MORPHOLOGICAL STUDIES OF *ABELMOSCHUS ESCULENTUS* L. ON AMENDED CRUDE OIL POLLUTED SOIL

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Abstract

Crude oil pollution interferes with the natural state of the environment and causes loss of soil function leading to greater dependence of soil on fertilizer for plant optimal growth. The objective of this study was to compare the effectiveness of TOW and PRP to mitigate crude oil effect on the environment. This investigation was conducted using A. esculentus as a test plant in RCBC design, consisting of 7 blocks; each of the blocks contains 2kg of crude oil polluted soil in 6 replications. Block 1-3 was further amended with 200g, 400g and 600g of TOW, while 4 – 6 contains 200ml, 400ml and 600ml of PRP, respectively and block 7 served as the control unamended. Quantitative observation showed that amelioration treatments TOW recorded significant increase in plant height, root length, root fresh weight, root dry weight and shoot dry weight, which was significant at p=0.05. Data generated showed significant increase in plant height at 2 and 4 months in TOW 400g and 600g respectively. The highest increase in root length was found in 200g TOW treatment at 4 months whereas PRP 400ml showed increase at 2 months. 600g TOW increased root fresh weight and root dry weight at 2 and 4 months. Highest in shoot dry weight at 2 and 4 months was found with 600g TOW treatment. On the basis of the result obtained, TOW 600g locally generated is a suitable soil enhancer capable of improving the optimal growth of plants.

Keywords: Petroleum Remediation Product (PRP), *Telfairia occidentalis* Waste

Introduction

The environment is essential for our survival and has provided useful resources to meet our needs. Urbanization and population increase has resulted in the over-utilization of natural resources (Wilson and Mukhopadhyaya 2020). Crude oil, a major contributor to Nigeria's economic growth has also caused severe pollution to the environment, affecting the livelihoods of locals. The Niger Delta region of Nigeria has been reported as one of the most polluted areas in world, due to crude oil pollution affecting the environment (water body, local income, employment, livelihood structures and community health). Changes to the natural state of any environment affects the lives within that environment. Thus, the continual existence, growth and reproduction of plant are dependent on favourable environmental conditions (Tashome *et al.,* 2020; Bashir 2021).

Crude oil pollution which are majorly by anthropogenic activities occurs when crude oil leaks from tankers, offshore platforms, drilling rigs, well and so on, into the environment and causing pollution on it. Crude oil contains hydrocarbon (carcinogenic and hazardous) and heavy metals (toxic, carcinogenic, non-degradable and persist in the environment for a long time). These heavy metals can be accumulated into the food chain causing serious health risk in humans and animals (Freije 2015; Yuan *et al.,* 2019; Xia *et al.,* 2022). Crude oil toxicity on plants varies based on the concentration of the crude oil, soil composition, pH and plant species. High concentration of hydrocarbon in soil is toxic to plant affecting seed germination, plant growth, production, physiological and biochemical processes, genetic elements and alters plant defence mechanism leading to their death (Athar *et al.,* 2016) Odukoya *et al.,* 2019; Skrypnik *et al.,* 2021; Sagaya *et al.,* 2023). Some plants have been able to develop mechanism to survive crude oil pollution whereas others are unable to tolerate unstable environmental conditions and eventually die leading to loss of biodiversity and extinction of some economic plants (Albert *et al.,* 2018). Based on these effects, some scientists have developed methods to reduce crude oil pollution on the environment using chemical and physical methods (Niewiadomska *et al.,* 2020). Although these methods have shown to be effective, however it is expensive, energy intensive and causes secondary pollution. This calls for affordable and ecologically friendly methods to minimize the adverse effect of crude oil pollution on the environment. Two plants were used in this study, one as a test plant and the other as an amendment. *A. esculentus* (test plant) is an economic plant is used to make several delicacies, it is nutritious with numerous medicinal benefits and improved the livelihood of local farmers who cultivate them (Harris *et al.,* 2019; Ibitoye *et al.,* 2022; Tavakolizadeh *et al*., 2023). Crude oil pollution will affect the growth and development of *A. esculentus* leading to its scarcity in the market and affects the livelihood of local farmers. *T. occidentalis* tendrils (amendment) are always littered in the marketplaces after its leaves have been sold making the environment look untidy. Utilizing these wastes as amendment will keep the marketplace clean, create materials (waste to wealth) which are ecologically friendly and accessible to farmers for the improvement of the soil fertility and promote crop yield. Therefore, this study assessed the morphological effect of organic and synthetic amendment on *Abelmoschus esculentus* (L) grown in a crude oil polluted soil. The objective was to determine the effect of TOW and PRP amendment on the number of leaves, height, girth, root length, root fresh weight (RFW), shoot fresh weight (SFW), root dry Weight (RDW) and shoot dry weight (SDW).

