MICROBIAL RESPONSE TO VARYING CONCENTRATIONS OF
CRUDE OIL POLLUTION OF AGRICULTURAL SOILS IN
ONDO STATE, NIGERIA

ABSTRACT

This research investigated the effects of varying concentrations of crude oil on the population
of crude oil degrading microorganisms in crude oil polluted agricultural soils from Igodan-
Lisa, Oba-Ile and Ido-Ani areas of Ondo State, Nigeria. The soil samples were exposed to 1-
4% (w/w) crude oil and analyzed monthly for six periods using standard microbiological
techniques for the cultivation and enumeration of crude oil degrading bacteria and fungi.
Results indicated that the crude oil degrading microbial populations were significantly altered.
The population of crude oil degrading microbes were higher (1.03 x 10^5 - 1.10 x 10^6 cfu/g for
bacteria and 1.07 x 10^4 – 8.67 x 10^5 sfu/g for fungi) in polluted than unpolluted (1.53 x 10^4 –
9.40 x 10^3 cfu/g for bacteria and 1.17 x 10^3 – 5.17 x 10^5 sfu/g for fungi) soils and also varied
with increase in the amount of crude oil spilled and time. The mean count indicated that the
microbiological status of the soil samples were not negatively impacted at 1-4% crude oil
contamination and the effect on soil microflora is a function of both concentration and contact
time.

KEYWORDS: Varying concentrations, Crude oil, Agricultural soils, Microbial population,
Microflora, Contact time.
INTRODUCTION

Crude oil contamination of the environment in the form of pollution and the consequent ecological and environmental impact is a global threat to environmental sustainability and hence a concern to environmental researchers. Pollution of the environment by petroleum occurs when petroleum or its derivatives are introduced into the environment at levels harmful either directly to the environment or indirectly to the dependents of the environment.

The sources of crude oil spill into the environment differ and the amount spilled vary from minor to disaster. The Niger- Delta Region of Nigeria frequently experience crude oil spill resulting from diverse human activities of exploration, exploitation, distribution and indiscriminate use and discharge of crude oil and its derivatives, sabotage, negligence during production operations and fuel tanker loading processes, pipeline vandalisation, corrosion and leakage of pipe lines and oil tanker terrestrial accidents. Crude oil is toxic in nature and no matter its quantity and size (minor, medium, major or disaster) may cause minor or severe damages to the environment and all forms biomass(including indigenous micro flora and micro fauna) dependent on the environment (Agamuthu and Dadrasina, 2013; Odeyemi, 2014, Shurry et al., 2013). Pollution of the soil environment with crude oil results in the devastation of arable agricultural lands in terms of its productive capacity as crude oil can sterilize the soil for a varying period of time (Atlas and Bartha, 1992; Onifade et al., 2007; Ijah et al., 2008; Chorom et al., 2010, Odeyemi, 2014), creating nutritional imbalances (especially of carbon-nitrogen ratio) as well as imbalance in the biological ecosystem and may impair or impede important biochemical processes such as organic matter formation, ammonification, nitrification, symbiotic and non- symbiotic nitrogen fixation, denitrification and the geochemical circling of elements to render such soils agriculturally unproductive (Odeyemi, 2014). The enormous
damages to the environment by spilled oil is due to the presence of many toxic compounds such
as polycyclic aromatic hydrocarbons, benzene and its substituted and cycloalkane rings in
relatively high concentration (Agarry et al., 2012). The overall implication of crude oil on
agricultural land and the socio-economic lives of people residing in the affected areas is
increased unemployment, poverty and hunger among the people who depend predominantly on
sales from their farms for food and economy.

