



LEVELS OF HEAVY METALS AND GROWTH RESPONSE OF *Abelmoschus* IN MYCOREMEDIATED CRUDE OIL POLLUTED SOIL USING

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ABSTRACT

Background: Crude oil contains heavy metals together with other chemical components, which may adversely affect plant growth as well as pose serious risks to human health when the products from such plants are consumed. **Objectives:** This study was designed to evaluate the growth performance of *Abelmoschus esculentus* and iron, lead, zinc, copper and manganese contents of crude oil pollution and bioremediation treatments using *Pleurotus ostreatus*. **Methods:** Ten grams (10g) grams of fungal inocula of *P. ostreatus* were aseptically weighed and transferred into the already sterilized bottles containing the crude oil contaminated soil and sawdust substrate for the mushroom. The cultures were incubated at 25°C for three months. The following treatments were used for this study: Control-0ml (soil only), Pollution treatment (Soil + each concentration of crude oil - 5, 10, 15, 20, and 30mls, respectively), and Bioremediation treatment (Soil + each concentration of crude oil: 5, 10, 15, 20, and 30mls + spawns of *P. ostreatus*, respectively). The physico-chemical parameters of crude oil polluted spawn were determined before and after harvest using standard methods. The experimental set up was maintained for germination and growth studies of *A. esculentus*. **Results:** There were marked variations ($P < 0.05$) in iron, lead, zinc, copper and manganese contents of crude oil pollution and bioremediation treatments before and after harvest. During harvest, the plant height, root length, fresh weight, dry weight, leaf area and fruit number of the plants in bioremediation treatment with *P. ostreatus* recorded higher values than those of the pollution treatment. **Conclusions:** This study clearly indicates that *P. ostreatus* can grow optimally as well as detoxify contaminants in crude oil polluted soil, hence improving the soil conditions for the growth of *A. esculentus*.

Keywords: Trace metals, Okra, Petroleum oil, Pollution, Mushroom

1. INTRODUCTION

The consequence of crude oil pollution on soil fertility and plant growth requires serious concern, especially in the Niger Delta region of Nigeria [1,2]. The adverse effects of crude oil pollution have been reported to be a function of the concentration of pollution [3,4]. Crude oil contains heavy metals together with other chemical components. Trace metals are known to be essential in plant nutrition, however plants growing in media with high level of trace metals pose serious risks to human health when the products from such plants are consumed [5,6]. Reports also indicates that heavy metals may reduce the availability of nutrients to plants when present in excessive concentration and affect biochemical processes such as litter decomposition, soil respiration, nitrogen mineralization and activities of key microorganisms [7]. *Abelmoschus esculentus* L. Moench belongs to the Family Malvaceae, and is widely cultivated in the tropics mainly for its fruit, which is used as a vegetable in both green and dried state. The fruit has mucilaginous characteristic, hence are used in tropical cockery to thicken soups, sauces and stews [8,9,10]. In recent years, there has been increasing interest by researchers in the use of micro-organisms (fungi or bacteria), to degrade pollutants in the environment. Bioremediation involves the application of microorganisms for effective biodegradation of contaminants [11]. The use of fungi in this study for bioremediation of crude oil polluted soil is supported by the fact that they can degrade petroleum oil better than other traditional remediation techniques, as well as microorganisms such as bacteria [12,13].

This study becomes increasingly important due to the problems associated with crude oil pollution of the environment in the Niger Delta region of Nigeria, where this research was carried out. In consequence, petroleum oil pollution may affect the physical and chemical properties of agricultural soil with deleterious impacts on the growth and development of cultivated crops in the study area. Appropriate approaches with economical and eco-friendly measures are needed in order to ameliorate the negative impacts of petroleum oil pollution in the study area, which is one of the major objectives

of this research. Therefore, this study was carried out to evaluate the levels of heavy metals and growth response of *A. esculentus* in mycoremediated crude oil polluted medium using *P. ostreatus*.

2. MATERIALS AND METHODS

2.1 Source of materials: Fresh cultures of *Pleurotus ostreatus* were obtained and identified by the African Centre for Mushroom Research and Innovation, University of Benin, Benin City, Edo State. Soil samples collected at a depth of 1-45cm from University of Port Harcourt Botanical Garden, River State, Nigeria were air dried to constant weight and sieved with 2mm Mesh. Crude oil sample was collected from Nigerian National Petroleum Corporation (NNPC), River State, Nigeria.

2.2 Culture media: The potato dextrose agar (PDA) used in this study was sterilized by autoclaving at 15psi (121°C) for 15 minutes. Chloramphenicol at 0.02gm per 200ml of medium was introduced at pouring to inhibit the growth of bacteria. Inoculation and transfer of culture were carried out on sterile inoculating bench after wiping with methylated spirit.

