ISSN 2350-1030

International Journal of Recent Research in Physics and Chemical Sciences (IJRRPCS)
Vol. 9, Issue 2, pp: (1-10), Month: October 2022 – March 2023, Available at: www.paperpublications.org

Comparative Ameliorative Potentials of Surfactants on some Physico-Chemical properties of Soil Impacted with Crude oil in Okarki Community of Ahoada West Local Government Area of Rivers State, Nigeria

[1]Berezi, P. K., [2]Nwauche, K.T., [3]Berezi, E.P., [1]Igbokwe, E.C.

[1]Department of Integrated Science, Ignatius Ajuru University of Education, Rumuolumini, Port Harcourt, Rivers State.

[2]Department of Biochemistry, Faculty of Science, Rivers State University, Nkpolu-Oroworukwu, Port Harcourt.

[3]Department of Chemistry, Isaac Jasper Boro College of Education, Sagbama, Bayelsa State, Nigeria.

DOI: https://doi.org/10.5281/zenodo.7341049

Published Date: 21-November-2022

Abstract: **This study investigated the comparative remediation potentials of *Pleurotus ostreatus* and triton x-100 on some physico-chemical properties of crude oil impacted soil. Crude oil highly impacted soil excavated from an oil spill site at Okarki community in Ahoada West Local Government Area of Rivers State, Nigeria was used. Remediation was induced using white rot fungus (*Pleurotus ostreatus*) and chemical surfactant (Triton x-100). Five experimental cells (20cm diameter x 20cm high polypropylene bags) labelled A-G, each containing 2kg of polluted soil was used and the different surfactants added in various concentrations as shown in the experimental design. After amendment/inoculation, each of the polypropylene bags was thoroughly mixed and tied with masking tape. All the bags were incubated at 28 ± 2ºC for 60 days. After the incubation period the soil samples were analyzed for various parameters. The pH value of polluted soil sample (Cell A) was high (6.87± 0.67) when compared with other cells that were amended. A significant reduction was observed in cells C and D (5.70 ± 0.00 and 6.06 ± 0.01) amended with 200g of *Pleurotus ostreatus* and 3ml of Triton x-100 respectively. After 60 days of incubation with the amendments used, there was an observed significant difference ($p \leq 0.05$) when all the cells are compared with cell A for total organic carbon content with an observed remarkable increase down the cells. Cells B, C, D, and E had high values of 3.25 ± 0.05, 3.50 ± 0.07, 3.78 ± 0.08 and 3.74 ± 0.09 respectively when compared with cell A (1.70 ± 0.01). The same trend was observed for the total nitrogen content where all the cells were significantly different ($p \leq 0.05$) when compared with cell A and were increased down the cells. For PO_4^{3-} (mg/kg) and NO^-_3 (mg/kg), all the cells were significantly different ($p \leq 0.05$) and there was a recognized decrease in their values when compared with the control sample (Cell A). Organic Sulphur value reduced exponentially in all the cells (0.12 ± 0.00) when compared with the control cell (A) (18.00 ± 0.00). There were significant and swift increase in the soil total heterotrophic bacteria count (THB) in cells B to E with the highest increase seen in cell D (CISS + 3ml Triton x-100) ($12.0x10^7$) when compared with the control sample. Cells B to E had an increased THF count with cell B (CISS + 100g *Pleurotus ostreatus*) having the highest value when compared with the control cell.**

Keywords: ***Pleurotus ostreatus*****, mycoremediation, triton x-100, soil, pollution.**

ISSN 2350-1030

International Journal of Recent Research in Physics and Chemical Sciences (IJRRPCS)
Vol. 9, Issue 2, pp: (1-10), Month: October 2022 – March 2023, Available at: www.paperpublications.org

1. INTRODUCTION

The presence of oil and refined petroleum products in the soil can lead to toxic effects on plants and soil microorganisms and acts as a source of ground water contamination. Petroleum hydrocarbon contamination of soil occurs through extraction, accidents, pipeline, raptures, consumption and refining (Scott, 2003). Most of the crude oil reservoirs and oil refineries in Nigeria are located in areas with agricultural activities and urban areas in the Niger Delta. It is believed according to UN reports, that an average riverine dweller of the Niger Delta is exposed to polluted air, polluted water and polluted food, hence facing health hazard resulting to reduced life expectancy (UN Report, 2001).

The exploration of petroleum products has rendered agricultural lands less productive (Dabbs, 1996) and the creeks and the aquatic lives have become more or less dead (Okpokwasili and Odokuma; 1990). The Niger Delta region of Nigeria has experienced several civil unrests due to environmental degradation from oil exploration (Inoni *et al*., 2006), therefore the release of crude oil into the environment by oil spill is receiving worldwide attention.

