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Effects of red guinea corn and soybean plants growing on diesel polluted soil on the total heterotrophic and diesel degrading microbes

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Abstract

Effect of red guinea corn and soybean plants growing on diesel-polluted soil on the total heterotrophic and diesel degrading microbes were studied. Soil sample was collected from the Federal Polytechnic Nekede Owerri school farm with the aid of a spade and transported to the preparation ground in clean plastic buckets where it was sun-dried for 3 days. 3kg each of the sun-dried loamy soil sample was weighed out in triplicates and each set was mixed with 30ml, 150ml and 300ml of diesel to obtain 1% (set 1), 5% (set 2) and 10% (set 3) pollutions, respectively, which were dispensed into perforated plastic buckets. Each of these polluted soil samples was planted with the seeds of Red Guinea Corn and Soybean plants. Controls were also set up. Each sample was set up in duplicates. During the first and second week of the plant's growth, total heterotrophic count, total diesel-utilizing microbes, and rhizosphere enumeration were carried out. The results showed that the total heterotrophic count and the hydrocarbon-utilizing microbes increased with increasing concentration of the diesel oil mixture (10%>5%>1%) in both the polluted soil planted with Red Guinea Corn and Soybeans. Similarly, the rhizosphere effects on both diesel degrading microbes and total heterotrophic bacterial counts were found to have increased with increase in percentage of diesel concentrations for both plants. The microbial population and rhizosphere effects were more in the polluted samples than in unpolluted ones. This implied that by encouraging these plants to grow on diesel oil polluted soils, conditions for microbial degradation of the contaminants can be enhanced and can increase the microbiota of the soil.

Keywords: Diesel Oil; Polluted Soil; Soybean Seeds; Red Guinea Corn Seeds; Total Heterotrophic; Total Degrading Microbes; Rhizosphere

1. Introduction

The effects of environmental pollution have increased substantially since industrial revolution. The main sources of pollution are from organic and inorganic municipal wastes, residues from materials used in agriculture, burning of fossil fuel, its exploration, mining and smelting of metaliferrous ores (Shreya et. al., 2007). Cases of spillage of the petroleum and its refined products onto agricultural lands through various operations have been reported since petroleum production began in Nigeria in 1958 (Omorondion, 2004). Pollution arising from crude oil exploration, transportation and use has caused several ecological hazards. Hydrocarbon contamination of soil causes extensive damage to the ecosystem. Hydrocarbon fuels are characterized into three major classes:gasolines, kerosene and diesel corresponding to increasing heavy petroleum fractions (Czes and Radolf, 2005). Diesel oil is a hydrocarbon product of boiling point between 1500C and 4000C with carbon chain lengths of C5-C22 (Czes and Radolf, 2005). When diesel oil is present in soil, it limits soil aeration and alters the general biological balance of soil. It creates an unconducive environment for plants and microorganisms, displacement of oxygen by the oil and blocking air spaces in the soil (Seklemora et al., 2001). The necessity to decontaminate polluted sites is recognized, both socially and politically, because of the increasing

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importance placed on environmental protection and human health. As the levels of contaminations and number of sites increase, so does the need to develop effective and affordable methods for decontamination (Shreya et al., 2007).

Scientist are investigating phytoremediation's potential by using plants such as sunflower, geranium, ragweed and cabbage, as well as other less known technologies (Steven and Jerald, 2003). According to Meagher (2000), this passive remediation technique is based on the nutrients which are transported by capillary action from the soil and ground water through a plant's root system.

Soybean is the richest natural vegetable food. It can withstand drought and heat and the plant may vary in growth and height. It contains about 162kg/357lb of protein (Omoni and Aluko, 2005). Red Guinea Corn is known as Sorghum and it dominates in hot and arid regions because of its ability to withstand heat and drought. Being a strong grass, it grows to a height of 2 to 8 feet (0.5-2.5m) and the leaves are about 5cm wide and 0.75m long. Guinea Corn can withstand a range of soil acidities (from pH 5.0-8.5) and tolerates salinity better than maize. It tolerates amazing array of soils. The aim of this work is to determine the effects of Red Guinea Corn and Soybean plants growing on diesel polluted soil on the total heterotrophic and diesel degrading microbes.

