ASUU JOURNAL OF SCIENCE
A Journal of Research and Development
Vol 3, No. 2, December, 2016; pp. 65-78

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- 6.8×10^4 cfu/g for oil free soil (OFS), 0.8×10^3 - 9.8×10^4 cfu/g for mechanic workshop soil (MS) and 1.24×10^4- 4.16×10^4 cfu/g for mechanic workshop soil amended with lime extract (MSAL). The fungal count ranged from 2.1×10^3 - 2.7×10^3 cfu/g for amended soil, 2.5×10^3 - 3.0×10^4 cfu/g for unpolluted and 2.2×10^3 - 4.0×10^4 cfu/g for polluted soil. There were significant differences in both bacteria and fungi counts 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 (<0.05) in the pH, phosphorus, organic matter, organic carbon between OFS, MS and

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MSAL. This study revealed that lime (Citrus aurantifolia) extract has a potential for remediating mechanic workshop soil.

Keywords: *Bioremediation, Lime extract, Citrus aurantifolia, 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, especially in Asia (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 them are liberated into the environment when motor oil is changed and disposed into the soil which is a common practice 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 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 can not 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 alkaline substance that raises the pH of the soil. When Lime is added to soil it increases the pH of the soil. Most lime is calcium carbonate ($CaCO_3$), and is often called "agricultural lime" or "ground limestone" (Sarah, 2006). Sarah (2006) is also of the view that adding lime to soil alleviates the dangers of low acid as well as addition of necessary calcium which may helps microorganisms in their breakdown of contaminants increases the rate of organic matter decomposition, decrease the solubility of certain toxic elements, improve soil aggregation and aid in the release of soil phosphorus.

Lime tree (*Citrus aurantifolia*) is widely grown in tropical and subtropical areas and its edible acid fruits. The tree seldom grows more than 5 m (16 feet) high and if not pruned becomes shrub like. Its branches spread and are irregular, with short, stiff twigs, small leaves, and many small, sharp thorns. The leaves are pale green; the small white flowers are usually borne in clusters. The fruit is about 3 to 4 cm (1 to 1.5 inches) in diameter, oval to nearly globular in shape, often with a small apical nipple; the peel is thin and greenish yellow when the fruit is ripe. The pulp is tender, juicy, yellowish green in colour, and decidedly acid. Limes exceed lemons in both acid and sugar content (Morton, 1987). Limes probably originated in the Indonesian archipelago or the nearby mainland of Asia. The Arabs may have taken limes, as well as lemons, from India to the eastern Mediterranean countries and Africa around AD 1000 (Morton, 1987). According to Morton (1987), Brazil leads in lime production, producing around 700,000 metric tons per year. Lime is grown widely in Mexico, the United States of America and even in Nigeria.

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, called petrophiles by enhancing and fertilizing them in their natural habitats. According to Pepper (2004), Petrophiles are organisms that 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 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. The samples were collected at a depth of 0-5 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. In addition to the 6.0 kg of soil (mechanic workshop soil) in A, 1500 ml (1.5liters) of lime extract used as amendment was incorporated into the soil. Pot B contained only mechanic workshop soil while C contained prestine 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

Sample Analysis

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). pH was determined as described by Thomas (1996). Nitrate was determined by the micro Kjedahl method (AOAC, 2005). The phosphorus content and moisture were determined using the Survey laboratory (1996) method. The ignition method of Akinsanmi (1975). Descriptive statistics and analysis of variance (ANOVA) was performed using procedure of SPSS version 16 (2007). Experimental precision achieved was reported at $p \leq 0.05$ level.

RESULTS

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 counts ranged from 6.3×10^3 - 6.8×10^4 cfu/g for OFS , 8.0×10^3 - 9.8×10^4 cfu/g for MS and 1.24×10^4 - 4.16×10^5 cfu/g for MSAL. The highest bacteria count was recorded in MSAL at day 28. 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.*

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 - 3.0×10^4 cfu/g for OFS, 2.2×10^3 - 4.0×10^4 cfu/g for MS and 2.1×10^3 - 2.7×10^3 cfu/g for MSAL. The highest fungi count was observed in MSAL at day 14 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.

Table 1 shows the frequency and occurrence of bacteria and fungi isolated from OFS, MS and MSAL. *Mucor sp* had the highest frequency of occurrence with 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%.

