

Preliminary study of mechanic workshop soil amended with juice extracted from Lime (*Citrus aurantifolia*)

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ABSTRACT: Bioremediation potential of lime extract (*Citrus aurantifolia*) on mechanic workshop soil was studied for a period of 56 days. The microbial enumeration was carried out using serial dilution and pour plate method. The bacteria count ranged from 6.3×10^3 to 6.8×10^4 cfu/g for oil free soil (OFS), 0.8×10^3 to 9.8×10^4 cfu/g for mechanic workshop soil (MS) and 1.24×10^4 to 4.16×10^4 cfu/g for mechanic workshop soil amended with lime extract (MSAL). The fungal count ranged from 2.1×10^3 to 2.7×10^3 cfu/g for amended soil, 2.5×10^3 to 3.0×10^4 cfu/g for unpolluted and 2.2×10^3 to 4.0×10^4 cfu/g for polluted soil. There were significant differences in both bacteria and fungi count at 5% probability level. *Micrococcus* sp, *Staphylococcus* sp, *Pseudomonas* sp, *Bacillus* sp, *Proteus* sp, *Mucor* sp, *Microsporum* sp, *Aspergillus niger*, *Aspergillus flavus*, *Saccharomyces* sp, *Rhizopus* sp and *Aspergillus fumigatus* were isolated in this study. MSAL had higher moisture content, phosphorus, organic matter, organic carbon and nitrate compared to OFS and MS. There were significant differences ($p < 0.05$) in the pH, phosphorus, organic matter, organic carbon between OFS, MS and MSAL. This study revealed that lime (*Citrus aurantifolia*) extract has a potential for remediating mechanic workshop soil.

Keywords: Bioremediation, *Citrus aurantifolia*, lime extract, organic carbon.

INTRODUCTION

Contamination of soils with oil in the environment has been reported by Yakubu (2007) as a well recognised problem in today's world. Oil spills affect many species of plants and animals in the environment as well as humans. Contamination of soils with petroleum hydrocarbons is a growing concern in many countries (Agamuthu et al., 2010). The indiscriminate disposal of spent lubricating oil by motor mechanics is a common source of spent lubricating oil contamination of soil in countries like Nigeria that do not enforce strict compliance to environmental laws (Ogbo et al., 2009).

Spent engine oil is a common and toxic environmental contaminants not naturally found in the environment (Dominguez-Rosado and Pichtel, 2004). large amount of spent oil are liberated into the environment when motor oil is changed and disposed into the soil by motor mechanics and generator artisans including small scale lubricating oil merchants along the road (Odjegba and Sadiq, 2002; Achuba and Clarke- Peretiemo, 2008).

Oil is released into the environment from exhaust system during engine use and also due to engine leaks (Osubor and Anoliefo, 2003). According to Ogbo et al. (2009), oil contamination causes low rate of germination in plants. Adam and Duncan (2002) also reported that this effect could be due to the oil which acts as a physical barrier preventing or reducing access of the seeds to water and oxygen. Oil released into the environment affects many plants, animals, microorganisms and humans within the oil impacted environment. Additionally, prolonged exposure to oil as well as high concentration of oil could cause the development of liver or kidney disease, possible damage to the bone marrow and an increased risk of cancer (Mishra et al., 2001).

Petroleum products such as engine oil, petrol and diesel are used daily in various forms in mechanic workshops. These products tends to harden and change the colour of the soil, which may have untold hazards to automobile technicians (Udeani et al., 2009). Abandoned

mechanic workshop soil cannot be used for agricultural purposes because of the different hydrocarbon products used and discarded over a long time. Hence there is the need to reclaim such polluted soil.

Application of lime to contaminated soils has been reported to significantly improve soil fertility (Yamoah et al., 1996). The effect of lime is long lasting but not permanent (Fageria et al., 2008). Lime is readily available and it is not expensive. The application of lime significantly increased soil pH, available phosphorus and the amount of soil exchangeable aluminium (Ruganzu, 2009).

Lime is an acidic substance that raises the pH of the soil. When Lime is added to soil it increases the pH of the soil.

Lime tree (*Citrus aurantifolia*) is widely grown in tropical and subtropical areas. Limes exceed lemons in both acid and sugar content (Morton, 1987).

Different methods have been employed in remediating contaminated soil with each having one form of disadvantage or the other. Bioremediation makes use of indigenous oil-consuming microorganisms. According to Gentry et al. (2004), these indigenous organisms can naturally degrade large hydrocarbons and utilise them as source of food. Microbial remediation of hydrocarbon contaminated sites is accomplished with the help of a diverse group of microorganisms, particularly indigenous bacteria present in soil. These microorganisms can degrade a wide range of target constituents present in oily sludge (Barathi and Vasudevan, 2001; Mishra et al., 2001).

