



RESEARCH ARTICLE

BIOSTIMULATION OF CRUDE OIL POLLUTED SOIL USING POULTRY MANURE

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Authors' contributions

This research was carried out in collaboration among all authors. Amadi N and Chikere L.C collected the scientific data and wrote the manuscript. While Yabrade revised and edited the manuscript. Literature research was conducted by Amadi, N. All authors read and approved the final manuscript.

ABSTRACT

The negative impacts of crude oil on the environment have attracted concern because of their toxicity nature which endangers the optimal growth and other soil biotic component. Phytodegradation is more suitable techniques than conventional methods but is being limited on the foundation of slow growth, decrease nutrient and microbial population. This study was aimed at harnessing the nutrient quality of poultry manure in addressing the pitfall in the use of phytodegradation techniques. Adopting Pere's Method of treatment application, treatment were applied after a week as: Block A: 100g of poultry manure, Block B: 200g of poultry manure, Block C: 300g poultry manure while Block D and E contain 0g of poultry manure which is designated a control (unpolluted soil) and double control (diesel polluted soil). Soil pH, conductivity, total petroleum hydrocarbon, total organic carbon, nitrogen, phosphorus, potassium in soil and plant height, fresh and dry weight were monitored for plant at 30 and 60 days. TOC decreased from 2.5% to 2% at 30days and 1% at 60 day, TPH decrease from 1450mg/kg to 200mg/kg at 30days and 98mg/kg at 60 days, pH decreased from 8.9 to 6.5 (optimal for bacteria activity) at 30days and 6.2 at 60 days, phosphorus and potassium increased from 0.05mg/kg to 26 mg/kg at 30 days ;38mg/kg at 60 days: 12mg/kg to 45mg/kg at 30days; 58mg/kg at 60 days respectively. Nitrogen content also increased from 7.8mg/kg-12mg/kg at 30days and 28mg/kg at 60days. In conclusion, the integration of poultry manure and *Platostoma africanum* presents a holistic and environmentally friendly approach to address the challenges posed by crude oil contamination in agricultural soils. By harnessing the power of nature's resources, this innovative remediation strategy offers a ray of hope for restoring contaminated lands and fostering resilient ecosystems for generations to come.

Key Words: Biostimulation, Crude oil, *Platostoma africanum*, physicochemical, poultry manure,

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1.1. INTRODUCTION

Nigeria is currently facing a major environmental pollution ranging from crude oil exploration, transportation and uses (Onuoha *et al.*, 2020). Contamination of soil with petroleum hydrocarbon is on the increase due to continuous oil exploration and refining activities. The major part of the environment showing adverse effects of hydrocarbon pollution is the terrestrial and aquatic aspects of the ecosystem and its structure (Onuoha *et al.*, 2020). Crude oil is composed of aromatic, alkanes, alicyclic, paraffin and other heteroatoms and its' petroleum product (Ugochukwu *et al.*, 2020). These components present in crude oil threatens soil stability, resulting in loss of fertility of agricultural land also making it unproductive and unsafe for microbial growth due to depletion in soil nutrient content (Amadi *et al.*, 2023). Crude oil contaminated soil can remain unproductive for a long period until its effects on the environment is decreased (Odokuma *et al.*, 2002; Godleads *et al.*, 2015). The pollution of the environment with crude is the main cause of food scarcity since food production and other food chain processes is impeded (Odokuma *et al.*, 2002; Godleads *et al.*, 2015).

The effects of crude oil pollution on the terrestrial environment is on soil and its' physical and chemical properties as well as microbial composition (Odokuma *et al.*, 2002; Adams *et al.*, 2014). The negative impacts of crude oil on the environment endanger the optimal growth and performance of plants. Plant species growing in crude oil polluted environment exhibit various symptoms such as oxidative stress pattern resulting in over production of reactive oxygen species which is could lead to plant death (Amadi *et al.*, 2023). Decrease plant morphological properties and biomass production of plant are among other effects of hydrocarbon on plants. The presence of crude oil spillage on the environment is capable of contaminating ground water through leaching process (Godleads *et al.*, 2015; Amadi *et al.*, 2023).