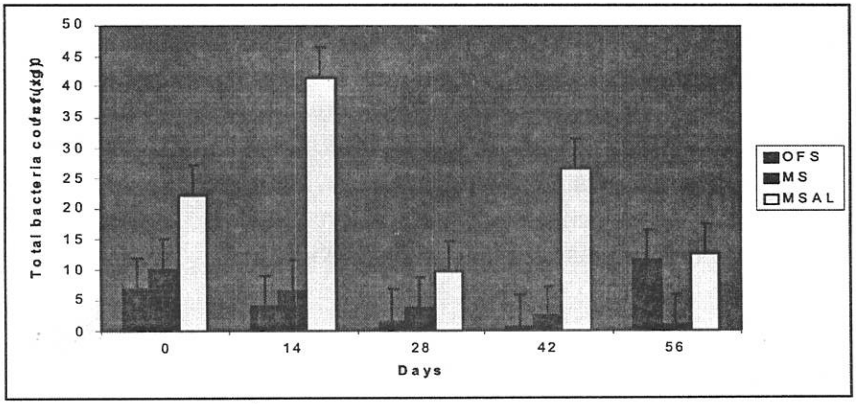

Fig 1.Total viable counts obtained from mechanic workshop soil undergoing Bioremediation

OFS: Unpolluted soil, MS: Mechanic workshop soil, MSAL: Mechanic workshop soil Amended wilt lime extract

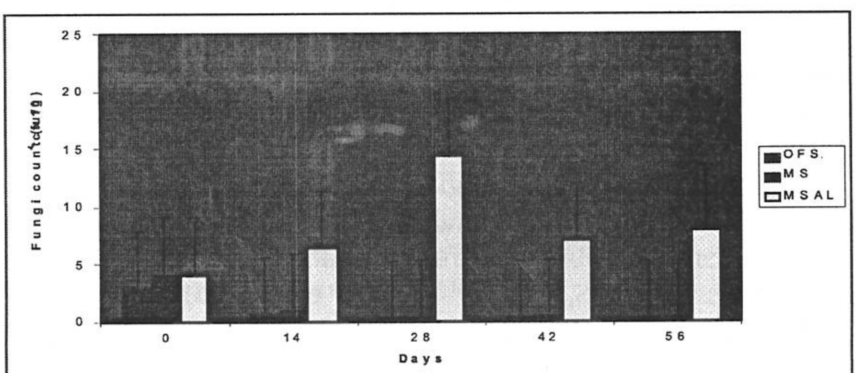

Fig 2. Total viable fungi counts obtained from mechanic workshop soil undergoing bioremediation.

OFS: Unpolluted soil, MS: Mechanic workshop soil, MSAL: Mechanic workshop soil Amended wilt lime extract

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

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

OFS: Unpolluted soil, MS: Mechanic workshop soil, MSAL: Mechanic workshop soil Amended wilt lime extract

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%

Table 2 shows the physicochemical parameters of the soil samples analyzed. 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 (<0.05) in the pH between OFS, MS and MSAL.

The highest moisture content was observed in MSAL followed by MS and OFS. It ranged from 7.01 ± 3.08-10.46 ± 1.80%. There was no significant difference (>0.05) in the moisture content between the treatments.

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 ± 0.92 %. There were significant differences between the treatments at 5% probability level.

Nitrate content was low in both OFS and MS compared to MSAL. It ranged from 0.09 ± 0.02 to 2.73 ± 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.

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 significant difference (<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 (<0.05) in the organic carbon between OFS, MS and MSAL.

The electrical conductivity (EC) was low in all soil samples regardless of treatment. The EC ranged from 0.50 ± 0.11Ms/cm to 0.91 ± 0.51Ms/cm. there was no significant difference in the electrical conductivity at 5% probability level between the treatments.

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

Parameter	OFS	MS	MSAL
pH	6.46 ± 0.29^{a}	$6.22 \pm 0.22^{a,b}$	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}	$10.40 \pm 1.00^{a,b}$	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 wilt lime extract, EC: Electrical conductivity

Discussion

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.

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.

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 (Chesworth, 2008).

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 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.

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.

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 (Sarah, 2006).

A similar result was reported by Atagana (2008) who worked on compost manure.

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 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 study showed that the use of Lime extract can improve the microbial activity and physicochemical properties of hydrocarbon polluted soil. 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 contain natural sugar which will enhance biodegradation of hydrocarbon polluted mechanic workshop soil.

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