Journal of Applied Life Sciences International

12(3): 1-7, 2017; Article no.JALSI.34679
ISSN: 2394-1103

Effect of Triton X-100 and White Rot Fungus (*Pleurotus ostratus*) on Physico-chemical Composition of Crude Oil Impacted Soil

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Authors' contributions

This work was carried out in collaboration between all authors. Author KTN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors FCA and CCIM managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JALSI/2017/34679
Editor(s):
(1) Shahira M. Ezzat, Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Egypt.
Reviewers:
(1) Chioma Nwakanma, Michael Okpara University of Agriculture, Nigeria.
(2) Arifa Tahir, Lahore College for Women University, Pakistan.
Complete Peer review History: http://www.sciencedomain.org/review-history/19779

Original Research Article

Received 5th June 2017
Accepted 21st June 2017
Published 30th June 2017

ABSTRACT

The study investigated the effect of triton x-100 and white rot fungus (*Pleurotus ostratus*) on physico-chemical composition of crude oil impacted soil. Crude oil highly impacted soil excavated from an oil spill site at Obeche community in Ogba/Egbema/Ndoni Local Government Area of Rivers State, Nigeria was used in this study. Remediation was induced by biostimulation using white rot fungus (*Pleurotus ostreatus*) and treatment with a chemical surfactant (Triton x-100). The pH value of polluted soil sample (Cell A) was high (6.81± 0.52) when compared with other cells that were amended. The result of the soil electrical conductivity shows a significant difference ($p \leq 0.05$) when cell A is compared with other cells. Only cell C amended with Triton x-100 had a lower value of 31.67 ± 1.52 when compared with cell A (polluted soil sample) (76.67 ± 1.52). Cells B and D had higher values of 161.00 ± 2.00, and 233.67 ± 4.04 respectively when compared with cell A (76.67 ± 1.52). Significant ($p \leq 0.05$) increase in the total organic carbon (TOC) and total nitrogen (TN) contents was observed amended cells after the 60 days period of incubation when compared with cell A. For PO_4^{3-} (mg/kg) and NO^-_3 (mg/kg) all the cells were significantly different ($p \leq 0.05$)

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and there was a recognized decrease in their values when compared with the control sample (Cell A). Organic Sulphur values decreased exponentially in all the cells amended (0.10 ± 0.00) when compared with the control cell (A) (15.00 ± 0.00).

Keywords: Surfactant; triton x-100; Pleurotus ostreatus; bioremediation.

1. INTRODUCTION

The nearness of oil and refined petroleum items in the soil can prompt harmful consequences for plants and soil microorganisms and acts as a source of ground water contamination. Pollution caused by petroleum and its derivatives is the most prevalent problem in the environment. Since commercial exploration of petroleum started in Nigeria in 1958 [1]. Petroleum has continuously grown to be mainstay of the Nigerian economy. However, the exploration of petroleum has led to the pollution of land and water ways.

The agricultural lands have become less productive [2] and the creeks and the fishing waters have become more or less dead [3,4]. Several civil unrests due to environmental degradation from oil exploration is reported in the tropics, especially, in the Niger Delta region of Nigeria [5], therefore the release of crude oil into the environment by oil spill is receiving worldwide attention.

Bioremediation, the basis of which may date back to the work of [6], is the use of microorganisms (bacteria and fungi) to accelerate the natural decomposition of hydrocarbon-contaminated waste into nontoxic residues. The use of different strains of bacteria such as *Pseudomonas aeruginosa*, Azotobacter and *Bacillus subtilis* (among others) in the bioremediation of hydrocarbon-contaminated soil, oily sludge and drill cuttings has been widely reported [7,8]. Similarly, different species of fungi including *Lentinus subnudus*, *Lentinus squarrosulus* Mont., *Pleurotus ostreatus*, *Pleurotus tuberregium* Fr. Singer, *Irpex lecteus* and *Phanerochaete chrysosporium* have been used in the bioremediation of engine-oil polluted soil, chemically polluted soil, crude-oil contaminated soil and wheat straw [9]. *P. ostreatus*, in particular, according to literature have the potentials to degrade PAHs.

Surfactants can help solubilize non-polar material in the liquid phase and increase its biodegradation. Triton x-100 is a non-ionic detergent widely used in extraction of membrane proteins. Surfactants have a micelle action such as observed in detergents when washing out dirt and stains from fabrics. Surfactants dislodge the oil from the soil particle making it possible for the oil to be washed off by rain or acted upon facilely by microorganisms. Sublette further expounded that the utilization of surfactants in soil to break up oil is not generally recommended because they can potentially interfere with cell membranes and enzymes, which in turn can reduce biodegradation rates. Surfactants should be pre-screened for toxicity to indigenous microbes. Dispersants, a type of surfactant, has been used prosperously in breaking up marine oil spills [10].

Therefore, the objective this study is to access the effect of single and combined application of triton x-100 and white rot fungus (*pleurotus ostratus*) on physico-chemical composition of crude oil impacted soil.

