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Organic fertilizer applications influence on the shoot and root biomass production and plant nutrient of *Calopogonium mucunoides* from crude oil-contaminated soils

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The impact of crude oil-contaminated soil on the shoot and root biomass yield and nutrients uptake of *Calopogonium mucunoides* Desv. using two types of composted manure (COM) as soil amendments were investigated. This was with a view to assessing the growth response of the test plant under different levels of crude oil soil contamination. Five levels [0, 2.5, 5, 10, and 20% (v/v)] of crude oil, each was replicated thrice to contaminate 3 kg of soil when 12 g pot^{-1} COM; 12 g pot^{-1} neem-fortified composted manure (NCM) and control, soil without manure application (C) were imposed as manure treatments. The mean fresh shoot biomass yield at zero crude oil soil contamination and with COM application was 2.67 g pot^{-1}. This value was significantly ($p < 0.05$) higher than 2.05 g pot^{-1} for NCM and 1.67 g pot^{-1} for the control. Also, the mean fresh root yield at zero crude oil soil contamination with COM application was 4.02 g pot^{-1}. This value was significantly ($p < 0.05$) higher than 2.41 g pot^{-1} for NCM and 1.71 g pot^{-1} for the control. The dry shoot and root biomass yield followed similar pattern. The shoot and root yield of *C. mucunoides* significantly ($p < 0.05$) reduced with increase in crude oil soil contamination. The nutrients uptake of *C. mucunoides*, particularly N, P, Ca, Mg, and Fe, were enhanced with COM fertilization having higher available P, K, and Na values; and by implication, suggesting the importance of adequately formulated composted manure usage in the rehabilitation studies of crude oil-contaminated soil.

Keywords: *Calopogonium mucunoides*; composted organic manure; crude oil-contaminated soil; neem-fortified organic manure; shoot biomass; root biomass

Introduction

The ecosystem pollution by organic and inorganic substances is increasing as a result of various forms of human activities such as smelting, oil exploitation, mining, quarrying, and use of agro-chemicals [1,2] often leading to human health problems. A various forms of kidney and liver problems may arise from the ingestion of Hg- and Cd-contaminated crops by animals, including humans.[3,4]

Shoot and root yields of plants have been found to be useful growth parameters in assessing the inherent potential of a green plant to remediate contaminated soil.[5] Several cultural operations, primarily agronomic, could be added advantages to enhance the effectiveness of phytoremediation techniques.[6] These operations include the application of fertilizers and/or green manures, surfactants, and tillage to the contaminated soil. The contributions of organic nutrient supplements enhance the effective rapid biodegradation of organic pollutants (with less toxic by-product) to augment the native soil fertility status, improve rate of oil recovery and crop performance and yield.[7]

A large percentage of cultivable land in the oil-producing South–South region of Nigeria had been degraded due to pollution from oil exploitation, spills, and pipeline leakages,[8] and thereby resulting to reduction in cultivable land area. Therefore, the need to restore these polluted soils cannot be over-emphasized. *Calopogonium mucunoides* Desv. is a tropical West African weed found dominant among other weeds that sparingly survive on the oil-spill-contaminated soils of South-South region of Nigeria. However, there is a dearth of information on the growth performance of *C. mucunoides*, if grown to restore the degraded crude oil-contaminated soils. This study therefore assessed the shoot and root biomass yield; plant nutrients' concentration of *C. mucunoides* when cultivated on crude oil-contaminated soils and different organic-based fertilizers was used as soil amendments.

Materials and methods

Experimental design, layout, and agronomic practices

The study was carried out in the greenhouse of the Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife, Nigeria. Bulk surface soil samples (0–15 cm) from a vacant farm land within the University was collected, air-dried for seven days, sieved using a 2-mm mesh to remove stones and analyzed to determine the physico-chemical characteristics of the soil prior to sowing of the viable seeds of *C. mucunoides*. Crude oil obtained from Nigeria National Petroleum Corporation, Eleme, Rivers State with chemical compositions (total hydrocarbon 173.20 mg l^{-1}, Zn 20.33 mg l^{-1}, and

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Fe 37. 03 mg l^{-1}) was added to contaminate the soils, manually mixed using glass rod and allowed to equilibrate for 1 week.

