Influence of Organic Wastes on Ecotoxicity of Petroleum Hydrocarbons in Contaminated Soil

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INTRODUCTION

Petroleum (crude oil) is a naturally occurring mixture of hydrocarbon and non-hydrocarbon compounds which is toxic to the ecosystem in high concentrations (Anderson and Labelle, 2000). The demand for petroleum is increasing globally, despite researches on green alternative energy. Nigeria is a leading petroleum producing and exporting country in the world. According to the portal of the Organization of the Petroleum Exporting Countries (OPEC), Nigeria produces about 2.4 million barrels per day (bpd), with its crude oil reserve standing at about 37 billion barrels (OPEC, 2020). Petroleum can be refined into various useful components like; diesel, petrol, lubricating oil, jet fuel, paraffin, etc. At times, these refined petroleum products are released into the ecosystems either through accidental discharge or indiscriminate disposal by the consumers. Spillage of petroleum products and their wastes into the environment constitutes a great danger to both plants and animals. This poisonous trend can pass through the food web and may eventually get to human beings who consume the plants and animals (Gibson and Parales, 2000).

Many researches have confirmed that, contamination of the ecosystems with petroleum and its products, had adverse effects on the animate and inanimate objects within the ecosystems. Hinojosa et al. (2004) and Akpokodje et al. (2019) observed that the contamination of the environment with petroleum products significantly altered the heavy metals concentrations and microbial...
activities in the soil. Citing Apokodje et al. (2019), petroleum products drastically increased the Copper, Lead, total hydrocarbon content, and the electrical conductivity by over 100%. Likewise, Udom et al. (2012) observed that excess petroleum hydrocarbons in the soil adversely affected the germination, growth performance and the yield of crops growing on the contaminated soil. Therefore, biodegradation of hydrocarbons in the ecosystem can cause cancer and possible damage to the bone marrow. According to Abioye et al. (2012), high concentrations of petroleum hydrocarbons in the ecosystem can cause inhibition of oil biodegradation due to oxygen limitation or through the toxic effects exerted by volatile hydrocarbons on microorganisms.

Since the harmful effects of petroleum and its products on the ecosystems have been scientifically established, many researchers are trying to proffer solutions to the problem. Gomez-Eyles et al. (2011) reported that biochar amendment of petroleum products contaminated soil, helped to reduce the petroleum hydrocarbons concentration within the soils, over time. Nitrogen rich amendment (biostimulation) has been recorded to improve microbial activity on petroleum contaminated soils and water samples; thereby, degrading (bioremediation) the petroleum hydrocarbons concentration in the soil samples (Riffaldi et al., 2006; Margesin et al., 2007; Walworth et al., 2007). According to Apokodje and Uguru (2019), compost manure significantly degraded the THC (from 957.21 mg/kg to 262.03 mg/kg) in a petroleum products contaminated soil, improving the soil’s physical characteristics in the process. Tanee and Jude (2017) observed that organic waste materials (sawdust, sewage sludge, cow dung, poultry waste, etc.) were able to bioremediate THC in petroleum contaminated soils to significant levels. In addition, Ogbogodo (2004) reported that the use of poultry waste to stimulate crude oil biodegradation in contaminated soil is an environmentally friendly remediation method, combating petroleum hydrocarbons pollution in the ecosystems.

Although there are several researches on bio-remediation, researches on the bioremediation of petroleum hydrocarbons contaminated Nigerian soils are still scanty. Therefore, the objectives of this study are: (i) to determine the potentials of cattle dungs, sawdust, and rice husks in the biodegradation of petroleum hydrocarbons in Nigeria soils, (ii) to evaluate the effect of the petroleum hydrocarbons concentration on the biodegradation efficiency of the organic waste materials.

**MATERIALS AND METHODS**

**Samples collection and preparation**

**Soil samples**

The top soil used for this study was collected from a fallow section of the irrigation and research farm of the Delta State Polytechnic, Ozoro, Nigeria. The soil was air-dried and then sieved with a 2 mm gauge stainless steel sieve.

**Cattle dungs**

The cattle dungs were collected from the local ranch located at Delta State Polytechnic, Ozoro, Nigeria. They were air-dried under ambient temperature (27±4°C) for one week.