2. MATERIALS AND METHODS

2.1 Collection of Soil Sample

The crude oil polluted soil samples used for this experiment were collected at a depth of 10- 15 cm from an oil spill site at Obeche community, location 1, Ogba/Egbema/Ndoni L.G.A., Rivers State.

2.2 Preparation of Culture Medium

The fungus *P. ostreatus* used for this study was obtained from the Mycology unit in the Department of Plant Science and biotechnology, University of Port Harcourt, Choba, Rivers State. The culture was subcultured in malt extract agar to get pure growing culture.

Malt Extract Agar (MEA) was prepared by dissolving 20 g of agar powder and 20 g of malt extract broth in 1000 ml of distilled water. The mixture was autoclaved at 121℃ for 15 min. On cooling to 45℃, the medium was dispensed into 9-cm Petri dishes to gel.

2.3 Spawn Production

Spawn was prepared following a modified method described by [11]. The guinea corn (*Sorghum bicolor*) grains used were thoroughly washed with tap water and soaked overnight. They were dispensed into spawn bottles and autoclaved at 121℃ for 1 h each day for three consecutive days. On cooling, the grains in each bottle were inoculated with four 9 mm mycelia discs taken from a 4-day-old agar culture of *P. ostreatus* and incubated at 28±2℃ for 14 days in darkness.

2.4 Fungal and Surfactant Inoculation

A modified method of [12] was employed. 2000 g aliquots of crude oil polluted soil were weighed into polypropylene bags (20 cm diameter × 30 cm high). Each bag was inoculated with 7 g of spawn of the test fungus and 3ml of triton x-100 and were subsequently tied with masking tape. All the bags were incubated at 28±2℃ for 60 days. Completely randomized design was used in the experiment.

2.5 Determination of Soil pH [13]

Five grams (5 g) of the soil sample was weighed into a clean beaker. 20 mls of distilled water was added to it and the sample was stirred with an electromagnetic stirrer for 10 mins and allowed to stand for 30 mins, the mixture was then stirred again for 2 mins, 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. pH model used is EQ-610 Equip Tronic pH meter.

2.6 Determination of Soil Electrical Conductivity

Soil conductivity was measured by meter method. 5g of the soil sample was weighed into a clean beaker and 20 mls of distilled water was added to it and the sample was stirred with a glass rod for 10 mins and allowed to stand for 30 mins, the mixture was then stirred again for 2 mins, the conductivity meter electrode was rinsed with distilled water and dipped into the sample in the beaker. Thereafter, the figures on the conductivity 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. The conductivity meter used is JENWAY Model 4010 conductivity meter.

2.7 Determination of Soil Nitrate

One gram (1 g) of soil sample was extracted with 50 ml of 2.5% acetic acid. The extract was filtered into a beaker. 1 ml of extract was pipette into a clean test tube with 0.5 ml 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.

2.8 Determination of Soil Phosphate [14]

One gram (1 g) of soil was extracted with 50ml of 2.5% glacial acetic acid. The extract was filtered into 250 ml capacity conical flask and 8ml of combined (0.42 g selenium powder + 14 g lithium sulphate was added to 350 ml 30% hydrogen peroxide and 420 ml concentrated H_2SO_4) reagent was added. A blank and standard phosphate ion concentration ranging from 0.0001 and 0.0007 was prepared. 8 ml of combined reagent was added respectively.

The blue colouration developed within 30mins interval was read at 840 nm wavelength in thermo-spectrometer. The volume of the extracted sample was also read at the same wavelength. The concentration of phosphate ion in the sample was extrapolated from the standard phosphate graph plotted with values in the table displayed. The phosphate ion (PO_4^-) in the soil samples were determined by multiplying the absorbent by 8.25 and 50. The PO_4^- values obtained were recorded in mg/kg.

2.9 Determination of Soil Total Organic Carbon [14]

The principal 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. The used K_2 Cr_2O_7, the difference between and residual K_2 Cr_2O_7 gives a measure of organic carbon content of the soil.

0.1 g of soil samples collected before and after the experiment was weighed into a 150 ml conical flask. 5 ml of K_2 Cr_2O_7 solution and 7.5 ml 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. The end point was a colour change from thick blue to green. The volume of ferrous ammonium sulphate used was recorded as titre value. The blanks were titrated and titre value also recorded.

TOC (%) = {(The blank titre value – sample titre value) x 0.195} / Weight of sample

2.10 Determination of Soil Total Nitrogen (Macro-Kjeldahl Method)

Ten grams (10 g) of dry soil sample was weighed into a macro-kjeldahl flask containg 20 ml of distilled water. The flask was stirred for a few minutes and allowed to stand for 30 minutes. One tablet of mercury tablet, 10 g of K_2SO_4 and 30 ml 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 100 ml of water was slowly added to the flask. The digest was carefully transferred into another clean macro-kjeldahl flask (750 ml). Distillation commenced when the flask was attached to the distillation apparatus and about 150ml of 10N NaOH was poured into the distillation flask opening the funnel stopcock. One hundred and fifty mililitres (150 ml) 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 25 ml burrette graduated at 0.1 ml intervals. The colour change at the end point is from green to pink. The percentage (%) Nitrogen content in soil was then calculated.