2. Materials and Methods

The loamy soil samples used for the study were collected from Federal Polytechnic Nekede, Owerri School farm. 6-12cm of the loamy top soil were collected with the aid of a spade and transported to the preparation ground using clean plastic buckets. The diesel oil was purchased from Vitaco oil located at Amalim Junction, along Owerri-Okigwe road, Owerri. Guinea corn and soybean seeds were purchased from Eke Atta Market, in Ikeduru LGA, Imo State.

2.1. Sample Preparation

The soil was dried in the sun for 3 days. 3kg each of the sun-dried loamy soil sample was weighed out in three sets and each set was mixed with 30ml, 150ml and 300ml of diesel to obtain 1% (set 1), 5% (set 2) and 10% (set 3) pollutions, respectively, which were dispensed into perforated plastic buckets. Each of these polluted soil samples was planted with the seeds of Red Guinea Corn and Soybean plants. Control 1 (without diesel and planted) and Control 2 (with diesel and unplanted) were also set up. Each sample was set up in duplicates. During the first and second week of plants growth total heterotrophic count, total diesel-utilizing microbes and rhizosphere enumeration were determined. All the samples were exposed to sunlight and rain throughout the study period (Kulakow et al., 2000).

2.2. Total Heterotrophic Count

The soil samples were collected in clean sterile glass bottles using a sterile spatula and analyzed within 1hr of collection. 10g of each soil was dispensed into 90ml of the dilution solution containing 0.5g of K2 HPO4 and 10.1g of NaCl per litre and was shaken vigorously. Ten fold serial dilution of the above setting was made. 0.1ml of the appropriate dilution was spread-plated on plates of nutrient agar in duplicates and incubated at 370C for 72hrs. Colonies growing on the plates were counted and used to calculate the colony forming units as follows:

cfu/g of soil = plate count X dilution factor x 10.

The mean of the duplicate colony counts was calculated and used to compute the number of heterotrophic bacteria in the samples (Ike et al., 2007).

2.3. Enumeration of Diesel-Utilizing Bacteria

Viable aerobic diesel-utilizing bacteria were counted using the vapour phase transfer method of Thijsse and van der Linden (1961). The mineral salts medium of Mills et al., (1978) as modified by Okpokwasili and Okorie (1988) was used. Fifteen grams of bacteriological agar was added to ensure solidification. Filter papers were saturated with diesel oil and placed in the lid of plates. The plates were incubated at 370C for 5 days. The microorganisms growing on the mineral salts agar plates were counted as diesel-utilizing bacteria. The colonies growing on the plates were counted and used to calculate the colony forming units as follows:

cfu/g of soil = plate count x dilution factor x 10.

2.4. Rhizosphere Enumeration

The roots and the thin layer of soil which adhered to them were carefully removed from the buckets. They were placed in 95ml of the dilution solution and shaken for 1 hour. Serial dilution of the series was made. In order to obtain the actual soil dilution in the first bottle, the soil materials were oven-dried in the bottles after removal of the roots and the weight of the soil was determined (Awasthi et al., 2011).

2.5. Rhizosphere Effects

The rhizosphere effect which is the ratio of the number of microbes in rhizosphere over the number of microbes in the unplanted soil was calculated using the ratio of the geometric mean of 2 replicates.

2.6. Statistical Analysis

Single factor analysis of variance (ANOVA) was used in determining the significance of differences among the means. Tables were used in interpreting results

3. Results

The total heterotrophic bacterial counts in the diesel polluted soils and the controls are presented in Table 1. It showed that there was an increase in the bacterial count with increasing concentration of the diesel oil mixture except in the control 1 (without diesel oil but planted) where the count decreased to 2.00 x 1010 (red guinea corn) and 1.86 x 1010 (soybean) which was lower than the counts obtained among the polluted sample. In control 2 (polluted with diesel oil but unplanted), there was a gradual decrease in the bacterial count obtained with increase in diesel oil concentration. The highest bacterial count was obtained from 10% diesel oil both in the polluted soil planted with soybeans and Red Guinea corn. The 10% unplanted polluted soil gave the lowest bacterial count. Statistical analysis showed a high statistical difference between the unplanted polluted samples (control 2) ($p>0.05$).