The significance of this preliminary study however, lies in the use of extract from *Citrus aurantifolia* which contains natural sugar and is completely biodegradable to amend mechanic workshop polluted soil.

MATERIALS AND METHODS

Sample Collection

Oil polluted soil sample was collected from a mechanic workshop opposite Kogi State University (KSU) first gate, Ankpa road, Anyigba. Anyigba lies between latitudes $7^{\circ} 29'$ North and longitude $7^{\circ} 11'$ East and falls within the rain forest belt of Nigeria (Stephen and Egene, 2012).

The samples were collected at a depth of 0 to 5 cm from three different points into two perforated plastic pots labelled A and B, while soil sample devoid of oil was collected from soil around the Faculty of Natural Sciences of Kogi State University Anyigba, into the third pot (C) which served as the control for this study. All the pots contained 6.0 kg of soil each.

Procedure

In the 6.0 kg of soil (mechanic workshop soil) in A, 1000

ml (1 litre) of lime extract used as amendment was incorporated into the soil to achieve 16.7% amendment level. Pot B contained only mechanic workshop soil while C contained pristine soil (control). The three soil samples were kept in Microbiology laboratory Kogi State University, and were watered twice with 500 millilitres a week for a period of eight (8) weeks. Sampling was conducted bi-weekly for period of 56 days (8 weeks) to determine the microbiological components and physicochemical properties of the soil. The soil samples from the three pots were analyzed microbiologically as described by Public Health England (2014). The pH was determined as described by Thomas (1996). Nitrate was determined by the micro Kjeldahl method (Association of Official Analytical Chemist, AOAC, 2005). The phosphorus content and moisture were determined as described by Kellogg Soil Survey laboratory Methods Manual (1996) method.

Sample Analysis

Descriptive statistics and analysis of variance (ANOVA) was performed using procedure of SPSS version 16 (2007). Experimental precision achieved was reported at 5% probability level.

RESULTS AND DISCUSSION

Figure 1 shows the total bacteria counts obtained from oil free soil (OFS), mechanic workshop soil (MS), and mechanic workshop soil amended with lime extract (MSAL). Higher bacteria counts were observed in MSAL than in OFS and MS. The bacteria count ranged from 6.3×10^3 to 6.8×10^4 cfu/g for OFS, 8.0×10^3 to 9.8×10^4 cfu/g for MS and 1.24×10^4 to 4.16×10^5 cfu/g for MSAL. The highest bacteria count was recorded in MSAL at day 14. There were significant differences in the bacteria count between the treatments at 5% probability level. The organisms identified were *Micrococcus* species, *Staphylococcus* species, *Pseudomonas* species, *Bacillus* species, *Proteus* species and *Escherichia coli*. Microbiological analysis revealed higher bacteria count in mechanic workshop soil amended with lime extract (MSAL) compared to mechanic workshop soil (MS) and oil free soil (OFS). This may be due to the amendment with lime extract (MSAL) which improved the nutrient content of the soil allowing more organisms to flourish. This is in agreement with the findings of Chorom et al. (2010). These authors observed high bacteria count in crude oil polluted soil amended with lime fertilizer.

Figure 2 shows the fungi count from oil free soil (OFS), mechanic workshop soil (MS) and mechanic workshop soil amended with lime extract (MSAL). The fungi counts ranged from 2.5×10^3 to 3.0×10^4 cfu/g for OFS, 2.2×10^3 to 4.0×10^4 cfu/g for MS and 2.1×10^3 to 2.7×10^3 cfu/g for