**Sawdust**

Sawdust used for this study was obtained from the local timber market, located along Ozoro-Oleh road, Delta State, Nigeria. The sawdust was air-dried under ambient temperature (27±4°C) for one week, to reduce its moisture content.

**Rice Husks**

The rice husks used for this study were collected from a local rice mill in Makurdi, Benue state, Nigeria. Rice husks are removed from the rice grains during the post-harvest processing operations of rice grains. The husk contains significant amount of basic nutrients, mostly Nitrogen, Phosphorus and potassium; therefore, it is used as a soil organic amendment to improve crop growth performance (Sharma et al., 1998). They were air-dried under ambient temperature (27±4°C) for one week, to reduce their moisture content.

**Cucumber (Cucumis sativus, L.) seeds**

The cucumber seeds (cv. Nandini) were collected from the National Centre for Agricultural Mechanization (NCAM), Ilorin, Kwara State, Nigeria. Cucumber was selected because it is very sensitive to the heavy metals and petroleum toxicity. According to Oleszczuk (2010) all the sativus family is highly sensitive to the presence of toxic substances, making them useful in the determination toxicity in soil samples.

**Petroleum products**

The Spent engine oil was collected from a mechanic workshop located at Oleh, Delta State, Nigeria; while the petrol, diesel and kerosene were obtained from a filling station located at Oleh, Delta State, Nigeria.
Methods

Soil sample preparation

Sixty (60) kg of the dried and sieved top soil was poured into three containers and coded A, B, and C. The soil in container A was contaminated with 5% weight/weight (w/w) of the mixed petroleum products; the soil in container B was contaminated with 10% (w/w) of the mixed petroleum products; while the soil in container C was contaminated with 15% (w/w) of the mixed petroleum products. The four petroleum products (spent engine oil, petrol, diesel and kerosene) were mixed together in a volumetric ratio of 1:1:1:1. All the containers with their contents were kept under a shade for one week, to enable acclimatization of the petroleum products with the soil. At the end of the seven day period, the contents of each container was poured separately unto a concrete platform, and mixed thoroughly to obtain a homogeneous mixture, and then left untouched for the next 24 hours.

Remediation set-up

The petroleum products contaminated soil from the three groups (5%, 10% and 15% contamination) was filled individually into plastic containers, in the quantities of 3 kg per container. Then the containers were arranged in nine rows, with four containers per row as designated in Table 1. All the containers in rows 1 to 3 contained no amendment, and were considered as the control. Containers in rows 4 to 6 contained 0.5 kg of cattle dungs and 0.5 kg of sawdust; while containers in rows 7 to 9 contained 0.5 kg of cattle dungs and 0.5 kg of rice husk.

Table 1: Remediation set up

<table>
<thead>
<tr>
<th>Row</th>
<th>% petroleum products</th>
<th>Amendment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Row 1</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Row 2</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Row 3</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>Row 4</td>
<td>5</td>
<td>CD + SD</td>
</tr>
<tr>
<td>Row 5</td>
<td>10</td>
<td>CD + SD</td>
</tr>
<tr>
<td>Row 6</td>
<td>15</td>
<td>CD + SD</td>
</tr>
<tr>
<td>Row 7</td>
<td>5</td>
<td>CD + RH</td>
</tr>
<tr>
<td>Row 8</td>
<td>10</td>
<td>CD + RH</td>
</tr>
<tr>
<td>Row 9</td>
<td>15</td>
<td>CD + RH</td>
</tr>
</tbody>
</table>

CD = Cattle dungs; SD = Sawdust; RH = Rice husks

Biodegradation process

All the containers with their content were carefully arranged under a shade for an experimental period of 56 days. At an interval of 7 days, the content inside each container was tilled properly, and 0.5 L of tap water added to it; to enhance the aeration and moisture content of the soil samples. This was done because soil microbes required adequate air and water to perform effectively.

At an interval of every 14 days, soil samples were randomly sampled from each container; at five different locations and at various depths. The THC concentration of the collected soil samples were determined in accordance with standard methods, at an interval of every 14 days; while at the end of the experimental period (56 days), seed germination toxicity tests of all the soil samples was carried out.

Laboratory analysis

Physicochemical analysis of the soil sample

The physicochemical analysis of the uncontaminated soil sample was carried out in accordance with ASTM and APHA standard recommended procedures. All the soil samples used for the physicochemical analysis were air-dried under ambient laboratory temperature, and sieved with a 2 mm stainless steel sieve.