The major mechanism for eliminating spilled oil from the environment is the microbial degradation (Atlas, 1995). This remediation option which involves the use of microorganisms to detoxify or remove organic and inorganic compounds from the environment also offers green technology solution to the problem of environmental degradation. The technological process relied upon microbial enzymatic activities to transform or degrade the contaminants from the environments (Phillip and Atlas, 2005). It is a form of bioremediation that harness fungal mycelium to transform complex or simple chemical compounds into non-hazardous forms thereby resulting in materials of higher nutritive value or simply reducing the final bulk of the product (Grady, 1985). Fungi use is expected to be relatively cheap as they can be cultivated on a number of inexpensive agricultural or forest wastes such as corncobs and sawdust (Lavrovsky, 2004). The natural degradative ability of white rot fungi can be used in the decontamination of polluted soil by ramifying the substratum and digesting it through the secretion of extracellular enzymes which are non-specific. The extracellular lignin-degrading enzymes such as manganese peroxidase, laccase and lignin peroxidase can be secreted by white rot fungi to decontaminated polluted soil (Croan, 2000). Okparanma *et al.*, (2011) stated that spent white-rot fungi (*Pleurotus ostreatus*) substrate can be used to biotreat Nigerian oil-based drill cuttings containing Polyaromatic Hydrocarbons (PAH's) under laboratory conditions. Adenipekun (2008) reported an improvement in the nutrient contents of the soil, bioaccumulation of heavy metals, degradation of total petroleum hydrocarbon (TPH), lignin, and increased activity of polyphenol oxidase and peroxidase due to biodegradation of spent cutting fluids by *Pleurotus tuberregium.*

Petroleum and its products are of specific concern in pollution studies due to their structural complexity, slow biodegradability, bio-magnification potential and above all the serious health hazards associated with their release into the environment. Inoni *et al*., (2006) opined that oil exploration and exploitation have over the last four decades impacted disastrously on the socio-physical environment of the Niger Delta oil bearing communities, massively threatening the subsistent peasant economy and the environment and hence, the entire livelihood and basic survival of the people.

Crude oil spills affect plants adversely by making essential nutrients like nitrogen and oxygen needed for plant growth unavailable to them. Oil contamination can be inferred to cause slow rate of germination and stunted growth in plants. According to Adam and Duncan (2002) this effect could be due to the oil which acts as a physical barrier thereby reducing or preventing access of the seeds to water and oxygen which aids germination.

Plants and soil micro-organisms compete continuously for the little nutrient available in soils that are not rich like those that has been polluted with crude oil thus suppressing the growth and development of plants in such soils. When soils not suitable for plant growth are augmented with manure, the growth and performance of plants in such soils are enhanced. Merkl *et al.*, 2005, observed that the addition of inorganic fertilizer in a crude oil polluted soil enhances the growth and performance of *Brachiaria brizantha* in crude oil polluted soil. The development of plants can also be enhanced in crude oil contaminated soil with fertilizer, thereby causing an increase in the cost of crop production in crude oil contaminated soil. Since manure like cow dung is cheaper and affordable to farmers than the inorganic fertilizers, it is necessary to investigate the impact or effect it will have on the growth and development of plants grown in crude oil contaminated soil.

Oil spillage continues to be a global concern not only due to the environmental impact but also due to the public health hazards associated with any spill. The rise in energy consumption worldwide is not without a price and the activities associated with crude oil production, exploration, transportation and marketing have led to increased number of oil spills both on land and into water bodies. The trend shows continuous increases in oil pollution which can be attributed to the increasing dependence on oil based technology such as fuels for aircrafts, automobiles and heating systems, although there

have been recent advances in alternative sources of energy such as production of biofuels (Wokocha *et al.*, 2011). It is difficult to draw a line between effects of oil pollution on man, soil and plants as these three are interwoven.

Surface active agents (biosurfactants) are amphiphilic molecules with both hydrophilic and hydrophobic moieties, which show a wide range of properties, including the lowering of surface and interfacial tension of liquids and the faculty to compose micelles and microemulsions between two different phases. The hydrophilic moiety of a surfactant is defined as the "head", while the hydrophobic one is referred to as the "tail" of the molecule which generally consists of a hydrocarbon chain of varying length. Surfactants are relegated as anionic, cationic, non-ionic and zwitterionic, according to the ionic charge of the hydrophilic head of the molecule (Christofi and Ivshina 2002). The most mundane hydrophobic components of chemically synthesized surfactants are paraffins, olefins, alkylbenzenes, alkylphenols and alcohols; the hydrophilic part is conventionally a sulphate, sulphonate or a carboxylate group in anionic surfactants, a quaternary ammonium group in cationic surfactants and polyoxyethylene, sucrose or a polypeptide in nonionic surfactants (Volkering *et al.*, 1998). A consequential descriptor of chemico-physical properties of surfactants is cognate to the balance between their hydrophilic and hydrophobic moieties. Thus, surfactants can additionally be relegated according to their Hydrophile-Lipophile Balance (HLB) (Tiehm, 1994). The HLB value denotes whether a surfactant will engender a dihydrogen monoxide-in-oil or oil-in-dihydrogen monoxide emulsion: emulsifiers with a lower HLB value of 3-6 are lipophilic and promote dihydrogen monoxide-in-oil emulsification, while emulsifiers with higher HLB values between 10 and 18 are more hydrophilic and promote oil-in-dihydrogen monoxide emulsions (Desai and Banat 1997). A relegation predicated on HLB values has been used to evaluate the congruousness of different surfactants for sundry applications. For example, it has been reported that the most prosperous surfactants in washing oil contaminated soils are those with a HLB value above 10 (Volkering *et al.*, 1998). As the designation suggests and due to their chemico-physical structure, "surfactants" partition preferentially at the interface between phases with different degrees of polarity and hydrogen bonding such as oil/dihydrogen monoxide and air/liquid interfaces. The presence of surfactant molecules at the interfaces results in a reduction of the interfacial tension of the solution. A number of different surfactants, both synthetic and of biological inception, are able to reduce the surface tension of dihydrogen monoxide from 72 mN m-1 to 27-30 mN m-1 (Christofi and Ivshina 2002, Tiehm, 1994). In the presence of a non-aqueous phase liquid (NAPL), the surfactant molecules withal aggregate at the liquid-liquid interface, thus reducing the interfacial tension (Volkering *et al.*, 1998).