The total diesel degrading microbes in the soil samples are presented in Table 2. It was observed that there was an increase in the count with increasing concentrations of the diesel oil among the polluted samples except in Control 1 where the count was much lower. In Control 2, the count decreases with increasing diesel oil concentrations. The highest total diesel degrading count was obtained from the 10% polluted samples. The least count was obtained from the 10% control 2 (5.00 x 105).

The rhizosphere effects on the total bacterial heterotrophic count in the polluted soils planted with soybeans and red guinea corn is presented in Table 3. It showed that the rhizosphere effect on the total heterotrophic count increased with increase in percentage of diesel concentration for both plants. The 10% diesel oil sample was observed in both plants to have the highest rhizosphere effect on the counts. The 10% diesel oil sample planted with soybeans had the highest rhizosphere effects.

The rhizosphere effects on diesel degrading microbes in the polluted soil samples planted with soybeans and red guinea corn is presented in Table 4. It showed that the rhizosphere effects on diesel-degrading microorganisms increases with an increase in the concentration of diesel for both plants. The highest effect was observed in 10% diesel oil. The highest effect was observed from the 10% diesel oil. The highest rhizosphere effect was obtained from the 10% diesel oil sample planted with soybeans (82.00).

Table 1 Total heterotrophic bacterial counts (CFU/g) for soybeans and red guinea corn

PLANT	SAMPLE PLATE (ml)	Cfu/g
RED GUINEA CORN	30	2.50×10^{10}
	150	4.20×10^{10}
	300	5.80×10^{10}
SOYBEANS	30	2.80×10^{10}
	150	4.85×10^{10}
	300	6.60×10^{10}

CONTROL 1 (RGC) (Without Diesel		2.00 x 10^{10}
planted) CONTROL 1 (SB)		1.86 x 10^{10}
CONTROL 2 (With Diesel and unplanted)	30	3.00 x 10^{10}
	150	2.21 x 10^{10}
	300	1.08x10^{10}

Table 2 Effects of different percentage of diesel oil on total diesel degrading microbes using vapour phase method

PLANT	SAMPLE PLATE(%)	Cfu/g
RED GUINEA CORN	1 5 10	2.20 x 10^7 3.12 x 10^7 4.00 x 10^7
SOYBEANS	1 5 10	2.50 x 10^7 3.84 x 10^7 4.10 x 10^7
CONTROL 1 (RGC) (Without Diesel and planted) CONTROL 1 (SB)		6.00 x 10^5 1.20 x 10^6
CONTROL 2 (With Diesel and unplanted)	1 5 10	7.50 x 10^6 1.00 x 10^6 5.00 x 10^5

Table 3 Rhizosphere effects on total heterotrophic bacterial count

Plant	Sample grade (ml)	Rhizosphere effects
SOYBEANS	30	0.93
	150	2.19
	300	6.1
RED GUINEA CORN	30	0.83
	150	1.90
	300	5.37

Table 4 Rhizosphere effects on diesel-degrading micro organisms

Plant	Sample grade (ml)	Rhizosphere effects
Soybeans	30	3.33
	150	38.40
	300	82.00
Red guinea corn	30 150	2.93 31.20
	300	80.00