Soil pH determination

Dried soil sample and distilled water were mixed in the ratio of 1:10 and stirred vigorously for 30 minutes using a glass rod. Then the electrode of an already standardized (calibrated with buffer solution of pH 4.10 and 9.20) pH meter was immersed into the partly settled suspension and the pH value read from the screen of the digital pH meter (Nwakaudu et al., 2012; Akpokodje et al., 2018). The pH meter’s electrode was rinsed with distilled water after each test, before another test was carried out.

Soil nutrients analysis

Ten (10) g of the dried and sieved soil sample was poured into a beaker, and digested with 15 ml of concentrated acids (HNO₃, HCl, and H₂SO₄) mixture (ratio of 5:1:1) at a temperature of 80°C in a water bath, until a clear solution was obtained. The digested sample was allowed to cool at ambient laboratory temperature, and filtered with a Whatman filter paper. The filtrate was poured inside a 100 ml volumetric flask, and diluted with distilled water up to the 100 ml mark. From the diluted digested solution, the Fe, Cu, Pb, and Ca concentrations were determined using an atomic absorption spectrophotometer. The potassium content of the soil was extracted by using 1N ammonium acetate (NH₄OAC) solution and determined by flame emission spectroscopy as outlined by Anderson and Ingram (1993). The total Nitrogen concentration of the soil samples was determined using the kjeldahl method (Enerijiofi and Ekhaise, 2019).

Total hydrocarbon content (THC) determination

The petroleum hydrocarbons concentrations in the uncontaminated and contaminated soils samples were determined, to characterize impacts of the volume of petroleum products (5%, 10%, and 15%) on the THC concentration in each soil group. The THC of all the soil samples collected was tested by using Soxhlet Extraction Method recommended by ASTM D 9071B – 7 (APHA, 1995), as described by Akpomrere and Uguru (2020).

All the laboratory tests were carried out under ambient laboratory temperature (26±5°C) and in triplicates.
Petroleum hydrocarbons ecotoxicity test

Seed germination and radicle growth inhibition test

Germination toxicity test of the control and remediated soils samples was carried out in accordance with Environmental Protection Agency (EPA 712-C-008) standard recommended procedures. This test helps to determine if the residual contaminant in the remediated soils is high enough to adversely affect the crops growing in the soils (U. S. EPA, 2012). Cucumber seeds were used for seed germination toxicity test; they were planted and closely monitored for an experimental period of 10 days.

During the test, 400 g of thoroughly mixed soil samples, from each remediation setup was poured into a sterilized plastic container. Then ten viable cucumber seeds were arranged evenly on top of the soil in each container; after which, they were uniformly covered with 50 g of uncontaminated top soil. All the containers were arranged under a shady environment, and kept moist by the daily addition of 50 ml of tap water. At the end of the toxicity test experimental period (10 days), the number of cucumber seeds that germinated was counted, and the length of the visible radicle per generated cucumber seed was measured with a flexible measuring tape. The mean radicle length for each remediation setup was used for further calculations.

The seed germination rate (SR), seed germination index (GI) and the radicle growth inhibition (GR) of the cucumber seeds growing in the soils samples were calculated by using the formula expressed by Millioli et al. (2009) and kader (2005), as presented in equations 1 to 3.

\[
SR = \frac{\text{number of seeds that germinated on contaminated soil}}{\text{number of seeds that germinated on uncontaminated soil}} \times 100
\]

(1)

\[
GR = \frac{\text{Length of radicle on contaminated soil}}{\text{Length of radicle on uncontaminated soil}} \times 100
\]

(2)

\[
GI = (10\times n1) + (9\times n2) + \cdots + (1\times n10)
\]

(3)

Where:
- SR = Seed germination rate;
- GR = Radicle growth inhibition;
- GI = Germination Index
- n1, n2 . . . n10 = No. of seeds that germinated on the 1st, 2nd, 3rd, until the 10th day
- 10, 9 . . . 1 = weights assigned to the number of seeds germinated on the 1st, 2nd, 3rd, until the 10th day (kader, 2005)

Statistical Analysis

The data obtained from this study were statistically analyzed using the Statistical Package for Social Statistics (SPSS version 20.0), and the Means were separated using The Duncan’s New Multiple Range (DNMR) Test (p ≤0.05). The Mean of the results were plotted in Microsoft Excel 2015, and the correlation relationship was determined by using the MS Excel 2015 (Microsoft Corporation Redmond, WA 98052).