Generally, anthropogenic activities are seen as the major contributor to crude oil pollution. This ranges from crude oil exploration, pipeline vandalism, transport and use of fossil fuels. These activities contribute an appreciable amount of hydrocarbon compounds on the environment (Onuoha *et al.*, 2020). The negative impact of crude oil pollution and in an attempt to protect and conserve the natural environment made Federal Government of Nigeria to enact laws against environmental polluted and created agencies that are saddled with the responsibilities to protect the environment, these agencies include OPA, FMENV, EGASPIN, NOSDRA etc. Since oil exploration is the major mains Federal Government of Nigeria gets her revenue and by this, environmental problems association with crude oil exploration cannot be eradicated hence developing alter ways in reducing the impact of crude oil pollution is now necessary (Adams *et al.*, 2014; Amadi *et al.*, 2023). Phytodegradation promises to be the best plant base technique to combat crude oil pollution problems. Phytodegradation is more suitable techniques than conventional methods but is being limited on the foundation of slow growth, decrease nutrient and microbial population. This factor affecting this process is lack of nutrient and microbial growth that is depleted in crude oil polluted soil hence the need for soil amendment. Soil amendments are material added to the soil to help stabilize soil physicochemical properties and microbial activities (Amadi *et al.*, 2022; Amadi *et al.*, 2023).

Biostimulation of crude oil-polluted soil using poultry manure holds immense significance for soil remediation, ecosystem restoration, and sustainable land management practices. By harnessing the synergistic effects of organic matter, nutrients, and microbial consortia present in poultry manure, stakeholders can expedite the degradation of petroleum contaminants, improve soil quality, and promote the recovery of polluted environments. Embracing the ecological and economic benefits of poultry manure-based biostimulation underscores its potential as a viable and eco-friendly strategy for addressing the environmental challenges posed by crude oil pollution, paving the way towards a greener and more resilient future. The aim of the study was to create a favorable condition that will enhance microbial growth, microbial diversity and plant optimal performance by enhancing depleted soil essential nutrients using poultry manure.

2.0. MATERIALS AND METHODS

2.1. Experimental Design

A Randomized Complete Block Design according to Pere's method (1981) and cited by Amadi *et al* 2018 was used for the experiment. The total bulk soil, weighing two kilograms, was measured using a Setra 480S weighing balance (USA) and divided into 15 polythene bags. Each bag, with dimensions of 18cm in height and 14cm in diameter, covered a surface area of 0.095 m². These bags were arranged into four blocks (labeled A, B, C, and E), with each block containing 12 replications. Additionally, a fifth block consisted of unpolluted soil sourced from a fallow farm land with no history of contamination.

2.2. Sources and Baseline Analyses of Materials

Soil sample suspected to be contaminated with waste diesel was collected from depth 0-10cm using soil auger behind the central generator plant house and unpolluted soil was obtained from a farm land situated 50m away from the generator plant in Rivers State University, Port Harcourt. After baseline analysis was done to ascertain the

pollution level (Table 1). The soils were air dried and sieved through a 2mm wire mesh to obtain a homogenous fine fraction of soil composites.

Table 1: Baseline analysis of soil

S/N	Parameter	Polluted	Control
1	TPH	1450.7	0.87
2	TOC (%)	3.81	2.5
3	pH	8.9	6.3
4	P (mg/kg)	0.05	0.05
5	K (mg/kg)	12	45
6	N (mg/kg)	7.8	20

The poultry manure was obtained from a local poultry farm located at Eliparanwo Village in Obio-Akpor Local Government Area, Rivers State, Nigeria. The poultry manure was dried mesh and sieved to obtain a uniform sized particle. The analysis of the poultry manure shows the following results: phosphorus 40.6mg/kg, potassium 483mg/kg, pH 6.5, total organic carbon 0.23%, nitrogen 20mg/kg.

2.3. Bio-amendment of Soil

Subsequently, 100 grams, 200 grams, and 300 grams of poultry waste were respectively incorporated into blocks A, B, and C. The mixture was meticulously blended to ensure thorough integration of the amendment and soil. Blocks D and E served as controls, with 0 grams of amendment added to block D for the unpolluted control and block E representing the polluted control and the bags were left undisturbed for a period of two weeks in accordance with Pere's method (1981) and cited by Amadi *et al* 2018

The summary of the design area follows:

Block A: contaminated soil + 100g of poultry waste + *Platostoma africanum*

Block B: contaminated soil + 200g of poultry waste + *Platostoma africanum*

Block C: contaminated soil + 300g of poultry waste + *Platostoma africanum*

Block D: uncontaminated soil + 0g of poultry waste + *Platostoma africanum*

Block E: contaminated soil + 0g of poultry waste + *Platostoma africanum*

2.4. Phytoapplication Process

Following that, two seedlings of *Platostoma africanum*, identical in size and vigor, were transplanted from the nursery unit into different blocks and their respective replications. The experiment was meticulously supervised, and watering was administered in accordance with the standard protocol, both in the morning and evening (50cl/planting bag). Monitoring of the experiment continued for duration of two months, with analysis conducted at monthly intervals.