2.11 Determination of Soil Extractable Sulfate

Five grams (5 g) of soil sample (air dried, passed 2 mm sieve) was weighed into a centrifuge tube and 25 ml of KH_2PO_4 solution was added. The tube was shaked on a mechanical shaker for 30 minutes whatman No. 42 filter paper was used to filter the suspension. The SO_4-S content in the solution was determined by the turbidity method.

2.12 Statistical Analysis of Data

Experimental data collected were analyzed for statistical differences by means of one-way ANOVA and post hoc LSD, on SPSS 20 to ascertain the level of significant difference between the control and the inoculated samples. In all, $p<0.05$ was considered significant. Data are presented as mean$\pm$S.D (standard deviation).

3. RESULTS AND DISCUSSION

Tables 1 and 2 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).

Table 1. Physicochemical characteristics 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	pH	Conductivity (µS/cm)
A	Crude oil impacted soil sample (CISS)	6.81 ± 0.52	76.67 ± 1.52[a]
B	CISS + *Pleurotus ostreatus*	6.09 ± 0.00	161.00 ± 2.00[a]
C	CISS + Triton x-100	6.26 ± 0.01	31.67 ± 1.52 [a]
D	CISS + *Pleurotus ostreatus* + Triton x-100	6.54 ± 0.00	233.67 ± 4.04 [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≤ 0.05 when compared with the contaminated soil sample

Table 2. Physicochemical characteristics 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	TOC (%)	Tot.N (%)	PO_4^{3-} (mg/kg)	SO_4^{2-} (mg/kg)	NO^-_3 (mg/kg)
A	Crude oil impacted soil sample (CISS)	1.67 ± 0.01[a]	0.14 ± 0.00[a]	2.15 ± 0.05[a]	15.00 ± 0.00	3.31 ± 0.05[a]
B	CISS + *Pleurotus ostreatus*	3.22 ± 0.05[a]	0.28 ± 0.00[a]	1.27 ± 0.04[a]	0.10 ± 0.00	0.80 ± 0.10[a]
C	CISS + Triton x-100	3.75 ± 0.08[a]	0.32 ± 0.00[a]	0.22 ± 0.01[a]	0.10 ± 0.00	0.27 ± 0.58[a]
D	CISS + *Pleurotus ostreatus* + Triton x-100	3.71 ± 0.09[a]	0.32 ± 0.00[a]	1.01 ± 0.03[a]	0.10 ± 0.00	1.03 ± 0.57[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≤ 0.05 when compared with the contaminated soil sample

The pH value of polluted soil sample (Cell A) was observed to be increased (6.81± 0.52) when compared with other cells that were amended.

The result of the soil electrical conductivity shows a significant difference ($p \leq 0.05$) when cell A is compared with other cells. Only cell C amended with Triton x-100 had a lower value of 31.67 ± 1.52 when compared with cell A (polluted soil sample) (76.67 ± 1.52). Cells B and D had higher values of 161.00 ± 2.00 and 233.67 ± 4.04 respectively when compared with cell A (76.67 ± 1.52).

The pH values obtained above in all the amended cells and the untreated cell is in line with the work of [15] and [16]. 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 [17] and [18] 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 [19]. 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.

There was an increase in the soil electrical conductivity in the crude oil impacted soil amended with *Pleurotus ostreatus* singly and in combination with other amendment materials when compared with the control cell suggesting that *Pleurotus ostreatus* in these soils enhanced the soil electrical conductivity. This could also be due to the observed pH values in these soils. Studies have shown that soils polluted with petroleum hydrocarbons are characterized by lower water holding capacity, moisture content and hydraulic conductivity compared with unpolluted soils [20,21,22].

The high organic carbon values recorded in all the cells except for cell C (CISS + *Costus afer*) 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 [23,22,24].

Asuquo et al. [25] in their research also observed significant increase 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, [26].

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 reports of Benka-Coker and Ekundayo [27]. They opined that low levels of nitrogen and phosphorous was observed from a crude oil spill site in the Niger delta region of Nigeria.

4. CONCLUSION

Crude oil pollution tends to persist in soils until remediation measures, involving the application of nutrients are resorted to because oxygen and nitrogen are limiting factors. The application of Pleurotus ostreatus singly or in combination with triton x-100 improved the selected Chemical properties (pH, TOC, total nitrogen) while sulphur, phosphorus and nitric oxide contents were significantly decreased. The results from this research strongly recommended the use of Pleurotus ostreatus to improve the physico-chemical parameters of crude oil polluted soil.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/19779