Another fundamental property of surfactants is the faculty to compose micelles which is responsible for the excellent detergency and dispersing properties of these compounds. When dissolved in dihydrogen monoxide in very low concentrations, surfactants are present as monomers. In such conditions, the hydrophobic tail, unable to compose hydrogen bonding, disrupt the dihydrogen monoxide structure in its vicinity, thus causing an incrementation in the free energy of the system. At higher concentrations, when this effect is more pronounced, the free energy can be reduced by the aggregation of the surfactant molecules into micelles, where the hydrophobic tails are located in the inner part of the cluster and the hydrophilic heads are exposed to the bulk dihydrogen monoxide phase. The concentration above which the formation of micelles is thermodynamically favoured is called Critical Micelle Concentration (CMC) (Haigh, 1996). The number of molecules compulsory to compose a micelle generally varies between 50 and 100; this is defined as the aggregation number. As a general rule, the more preponderant the hydrophobicity of the molecules in the aqueous solution, the more preponderant is the aggregation number (Rosen, 1989). CMC is commonly used to quantify the efficiency of a surface active agent (Desai and Banat 1997). The CMC of surfactants in aqueous solution can vary depending on several factors, such as molecule structure, temperature, presence of electrolytes and organic compounds in solution. At soil temperatures, the CMC typically varies between 0.1 and 1 mM (Volkering *et al.*, 1998). The size of the hydrophobic region of the surfactant is categorically consequential for the tenaciousness of the CMC: in fact the CMC decreases with incrementing hydrocarbon chain length, i.e. incrementing hydrophobicity. The additament of a CH2- group to the chain has been shown to decrement the CMC by a factor of 3, according to the Traube's rule (Fan *et al.*, 1997). However, anionic surfactants have higher CMCs than nonionic surfactants even when they apportion the same hydrophobic group. Electrolytes in solution can reduce the CMC by shielding the electrical repulsion among the hydrophilic heads of the molecules; such effect is more pronounced with anionic and cationic surfactants than with nonionic compounds (Haigh, 1996). At concentrations above the CMC, supplemental quantities of surfactant in solution will promote the formation of more micelles. The formation of micelles leads to a consequential increase in the ostensible solubility of hydrophobic organic compounds, even above their dihydrogen monoxide solubility limit, as these compounds can partition into the central core of a micelle. The effect of such a process is the enhancement of mobilization of organic compounds and of their dispersion in solution (Perfumo *et al.*,

2010). This effect is additionally achieved by the lowering of the interfacial tension between immiscible phases. In fact, this contributes to the engenderment of supplemental surfaces, thus ameliorating the contact between different phases (Christofi and Ivshina 2002). The reduction effect of interfacial tension is categorically pertinent when the pollutant is present in soil as a non-aqueous phase liquid. In summary, the main surfactant mediated mechanisms which may potentially enhance hydrophobic organic compound remediation include the reduction of interfacial tension, micellar solubilization and phase transfer between soil particles and the pseudo-aqueous phase.

2. RESEARCH METHODS AND MATERIALS

Study Area

Polluted soil sample were collected from a contaminated site in Okarki community of Ahoada west LGA of Rivers State. The community has a boundary with Bayelsa State and the major occupation of the people is fishing and farming. Okarki is located on latitude 4.98334 and longitude 6.42877. The community has a monsoon climate with very long and heavy rainy season and short dry season. December and January months of the year truly qualify the dry season of the community. Harmattan is very low in Okarki and its annual temperatures are typically between 25ºC – 28ºC.

Research Design

The study adopted experimental research design for soil amendment.

Five experimental cells (polypropylene bags 20cm diameter x 20cm high) labelled A-G, each containing 2kg of polluted soil was used. The amendment schedule using the different surfactants is shown in the table below:

Table 1: Experimental cells for soil amendment

Cell	Amendment
A	Crude oil Impacted soil sample (CISS) with no amendment
B	CISS + 100g of *Pleurotus ostreatus* spawn
C	CISS + 200g of *Pleurotus ostreatus* spawn
D	CISS + 3ml of Triton x-100
E	CISS + 100g of *Pleurotus ostreatus* spawn + 3ml of Triton x-100

After amendment/inoculation, each of the polypropylene bags was thoroughly mixed and tied with masking tape. All the bags were be incubated at 28 ± 2ºC for 60 days.

Collection of Samples

a) Soil sample

Polluted soil sample were collected from a contaminated site in Okarki community of Ahoada west LGA of Rvers State from the depth of about 0-25cm and 2kg weighed out into cellophane bag measuring 20cm in height and 20cm in width.

b) Mushroom (*Pluerotus ostreatus*) spawn

The fungus *P. ostreatus* used for this research were obtained from the mycology unit of the department of plant science and biotechnology, University of Port Harcourt, Choba campus. The culture was sub-cultured in malt extract agar to get pure growing culture.

c) Triton x-100.

