BIOTREATMENT OF CRUDE OIL CONTAMINATED SOIL

Abstract

The impact of petroleum prospecting and production operations on the environment has produced ecological problems of great dimensions. Biodegradation of hydrocarbons by microorganisms represents one of the primary mechanisms by which petroleum and other hydrogen pollutants are eliminated from the environment. The fate of petroleum hydrocarbons in the environment is largely controlled by abiotic factors, which influence rate of hydrocarbon biodegradation. This work was carried out on the effect of microorganisms on the biotreatment of oil in crude oil contaminated soil.

Microorganisms were isolated from two experimental soil samples contaminated with Bonny Crude and normal uncontaminated soil as a control over a period of seven months. The isolates were characterized and identified using standard methods,physico-chemical parameters of the soil samples were also analyzed using standard methods. Changes in total petroleum hydrocarbon level were measured appropriately. Treatments used were the microbial isolates.

Forty-four microorganisms were isolated from the contaminated soils and identified as species of *Pseudomonas* (7), *Flavobacterium* (6), *Bacillus* (8), *Proteus* (4), *Klebsiella* (1), *Pencillium* (5), *Aspergillus* (7), *Fusarium* (3), *Trichypton* (2) and*Neurospora* (1).Ten of the thirty isolates had ability to degrade crude oil in the laboratory. On contamination a value of $1.0X10^5$cfu/g in microbial counts were obtained followed by a subsequent increase in population levels after a period of 2months with a value of $1.0X10^6$ cfu/g. Oil application to the soil resulted in an increase in total petroleum hydrocarbon from 0.31 ppm to 5.53 ppm; organic matter from 0.426% to 0.426 %; available phosphorus from 1.75ppm to 2.84ppm. The treatment measures all showed progressive decrease in oil concentration in the soil. Mixture of bacterial and fungal isolates as a treatment measure proved to be more favourable above all others, it brought the concentration from 5.53ppm to 0.31ppm after a period of 5weeks of treatment, which is same value with the normal soil (uncontaminated).

Species of *Pseudomonas,Bacillus, Flavobacterium, Proteus, Klebsiella, Penicillium, Aspergillus, Fusarium, Trichyphyton* and *Neurospora* had potential for the degradation of bonny crude oil. They could therefore be employed in environmental cleanup of petroleum spill site.

Keywords: Biodegradation, Petroleum Spill Site, Bioaccumulation, Treatment.

INTRODUCTION

1.1 Crude Oil Chemical Composition and its Classification

Oil is an exceedingly complex substance composed literally of thousands of different kinds of organic molecules. It varies in composition depending on age as well as conditions of its formation. Despite the complexity and variability of crude oil, some generalizations about its composition can be made. According to James, C. 2018, crude oil is a naturally occurring, unrefined petroleum product composed of hydrocarbon deposits and other organic materials. Akiner and Aldis, 2004 say it consists of hydrocarbons of various molecular weights and other organic compounds, while Atlas, 1990 defines it as constituting mainly of parrafins (30-50%), cycloparrafins (20-50%) and Aromatics (6-14%). It also contains concentration of metals such as nickel, aluminium, magnesium, iron, cobalt, zinc, gold, mercury, chromium, molybdenium and lead.

Petroleum contains hundreds of individual compounds and their components are generally grouped into four classes according to their differential solubility in organic solvents: the saturates (N and branched chain alkanes and cycloparrafins), the aromatic compounds containing alkyl side chains and (or fused cycloparrafin rings), the resins (aggregates with a multitude of building blocks such as pyridines, quinolines, carbazoles, thiophenes, sulfoxides, and amides), and the asphaltenes (aggregates of extended polyaromatics, naphthenic acids, sulfides, polyhydric phenols, fatty acids and mettalophyrins) (Sugiura*et al*., 1997).

