Nigerian Journal of Soil Science

Journal homepage:www.soilsjournalnigeria.com

Effect of Particle Size Biochar (*Rhizophora racemose)* of Red Mangrove on Bacteria population in Crude oil-contaminated Soil.

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ARTICLE INFO

Article history:

Received December 24, 2019

Received in revised form December 30, 2019

Accepted January 12, 2020

Available online January 30, 2020

Keywords:

Biochar
Contamination
Bacteria
Amendment
Incorporation

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https://doi.org/10.36265/njss.2020.300101

ISSN-1597-4488 cpublishingrealtime.

ABSTRACT

Soils contaminated at different levels of crude oil were amended with two-particle-sized fractions of red mangrove biochar [mm (B1) and 4 mm (B2)] from locally pyrolyzed red mangrove in earthen kiln and incorporated at five different rates (1, 2, 3, 4 and 6 mg ha^{-1}) in the Teaching and Research Farm, Faculty of Agriculture, Niger Delta University, Bayelsa State, were assessed on the fate of bacteria population and its resultant effect on the soil organic matter, soil organic carbon, total nitrogen, and plant height. Total heterotrophic bacteria were analyzed using the pour plate method with Nutrient Agar and selective nutrient media such as Centramide Agar and Macconkey Agar for qualitative analysis, and the plates were incubated at 37^{o}C for 24 hours. Twelve (12) bacteria were isolated from the study, which is six Gram-stain-negative and six Gram-stain positive. The incorporation of B1 biochar was observed to influence the SOC significantly, SOM and TN reduced from 2.90 to 0.87 %, 5.00 to 1.50, and 0.13 to 0.4 % respectively while B2 recorded slight increases on same parameters. It is concluded that the application of locally pyrolyzed biochar for the amendment of crude oil contaminated soils had little influence on the bacteria population.

1.0. Introduction

Biochar is a form of black carbon (C) created by thermal degradation of organic material (e.g., wood, manure, leaves, etc.) in a low or zero oxygen environments (pyrolysis). It is distinguished from charcoal and similar materials by its use as a soil amendment (Lehmann and Joseph, 2009). Depending on the temperatures reached during pyrolysis, and the fundamental properties of the feedstock used, biochar's chemical and physical properties may vary (Gundale and DeLuca, 2006). Biochar application to soil is being considered as a mechanism for long-term storage of C and can play a crucial role in climate change mitigation by reducing atmospheric CO_2 concentrations (Lehmann et al., 2006). Biochar may also reduce soil greenhouse gas emissions, such as nitrous oxide (N_2O) or methane (CH_4). By trapping these gases in pores (Clough et al., 2010; Gaunt and Lehmann, 2008), biochar may contribute to the decrease or a slowing of the increase in global warming. Biochar is also being examined as a means to improve soil fertility. While biochar has been studied for its effects on soil chemical and physical properties, biochar's effects on soil microbial communities are understudied. In one of the few published studies, Thies and Rillig (2009) explained that biochar could have a positive effect on microbial community biomass by providing a habitat, where bacteria and fungi could escape from predators, as well as providing substrates to meet many of their diverse C, energy, and nutrient needs. Also, some research has suggested that changes in soil microbial community composition may occur due to the application of biochar.

Pollution of the environment by petroleum products is an inevitable consequence of oil production, transportation, and distribution activities. Large amounts of petroleum products handled on land every year create the possibility for land contamination. Contamination of soils by hydrocarbon and its derivatives has assumed great prominence in many countries, and this has become a global problem (Vidali, 2001). The prevalence of oil spillage and other activities exposes the ecosystem to hazardous substances. The toxicity of crude oil or petroleum products varies widely, depending on their composition, concentration, environmental factors, and on the biological state of the organisms at the time of the contamination. Biodegradation of hydrocarbons by the natural population of microorganisms represents one of the primary mechanisms of eliminating petroleum pollution from the environment (Leahy and Colwell, 1990). The ability to degrade and or utilize hydrocarbon substrates is exhibited by a wide range of bacteria and fungi (Atlas, 1981). The ability to isolate high numbers of certain oil-degrading microorganisms from the oil-polluted environment is commonly taken as evidence that these microorganisms are the active degraders of the pollutants in the environment (Okerentugba and Ezeronye, 2003).