4. Discussion

The results of the total heterotrophic bacterial count (cfu/g) obtained from the planted polluted soil samples, the unpolluted soil and the unplanted polluted soil samples showed that higher bacterial counts were obtained from the polluted samples than the unpolluted ones. This agrees with the work of Ike et al., (2007), which showed that phyto-remediation in the rhizosphere increases soil organic carbon, soil bacteria and mycorrhizal fungi. They also asserted that plants may also release exudates to the soil that help to stimulate the degradation of organic chemicals, including enzyme systems of existing bacterial populations, stimulating growth of new species that are able to degrade the waste, and/or increasing soluble substrate concentration for all microorganisms. This is also in line with the work of Adam and Duncan (2004) which showed that when plants grow on diesel oil contaminated site, conditions are improved for the microbial degradation of the contaminants. The result obtained from the unplanted polluted soil samples (1%; 5% and 10% diesel oil) showed decrease in the microbial counts with increase in diesel oil mixture. This implies that higher percentage of diesel oil mixture reduces the total microbial population.

Results obtained from the enumeration of diesel-degrading microbes using the vapour phase proved that increase in the diesel contamination of soil increases the diesel - degrading microbial counts. This is in line with the work of Juhasz and Naidu, (2000) which showed that microorganisms decompose organic substances to generate energy and nutrients for their growth and thus, a usual consequence of biodegradation of a compound is an increase in the number of microbes degrading that substance.

The highest count was obtained from the 10% diesel oil mixture. The result also proved that presence of plant's root increase the number of diesel utilizing microbes in the soil. This can be compared to the unplanted polluted soil samples (control 2) that had lowest counts. However, it was observed that the unplanted 10% diesel oil sample mixture had higher counts than the planted unpolluted soil samples. Generally, it was observed that the Soybeans plant had the highest number of total heterotrophic counts and diesel degrading microbes. From the result it can be concluded that the microbes are actually degrading and mineralizing the substrate by increased microbial activity. Plants help with microbial transformation through mycorrhizal fungi and bacteria association with plants root which metabolize the organic pollutants. Plants exudates stimulate bacterial transformation. It increases microbial mineralization rate by building-up organic carbon (Garbisu et al., 2002). It is obvious that plants provide habitat for increased microbial populations (microbiota) and activity.

Red Guinea corn proved to be excellent candidates for phyto-remediation in that they possess long fibrous roots which provide a large surface area for root soil contact and therefore very rich in rhizosphere soil. This enhances phyto-transformation and rhizosphere bioremediation in sites contaminated with organic pollutants. This is in line with the work of Kulakow et al., (2000), which state that grasses are thought to be excellent candidates because their fibrous root systems can stabilize soil and provide a large surface area for root soil contact. This is also in line with the work of Chan and Chu (1985) which stated that plants have been shown to encourage organic contaminant reduction principally by providing an optimal environment for microbial proliferation in root zone (rhizosphere) and these degradative processes are influenced not only by rhizosphere organisms but also by unique properties of the host plant.

Similarly, legumes such as Soybeans possess root nodules formed by nitrogen fixers such as Rhizobium sp. The root nodules of leguminous plants can be said to be a microbial ecosystem where different populations of microorganisms exist and derive nutritional benefits from the symbiotic associations between the root of leguminous plants and nitrogen fixing organisms. Alternatively, a chemical may completely be degraded by series of sequential co-metabolic attacks, by various microbial species. It is also worthy of note that microbial communities exposed to hydrocarbon become adapted exhibiting selective enrichment and genetic changes resulting in increased proportion of hydrocarbon-degrading bacteria and bacterial plasmids encoding hydrocarbon catabolic genes (Gentry et al., 2002). Hence planting of legumes such as Soybeans in sites contaminated with organic pollutants such as diesel oil is tremendously helpful in the treatment of such areas.

Results obtained from rhizosphere effects showed that the microbial populations in the polluted sample were affected more by the presence of the rhizosphere and this led to their increase in numbers.

5. Conclusion

These plants, therefore, can be useful in diesel oil contaminated soil for bioremediation purposes. By encouraging these plants to grow on diesel oil polluted soils, conditions for microbial degradation of the contaminants can be enhanced and can increase the microbiota of the soil. Hence Red Guinea corn and Soybeans are recommended for diesel oil pollution control.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interests.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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