ORIGINAL ARTICLE

PLANT - SOIL STATUS IN ENGINE OIL POLLUTED SOIL AND AMELIORATION TREATMENT USING ORGANIC MANURE FROM *Calopogonium mucunoides* Desv. and *Amaranthus hybridus* L. AS TEST CROP

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ABSTRACT

Background: Plants interaction with contaminated soil due to petroleum oil pollution as well as appropriate management practices are major concerns in plant growth and development. **Objectives:** This study was designed to assess the chlorophyll contents and growth parameters of *Amaranthus hybridus* L. in engine oil polluted soil amended with organic manure from *Calopogonium mucunoides.* **Methods:** A single pollution level of 80 ml of engine oil was used, while organic manure (from leaves of *C. mucunoides*) was added at the rate of 0.5, 1.0 and 1.5 kg to 2kg of sandy loam soil. The physico-chemical properties of the experimental soil were examined. **Results:** Results showed that the contents of chlorophyll a, b, and ab recorded in amended soil were comparatively higher than those of the pollution treatment, but lower than those of the control. The moisture content, fruit number, fruit weight and root length increased with increase in the levels of organic amendment with the highest values of 67.49%, 12.66, 3.62g and 28.21cm, respectively, relative to the control treatment. These values were significantly ($P < 0.05$) higher than those recorded in the pollution treatment. **Conclusions:** The use of organic manure from *C. mucunoides* to restore engine oil polluted soil could provide favourable conditions for biodegradation as well as provide environmentally friendly approach and nutrient source for abating petroleum hydrocarbon, and enhancing plant growth.

Keywords: *Interaction, growth, contamination, remediation, organic supplement.*

1. INTRODUCTION

Petroleum oil pollution of both aquatic and terrestrial environment, generated either naturally or through anthropogenic activities pose a major threat to viable habitats [1,2,3]. Physiological activities in living organisms are greatly influenced by petroleum oil pollution, thus, inhibiting or enhancing some metabolic pathways [4]. The specific effects of crude oil pollution on plants include among others, chlorosis, bleaching, spotting of leaves and necrosis [5,6]. The test crop *Amaranthus hybridus* L. is a robust annual herb that grows up to 2-3m high. It is sometimes found growing on waste places farms and barnyards. The leaves and young seedling are utilized as vegetables in soups [7,8].

The source of organic manure, *Calopogonium mucunoides* Desv. is an hairy annual or short-lived perennial trailing legume that can grow up to several meters in length [9, 10]. It is mainly used as cover crop, either alone or in combination with other legumes, as well as in forest plantation with rubber, and oil palm. It is also used as green manure, soil protection against erosion, reduces soil temperature, improves soil fertility and controls weeds [10, 11]. It is also utilized as a Nitrogen-fixing legume without specific requirement for rhizobium and nodulates readily [11].

Rehabilitation of crude oil polluted sites involves the use of physical, chemical and biological methods. Several studies have been conducted using bioremediation approaches to restore petroleum oil polluted sites. However, this research was aimed at using eco-friendly and economically as well as locally sourced organic material (organic manure from *C. mucunoides*) to restore engine oil polluted soil as well as assessed the chlorophyll contents and growth parameters of the test crop in polluted and amended soils.

2. MATERIALS AND METHODS

Materials and Methods are written in this area. Describe in detail the technic used, the Name and the references of laboratory materials used should be cited.

2.1 Study area: This research was conducted in Cross River University of Technology, Calabar, Nigeria.

2.2 Collection and analysis of soil samples: Sandy-loam soil were obtained from the study sites, and analysed for physico-chemical properties using standard procedures [12]. The pH values of the soil were measured in 1:2 soil to liquid suspension with electro pH meter. Organic carbon was determined by dictrometric wet oxidation method. Total nitrogen was determined by the Macro-kjeldal's method. Available phosphorus was estimated by the Bray P-I method. Exchangeable bases were extracted with 1M ammonium acetate solution. The potassium and sodium extracts were determined by flame photometry, while calcium and magnesium were determined by the EDTA filtration method [12].