Microbial degradation of the hydrocarbon-contaminated site is performed with the help of a diverse group of microorganisms, particularly the indigenous bacteria present in the soil. A large number of *Pseudomonas* strains capable of degrading. Polyaromatic hydrocarbons have been isolated from soil and aquifers (Kiyohara *et al.* 1992; Johnson *et al*. 1996). Other petroleum hydrocarbon-degrading bacteria include *Bacillus, Micrococcus*, *Alcaligenes* spp., *Flavobacterium*, *Corynebacterium* spp. and *Streptococcus* spp. (Antai, 1990; Bhattacharya *et al*.

2002).

Plants and animals are also known recipients of the adverse effects of crude oil spillage and pollution. Such spillage influences soil and groundwater wellness. It is reported that oil spillage has caused a constant threat to farmlands, crop plants, forest tree species, and other vegetation in oil-producing areas in Nigeria and other parts of the world (Agbogidi, 2003; Ogri, 2001).

The objectives of the study are: (1) determine the effect of locally pyrolyzed biochar on the amelioration of soils polluted with crude oil considering cost-effectiveness and (2) assessing the capability of variation in particle size fractions of red mangrove biochar in the population of bacteria and assessing its effect on plant in soils polluted with crude oil.

2.0. Materials and Methods

2.1. Local pyrolysis

The feedstock of Red Mangrove (Rhizophora racemose) tree was locally sourced and pruned before subjected to slow pyrolysis in a mini pit (Kiln) sunk at the Teaching and Research Farm, Niger Delta University, Wilberforce Island (Amassoma, Bayelsa State; 4°58'52.8" N.6° 06'27.2"E). The kiln was adequately covered with earthen mud over thin zinc materials to limit oxygen availability with an outlet at the top for fume emission after the heat was applied and left for 48 hrs. Pyrolysed material was collected, and woody material separated before let to cool in a desiccator. The biochar derived was ground and screened through 4 and 2 mm sieves to collect two different particle sizes of < 2 mm and between 2 – 4 mm, respectively. The two amendment separates were denoted as B1 and B2, respectively.

2.2. Experimental Design

A plot size of 216 m^2 was mapped out, and six sub-plots delineated with a 1 m walk path between sub-plots for the study. Four sub-plots were contaminated with Bonny Light Crude Oil (BLCO) at levels of 10,000, 20,000, 30,000, and 40,000 L/ha while biochar amendments were incorporated in five sub-plots at levels of 1, 2, 3, 4 and 6 Mg/ha respectively. The control plot was neither contaminated with crude oil nor amended with biochar, whereas the first subplot was amended with biochar alone with no crude oil contamination to check for the effect of the amendment in uncontaminated soils. All treatment levels were replicated three times. The contaminated plots were denoted as P0, P1, P2, P3, and P4, respectively.

2.3.Soil physicochemical Analysis

Soil pH was determined using 10g of soil samples mixed with 25 mL distilled water, which were stirred and left for 30 min at room temperature (23^{o}C). Soil (pH) meter (electrode) rod was inserted in the partly settled suspension of each sample after calibration with buffers 4 and 7. Soil organic carbon (SOC) content was determined by the Walkey-Black wet oxidation method, whereas organic matter (SOM) was determined by adopting the Van Bemmelen's factor of multiplying organic carbon values by 1.724 (Pribyl, 2010). Total nitrogen was determined by the Kjeldahl method, as described by (Bremner et al., 1996).

2.4. Cultivation of Soil Bacteria

The isolation of bacteria present in the sampled soils was done by culture-dependent methods. Plating was done in triplicates using the pour plate method. The incubation of the plates was done under the aerobic condition at 37^{0} C for 24 hours. The total heterotrophic plate count was done in Nutrient Agar media. Selective nutrient media such as Macconkey Agar and Centramide Agar were used for the qualitative analysis, the presence or absence of pseudomonads, and coliform bacteria.

2.5. Biochemical characterization of bacterial isolates

The characterization of bacterial isolates was done with biochemical tests. Gram stain, catalase test, citrate utilization test, indole test, gas, and hydrogen sulphide production test, motility test, and oxidase tests were done to aid in identification.

2.6. Statistical Analysis

ANOVA analysis of pH, SOC, SOM and total nitrogen was used to determine the effects of the different particle sizes of biochar on the amelioration of the contaminated soils and its effect on the plant height, whilst Pearson correlation was used to check for relationships between crude oil contamination and biochar amendments. All analyses were carried out using MinitabTM v.17 and graphs plotted using SigmaPlotTM v.10.0 for Windows.

3.0 Results and Discussion

The result in Table 1 shows the total composition or properties of the biochar used in the experiment.

