Bioremediation of Crude Oil Polluted Soil: Using Poultry Droppings

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Abstract: The use of poultry manure for bioremediation was evaluated ex situ during a period of 6 weeks remediation at the same crude oil contamination levels but at different time intervals. This research was experimentally designed in five replicates including the control sample with each representing various time intervals of 1, 2, 4 and 6 weeks. 30g of poultry droppings were added to the contaminated soil except the control. The soil was in the same weight of 150g and spiked with 10% (w/w) bonny light crude oil. The soil was tilled and watered daily for aeration of the micro organisms capable of degrading hydrocarbons. The soil and the amendment agent (poultry droppings) were characterized so as to know their physicochemical properties before pollution and remediation. At the first, second, fourth and sixth week of remediation, various sample representatives were sampled and analyzed for total petroleum hydrocarbon (TPH) and polycyclic aromatic hydrocarbons (PAHs) to know the level of remediation. From the results obtained, it really certified that remediation has occurred with poultry droppings being very effective because of high reduction of total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAHs) values from 25,450mg/kg to 7820mg/kg (69.3%) and 52.78mg/kg to 7.67mg/kg (85.5%) respectively at the sixth week of remediation. The result of this research work suggest that the application of poultry droppings will be environmentally friendly since it helps microbial utilization of hydrocarbon content of the soil and degrade it to less toxic condition.

Keywords: Crude Oil, Poultry droppings, Amendment, Bioremediation, Polycyclic Aromatic Hydrocarbon, Total Petroleum Hydrocarbon.

1. INTRODUCTION

Crude petroleum oil spill has been the most prevalent problem in the environment. The release of crude petroleum into the environment due to oil spill has recently drawn worldwide attention (Korie-Sinkepere et al; 1998). Oil spillage has dislocated many, living within the oil producing area as a result their polluted water, air, damaging of farmlands (Ekpu,2008). In Niger Delta area of Nigeria there has been over 500 reported cases of crude petroleum oil spillage since 1976, releasing about 2.5 million barrels of...
crude into the environment, (Odite, 1999; Korie-siakapere, et al, 1998). On the other hand soil is the key component of natural ecosystem and environmental sustainability (Adenipekun, 2008; Onuha, et al, 2008; Adelokun and Ataga, 2007). Crude oil pollution adversely affects the soil ecosystem through absorption to soil particles, provision of an excess carbon that might be unavailable for microbial use and an induction of a limitation in soil nitrogen or phosphorus (Baker and Herson, 1994; Atlass, 1981). These process which effect drastically soil enzymatic activities result in a very slow biodegradation of crude oil polluted soils (Ljah, et al, 2008; Okolo, et al, 2005). Consequently, various soil amendments have been used in bioremediation to hasten the process for the actualization of sustainable ecosystem.