2.5. Post- phytoapplication process and assessment

At one month interval, the soil from all the blocks with their replicates were collected, air-dried for 3 days and *Platostoma africanum* from each block was harvested by uprooting them from the bag. This was achieved by shaking and destroying the bags so that the soil will lose grip of the plant. The plant samples were washed; separated and air dried while soil samples were ground and sieved (500µm sieve) and then dried in an oven at 65°C for 16 hrs, using the air oven method and kept in clean polythene bags for further analysis. The following phytoindices were used to monitor the restoration performance of depleted soil. Soil pH and conductivity were determined electronically using a glass electrode pH meter (Jennway 3015 model) and conductivity (HACH model) respectively. Total hydrocarbon, phosphorus and nitrogen content were determined by Oxidation, Ascorbic acid and Kjeldahl methods respectively (Stewart *et al.* 1974). Potassium was determined using atomic absorption spectrophotometry after digestion. The plant height was accessed at two weeks interval using a meter rule which was placed from the base of the plant to its shoot apex and fresh and dry weight were obtained using a digital weighing balance (Ohaus, scout HSC4010), fresh weight determination was done immediately after harvest after drying off excess water using absorbent paper while the dry weight was determined after subjecting the plant to hot air oven drying process for 7 days. All data generated were subject to statistical analysis using SAS 9.1 version (2002).

3.0. RESULTS AND DISCUSSION

The effect of poultry manure on soil chemical properties are presented in Figures 1-7 with data obtain at the end of the experiment. Result showed reduction in total organic carbon content (TOC) (Fig. 1) in the remediation treatment (different concentration of poultry manure). The effects of biostimulants on total organic carbon decrease was significant between and within treatment at ($p=0.05$). The least reduction in TOC content was in soil biostimulated with 200g poultry manure at 30 and 60 days. The decrease in TOC content could be attributed to the concentration of biostimulants (poultry manure) added. TOC is a measure of the amount of carbon present in organic matter in the soil and the addition of organic amendment tends to improve it concentration and enhance TOC level. However, the concentration of organic matter added was insufficient to triggered increase in TOC hence low concentration. In addition the concentration and type of organic matter addition is also capable of influencing TOC levels, different contains different carbon content and their rate of decomposition varies as such organic amendment with lower carbon content can be easily decomposed by microorganisms, which can result in decrease TOC over a given period. This result agrees with (Tanee and Kinako 2008) who reported a decrease in TOC content with addition of biostimulants of different type and concentration and a highest reduction was recorded with the application of inorganic amendment. This result was not in agreement with the findings of (Debska *et al.* 2022) who reported an increase in TOC in soils amended with different concentration of sawdust, this was possible because sawdust is not readily decomposed by soil organisms and the rate of decomposition and influence TOC concentration in amended soil. Highest increment of TOC in double control soil (polluted soil with 0g amendment) could be attributed to the concentration of carbon present in crude oil and its products. This increment is envisage since crude oil polluted soil usually comes with decrease in microbial population as such the breakdown of the carbon content in crude oil is impeded hence the increase in TOC. This finding corroborated with the report of (Tanee and Kinako 2008; Egobueze *et al.* 2019) who reported high increase in TOC content in crude oil polluted plots compared with control plots.

Soil phosphorus (P) increased significantly between and within biostimulants with time ($p=0.05$). At the termination of the experiment, highest increment in soil phosphorus was recorded in soil amended with 300g and 200g of poultry waste manure at 30 and 60 days respectively (Fig.2). The least decrease in phosphorus content was recorded for double control (polluted soil with 0g biostimulants).

