ORIGINAL ARTICLE

PHYSICO-CHEMICAL PROPERTIES AND GROWTH RESPONSE OF *Zea Mays L.* IN MYCOREMEDIATED CRUDE OIL POLLUTED SOIL USING *Lentinus Squarrosulus*

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ABSTRACT

Background: *Zea mays* L. is one of the important economic and stable food crops cultivated in the Niger Delta region of Nigeria, where crude oil pollution occurs. The pollution of agricultural lands in this region becomes a major concern to the inhabitants.
Objectives: This study was designed to evaluate the pH, and mineral nutrients contents of the experimental soils as well as examine the growth response of *Z. mays* in mycoremediated crude oil polluted soil using *Lentinus squarrosulus.* **Methods:** Fresh cultures of *Lentinus squarrosulus* were cultured on potato dextrose agar. A basal medium of sawdust in bottle was inoculated with four 0.5mm mycelial disc of the mushroom species under aseptic condition. Crude oil contamination levels of 5, 10, 15, 20, 25 and 30mls were used. The experimental set up consisted of control, pollution, and bioremediation treatments. Viable seeds of *Z. mays* were sown based on treatment, and plant growth parameters were examined. The physico-chemical parameters of crude oil polluted and unpolluted soils were determined before and after harvest using standard methods. **Results:** The contents of calcium, sodium, magnesium, nitrogen, phosphorus and potassium increased ($P < 0.05$) with increase in the concentration of crude oil in soil remediated with *L. squarrosulus,* while the contents of nitrogen and phosphorus decreased with increase in the concentration of crude oil in pollution treatment. At harvest, the pH values of experimental soil in the crude oil pollution treatment were lower than those recorded before harvest, while the pH values of treatments with *L. squarrosulus* were higher during harvest than those recorded before harvest. The plant height, stem diameter, leaf number, moisture content and grain yield of the crop in bioremediation treatment with *L. squarrosulus* were higher than those of pollution treatment at all levels of concentration of crude oil, but lower than those of the control treatment. **Conclusions:** The *L. squarrosulus* remediated soil improved the mineral nutrient contents and pH values of crude oil polluted soil as well as enhanced the growth performance of *Z. mays*. This study suggests that *L. squarrosulus* can be used in remediating the adverse effect of crude oil contaminated sites.
Keywords: pH, *Mineral nutrients, Maize, Petroleum oil, Pollution, Mushroom*

1. INTRODUCTION

Zea mays belongs to the family Poaceae and is cultivated as an important economic and food crop in the Niger Delta region of Nigeria [1,2]. It is commonly utilized as a stable food crop and as a key constituent in various dishes, as well as a raw material in some industries [1]. This crop is cultivated together with other useful plant species in the study area, where petroleum oil pollution occurs. Petroleum hydrocarbon pollutant is one of the toxic organic chemicals, which are introduced through various sources into the environment [3,4]. The contamination of viable lands has generated growing public concerns due to the use of such lands for agricultural activities [5]. The negative impacts of petroleum oil pollution on the environments include; increase in organic carbon contents of soil, reduction in soil nitrate and phosphorus, and distortion to microbial activities as well as reduction in plant growth and development [6]. These adverse effects together with destruction of soil structure and texture result in deteriorating soil conditions that make degradation of oil in the soil difficult [6,7]. Several researches conducted in recent times on a large scale bioremediation approaches utilize bacteria with only few attempts to use white rot fungi [8]. White rot fungi however, have been reported to possess the ability to oxidize a wide diversity of compounds compared to bacteria [9]. In addition, they exhibit a high degree of tolerance to high concentration of polluting chemicals than bacteria [10], hence, a valuable tool in mycoremediation studies [8].

This research was carried out in view of the fact that the Niger Delta region of Nigeria has been under the threat of petroleum pollution over the years [3,4]. Similarly, the study area has been subjected to oil exploration activities with corresponding effects of contamination on the ecosystem [7]. Although, various remediation approaches have been adopted and reported, very few examine the response of plants to crude oil polluted soil remediated with mushroom.

