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Comparative evaluation of the physiochemical parameters of crude oil polluted soil remediated with mushroom (*Pleurotus tuberregium*)

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Abstract
The impact of crude oil pollution on the physicochemical parameters of the polluted soil and the influence of remediating the soil with mushroom were investigated. The treatments were three different concentrations of crude oil pollution (150, 300 and 450 ml). The polluted soil was amended 14 days after pollution with sclerotia, while sawdust was added as a substrate for the sclerotia, with two sets of control: polluted soil without amendment and unpolluted soil. Results indicated that crude oil pollution significantly affected the physicochemical parameters at ($P < 0.05$). The percentage total organic carbon were significantly increased two weeks after crude oil pollution while the pH, percentage total nitrogen, phosphorus significantly decreased. The results also showed that the amendment treatments significantly decreased the toxic effect crude oil at different degrees by improving the nutrient content of the soil. The C: N ratio was significantly reduced in the different concentrations of the polluted soil compared to the control. Therefore, mushroom helps in restoration of soil fertility by increasing the nutrient content after remediation.

Keywords: bioremediation, physicochemical parameters, mushroom, crude oil, pollution

Introduction
Soil is a basic constituent of the ecosystem and the maintenance of ecological integrity that included the soil largely depends on the sustainable ecosystem [1]. Crude oil pollution has become a serious problem in the Niger delta, Nigeria and all over the globe. Thus, this resulted in loss of soil fertility, soil degradation and wasteland. this is because the nutrient in the soil are no longer available for plant and micro organism due to pollution[2]. Bioremediation which has been defined as the use of living organism, living system or its derivatives to detoxify and degrade environmental pollution[3]. The long-term use of bioremediation is to achieve a cost effective way of remediation using locally available materials and to maintain the integrity of the environment (environmental friendly process). To achieve this, researchers has used various amendments and supplements such as organic matter, mushroom, biosurfactants and natural attenuation for bioremediation.
Mushroom as one of the amendments have been used to enhance the biodegradation of crude oil polluted soil. White rot fungi (*Pleurotus tuberregium*) has been reported to be effective in the bioremediation process[4]. Crude oil pollution affects the physiochemical properties of the soil. [5] reported that crude oil pollution reduces soil nitrate and phosphorus content while the carbon content increase. Also crude oil pollution increase the carbon to nitrogen content in the soil[2]. Micro organism are also affected negatively by crude oil pollution thereby destroying the microbial flora of the soil [3]. The release of these crude oil pollutant either accidently or due to human activities causes disruption of the dynamics of the ecosystem [6].
The ability of mushroom to degrade crude oil polluted soil is well documented but the effect of the mushroom on the physiochemical properties of the remediated soil is not discussed. This investigation therefore propelled the aim of this study, to find out the effect of mushroom on the physiochemical parameters of the crude oil polluted soil.

Materials and Methods
Study Area
The study was carried at the University of Benin Botanic Garden, Benin City, Edo State of

Nigeria. The study site is along the Lagos-Benin road between 06^{o} 2358.67^{i} N latitude, 05^{o} 36.486^{i} E longitude of the tropical rainforest belt of southern Nigeria.

Sample Collection
Soil samples for the experiment were collected randomly with at the surface soil (loamy clay) between the depths of 0 to 10 cm from the garden in the Botanic Garden, University of Benin, Benin City Edo state. The soil samples were bulked together, homogenized and 5000 g was put into perforated labeled bags[7]. This perforation is to avoid water logging and good aeration of the experimental soil. A total number of 21 bags filled with homogenized experimental soil were used for the experiment. The sclerotia were purchased from Uselu market in Benin City and they were cut in small bits before application to the polluted soil. Saw dust was got from the sawmill at Uselu shell located at Benin City and the plant species used for the sawdust was *Brachystagia nigerica.* This serve as a substrate for the sclerotia. The crude oil was obtained from Nigerian National Petroleum Corporation (NNPC), Eleme, Port-Harcourt, Rivers State and was applied as pollutant to the soil.