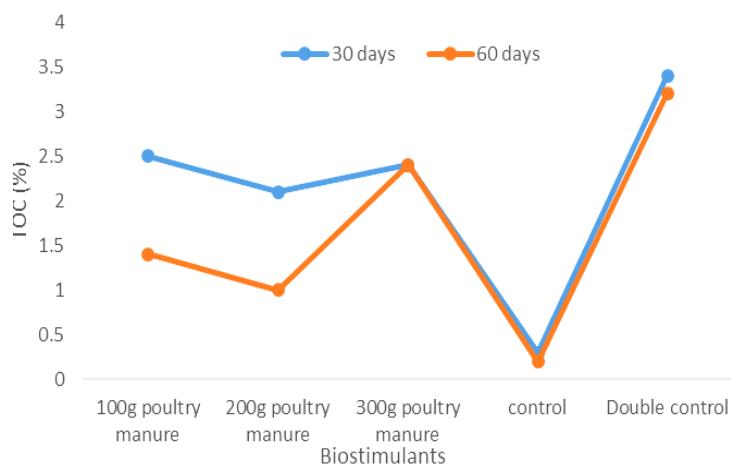


Fig 1 Soil Total Organic Carbon (TOC) (%) in different Remediation Treatment

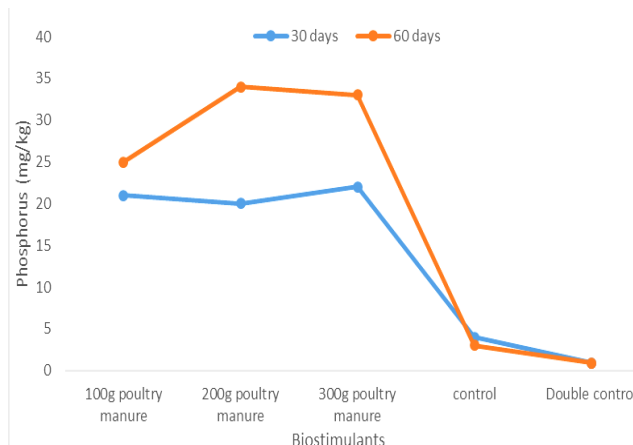


Fig 2. Soil Phosphorus content in different Remediation Treatment

Increase in potassium content (Fig 3) was also found in the biostimulated soils, with 300g poultry amended soil having the highest increment at 30 and 60 days. Increase in soil nitrogen content was observed with different concentrations of poultry manure waste which was significant at ($p=0.05$). Highest increment in nitrogen content was found in 100g and 300g poultry manure biostimulated soil at 30 and 60 days respectively with double control (polluted 0g poultry manure) showed least decrease (Fig. 4). The increase in phosphorus, potassium and nitrogen contents in biostimulated soils may have resulted from the composition of these nutrients present in poultry manure

biostimulants may have triggered the concentrations of these nutrients in the soil. Phosphorus is an essential nutrient required for adequate plant growth and is usually found in organic matter. When these organic amendments are incorporated soil stimulate microbial activity through mineralization process they release phosphorus in the soil and also limits its retention capacity. The application of 100g and 300g of poultry manure triggered increment in nitrate content this increase is also attributed to the organic amendment used. Organic amendments contain nitrogen which is seen in form of protein and amino acids and during mineralization microorganisms breakdown and convert nitrogen into nitrate and ammonium which further undergo nitrification by nitrifying bacteria resulting in increased soil nitrate. Potassium is an important macronutrient which play and crucial role in growth and performance of plant development. The addition of 300g poultry manure triggered an increase in potassium content, this is true because organic amendments often contain certain amount of potassium and increase in potassium is recorded during the decomposition process of the amendment. This finding corroborated with the report of (Amajuoyi and Wemedo 2015) who associated the increase in mineral nutrients (nitrate, phosphate and potassium) in diesel oil polluted soil with the addition of PBA amendment. This report also agrees with (Amadi *et al.* 2023) who reported an increase in nutrient content in organic enhanced soil. The least decrease in nitrogen, phosphorus and potassium in double control with (0g biostimulants) could be attributed to the depletion of soil nutrients due to toxic nature of the pollutant which influence microbial population leading to decrease in mineralization process.