One hundred milliliters (100ml) of Triton x-100 was purchased from Sigma-Aldrich company, Germany through Bristol Scientific Company Limited, Apapa, Lagos State, Nigeria.

Determination of Soil pH

Five grams (5g) of the soil sample was weighed into a clean beaker. 20mls of distilled water added to it and the sample stirred with an electromagnetic stirrer for 10mins and allowed to stand for 30mins, the mixture was stirred again for 2mins, the pH meter electrode was rinsed with distilled water and dipped into the sample in the beaker. Thereafter, the figures on the pH meter screen were allowed to stabilize before the reading was taken. This was done for the polluted samples at the beginning of the experiment and at the end of the experiment.

Determination of Soil Nitrate

One gram (1g) of soil sample was extracted with 50 ml of 2.5 % acetic acid. The extract was filtered into a beaker. One (1) ml of extract was put into a clean test tube using pipette with 0.5ml of Brucine reagent. 2 ml of concentrated sulphuric acid (H_2S0_4) was added to develop a yellowish colour in the presence of NO_3^- ion. The colour produced was absorbed at 400 nm using water as blank. Standard nitrate (NO_3^-) was prepared by dissolving 0.7216 g of potassium nitrate in 100 ml distilled water and was diluted to obtain a working standard of 0.1 mg NO_3^-/ml.

CALCULATION

NO_3^- = N mg/l = Absorbent x standard nitrate graph gradient

Determination of Soil Total Organic Carbon

The principle behind this determination is based on the fact that organic carbon is determined by sulphuric acid (H_2SO_4) and aqueous potassium dichromate ($K_2 Cr_2O_7$) mixture.

One gram (1g) of soil samples collected before and after the experiment was weighed into a 150ml conical flask. 5ml of $K_2 Cr_2O_7$ solution and 7.5ml concentrated sulphuric acid was added into the sample. The solution was heated for about 30mins and allowed to cool. Blanks were also set up with only the reagents excluding samples. A magnetic stirrer was used to ensure proper mixing. The digest was titrated with ferrous ammonium sulphate solution.

$$\text{TOC (\%)} = \frac{\text{(The blank titre value – sample titre value) x 0.195}}{\text{Weight of sample.}}$$

Determination of Soil Total Nitrogen

Ten grams (10g) of dry soil sample was weighed into a macro-kjeldahl flask containing 20ml of distilled water. The flask was stirred for a few minutes and allowed to stand for 30 minutes. One tablet of mercury tablet, 10g of K_2SO_4 and 30ml of H_2SO_4 was added to the flask. The flask was heated cautiously at low heat on the digestion stand until the water was removed and frothing ceased. The mixture was then boiled for 5 hours. The flask was allowed to cool and 100ml of water slowly added to the flask. The digest was carefully transferred into another clean macro-kjeldahl flask (750ml). Distillation was commenced when the flask is attached to the distillation apparatus and about 150ml of 10N NaOH poured into the distillation flask opening the funnel stopcock. One hundred and fifty mililitres (150ml) of the distillate was collected and the distillation was stopped. The NH_4-N in the distillate was determined by titrating with 0.01N standard HCl using 25ml burrette graduated at 0.1ml intervals. The colour change at the end point was observed. The percentage (%) Nitrogen content in soil was then calculated.

Microbial Analysis

For soil analysis, soil sample from the treatment cells was collected after the 60 days period of incubation and placed on sterile containers, labelled appropriately and taken to the laboratory for analysis.

a) Preparation of Nutrient agar and Potato dextrose agar

The soil sample was mixed, and a suspension of 1g (dry weight equivalent) in 10 ml of sterile distilled water was prepared. One ml of the soil suspension was then diluted serially (ten-fold) and used in the estimation of aerobic heterotrophic bacterial and fungal populations by standard spread-plate dilution method in triplicate. Nutrient agar (prepared by dissolving 28g in 1 litre of distilled water and autoclaved for 15mins at 121^{o}C and 15psi) containing 0.015% (w/v) nystatin (to inhibit fungal growth) was used for bacteria isolation.

Potato dextrose agar (prepared by dissolving 65g in 1 litre of distilled water and autoclaved under same conditions) to which 0.05% (w/v) chloramphenicol has been added (to inhibit bacteria growth) was used for fungal isolation.

b) Sample inoculation.

Various dilutions of the soil suspensions (0.1ml each) were poured and spread over the nutrient agar/ potato dextrose agar plates respectively using a sterile L rod. The inoculated nutrient agar plates (in triplicates) were incubated at 35^{o}C for 24 – 48hrs. After 48hrs of incubation, individual colonies were sub-cultured daily to obtain pure isolates for characterization.

The inoculated Potato dextrose agar (PDA) plates were left at room temperature for between 3 – 5 days for the fungal characterization.

c) Preservation of isolates

Pure isolates were inoculated into freshly prepared NA and PDA slants in McCartney bottles for bacteria and fungi preservation respectively and stored at 4^{o}C in a refrigerator.