1.2 Environmental Pollution Due To Petroleum Hydrocarbon Spillage and Fate in Soil

The impact of petroleum prospecting and production operations has produced ecological problems of great dimensions. The significance of any given spill is dependent on the amount of oil spilled and on the impact on the environment. But the transmission of the oil from the point of contamination to other points depends on many factors, which includes the spill volume, hydrocarbon viscosity, temperature, wind speed, land contouring, rainfall, extent of cultivation, fluctuations in the level of water table, and the nature of the hydrocarbon and soil (Atlas, 1984)

Spilled oil has deleterious effects on Flora and Fauna of the ecosystem. The economic life of the people in the affected areas is usually disrupted, such as farmlands, navigational activities and fishing efforts as well as the disruption of the eco balance in the affected ecosystem. Also, pollution of the environment due to accidental seepages rupture of pipelines, blow out of terrestrial oil wells and sabotage has been reported (National Academy of Sciences, 1975; Awobajo, 1983). The resulting spillages have brought about economic losses as well as contamination of the aquatic and terrestrial ecosystems.

The soil microflora generally responds to changes induced due to petroleum hydrocarbon spillage, such as a rapid increase in size of hydrocarbon metabolizing portion of the

community,possible concomitant increase in the non-hydrocarbon utilizing population, inhibition of ATP production, dehydrogenase activity, nitrogen fixation and microbial respiration. (Morgan and Watkinson, 1989). Therefore to reduce the hazardous effect of petroleum hydrocarbon, their control and treatment strategies are required.

1.3 Treatment of Petroleum Hydrocarbon Contaminated Soil

The problem of petroleum hydrocarbon contaminated soil could be solved by removal of the contaminated soil or reclean in site.

A variety of techniques are available for the physiochemical treatment of hydrocarbon-contaminated soil, this includes flooding; excavation; thermal disruption and incineration (Morgan and Watkinson, 1989). Also, the use of seeding or biodegradation, which shows a promising level of success have been reported (Kaneez, F., Asma, I. Imran, A., Qaiser, M.K. and Muhammed, A., 2018). Other techniques include use of straw or plant material as an absorbent for oil; bio surfactants to clean oiled surfaces (Alfred, O.U. 2011) and addition of material to encourage microbiological biodegradation of oil (http://www.nap.edu.>read>chapter). Others have produced materials even in commercial and patented products.

Bioremediation, which is the productive use of microorganism to remove or detoxify pollutants, usually as contaminants of soils, water or sediments that otherwise threaten public health (Samina, W., Shams, T. and Masood, A., 2013), is one of the other biological methods for treatment of all pollutants.

Biodegradation is the degradation caused by biological activities especially by enzymatic action, leading to a significant change in the chemical structure of the exposed material and resulting in the production of carbon dioxide water, mineral salts,(mineralization) and new microbial cellular constituents (biomass) (Gosh, S.B and Sain., 2015). Bartha and Atlas (1972) define biodegradation in relation to petroleum and chemical changes in parent hydrocarbon to petroleum and chemical changes in parent hydrocarbon of spilled oil usually accompanied by a reduction in p^H, but the product are not necessarily harmless.

The breakdown of hydrocarbon mixtures depends on the nature of the petroleum hydrocarbon, nature of the microbial community, and on a variety of environmental factors which influences microbial activities (Atlas, 1981). Microbial degradation involves the interactive effects of mixed populations of microorganisms and relies on the metabolic versatility of bacteria and other microbes.

1.4 Hydrocarbon Degradation by Microorganisms and Environmental Conditions Influencing Biodegradation.

Hydrocarbons in the environment are biodegraded primarily by the bacteria and fungi, although ubiquitous in terrestrial and aquatic ecosystem, the fraction of the total heterotrophic community represented by the hydrocarbon utilizing bacteria and fungi is highly variable, with reported frequencies ranging from 6%-82% for soil fungi, 0.13%-50% for soil bacteria and 0.003%-100% for marine bacteria. Individual organisms can metabolize only a limited range of hydrocarbon substrates, so that assemblages of mixed populations with overall broad enzymatic capacities are required to degrade complex mixtures of hydrocarbon such as crude oil in soil, fresh water, and marine environments.

The ability to degrade and/or utilize hydrocarbon substrates is exhibited by a wide variety of bacterial and fungal genera. Floodgate (1984) lists 25 genera of hydrocarbon degrading fungi which have been isolated from the marine environment. A similar compilation by Bossert and Bartha, 1984) for soil isolates includes 22 genera of bacteria and 31 genara of fungi. Based on the number of published reports, the most important hydrocarbon degrading bacteria in both marine and soil environments are; *Achromobacter, Acinetobacter, Alcaligenes, Arthrobacter, Bacillus, Flavobacterium, Nocardia and Pseudomonas sp* and coryneforms. Among the fungi *Aureobasidum, Candida, Rhodutorula,* and *Sporobolomycessp* are the most common marine isolates and *Trichoderma* and *Mortierellasp* are most common soil isolates. Hydrocarbons degrading *Aspergillus*and*Penicilliumsp* have been frequently isolated from both environments.