Pollution Treatment and Amendment
Crude oil was added to the soil in the bags at various levels (0, 150, 300 and 450 ml) and thoroughly mixed with the 5000g of soil per bag. This corresponds to 3%, 6% and 9% concentrations of the polluted soil respectively. This was done in 3 replicates. The polluted soils were allowed to stand under natural environment for 14 days before application of sclerotia and sawdust as amendments. During this period, the soil samples were watered at intervals of two days with 500ml of water. After 14 days of pollution treatments, the carefully small bits of sclerotia and sawdust were carefully weighed into the bags containing the crude oil treated and in the ratio of 1:3 of soil contents

Sampling
Soil samples were collected from the bag at three different times. First was before crude oil application to ascertain the physicochemical nature of the unpolluted soil. Second was at the 14th day after crude oil pollution and third was at 150th day after amendments of crude oil polluted soil. The experiment was terminated on the 150th day.

Determination of Physiochemical Parameters of the Soil
The pH were determined by the method outlined by [8] using an electronically Jenway 3015 pH meter. The organic carbon and organic matter was determined by the titrimetric method [9]. Phosphate determination was done by ascorbic acid method by AOAC [10]. Total nitrogen was determined by Kjaldahl method as outlined by AOAC [10]. Total hydrocarbon contents were determined according to the method as outlined in [9] while the determination of, potassium was done by mixed acid digestion method AOAC [10].

Experimental Setup
5000g UNPOLLUTED SOIL (3 Replicate)
5000g SOIL+ 3%CRUDE OIL (control 1) 3 replicates
5000g SOIL+ 6%CRUDE OIL (control 2) 3 replicates
5000g SOIL+ 9%CRUDE OIL (control 3) 3 replicates
5000g SOIL+ 3%CRUDE OIL + 10% SD + 10%MR (3 replicates)
5000g SOIL+ 6%CRUDE OIL+ 10% SD + 10% MR (3 replicates)
5000g SOIL+ 9% CRUDE OIL + 10% SD + 10%MR (3 replicates)
Key: SD = saw dust; MR= Mushroom

Statistical Analysis
The results were expressed as mean ± standard error for three replicates. Analyses of variance (ANOVA) and 2 sample t- Test were carried out using PAST statistical software and mean values were separated using the Duncan multiple range test (DMRT) at ($P < 0.05$).

Results
Effect of crude oil on the physiochemical parameters of the soil before and at the point of pollution

Table 1: Physiochemical parameters of the unpolluted soil and polluted soil after 14 days of pollution

Concentrations of crude oil pollution/parameters	0%(Unpolluted Soil)	3%(150ml)	6%(300ml)	9%(450ml)
p H	5.70 ± 0.01^{a}	4.98±0.01^{b}	4.97±0.03^{c}	4.90±0.01^{d}
Organic C (%)	3.33 ± 0.11^{b}	7.86 ±0.13^{a}	7.90 ±0.01^{d}	8.00 ±0.01^{c}
Nitrogen (%)	0.15 ± 0.03^{c}	0.03±0.01^{b}	0.02±0.07^{d}	0.02±0.15^{a}
Potassium (mg/kg)	1.32 ± 0.02^{d}	0.85±0.01^{a}	0.84±0.01^{c}	0.80±0.02^{b}
Phosphorus (mg/kg)	1.03 ± 0.04^{b}	0.35±0.05^{c}	0.33±0.03^{d}	0.31±0.03^{a}
C:N Ratio	22.20 ± 5.60^{a}	262.00 ±8.68^{b}	395.00 ±10.34^{c}	400.00 ± 11.20^{d}

Values are means of three replicates ± standard error. Same alphabets in a row are not significantly different at P = 0.05 using Duncans multiple range test.

The result of the physicochemical properties of the unpolluted soil and the polluted soil after 14 days is shown in Table 1. The pH ranges from 4.90 to 4.98 for the crude oil polluted soil at various concentrations. While that of the unpolluted soil has the pH mean value of 5.70. The crude oil-polluted soil indicated acidity. The results also showed significant increase in organic carbon as the concentration of crude oil in the polluted soil increased. Table 1 also showed that the percentage of nitrogen significantly decreased as pollution concentration increased. The results of Table 1 showed a corresponding significant increase ($P = 0.05$) in carbon: nitrogen(C: N) ratio as the crude oil pollution levels increased. For instance, the carbon to nitrogen ratio in the unpolluted soil was 22.20± 5.60. The ratio increase to 262.00± 8.68 in 3% polluted soil to 395± 10.34 in 6% polluted soil then to 400.00 ± 11.20 for 9% polluted soil.