Therefore, this study was designed to evaluate the physico-chemical properties and growth response of *Zea mays* in mycoremediated crude oil polluted soil using *Lentinus squarrosulus.*

2. MATERIALS AND METHODS

2.1 Collection of materials: Soil samples used for this experiment were collected (1-45cm depth) from University of Port Harcourt Botanical Garden, River State, Nigeria. The soil samples were air dried to constant weight and sieved with 2mm Mesh. Pure cultures of *Lentinus squarrosulus* were obtained from African Centre for Mushroom Research and Innovation, University of Benin, Benin City, Edo State, Nigeria. Crude oil was collected from Nigerian National Petroleum Corporation, River State, Nigeria.

2.2 Method of spawn preparation: Fresh cultures of *L. squarrosulus* were by flaming an inoculating loop to be red hot, after which, it was used to cut a portion of the fresh mushroom into freshly prepared PDA. The cultures were then incubated for about seven days in an incubator. The centre of each PDA plate containing each of the mushroom isolates was punched with a cork borer with a diameter of 0.5 mm to form a fungal mycelia disc. This was done according to the method of [11]. 40g of saw dust was measured in a weighing balance and then transferred to a clean bowl where it was filtered to remove chaff and other particles. The saw dust was then transferred again into a clean bowl and mixed with water to make it moist. The moist saw dust was then put into a spawn flask. After which it was autoclaved at 121^{0}C for 30 minutes for 3 days. The saw dust in the bottles was inoculated with four 0.5mm mycelial discs of *L. squarrosulus* under aseptic conditions [12]. These were then incubated at room temperature (28±2^{0}C) for three months.

2.3 Preparation of the crude oil contaminated substrate: The technique used for the screening of the bioremediative properties of *L. squarrosulus* was done by modifying the method of [13]. Two hundred grams (200g) of soil were measured into locally available bottles and mixed thoroughly with the crude oil based on the concentration. The concentration levels of 5, 10, 15, 20, and 30mls were used alongside a control treatment (0ml).Thirty grams (30g) of sawdust were laid on the contaminated soil in each bottle separated with wire gauze. The bottles containing the soil, saw dust and crude oil were then sterilized in an autoclaved at 115^{0}C for 30 minutes. Ten grams (10g) of fungal inocula of *L. squarrosulus* were aseptically weighed and transferred into the already sterilized bottles containing the soil and sawdust substrate for the mushroom. The cultures were incubated at 25^{0}C for three months. The experimental set up consisted of the following treatment: Control (soil only), Pollution treatment (Soil + each concentration level of crude oil: 5, 10, 15, 20, and 30mls, respectively), and Bioremediation treatment (Soil + each concentration of crude oil + spawns of *L. squarrosulus*). The experimental set up was replicated five times, and further used for assessment of test crop growth response in the bioremediated crude oil polluted soil.

2.4 Germination studies: Viable seeds of *Zea mays* were sorted out, sterilized with 0.01% mercuric chloride solution for 30 seconds, washed several times with distilled water and air dried. Five (5) seeds of the test crop were sown in plastic containers containing one-quarter level of spawn of crude oil polluted soil colonized by *L. squarrosulus* based on treatment: Control (soil only), Pollution treatment (Soil + each concentration level of crude oil - 5, 10, 15, 20, and 30mls, respectively), Bioremediation treatment (Soil + each concentration of crude oil + spawns of *L. squarrosulus*). The seedlings were thinned to three (3) per container. Five replicates were used for each treatment using randomized complete block design. The experimental set up was maintained at a mean minimum temperature of 22.32^{o}C and mean maximum temperature of 34.18^{o}C, under natural light condition for four (4) months.