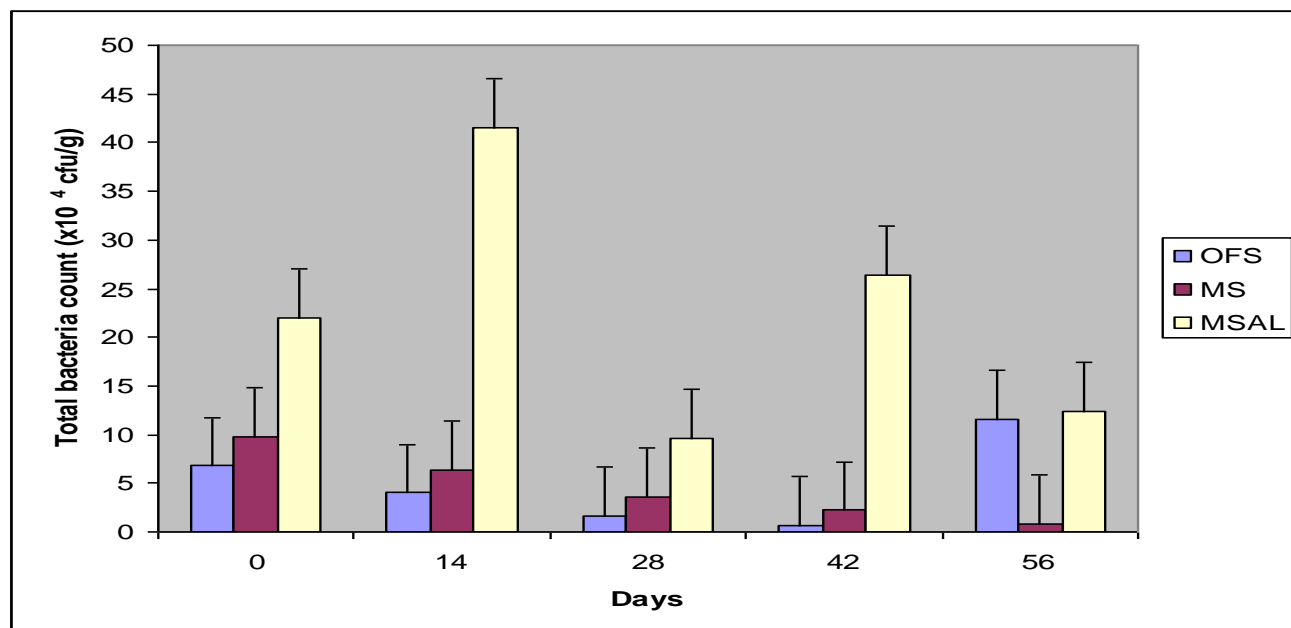


Figure 1. Total viable counts obtained from mechanic workshop soil undergoing Bioremediation. OFS, Unpolluted soil, MS, Mechanic workshop soil, MSAL, Mechanic workshop soil Amended with lime extract.

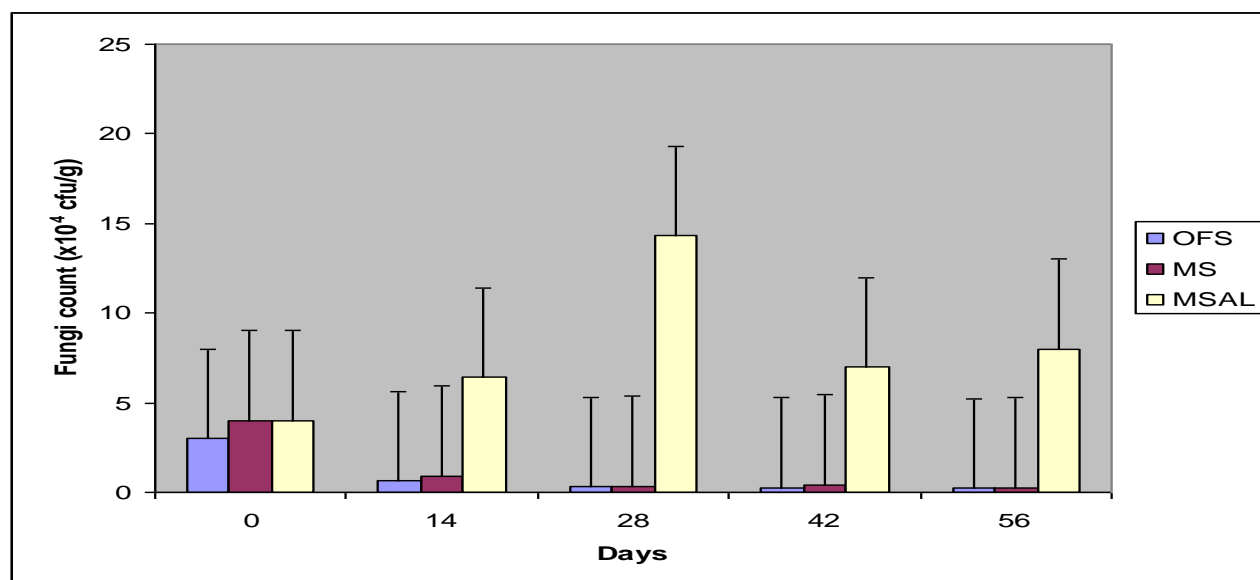


Figure 2. Total viable fungi counts obtained from mechanic workshop soil undergoing bioremediation. OFS, Unpolluted soil, MS, Mechanic workshop soil, MSAL, Mechanic workshop soil Amended with lime extract.