The effect of crude oil pollution on the properties of soil have been the subject of many studies. Okolo, et al. (2005) reported that oil pollution increases carbon and reduces soil nitrate and phosphorus. Similarly Adedokun and Ataga (2007) reported that soil with crude oil result in damage to the soil micro organisms and plants while Onuha et al, (2003) among others have shown that crude oil pollution prevent oxygen exchange between soil and the atmosphere due to hydrophobic properties of oil. Also an uncontrolled release of crude petroleum hydrocarbons into the soil and ground water are frequent as a result of leakages from an underground storage tanks and piping containing diesel, heating oil, fuel and gasoline this leakages is in droplets but in large quantity causes acute and long time damages to agricultural soil and other works done on the soil which have greatly affected our environments and societies as polluted soils are great threat to both plants, and animals, human and micro-organism. Crude petroleum oil is physically, chemically and biologically harmful to soil due to high concentration of many toxic component e.g. polycyclic aromatic hydrocarbons, benzene and its substitute cycloalkane rings (Franco et al, 2004). The presence of higher molecular weight components with very low solubility in water prevents natural biodegradation process from working efficiently in hydrocarbon contaminated soils. These components also penetrate macro and micro pores in soil and thus limit water and air transport that would be necessary for organic matter conversion (Cavavaca and Roida, 2003). Soil remediation is a collective term for various methods that are used to purify and revitalize the soil. This method of clean up is a part of a broader effort to purify the soil and otherwise repair damage done to the ecosystem. Examples of countries that practice soil remediation are Canada, United state and Australia (Online/www.soil remediationtech.com). The conventional technique used for bioremediation has been to dig up contaminated soil and move it to a land fill, but this method simply moves the contaminated soil elsewhere and may cause significant risk in the excavation, handling and transportation of hazardous materials. Additionally, it is very difficult and expensive to find new land fill site for final disposal of the excavated material (Ayotamuno et al, 2013). A better approach than this traditional method is to completely destroy the pollutant if possible or at least to transform them to harmless substances. Some technologic that have been used are high temperature incineration and various types of chemical decompositions (e.g. base catalyzed dechlorination, oxidation). They can be very effective at reducing several drawbacks, principally, their technology, complexity, the cost for both workers at site and nearby residents (Okoh, 2003). The pollution of the environment draw the attention of pollution control regulation in the oil and gas industries governed by the principal legislation of petroleum Act.1967. Regulatory bodies such as federal ministry of environment (FMENV) and Department of petroleum Resources (DPR) here in Nigeria required that operators should treat and control the discharge of these effluents to ensure that the environment are friendly to man and other living things here on earth (Ayotamuno et al, 2006).

The bioremediation of crude oil polluted soil using poultry manure as a nutrient source offer an alternative measure over the other conventional methods of bioremediation technologies, because it does not involve the use of inorganic chemicals which may have adverse effect on plant and it destroys the contaminants rather than transferring them elsewhere. It will not only be effective in bioremediation of the polluted site, but would be less expensive and as well environmentally friendly. Therefore the aim of this work is to determine the effectiveness of remediating crude petroleum oil polluted soil with poultry droppings to ascertain the level of bioremediation and make recommendations based on the findings.
2. MATERIALS AND METHODS

2.1 Sample Collection

Soil sample were collected randomly with a shovel from the top surface soil (0-15cm) behind physics laboratory, science block, Abia state university, Uturu, Abia state, Nigeria. The soil sample were air dried for two weeks, homogenized, passed through a 2-mm (pore size) sieve and stored in a polyethylene bag in the laboratory prior for use.

The poultry dropping was obtained from a local poultry farm in Okigwe, Imo state Nigeria, the poultry droppings was air dried ground and sieved to obtain uniform particle size. The crude oil used for this study was Bonny light crude obtained from the core analysis laboratory of the Nigerian National Petroleum Corporation (NNPC), Moscow Road, Port-Harcourt, Nigeria. The bioreactors (plastic containers) were obtained from a local market in Okigwe, Imo state Nigeria, and the amendment agent was stored in a polyethylene bag and kept in the laboratory prior to use.

2.2 Characterization of Samples And Amendment Agents

The soil sample and amendment agent were characterized for total organic carbon (TOC), total nitrogen (N) total phosphorus (P), moisture content (MC) and pH according to standard methods. The pH was determined by glass electrode pH meter in (1:2.5) soil: water ratio, (Bates,1954), total organic carbon was determined by the modification wet combustion method (Nelson and Sommers,1982). Total nitrogen was determined by the semi-micro Kjeldhal method (Bremner and Mulvaney, 1982). Available phosphorus was determined by Brays No.1 methods (Olsen and Sommers 1982).

2.3 Slurry-Phase Experimental Design

The remediation work was carried out for six weeks between October and December 2014. The soil sample was divided into five (5) treatment reactors as shown below.

A= as the control soil sample (polluted) without amendment agent.

P1 (for 1 week) = polluted soil applied with 30g of poultry manure made up to 50% vol. by distilled water and tilling was done daily.