Fig 3. Soil Potassium content in different Remediation Treatment

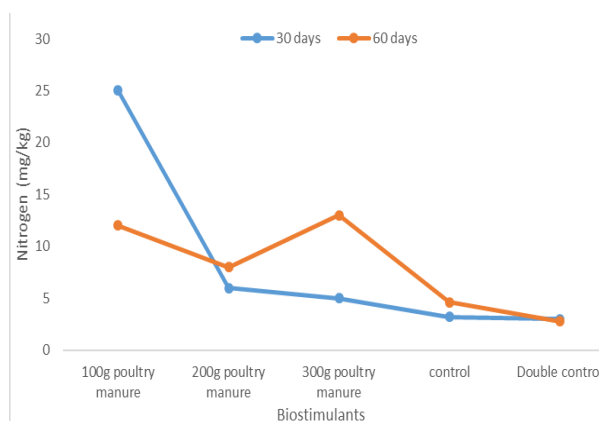


Fig 4. Soil nitrogen content in different Remediation Treatment

Total hydrocarbon content and pH decreased in biostimulated soil (Figs.5 & 6) and there was significant difference within and between treatment at ($p=0.05$). The least decrease in THC and pH was in 300g poultry manure biostimulated soil at 30 and 60 days while the highest increment in THC and pH was in polluted soil with 0g biostimulants (double control). The high THC content in the double control soil is attributed to lack of organic amendment which is able to stimulate microbial activities since the population of microorganisms with the ability to degrade hydrocarbon is always limited hence optimum activity of microorganism is impeded resulting in increasing soil THC content. This result agrees with (Brown *et al.* 2017) who accessed waste diesel oil spilled sites and discovered that increase in total hydrocarbon content is function of concentration of hydrocarbon present in crude oil (Brown *et al.* 2017; Amadi *et al.* 2022) also reported that the increase in THC is due to the concentration of carbon present in the crude oil and its products. The decrease in THC observed in biostimulated soil is understandable since the addition of amendments may have triggered the microbial population responsible for hydrocarbon degradation leading to the breakdown of THC content within a specific period. In addition, the amendment composition is also capable of influencing the concentration of THC hence the concentration of THC in the amendment used was insignificant. This assertion is in line with the report of (Speight 1999; Amadi *et al.* 2023) who reported that stimulants can cause an increase in microbial population and activities leading to high energy demand by the oil degrading microbes. The optimal pH level necessary for the growth of oil degraders was achieved with the addition of biostimulants. This finding agrees with (Speight 1999; Amadi *et al.* 2023) who reported similar result that organic amendments have the capacity to alter pH of a growth medium.