Identification of isolates was based on cultural, microscopic characterization, Gram reactions and biochemical reactions for the bacteria isolates. The fungal isolates were dissolved into Lactophenol cotton blue solution dropped onto a clean grease-free slide, covered with a cover slip, and examined under the microscope with the 10X and 40X objectives. The fungal isolates were identified based on the colour of aerial hyphae and substrate mycelium, arrangement of hyphae, conidial arrangement as well as cultural morphology.

d) Total Fungi (TF)

One gram (1g) of soil sample was weighed into 9ml sterile diluents (0.85% Nacl) under aseptic conditions. It was then shaken vigorously and serially diluted. 0.1ml Aliquot of inoculums was inoculated on Potato Dextrose Agar (PDA) acidified on 0.05% (w/v) chloramphenicol to inhibit growth of bacteria and allow for only the growth of fungi. The inoculated plates was incubated at room temperature for 5-7 days. Thereafter the numbers of visible colonies was enumerated to obtain the colony forming unit per gram (cfu/g) of samples.

e) Total Heterotrophic Bacteria (THB)

One gram (1g) of soil sample was weighed into 9ml sterile diluents (0.85% Nacl) under aseptic conditions. It was then shaken vigorously to homogenize and serially diluted. Then 0.1ml aliquot of inoculums was collected using a sterile pipette, inoculated on Nutrient Agar (NA) surface. The inoculum was spread evenly with a sterile hockey stick. The plates were incubated at room temperature for 24hours. Thereafter the numbers of colonies were counted to obtain the colony forming unit (cfu/g) gram ml of the sample.

Nature/Sources of Data

Data of this study were obtained from primary source mainly through laboratory work and through other literatures.

Method of Data Analysis

All data for soil analysis were analyzed for statistical differences by means of one-way ANOVA and post hoc LSD, on SPSS 20. In all, $p<0.05$ was considered significant. Data was presented as mean$\pm$S.D (standard deviation).

3. RESULTS

Soil Physico-Chemical Parameters

Table 2a below shows the mean pH values of crude oil polluted soil sample and the polluted soil samples amended/inoculated with white rot fungus (*Pleurotus ostreatus*) and non-ionic surfactant (Triton-x-100).

The pH value of polluted soil sample (Cell A) was high (6.87± 0.67) when compared with other cells that were amended. A significant reduction was observed in cells C and D (5.70 ± 0.00 and 6.06 ± 0.01) amended with 200g of *Pleurotus ostreatus* and 3ml of Triton x-100 respectively.

Table 2b below shows the mean values of some physicochemical characteristics (TOC (%), Tot.N (%), PO_4^{3-} (mg/kg) SO_4^{2-} (mg/kg) and NO^-_3 (mg/kg)) of crude oil polluted soil sample and the polluted soil samples amended/inoculated with white rot fungus (*Pleurotus ostreatus*) and non-ionic surfactant (Triton-x-100).

After 60 days of incubation with the amendments used, there was an observed significant difference ($p\leq 0.05$) when all the cells are compared with cell A for total organic carbon content with an observed remarkable increase down the cells. Cells B, C, D, and E had high values of 3.25 ± 0.05, 3.50 ± 0.07, 3.78 ± 0.08 and 3.74 ± 0.09 respectively when compared with cell A (1.70 ± 0.01). The same trend was observed for the total nitrogen content where all the cells were significantly different ($p\leq 0.05$) when compared with cell A and were increased down the cells. For PO_4^{3-} (mg/kg) and NO^-_3 (mg/kg), all the cells were significantly different ($p\leq 0.05$) and there was a recognized decrease in their values when compared with the control sample (Cell A). Organic Sulphur value reduced exponentially in all the cells (0.12 ± 0.00) when compared with the control cell (A) (18.00 ± 0.00).

ISSN 2350-1030

International Journal of Recent Research in Physics and Chemical Sciences (IJRRPCS)
Vol. 9, Issue 2, pp: (1-10), Month: October 2022 – March 2023, Available at: www.paperpublications.org

Table 2a: Effect of amendments on soil pH

Cell	Treatment	pH
A	Crude oil impacted soil sample (CISS)	6.87 ± 0.67
B	CISS + 100g of *Pleurotus ostreatus*	6.19 ± 0.00
C	CISS + 200g of *Pleurotus ostreatus*	5.70 ±0.00
D	CISS + 3ml Triton x-100	6.06 ± 0.01
E	CISS + 100g of *Pleurotus ostreatus* + 3ml of Triton x-100	6.55 ± 0.00

Each value is a mean of three replicates expressed as mean ± S.D. Values in the same column with common superscript letters (a, b,…) are significantly different at $p \leq 0.05$ when compared with the contaminated soil sample.

Table 2b: Effect of amendments on Soil Physico-chemical Parameters

Cell	Treatment	Total Organic Carbon (%)	Total Nitrogen (%)	PO_4^{3-} (mg/kg)	SO_4^{2-} (mg/kg)	NO^-_3 (mg/kg)
A	Crude oil impacted soil sample (CISS)	1.70 ± 0.01^a	0.16 ± 0.00^a	3.78 ± 0.05^a	18.00 ± 0.00	3.91 ± 0.03^a
B	CISS + 100g *Pleurotus ostreatus*	3.25 ± 0.05^a	0.30 ± 0.00^a	2.27 ± 0.04^a	0.12 ± 0.00	0.85 ± 0.12^a
C	CISS + 200g *Pleurotus ostreatus*	3.50 ± 0.07^a	0.32 ± 0.00^a	3.01 ± 0.08^a	0.12 ± 0.00	1.33 ± 0.10^a
D	CISS + 3ml Triton x-100	3.78 ± 0.08^a	0.34 ± 0.00^a	1.22 ± 0.01^a	0.12 ± 0.00	0.30 ± 0.57^a
E	CISS + 100g *Pleurotus ostreatus* + 3ml Triton x-100	3.74 ± 0.09^a	0.34 ± 0.00^a	2.01 ± 0.03^a	0.12 ± 0.00	1.03 ± 0.52^a

Each value is a mean of three replicates expressed as mean ± S.D. Values in the same column with common superscript letters (a, b,…) are significantly different at $p \leq 0.05$ when compared with the contaminated soil sample.