P2 (for 2 weeks )= polluted soil 30g of poultry manure made up to 50% vol. by distilled water and tilling was done daily.

P3 (for 4 weeks ) = polluted soil applied with 30g of poultry manure made up to 50% vol. by distilled water and tilling was done daily.

P4 (for 6 weeks ) = polluted soil applied with 30g of poultry manure, made up to 50% vol. by distilled water and tilling was done daily.

2.4 Method of Remediation

150g of each of the soil sample was measured out, placed in plastic containers labeled A,P1,P2,P3, and P4 respectively. Sample A was used as the control while sample P1,P2,P3 and P4 represent different time interval of 1, 2, 4 and 6 weeks respectively as indicated above. The soil in each plastic container was spiked with 10% (w/w) bonny light crude oil and thoroughly mixed together to achieve severe contamination because above 3% concentration, oil has been reported to be increasingly deleterious to soil biota and crop growth (Osuji et al, 2005). These sample were allowed for one week for proper infiltration after which 30g of poultry manure was added to P1,P2, P3, and P4 representing each time interval. Each container except A (the control) was made up to 50% volume by distilled water for proper percolation and the contents of each container was tilled daily to ensure maximum aeration, adequate mixing of nutrients, microbes and water that was added. Water was sprinkled on P1, P2, P3 and P4 sporadically whenever the water level gets low. And this were in line of the work of (Odokuma and Dickson, 2005; Ayotanmuno et al ,2006 and Agarry et al, 2013).
2.4 Laboratory Analysis And Procedure

Sample A was analyzed for total petroleum hydrocarbon (TPH), polycyclic aromatic hydrocarbons (PAHs). After each time interval the sample with amendments (P1, P2, P3 and P4) were analyzed for changes in level of polycyclic aromatic hydrocarbons and total petroleum hydrocarbons to define the level of remediation achieved in the research work. Sample A was analyzed one week after pollution was done, P1, P2, P3 and P4 were analyzed 1, 2, 4 and 6 weeks respectively in the course of the remediation. The total petroleum hydrocarbon (TPH) content of the soil sample was determined gravimetrically by solvent extraction method of Adesodun and Mbagwu (2008). The polycyclic aromatic hydrocarbons (PAHs) were determined by gas chromatography/mass spectroscopy (GC/MS).

3. RESULTS

Table 1 and 2 shows the physicochemical characteristics of an unpolluted soil sample collected behind physics laboratory, science block Abia state university Uturu and poultry droppings collected form a local poultry In Okigwe, Imo state Nigeria respectively.

Table 1 physicochemical characteristics of unpolluted soil sample collected behind Physics laboratory

<table>
<thead>
<tr>
<th>PH</th>
<th>EC µc/cm</th>
<th>MC %</th>
<th>TOC %</th>
<th>TN Mg/kg</th>
<th>Potassium Mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.60</td>
<td>8.74</td>
<td>4.33</td>
<td>2.87</td>
<td>0.196</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Table 2: physicochemical characteristics of poultry droppings (amendment agent) collected from a local poultry in Okigwe

<table>
<thead>
<tr>
<th>pH</th>
<th>EC µc/cm</th>
<th>MC %</th>
<th>TOC %</th>
<th>TN Mg/kg</th>
<th>Potassium Mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.55</td>
<td>7000</td>
<td>6.04</td>
<td>5.98</td>
<td>2140</td>
<td>996.87</td>
</tr>
</tbody>
</table>

Table 3: concentrations of polycyclic aromatic hydrocarbons (PAHs) and total petroleum hydrocarbons (TPH) of the control (without amendment agents) and the remediated sample (with amendment agent) within six weeks of remediation