The fate of petroleum hydrocarbons in the environment is largely controlled by abiotic factors, which influence rate of microbial growth and enzymatic activities that determines the rate of hydrocarbon biodegradation.

Many reports on laboratory testing of the biodegradation of crude oil have not been tested in field conditions because laboratory products tests may not be attained within the natural environment where complex physical, chemical and biological interactions occur.

Hence, this study examines the responses of the indigenous microbial communities in experimentally crude oil contaminated soils, assess the changes in soil physio-chemical properties and evaluate some biological treatments with a view to assessing their usefulness in bio treating soil contaminated with crude oil.

MATERIALS AND METHODS

Collection of samples

Two experimental plots (1m by 1m each) were mapped out at the nursery unit of the department of microbiology, University of Ibadan. One of the plots was deliberately contaminated with 400mls of Bonny crude oil and the second plot served as the control plot, which is the uncontaminated reference plot. The oil was thoroughly mixed with the upper 15cm of the soil and tillage was done repeatedly for aeration every other day throughout the experimental period. Soil samples were collected at regular intervals (7 days), the collection was made at random on each of the plot before they were taken for physico- chemical analysis and collection was also taken monthly for microbial analysis.

Soil Analysis:

Physico-chemical analysis:

Uncontaminated and contaminated soil samples with crude were analyzed for its physic-chemical properties. Parameters such as p^H in water, % organic Carbon, Exchangeable Cations (Na, k, Ca, and Mg) and Total Nitrogen were all analysed using their standard methods.

Microbial Analysis:

Isolation and Identification Organisms

Isolations of all the isolates were by the method of (Harrigan and McCance, 1976). 1ml inoculum was aseptically transferred into a sterile petri-dish and pour-plated with the appropriate agar medium, nutrient agar for bacterial isolation and potato dextrose agar for fungi isolation. The plates were incubated at room temperature $27^oC_{+\_}$ 2^oC) for 24 hours in the case of bacterial isolates, while fungal isolates were incubated for 3to5 days. Morphological appearances of the inoculated plates were observed and distinct colonies were sub-cultured to obtain pure isolates which were then maintained on nutrients agar and potato dextrose agar slants for bacterial and fungal isolates respectively and preserved at 4^oC.

Bacterial isolates were identified using morphological procedures and bio chemical tests, with reference to Bergey'smanual of Systematic Bacteriology (Sneath, 1986)

The isolated fungi were identified according to their micro morphology as well as color morphology of sporulatingstructures;reference was made to compendium of soul fungi.

Total Oil Degraders Count (TOD)

Total oil degraders counts were carried out on the contaminated experimental plot and the control plot. Serial dilution method was adopted using modified oil agar of Jensen (1975). The oil agar medium consist of basal (mineral salt) medium which consists (g/l) of K_2HPO_4, 1.8; K_2HPO_4, 1.2; NH_4cl, 4.0; $MgSO4.7H_2O$, 2.0; NaCl, 0.1; $FeCl_2.4H_2O$, 0.05; yeast extract, 0.1; and trace elements H_3BO_3,0.1; $ZnSO_4.7H_2O$, 0.1; $CuSO_4.5H_2O$, 0.05; and MnSO4.H20, 0.04. Two percent

of agar was added to the basal medium before autoclaving, after which 2ml of crude oil sterilized through membrane pore filtration as sole carbon source was added to the basal medium. Trace elements solutions was also sterilized separately before a small portion of it was added to the basal medium and mixed together aseptically.

Serial dilution method was carried out and pour plate method was used and the plates were incubated at 35^{o}C, visible bacterial colonies were counted after seven days of incubation. Growths of fungi degrading the oil were also seen after the incubation at room temperature for seven days.
Testing of Bacterial and Fungal Isolates for Crude Oil Utilization.

