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EFFECT OF ORGANIC MANURES AND INORGANIC FERTILIZER ON SOIL MICROBIAL BIOMASS AND FLUTED PUMPKIN (*Telefeira occidentalis*) IN THE REMEDIATION OF PETROLEUM POLLUTED SOILS

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Abstract: **The soils were analyzed to access the effect of soil microbial biomass on the growth of fluted pumpkin (*Telfairia occidentalis*) in the remediation of crude oil polluted soils of Bayelsa State. Two (2) locations, Imiringi in Ogbia Local Government Area with latitudes 4. 8519 N and longitudes 6.3745 E and Koloama in Southern Ijaw Local Government Area with latitudes 4.582222 N and longitudes 6.064722 E, were selected for this research. Soil samples were collected at a depth of 0 – 30 cm, bulked for pot experiment in the screen house. Five (5) kilograms of soil were weighed into twenty-four (24) plastic buckets of seven (7) liters each for Imiringi and Koloama, making a total of forty-eight (48) plastic buckets used. The experimental design was a 2 x 8 factorial experiment laid out in a Complete Randomized Design (CRD) replicated three times. Results obtained were subjected to statistical analysis of variance using the Tukey's Test to separate means of significant difference at 5 % probability level. The amendment materials used were organic manures [cow dung (CD) and poultry dropping (PD)] inorganic fertilizer (NPK) and their combinations (CD + PD, PD + NPK, NPK + CD and CD + PD + NPK). The study revealed the relationship between soil microbial biomass and growth of the plant (*Telfairia occidentalis*) in Imiringi and Koloama, on application of the amendments (treatments) with the release of the essential element – Carbon, Nitrogen and Phosphorus to the plant, showing that both are significantly different with few variations on the plant growth parameters (vine length, girth and number of branches) at different times (4 WAP, 8 WAP and 12 WAP), indicating the effectiveness of the application of these amendment materials.**

Keywords: **Crude Oil, Fluted Pumpkin (*Telfairia occidentalis*), Imiringi, Inorganic Fertilizer (NPK), Koloama, Organic manures, Polluted Soils, Soil Microbial Biomass.**

1. INTRODUCTION

Petroleum or crude oil is a naturally occurring flammable liquid that is found in geological formations beneath the Earth's surface. The components of petroleum and its products number in thousands. The toxicity of the components varies immensely, they are derived from hydrocarbons [hydrocarbon functional groups – carbon and hydrogen atoms (C-H)]. The constituent hydrocarbon compounds are present in various proportions resulting in great variability in crude oils from

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different sources. The relative proportions of these fractions are dependent on many factors such as the source, geological history and age of crude oil (Balba *et al.*, 1998).

Soils of Bayelsa State are affected adversely with serious ecological problems associated with the activities being carried on the land such as oil exploration, spillage and disposal of petroleum products resulting in the pollution of the aquatic and soil environment (Teknikio *et al.*, 2018). The environment polluted with hydrocarbon compounds causes serious health risk due to their carcinogenic and mutagenic effects (Mishra *et al*., 2010). Hence, the release of petroleum products to the environment has led to changes in the abiotic and biotic properties of the soils (Barua *et al*., 2011).

However, soils polluted with petroleum products poses significant threats to agricultural productivity and environmental sustainability. In polluted soils, the activities of soil microorganisms, including microbial biomass, play a crucial role in soil fertility restoration and plant growth. The effects of these factors differ in different soil zones, horizons and climatic zones (Garbeva *et al*., 2004).

Soil microbial biomass refers to the living fraction of microorganisms present in the soil, including bacteria, fungi, and other microorganisms. These organisms contribute to crucial soil functions, such as nutrient cycling, organic matter decomposition, and the suppression of soil-borne diseases. The microbial biomass is considered a sensitive indicator of soil health and fertility.