2.3 Sterilization: All glass wares used in this study were properly washed in OMO detergent, rinsed in several changes of tap water and finally with distilled water and allowed to dry. They were sterilized in an electric oven at a temperature of 60°C for 24 hours.

2.4 Preparation of spawn: This was done according to the method of [14]. Fresh *Pleurotus ostreatus* were aseptically cut and transferred into freshly prepared PDA and the cultures were then incubated for about seven days in an incubator. 40g of saw dust was measured using a weighing balance and then transferred to a clean bowl where filtration was carried out to remove unwanted particles. The saw dust was then moistened by mixing with water in a clean bowl. The moist saw dust was then transferred to a spawn flask, and autoclaved at 121°C for 30 minutes for 3 days. The saw dust in the bottles was inoculated with four 0.5mm mycelial discs of *P. ostreatus* under aseptic conditions [15], and incubated at room temperature (28±2°C) for three months.

2.5 Preparation of the crude oil contaminated substrate: The preparation of crude oil pollution and bioremediation treatment using *P. ostreatus* spawns, sawdust and soil samples were carried out by modifying the method of [16]. The following treatments were used for this study: Control-0ml (soil only), Pollution treatment (Soil + each concentration of crude oil: 5, 10, 15, 20, and 30mls, respectively), and Bioremediation treatment (Soil + each concentration of crude oil: 5, 10, 15, 20, and 30mls + spawns of *P. ostreatus*, respectively). For pollution treatment, 200g of soil were measured into locally available bottles and mixed thoroughly with the crude oil based on the concentration. For bioremediation treatment, 30g of sawdust were laid on the crude oil 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°C for 30 minutes. Ten grams (10g) grams of fungal inocula of *P. ostreatus* 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°C for three months. Five replicates were used, and the experimental set up maintained for germination and growth studies of *Abelmoschus esculentus*.

2.6 Green house experiment: Seeds of *A. esculentus* obtained from local farmers in River State were sterilized with approximately 0.01% mercuric chloride solution for 30 seconds, thoroughly washed several times with distilled water and air dried. Five (5) seeds of the test crop were sown directly in each plastic container containing one-quarter level of spawn of crude oil polluted soil colonized by *P. ostreatus* based on treatment: Control-0ml (soil only), Pollution treatment (Soil + each concentration of crude oil: 5, 10, 15, 20, and 30mls, respectively), Bioremediation treatment (Soil + each concentration of crude oil: 5, 10, 15, 20, and 30mls + spawns of *P. ostreatus*, respectively). The seedlings were thinned to three (3) per container. Each level of treatment was replicated five times using randomized complete block design. The experimental set up was maintained at a mean minimum temperature of 22.32°C and mean maximum temperature of 34.18°C, under natural light condition for four (4) months.

2.7 Growth Studies: Growth parameters such as plant height, root length, leaf area, fresh weight, dry weight, moisture content, and fruit number were determined after harvest.

2.8 Analysis of pH and heavy metals in experimental soil: The pH values of soils were determined in a 1:2 soil to liquid suspension using an electro pH meter [17]. Soil samples were digested using wet digestion method of [18]. 0.5g of air dried, ground and sieved soil samples was measured into a digestion tube. 6 ml aqua regia and 1.5 ml H₂O₂ were accurately measured into the digestion tube and shaken gently to homogenize the mixture. The digestion tubes were transferred to digestion furnace, maintained at 180°C for 3h. Whatman No.42 filter paper was used to filter the digest after cooling, and then diluted to 50ml by double distilled water. Samples were transferred to acid-washed stoppered glass bottle, and kept for metal analysis. The required instrument was calibrated using calibration blank and series of working standard solutions of each metal to be analysed. Flame atomic absorption spectrophotometer was used to

determine the concentration of heavy metals (Fe, Zn, Mn, Cu, and Pb) from the digested samples. The final concentrations of the metals in the soil samples are calculated using the following formula:

$$\text{Concentration (mg/kg)} = \frac{\text{Concentration (mg/l)} \times V}{W} \quad (1)$$

Where **V**= final volume (50ml) of solution, and **W**= initial weight (0.5g) of sample measured.

2.9 Statistics: The data generated from the study were subjected to analysis of variance (ANOVA) where the differences in the means were tested using Least Significant Difference (LSD), according to the method of [19].