MSAL. The highest fungi count was observed in MSAL at day 28 while the lowest fungi count was observed in OFS at day 56. The fungi isolated were *Aspergillus niger*, *A.flavus*, *A. fumigatus*, *Sacchromyces* sp, *Rhizopus* sp, *Microsporum* sp and *Mucor* sp. The fungi count was also higher in mechanic workshop soil amended with lime extract (MSAL) than the unamended mechanic soil (MS)

and the oil free soil (OFS). This result is in agreement with earlier works by Stephen et al. (2013a,b). These authors observed higher fungi counts in soils amended with plant remains.

The frequency and occurrence of bacteria and fungi isolated from OFS, MS and MSAL is shown in Table 1. *Mucor* sp had the highest frequency of occurrence with

Table 1. Frequency and Occurrence of Isolates obtained from mechanic workshop soil undergoing bioremediation.

| Organisms | OFS | MS | MSAL | Total | % Total |
|------------------------------|-----|----|------|-------|---------|
| <i>Aspergillus flavus</i> | 1 | - | 1 | 2 | 3.45 |
| <i>Aspergillus fumigatus</i> | - | 2 | 2 | 4 | 6.90 |
| <i>Aspergillus niger</i> | 2 | 1 | 2 | 5 | 8.62 |
| <i>Bacillus sp</i> | 5 | 4 | 2 | 11 | 18.97 |
| <i>Micrococcus sp</i> | 1 | 1 | 3 | 5 | 8.62 |
| <i>Mucor sp</i> | 5 | 4 | 4 | 13 | 22.41 |
| <i>Pseudomonas sp</i> | 1 | 2 | 3 | 6 | 10.34 |
| <i>Proteus sp</i> | - | 3 | 1 | 4 | 6.90 |
| <i>Rhizopus sp</i> | 1 | 2 | 1 | 4 | 6.90 |
| <i>Saccharomyces sp</i> | - | 1 | - | 1 | 1.72 |
| <i>Staphylococcus sp</i> | 1 | 1 | 1 | 3 | 5.17 |
| Total | 17 | 21 | 20 | 58 | 100 |

OFS, Unpolluted soil, MS, Mechanic workshop soil, MSAL, Mechanic workshop soil Amended with lime extract.

22.41% followed by *Bacillus sp* with 18.97%, *Pseudomonas sp* 10.34%, *Staphylococcus sp* and *Aspergillus niger* had 8.62%, *Proteus spp*, *Rhizopus spp* and *Aspergillus fumigatus* 6.90%, *Micrococcus spp* had 5.17%, *Aspergillus flavus* 3.45% while *Saccharomyces spp* had the least frequency with 1.72%. Similar result was obtained in an earlier study by Stephen et al. (2016). However, in addition to the above organisms, the authors reported the occurrence of *Candida tropicalis* (1.72%) in mechanic workshop soil undergoing bioremediation amended with lime fertilizer. In this study, the total microbial load was more in the unamended mechanic workshop soil (MS) followed by the amended soil (MSAL) and OFS. This may be due to the remediation process going on and probably with time, the amended soil (MSAL) may have more microbial load than OFS and MS.

The physicochemical qualities of the lime extract (juice) revealed an acidic pH of 2.64, nitrate concentration of 0.80% and phosphorus content of 21.30%. Lime extract is acidic, poor in nitrogen but contains high phosphorus content. This finding is in agreement with Ruganzu (2009) who reported that lime contains high amount of phosphorus which may increase microbial activity in the soil.

The physicochemical parameters of the soil samples analyzed were shown in Table 2. The pH ranged from 5.54 ± 0.23 to 6.46 ± 0.29 . The highest pH was observed in oil free soil while the least pH was observed in MSAL. There were significant differences ($p < 0.05$) in the pH between OFS, MS and MSAL. The pH in the amended soil was lower than unamended mechanic workshop soil and oil free soil. This may be due to the acidic juice (2.64) incorporated into the soil. This result is in sharp contrast to the result of Stephen et al. (2013a) who observed a higher pH in mechanic workshop soil amended with cow

pea chaff.

The highest moisture content was observed in MSAL followed by MS and OFS. It ranged from 7.01 ± 3.08 to $10.46 \pm 1.80\%$. There was no significant difference ($p > 0.05$) in the moisture content between the treatments. The moisture contents of the samples were high. This may be due to the consistent watering of the soil. This is in agreement with Stephen and Egene (2012). They observed high moisture content in spent lubricating oil polluted soil receiving constant water.