Effect of amendment on Soil Total Heterotrophic Bacteria (THB) count and Total fungi (THF) count

Results for the Total Heterotrophic Bacteria (THB) and Total Heterotrophic fungi (THF) of crude oil polluted soil sample and the polluted soil samples inoculated with white rot fungus (*Pleurotus ostreatus*) and non-ionic surfactant (Triton-x-100) are shown in table 2 below.

There was an increase in the soil THB in cells B to E with the highest increase seen in cell D (CISS + 3ml Triton x-100) ($12.0x10^7$) when compared with the control sample. Cells B to E had an increased THF count with cell B (CISS + 100g *Pleurotus ostreatus*) having the highest value when compared with the control cell.

Table 3: Total microbial count of crude oil Impacted soil sample and the impacted soil samples inoculated with white rot fungus (*Pleurotus ostreatus*) and non-ionic surfactant (Triton-x-100).

Cell	Treatment	Total Heterotrophic Bacteria count (THB) (CFU/g)	Total fungal count (CFU/g)
A	Crude oil Impacted soil sample (CISS)	$5.3x10^7$	$4.3\ x10^2$
B	CISS + 100g *Pleurotus ostreatus*	$10.7x10^7$	$6.6x10^2$
C	CISS + 200g *Pleurotus ostreatus*	$8.2x10^7$	$4.9x10^2$
D	CISS + 3ml Triton x-100	$12.0x10^7$	$4.7x10^2$
E	CISS + 100g *Pleurotus ostreatus* + 3ml Triton x-100	$5.7x10^7$	$5.4x10^2$

4. DISCUSSION

Soil Physico-Chemical Parameters

Tables 2a and 2b above shows the mean values of some physicochemical characteristics of crude oil polluted soil sample and the polluted soil samples amended/inoculated with white rot fungus (*Pleurotus ostreatus*) and non-ionic surfactant (Triton-x-100).

The pH value of polluted soil sample (Cell A) was high (6.87± 0.67) when compared with other cells that were amended. A significant reduction was observed in cells C and D (5.70 ± 0.00 and 6.06 ± 0.01) amended with 200g of *Pleurotus ostreatus* and 3ml of Triton x-100 respectively.

The pH values obtained above in all the amended cells and the untreated cell are in line with the work of Dibble and Bartha, 1979 and Greenfield 1991. They reported that a pH range of 6.5-8.0 is favourable for optimal mineralization of hydrocarbons. The increase in pH observed in the untreated cell could be attributed to the fact that crude oil increases the pH of a soil. This is similar to the findings of Andrade *et al*., (2004) and Ayotamuno *et al.*, (2004) who observed increase in the pH of soils polluted with crude oil. The pH is a function of the accumulated acid production and the decomposition of acids to produce CO_2 and heat (Sundberg, 2005). It is assumed that when the rate of acid production is faster than the rate of acid decomposition, acids accumulate so the pH falls, and when the rates of both acid production and decomposition are equal, the pH reaches equilibrium. Thus, pH values recorded throughout the study period for all the cells were optimal for microbial activity is soils and suitable for bioremediation as most tropical crops perform optimally at the range of 5.5 to 6.5 (Ahn, 1979).

After 60 days of incubation with the amendments used, all the cells amended (B to E) had significant difference ($p \leq 0.05$) and were increased when compared with cell A for total organic carbon (%) content. The same trend was observed for the total nitrogen (%) content where all the cells were significantly different ($p \leq 0.05$) when compared with cell A. For PO_4^{3-} (mg/kg) and NO^-_3 (mg/kg) all the cells were significantly different ($p \leq 0.05$) and there was a recognized reduction in their values when compared with the control sample (Cell A). Organic Sulphur (mg/kg) value reduced exponentially but not significantly in all the cells (0.12 ± 0.00) when compared with the control cell (A) (18.00 ± 0.00). The high organic carbon (%) values recorded in all the ameneded cells could be as a result of greater percentage of phenolic compounds facilitated by the amendment substances which will stimulate fungus to secrete more enzymes than in the untreated cell. Though these microorganisms are beneficial for crop growth, the reverse is the case when they are present in large quantities as they compete with crops for available soil nutrients thus reducing the nutrient of the soil and indirectly contributing to a reduction in crop growth (Trofimov and Rozanova, 2003).

Asuquo *et al.*, (2001) and Anacletus *et al.*, (2017) in their research also observed significant increases in organic carbon in contaminated soil following an initial scarcity with contamination. This study has shown that crude oil pollution resulted in an imbalance in the carbon-nitrogen ratio in the crude oil impacted soil sample compared to the ameliorated soil sample because crude-oil is essentially a mixture of carbon and hydrogen. This causes a nitrogen deficiency in an oil-soaked soil, which retards the growth of bacteria and the utilization of carbon source(s), as well as deficiency in certain nutrients like phosphorus which may be growth-rate limiting, (Atlas and Bartha, 2005).

