A STUDY ON MECHANIC WORKSHOP POLLUTED SOIL AMENDED WITH GREEN WASTE (*Citrus aurantifolia* POWDER)

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Abstract: Bioremediation potential of 400 g of lime powder (*Citrus aurantifolia*) on mechanic workshop soil was studied for a period of 56 days. Mechanic workshop soil samples were collected from three different points into two perforated plastic pots labelled AS (amended soil) and PS (un-amended soil), while pristine soil sample served as the control. Soil amended with green waste (*Citrus aurantifolia* powder) AS, contained 400 g of pulverized lime peelings to achieve a 13.30% amendment level. PS contained only mechanic workshop soil without green wastes while OFS contained pristine soil (control). Sampling was conducted bi-weekly to determine the microbiological components and physicochemical properties of the soil. The bacterial count ranged from 1.72×10^4 to 1.22×10^5 cfu/g for mechanic workshop soil amended with lime powder, AS, 1.68×10^4 to 7.4×10^4 cfu/g for oil-free soil, OFS and 2.1×10^4 to 8.7×10^4 cfu/g for mechanic workshop soil without amendment, PS. The highest bacterial count (1.22×10^5 cfu/g) was recorded at day 28 in AS while the lowest count (1.68×10^4) was recorded in OFS at day 56. The fungal count ranged from 2.6×10^3 to 4.0×10^4 cfu/g for amended soil (AS), 2.3×10^3 to 2.9×10^4 cfu/g for unpolluted soil (OFS) and 2.4×10^3 to 2.7×10^3 for mechanic workshop soil without amendment (PS). The highest fungal count (4.0×10^4 cfu/g) was observed in the amended soil at day 14 while the lowest fungal counts (2.3×10^3 cfu/g) was recorded at day 0 in the oil-free soil. The organisms isolated were *Proteus* species, *Pseudomonas* species, *Staphylococcus* species, *Bacillus* species and *Micrococcus* species. The fungi groups were *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Rhizopus* species, *Mucor* species and yeast. Mechanic workshop soil amended with lime powder had the highest moisture content, organic matter, organic carbon and nitrate compared to OFS and PS. There were significant differences in the pH, organic matter content, organic carbon and nitrate concentrations. This study revealed that lime powder (*Citrus aurantifolia*) had a potential for remediation of mechanic workshop soil as it helps to condition the soil for enhanced microbial activity.

Keywords: Amended soil bioremediation, *Citrus aurantifolia*, microbial counts, polluted soil

Introduction

Pollution has been described by Aboribo (2001) as an undesirable change in the physical, chemical, and biological characteristics of all the components of an environment, which can threaten human health and that of beneficial organisms. Hydrocarbon pollution has been reported to have deleterious effects on plant germination and seedling growth (Kyung-Hwu *et al.*, 2004). Ogbo *et al.* (2009) reported that hydrocarbon pollution creates conditions that limit the availability of essential nutrients like nitrogen and oxygen required for plant growth. Vwioko *et al.* (2006) are of the opinion that oil pollution of soil often leads to build up of both essential elements (organic C, P, Ca, Mg) and non-essential elements (Mg, Pb, Zn, Fe, Co, Cu) in the affected soil which may be translocated in plant tissues.

Petroleum products such as lubricating oil, petrol and diesel are used in various forms in mechanic workshops. They also tend to be spilled accidentally during transport. These products tend to harden and change the colour of the soil. It may also have adverse effect on soil microbiota as well as the physicochemical properties (e.g. pH) of the soil (Stephen *et al*., 2013a).

The presence of different types of machinery and automobiles has resulted in an increase in the use of lubricating oil. A seemingly inescapable consequence of these transport activities is the accidental spill of the oil into both land and water (Akaniwor *et al*., 2007; Stephen *et al*., 2013a).

Bioremediation is considered to be a more economical and safe method for the treatment of oil contaminated site (Gallego *et al*., 2001). It involves the addition of specific microorganisms (bacteria, cyanobacteria, algae and fungi) or stimulation of microorganisms already present in polluted soil to improve their biodegradation activities.