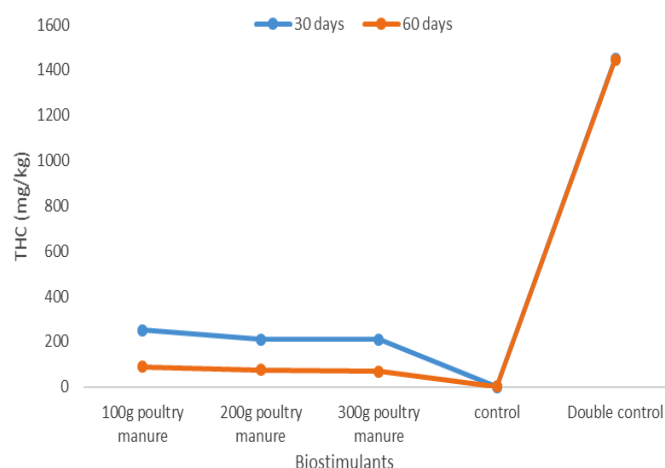


Fig 5. Soil Total Hydrocarbon Content (THC) in different Remediation Treatment

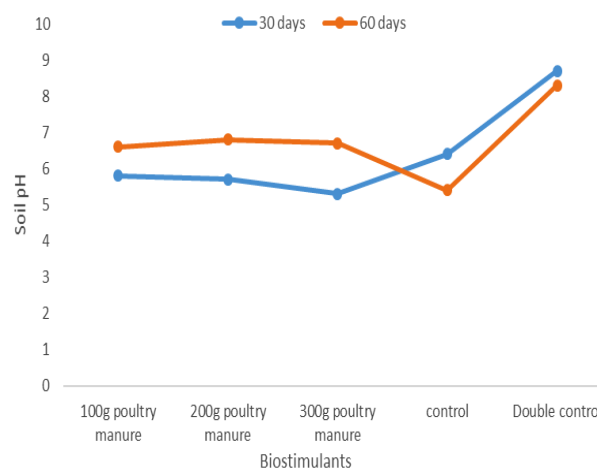


Fig 6. Soil pH in different Remediation Treatment

Table 2 and 3 Result showed Pearson's Correlation coefficient between different levels of biostimulants and soil properties at 30 and 60 days. At 30th day strong positive correlation was established between TOC-THC ($r = 0.7$), P- TOC ($r = 0.9$); P-K ($r = 0.8$), N-TOC ($r = 0.8$) THC-N ($r = 0.6$) with pH having weak positive correlation. Strong positive correlation was also recorded between P and N while pH showed strong negative correlation. Pearson Correlation coefficient was also estimated to examine of relationship between physicochemical properties at 60 days. Positive coefficient were observed for TOC-THC, pH, P, K, N as ($r = 0.6$; 0.8 ; 0.6 ; 0.7 ; 0.9) respectively.

Table 2: Pearson Correlation between Different Levels of Treatment Application at 30days

	TOC	P	K	N	THC	pH
TOC	1***	0.94106***	0.45323	0.84369***	0.1081	0.32342
P	-0.04631	1***	0.062873	0.38965	0.38239	0.2426
K	-0.44454	0.85796***	1***	0.56128*	0.058526	0.020598
N	-0.12308	0.50123*	0.35198	1	0.65571**	0.61727**
THC	0.79476***	-0.50783*	-0.86468***	-0.27387	1	0.014014
pH	0.56267*	-0.64226**	-0.93302***	-0.30541	0.94827***	1

** and *** indicate significance level at 0.05

Table 3: Pearson Correlation between Different Levels of Treatment Application at 60days

	TOC	P	K	N	THC	pH
TOC	1***	0.67084**	0.71081***	0.89357***	0.24797	0.078611
P	0.26154	1***	0.060144	0.085415	0.33785	0.6212**
K	-0.22915	0.86216***	1***	0.061524	0.041134	0.16387
N	0.08369	0.82517***	0.86003***	1***	0.23562	0.46841
THC	0.63677**	-0.54904*	-0.89335***	-0.64943	1	0.011588
pH	0.83475**	-0.30217	-0.72719**	-0.43128	0.95446***	1***

** and *** indicate significance level at 0.05

Result in Fig. 7, showed the effects of organic amendments on total petroleum hydrocarbon (TPH). Decreased in TPH was found in biostimulated soil of various concentrations and there was significant difference within and between treatment at ($p=0.05$). The least decrease in TPH was in 300g poultry manure biostimulated soil at 30 and 60 days while the highest increment in TPH was in polluted soil with 0g biostimulants (double control). Increase in total petroleum hydrocarbon (TPH) levels is usually attributed either to the concentration of TPH present in the amendments or the polluted environment. Since TPH concentration in the amendments used was insignificant however, increase in TPH concentration may be attributed to its level present in the studied soil. This result

corroborated with the findings of (Brown *et al.* 2017) who sampled a crude oil waste sites an increase in total petroleum hydrocarbon and concluded that the concentration of carbon present in the crude is responsible for the increase in TPH. This assertion also agrees with (Amadi *et al.* 2022; Brown *et al.* 2017) also reported that the increase in TPH is due to the concentration of carbon present in the crude oil and its products. The decrease in TPH recorded in amended soil could be attributed to the amendment used may have reduced TPH levels. This proposition is possible since the added amendment may have promoted the microbial population and this stimulant may have triggered the activity of hydrocarbon degraders which are capable of breaking down hydrocarbons into less toxic compound and the efficacy this microbial activity can result in a decrease in TPH levels. This findings further suggest that organic amendments not only increase microbial population and activity, which in turn increases energy demand by oil-degrading microbes, but they also have the ability to sequester Total Petroleum Hydrocarbons (TPH) molecules. This sequestration reduces the mobility and availability of TPH in the soil. As a result, the immobilization process leads to a decrease in total petroleum content in the soil. The findings of Speight (1999) and Amadi *et al.* (2023) corroborate each other's conclusions on the benefits of organic amendments in hydrocarbon-contaminated environments. This suggests that incorporating organic amendments could be an effective strategy for remediation efforts in such environments.