The low levels of total nitrogen, phosphorous, Sulphur and nitrate recorded in all the amended cells suggests that the microorganisms must have utilized them for growth during the incubation period. This is in agreement with the report of Anacletus *et al.*, (2017). They opined that low levels of nitrogen and phosphorous was observed from a crude oil spill site in the Niger delta region of Nigeria.

Effect of amendment on Soil Total Heterotrophic Bacteria (THB), Total fungi (TF) of crude oil impacted soil samples and Hydrocarbon Utilizers.

Results for the Total Heterotrophic Bacteria (THB) and Total fungi (TF) (CFU/g) of crude oil polluted soil sample and the polluted soil samples inoculated with white rot fungus (*Pleurotus ostreatus*) and non-ionic surfactant (Triton-x-100) are shown in table 3 above.

There was an increase in the soil THB (CFU/g) in cells B to E with the highest increase seen in cell D (CISS + 3ml Triton x-100) ($12.0x10^7$) when compared with the control sample. Cells B to E had high TF (CFU/g) count when compared with the control cell.

The enumeration of microbial population will provide information concerning the biodegradation of hydrocarbons and/ or test biodegradation efficiency as carried out in this study. The bacterial diversity of crude oil polluted soil was investigated. Cells D (PSS + 3ml Triton x-100) and B (CISS + 100g *Pleurotus ostreatus*) had the highest Total Heterotrophic Bacteria (THB) count. This result indicates that bacteria can grow rapidly on culture containing hydrocarbon compounds and degrade the hydrocarbon source. *Arthrobacter* spp and *Pseudomonas* spp have a remarkable capacity for the degradation of a broad range of organic pollutants including PAH, halogenated derivatives and recalcitrant organic residues. *Pseudomonas*

fluorescens and *Pseudomonas putida* are two examples of well-known species capable of PAH degradation frequently reported in PAH impacted soil (Francisco *et al.*, 2010). The bacteria belonging to *Arthrobacter* spp have been reported to consume organic waste thousands of time faster than the types of bacteria that are naturally present in waste. They grow and reproduce easily, do not produce foul odors or gas and are non-pathogenic. *Pseudomonas aeruginosa* strains isolated from a petroleum contaminated soil sample of North-East India (ONGC oil Weld) grew on a large number of hydrocarbon compounds as a source of carbon and energy demonstrating these strains might be efficient hydrocarbon degraders (Das and Mukherjee, 2005). These strains have been reported to be efficient producers of biosurfactants in hydrocarbon rich culture medium. Abalos *et al.*, 2004 reported that exogenously added microbial biosurfactants enhanced the bioremediation of crude oil polluted soils by indigenous microbes.

5. CONCLUSION

Crude oil pollution tends to persist in soils until remediation measures, involving the application of nutrients and other bioremediating agents are resorted to because oxygen and nitrogen are limiting factors. The application of biological surfactant alone and in combination with the non ionic surfactant (triton x-100) improved the selected chemical properties (pH, TOC, total nitrogen) while sulphur, phosphorus and nitric oxide contents were significantly decreased.

REFERENCES

[1] Abalos, A., Vinas, M., Sabate, J., Manresa, M.A. and Solanas, A.M. (2004). Enhanced biodegradation of Casablanca crude oil by a microbial consortium in presence of a rhamnolipid produced by *Pseudomonas aeruginosa* AT 10. *Biodegradation* **15**:249-260.

[2] Adam, G. and Duncan, H.J. (2002). "Influence of diesel fuel on seed germination". *Environmental Pollution*, **20;**363-370.

[3] Adenipekun, C.O and Isikhuemhen, O.S. (2008). Bioremediation of engine oil polluted soil by by a tropical white rot fungus, *Lentinus squarrosulus* Mont. (Singer). *Pakistan Journal of Biological Science.* **11**(12); 1634 - 1637.

[4] Ahn, P. M. (1979). West African Soils. *Oxford University Press*. London.

[5] Anacletus F. C., Nwauche K. T. and Ighorodje-Monago C. C. (2017). Effect of Triton X-100 and White Rot Fungus (*Pleurotus ostratus*) On Physico-Chemical Composition of Crude Oil Impacted Soil**.** *Journal of Applied Sciences International,* **12** (3): 1-7

[6] Andrade, L.R, Farina M, and Amado Filho, G.M., (2004). Effects of copper on *Enteromorpha flexuosa* (Chlorophyta) in vitro. *Ecotoxicology and Environmental Safety* **58**: 117-125.

[7] Asuquo, F.E., Ibanga, I.J. and Hungafa, N. (2001). Effects of Quaiboe crude oil contamination on germination and growth of Okra (*Abelmoschus esculentus*. L) and fluted pumpkin (*Telfairia occidentalis L) Proc. of the 27th Annual Conf. of the soil science of Nig.* Nov 5-9, 2001. University of Calabar.

[8] Atlas, R.M and Bartha, R. (1998). Fundamentals and Applications. In: microbial Ecology. 4th edition. *Benjamin/Cumming Publishing Company*, Inc. California, USA, **pp** 523-530.

[9] Atlas, R.M. (1995). Bioremediation. *Chemical Engineering News*, **73**; 32-42

[10] Christofi, N., and Ivshina, I.B. (2002) Microbial surfactants and their use in field studies of soil remediation. *Journal of Applied Microbiology* **93**: 915-929.