Several physicochemical and biological strategies have been applied to remediate
polluted soil and water environments. The effects of crude oil spill and time for reclamation of
crude oil polluted soil depends on the quantity and the concentrations of the pollutant (Ikpeme
et al., 2007; Onuoha et al., 2014). Among the remediation techniques, bioremediation which
relies on the use of microorganisms with diverse metabolic capabilities is an evolving and
effective method that holds promise for the degradation and removal of many environmental
pollutants. Microbial biodegradation can be carried out by autochthonous and allochthonous
population. Microbial degradation of hydrocarbons by natural populations of microorganisms is
the major and ultimate natural mechanism by which the petroleum hydrocarbon pollutants can
be cleaned up from the environment (Farag and Soliman, 2011; Jain et al., 2011; Odeyemi;
2014). Ikuesan (2015) reported that in a six months laboratory study, native microbial
population was responsible for 52.33-58.74% crude oil removal in soil samples contaminated
with 5% (w/w). This method of biological mineralization of petroleum hydrocarbon involving
primarily bacteria and fungi is ecosystem friendly, cost effective and simple relative to the
physico-chemical methods (Marinescu et al., 2011; Jain et al., 2011; Onuoha et al., 2014,
Odeyemi, 2014). The microorganisms involved in bioremediation of crude oil polluted soil use
petroleum hydrocarbon as carbon and energy source. However, this process is complex because
it depends not only on the biodegrading capabilities of native microbial populations but also on
other parameters such as environmental factors, soil type, nutrients, chemical composition, concentration and physical state of the pollutant the nature and amount of hydrocarbon present (Diaz-Ramirez et al., 2008; Das and Chandran, 2011, Odeyemi, 2014).

In order to optimize agricultural yield and improve the livelihood of the people in the oil producing region where agricultural lands have been devastated due to crude oil pollution, it is expedient to return polluted soils to their pre-contamination status to support agricultural activities which is the main occupation of the people. Ikuesan(2017) evaluated the crude oil biodegradation potentials of some indigenous soil microorganisms but the study does not focus on the effect of crude oil concentrations on the microbial population associated with crude oil degradation. The use of bioremediation strategy to return crude oil contaminated environment to its pristine state requires a thorough understanding of the effects of crude oil concentrations on the native microbial population with ability to metabolize petroleum hydrocarbon in order to optimize crude oil mineralization. Therefore, the overall objective of this research is to evaluate the effects of varying concentrations of crude oil on the population of crude oil degrading microorganisms associated with agricultural soils in order to develop appropriate bioremediation approach of reclaiming polluted agricultural soil.

MATERIALS AND METHODS

Sample Collection

The samples used in this study were arable agricultural soils collected from Igodan-Lisa, Oba- Ile and Ido-ANI all in Ondo State, Nigeria. The samples were collected using the
hand auger at depth of 15-20 cm into sterile black cellophane bags and transported to the laboratory for analysis within 48 hours (Onifade et al., 2007)

MICROBIOLOGICAL ANALYSIS OF SOIL SAMPLES

(i). Enumeration of microbial population: All media, distilled water and diluents were sterilized by autoclaving at 121°C for 15 minutes at 1.1 kg/cm² pressure. Glassware were sterilized in a hot air-oven at 160°C for two hours. One gramme of each soil sample was serially diluted to $10^{10}$ using nutrient broth as diluent. Each dilution (1 ml) was cultured using pour plate method on Bushnell-Hass (MSM) broth incorporated with 1.5% agar (for bacteria), 1.2% agar (for fungi). The media were also fortified with fungisol (10 mg/lt) for bacteria and 50 mg/lt of streptomycin for fungi after sterilization to determine the loads of Total Crude oil Degrading Bacteria and Fungi. Crude oil (2%) sterilized using 0.45 µm millipore filter served as carbon source. The pH of the medium was adjusted to 7.2 and 5.6 respectively for bacteria and fungi estimation. The MS-oil medium for crude oil degrading bacteria and fungi were then incubated at 28 ± 2°C respectively for 14 and 21 days. The colonies which developed on the plates were counts in which the number of colonies were less than 300 (Odokuma and Dickson, 2003) and its triplicate for each sample was selected. The averaged count was then multiplied by the dilution factor at that dilution and expressed as colony forming unit (cfu/g) or spore forming unit (sfu/g) per gramme of sample bacteria and fungi respectively.