Oil agar was used for testing according to a modified method of Jensen (1975). Fifteen millitre of molten sterile oil agar was aseptically poured into each petri dish and allowed to solidify. Testing of each isolate was done by streaking a portion of the colony (using a sterile inoculating loop) from a previous activated 24 to 48 hours culture of the isolate. Bacterial ability to utilize the oil was indicated by the plates on which bacteria grew. Plates on which fungi grew in the case of fungal isolation were also taken as indicators of the fungal ability to utilize crude oil.Pure cultures of each isolate capable of utilizing crude oil weresubjected to various microbiological tests to determine their probable identity.

Bacterial isolates were identified using morphological procedures and various biochemical tests. The result of each test was recorded and the probable identity of the isolate determined using Bergey's Manual of systematic Bacteriology (Sneath, 1986).

Fungal isolates were identified according to their micro morphology, as well as colour and morphology of sporulatingstructures glass slides preparations were done using lactophenol blue (Harrigan and McCance, 1966). Microscopic examination of prepared slide was done using low power objective, followed by 40 X magnification objective lens. The probable identity was determined using compendium of soil fungi.

Biotreatment Experiment

Soil bag preparation

Soil was collected at a site located at the nursery unit of the department of microbiology, university of Ibadan. The soil was collected to a depth of 10cm different points on the site, the soil samples were then mixed together thoroughly. Soil textural class was determined to be loamy sand.

Seed bags were used as experimental units, they were 16cm by 12cm. 500g each of five soil samples were weighed and then, thoroughly mixed with 10mls each of the crude oil, one of the samples served as the experimental control and one of the samples was not contaminated at all

with crude oil, it serves as the control. The remaining three (3) samples were treated with various treatments.

Preparation of Inocula, Treatments and Seeding

Test isolates were selected based on their ability to grow well on oil agar medium. Five bacterial and five fungal isolates each a mixture of the cultures were used for the study.

Bacterial and fungal isolates were first cultured in 100ml. Erlenmeyer Flasks containing nutrient and potato dextrose broths at room temperature and 100 rpm in a shaker for 48 hours to increase the inoculum size.

The various treatment measures used were as follows:

- Seed bag 1 was treated with 20mls of the bacteria isolated, designated as (B).
- Seed bag 2 was treated with 20mls of the fungi isolated, designated as (F)
- Seed bag 3 was treated with 40mls of the mixture of bacteria plus fungi designated as (B+F).

All the samples were duplicated. Changes in total petroleum hydrocarbon level and physico-chemical analysis were carried out at two weeks interval for two months.

RESULTS AND DISCUSSION

Forty-four microbial isolates were obtained from the contaminated soil; these isolates were identified for bacteria as species of *Pseudeomonas, Flavobacterium, Bacillus, Proteus* and *Klebsiella* while for fungi as species of *Penicillium, Aspergillus, Fusarium, Trichypton* and *Neurospora*allthese are shown on Table 1 in their frequency of occurrence. Several researchers have also reported similar isolates from petroleum hydrocarbon contaminated soil samples (Atlas, 1981; Bartha and Bossert, 1984;Althalb, H. and Singleton, I., 2017;Ajayi, and A.O Abiola, A.K., 2018)

Table 1:Frequency of occurrence of bacterial and fungal isolates from artificially contaminated soil

Isolate Name	Frequency of Occurrences

Pseudomonas sp	7 (15.71)
Flavobacteriumsp	6 (13.64)
Bacillus sp	8 (18.18)
Proteus sp	4 (9.09)
Klebsiellasp	1 (2.27)
Penicillumsp	5 (11.36)
Aspergillussp	7 (15.91)
Fusariumsp	3 (6.82)
Trichyptonsp	2 (4.55)
Neurosporasp	1 (2.27)
Total	44 (100)

Values in parentheses represent percentage of occurrence.