RESULTS AND DISCUSSION

The results of the physicochemical properties and the THC of the uncontaminated soil sample are presented in Table 2. While Table 3 shows the new THC in the soil samples after the contamination by the various volumetric weight (5%, 15% and 15%) of petroleum products. Despite the initial value of THC recorded in the uncontaminated soil sample, the results (THC) revealed that the petroleum products significantly increased the THC of the soils samples.

Table 2: Physicochemical properties of the uncontaminated soil sample

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.32</td>
</tr>
<tr>
<td>Nitrogen (mg/kg)</td>
<td>1.9</td>
</tr>
<tr>
<td>Phosphorus (mg/kg)</td>
<td>24.5</td>
</tr>
<tr>
<td>Potassium (mg/kg)</td>
<td>62.8</td>
</tr>
<tr>
<td>Calcium (mg/kg)</td>
<td>216.1</td>
</tr>
<tr>
<td>Sodium (mg/kg)</td>
<td>350.6</td>
</tr>
<tr>
<td>Iron</td>
<td>1413</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>53.7</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>11.9</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>34.4</td>
</tr>
<tr>
<td>THC (mg/kg)</td>
<td>26.72</td>
</tr>
</tbody>
</table>

Table 3: Contaminated soils samples (THC level)

<table>
<thead>
<tr>
<th>Row</th>
<th>% petroleum products</th>
<th>THC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A</td>
<td>5</td>
<td>1277\textsuperscript{a} (mg/kg)</td>
</tr>
<tr>
<td>Sample B</td>
<td>10</td>
<td>3082\textsuperscript{b} (mg/kg)</td>
</tr>
<tr>
<td>Sample C</td>
<td>15</td>
<td>4933\textsuperscript{c} (mg/kg)</td>
</tr>
</tbody>
</table>

Columns with the same common letter (superscript) are not significantly different (p ≤0.05) according to Duncan’s Multiple Ranges Test

Biodegradation process

The results show that the bioremediation of petroleum hydrocarbons in a contaminated soil differs in relation to the type of organic waste used, and the THC level in the contaminated soil. Figure 1 shows the relationship between the THC biodegradation, the organic wastes remediation efficiency, and the THC levels in the contaminated soil samples. Sawdust and rice husks were
used in this study due to their good THC adsorption properties. According to Abioye et al. (2009) and Sumathi et al. (2015), plant-based adsorbents play an important role during the bioremediation of contaminated soil, by absorbing the harmful chemicals from the soil. In addition, plant-based adsorbents are ecofriendly, cost-effective and naturally abundant. It was observed that the THC concentration in the soil samples has a significant effect on the biodegradation rate of the petroleum hydrocarbons. The highest biodegradation rate was recorded at 5% (w/w) contamination when compared to the results obtained at 10% (w/w) and 15% (w/w) contaminations. Soil samples contaminated with 5% petroleum products amended with CD + SD, recorded a 59.85% biodegradation rate, while the soil samples amended with CD + RH recorded a biodegradation rate of 68.04%. A biodegradation rate of 48.42% was recorded in the soil with 10% petroleum products, but amended with CD + SD, while 60.5% biodegradation rate was recorded in the soil samples amended with CD + RH. Furthermore, at 15% contamination, a 24.09% biodegradation rate was observed in the CD + SD amended soil samples; while 33.32% was recorded in the CD + RH amended soil samples. This indicated that petroleum hydrocarbons concentration in the soil, could significantly affect the performance of the microorganisms dwelling within the soil. Bossert and Bartha (1984) and Abioye et al. (2012) stated that the performance of soil microbes in a petroleum hydrocarbons contaminated soil is highly dependent on the volume of the crude oil spilled, the quality of the petroleum product spilled, and/or the petroleum hydrocarbons contamination history of the ecosystem. According to Abioye et al. (2012), the biodegradation rate was higher (92%) in soils contaminated with 5% (w/w) of spent motor engine oil, when compared to soil contaminated with 15% (w/w) of spent motor engine oil (55%), at the end of a period of 84 experimental days using spent brewery grains. Likewise, Schaefer and Juliane (2007) stated that the bioremediation of soil samples contaminated with Total Petroleum Hydrocarbons (TPH) is highly dependent on the TPH concentrations; the bioremediation rate was higher in soil samples containing moderate TPH concentrations. Ijah and Antai (2003) observed that biodegradation of petroleum hydrocarbons was higher in soils contaminated with 10% crude oil, when compared with the results of the soil samples contaminated with 30% or 40% crude oil. Likewise, Rahman et al. (2002) reported that the bacterial consortium (biodegradation) efficiency of a petroleum contaminated soil decreased from 78% to 52%, as the volume of crude oil increased from 1 to 10%. Furthermore, Akpokodje and Uguru (2019) observed that petroleum products significantly decreased the aeration and water holding capacity of soil samples, which are required by soil microbes for growth and development.