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Time (weeks)</th>
<th>TPH (mg/kg)</th>
<th>PAH (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (control)</td>
<td>0</td>
<td>25450</td>
<td>52.78</td>
</tr>
<tr>
<td>P1</td>
<td>1</td>
<td>16230</td>
<td>19.48</td>
</tr>
<tr>
<td>P2</td>
<td>2</td>
<td>12600</td>
<td>16.50</td>
</tr>
<tr>
<td>P3</td>
<td>4</td>
<td>10650</td>
<td>13.35</td>
</tr>
<tr>
<td>P4</td>
<td>6</td>
<td>7820</td>
<td>7.67</td>
</tr>
</tbody>
</table>
Table 4: changes in the concentrations of PAHs during the 6 weeks of remediation

<table>
<thead>
<tr>
<th>PAHs</th>
<th>0 week</th>
<th>1 week</th>
<th>2 Week</th>
<th>4 Week</th>
<th>6 week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>0.98</td>
<td>0.06</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td>2-methylenaphthalene</td>
<td>1.14</td>
<td>0.38</td>
<td>0.28</td>
<td>0.14</td>
<td>BDL</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>1.05</td>
<td>0.52</td>
<td>0.42</td>
<td>0.37</td>
<td>0.11</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>2.88</td>
<td>0.96</td>
<td>0.91</td>
<td>0.63</td>
<td>0.08</td>
</tr>
<tr>
<td>Fluorene</td>
<td>2.23</td>
<td>0.78</td>
<td>0.75</td>
<td>0.71</td>
<td>0.18</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>2.24</td>
<td>1.12</td>
<td>0.99</td>
<td>0.64</td>
<td>0.39</td>
</tr>
<tr>
<td>Anthracene</td>
<td>4.66</td>
<td>1.14</td>
<td>1.07</td>
<td>0.96</td>
<td>0.60</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>8.77</td>
<td>2.39</td>
<td>1.98</td>
<td>1.48</td>
<td>0.52</td>
</tr>
<tr>
<td>Pyrene</td>
<td>5.22</td>
<td>2.65</td>
<td>2.10</td>
<td>1.75</td>
<td>0.78</td>
</tr>
<tr>
<td>Benzo(a)anthracene</td>
<td>3.77</td>
<td>0.88</td>
<td>0.86</td>
<td>0.77</td>
<td>0.57</td>
</tr>
<tr>
<td>Chrysene</td>
<td>3.01</td>
<td>1.38</td>
<td>1.22</td>
<td>1.08</td>
<td>0.92</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>4.55</td>
<td>2.09</td>
<td>1.66</td>
<td>1.33</td>
<td>0.99</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>2.96</td>
<td>1.34</td>
<td>1.13</td>
<td>1.01</td>
<td>0.69</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>2.56</td>
<td>1.28</td>
<td>1.01</td>
<td>0.73</td>
<td>0.58</td>
</tr>
<tr>
<td>Dibenzo(a,h)anthracene</td>
<td>3.77</td>
<td>1.45</td>
<td>1.22</td>
<td>1.03</td>
<td>0.67</td>
</tr>
<tr>
<td>Benzo (g,h,i) ppylene</td>
<td>2.56</td>
<td>0.92</td>
<td>0.84</td>
<td>0.70</td>
<td>0.58</td>
</tr>
<tr>
<td>Indeno(1,2,3,-cd)pyrene</td>
<td>0.43</td>
<td>0.14</td>
<td>0.06</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Total mg/l</td>
<td>52.78</td>
<td>19.48</td>
<td>16.50</td>
<td>13.35</td>
<td>7.67</td>
</tr>
</tbody>
</table>