2.3 Remediation studies: Leaves of *C. mucunoides* were obtained from secondary forest habitat in Cross River State, Nigeria. Polluted soils were obtained by mixing thoroughly 2kg of sandy-loam soil with 80ml of engine oil and left undisturbed for one week. 0ml (unpolluted soil) was used as control. The amelioration treatments were carried out by adding organic supplements at the rate of 0.5, 1.0, and 1.5 kg to 80ml level of engine oil polluted soils. The soil samples with or without organic material were left undisturbed for another four weeks before being placed in perforated plastic buckets.

2.4 Germination studies: Seeds of *Amaranthus hybridus* L obtained from local farmers in the study area were sterilized with 0.01% mercuric chloride solution for 30 seconds, thoroughly washed several times with distilled water and air dried. Six (6) seeds of the test crop were sown directly in each plastic bucket containing the various level of organic manure mixed with 2kg sandy-loam soil (with or without engine oil). The seedlings were thinned to three (3) per bag. Each level of treatment was replicated five times using randomized complete block design. The experiment was maintained at a mean minimum temperature of 22.42^{0}C and a mean maximum temperature of 33.06^{0}C, under natural light condition for four (4) months.

2.5 Determination of shoot length and root length: The shoot length and root length of the test crop were measured with a meter rule and expressed in centimeters.

2.6 Determination of moisture content: The plant materials were harvested and repeatedly washed with sterile-distilled water using a sieve to avoid loss of plant parts. Blotting papers were used to dry the seedlings and the fresh weight measured using mettler-p-165 weighing balance. The fresh plant materials were dried in a Gallen Kamp oven maintained at 65^{o}c for 2 days to a constant weight in order to determine the dry weight. The difference between the fresh weight and dry weight of the plantlets multiplied by 100 over the fresh weight was taken as the percentage moisture content of the plant [13].

2.6 Determination of chlorophyll content: Standard methods were used for the determination of chlorophyll content in leaves of the test plant [14, 15]. 2g of leaf samples were crushed with a mortar and homogenization of the tissue was carried out by adding appropriate quantity of 80% acetone. The supernatant was extracted with a filter paper into a 100ml volumetric flask. Repeated addition of acetone to the residue in the mortar and the extraction process was carried out. A 100ml mark was attained in the volumetric flask by using additional acetone to wash off the chlorophyll. The solution was appropriately mixed and 5ml pipette into a 50ml flask. The final solution was made to volume with 80% acetone. 80% acetone was used as blank for measurement of absorbance of the extract at 645, 663, and 652nm wavelengths using spectrophotometer for chlorophyll a, b, and ab respectively. The calculation of concentration of chlorophyll (mg/g fresh leaf weight) was carried out.

2.7 Statistical analysis: Data analysis was carried out using analysis of variance (ANOVA) ($P < 0.05$) using appropriate statistical method [16].

3. RESULTS

Table 1 shows the pH and chemical properties of experimental soils. There were marked variations ($P < 0.05$) in pH and chemical properties of experimental soil in the control, engine oil pollution and remediation treatment (Table 1). Table 2 shows the chlorophyll contents in leaves of *Amaranthus hybridus* in the control, engine oil pollution and remediation treatments. The contents of chlorophyll a, b, and ab recorded in amended soil were comparatively higher than those of the pollution treatment, but lower than those of the control (Table 2). Table 3 shows the growth parameters of *A. hybridus* in engine oil polluted soil and amelioration treatment. The highest plant height (128.72cm) was recorded in 1.50kg level of organic manure, while the lowest value (120.30cm) was recorded in pollution treatment relative to the control (Table 3). The moisture content, fruit number, fruit weight and root length increased with increase in the levels of organic amendment with the highest values of 67.49%, 12.66, 3.62g and 28.21cm, respectively, relative to the control treatment (Table 3).

Table 1: The table presents the chemical properties of experimental soil.

Properties	**Garden Soil (0-Control)**	**Polluted soil**	**Amended soil- Levels of *Calopogonium mucunoides* (kg)**		
			0.5	**1.0**	**1.5**
Ph	5.20±0.33	4.90±0.78	5.30± .93	5.40±0.23	5.50±0.67
Available-P (mg/100g)	8.50±0.64	0.21±0.06	0.67±0.02	1.84± 0.63	2.14±0.22
Ca (mg/100g)	2.63±0.24	2.42±0.62	3.26±0.43	3.38± .91	3.49±0.37
Organic-C (%)	2.66±0.24	3.42±0.46	3.70±0.29	3.83±0.46	3.98±0.17
Total N- (%)	2.02±0.34	0.31±0.02	0.52± 0.06	0.82±0.03	1.22±0.78
Na (mg/100g)	3.45±0.46	5.30±0.32	2.22±0.17	2.77 ±0.52	2.88 ±0.15
Mg (mg/100g)	2.02±0.43	2.11±0.53	2.29±0.44	2.43±0.96	2.63±0.25
K (mg/100g)	1.72±0.15	2.05±0.34	1.84±0.42	1.93±0.40	2.02 ±0.28

Mean value ± Standard error of 3 replicates.