Most oil-polluted soils are acidic and this acidity alters the microbiological and physicochemical properties of such soils which consequently affects soil fertility (Akaniwor *et al.*, 2007). This may result in a long term low agricultural productivity if not amended.

Lime orange (*Citrus aurantifolia)* is a common food industry by-product and a common agricultural waste. These kinds of wastes are referred to as "Green waste". Green waste (food, agro-industrial and forest residues, compost etc) is a valuable resource of polyphenols. Wiltse *et al.* (1998) reported that natural polyphenols are relatively efficient in the clean-up of environmental pollutants based on their unique traits of chelation, adsorption, reduction, nutrient cycling, and plant growth promotion. These significant traits have found emerging applications in the removal of heavy metals, and dyes from contaminated soil and water through existing bioremedial techniques such as biosorption, phytoextraction and coagulation (Blodgett, 2001).

Green wastes such as *Citrus aurantifolia* powder is a natural, biodegradable, environmentally-friendly, noninvasive and not expensive product. Most people utilize *Citrus aurantifolia* juice only. It has been reported that when *Citrus aurantifolia* juice is added to hydrocarbon-polluted soil, it increases the pH of the soil (Stephen *et al.*, 2016a). Hence, the use of green waste product (*Citrus aurantifolia* powder) in this study is calculated to increase the pH of the mechanic workshop soil to almost neutral thereby enhancing its restoration by hydrocarbon utilizing organisms.

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 7^0 29^1 North and longitude 7^0 11^1 East and falls within the rain forest belt of Nigeria (Stephen and Egene, 2012).

The samples were collected at a depth of 0-5cm from three different points into two perforated plastic pots labelled AS (amended soil) and PS (un-amended soil), while pristine soil sample collected from soil around the Faculty of Natural Sciences of Kogi State University Anyigba, into the third pot OFS (pristine soil) served as the control for this study. All the pots contained 3000 g of soil each.

Sample analysis

Procedure

Mechanic workshop soil amended with green waste (*Citrus aurantifolia* powder) AS, contained 400 g of pulverized lime peelings to achieve a 13.30% amendment level. The bowls were perforated as described by Stephen *et al*. (2016a). PS contained only mechanic workshop soil without green wastes while OFS contained pristine soil (control). The three soil samples were kept in Microbiology laboratory Kogi State University, and were watered with 500 millilitres once 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 Kjedahl method (Association of Official Analytical Chemist – AOAC, 2005). The phosphorus content and moisture were determined as described by Kellog Soil Survey Laboratory Methods Manual, (1996).