It was observed in this study that the contaminated soil samples amended with CD+RH had a better THC biodegradation rate, when compared with the contaminated soil samples amended with CD+SD (Figure 1). This could be attributed to the higher nitrogen content and adsorption properties of the rice husks compared to those of sawdust (Nwite et al., 2014). Researches have shown that nitrogen is a powerful component during bioremediation or phyto remediation of petroleum products contaminated soils. Soil microbes prefer nitrogen rich soil to nitrogen deficient soil during the degradation of petroleum hydrocarbons contaminated soil (Ijah and Safiyanyu, 1997; Walworth et al., 2007; Schaefer and Juliane, 2007). Likewise, Nwite et al. (2011) stated that due to the significant concentration of nitrogen in rice husk over sawdust, it can be effectively used as a soil amendment in remediating contaminated soils. In terms of the nitrogen concentration in sawdust and rice husks; Abbas et al. (2012) and Oluchukwu et al. (2018) reported that rice husks contain 0.476% of Nitrogen, 0.0504% of prosperous, and 25.986% of potassium; while sawdust contains 0.38 % of Nitrogen and a Carbon: Nitrogen (C:N) ratio of 101:3. Likewise, Thiyageshwari et al. (2018) reported that the nitrogen concentration of rice husk increases from 0.31% to 0.63%, with a C:N ratio of 36:1, as it decomposes within an experimental period of 90 days under ambient atmospheric condition.

This study revealed a relatively lower biodegradation rate, during the initial phrase (Day 0 to Day 28) of the experimental period. At day 28 of the experimental period, it was observed that a biodegradation rate of 16.99% was recorded in the contaminated soil amended with CD+SD, while a biodegradation rate of 24.58% was recorded in the soil amended with CD+RH; in the soil samples contaminated with 5% petroleum products. In contrast, at day 56, the soil samples amended with CD+SD recorded a mean biodegradation rate of 59.85%, while the soil amended with CD+RH recorded a mean biodegradation rate of 68.04%; in soil samples contaminated with 5% petroleum products. This could be attributed to the high toxicity of the petroleum hydrocarbons on the microbial activities within the contaminated soil, during the initial stage of the experimental period; and the lower nitrogen content in the remediation setups. It had been reported that high petroleum hydrocarbons concentration in soils can be inhibitory to the indigenous microorganisms (Ijah and Antai, 2003). Studies have shown that during the decomposition of rice husks and sawdust, more nitrogenous compounds are released into the soil (environment). According to Abbas et al. (2012), the nitrogen content of rice husks and sawdust generally increases as it decomposes. In addition, Walworth et al. (2007) reported that remediating petroleum contaminated soil with Nitrogen rich amendments will increase microbial growth rate. This will help to maintain the microbial activities; thereby, increasing the rate of petroleum hydrocarbons dilapidation (Braddock et al., 1999). The differences observed in the biodegradation results of this
research when compared with others research studies, could be attributed to the different composition of petroleum products used. Furthermore, the difference between the chemical compositions and nutritional values of the different amendment (cattle dungs, sawdust, rice husks, etc.) used for bioremediation, can affect the bioremediation results. Similar results were obtained by Abioye et al. (2012) on the biodegradation of spent vehicle engine oil, using banana peels. Citing Abioye et al. (2012), the germination rates of Lettuce (Lactuca sativa) planted in soil contaminated with 5% and 15% spent vehicle engine oil, but remediated with banana peels were 80% and 40% respectively, after an 84 day experimental period.