Soil Treatments: For the preparation of crude oil contaminated soils, the method of Ekpo and Ebeagwu, 2009; Njoku et al., 2009 was adopted. Contamination with crude oil was done by thoroughly mixing crude oil with the soils in their respective plastic container to obtain 0-4% (w/w) crude oil contamination. The untreated samples (0% w/w) were the controls.
Effects of Crude oil Contamination on the Population of Crude Oil Degrading Microorganisms of Soil:

The agricultural soil samples treated (0-4%) as described above were used to study the effects of varying concentrations of crude oil on the population of crude oil degrading bacteria and fungi. Samples were incubated at 28°C ± 2°C for 7 days to allow for acclimatization between oil and soil and then enumerated for crude oil degrading bacteria and fungi as day zero. Changes in microbial population of crude oil degrading microbes were monitored monthly for six periods using standard microbiological techniques for the enumeration of crude oil degrading microorganisms. The counts of bacteria and fungi were thus expressed as colony forming unit (cfu/g) and spore forming unit per gram sfu/g respectively.

Statistical Analysis

Data obtained on microbial counts were analyzed by one way Analysis of Variance (ANOVA) using SPSS version 18.0 (2010).

Note: Values are means of triplicate determinations

RESULTS

Effects of varying concentration of crude oil on the population of crude oil degrading microorganisms

The results of this study revealed the effects of varying concentration of crude oil on the population of crude oil degrading microbes of soil samples. Treatment of soil samples with crude oil at 1 - 4% (w/w) contamination level showed that the population of crude oil degrading microorganisms of the agricultural soils were significantly altered.
The population of crude oil degrading microbes were higher \((1.03 \times 10^5 - 1.10 \times 10^6\) cfu/g for bacteria and \(1.07 \times 10^4 - 8.67 \times 10^5\) sfu/g for fungi) in polluted than unpolluted \((1.53 \times 10^4 - 9.40 \times 10^5\) cfu/g for bacteria and \(1.17 \times 10^3 - 5.17 \times 10^5\) sfu/g for fungi) soils. Tables 1 (a-c) show the trend of the effects of varying concentration of crude oil on the bacterial population within the sampling period of 150 days. The bacterial mean counts ranged \(1.80 \times 10^4\) cfu/g – \(3.45 \times 10^5\) cfu/g, \(3.00 \times 10^4\) cfu/g – \(9.40 \times 10^5\) cfu/g and \(1.5 \times 10^4\) cfu/g – \(3.40 \times 10^5\) cfu/g respectively for Igodan-Lisa, Oba-Ile and Idoani control samples. The counts of bacteria at 1% (w/w), 2% (w/w), 3% (w/w) and 4% (w/w) crude oil treated soils were \(1.30 \times 10^5\) cfu/g – \(1.1 \times 10^6\) cfu/g, \(1.40 \times 10^5\) cfu/g – \(9.77 \times 10^5\) cfu/g, \(1.03 \times 10^5\) cfu/g – \(8.70 \times 10^5\) cfu/g and \(1.47 \times 10^5\) cfu/g – \(8.37 \times 10^5\) cfu/g respectively. The fungal counts ranged between \(1.67 \times 10^4\) sfu/g – \(8.67 \times 10^5\) sfu/g, \(1.83\) sfu/g – \(6.60 \times 10^5\) sfu/g, \(1.20 \times 10^4\) sfu/g – \(4.20 \times 10^5\) sfu/g and \(1.07 \times 10^4\) sfu/g – \(4.50 \times 10^5\) sfu/g for soils exposed respectively to 1% (w/w), 2% (w/w), 3% (w/w) and 4% (w/w) of crude oil (Tables 2a - c). The highest counts of bacteria and fungi were observed at 1 - 3% (w/w) of crude oil pollution. The effect of contact time on microbial population was also significant. The highest population count was at days 60 - 90 for bacteria and 90 - 120 for fungi.
Table 1(a) Effects of varying concentrations of crude oil on the bacterial counts ($x10^5$ cfu/g) of Igodan-Lisa soil sample