Fluted pumpkin (Telfairia occidentalis) is a popular leafy vegetable crop in tropical regions known for its nutritional and economic value. However, the growth of fluted pumpkin can be significantly affected by petroleum pollution in soil. The growth of fluted pumpkin is highly dependent on the soil microbial community. Soil microbial biomass contributes to nutrient mineralization, organic matter decomposition, and the release of plant growth-promoting substances. Therefore, an increase in microbial biomass can positively impact on the growth and yield of fluted pumpkin in petroleum polluted soils. The addition of organic manures and inorganic fertilizers to petroleum-polluted soils can alleviate these negative effects and promote the growth of fluted pumpkin. Organic amendments contribute to the improvement of soil structure and nutrient availability, while inorganic fertilizers supply essential nutrients required for plant growth. Furthermore, the stimulation of soil microbial biomass through organic amendments enhances the degradation of petroleum hydrocarbons, reducing their toxic effects on plant growth. Soil microbial biomass plays a crucial role in plant-soil interactions and nutrient cycling. Microorganisms in the soil help break down organic matter, release essential nutrients, and create a favorable rhizosphere environment for plant roots. The objective of this study accessed the effect of soil microbial biomass on the growth of fluted pumpkin (Telfairia occidentalis) in petroleum polluted soils amended with organic manures and inorganic fertilizers.

2. MATERIALS AND METHODS

Two (2) crude oil polluted locations were selected for this research namely; Imiringi with latitudes 4º.52' – 4º.85' N and longitudes 6º.23' – 6º.37' E in Ogbia Local Government Area and Koluama I with latitudes 4º.58' - 52.8'N and longitudes 6º.06' - 27.2' E in Southern Ijaw Local Government Area both in Bayelsa State.

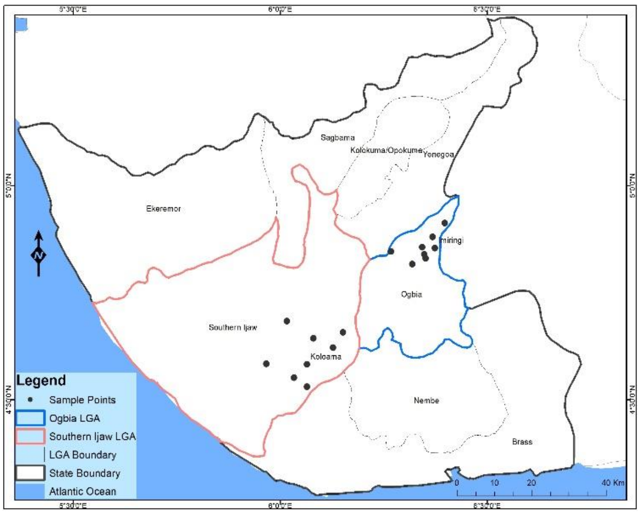

Fig. 1 Map of Bayelsa State showing the sampling points in the two study locations (Imiringi and Koloama) of the crude oil polluted soils

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Experiments / Material Used

The study was carried out in the screen house. The materials used for the experiment were sourced locally and included the remediation materials (cow dung, poultry droppings and NPK) and their combinations. The soils were randomly collected at a depth of 0 – 30 cm from the oil spilled polluted sites for the experiment. A total of 48 samples comprising of the control, treatments and their combinations were analyzed in this experiment. The soils were bulked and 5kg of soil were measured into 48 plastic buckets of 7 liters each according to the controls, treatments and treatment combinations. A fixed rate of 0.5 kg of the amendments (cow dung, poultry dropping and NPK 20 -10 -10) were applied to the polluted soils 3 weeks before planting. A total of 48 plastic buckets of 7 liters each were filled with soils collected from the two (2) polluted locations (Imiringi and Koloama) at a fixed weight of 5 kg into each bucket, 24 plastic buckets per location. A fixed rate of 0.5 kg of the amendments and their combinations (CD, PD, NPK, CD + PD, PD + NPK, CD + NPK and CD + PD + NPK) was applied to the polluted soils except for the control bucket.

Fluted pumpkin (*Telfaria occidentalis*) seeds were planted 2 seeds per pot to accesse the effect of soil microbial biomass on the growth of fluted pumpkin (*Telfairia occidentalis*) in petroleum polluted soils amended with organic manures and inorganic fertilizers.

Soils were collected at different times (30 days, 60 days and 90 days) for analysis in the laboratory, while the growth parameters of the plants were measured at different times (4 weeks, 8 weeks and 12 weeks) after planting.