2.5 Analysis of experimental soils: The physico-chemical properties (pH, organic carbon, calcium, magnesium, sodium, nitrogen, potassium, and phosphorus) of the experimental soils were analyzed using standard procedures [14]. Experimental soils (control, pollution, and bioremediation treatments) were analyze before and after harvest.

2.6 Growth Studies: Plant height, leaf number, stem diameter, moisture content, and grain yield were measured at the end of the experiment.

2.7 Statistical Analysis: Data analysis was carried out using analysis of variance (ANOVA) ($P < 0.05$) using the method of [15].

3. RESULTS

In Table 1, the pH values, and mineral nutrient contents of the experimental soil are presented. The pH values of the soil decreased with increase in the concentration of crude oil in all treatments (Table 1). The calcium, magnesium, phosphorus, sodium, and potassium contents in crude oil pollution and bioremediation treatments were relatively higher ($P < 0.05$) than those of the control treatment. The contents of calcium, sodium, magnesium, nitrogen, phosphorus and potassium increased with increase in the concentration of crude oil in soil remediated with *Lentinus squarrosulus* (Table 1). The contents of nitrogen and phosphorus decreased with increase in the concentration of crude oil in pollution treatment (Table 1). At harvest, the pH values of experimental soil in the crude oil pollution treatment were lower than those recorded before harvest, while the pH values of treatments with *L. squarrosulus* were higher during harvest than those recorded before harvest (Table 2). The calcium, magnesium, sodium, nitrogen, phosphorus and potassium contents increased ($P < 0.05$) with increase in the concentration of crude oil in soil remediated with *L. squarrosulus*, while nitrogen and phosphorus decreased with increase in the concentration of crude oil pollution in pollution treatment (Table 2). The plant height, stem diameter, leaf number, moisture content and grain yield of the crop decreased with increase in the concentration of crude oil in all treatments (Table 3). The crop growth parameters were higher in the bioremediation treatment with *L. squarrosulus* than those of pollution treatment at all levels of concentration of crude oil, but lower than those of the control treatment (Table 3).

Table 1: The table presents the physico-chemical properties of the experimental soils (Pollution treatment-PT, Bioremediation treatment-BT) before harvest.

Conc. of crude oil (ml):		**0**	**5**	**10**	**15**	**20**	**25**	**30**
Parameters								
pH	PT	6.20±0.23	5.34±0.37	5.16±0.53	5.10±0.57	4.84±0.66	4.90±0.42	4.86±0.20
	BT	6.20±0.23	5.72±0.63	5.64±0.98	5.63±0.30	5.60±0.27	5.57±0.39	5.50±0.75
Ca	PT	0.08±0.02	0.12±0.03	0.16±0.07	0.20±0.01	0.35±0.04	0.62±0.03	0.74±0.02
(mg/100g)	BT	0.08±0.02	0.28±0.08	1.42±0.24	1.60±0.32	2.07±0.67	2.72±0.13	2.86±0.16
Mg	PT	0.15±0.03	0.82±0.02	0.96±0.04	1.34±0.37	1.48±0.75	2.27±0.42	2.52±0.17
(mg/100g)	BT	0.15±0.03	1.72±0.16	3.21±0.53	3.47±0.22	4.20±0.34	4.32±0.21	4.46±0.40
Na	PT	0.16±0.02	1.73±0.21	4.66±0.90	4.76±0.64	5.92±0.23	6.72±0.47	6.96±0.50
(mg/100g)	BT	0.16±0.02	1.75±0.33	3.12±0.67	3.22±0.53	4.88±0.36	4.92±0.39	5.36±0.32
N	PT	0.59±0.07	0.21±0.04	0.16±0.01	0.14±0.03	0.12±0.01	0.10±0.02	0.09±0.02
(%)	BT	0.59±0.07	0.36±0.02	0.47±0.03	0.54±0.02	0.61±0.03	0.88±0.04	0.97±0.03
P	PT	7.37±0.67	4.27±0.56	4.20±0.33	3.07±0.44	2.12±0.52	1.76±0.41	0.17±0.04
(mg/100g)	BT	7.37±0.67	6.17±0.45	7.07±0.21	7.26±0.27	8.18±0.32	8.40±0.26	8.72±0.43
K	PT	0.47±0.03	0.72±0.04	0.76±0.06	0.87±0.02	0.98±0.03	1.24±0.02	1.37±0.04
(mg/100g)	BT	0.47±0.03	0.61±0.07	0.63±0.03	0.74±0.06	0.78±0.01	0.82±0.03	0.84±0.02