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>SS$_3$</th>
<th>SS$_3$A</th>
<th>SS$_3$B</th>
<th>SS$_3$C</th>
<th>SS$_3$D</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.18±0.00$^a$</td>
<td>1.53 ± 0.15$^a$</td>
<td>2.26 ± 0.88$^b$</td>
<td>4.57± .0.29$^e$</td>
<td>1.50± 0.15$^a$</td>
</tr>
<tr>
<td>30</td>
<td>3.07±0.12$^d$</td>
<td>1.30 ±0.20$^a$</td>
<td>1.60 ± 0.17$^a$</td>
<td>4.00± 0.12$^d$</td>
<td>1.93 ± 0.20$^b$</td>
</tr>
<tr>
<td>60</td>
<td>3.10 ±0.21$^d$</td>
<td>2.47 ±0.35$^b$</td>
<td>2.87 ± 0.14$^c$</td>
<td>2.30± 0.12$^b$</td>
<td>3.17± 0.12$^d$</td>
</tr>
<tr>
<td>90</td>
<td>2.53± 0.09$^c$</td>
<td>2.80 ±0.20$^c$</td>
<td>3.27± 0.15$^d$</td>
<td>2.57± 0.07$^c$</td>
<td>2.50± 0.12$^c$</td>
</tr>
<tr>
<td>120</td>
<td>1.67 ±0.15$^b$</td>
<td>2.30 ±0.20$^b$</td>
<td>3.03± 0.03$^c$</td>
<td>1.93± 0.07$^b$</td>
<td>1.63± 0.15$^a$</td>
</tr>
<tr>
<td>150</td>
<td>3.45±0.15$^d$</td>
<td>2.37 ±0.15$^b$</td>
<td>1.60± 0.06$^a$</td>
<td>1.03± 0.08$^a$</td>
<td>2.27± 0.37$^{ab}$</td>
</tr>
</tbody>
</table>

Legend: SS$_3$; Igodan- Lisa soil without 0%(w/w) crude oil contamination (Control)

SS$_3$A; Igodan- Lisa soil with 1% (w/w) crude oil contamination

SS$_3$B; Igodan- Lisa soil with 2% (w/w) crude oil contamination

SS$_3$C; Igodan- Lisa soil with 3% (w/w) crude oil contamination

SS$_3$D; Igodan- Lisa soil with 4% (w/w) crude oil contamination
Table 1(b) Effects of varying concentrations of crude oil on the bacterial counts (x10^5 cfu/g of Oba-Ile soil sample)

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>SS4</th>
<th>SS4A</th>
<th>SS4B</th>
<th>SS4C</th>
<th>SS4D</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.30± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.17 ± 0.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.50± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.63 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.50± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>30</td>
<td>2.83± 0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.80 ± 0.28&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.00± 0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.13 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.47± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>60</td>
<td>1.26± 0.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.00± 1.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.77± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.70± 0.12&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.07± 0.09&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>90</td>
<td>9.40± 0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.27± 0.15&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.53± 0.09&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.10 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.70± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>120</td>
<td>6.60± 1.53&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.33± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.20 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.47 ± 0.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.20± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>150</td>
<td>1.38 ± 0.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.70± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.40 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.00± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.60± 0.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Legend: SS4; Oba-Ile soil without crude oil contamination (Control)

SS4A; Oba-Ile soil with 1% (w/w) crude oil contamination

SS4B; Oba-Ile soil with 2% (w/w) crude oil contamination

SS4C; Oba-Ile soil with 3% (w/w) crude oil contamination

SS4D; Oba-Ile soil with 4% (w/w) crude oil contamination
Table 1(c) Effects of varying concentrations of crude oil on the bacterial counts ($10^5$ cfu/g) of Ido-Ani soil sample