The effects of different concentration of biostimulants influence the morphological properties of *Platostoma africanum*. (Table 4). Increase in shoot growth, fresh weight and dry weight was recorded in the biostimulated soil when compared to control soils. There was significant difference between and within treatment at ($p=0.05$). The highest increase in shoot growth, dry weight and fresh weight were recorded in plants grown in 100g and 300g poultry manure stimulated soil respectively and the least decrease in shoot growth and other morphological properties were found for plant in grown 0g poultry biostimulated soil (double control). The decrease in shoot length, fresh weight and dry weight can be attributed to the effects of the toxic compounds such as polycyclic aromatic hydrocarbon (PAHs) and volatile organic compound (VOCs) present in the crude. Direct impact of hydrocarbon on plants lead to cell damage, impaired photosynthetic action, depletion of plant essential nutrient and the combine force of these action disrupt the normal functioning of plant tissues and this can lead to reduced growth rate, chlorosis, decrease in plant weight and plant death. This result agrees with (Odokuma and Ibor 2002; Fidanza *et al.* 2010) reported a decrease in shoot length of plant grown in crude oil polluted soil and concluded that the reduction in plant morphology is attributed to the negative impact of crude oil on plant which is achieved through reduces soil water holding capacity, inadequate aeration and nutrient availability. The increase in plant morphological properties could be attributed to the type and concentration of amendment used. Since crude oil contaminated soil cause a serious challenges to plant growth the added organic amendment may have enhanced soil structure and fertility by its increasing aggregation and porosity the resultant effects create an atmosphere for better root penetration, water retention, nutrient availability and absorption, it also contribute essential nutrients, increase organic matter content and stimulant the microbial activity of hydrocarbon degrader. This assertion is in line with the findings of (Speight 1999) who conducted an investigation on the effects of organic amendment on soil chemical properties, microbial activities and growth of plant and concluded that the addition of organic biostimulants is effective in restoring soil nutrient deficits, enhance microbial growth and also capable of promoting the growth of plants in a polluted environment.

Table 4. Effects of different levels of biostimulants on plant growth, fresh weight and dry weight.

S/N	Biostimulants	Shoot growth(cm)	Fresh weight (g)	Dry weight (g)
1	100g poultry manure	52.0 \pm 2.1 ^a	45.9 \pm 3.01 ^a	15.02 \pm 1.20 ^a
2	200g poultry manure	34.8 \pm 0.01 ^b	41.3 \pm 2.0 ^{ab}	12.8 \pm 0.1 ^{ab}
3	300g poultry manure	35.0 \pm 0.01 ^b	46.3 \pm 2.0 ^a	11.6 \pm 0.1 ^{ab}
4	Control (unpolluted soil)	13.5 \pm 0.02 ^c	15.0 \pm 1.02 ^c	7.6 \pm 0.002 ^c
5	Double control (polluted soil)	6 \pm 0.002 ^d	4.8 \pm 0.001 ^d	2.1 \pm 0.004 ^c
	LSD	1.066	2.262	2.76

CONCLUSION

The study emphasizes the detrimental effects of crude oil on soil nutrient levels and physicochemical stability, emphasizing the urgent need for effective remediation strategies. Remarkably, the research confirms that a synergistic approach involving poultry manure and *Platostoma africanum* holds immense potential in accelerating the degradation of crude oil contaminants in soil. Poultry manure emerges as an ideal soil enhancer in this remediation method, thanks to its rich nutrient composition that not only nourishes the soil but also fuels microbial

activity essential for biodegradation processes. The integration of *Platostoma africanum* further enhances this process, potentially through mechanisms such as phytoremediation, thereby amplifying the remediation efficacy. One of the most noteworthy findings of the study is the optimal dosage of poultry manure 300g which yielded the highest hydrocarbon degradation and nutrient enrichment compared to other concentrations. This result not only highlights the effectiveness of poultry manure as a soil amendment but also provides practical insights for end users and policymakers. The implications of this research extend far beyond the confines of the scientific community. By advocating for the widespread adoption of poultry manure-based remediation methods, the study paves the way for sustainable soil management practices that mitigate the environmental impacts of crude oil contamination while simultaneously promoting agricultural productivity. In conclusion, the integration of poultry manure and *Platostoma africanum* presents a holistic and environmentally friendly approach to address the challenges posed by crude oil contamination in agricultural soils. By harnessing the power of nature's resources, this innovative remediation strategy offers a ray of hope for restoring contaminated lands and fostering resilient ecosystems for generations to come.

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CONFLICT OF INTEREST

All authors have no known or potential conflict of interest as they declared that no conflicts of interests exist.

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