There is no universal recommended incubation time (ecotoxicity experimental period) for the generality of all seed species; this is because, the germination period for each seed species is dependent on the environmental conditions and the seed species itself (Luo et al., 2017). A ten day experimental period was adopted for the cucumber seeds because if the experimental period were prolonged, there will be a high possibility that secondary roots will start to grow. And therefore, the radicle length of the cucumber seed will not precisely reflects the toxicity of the soil sample.

Analysis of the ecotoxicity result further revealed that it was highly influenced by the contaminant concentration and the type of organic waste used for the biodegradation. Soil samples contaminated with 15% petroleum products exhibited a severe ecotoxicity response (radicle growth), when compared with the soil samples contaminated with 10% petroleum products. At the end of the experimental period, soil samples contaminated with 15% petroleum products and remediated with CD+SD had the highest sensitivity level (53.3% radicle growth inhibition). A lower sensitivity level of 48% radicle growth inhibition, was recorded in the same contaminated soil samples (15% petroleum products) remediated with CD+RH (Figure 3). Likewise, the soil samples contaminated with 5% petroleum products exhibited a mild sensitivity on plant radicle growth when compared with the stronger sensitivity recorded in the soil samples contaminated with 10% and 15% petroleum products. In the control samples, sensitivity levels of 83.3%, 88.3%, and 90% were recorded respectively for the soil samples containing 5% 10% and 15% petroleum products (Figure 3). Similar results were obtained by Abioye et al. (2012) during the bioremediation of petroleum hydrocarbons (5% and 15% concentration) contaminated soil, using brewery grain, banana peels, and mushroom compost. According to Abioye et al. (2012), the biodegradation of soils contaminated with high concentration of petroleum hydrocarbons required a longer duration and a large volume of biological materials, to degrade the petroleum hydrocarbons and restore the soils back to agricultural production. According to Adam and Duncan (2002), the toxicity nature and hydrophobic properties of petroleum hydrocarbons negatively affect the germination index of plant seeds. Due to the hydrophobic properties of the petroleum hydrocarbons, they are able form impermeable coating around the roots surface. This reduces the nutrient absorption and respiration rate of the plant’s roots; thereby, leading to a decrease in the seed germination rate. This shows that as the THC concentration in the soil increases; the seeds germination rates decreases. Seed germination rates of 99%, 72%, and 65% were recorded in the contaminated soil with 5%, 10%, and 15% petroleum products amended with CD+RH. However, germination rates of 81%, 68% and 55% were recorded in contaminated soil with 5%, 10%, and 15% petroleum products and amended with CD+SD. Germination rates of 19%, 11%, and 5% were recorded in the control soil samples contaminated with 5%, 10%, and 15% petroleum products at the end of the 56 day experimental period.

**Residual petroleum hydrocarbons ecotoxicity**

Cucumber is an important leguminous crop, wildly cultivated for its high vitamins content, but it is highly sensitive to petroleum hydrocarbons toxicity. Figures 2 and 3 illustrate the ecotoxicological results of the remediated and control soil samples. The ecotoxicological results showed that the control soil samples had the least seed germination rate index (Figure 2) and highest radicle growth inhibition index (Figure 3), when compared with the results obtained from the remediated soil samples. The results of the seed germination rate revealed that the contaminated soil samples remediated with CD+RH had a better seed germination rate, than the soil remediated with CD+SD (Figure 2). The results further revealed a strong correlation \( r \geq 0.86 \) between the THC concentration in the contaminated soils samples and the seed radicle inhibition. This shows that as the THC concentration in the soil increases; the seeds germination rates decreases. Seed germination rates of 89%, 72%, and 65% were recorded in the contaminated soil with 5%, 10%, and 15% petroleum products amended with CD+RH. However, germination rates of 81%, 68% and 55% were recorded in contaminated soil with 5%, 10%, and 15% petroleum products and amended with CD+SD. Germination rates of 19%, 11%, and 5% were recorded in the control soil samples contaminated with 5%, 10%, and 15% petroleum products at the end of the 56 day experimental period.