3. DISCUSSION

From table 3, it was observed that the control which is the crude oil polluted soil without amendment agent has a TPH concentration of 25450mg/kg. However, after 1 and 2 weeks of bioremediation, there was a reduction in TPH to 16230mg/kg (36.2%) and 12600mg/kg (50.5%) respectively. TPH reduction was significantly higher (<0.05) in treatment categories compared to the control sample. Crude oil comprises of complex hydrocarbons and other organic compounds and organometallic constituents (Ojo, 2006). Improperly discarded crude oil renders impacted environment unfit for purpose, alters soil microbial properties, Oxygen, content and nitrogen availability. In this study the TPH reduction was gradual and faster in the first two weeks of when compared to the final weeks. Anyasi and Atagana(2011) reported that degradation of crude oil was highest in the first two weeks of their experiment, and generally slowed down as time progressed not directly dependent on quantity of micro organisms present. They suggested that slow down of degradation rate might be attributed to the presence of higher reaction intermediates present after initial hydrocarbon degradation. However, the reduction of TPH as obtained in figure 1, below shows that organic nutrient and as well tillage could improve positivity on biodegradation of crude oil polluted soil. The reduction in TPH values from 25450mg/kg to 7820 mg/kg really shows effective utilization of nutrients and support the claim that nitrogenous nutrient supplied, provides a suitable environment for decontamination. This is in line with the findings of lielani (2004); Ayotamuno et al; (2013) and Tibiene et al; (2011). TPH reduced significantly within the period of six weeks as seen in figure 1 (<0.05) by up to 69.3% which is approximately equal to the result of similar researchers (Adelowo et al; 2008; Akinde et al; 2008; Udeani 2009) who achieved about 60-79% of TPH degradation.
The results show that there was a significant difference between the remediated sample and the control sample (table 3 and 4). After one week of remediation, the PAH value reduced drastically from 52.78mg/kg to 19.48mg/kg (63.1%). This is an evidence that poultry manure micro flora can remove PAHs faster (Atanga 2004). Poultry manure has also been reported to enhance the degradation of hydrocarbons in soil composted mixtures (Hils and McCarty 1967; Wilson et al; 1983). The rapid degradation observed during the first week of bioremediation was expected as poultry manure is rich in carbon and mineral nutrients particularly nitrogen (Combs et al, 2001; Schmitt and Rhem 2002). The organisms growing on the nutrients present in the poultry manure were found to readily metabolize the contaminant PAH while still growing on the manure. The manure afforded the organisms the opportunity to grow while adapting to

Figure 1: Changes in the concentration of total petroleum hydrocarbon during the study period
the hydrocarbons, it also afforded the organisms the opportunity to produce enzymes that are required to metabolize hydrocarbons (Sutherland et al; 1995; Bardos et al 1996; Diaz et al ; 1996).

![Figure 2: Changes in the concentrations of polycycle aromatic hydrocarbons (PAHs)](image)

After six weeks of bioremediation the PAH values was observed to have reduced by 85.5% (7.67mg/kg) as expected and this is in agreement with the works of Rolf and Bjom (2003) and Atagana (2004). From table 4, it was observed that 2-membered rings were removed below the remediation target of 1mg/kg by the 2\textsuperscript{nd} and 6\textsuperscript{th} week (naphthrene and 2- methyl naphthene) respectively. The 3,4,5 and 6-membered rings (anthracene, phenanthrene, fluorene, pyrene, chrysene, benzo-(a) pyrene, and indeno(1,2,3,-cd) pyrene became slower to degrade after one week of remediation. fluoranthene, pyrene, chrysene, benzo(b)floranthene, and Dibenzo(a,h) anthracene continued to be above 1mg/kg until the 6 sixth week when drastic reduction was observed. This results were similar to the findings of Atagana (2004); Rolf and Bjom (2003)

4. CONCLUSION
The results of this study demonstrated the potential of poultry manure (an organic waste amendment) to remediate crude oil contaminated soil. From the performance of individual treatment P1, P2, P3, and P4 at 1,2,4 and 6 weeks intervals respectively, higher values of total petroleum hydrocarbons and polycyclic aromatic hydrocarbons (PAHs) degradation of 69.3% and 85.5% respectively was observed compared to the control. The study therefore proves the viability of using poultry manure as an amendment agent in remediating hydrocarbon contaminated soil. This affords an alternative method in removing oil contaminants from soil.

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6. REFERENCES