Methodology

This research was carried out in Rivers State University Ecological Centre, Rivers State, Nigeria. This experimental area lies within latitude 4^{o} 48$^{'}$4.67856" N and Longitude 6^{o} 58$^{'}$53.35032" E. This area is located in the tropical rainforest region of southern part of Nigeria popularly called Niger Delta region. It has two main seasons; The raining season starting from February - November while the dry season starts from December - March, with annual temperature of 28^{o} C and annual rainfall of 2400mm (Tamunobereton-Ari *et al.,* 2013).

Abelmoschus esculentus L. (NKOKO 195), used as the test plant was obtained from the National Institute for Horticultural Research (NIHORT), Idi-Shin Ibadan, Nigeria. Crude oil polluted soil was obtained from a Hydrocarbon Pollution Remediation Project (HYPREP) site at N 533181 and E293746 in Ogale, Eleme, Rivers State. The collected soil was transported to the Rivers State University Ecological unit and samples were bottled and taken to the laboratory for proximate analysis (Table 1). *Telfairia occidentalis* wastes (TOW) locally called the ugu tendril is an organic material, procured from a local market in Port Harcourt, Nigeria. The sourced *T. occidentalis* was cut in bits, dried and processed into powdered form, which was subjected to Chemical analysis (Table 2). Petroleum Remediation Product (PRP) is a chemical stimulant composed of filtered beewax, filtered soy wax and corncob fraction. PRP was sourced from an Environmental Company located in Port Harcourt, Rivers State. The samples were taken to Rivers State University Ecological centre, where they were prepared and used as one of the treatments for the polluted soil.

Table 1: Proximate analysis of Polluted Soil

Parameter	**Unit**	**Polluted soil**
pH	-	5.90
Temperature	^{O}C	27
Electrical Conductivity (Ec)	µS/cm	10
Sand	%	72.32
Clay	%	3.08
Silt	%	24.56
Particle Size/ textural class		Sandy Silt

Moisture Content	%	12.0
/Phosphorus (P)	mg/kg	3.880
Potassium (K)	mg/kg	24.52
Total Organic carbon (TOC)	%	1.00
Total Nitrogen	%	0.77
Total Heterotrophic Bacteria (THB)	cfu/g	1.48
Total Heterotrophic Fungi (THF)	cfu/g	1.17
Total Hydrocarbon Utilizing Bacteria (THUB)	cfu/g	3.50
Total Hydrocarbon Utilizing Bacteria (THUF)	cfu/g	2.30
Total Petroleum Hydrocarbon (TPH)	Mg/kg	2350
Cation Exchange Capacity (CEC)	Meg/100g	2.97

Source: Field Survey, 2024

Table 2: Chemical Analysis of *T. occidentalis* Waste

Parameter	**Unit**	**Sample** *Telfairia occidentalis* **waste**
Nitrogen	%	2.10
Phosphorus	mg/kg	281.39
Ph		7.50
Electrical Conductivity (Ec)	µS/cm	4520
Calcium (Ca)	mg/kg	121.50
Magnesium (Mg)	mg/kg	3650.50
Sodium (Na)	mg/kg	128.45
Potassium (K)	mg/kg	686.50

Source: Field Survey, 2024

Determination of the morphological effect of amendments on *A. esculentus* grown in polluted soil

The morphological properties of *A. esculentus* were determined using the following parameters: Number of leaves, height, girth, root length, root fresh weight (RFW), shoot fresh weight (SFW), root dry Weight (RDW) and shoot dry weight (SDW). The number of leaves was collected weekly for T1-T7 using a measuring tape, from the base of the stem to the highest part of the plant and recorded in centimeters (cm). The number of leaves was done weekly by physically counting the number of leaves on each plant and recording in the logbook. Measurements of the girth was taken at 2 months interval, with a vernier calliper around 2m above root and the observed readings were recorded. At 2 months, the root of *A. esculentus* was harvested, rinsed, and drained, then separated from the shoot using a clean blade and weighed using an electronic compact scale balance (in grams) with the root fresh weight recorded. The shoot fresh weight was measured by cutting the shoot with a razor blade and weighing it on a calibrated electronic compact scale balance in grams (g) and result recorded. The root dry weight was determined by drying the cut roots for one week, then weighing them on a calibrated balance in grams (g) and recording the result. Shoot Dry Weight was measured by drying the cut shoot for two weeks, then weighing it on a calibrated balance and recording the result in grams in the field log.