Table 2:Physico-chemical Analysis of Soil Sample (field study)

Parameters	Sample A	Sample B
	Uncontaminated soil	contaminated soil

	1wk	4wks	7wks	1wk	4wks	7wks
pH	7.40	6.10	6.50	7.30	6.10	5.80
Calcium (mg/kg)	3.12	1.43	1.20	7.30	2.00	2.00
% Nitrogen	0.30	0.14	0.10	0.41	0.34	0.40
TPH (PPM)	1.80	1.78	1.50	6.50	4.55	4.10
Sulphur (mg/kg)	9.50	9.23	8.70	22.40	24.70	24.10
Magnesium (mg/kg)	0.48	0.46	0.35	0.38	0.45	0.48
Av.P (ppm)	1.89	1.83	1.80	3.18	3.10	3.00

Overall Temperature range 29-31^{o}C

Key

1, 4, 7, wks. – Numbers of weeks on analysis

TPH (ppm) – Total Petroleum Hydrocarbon (parts per million)

Av.p – Available phosphorus

Table 3:Physico-Chemical Analysis Of Some Heavy Metals In Soil Sample (Field Study)

	SAMPLE A	SAMPLE B
PARAMETERS	Uncontaminated soil	Contaminated soil

	i	1	7	i	1	7
Copper (ppm)	11.00	13.00	9.00	17.00	14.00	10.00
Lead (ppm)	7.00	7.00	6.00	10.00	6.10	6.00
Iron (ppm)	8.00	9.00	6.00	12.00	10.00	8.00
Chromium (ppm)	6.00	8.00	7.00	10.00	7.00	8.00
Cadmium (ppm)	6.20	7.00	7.00	9.00	6.10	6.00
Nickel (ppm)	9.00	7.00	8.00	9.00	7.00	6.00

Key

Ppm – parts per million

i - Day soil was contaminated

1, 7- Numbers of week on analysis

Table 4: Total Petroleum Hydrocarbon In Ppm Present In Soil Sample (Field Study)

SAMPLE	DAY OF SAMPLE ANALYSIS						
	i	1	2	3	4	5	6	7

A	1.82	1.80	1.72	1.82	1.78	1.68	1.53	1.50
B	6.78	6.50	5.98	4.73	4.55	4.50	4.30	4.10

Key

A – Uncontaminated soil sample

B – Contaminated soil sample

i – Day of contamination

1 2 3 4 5 6 7 – Numbers of weeks on analysis

Table 5:Physico-Chemical Parameters of Polluted Soil Sample

	PARAMETERS					
SAMPLE	PH	TPH (ppm)	OM (%)	%OC	%N	Av.p (ppm)
NS	6.20	0.31	0.41	0.24	0.024	1.75
CS	6.00	5.53	7.34	4.26	0.426	2.84

Key

NS – Uncontaminated soil (day 0)

CS – Contaminated soil (day 0)

Table 6: Total Culturable Heterotrophic Bacterial (Thb) And Total Culturable Fungal Count (Thf) In Soil

SAMPLE A, B /	SEASON /DAY/Cfu/g			
THB, THF, TOD.	Day 1	I month	2months	7months

A (THB)	$1.1x10^5$	$5.2x10^5$	$2.8x10^5$	$3.4x10^4$
B (THB	$1.0x10^5$	$4.8x10^5$	$1.1x10^6$	$2.4x10^4$
A (THF)	$2.1x10^4$	$5.2x10^4$	$5.6x10^4$	$2.5x10^3$
B (THF)	$2.3x10^5$	$5.4x10^4$	$5.8x10^4$	$1.8x10^3$
A (TOD)	$1.0x10^5$	$1.0x10^5$	$1.0x10^5$	$1.7x10^4$
B (TOD)	$6.1x10^4$	$1.2x10^5$	$1.3x10^5$	$1.8x10^4$