3. RESULTS

The iron, lead, zinc, copper and manganese contents of the crude oil pollution treatment and crude oil polluted soil remediated with *P. ostreatus* increased with increase in the concentration of crude oil. The values recorded in crude oil pollution treatment were significantly ($P < 0.05$) higher than those of the bioremediation treatment with *P. ostreatus* (Table 1). During harvest, the iron, lead, zinc, copper and manganese contents of the crude oil pollution and bioremediation treatments increased with increase in the concentration of crude oil in all treatments (Table 2). There were marked variations ($P < 0.05$) in iron, lead, zinc, copper and manganese contents of crude oil pollution and bioremediation treatments before and after harvest (Table 2). During harvest, substantial amount of heavy metals were recorded in pollution treatment than those of bioremediation treatment (Table 2). During harvest, the plant height, root length, fresh weight, dry weight, leaf area and fruit number of the plants in bioremediation treatment with *P. ostreatus* recorded higher values than those of the pollution treatment. Although, these values were lower than those of the control treatment, the crop growth parameters in bioremediation treatment with *P. ostreatus* competed favourably with those of the control treatment, mostly at 5ml concentration of crude oil remediated soil (Table 3). At 5ml concentration in crude oil polluted soil remediated with *P. ostreatus*, the fruit number was unaffected relative to the control treatment, while at 20, 25 and 30ml concentration of crude oil pollution, there were no fruiting (Table 3).

Table 1: The table presents heavy metals contents crude oil polluted soil remediated with *Pleurotus ostreatus* before harvest

Conc. of crude oil (ml):		0	5	10	15	20	25	30
Parameters								
Fe (mg/100g)	PT	0.56±0.03	0.82±0.05	0.97±0.10	1.08±0.24	1.16±0.28	1.27±0.74	1.46±0.64
	BT	0.56±0.03	0.62±0.07	0.76±0.21	0.92±0.13	1.01±0.32	1.18±0.29	1.24±0.33
Pb (mg/100g)	PT	0.30±0.02	0.61±0.10	0.77±0.06	0.92±0.02	1.52±0.54	1.73±0.02	1.86±0.56
	BT	0.30±0.02	0.42±0.03	0.56±0.04	0.67±0.05	0.74±0.11	0.92±0.15	1.06±0.27
Zn (mg/100g)	PT	0.14±0.01	0.22±0.02	0.32±0.06	0.46±0.05	0.54±0.02	0.66±0.30	1.27±0.44
	BT	0.14±0.01	0.17±0.04	0.21±0.03	0.24±0.02	0.26±0.03	0.35±0.14	0.42±0.16
Cu (mg/100g)	PT	0.54±0.03	0.76±0.05	0.82±0.13	0.86±0.02	0.92±0.02	1.17±0.33	1.21±0.02
	BT	0.54±0.03	0.62±0.04	0.68±0.02	0.74±0.02	0.83±0.05	0.90±0.16	1.06±0.03
Mn (mg/100g)	PT	0.48±0.06	0.87±0.04	1.07±0.53	1.67±0.34	1.92±0.42	2.07±0.62	2.18±0.56
	BT	0.48±0.06	0.56±0.09	0.62±0.26	0.68±0.13	0.70±0.11	0.76±0.16	0.85±0.65

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

Table 2: The table presents heavy metals contents crude oil polluted soil remediated with *Pleurotus ostreatus* after harvest

Conc. of crude oil (ml):		0	5	10	15	20	25	30
Parameters								
Fe (mg/100g)	PT	0.36±0.02	0.61±0.02	0.77±0.17	0.82±0.15	0.97±0.28	1.16±0.48	1.27±0.33
	BT	0.36±0.02	0.43±0.06	0.52±0.02	0.76±0.19	0.82±0.32	0.90±0.21	0.98±0.24
Pb (mg/100g)	PT	0.16±0.04	0.30±0.12	0.52±0.03	0.67±0.13	0.82±0.24	0.91±0.03	1.06±0.06
	BT	0.16±0.04	0.26±0.04	0.31±0.02	0.42±0.05	0.48±0.04	0.52±0.05	0.86±0.07
Zn (mg/100g)	PT	0.09±0.01	0.17±0.02	0.19±0.04	0.27±0.03	0.38±0.04	0.52±0.04	0.70±0.14
	BT	0.09±0.01	0.10±0.03	0.13±0.02	0.16±0.02	0.17±0.02	0.20±0.10	0.23±0.04
Cu (mg/100g)	PT	0.43±0.02	0.63±0.02	0.70±0.10	0.77±0.04	0.86±0.10	0.94±0.24	1.07±0.04
	BT	0.43±0.02	0.55±0.03	0.59±0.04	0.61±0.12	0.67±0.06	0.72±0.12	0.82±0.05
Mn	PT	0.31±0.03	0.61±0.02	0.72±0.33	0.86±0.42	1.02±0.02	1.20±0.24	1.30±0.60

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

Table 3: The table presents growth parameters of *Abelmoschus esculentus* in crude oil polluted soil remediated with *Pleurotus ostreatus* after harvest.