Effect of mushroom on the physiochemical parameters of the soil after 150days of bioremediation

Table 2: Physicochemical properties of 150 ml (3%) polluted soil 5 months after amendments with mushroom

PARAMETER/TREATMENT	CONTROL(unamended)	MR+SD+SOIL
p H	5.11±0.11[a]	5.33±0.03[b]
Organic C (%)	7.20±0.02[b]	8.00±0.43[e]
Nitrogen (%)	0.04±0.31[e]	0.35±0.01[b]
Potassium (mg/kg)	0.80±0.01[d]	0.90±0.01[a]
Phosphorus (mg/kg)	0.43±0.01[a]	0.58±0.01[c]
C:N ratio	180.00±6.50[c]	22.87±5.00[d]

Table 3: Physicochemical properties of 300 ml (6%) polluted soil 5 months after amendments with mushroom

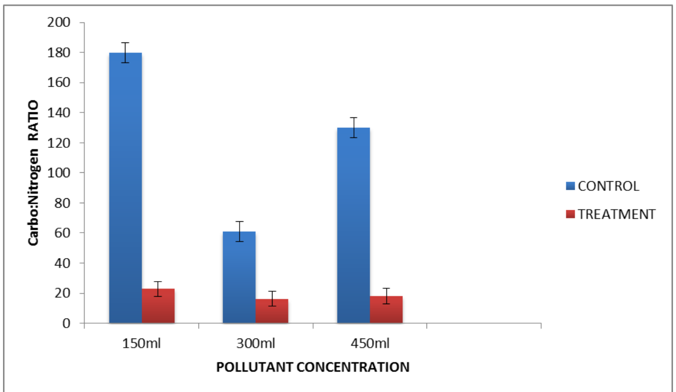
PARAMETERS/TREATMENT	CONTROL(unamended)	MR+SD+SOIL
p H	5.03 ± 0.11[a]	5.13±0.07[b]
Organic C (%)	7.94± 0.02[b]	7.96±.027[a]
Nitrogen (%)	0.13± 0.01[c]	0.49±0.01[d]
Potassium (mg/kg)	0.85± 0.05[d]	0.88±0.02[c]
Phosphorus (mg/kg)	0.41± 0.13[a]	0.57±0.01[b]
C:N ratio	61.00±6.56[b]	16.24±.5.01[c]

. *MR- Mushroom, SD- Saw dust MR- Mushroom, SD- Saw dust *Values are means of three replicates ± standard error. Same alphabets in a row are not significantly different at P = 0.05 using Duncans multiple range test.*

Table 4: Physicochemical properties of 450ml (9%) polluted soil 5 months after amendments with mushroom

PARAMETER/TREATMENT	CONTROL(unamended)	MR+SD+SOIL
PH	5.55±0.02[a]	5.54±0.02[b]
Organic C (%)	7.94±0.05[b]	9.02±0.11[a]
Nitrogen (%)	0.06±0.41[c]	0.50±0.05[d]
Potassium (mg/kg)	0.76±0.11[d]	0.92±0.01[c]
Phosphorus (mg/kg)	0.40±0.04[e]	0.60±0.05[a]
C:N ratio	130.00±6.66[c]	18.04±5.32[b]

*MR- Mushroom, SD- Saw dust *Values are means of three replicates ± standard error. Same alphabets in a row are not significantly different at P = 0.05 using Duncans multiple range test.*