Key

A – Uncontaminated soil sample

B – Contaminated soil sample

1,2,7 – Days of Culturing

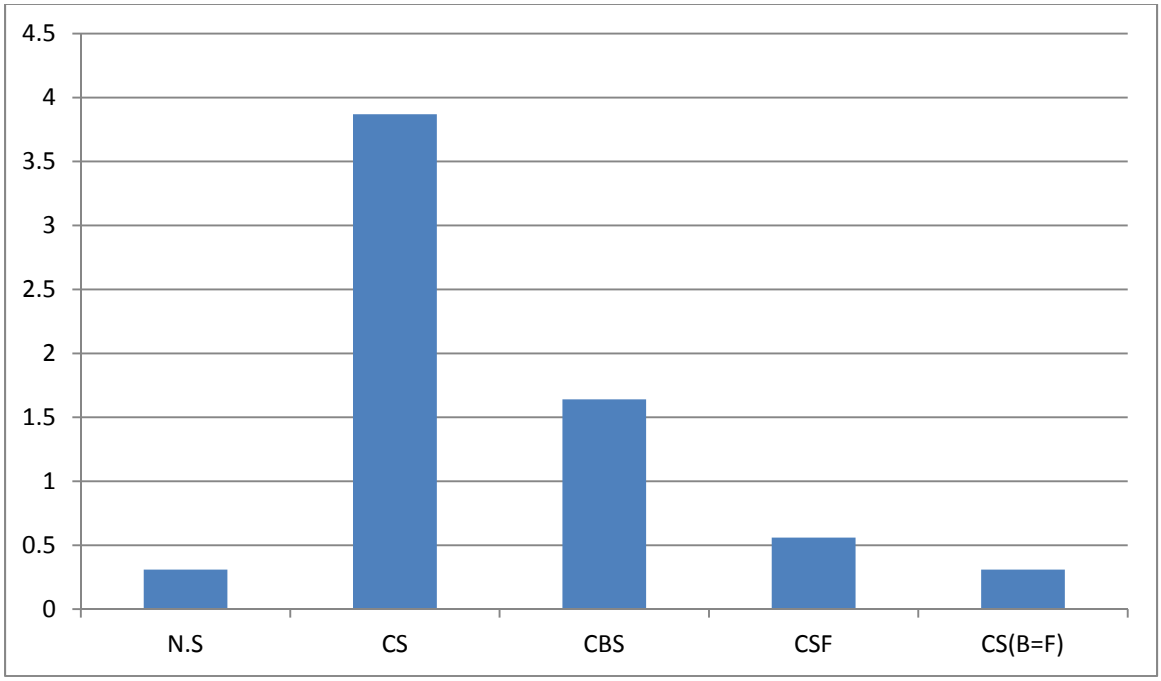

Figure 1: Effect of various treatments on the total petroleum hydrocarbon in the contaminated soil sample in the soil bags after five weeks.

The physico-chemical analysis of soil samples taken from the field study are shown on Table 2, the pH values for the contaminated soil decreased over the 7 weeks period of contamination from 7.30 on the first week to 5.80 on the seventh week of contamination, this corroborates the work of Osuji and Nwoye,2007, who found that oil contamination decrease both surface and subsurface soil pH. Wang et al, 2013 however observed that crude oil contamination significantly increases the soil pH up to 8.0 but on the other hand reduced available phosphorus concentration. The oil may have had some direct impact in lowering the pH, it is more likely that production of organic acids by microbial metabolism is responsible for the difference. Calcium present in the soil also decreases from 7.30mg/kg to 2.00mg/kg (first week to seventh week). Table 3 also shows some heavy metals in the soil sample, there were changes in the measures of the different parameters from the day of contamination to the 7th week of contamination. The presence of heavy metals in some environment has attributed to petroleum prospecting and mining as well as oil spills (Osuji and Onojake, 2004).

Changes in physico-chemical and microbiological properties of soil after the addition of hydrocarbons as observed in this investigation seem to be a universal phenomenon. As seen in tables 4 and 5, the deliberately contaminated soil sample contained higher amount of total petroleum hydrocarbon 6.78PPM compared to the uncontaminated soil sample in the case of the

biotreatment experiment. This showed that petroleum hydrocarbon can also be found in an environment that is not contaminated with crude oil. This was in accordance to the report of Ragheb, (2019) that hydrocarbon are produced biogenically from the decay of organic material and thus, have origins from sources other than crude oil and petroleum deposits.

Soils contaminated with petroleum product have been observed to show large increase in organic matter, total carbon and nitrogen compared with uncontaminated soils. (Ellis and Adams 1961; odu, 1972), similar observations were made in this study. This was probably due to effect of contamination with petroleum hydrocarbon.