Table.1: Properties of biochar (*Rhizophora racemose).*

Biochar	**Value**
pH	8.9
Ash (%)	4.58
C (%)	72.05
H (%)	3.36
N (%)	1.07
Surface Area (m^2g^{-1})	24.8

The result in Table 1, shows the properties of the biochar used in the experiment, with carbon having the highest value and the surface area of the biochar following next. The pH value indicates the alkaline nature of the biochar which can be used as a liming material for soil remediation.

The result in Table 2 shows the Interaction effects of the particle size biochar and the pollution levels on the bacteria population (CFU/g x 10^6)

Table 2: Effects of particle size biochar and pollution levels on bacteria population (CFU/g x106)

Biochar particle size fractions	**Pollution**	**Bacterial count**
2mm	Control	1.18 x 10^6b
	P0	9.40 x 104^4b
	P1	6.37 x 10^4b
	P2	7.43 x 10^4b
	P3	1.10 x 10^6b
	P4	1.63 x 10^6b
4mm	Control	4.06 x 10^6a
	P0	8.50 x 10^4b
	P1	4.97 x 10^4b
	P2	4.27 x 10^4b
	P3	6.50 x 10^5b
	P4	1.07 x 10^6b

Same alphabets = Not significantly different; Different alphabets = Significantly different

From Table 2, the interactions of the biochar (2mm and 4mm) and the pollution levels (Control, P0, P1, P2, P3, and P4) on the bacteria population show no significant difference ($p \leq 0.05$) as the values indicate. The plot without crude oil pollution but amended with biochar (2mm and 4mm) P0, records low as seen in the result, indicating that the addition of biochar has an impact on the population of bacteria, while the plot with pollution level at P4 with biochar application (2mm and 4mm) recorded highest in value. But there was a significant difference ($p \leq 0.05$) in the control of both main plots for the amendments (2mm and 4mm).

Biochar effects on the soil chemical properties as shown in Table 3, indicates significant difference ($p>0.5$) in soil organic carbon and soil organic matter with no difference at all in the total nitrogen content of the soil.

Table 3: Chemical characteristics of soil after contamination with crude oil and amendment with biochar. (n = 3; ± S.E).

Sample	pH-H2O	SOC	SOM	Total N	C:N
	-	%	%	%	-
Cntrl	4.78	2.90 (0.54) a	5.00 (0.71) a	0.13 (0.36)	22.3
P0 B1	4.66	2.06 (0.45)	3.55 (0.60) b	0.09 (0.30)	22.9
P0 B2	4.68	2.61 (0.51)	4.50 (0.67) ab	0.11 (0.33)	23.7
P1 B1	5.03	2.64 (0.51)	4.55 (0.67)	0.12 (0.35)	22.0
P1 B2	4.96	2.49 (0.50)	4.30 (0.66)	0.11 (0.33)	22.6
P2 B1	5.12	1.74 (0.42)	3.00 (0.55) b	0.08 (0.28)	21.8
P2 B2	4.84	2.41 (0.49)	4.15 (0.64) a	0.10 (0.32)	24.1
P3 B1	5.14	1.62 (0.40)	2.80 (0.53) b	0.07 (0.26)	23.1
P3 B2	5.16	1.91 (0.44)	3.30 (0.57)	0.08 (0.28)	23.9
P4 B1	5.22	0.87 (0.29) b	1.50 (0.39) b	0.04 (0.20)	21.8
P4 B2	5.09	1.45 (0.38) b	2.50 (0.50) c	0.06 (0.24)	24.2

Same alphabets = Not significantly different; Different alphabets = Significantly different. SOC = soil organic carbon; SOM = soil organic matter.

The result in Table 3, shows significant differences between the control and P3 and P4 for OM content while OC was only at P4. Furthermore, there was no significant difference recorded between B1 and B2 for OC content in the soil at all pollution levels regardless of the high C content in the char material, but significant difference was recorded between B1 and B2 at P4 for OM. It was observed that biochar at both particulate sizes was seen to be ineffective in influencing total nitrogen content in the soil, as seen in the result.

Figures 1 to 3 shows the rate of the effect of biochar on the bacteria population, the frequency of occurrence of the bacterial isolates as well as the effect of the biochar amendment on plant height .