The results are presented as mean ± standard error from 5 replicates; **PT**: Pollution treatment; **BT**: Bioremediation treatment.

Table 2: The table presents the physico-chemical properties of the experimental soils (Pollution treatment-PT, Bioremediation treatment-BT) After harvest.

Conc. of crude oil (ml):		**0**	**5**	**10**	**15**	**20**	**25**	**30**
Parameters								
pH	PT	6.08± 0.34	5.21±0.45	5.14±0.21	5.08±0.34	4.62±0.23	4.50±0.36	4.48±0.24
	BT	6.08± 0.34	5.83±0.56	5.76±0.43	5.72±0.27	5.66±0.35	5.30±0.20	5.24±0.43
Ca	PT	0.04±0.01	0.07±0.02	0.09±0.03	0.12±0.03	0.17±0.02	0.20±0.02	0.26±0.04
(mg/100g)	BT	0.04±0.01	0.21±0.03	0.47±0.08	0.58±0.09	0.72±0.01	0.94± 0.05	1.06±0.21
Mg	PT	0.10±0.01	0.34±0.03	0.54±0.02	0.74±0.07	0.87±0.06	1.19±0.22	1.24±0.33
(mg/100g)	BT	0.10±0.01	0.12±0.05	0.66±0.06	0.70±0.04	0.84±0.02	1.17±0.34	1.33±0.56
Na	PT	0.26±0.02	1.10±0.32	1.46±0.23	2.72±0.87	3.96±0.42	4.62±0.35	4.98±0.66
(mg/100g)	BT	0.26±0.02	1.07±0.22	1.32±0.56	1.63±0.43	2.62±0.33	2.72±0.64	2.92±0.31
N	PT	0.24±0.03	0.14±0.02	0.10±0.03	0.09±0.02	0.07±0.02	0.05±0.01	0.03±0.02
(%)	BT	0.24±0.03	0.20±0.06	0.25±0.04	0.33±0.04	0.37±0.05	0.42±0.02	0.56±0.03
P	PT	6.23 ±0.26	4.01±0.44	3.72±0.61	2.96±0.73	1.96±0.22	1.73±0.12	0.12±0.02
(mg/100g)	BT	6.23±0.26	5.10±0.49	6.42±0.26	6.61±0.33	7.18±0.31	7.33±0.41	7.27±0.54
K	PT	0.22±0.02	0.84±0.07	0.87±0.04	0.92±0.03	1.21±0.72	1.30±0.34	1.39±0.43
(mg/100g)	BT	0.22±0.02	0.62±0.05	0.74±0.02	0.86±0.06	0.96±0.02	1.02±0.28	1.28±0.55

The results are presented as mean ± standard error from 5 replicates; **PT**: Pollution treatment; **BT**: Bioremediation treatment.

Table 3: The table presents the growth parameters of Zea *mays* in crude oil polluted soil remediated with *Lentinus squarrosulus* after harvest.