Three kilogram each of the soil was filled into pots perforated at the base to avoid water logging and to increase the soil aeration. Each treatment was replicated thrice and arranged in a 5 × 3 × 3 completely randomised design to give a total of 45 pots. Equal volume (340 ml) of distilled water was initially added to the soil inside each pot to attain field moisture capacity. Different contamination levels of 0.0, 2.5, 5.0, 10.0, and 20.0% (v/v) were then added to give a total of five treatment contaminants. The two manures were locally produced by different organic manure factories and were procured from an open market in Ibadan, Nigeria were used in this study. The manure management levels, viz: 12 g pot^{-1} composted manure (COM); 12 g pot^{-1} neem-fortified composted manure (NCM) and control, soil without manure application (C) were applied at planting using dump and bury method. The N: P: K per cent in the manure was: 4.06, 1.40, and 0.71 for COM; 6.40, 0.64, and 0.46 for NCM, respectively. Four seeds of *C. mucunoides* were planted in each of the pots and thinned to two seedlings per pots at 2 weeks after planting (WAP). The uprooted stands were put back into their pots to ensure that nothing was lost through the excess uprooted stands. The pots were regularly watered with distilled water whenever the soil dryness was noticed. At the end of 12 WAP, the experiment was terminated.

Each pot containing stands of *C. mucunoides* plants was brought under a running tap for water to remove the soil attached to the roots of the test plant. The roots were thereafter rinsed with distilled water. A clean and sharp knife was used to separate the test plant into roots and shoots. Weight of the roots and shoots from each pot was determined.

Laboratory analyses

The particle size analysis was determined using 5% sodium hexametaphosphate as the dispersing agent as highlighted by Odu et al. [9]. Soil pH was determined potentiometrically in 1:1 soil–water ratio.[10] Organic carbon content of the soil was determined using Walkley–Black method.[11] Total nitrogen of the soil was determined by the macro-Kjeldahl method.[12] The available phosphorus of the soil was determined by Bray P1 method.[13] The exchangeable cations (Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}) were determined using 1 M NH_4OAc buffered at pH 7.0 as extractant.[14] The Na^{+} and K^{+} concentrations in the soil extracts were read on Gallenkamp flame photometer while Ca^{2+} and Mg^{2+} were read using a Buck Scientific Model 210 VGP (Norwalk, Connecticut, USA) atomic absorption spectrophotometer (AAS). The micronutrients (Fe and Zn) in the soil were extracted with 0.1 M HCl,[15] and their concentrations in the extracts were read using AAS.

All the roots and shoots were oven-dried for 48 h at 70 °C, weighed again, ground, and stored. Each plant sample (0.5 g) was digested using 5 ml of the mixture (conc. HNO_3 and conc. $HClO_4$ in the ratio 2:1) for 2 h at 150 °C,[15] and Ca, Mg, Fe, and Zn concentrations in the extracts were read on AAS.

Statistical analysis

Data collected were subjected to descriptive and analysis of variance to test their treatment effects. Differences in the treatment means were separated using the Duncan range test at 95% level of probability.

Results and discussion

The topsoil used during the experiment was slightly acidic with a pH of 6.00 in 1:1 soil–water ratio (Table 1). The organic carbon content was 29.7 g kg^{-1}, while the exchangeable cations (Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+}) were 2.75 cmol kg^{-1}. The soil used was loamy sand; with sand, silt, and clay proportions of 858.00, 60.00, and 82.00 g kg^{-1}, respectively. The effect of crude oil contamination and organic manure applications to soil on the shoot biomass yield of *C. mucunoides* is presented in Table 2.

Table 1. Selected properties of soil and organic-based manures used.

Characteristics	Soil	Composted manure	Neem-fortified manure
pH in water (H_2O) 1:1	6.00	–	–
Organic carbon (g kg^{-1})	29.71	38.21	54.03
Nitrogen (g kg^{-1})	2.56	4.06	6.40
Available phosphorus (mg kg^{-1})	9.40	1.40	0.64
K^{+} (cmol kg^{-1})	0.39	0.71	0.46
Na^{+} (cmol kg^{-1})	0.40	0.35	0.27
Mg^{2+} (cmol kg^{-1})	0.72	0.16	0.59
Ca^{2+} (cmol kg^{-1})	1.24	1.60	12.42
Exchangeable cations (cmol kg^{-1})	2.75	–	–
Zn (mg kg^{-1})	6.02	–	–
Fe (mg kg^{-1})	25.10	–	–
Total hydrocarbon content (mg kg^{-1})	0.17	–	–
Textural class	Loamy sand	–	–

Table 2. Effects of crude oil contamination and manure applications to soils on the shoot biomass yield (in g) of *C. mucunoides*.