![Figure 1: Bioremediation rate of petroleum products contaminated soils](image)

**Figure 1: Bioremediation rate of petroleum products contaminated soils**

**Figure 2:** The results of the biodegradation of petroleum products (5% and 15% concentration) in cultured mushroom compost. They showed that the biodegradation of petroleum products with CD+RH (control) was lower than that of petroleum products with CD+SD. This is because, the soil samples remediated with CD+RH had a lower seed germination rate index (Figure 2) and highest radicle growth inhibition index (Figure 3), when compared with the results obtained from the remediated soil samples. The results of the seed germination rate revealed that the contaminated soil samples remediated with CD+RH had a better seed germination rate, than the soil remediated with CD+SD (Figure 2). The results further revealed a strong correlation \( r \geq 0.86 \) between the THC concentration in the contaminated soils samples and the seed radicle inhibition. This shows that as the THC concentration in the soil increases; the seeds germination rates decreases. Seed germination rates of 89%, 72%, and 65% were recorded in the contaminated soil with 5%, 10%, and 15% petroleum products amended with CD+RH. However, germination rates of 81%, 68% and 55% were recorded in contaminated soil with 5%, 10%, and 15% petroleum products and amended with CD+SD. Germination rates of 19%, 11%, and 5% were recorded in the control soil samples contaminated with 5%, 10%, and 15% petroleum products at the end of the 56 day experimental period.
germination index and increase in radicle growth inhibition (Adam and Duncan, 2002; Abioye et al., 2012).

The results of this study corroborate earlier findings by Tamada et al. (2012), which observed that after a 180 day biodegradation period, seed germination and performance were highly inhibited by the initial concentration of hydrocarbons in the contaminated soil samples. Likewise, Banks and Schultz (2005) reported that the germination index of lettuce plant was highly dependent on the concentration of the petroleum contamination in the soil samples in which the lettuce plants were cultivated. Findings by Lors et al. (2009), showed that the growth inhibition of plants growing in un-remediated soils with petroleum hydrocarbons contamination, was higher (79%) as against the 0% inhibition level (no phytotoxicity) recorded in the remediated soil samples, at the end of the remediation period. Horticultural crops depend mainly on soil for germination and growth performance; therefore, any alteration in the soil’s physical characteristics and biochemical properties (through toxic substances) reflects on the seed performance and development (Cruz et al., 2013). According to Haeseler et al. (2001), incomplete biodegradation of petroleum hydrocarbons during remediation could cause the formation of toxic intermediary metabolites within the soil, which may lead to an increment in the soil’s toxicity level.

The results of this study provide important information on the selection and combination of organic wastes, during the remediation of contaminated areas. This is because a significant relationship between type of organic waste used for the soil amendment and cucumber seeds germination toxicity was established. The toxicity level of petroleum hydrocarbons on the soil can be forecasted by appropriate seed germination and radicle growth indices of different plants species.

**Figure 2: Table 2: Seed germination rate**

**CONCLUSION**

This study statistically revealed that organic materials can successfully biodegrade petroleum hydrocarbons, and reduce petroleum hydrocarbons ecotoxicity in the soil. The study showed that the treatment of soil samples contaminated with various volumes (5%, 10%, and 15% w/w) of petroleum products, with CD + SD and CD + RH improved the THC biodegradation rate in the samples. The volume of petroleum products present in the soils samples was discovered to influence the bioremediation rate of the THC in the soil samples. The lowest biodegradation rate was recorded in the soils with 15% (w/w) petroleum products when compared to results obtained at 10% (w/w) and 5% (w/w) contaminationResults obtained from the laboratory tests revealed that after a 56 day experimental period, soil samples contaminated with 5% petroleum products and amended with CD+SD, recorded a 59.85% biodegradation rate, while the soil samples amended with CD+RH, recorded a 68.04% biodegradation rate. A biodegradation rate of 48.42% was recorded in the soil with 10% petroleum products contamination amended with CD + SD, while a 60.5% biodegradation rate was recorded in the soil samples amended with CD + RH. Furthermore, at 15% petroleum products contamination, a 24.09% biodegradation rate was observed in the CD + SD amended soil samples; while 33.32% was observed in the CD + RH amended soil samples. In addition, the contaminated soil samples amended with CD + RH generally recorded the least radicle growth inhibition rate when compared to the soil samples amended with CD + SD and the control soil samples. This showed that contaminated soils amended with CD + RH had lower toxicity than soils amended with CD + SD. This study revealed that rice husk which is a waste material from rice production, can be utilized in the remediation of petroleum contaminated soils.
REFERENCES