Soil Microbial Biomass Determination

Soil Microbial Biomass (Carbon– C); Fumigation-extraction method as per the procedure given by (Tate *et al.*, 1988; Joergensen and Brookes 1990).

$$C\ \mu g\ g^{-1}\ OD\ soil = C\ (\mu g\ ml^{-1}) \times \frac{EX + MC}{DWt\ soil}$$

Where:

EX = quantity of extractant

MC = moisture content of soil used

DWt = dry weight of soil used

Biomass carbon is calculated from:

C = 2.64 Ec

Whre Ec is the difference between the carbon extracted from the fumigated sample and the carbon extracted from the control sample.

Soil Microbial Biomass (Nitrogen – N); Fumigation-extraction method as per the procedure given by (Joergensen and Brookes 1990).

Biomass Nitrogen (BN)

$$B_N = \frac{F_N}{K_N}$$

Where:

BN = Biomass Nitrogen

F_N = the N mineralized from the biomass (polluted N - control)

K_N= the fraction of biomass N mineralized = 0.68 x 0.79 = 0.54

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Soil Microbial Biomass (Phosphorous – P); Fumigation-extraction method of Vance *et al.*, (1987).

Dilution Factor (D) is calculated from:

$$D = \frac{\text{Volume of Olsen's reagent used to extract from soil + soil moisture content (ml)}}{\text{Volume of extractant used in test x weight of soil (g)}}$$

Correction Factor is the product of the molar coefficient of phosphorus and the dilution factor. $\mu g\ P\ l^{-1}$ x D.

The results were calculated from:

(abs. of sample – abs. of blank) x ($\mu g\ P\ l^{-1}$ x D) = $\mu g\ P\ g^{-1}$ of soil

Therefore, microbial biomass of P:

a ($\mu g\ P\ g^{-1}$ of soil) from non-fumigated soil (control)

b ($\mu g\ P\ g^{-1}$ of soil) from fumigated soil (polluted soil)

c ($\mu g\ P\ g^{-1}$ of soil) from spiked soil

Then microbial biomass P = 25 (b-a) / 0.4 (c-a) $\mu g\ g^{-1}$ of soil

Experimental design and data collection: Experimental design was a 2 x 8 factorial experiment in a complete randomized design (CRD) where locations and treatments are factors replicated three (3) times. Soil samples were collected at 30 days, 60 days and 90 days respectively during the research period.

Statistical Analysis: All data collected were subjected to statistical analysis of variance (ANOVA) and data analysis of General Linear Model (GLM) was used to evaluate the effects of treatments on crude oil polluted soils. Tukey test was used to separate all the means. All analyses were performed using Minitab Statistical Software Release 17.1.

3. RESULTS

KEY:

IMI = Imiringi

KLM = Koloama 1

C = Control (Polluted Soil Not Treated)

CD = Polluted Soil Treated with Cow Dung Manure

PD = Polluted Soil Treated with Poultry Dropping Manure

NPK=Polluted Soil Treated with Nitrogen Phosphorous Potassium Fertilizer (Inorganic Manure)

CD + PD = Polluted Soil Treated with Cow Dung Manure + Poultry Dropping Manure

PD + NPK = Polluted Soil Treated with Poultry Dropping Manure + Nitrogen Phosphorous Potassium Fertilizer (Inorganic Manure)

CD + NPK = Polluted Soil Treated with Cow Dung Manure + Nitrogen Phosphorous Potassium Fertilizer (Inorganic Manure)

CD + PD + NPK = Polluted Soil Treated with Cow Dung Manure + Poultry Dropping Manure + Nitrogen Phosphorous Potassium Fertilizer (Inorganic Manure)

SMBC = Soil Microbial Biomass - Carbon

SMBN = Soil Microbial Biomass - Nitrogen

SMBP = Soil Microbial Biomass - Phosphorus

PLANT PARAMETERS = Vine Length, Girth, No. of Branches, No. of Leaves and Leaf Area.

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Main and interaction effects of locations and treatments on the soil microbial biomass carbon, nitrogen and phosphorus (SMBC, SMBN and SMBP) in the soils during the remediation period

Table 1, is the main and interaction effect of locations and treatments on the soil microbial biomass carbon, nitrogen and phosphorus, showing the significant difference ($p \leq 0.05$) in both locations and treatments.