The increase in nitrogen content of oil-contaminated soils could be attributed to the activities of nitrogen-fixing bacteria whose presence have been reported in oil-polluted soils (Odukuma and Inor, 2002). In situ nitrogen-fixing capabilities of heterotrophic hydrocarbon-degrading bacteria have also been demonstrated (Odukuma and Inor, 2002). The increase observed in available phosphorus in oil-contaminated soils might be due to the existence of reducing conditions at the experimental sites that made iron phosphates more soluble and which brought some phosphorus into solution. The positive correlation between nitrogen content, available phosphorus and the population density of hydrocarbon utilizers in contaminated soils observed in this study corroborated the observation of other workers which promoted the use of N+P fertilizers to increase the rate of oil biodegradation in polluted soils (Greei*et al*., 2003, MinaiTehrani and Herfatmanesh, 2007).

Decrease in microbial numbers in soils immediately after oil application as seen in Table 4 is not an unusual occurrence and has been attributed to the toxic effect or other unfavorable conditions which may occur as a result of the introduction of the oil (Jensen, 1975). The gradual increase in microbial population counts after the initial repression could indicate the adaptation of the organisms to the new environment. In addition, the pollutant oil could have stimulated the growth of the adapted strains. Similar population increases have been demonstrated in soils from temperate and arctic climatic regions using gaseous hydrocarbons and mineral oils (Wegeberg*et al*., 2018).

Treatment of the contaminated soil with the mixture of bacteria and fungi favoured the biodegradation of the oil above all others. Similar evidence has been found that microbial degradation involves the interactive effects of mixed populations of microorganisms and relies on the metabolic versatility of bacteria and other microbes (Dibble and Bartha, 1979).

Pinholt*et al*. (1979) observed an increase from 60% to 80% in oil utilizing fungi and an increase from 3% to 50% in oil degrading bacteria during oil decomposition in soil after a fuel oil

spill. This can be reflected in this work, which shows that the contaminated soil treated with fungal isolates alone favoured degradation than that treated with bacteria alone.

The trend of degradation observed in this study were as follows, most degraded normal soil {NS (control 1), contaminated soil, treated with bacterial and fungal isolates (CS (B+F)), contaminated soil, contaminated soil, treated with fungal isolates (CSF), contaminated soil, treated with bacterial isolates (CSB), and the least degraded being the contaminated soil, (CS (control 2)}

However many reports on laboratory testing of biodegradation of crude oil have not been tested under field conditions because laboratory products tests may not be attained within the natural environment where complex, chemical and biological interactions occur.

REFERENCE

Ajayi, A.O and Abiola, A.K. 2018. Microbial Diversity of petroleum polluted soil at Ayetoro community in Ilaje Riverine Oil Producing Areas ofOndo State, Nigeria. Progress Petroleum Soil (5).pps. 000525. Doi: 10.31303/pps.2018.01.000524

Alfred O. U, 2011. Bioremediation strategies for oil polluted marine ecosystems.Australian Journal of Agricultural Engineering. 2 (6): 160-168.

AlthalbHakima and Singleton Ian.2017.Isolation of Indigenous Hydrocarbon Transforming Bacteria from oil contaminated soils in Libya; selection for use as potential Inocula for soil Bioremediation.International Journal of Environmental Bioremediation and Biodegradation. 5(1): 8-17.

Atlas, R.M. (1981). Microbial degradation of petroleum hydrocarbon. An environmental perspective: Microbiol Rev. 180-209.

Atlas, R.M. and Bartha, R. (1972). Degradation and mineralization of petroleum in sea water: limitation by nitrogen and phosphorus. Biotechnol.Bioeng. 14:309-317.

Awobajo, A.O. (1983). An analysis of oil spills incidents in Nigeria: In: Proceedings of the international seminar on petroleum industry and the Nigeria environment F.P.M.M. NNPC Lagos, pp 51-63

Bandyopadhyay-Ghosh, S. and Sain, M., 2015.The use of bio-based nanofibres in composites.Biofiber Reinforcements in composite material pp 571- 647.

Bartha, Rand Bossert, I. (1984). The treatment and disposal of petroleum wastes, pp. 553-578.In R.M. atlas (ed.), petroleum microbiology. Macmillan publishing Co., New York

Dibble, J.T and Bartha, R. (1979).Effect of environmental parameters on the biodegradation of oil sludge.Appl.Environ. Microbial.37:729-739.

https//www.nap.edu>read>chapter 500 fifth st.nw/Washington, DC 2001, 2019 National Academy of Sciences.