Conc. of crude oil (ml):		0	5	10	15	20	25	30
Parameters								
Plant	PT	39.40±0.27	29.27±0.42	25.21±0.21	21.72±0.54	18.26±0.82	16.72±0.87	15.07±0.33
Height (cm)	BT	39.40±0.27	41.36±0.36	38.72±0.42	37.21±0.93	35.14±0.21	33.61±0.17	31.64±0.49
Root Length (cm)	PT	19.40±0.34	16.32±0.20	13.47±0.32	11.32±0.37	9.60±0.40	9.40±0.34	8.46±0.61
	BT	19.40±0.34	19.38±0.41	18.56±0.23	17.80±0.50	17.36±0.45	16.40±0.46	16.27±0.73
Fresh weight (g)	PT	9.63±0.16	6.27±0.28	4.39±0.49	4.07±0.36	3.22±0.43	3.16±0.47	2.92±0.20
	BT	9.63±0.16	10.37±0.33	10.07±0.24	9.40±0.22	7.86±0.29	7.42±0.19	7.04±0.33
Dry weight (g)	PT	3.14±0.20	2.06±0.43	1.47±0.29	1.76±0.44	1.34±0.42	1.23±0.40	1.06±0.12
	BT	3.14±0.20	3.03±0.36	2.94±0.43	3.90±0.64	2.46±0.62	2.07±0.26	1.82±0.52
Leaf area (cm ²)	PT	128.40±0.37	74.67±0.26	42.17±0.53	38.24±0.77	18.20±0.24	16.21±0.83	15.33±0.63
	BT	128.40±0.37	128.54±0.61	121.52±0.42	109.36±0.21	76.44±0.62	65.37±0.51	62.25±0.18
Fruit number	PT	2.00±0.30	1.32±0.02	1.00±0.03	0.89±0.02	0.00±0.00	0.00±0.00	0.00±0.00
	BT	2.00±0.30	2.00±0.07	1.67±0.16	1.56±0.26	1.44±0.13	1.33±0.10	1.22±0.02

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

4. DISCUSSION

The contents of iron, lead, zinc, copper and manganese recorded in crude oil pollution treatment were significantly ($P < 0.05$) higher than those of the bioremediation treatment with *P. ostreatus*. Similarly, there were marked variations in iron, lead, zinc, copper and manganese contents of crude oil pollution and bioremediation treatments before and after harvest. These variations in the contents of heavy metals in crude oil polluted soil relative to unpolluted soil and remediated soil with *P. ostreatus* may be attributed to changes in soil physical, chemical and biological properties usually associated with crude oil pollution. These changes in soil physico-chemical properties may contribute significantly to bioavailability of metallic ions in soils [20]. This explains the reason for high contents of heavy metals in crude oil pollution treatment compared to those in unpolluted and bioremediation treatments as indicated in this study. The soil structure, texture and moisture greatly influence the movement of solute, salt solubility, chemical reactions and microbial activities as well as bioavailability of the metal ions [21,22]. Although, the innate capacity of the plant species to absorb metals affect the contents of metals in plant tissue, availability of metallic ions in soil may depend on the pH, binding or ion exchange with the soil medium [22,23].

The plant height, root length, fresh weight, dry weight, leaf area and fruit number of the crop in bioremediation treatment with *P. ostreatus* recorded higher values than those of the pollution treatment. The heavy metal contents as well as other adverse conditions associated with crude oil pollution might have contributed to the poor growth performance of *A. esculentus* in the pollution treatment relative to those of the control and bioremediation treatments. Heavy metals have been reported to persist in the environment without being subjected to biological destruction, rather they are transformed from one oxidation state or organic complex to another [24,25]. The presence of high contents of heavy metal in soils has been shown to adversely affect crop growth due to the interference of these metals with physiological and biochemical activities, inhibition of photosynthesis, respiration, activities of organelles and plant survival [26,27]. However, treatments containing *P. ostreatus* remediated the adverse effects of crude oil pollution. This further proves that fungi can degrade petroleum oil better than other traditional remediation techniques, as well as microorganisms such as bacteria [11,12]. Fungi are able to grow optimally in the presence of harmful contaminants and are able to detoxify such contaminants [28], as revealed in this study.

5. CONCLUSION

In this study, the contents of iron, lead, zinc, copper and manganese recorded in crude oil pollution treatment were significantly higher than those of the bioremediation treatment with *P. ostreatus*. There were marked variations in iron, lead, zinc, copper and manganese contents of crude oil pollution and bioremediation treatments before and after harvest. The plant height, root length, fresh weight, dry weight, leaf area and fruit number of the test crop in bioremediation treatment with *P. ostreatus* recorded higher values than those of the pollution treatment. This study clearly indicates that *P. ostreatus* can grow optimally as well as detoxify contaminants in crude oil polluted soil, hence improving the soil conditions for the growth of *A. esculentus*.

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