Table 2: The table demonstrates the chlorophyll contents of *Amaranthus hybridus* in engine oil polluted soil amended with organic manure from *Calopogonium mucunoides.*

Chlorophyll Type	Garden Soil (0-Control)	Polluted soil	Amended soil- Levels of *Calopogonium mucunoides* (kg)		
			0.5	1.0	1.5
Chlorophyll a (mg/l)	0.921±0.03	0.436±0.08	0.643±0.09	0.727±0.10	0.836±0.07
Chlorophyll b (mg/l)	0.830±0.12	0.321±0.04	0.564±0.03	0.659±0.02	0.745±0.05
Chlorophyll ab (mg/l)	1.748±0.37	0.754±0.18	1.205±0.32	1.384±0.27	1.578±0.73

Mean value ± Standard error of 3 replicates.

Table 3: The table shows the growth parameters of *Amaranthus hybridus* in engine oil polluted soil amended with organic manure from *Calopogonium mucunoides.*

Growth Parameters	Garden Soil (0-Control)	Polluted soil	Amended soil- Levels of *Calopogonium mucunoides* (kg)		
			0.5	1.0	1.5
Plant height	130.52±0.73	120.30±0.86	124.22±0.95	126.37±0.32	128.72±0.78
Moisture content (%)	76.02±0.26	47.97±0.46	52.14±0.38	60.41±0.27	67.49±0.58
Fruit number Per plant	14.26±0.74	5.67±0.55	8.26±0.22	10.34±0.75	12.66±0.39
Fruit weight (g)	4.2±0.41	2.60±0.58	2.70 ±0.34	3.21±0.56	3.62±0.74
Root length (cm)	31.30±0.48	24.33±0.87	25.76 ±0.64	27.33±0.50	28.21±0.90

Mean value ± Standard error of 3 replicates.

4. DISCUSSION

In this study, the crop growth parameters were poor in pollution treatments relative to the control treatment. Deleterious impacts of petroleum oil pollution on plants have been reported in other studies [3-6]. The negative impacts of petroleum oil pollution on plant growth have been attributed to the poor soil conditions created by the crude oil [17, 18]. The differences or fluctuations in the soil nutrients and pH value among the various treatments may be due to variation in microbial activities in the various treatments. Similarly, deficiency in soil nitrogen and phosphorus were observed in engine oil polluted soils. This result agrees with the work of Asuquo et al., (2001) that increases in organic carbon content in crude oil contaminated soils were observed following an initial decrease [19]. Thus, generating nitrogen deficiency in an oil-soaked soil, which ultimately limit the activities of bacteria and the utilization of carbon sources [20].

The addition of organic manure of *C. mucunoides* improves the soil conditions as well as enhanced the growth and development parameters of the test plant. Organic manure act as a chemical and base microbial base that initiate biodegradation of polluted soil as well as provides the pH range, which is optimal for the growth of known oil-utilizing bacteria [21]. Effective biodegradation process involves enhansing and speeding the activities of petroleum oil degraders [21, 22]. The addition of limiting nutrients such as nitrogen and phosphorus has been shown to enhance the rate of biodegradation [23, 24]. Therefore, the use of organic manure from *C. mucoinoides* in this study provides the nutrient base for biodegradation of engine oil pollution. According to Eneje, et al., (2012) the addition of organic material to petroleum oil polluted soil either singly or in combination would not only improve the chemical properties of the site, but can also enhance the solubility and removal of this contaminant as well as improve oil biodegradation rates [25].

5. CONCLUSION

The use of organic manure from *C. mucunoides* to restore engine oil polluted soil could provide favourable conditions for biodegradation as well as provide environmentally friendly approach and nutrient source for abating petroleum hydrocarbon, and enhancing plant growth.

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