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>SS$_S$</th>
<th>SS$_{SA}$</th>
<th>SS$_{SB}$</th>
<th>SS$_{SC}$</th>
<th>SS$_{SD}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.15± 0.01$^a$</td>
<td>9.40± 1.15$^f$</td>
<td>4.40± 1.53$^c$</td>
<td>5.70 ± 0.12$^d$</td>
<td>8.37± 0.09$^c$</td>
</tr>
<tr>
<td>30</td>
<td>2.00± 1.73$^b$</td>
<td>2.83 ± 0.12$^b$</td>
<td>3.47± 1.45$^b$</td>
<td>3.10 ± 0.00$^a$</td>
<td>5.83± 0.60$^b$</td>
</tr>
<tr>
<td>60</td>
<td>2.40± 1.15$^b$</td>
<td>6.43 ± 1.20$^c$</td>
<td>4.27± 1.45$^c$</td>
<td>4.47 ± 0.12$^c$</td>
<td>4.07± 0.07$^a$</td>
</tr>
<tr>
<td>90</td>
<td>2.63± 1.45$^c$</td>
<td>7.07± 0.67$^d$</td>
<td>4.23± 1.45$^c$</td>
<td>3.90 ± 0.12$^b$</td>
<td>3.80± 0.12$^a$</td>
</tr>
<tr>
<td>120</td>
<td>3.13± 1.45$^c$</td>
<td>8.20 ± 0.12$^c$</td>
<td>1.52 ± 0.64$^a$</td>
<td>2.97 ± 0.15$^a$</td>
<td>4.27 ± 0.15$^a$</td>
</tr>
<tr>
<td>150</td>
<td>3.40 ± 0.12$^c$</td>
<td>1.77± 0.07$^a$</td>
<td>6.53 ± 0.12$^d$</td>
<td>2.87± 0.15$^a$</td>
<td>4.13 ± 0.09$^a$</td>
</tr>
</tbody>
</table>

Legend: SS$_S$; Ido- Ani soil without crude oil contamination (Control)

SS$_{SA}$; Ido- Ani soil with 1% (w/w) crude oil contamination

SS$_{SB}$; Ido- Ani soil with 2% (w/w) crude oil contamination

SS$_{SC}$; Ido- Ani soil with 3% (w/w) crude oil contamination

SS$_{SD}$; Ido- Ani soil with 4% (w/w) crude oil contamination
Table 2(a) Effects of varying concentrations of crude oil on the fungal counts (x10^4 sfu/g) of Igodan-Lisa soil sample

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>SS₃</th>
<th>SS₃A</th>
<th>SS₃B</th>
<th>SS₃C</th>
<th>SS₃D</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.33 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.33 ± 0.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.77 ± 0.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.33 ± 0.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.07 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>30</td>
<td>3.50± 0.29&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.67 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.83 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.33 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.43 ± 0.30&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>60</td>
<td>4.33 ± 0.33&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.33± 0.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.50 ± 0.29&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.83 ± 0.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.33 ± 0.35&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>90</td>
<td>3.67± 0.67&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.50± 0.29&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.53 ± 0.29&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.20 ± 0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.27 ± 0.15&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>120</td>
<td>1.50± 0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.50± 0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.53 ± 0.29&lt;sup&gt;f&lt;/sup&gt;</td>
<td>7.87 ± 0.13&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.60± 0.30&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>150</td>
<td>3.33± 0.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.33 ± 0.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.13± 0.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.43± 0.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.33 ± 0.67&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Legend: SS₃, Igodan-Lisa soil without crude oil contamination (Control)

 SS₃A, Igodan-Lisa soil with 1% (w/w) crude oil contamination

 SS₃B, Igodan-Lisa soil with 2% (w/w) crude oil contamination

 SS₃C, Igodan-Lisa soil with 3% (w/w) crude oil contamination

 SS₃D, Igodan-Lisa soil with 4% (w/w) crude oil contamination
Table 2(b) Effects of varying concentrations of crude oil on the fungal counts \( \times 10^4 \) fu/g of Oba-Ile soil sample