Treatment	Concentration level (%) of crude oil	Weight		% moisture content
		Fresh	Dry	
COM	0.00	2.67a	0.87a	67
	2.50	0.52d	0.15d	71
	5.00	0.13ef	0.03e	77
	10.00	0.04f	0.01e	75
	20.00	0.01f	0.004f	60
NCM	0.00	2.05b	0.72b	65
	2.50	0.20e	0.05e	75
	5.00	0.14ef	0.04e	71
	10.00	0.11f	0.03e	73
	20.00	*	*	*
Control	0.00	1.67c	0.54c	68
	2.50	0.18e	0.04e	78
	5.00	0.12ef	0.03e	75
	10.00	0.03f	0.009f	70
	20.00	*	*	*

Note: Means followed by the same letter(s) within a column are not significantly different at $p < 0.05$ by Duncan multiple range test.
COM = composted manure.
NCM = neem-fortified composted manure.
*The plants died before harvesting.

The *C. mucunoides* plants survived in all the crude oil soil contamination levels when COM was added. However, for NCM and control treatments, the test plant could only survive in all; except at 20% crude oil soil contamination, as the leaves of the test plant changed color as a sign of withering from green to brown at 8 WAP and eventually died after 10 WAP. Merkl et al. [16] observed similar death of *Brachiaria brizantha*, *Cyperus aggregatus,* and *Eleusine indica* within 6–8 WAP at high concentration of crude oil contamination on sandy soil. High concentration of crude oil as contaminant could have affected the physiology of *C. mucunoides*, probably because the tolerable level had been exceeded. Crude oil soil contamination may affect plants by retarding seed germination, photosynthetic rate or may result to complete mortality.[17]

The mean fresh shoot biomass yield at zero crude oil soil contamination with composted manure application was 2.67 g pot^{-1}. This value was significantly ($p < 0.05$) higher than 2.05 and 1.67 g pot^{-1} mean fresh shoot biomass yields when neem-fortified composted and zero organic manures were applied, respectively. Fresh shoot biomass yield of *C. mucunoides* significantly ($p < 0.05$) reduced with increase in crude oil soil contamination. This observation was an indication of adverse effect of crude oil soil contamination on the stem and shoot biomass yield of the test plant.

Reduced access to water and oxygen could have caused shoot biomass yield reduction.[18] Lee et al. [19] considered reduced access to water and soil nutrients in diesel oil soil contamination as the major factors for reduced leaf biomass yield of *Scirpus mucronatus.* The dry shoot biomass yield obtained at various crude oil concentration and manure applications followed similar pattern of the fresh shoot biomass yield. The percent moisture contents of the shoot biomass of *C. mucunoides* across the crude oil soil contamination varied from 60 to 78% (Table 2), with no definite pattern irrespective of the manure treatments. This was an indication that the applied manures had no noticeable influence on the moisture content of *C. mucunoides* shoot biomass production.

The effect of crude oil contamination and organic manure applications to soil on the root yield of *C. mucunoides* is presented in Table 3. The mean fresh root yield at zero crude oil soil contamination with composted manure application was 4.02 g pot^{-1}. This value was significantly ($p < 0.05$) higher than 2.41 and 1.71 g pot^{-1} mean fresh root yields when neem-fortified composted and zero organic manures were applied, respectively. The root yield of *C. mucunoides* significantly ($p < 0.05$) reduced with increase in crude oil soil contamination. It was observed that as the concentration of crude oil in the soil increased, the root yield reduced. Roots of plants are living organisms; therefore, inadequate access to water and oxygen earlier suggested by Ogbo et al. [18] could cause reduced root yield of *C. mucunoides*.

Plants do not have good vegetative and root development in soil with poor organic matter and plant nutrients. So, addition of manure inputs leads to increase in soil organic matter and nutrient release for enhanced plant growth performances. Rawat [20] obtained higher fine root biomass due to an increase in soil organic matter which was brought about by increase in litter decomposition in a community-managed forest in India. The composted manure application significantly ($p < 0.05$) enhanced both the shoot and root biomass yields of

Table 3. Effect of crude oil contamination and manure applications to soils on the root yield (in g) of *C. mucunoides*.