Main and interaction effects of locations and treatments on growth parameters of Fluted pumpkin *(Telfairia occidentalis)* plant during the remediation period

In Table 2, the growth parameters of the plant *(Telfairia occidentalis)* were significantly different ($p \leq 0.05$) with few variations as seen in the results below.

Table 1. Main and interaction effects of locations and treatments on the soil microbial biomass carbon, nitrogen and phosphorous (SMBC, SMBN and SMBP)

Factors	SMBC (µg/g)			SMBN (µg/g)			SMBP (µg/g)		
	30 Days	60 Days	90 Days	30Days	60 Days	90 Days	30 Days	60 Days	90 Days
Locations									
IMI	0.86a	0.49a	0.19c	0.84b	0.63a	0.53b	7.61c	8.30b	9.74b
KLM	0.70b	0.42b	0.52b	0.88a	0.46c	0.49c	17.87b	10.86a	6.71c
Treatments									
CONTROL	0.79a	0.53a	0.43bc	0.58e	0.99a	0.56ab	0.44f	3.49d	9.10de
CD	0.57d	0.32c	0.47ab	0.48f	0.32e	0.59a	10.10e	5.48d	7.40e
PD	0.76ab	0.43b	0.33c	1.17a	0.49cd	0.54ab	25.36a	13.27a	26.20a
NPK	0.75ab	0.40bc	0.56a	0.75d	0.62b	0.48c	15.98c	11.74ab	10.24d
CD + PD	0.67c	0.38bc	0.41bc	0.83c	0.40de	0.57ab	17.85c	9.38c	16.80bc
PD + NPK	0.76ab	0.42b	0.45b	1.07b	0.56bc	0.51bc	20.67b	12.51ab	18.23b
CD + NPK	0.66c	0.36bc	0.49ab	0.72d	0.47cd	0.54ab	13.02d	8.61c	8.82de
CD + PD + NPK	0.70bc	0.38bc	0.46b	0.85c	0.48cd	0.55ab	17.65c	10.39bc	14.61c
P value									
Locations	*	*	*	*	*	*	*	*	*
Treatments	*	*	*	*	*	*	*	*	*
Locs. x Trts	*	*	*	*	*	*	*	*	*
CV (%)	4.84	7.47	17.10	4.59	12.88	7.04	12.39	16.45	15.28
R^2 (%)	98.09	91.81	94.97	98.40	94.83	92.73	99.00	90.34	99.04

The means with same letters in the columns separated using Tukey's Test are not significantly different at $p \leq 0.05$ level test. Same letters, NS = Not Significantly different and different letters, * = Significantly different.

Table 2. Interaction effects of locations and treatments on the growth parameters of Fluted pumpkin *(Telfairia occidentalis)*.