The results of the physicochemical parameters of the crude oil polluted soil after 150days amendments with mushroom are shown in (Tables 2 to 4). The results indicated that mushroom significantly influenced the physicochemical parameters of the polluted soils. The increase in pH values on application of mushroom differed significantly ($P < 0.05$). The results also showed that the organic carbon contents of the crude oil polluted soil increased significantly ($P < 0.05$) on addition of mushroom. There is significant increase in the percentage nitrogen and phosphorus in the crude oil polluted soils ($P < 0.05$). There were also decreases in the carbon: nitrogen ratio (Tables 2 to 4) in all the crude oil polluted soils on the application of the mushroom. The ratio decreased compared to the value obtain at the 14th day after pollution. The carbon to nitrogen ration for 3% polluted soil control was 180± 0.16 while that of the remediated soil was 22.87 ± 0.30. There is a significant difference in carbon to nitrogen ratio in all the 3 different concentrations of the polluted soil compared to that of the control ($P< 0.05$).

Discussion

The pH, which is the degree of acidity or alkalinity of soil, affects not only the physicochemical properties but also the flora and fauna of soil. Thus, it determines the availability of many nutrients for plant growth and maintenance. The results showed a decrease in the pH value as the concentration of crude oil polluted soil increased, this agree with the reports of [11] who observed increased soil acidity following increased crude oil pollution but this study contradicts the reports of [7] who observed an increase in pH as the levels of crude oil pollution increased. Strong acidic soils (pH 4 to 5) have been reported to toxic to plants. Consequently, the lowered pH values observed in the polluted soils can be raised by liming through appropriate application of calcium and magnesium compounds.[7]. This is what the mushroom supply to the soil.

At the 14th day after pollution, percentage organic carbon content of the soil samples increased with increase in the concentration of crude oil pollution (Table 1). The increase in the percentage organic carbon content observed in this study had been reported earlier[11] and may be attributed to the activities of microorganism in mineralizing the crude oil in the polluted soil. Available percentage nitrogen and phosphorus of the soil decreased with increase in the concentration of crude oil polluted soil. [7] and [3] had also in their reports observed a decrease in nitrogen availability with increased concentrations of crude oil polluted soil. Similarly, a decrease in phosphorus availability with increased levels of crude oil pollution had been reported [12] The decrease in the available nitrogen and phosphorus with increased levels of crude oil pollution may be attributed to the limitation induced by the introduction of excess carbon to the soil since crude oil is a rich source of hydrocarbon [13]. Total extractable hydrocarbon content (THC) of soils is frequently used to assess and ascertain the extent of contamination on sites [12]. Several reports have shown that high concentration of THC in soils is detrimental to the growth and productivity of plants and animals [5]. The results obtained are in consonance with those obtained by [14]. The results also confirm earlier findings [15] that organic manures (for example, chicken droppings) have buffering effect on crude oil polluted soil. This rise in the pH of the amended soils may favour oil degradation by microorganisms as observed in similar studies that higher pH range (6 to 9) provides better conditions for mineralization of hydrocarbons since most bacteria capable of metabolizing hydrocarbons develop best at pH conditions close to neutrality [16, 18].

The results also showed a significant increase in the percentage organic carbon crude oil polluted soils amended with mushroom. Organic carbon affect soil properties such as their water holding capacity, bulk density and mobilizes nutrients for plants [19]. [13] Also reported that organic carbon when present in sufficient quantity have beneficial effect on soil chemical and physical properties. The results indicated that C : N ratios were significantly reduced in all the treatments. These results compared favourably with those documented by [15] using inorganic fertilizer, NPK and poultry manure. The fact that lower C : N ratio were recorded with poultry manure and poultry manure and cow dung manure treatments indicated that some nutrients were lacking [20]. This means that hydrocarbon loss increased with smaller C: N ratios justifying the use of organic nitrogenous nutrient sources to aid biodegradation.

Conclusion

The results indicated that crude oil adversely affect the physicochemical parameters of the soil. Results from this study also showed improved soil physicochemical parameters on crude oil contaminated soils supplemented with mushroom compared to the control that was not amended with mushroom. The increase nutritional status of the soil because of mushroom was significantly difference at (P<0.05). Consequently, the results provided evidence that showed that mushroom supplements modify the physical, chemical and biological properties of crude oil polluted soils and improve their nutritional status thereby restoring the fertility of the soil for agricultural purposes.

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