[11] Croan, S.C. (2000). Conversion of wood waste into value-added products by edible and medicinal (Fr.) *P. Karst.* Species (Agaricales S. I., Basidiomycetes). **pp** 73-80.

[12] Dabbs, W.C. (1996). Oil Production and Environmental Damage. *http://www.american.edu.TED/hpl.htm*.

[13] Das, K. and Mukherjee, A.K. (2005). Characterization of biochemical properties and biological activities of biosurfactants produced by *Pseudomonas aeruginosa* mucoid and non-mucoid strains isolated from hydrocarbon contaminated soil samples. *Applied Microbiology and Biotechnology* **69**: 192-199.

[14] Desai, J.D., Banat, I.M. (1997). Microbial production of surfactants and their commercial potential. *Microbiology and Molecular Biology Reviews*, **61**: 47.

[15] Fan, A., Somasundaran, P., and Turro, N.J. (1997). "Adsorption of Alkyltrimethyl-ammonium Bromides on Negatively Charged Alumina" *Langmuir*, **13**: 506.

[16] Francisco, J.R.C., Sara, S., Luisa S., Ana, L.S., Adelaide, A., Newton, C.M.G. and Angela, C. (2000). PAH degrading bacteria in an Estuarine System, Interdisciplinary Studies on Environmental Chemistry-Biological Responses to contaminant. **pp** 77-78.

[17] Grady, F. (1985). Biodegradation. Its Measurement and Microbiological basis. *Biotechnology and Bioengineering*, **27**: 660-671.

[18] Greenfield, J.H. (1991). In stitu comparison of Bioremediation Methods for a Number 6 Residual Fuel Oil Spill-in Lee County, Florida. *Proceedings of the 1991 Oil Spill Conference*. American Petroleum Institute, Washington, D. C.

[19] Haigh, S.D. (1996). A review of the interaction of surfactants with organic contaminants in soil, *Science of the Total Environment*, **185**: 161.

[20] Inoni, O.E., Omotor, D.G. and Adun, F.N. (2006). The effect of oil spillage on crop yield and farm income in Delta State, Nigeria. *Journal of Central European Agriculture*, **7**(1): 41-49.

[21] Lavrovsky, V. (2004). Microencapsulated enzyme systems. Enhanced oil recovery and bioremediation. Paper presented at the 2004 Calgary youth science fair. Retrieved on July 18, 2012.

[22] Merkl N, Schutze-Kraft, R. and Arias, M. (2005). Influence of Fertilizer Level on Phytoremediation of Crude Oil-contaminated Soils with the Tropical Grass *Brachiaria brizantha* (Hochst. ex A. Rich.) Stapf. In: Merkl N (Eds). Phytoremediation of Petroleum-contaminated Soil. *Margraf Publisher*, Weikershim; **pp** 71 – 83.

[23] Okparanma, R.N., Ayotamuno, J.M., Davis, D.D. and Allagoa, M. (2011). Mycoremediation of polycyclic aromatic hydrocarbons (PAH) – contaminated oil based drill cutting. *African Journal of Biotechnology*, **10**(26): 5149-5156.

[24] Okpokwasili, G.C. and Odokuma, L.O. (1990). Effect of salinity on Biodegradation of oil spills dispersants. *Waste Management*, **10:**141 – 146.

[25] Perfumo, A., Smyth, T.J.P., Marchant, R. and Banat, I.M. (2010). Production and roles of biosurfactants and bioemulsifiers in accessing hydrophobic substrates. In Handbook of Hydrocarbon and Lipid Microbiology ed Timmis, K.N. Chapter 47 **2**(7): 1501-1512. *Springer-Verlag*, Berlin Heidelberg.

[26] Phillip, J.C. and Atlas, R.M. (2005). Bioremediation of contaminated soil and aquifers. In: Bioremediation: *Applied Microbial Solution for Real- World Environmental Clean Up.*

[27] Rosen, M.J. (1989). Surfactants and Interfacial Phenomena 2nd Edition. *Wiley Interscience*, New York, USA.

[28] Scott, S. L. (2003). Biodegradion and Toxicity of Total Petroleum Hydrocarbon Leachate from Land Treatment Units. Department of Engineering, *California Polytechnic State University*, **pp.** 52

[29] Sundberg, C. (2005). Improving compost process efficiency by controlling aeration, temperature and pH. Doctoral thesis, Swedish University of Agricultural Science, Uppsala.

[30] Tiehm, A. (1994). Degradation of polycyclic aromatic hydrocarbons in the presence of synthetic surfactants. *Applied Environmental Microbiology*, **60**: 258.

[31] Trofimov, S. Y., and Rozanova, M. S., (2003), Transformation of soil properties under the impact of oil pollution, Eurasian *Soil Science*, 36: 82-S87.

[32] United Nations (UN) Report. (2001). Protecting Ecosystem for people and planet. *United Nations Environmental Programme*, **pp.** 130-147.

[33] Volkering, F., Breure, A.M., and Rulkens W.H. (1998). Microbiological aspects of surfactant use for biological soil remediation. *Biodegradation*, **8:** 401.

[34] Wokocha, G. A., Emeodu, D. and Sopruchi, I. (2011). Impact of crude oil spillage on soil and food productions in RiversState, Nigeria. *Journal of Money, Investment and Banking*, 31 -34.