Conc. of crude oil (ml):		0	5	10	15	20	25	30
Parameters								
Plant Height (cm)	PT	260.17±0.27	162.21±0.23	158.10±0.47	152.70±0.65	143.26±0.81	139.24±0.36	133.63±0.33
	BT	260.17±0.27	242.30±0.54	227.42±0.38	214.66±0.32	206.13±0.55	192.17±0.43	184.32±0.93
Stem diameter (cm)	PT	2.96±0.27	1.92±0.47	1.70±0.54	1.45±0.70	1.21±0.41	1.09±0.26	1.04±0.11
	BT	2.96±0.27	2.30±0.66	2.20±0.18	1.80±0.20	1.40±0.59	1.38±0.32	1.32±0.53
Leaf Number	PT	17.24±0.40	12.42±0.49	9.50±0.68	8.21±0.76	6.17±0.52	5.20±0.38	5.07±0.29
	BT	17.24±0.40	14.80±0.90	12.26±0.72	10.17±0.25	8.23±0.20	7.21±0.45	7.06±0.34
Moisture content (%)	PT	69.30±0.22	52.36±0.75	42.17±0.26	42.07±0.61	40.81±0.66	40.02±0.40	38.07±0.12
	BT	69.30±0.22	67.21±0.60	64.36±0.33	62.18±0.95	61.34±0.72	58.02±0.26	54.21±0.28
Grain yield (g)	PT	197.47±0.50	161.41±0.62	154.33±0.34	132.77±0.99	120.16±0.40	114.21±0.37	107.26±0.30
	BT	197.47±0.50	180.21±0.23	172.18±0.20	160.42±0.18	147.23±0.26	138.18±0.19	128.21±0.26

The results are presented as mean ± standard error from 5 replicates; **PT**: Pollution treatment; **BT**: Bioremediation treatment.

4. DISCUSSION

In this study, the pH values and mineral nutrients were negatively affected in the pollution treatments. These adverse effects on soil properties may be due to destruction of soil structure and texture that result in deteriorating soil conditions. Petroleum oil pollution has been shown to adversely affect soil physical and chemical properties as indicated in this study. The negative impacts of petroleum oil pollution on the environments include; increase in organic carbon contents of soil, reduction in soil nitrate and phosphorus, and distortion to microbial activities as well as reduction in plant growth and development [2-6]. *Zea mays* recorded low performance in plant height, leaf number, stem diameter, moisture content and grain yield in crude oil pollution treatment relative to the control and *L. squarrosulus* remediated soil. These reductions may be due to distortion of the soil-plant -water relationship resulting from the deteriorating soil conditions in the crude oil pollution treatment. Crude oil polluted soil is characterized by reduction in oxygen and water holding capacity of the soil as well as water permeability through the soil due to partial coating of soil surface by hydrocarbons [6-16]. Plants subjected to crude oil pollution may exhibit reduction in growth, photosynthetic rate, plant height, density and above ground biomass as well as death of the whole plant [17,18]. The reduction in pH values of the crude oil polluted soil might have contributed to the low growth performance of the test crop in the pollution treatment. Petroleum oil polluted soil has been reported to affect plant nutrition with the promotion of aluminium toxicity, and the distortion of metabolic processes due to increased osmotic potential as well as decreased rhizobial growth as a result of acidity [17-19].

In crude oil polluted soil remediated with *L. squarrosulus,* the resulted favourably soil conditions and pH range of the soil enhanced the nutrient base and microbial activities than those of pollution treatment. Therefore, the enhanced growth of *Z. mays* in crude oil polluted soil remediated with *L. squarrosulus* as observed in this study may be attributed to the important role played by the mushroom species in degradation of the petroleum pollutant. Fungi, have been reported to possess the ability to oxidize a wide diversity of compounds [9], and exhibit a high degree of tolerance to high concentration of polluting chemicals than bacteria [10], hence, a valuable tool for remediation of crude oil polluted soil [8], as used in this study.

5. CONCLUSION

The growth parameters of *Z. mays* were enhanced in crude oil polluted soil ameliorated with *L. squarrosulus* than those of the pollution treatment. The *L. squarrosulus* remediated soil improved the mineral nutrient contents and pH values of crude oil polluted soil. This study suggests that *L. squarrosulus* can be used in remediating the adverse effect of crude oil contaminated sites.

6. REFERENCES

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