Factors	Vine Length (cm)			Girth (cm)			No. of Branches			No. of Leaves			Leaf Area (cm^2)		
	4 WAP	8 WAP	12 WAP	4 WAP	8 WAP	12 WAP	4 WAP	8 WAP	12 WAP	4 WAP	8 WAP	12 WAP	4 WAP	8 WAP	12 WAP
Locations															
IMI	17.44c	82.79a	77.81b	0.37b	0.49b	0.52b	0.04b	0.96c	1.62b	8.56b	15.40b	23.53c	13.55b	18.78b	26.28b
KLM	48.39a	44.96a	74.18b	0.48ab	0.56b	0.54b	0.81a	3.11a	3.00a	19.15a	30.29a	35.92b	20.20a	25.67ab	34.64ab
Treatments															
CONTROL	13.33d	32.56a	51.56d	0.51ab	0.54ab	0.61a	-0.00a	0.33d	1.22c	8.78c	15.56d	20.44c	11.67cd	12.82c	21.14cd
CD	33.78bc	56.89a	88.88bc	0.45ab	0.71ab	0.75a	0.33a	1.11bc	1.77c	11.67bc	23.11cd	28.67b	14.28c	21.78c	32.06bc
PD	57.67a	106.33a	151.88a	0.63a	0.80a	0.80a	0.55a	3.22a	3.33ab	20.44ab	43.22a	52.66a	34.08a	48.49a	62.42a
NPK	5.44e	35.22a	66.00cd	0.11c	0.49b	0.54a	0.00a	0.67cd	2.22bc	2.89d	16.22d	23.33c	3.28d	14.77c	19.34cd
CD + PD	49.22ab	84.77a	122.66ab	0.63a	0.68ab	0.66a	0.55a	3.11a	4.44a	23.78a	37.22ab	53.77a	23.46ab	37.79ab	50.40ab
PD + NPK	41.89ab	89.77a	102.11bc	0.56a	0.69ab	0.70a	0.33a	3.11a	3.55ab	23.44a	34.55ab	46.11a	28.26ab	25.14bc	38.12bc
CD + NPK	40.67abc	49.83a	80.00bc	0.18bc	0.63ab	0.48a	0.00a	2.00ab	1.50c	18.67ab	22.67bc	28.00bc	7.62cd	26.21bc	27.87cd
CD + PD + NPK	43.22ab	136.00a	93.33bc	0.59a	0.62ab	0.65a	0.89a	2.44ab	3.33ab	25.44a	25.67b	42.55ab	18.60bc	28.64bc	30.81cd
P value															
Locations	*	NS	*	*	NS	NS	*	*	*	*	*	*	*	*	*
Treatments	*	NS	*	*	*	NS	NS	*	*	*	*	*	*	*	*
Locs. x Trts	*	NS	*	*	*	*	NS	*	*	*	*	*	*	*	*
CV (%)	51.97	53.15	36.79	40.43	37.09	37.06	42.57	30.16	31.15	48.08	43.57	39.53	47.16	51.63	53.75
R^2 (%)	81.47	35.84	83.51	76.51	52.11	54.10	69.01	82.19	81.09	88.16	77.96	83.42	77.57	73.71	74.94

The means with same letters in the columns separated using Tukey's Test are not significantly different at $p \leq 0.05$ level test. Same letters, NS = Not Significantly different and different letters, * = Significantly different.

4. DISCUSSION

From this study, it was observed that soil microbial biomass played an important role in the successful remediation of petroleum polluted soils. The presence of microbes in the soil aids in the breakdown of petroleum hydrocarbons, facilitating the biodegradation process. Additionally, the microbial activity enhances nutrient cycling and availability, which are crucial for plant growth and development. From the results shown above, organic manures and inorganic fertilizers acted as nutrient sources for both the soil microbial community and the plants. The organic amendments used in this study improved the soil structure and enhanced the microbial activity, leading to increased nutrient release and uptake by plants. Inorganic fertilizer provided readily available nutrients that supplemented the microbial biomass and supported plant growth. The combination of the treatments, involving the simultaneous application of organic manures and inorganic fertilizer, resulted in the growth of the plant. The synergistic effects of organic matter and nutrient supplements contributed to improved soil conditions and nutrient availability. This supports the findings of Odu *et al.,* (2018) in their assessment of the impact of compost and inorganic fertilizer on soil microbial biomass in a crude oil-contaminated soil, and found that the combined application of compost and inorganic fertilizer significantly increased microbial biomass carbon, nitrogen, and phosphorus compared to the control. Owei *et al.,* (2018) also attested to it in their findings on the impact of compost and inorganic fertilizer amendments on microbial biomass and soil quality parameters in oil-polluted soils. The results demonstrated that both amendments significantly increased soil microbial biomass carbon, nitrogen, and phosphorus content, indicating improved soil health and fertility which support the results in this study. To this end, the results showed that the application of these amendments (organic manures, inorganic fertilizer and their combinations) proved to be effective in the bioremediation process.

5. CONCLUSION

Crude oil pollution has had detrimental effects on soil microbial biomass and the overall soil health. However, from this study conducted in Imiringi and Koloama, it was shown that the application of organic and inorganic amendments mitigated the negative effects of petroleum hydrocarbons pollution by improving soil fertility, nutrient availability, and microbial activities demonstrating the positive effects of organic and inorganic amendments on soil microbial biomass carbon, nitrogen, and phosphorus content which was reflected in the growth of the plant. However, further research is needed to optimize amendment application rates, determine long-term effects, and assess the overall ecological impact of the remediation strategies in Bayelsa State's crude oil polluted soils.

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