Phosphorus content was high in all treatment. However, it was higher in MSAL compared to MS and OFS. The phosphorus content ranged from 8.06 ± 0.94 to $11.26 \pm 0.92\%$. There were significant differences between the treatments at 5% probability level. The phosphorus content was higher in the MSAL and MS. This agrees with the findings of Ijah and Abioye (2003) who reported increase in phosphorus contents in soil polluted with hydrocarbon products. Moreover, the lime extract had high phosphorus content which may also be responsible for the higher phosphorus result obtained in MSAL.

Nitrate content was low in both OFS and MS compared to MSAL. It ranged from 0.09 ± 0.02 to $2.73 \pm 2.09\%$. The highest nitrate content was observed in MSAL followed by MS. There was no significant difference in the nitrate concentration at 5% probability level. The nitrate content was higher in MSAL compared to MS and OFS. This may be due to increase rate of organic matter decomposition as a result of the amendment (Stephen et al., 2016). A similar result was reported by Atagana (2008) who worked on compost manure.

There was a linear increase in the organic matter content of the soil samples from OFS to MSAL. The least organic matter content was observed in OFS while the highest value was recorded in MSAL. There was a

Table 2. Physicochemical qualities of mechanic workshop polluted soil undergoing bioremediation (M+SE)

| Parameter | OFS | MS | MSAL |
|--------------------|--------------------------|-----------------------------|---------------------------|
| pH | 6.46 ± 0.29 ^a | 6.22 ± 0.22 ^{a,b} | 5.54 ± 0.23 ^a |
| Moisture (%) | 7.01 ± 3.08 ^a | 8.12 ± 1.66 ^a | 10.46 ± 1.80 ^a |
| Nitrogen (%) | 0.09 ± 0.02 ^a | 0.19 ± 0.03 ^a | 2.79 ± 2.09 ^a |
| Phosphorus (%) | 8.06 ± 0.94 ^a | 10.40 ± 1.00 ^{a,b} | 11.26 ± 0.92 ^a |
| Organic carbon (%) | 0.60 ± 0.04 ^b | 1.04 ± 0.05 ^b | 3.27 ± 0.42 ^a |
| Organic matter (%) | 1.04 ± 0.04 ^b | 1.60 ± 0.26 ^b | 4.0 ± 0.86 ^a |
| EC(Ms/cm) | 0.50 ± 0.11 ^a | 0.49 ± 0.07 ^a | 0.91 ± 0.51 ^a |

a,b,c, means denoted by different superscripts along the same row are significantly ($p < 0.05$) different. Values are mean of five replicates. OFS, Unpolluted soil, MS, Mechanic workshop soil, MSAL, Mechanic workshop soil Amended with lime extract, EC, Electrical conductivity.

significant difference ($p < 0.05$) in the organic matter content between the treatments. Similar trend in organic matter content was observed in the organic carbon. The highest value of organic carbon was recorded in MSAL while the least value was observed in OFS. There was a significant difference ($p < 0.05$) in the organic carbon between OFS, MS and MSAL. The organic matter and organic carbon contents were higher in MSAL compared to MS and OFS. This may be due to accumulation of organic compounds in oil polluted soil (Ayotamuno et al., 2006). According to Tester (1990) and Darby et al. (2006), repeated applications of organic materials and compost can increase the level of soil organic matter which can also enhance microbial activity (Darby et al., 2006).

The electrical conductivity (EC) was low in all soil samples regardless of treatment. The EC ranged from 0.50 ± 0.11 Ms/cm to 0.91 ± 0.51 Ms/cm. there was no significant difference in the electrical conductivity at 5% probability level between the treatments. The higher electrical conductivity observed in MSAL compared to MS and OFS may be due to the bioremediation process resulting in the release of salts and ions from the oil polluted soil (Stephen et al., 2013a). The values of electrical conductivity in this study were higher than earlier works by Stephen et al. (2013a). The reason may be due to the high biodegradable liquid nature of the lime extract used in this study compared to cowpea chaff.

Conclusion

The results of this preliminary study showed that the use of lime extract can improve the microbial activity and physicochemical properties of hydrocarbon polluted soil time. Phosphorus, organic matter content and organic carbon were higher in the amended soil compared to the unamended soil and the free soil suggesting that lime extract has a potential for remediating mechanic

workshop polluted soil as it contains natural sugar which probably enhanced biodegradation of hydrocarbon polluted mechanic workshop soil.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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