concentration of hydrocarbons. The microorganisms isolated have been implicated in hydrocarbon degradation and are among such organisms. The hydrocarbon-utilizing bacteria isolated were dominated by Gram negative bacteria belonging to a wide range of taxa. The existence of wide taxa of hydrocarbon degraders might result in co-oxidation, commensalitic or complimentary degradation of the hydrocarbons in the ecosystems. Such co-oxidation may be a process through which undegraded and otherwise recalcitrant hydrocarbon can easily be removed from the oil contaminated ecosystem (Westlake *et al.*, 1976; West *et al*., 1984; Perry, 1989; Eze *et al*., 2006). The genera of hydrocarbon-utilizing fungi isolated from the polluted soil of

were *Aspergillus* species, *Penicillum* species, *Yeast* and *Mucor* However, various strains *Aspergillus*, *Penicillium*, *Cladosporium* and *Yeast- Candida* and *Rhodotorula* have been implicated in hydrocarbon biodegradation (Bossert and Bartha, 1984; Okpokwasili and Amanchukwu, 1988; Amanchukwu *et al*., 1989)

Bartha and Atlas (1977) reported that when natural environments are contaminated with pollutants the indigenous microbial communalities are likely to contain microbial populations of different taxonomic characteristics which are capable of degrading the contaminating waste. Degradation of macromolecules in waste to smaller molecules is enhanced by soil microorganisms which produce a tremendous range of potentially useful enzymes that help in breaking down or decomposition of these macromolecules. Calomiries (1976) reported that the medium employed for isolation of degrading microorganisms may have a significant selective effect on the microbial population being investigate Oxidation by the microflora on the hydrocarbon is considered as another source of carbon for use in the ecosystem (Amund *et al*., 1981; Bhattacharya *et al*., 2002). There is a general increase in the fertility of the soil after oil pollution, but since generating plants are most stationary, the soil remain polluted and infertile which means that it is rare to find a good vegetation around it. The changes in the physicochemical parameters were as a consequence of the build up in the soil of the breakdown products of oil degradation. An increase in the cation exchange capacity (Na^+, K^+, Ca^{2+}, Mg^{2+}) is a reflection of increased soil conditions with regard to the agricultural potential of soils at the study sites. Such changes in soil condition are favourable since they promote the increase of organic colloids and humic acids and better nutrient availability (Bleams *et al*., 1987).

The pH of the soil samples was low. It has been observed that mineralization of hydrocarbons proceeds most rapidly at pH values between 6.5 and 8.0 (Dibble and Bartha, 1979a). The type of microorganisms that participate in hydrocarbon degradation is determined by the pH of the soil (Bossert and Bartha, 1984). The bacteria have limited tolerance for acid condition and fungi are more tolerant (Alexander, 1981). Since the pH in this study was at low pH, it could be assumed that fungi were more involved in the degradation of the oil. The nitrate, phosphate and sulphate were higher in value than the control. The increase in the sulphate content can be attributed to the fact that as sulphate is being removed from the soil, it is being replaced. It has been shown that some bacteria particularly species of *Thiobacillus* (*T. thioparus*, *T. novellas*) oxidizes H_2S and other sulphur compounds and because they have a low acid tolerance, deposit elemental sulphur rather than generate sulphuric acid by further oxidation. Other members of the genus *Thiobacillus* produce sulphate from the oxidation of elemental sulphur and other organic sulphur compounds (Kuenem *et al*., 1985). Phosphorus is not an abundant component of the ecosphere (Atlas and Bartha, 1992). It had been shown that its availability is further restricted by its tendency to precipitate in the presence of bivalent metals (Ca^{2+}, Mg^{2+}) and ferric (Fe^{3+}) ion at neutral to alkaline pH. Phosphates are combined with calcium within many habitats rendering them insoluble and unavailable to plants and many microorganisms are capable of solubilizing phosphates from such sources and assimilate and release them for use by other organisms (Atlas and Bartha, 1992). Nitrogen is an important constituent of protein and nucleic acid. In most microorganisms and plants, inorganic nitrogen is taken up as nitrate (NO_3^-) or ammonium (NH_4^+) ions. It has been shown that nitrogen can be lost from the soil because some species of bacteria convert nitrate to gaseous nitrogen by using nitrate as a metabolic electron acceptor in place of oxygen (Nester *et al*., 2001). Nitrates are important nutrient in the soil and can cause eutrophication in aquatic environment.

The increases in the hydrocarbon-utilizing bacterial and fungal counts suppose to have resulted in the removal of the hydrocarbon resulting in the decrease in the value. But because the oil is discharged continuously into the surrounding environment the level of the hydrocarbons was not affected hence the difference in the value between the contaminated soil and the control. Other factors that can affect hydrocarbon removal in the soil include runoff, flood and leaching, evaporation and photo oxidation (Bartha and Bossert, 1984). The high values of the total organic carbon show how richly the soil is enriched with organic matter. The presence of organic matter shows the presence of other nutrients in the soil. This also encouraged the growth of the microbial population. Contaminated soil contains heavy metals. When plants are grown in soil contaminated with used oil, they bioaccumulate hwigh concentration of heavy metals. One of the indirect risks of such environmental dangers is the poisoning of the food chain, which ultimately affects human health (Colombo *et al*., 1996). The high levels of these metals must be controlled because some of them are neurotoxins. Acute lead poisoning in humans causes server dysfunction in the kidneys, reproductive systems, liver, brain and central nervous system. The effect of cadmium poisoning in human is very serious. These include high blood pressure, kidney damage, destruction of testicular tissue and red blood cells. Cadmium replaces zinc biochemically thereby altering the stereo structure of the enzyme and impairing its catalytic activity. This ultimately results in disease symptoms (Manahan, 1993; Oze *et al*., 2006). Manganese is neuro-toxic and manifests in the form of impulsive and aggressive behaviour, in some cases euphoria and sexual stimulation. Manganese selectively accumulates in the central nervous system, bones, liver and kidney. Chromium is associated with the cancer of lungs and kidney. Zinc causes neuronal cell death (Manahan, 1993; Kiely, 1998). The used generating plant oil should therefore be properly channeled to a collecting vessel for proper disposal so as to reduce the pollution caused by it.

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