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>SS(_4)</th>
<th>SS(_{4A})</th>
<th>SS(_{4B})</th>
<th>SS(_{4C})</th>
<th>SS(_{4D})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.12 ± 0.02(^a)</td>
<td>30.00 ± 0.12(^b)</td>
<td>17.67 ± 0.15(^c)</td>
<td>1.20 ± 0.12(^a)</td>
<td>6.00 ± 0.59(^a)</td>
</tr>
<tr>
<td>30</td>
<td>5.33 ± 0.33(^e)</td>
<td>12.67 ± 0.15(^a)</td>
<td>10.67 ± 0.88(^a)</td>
<td>12.33 ± 0.17(^c)</td>
<td>10.67 ± 0.67(^b)</td>
</tr>
<tr>
<td>60</td>
<td>2.53 ± 0.12(^c)</td>
<td>46.67 ± 0.15(^d)</td>
<td>29.67 ± 1.45(^d)</td>
<td>15.00 ± 0.20(^d)</td>
<td>15.67 ± 0.18(^c)</td>
</tr>
<tr>
<td>90</td>
<td>3.67 ± 0.33(^d)</td>
<td>80.67 ± 0.07(^e)</td>
<td>65.00 ± 0.28(^e)</td>
<td>35.33 ± 0.29(^e)</td>
<td>6.60 ± 0.64(^a)</td>
</tr>
<tr>
<td>120</td>
<td>4.10 ± 0.10(^d)</td>
<td>86.67 ± 0.33(^f)</td>
<td>13.00 ± 2.31(^b)</td>
<td>2.10 ± 0.59(^b)</td>
<td>31.33 ± 0.09(^e)</td>
</tr>
<tr>
<td>150</td>
<td>2.33 ± 0.33(^b)</td>
<td>40.00 ± 0.58(^c)</td>
<td>66.00 ± 0.31(^f)</td>
<td>1.90 ± 0.06(^b)</td>
<td>18.00 ± 1.25(^d)</td>
</tr>
</tbody>
</table>

Legend: SS\(_4\); Oba-Ile soil without crude oil contamination (Control)
SS\(_{4A}\); Oba-Ile soil with 1% (w/w) crude oil contamination
SS\(_{4B}\); Oba-Ile soil with 2% (w/w) crude oil contamination
SS\(_{4C}\); Oba-Ile soil with 3% (w/w) crude oil contamination
SS\(_{4D}\); Oba-Ile soil with 4% (w/w) crude oil contamination
Table 2(c) Effects of varying concentration of crude oil on the fungal counts (x10^4 sfu/g) of Ido-Ani soil sample

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>SS5</th>
<th>SS5A</th>
<th>SS5B</th>
<th>SS5C</th>
<th>SS5D</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>32.33 ± 1.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.67 ± 1.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>35.67 ± 4.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.50 ± 0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.00 ± 2.89&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>30</td>
<td>13.00 ± 1.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.33 ± 1.20&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.67 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.00 ± 2.89&lt;sup&gt;d&lt;/sup&gt;</td>
<td>36.00 ± 6.11&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>60</td>
<td>11.67 ± 1.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.50 ± 0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.33 ± 0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.00 ± 2.89&lt;sup&gt;c&lt;/sup&gt;</td>
<td>45.33 ± 1.76&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>90</td>
<td>51.67 ± 1.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.27 ± 0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.67 ± 1.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.33 ± 1.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>27.00 ± 1.76&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>120</td>
<td>13.67 ± 1.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.50 ± 0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.00 ± 1.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.83 ± 0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.33 ± 4.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>150</td>
<td>11.67 ± 0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.67 ± 0.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>60.03 ± 1.76&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.00 ± 0.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.00 ± 1.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Legend: SS5; Ido-Ani soil without crude oil contamination (Control)

SS5A; Ido-Ani soil with 1% (w/w) crude oil contamination

SS5B; Ido-Ani soil with 2% (w/w) crude oil contamination

SS5C; Ido-Ani soil with 3% (w/w) crude oil contamination

SS5D; Ido-Ani soil with 4% (w/w) crude oil contamination
One of the considerations critical for returning environmentally polluted soils to its pristine state is a thorough knowledge of the impact of oil pollution on the technological parameters for its elimination. In this study, the effect of varying concentrations of crude oil on the population of crude oil degrading microbes of soil was evaluated. Crude oil contamination of the three arable experimental agricultural soil samples of Igodan-Lisa, Oba- Ile and Ido- Ani at 1-4% (w/w) caused alterations in the bacterial and fungal counts of the soils. This finding is in line with those of Ijah and Antai; (2003 ) that oil spills cause alterations in microbiological properties of the soil.