Ellis, R.S and Adams, R.S. (1961).Contamination of soils by petroleum hydrocarbons.Adv.Agron. 13:197-216.

Floodgate, G. (1984).The fate of petroleum in marine ecosystems, pp 335-398. In R.M. atlas (ed.),petroleum microbiology. Macmillan publishing Co., New York

Greer, C.W, Fortin, N., Roy, R., whyte, L.G and Lee, K. 2003.Indigenous sediment microbial activity in response to nutrient enrichment and plant growth following controlled oil spill on a fresh water wetland.Bioremediat. J:7:69-80.

Harrigan, W.F. and Mc Cane, M.E. (1976).Laboratory methods in food and diary microbiology. Academic press Inc. Ltd. London

Jensen, V. (1975).Bacterial flora of soil after application of oily waste.Oikos 26: 152-158

Kaneez Fatima.,AsmaImran; Imran Amin; Qaiser M. Kan and MuhammedAfzal. 2018. Successfully phytoremediation of crude-oil contaminated soil at an oil exploration and production company by plats bacterial synergism.International journal of phytoremediation. 20 (7): 675-681.

Leo C. Osuyi and IrukaNwoye, 2007. An appraisal of the impact of petroleum hydrocarbons on soil fertility: the owaza experience African Journal of Agricultural Research . 2 (7):318-324.

Minai-Tehrani D. and Herfatmanesh, A. 2007.Biodegradation of aliphatic and aromatic fractions of heavy crude oil-contaminated soil.A pilot study. Bioremediation 11: 71-76

Morgan P. and Watkinson R.J. (1989).Hydrocarbon degradation in soils and methods for soil biotreatmentCrit.Rev. Biotechnical. 8:302-33.

mragheb.com (Ragheb, M. 2019).

National Academy of Sciences (1975).Petroleum in the marine Environment. National Academy of Science, Washington D.C

Odokuma, L.O andInor, M.N. 2002. Nitrogen fixing bacteria enhanced bioremediation of a crude oil polluted soil. DOI:10.4314/gjpas.v814.15993.

Ogbo, E.M and Okhuoya, J.A. 2011. Bioavailability of some Heavy Metals in Crude Oil contaminated soils remediated with pleurotus tuber-regiumFr.singer. Asian Journal of Biological Sciences.4(11): 53-61

Osuji, L.C and Onojake C.M. 2004. Trace heavy metals associated with crude oil: A case study of Ebocha – 8 – al – spill- polluted sites in Niger Delta, Nigeria. Chem. Biodiversity, 1:1708-1715

Pinholt, Y.; Struwe, S. and Kjoller, A. (1979). Microbial changes during oil decomposition in soil. Holarct.Ecol. 2:195-200

Samina, Wasi., Shams, Tabrex and Masood Ahmed. 2013. Use ofPseudomones spp. For the bioremediation of environmental pollutants: A Review in Environmental Monitoring and Assessment. Doi 10.1007/s/0661-013-3163-x

Saminwa, W., Shams, T. and Masood, A. 2013. Use of Pseudomonas spp. for the bioremediation of environmental pollutants: A review in Environmental Monitoring and Assessments. Doi.10.1007/s/0661-013-3163-X

Sugiura, K.; Ishihara, M.; Shimauchi, T. and Shigeakiharayama.(1997). Physic-chemical Environ. Sci. technol. 31:45-50

Wang Yang., Feng Jiang., Lin Qianxin., LyuXianguo., Wang Xianoyu.,and Wang Guoping. 2013. Effects of crude oil contamination on soil physical and chemical properties in momage wetland of china. China. Chinese Geographical Science, 23 (6): 708-715. doi:10.1007/s11769-013-0641-6.

Wegeberg, S., Johnsen, A., Aamand, J., Lassen, P.,Gosewinkel, U., Fritt-Rasmussen, J., Riget, F.,Gustavon, K.andMosbech, A. 2018.Arctic marine potential of microbial oil degradation. Aarhus University, DCE-Danish centre for Environment and Energy, pp 54. Scientific Report from DCE-Danish Centre for Environment and Energy NO.271 http://dce 2.au.dk/pub/SR271.pdf.

www.investopedia.com (James, C. 2018).