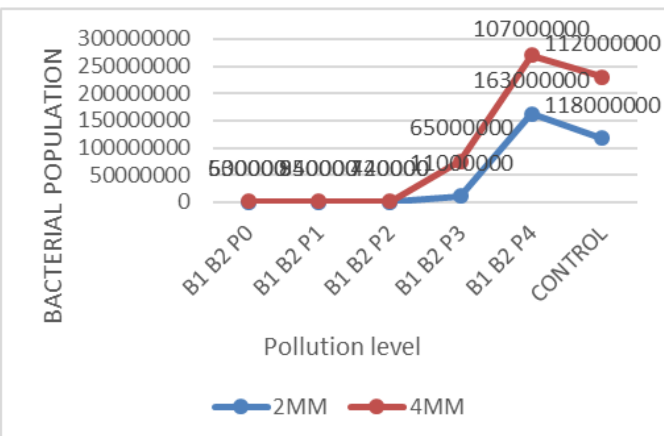

Fig 1: Rate of biochar effect on the bacteria population

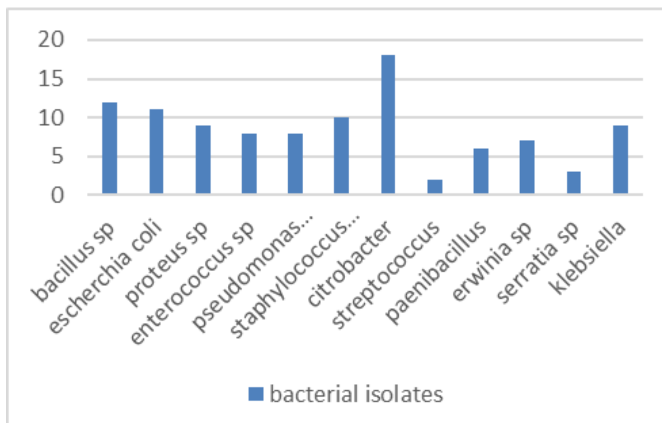

Fig. 2: Frequency of occurrence of bacterial isolates

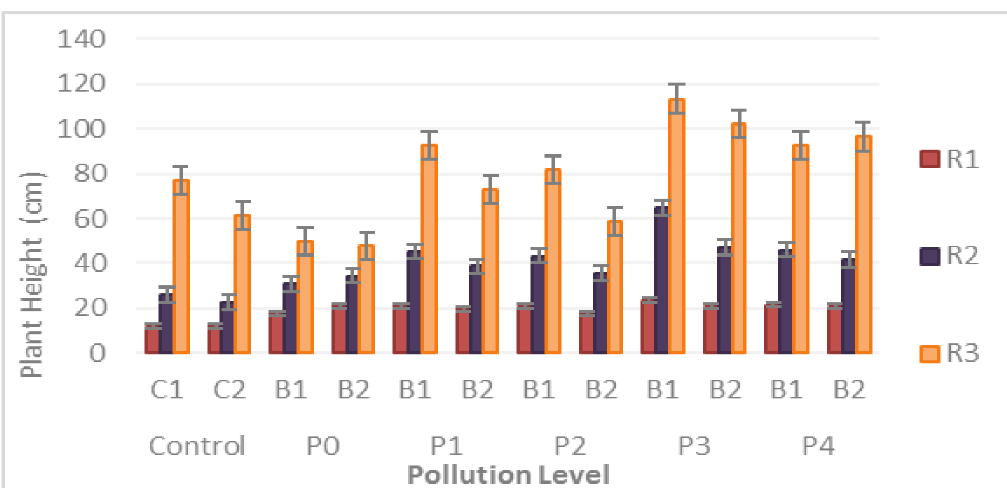

Fig. 3: Biochar effect on plant height

Figure 1 shows the population rate of the bacteria in the soil without pollution (control) and when the soil is polluted and amended with the biochar (2mm and 4mm). Soils amended with 2mm biochar recorded a high amount of bacteria present. Figure 2 shows the frequency of occurrence of the bacteria that were identified and a total of twelve (12) bacteria isolates were identified via six (6) gram-negative and six (6) gram-positive bacteria and Citrobacter as the highest and figure 3 shows the effect of the biochar on the plant height. The highest height of the plant (maize) was observed on P3B1 at 2 weeks, 4weeks, and 6 weeks after planting, respectively.

4.0 Discussion

The study showed that bacterial community increased with levels of biochar amendment of the polluted soils which agrees with (O'Neill et al., 2009; Jin, 2010), that bacterial community in soils high in biochar differs significantly from that in unmodified soils with the same mineralogy. Jin (2010) demonstrated that increasing rates of biochar addition to soil led to an increasing divergence in the bacterial community. It, therefore, becomes imperative that a whole bacterial community changed with biochar additions.