The findings in this research revealed that the ecosystem from which samples were collected for this study harbour considerable numbers of crude oil degrading bacteria and fungi. The counts of petroleum hydrocarbon degrading microbes in samples is suggestive of previous exposure to crude oil or other forms of hydrocarbon as a result of various anthropogenic activities which undoubtedly boosts the supply of carbon in the soils hence favours the growth and multiplication of these microbes. This assertion corroborates the report of Atlas(1981) that the microbial populations of hydrocarbon degrading microorganisms in an ecosystem quantitatively reflect the degree or extent of exposure of that ecosystem to hydrocarbon contamination.

Result showed that there was a gradual increase in the population of both bacteria and fungi showing response to moderate levels of crude oil addition. The populations of crude oil degrading microbes were higher in polluted than unpolluted soils. Microbial population varied with increase in the amount of crude oil spilled and contact time. The changes in microbial
counts may be the presence of crude oil which boosts the carbon supply in the soils, hence favour the growth of these organisms or due to changes in the physico-chemical properties of the soils especially the provision of carbon and nitrogen. The increased microbial counts suggestively represented an immediate response to the added organic carbon present in petroleum hydrocarbon which acted as additional carbon substrate for microbial growth, activity and multiplication. This finding supports the reports of Linkins et al., (1978); Ekpo and Ebeagwu (2009) that microbial population show rapid increase in response to moderate level of oil with a more delayed increase to high level of oil. Prolonged contact with crude oil resulted in an unstable rise and fall in microbial population. This implies that prolonged contact of microorganisms with petroleum hydrocarbon may have deleterious effects on microbial population at any given concentration. This unstable trend of microbial population may be attributed to toxic components of petroleum or other metabolic products which selectively inhibit microorganisms resulting in a shift in population size and species diversity within the microbial community. Odu (1981) also noted that this unstable trend in microbial population may also arise from selective destruction of aerobic microorganisms leaving the resistant and adaptive microbial strains to multiply. It has been reported that population levels of hydrocarbon utilizers and their population within the microbial community appear to be a sensitive index of environmental exposure to hydrocarbon (Rahman et al., 2002). The findings from this study therefore revealed that crude oil contamination of soils does not negatively impact on soil micro flora at moderate levels of contamination (1-4%) as microbial counts at the end of the study period were even at 4% higher than the control. This finding however deviates from the assertion of Osuji et al., (2005) that beyond 3% concentration, oil has
increasing deleterious effects on soil biota. Also, the highest counts of bacteria and fungi were obtained at days 60-90 and 90-120 of contact for bacteria and fungi respectively.

CONCLUSION AND RECOMMENDATION

This study concludes that the microbiological status of the soil samples were not negatively impacted at 1-4% crude oil contamination. The population of both bacteria and fungi were significantly increased at the end of experiment at 1-4% w/w crude oil addition. Also, this study asserts that the effect of crude oil on soil micro biota is a function of both concentration and contact time. The occurrence of these bacteria and fungi with capacity to actively metabolize crude oil is indicative of potential use of indigenous microorganisms in the cleanup of hydrocarbon polluted sites.

Apart from the type and population of indigenous microorganisms in soil, other technological parameters critical for the application of bioremediation strategy in the elimination of hydrocarbon pollutant in soil are environmental factors, soil type, nutrients, chemical composition, concentration and physical state of the pollutant. Therefore, future study should investigate the effect of crude oil concentrations on soil physicochemical properties such as those that directly affect the growth, survival and multiplication of microorganisms in order to optimize their efficiency in bioremediation.
REFERENCES