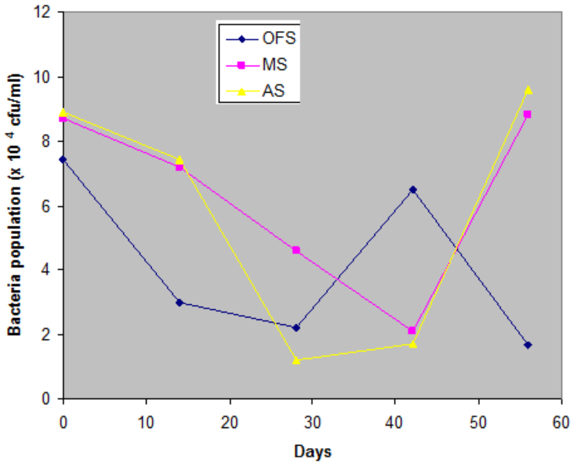
Statistical analysis

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

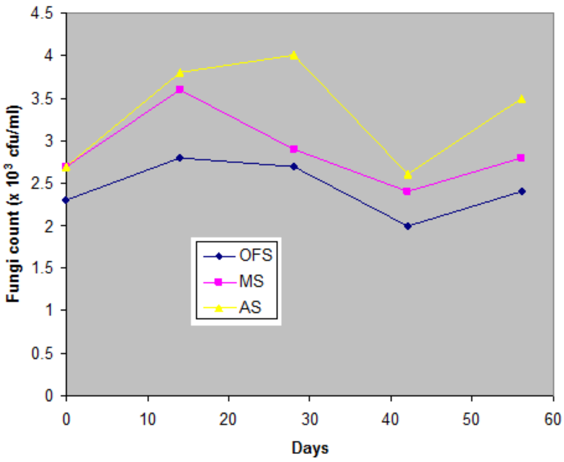
Figure 1 shows the total bacteria counts obtained from oil-free soil (OFS), polluted soil (PS), and polluted soil amended with green waste (AS). The result shows a higher bacteria count in AS than in OFS and PS. The bacteria counts ranged from 1.72 $\times 10^4$ – 9.6 $\times 10^4$ cfu/g for AS, 1.68 $\times 10^4$ – 7.4$\times 10^4$ cfu/g for OFS, and 2.1 $\times 10^4$ – 8.7 $\times 10^4$ cfu/g for PS. The highest count (1.22 $\times 10^5$ cfu/g) was recorded at day 28 in the amended soil (AS) and the lowest count (1.68 $\times 10^4$) was recorded in the oil-free soil (OFS) at day 56. There were no significant differences in the bacteria counts obtained from three treatments at 0.05 probability limit. The bacteria isolated were *Pseudomonas* species *Bacillus* species, *Micrococcus* species, *Staphylococcus* species, and *Proteus* species. These bacteria isolates were also observed in earlier studies by Stephen *et al*. (2016a,b,c) using lime extract (juice) on mechanic workshop soil, lime fertilizer to amend spent lubricating oil polluted soil and mechanic workshop polluted soil, respectively.

Bacteria population was higher in mechanic workshop soil amended with green waste (AS) compared to polluted mechanic workshop soil (PS) and oil-free soil (OFS). This may be attributed to the treatment of the mechanic workshop soil with lime powder which may be responsible for increase in the nutrient content of the soil allowing more organisms to flourish, and consequently, increased microbial activity. This agrees with the findings of Chorom *et al.* (2010). These researchers observed increased bacteria population on application of lime in form of fertilizer to hydrocarbon-polluted soil.



OFS: Oil-free soil, PS: Mechanic workshop polluted soil, AS: Amended soil

Fig. 1: Total bacteria counts (cfu/g) obtained from mechanic workshop polluted soil amended with green waste



OFS: Oil-free soil, PS: Mechanic workshop polluted soil, AS: Amended soil

Fig. 2: Total fungi counts (cfu/g) obtained from mechanic workshop polluted soil amended with lime powder

Figure 2 shows the total fungi count in the oil-free soil (OFS), the polluted soil (PS) and the amended soil (AS). The fungi counts ranged from 2.6 $\times 10^3$ – 4.0 $\times 10^4$ cfu/g for amended soil (AS), 2.3 $\times 10^3$ – 2.9 $\times 10^4$ cfu/g for oil-free soil (OFS) and 2.4 $\times 10^3$ – 2.7 $\times 10^3$ for polluted soil (PS). The highest fungi counts (4.0 $\times 10^4$ cfu/g) was recorded at Day 14 in the amended soil (AS) while the lowest fungi counts (2.3$\times 10^3$ cfu/g) was recorded at day 0 in the oil-free soil (OFS). There were no significant differences in the fungi counts obtained from three treatments at 5% probability limit. Similar pattern in the bacteria population was observed in the fungi count. The presence of the green waste in the mechanic workshop soil may be responsible for the availability of nutrients in the soil which allowed more fungi to flourish. This result is in agreement with earlier works by Stephen *et al.* (2013a) and Stephen *et al.* (2013b). They observed higher fungi counts in amended soil compared to oil polluted and oil-free soil. The hydrocarbon-utilizing fungi identified in this study include: Yeast, *Mucor* species, *Aspergillus fumigatus, Aspergillus flavus* and *Aspergillus niger.* These fungi isolates were isolated by Eze and Okpokwasili (2010) and Stephen *et al.* (2013a) from hydrocarbon polluted soils.

The physicochemical analysis of ground lime peelings before it was incorporated into the soil is shown in Table 1. The lime powder had a pH of 2.60, nitrate content of 0.90% and a high phosphorus composition of 22.10%.