Soil microbial communities keep continually changing in response to soil characteristics, climatic, and management factors (Thies and Rillig, 2009). The

addition of biochar brings about changes to both soil

physical and chemical properties such as soil pH (Granatstein *et al*., 2009; Chintala *et al*., 2013), cation exchange capacity (Joseph *et al*., 2009) and soil aggregation (Major *et al*., 2010). Changes in soil properties are mediated by the inherent properties of biochar, *e.g*., the surface area, density, and pore size distribution, which are dependent on the nature of feedstock and pyrolysis conditions. Therefore, the soil which is directly influenced by the chemical and physical properties of biochar may ultimately affect soil-plant-microbe interactions (Quilliam *et al*., 2013). The relationship between biochar and the soil biota and their implications on different soil processes have yet not been adequately described. At the moment, there is a wide gap in our knowledge of interactions between the soil biota and biochar. This calls for systematic and strategic investigation of soil-biochar dynamics to evaluate the potential consequences of the widespread application of a seemingly excellent product.

The response of soil microorganisms (bacteria) to biochar depends on the chemical composition of biochar produced and the application rate (Chan *et al*., 2008; Weyers *et al*., 2009). Chan *et al*. (2008), however, noted that the underlying mechanisms driving these preferences required further work. It also depends on the soil type. Therefore, predicting the effects of biochar on the soil microorganisms (bacteria) whilst very important is inherently very difficult.

The main effect of different particle sizes of locally pyrolyzed biochar on the decomposition process of organic matter is not related to soil organic carbon amounts that may, in turn, affect the C:N ratio as biochar is known to contain high aromatic carbon that is highly recalcitrant.

It was observed from the study that high SOC in the control plot affected the amounts of SOM (Table 3). However, it did not translate into a low C:N ratio as nitrogen was below 0.5 % in the soil. If OM is lost and particulate biochar influences N amount, one would expect a substantial decrease in C:N ratio as reported by (Vandecasteele, et al., 2014). Results from this study, however, indicates that there was no significant difference between the control and the biochar incorporated soils for OM content. This could be a result of either crude oil pollution-reducing OM accumulation or the method of preparation of the biochar, thereby inhibiting mineralization. It could also be observed that the OC and OM amount decreased with increasing crude oil levels. However, there were significant differences between the control and P3 and P4 for OM content, while OC was only at P4 (Table. 3), indicating an increase in bacteria population due to the addition of the biochar. Furthermore, there was no significant difference recorded between B1 and B2 for OC content in the soil at all pollution levels regardless of the high C content in the char material (Table 3), but significant difference was recorded between B1 and B2 at P4 for OM. Since, biochar addition to soil has the capability of affecting soil quality based on inherent soil and biochar properties (Singh & Singh, 2010), the amount of SOC could be attributed to the type of material used, the temperature and or the method of pyrolysis which likely was not suitable for soil application by contributing insignificantly to the nutrient status and further adding recalcitrant C to the soil. A similar result was reported by (Bera et al., 2016) when soils treated with only biochar could not influence total organic carbon (TOC) due to the temperature of pyrolysis but not the method. Similarly, biochar at both particulate sizes was seen to be ineffective in influencing total nitrogen content in the soil (Table. 3).

The increase in height recorded in the maize grown in biochar amended soil could be attributed to the increased pH and soil organic matter due to the increase in nutrients released from the biochar as the microorganisms (bacteria) fed on it following application of biochar amendments. Oguntunde et al., (2004), studied the effects of charcoal on growth and yield of maize in a Ghanaian soil and attributed the significant maize growth rate they observed in the biochar-amended soils compared to the control to biochar's ability to increase the soil pH. Hence, it was observed from the study that the growth and height of the plant are dependent on the rate of biochar application.

5.0. Conclusion

The study proved that the application of locally pyrolyzed biochar had considerable influence on the quantity (population) of bacteria found in the soil, on some chemical properties, and on the growth of the plant (plant height), as seen from the result. Within the period of study, it was also found that soil pH plays a significant role in the immobilization of minerals. Comparatively, while soil pH increased with increased biochar application regardless of crude oil quantity, organic matter and organic carbon decreased with increased biochar application in the soil. On the other hand, TN was found not to be influenced by the biochar application. As evident from our experimental finding, B1 incorporation generally influenced all the chemical properties more than B2 except for TN. Low C:N ratio for both particulate sizes of biochar regardless of its insignificant effect on TN over the study period indicated substantial soil ecological functioning with substantial Carbon sequestration potential through biochar amendment in agricultural soils polluted with crude oil. Therefore, further studies are required to ascertain extents to which different particle sizes of biochar pyrolyzed locally by low-earning farmers can improve soil wellness.

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