		Weight	
Treatment	Concentration level (%) of crude oil	Fresh	Dry
COM	0.00	4.02a	0.64a
	2.50	0.81d	0.13d
	5.00	0.41ef	0.06e
	10.00	0.27f	0.04e
	20.00	0.06g	0.01e
NCM	0.00	2.41b	0.47b
	2.50	0.52e	0.10d
	5.00	0.31f	0.06e
	10.00	0.18g	0.03e
	20.00	*	*
Control	0.00	1.71c	0.30c
	2.50	0.24f	0.04e
	5.00	0.14g	0.02e
	10.00	0.05g	0.007ef
	20.00	*	*

Note: Means followed by the same letter(s) within a column are not significantly different at $p < 0.05$ by Duncan multiple range test.
COM = composted manure.
NCM = neem-fortified composted manure.
*the plants died before harvesting.

Table 4. Effect of crude oil contamination and manure application to soils on the selected nutrient of *C. mucunoides* plant.

		Plant nutrient					
Treatment	Concentration level (%) of crude oil	N	P	Ca	Mg	Fe	Zn
COM	0.00	24.56a	5.17a	27.50b	23.88b	8.51c	0.03 ns
	2.50	21.95ab	3.56b	47.58a	37.01a	8.91c	0.04 ns
	5.00	18.45c	3.09b	37.69b	34.87a	11.58bc	0.06 ns
	10.00	14.51d	1.90c	3.88d	26.39b	18.66a	0.07 ns
	20.00	*	*	*	*	*	*
NCM	0.00	20.47b	4.01b	22.82c	19.89b	7.08c	0.03 ns
	2.50	18.29c	2.97b	39.66b	30.40a	7.41c	0.03 ns
	5.00	15.98d	2.54b	31.40b	22.84b	9.65c	0.06 ns
	10.00	13.71d	1.58c	3.24d	16.99c	15.60b	0.06 ns
	20.00	*	*	*	*	*	*
Control	0.00	17.80c	2.75b	17.19c	15.89c	5.44c	0.02 ns
	2.50	13.72d	2.23bc	29.76b	16.80c	5.57c	0.03 ns
	5.00	11.53d	1.90c	23.55c	23.12b	7.24c	0.05 ns
	10.00	9.07d	1.19c	2.44d	9.39d	11.70bc	0.05 ns
	20.00	*	*	*	*	*	*

Note: Means followed by the same letter(s) within a column are not significantly different at $p < 0.05$ by Duncan multiple range test.
COM = composted manure.
NCM = neem-fortified composted manure.
*The plants died before harvesting.
ns = not significant.

C. mucunoides in crude oil-contaminated loamy sand soil; an indication that composted manure enhanced the agronomic performance of the test plant on crude oil-contaminated soil.

Nutrient elements, particularly N and P, stimulate root development. Significantly ($p < 0.05$) higher root yield obtained could be directly attributed to better root development obtained with composted manure application that had twice P composition when compared with neem-fortified manure (Table 1). Atayese et al. [21] obtained similar higher shoot and root yields of woody legumes, though with vesicular–arbuscular mycorrhiza (VAM) fungi as inoculants. The VAM fungi inoculation enhances the availability of plant nutrients, particularly N and P for root development.

The effect of crude oil contamination and manure application to soils on the selected nutrient content of *C. mucunoides* plant is presented in Table 4. Except for Zn, other plant nutrients such as N, P, Ca, Mg, and Fe were significantly ($p < 0.05$) different under different manure treatments and contamination levels. For instance, the zero crude oil contamination had significantly ($p < 0.05$) highest values of 24.5 mg N kg^{-1} and 5.17 mg P kg^{-1} plant nutrients when compost manure was applied. Generally, the two manures (COM and NCM) applied enhanced plant nutrients uptake with or without crude oil contamination of the soil. However, crude oil soil contamination lowered nutrients uptake of *C. mucunoides*. Since plants absorb nutrients and water from the soil through the roots [22] which had been poorly developed as a result of crude oil contamination, low nutrients uptake may therefore not be in doubt.

Conclusion

We concluded that nutrient compositions of the applied composted manures played significant role in the agronomic development, vis-a-vis the shoot and root of *C. mucunoides* in a loamy sand soil of the humid tropics. Higher nutrient values of organic C, N, Mg, and Ca in NCM could not adequately enhance the shoot and root development of *C. mucunoides*, but COM with higher available P, K, and Na. However, at higher level of crude oil soil contamination, with or without manure fertilization, *C. mucunoides* general growth performance was retarded. Also from this study, the plant nutrients uptake of *C. mucunoides*, particularly N, P, Ca, Mg, and Fe, was enhanced with manure fertilization; and by implication, suggesting its usefulness in the rehabilitation of crude oil associated metals at low level of soil contamination.

Disclosure statement

No potential conflict of interest was reported by the authors.

Notes on contributors

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