Table 1: Physicochemical analysis of ground lime peelings

pH	%Nitrate	%Phosphorus
2.60	0.90	22.10

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

Parameter	OFS	AS	PS
pH	6.21±0.33^{a}	5.65±0.22^{b}	7.36±0.56^{a}
Moisture(%)	6.33±1.20^{a}	9.39±1.66^{a}	11.14±2.30^{a}
Phosphorus(%)	15.87±1.40^{a}	13.10±0.97^{a,}	12.87±0.81^{a}
Nitrate(%)	0.03±0.01^{b}	0.14±0.03^{a}	0.04±0.01^{b}
Organic matter(%)	1.25±0.22^{b}	1.71±0.19^{b}	2.76±0.21^{a}
Organic carbon(%)	1.37±0.06^{b}	1.91±0.10^{a}	1.73±0.01^{b}

a: same superscripts along the same row are not significantly ($p<0.05$) different. Values are mean of five replicates; **%** (percentage), **OFS**: Oil free soil, **PS**: Polluted soil, **AS**: Amended soil

The physicochemical qualities of the mechanic workshop soil undergoing bioremediation are shown in Table 2.
The pH ranged from 5.65 ± 0.22 - 7.36 ± 0.56. There were significant differences in the pH obtained from the three treatments at 5% probability level. The soil samples were either weakly acidic or weakly alkaline. Mechanic workshop soil without amendment had a weak alkaline pH while the amended soil (AS) had weak acidic pH. The values of pH obtained in this study have been reported by other researchers to favour biodegradation of hydrocarbon polluted soil (Ijah and Antai, 2003; Eze and Okpokwasili, 2010).
The highest moisture content was observed in the polluted soil (PS) followed by AS and OFS had the least moisture content. There was no significant difference in the treatments at 0.05 probability level. According to Ijah and Antai (2003), moisture content in polluted soil is usually high compare to oil free soil.
The mean phosphorus content ranged from 12.87 ± 0.81% – 15.87 ± 1.40%. The highest phosphorus concentration was observed in oil free soil (15.87 ± 1.40 %), followed by amended soil (13.10 ± 0.97%) while the polluted unamended soil had the least phosphorus content (12.87 ± 0.81 %). There were no significant differences in the phosphorus content of AS, PS and OFS at 5% probability level. This agrees with the findings of Ijah and Antai (2003). They reported increase in phosphorus content of soil polluted by hydrocarbon products. Moreover, high phosphorus content in the mechanic workshop soil amended with lime powder could also be attributed partly to the high phosphorus content present in the lime powder (Table 1).
The nitrate concentration in amended soil (AS) was higher than those in polluted soil (PS) and oil-free soil (OFS). This may be due to degradation of the lime powder incorporated into the soil. Atagana (2004) obtained similar result from the addition of compost manure to hydrocarbon polluted soil. There were significant differences in the nitrate concentration in the three (3) soil samples.
The organic matter content was higher in the polluted soil compared to amended and oil free soil. There was a significant difference between AS, PS and OFS at 95% confidence interval. This implies that lime powder had effect on the polluted soil. High organic matter has also been reported in hydrocarbon polluted soils (Atagana, 2008; Chorom *et al*., 2010; Stephen *et al.*, 2013b).
The organic carbon ranged from 1.37 ± 0.06% - 1.91± 0.10%. Amended soil had the highest organic carbon compared to unpolluted soil and oil free soil. This, according to Ayotanumo *et al.* (2006), may be due to accumulation of organic compounds in oil-polluted soils. The high organic carbon content in AS could be attributed to the treatment using lime powder.

Conclusion

Mechanic workshop polluted soil is a common environmental pollution that results from oil spills and from the use of hydrocarbon related compounds. The result of the microbiological parameters of the soil samples showed that higher microbial counts were recorded in mechanic workshop polluted soil amended with lime powder (AS) than polluted (PS) and in the oil free. The pH, organic carbon, organic matter, phosphorus content, moisture content and nitrate were higher in the soil amended with lime powder (AS) than in the polluted soil (PS) and the oil-free soil. Hence, lime powder which is biodegradable green waste can be used to stimulate hydrocarbon degrading microorganisms to enhance the bioremediation of hydrocarbon polluted soil. This could make these hydrocarbon polluted soil fit for other purposes such as farming and easy access to unpolluted ground water supplies.

Conflict of Interest

Authors declare that there is no conflict of interest reported on this work.

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