A Randomized Complete Block Design (RCBD) was used in this study. Homogenized soil from the HYPREP site at Ogale, Eleme, was weighed (2kg) using a Setra 480 balance and filled into planting bags (18cm x 14cm x 0.095m2). The bags were arranged in 7 rows with 6 replicates each, and treatments were then applied as:

Table 3: Experimental design for the study

Treatment	Contents
TOW 200	2kg Crude oil polluted soil + 200g of *T. occidentalis* wastes + Test Plant
TOW 400	2kg Crude oil polluted soil + 400g of *T. occidentalis* wastes + Test Plant
TOW 600	2kg Crude oil polluted soil + 600g of *T. occidentalis* wastes + Test Plant
PRP 200	2kg Crude oil polluted soil + 200mls of PRP + Test Plant
PRP 400	2kg Crude oil polluted soil + 400mls of PRP + Test Plant
PRP 600	2kg Crude oil polluted soil + 600mls of PRP + Test Plant
Polluted (Control)	2kg Crude oil polluted soil + 0 treatments + Test Plant

Source: Field Survey, 2024

Two weeks after treatment, two moistened *A. esculentus* seedlings were planted in each bag. The plants were watered and weeded as needed and monitored at 2-month intervals. Morphological properties such as height, plant grith and number of leaves were estimated weekly, soil baseline analysis was carried out at 2 months, and 4 months to assess hydrocarbon content, microbial load, and physicochemical properties. At 2 and 4 months, three replicates were harvested and analyzed for root length, root and shoot biomass. All samples were properly labelled and taken to the laboratory for analysis. The research results were analyzed using SAS version 20, with Analysis of Variance (ANOVA) to determine treatment effectiveness. Significant differences were separated using Fisher's LSD at a 5% probability level to identify specific treatment means.

Results and Discussion

Morphological effect of amendments on *A. esculentus*

The analysis of the morphological result obtained from *A. esculentus* grown in polluted soil treated with TOW and PRP at 2 month and 4 months showed significant differences at $P<0.05$ interaction between and within treatment in the height, root length, root fresh weight, root dry weight and shoot dry weight. The height increased most with 400g TOW amendment at 2 months, while at 4 months, it increased most with 600g TOW amendment, with the least decrease observed in the polluted control (0g amendment) at 2 months and in 600ml synthetic PRP soil amendment at 4 months (Fig 1). At 2 months, root length increased most with 400ml synthetic PRP soil amendment, while at 4 months, it increased most with 200g TOW amendment, with the least decrease consistently observed in 600ml synthetic PRP soil amendment at both time points (Fig 2). Root fresh weight increased most in the polluted control (0g amendment) at 2 months, while at 4 months, it increased most with 600g TOW amendment, with the least decrease observed in 200ml synthetic PRP soil amendment at 2 months and in 600ml synthetic PRP soil amendment at 4 months (Fig 3). The 600g TOW amendment showed the highest increase in root dry weight at both 2 and 4 months, while the least decrease was observed in 400ml synthetic PRP soil amendment at 2 months and in 600ml synthetic PRP soil amendment at 4 months (Fig 4). The shoot dry weight showed the highest increase in 600g TOW amendment at both 2 and 4 months, with the polluted control (0g amendment) consistently showing the least decrease at both time points (Fig 5).

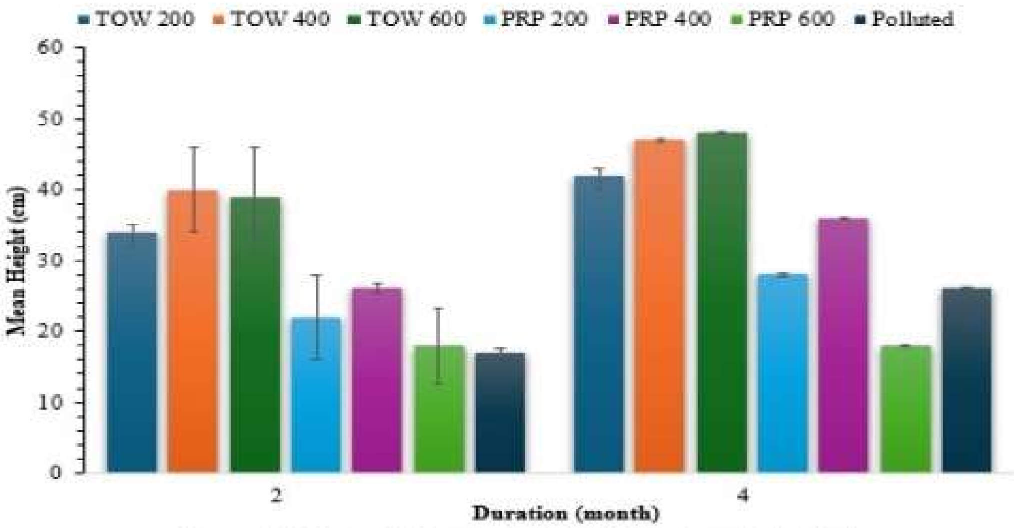

Figure 1: Effect of Different Levels of Organic and Synthetic Amendments on the Height

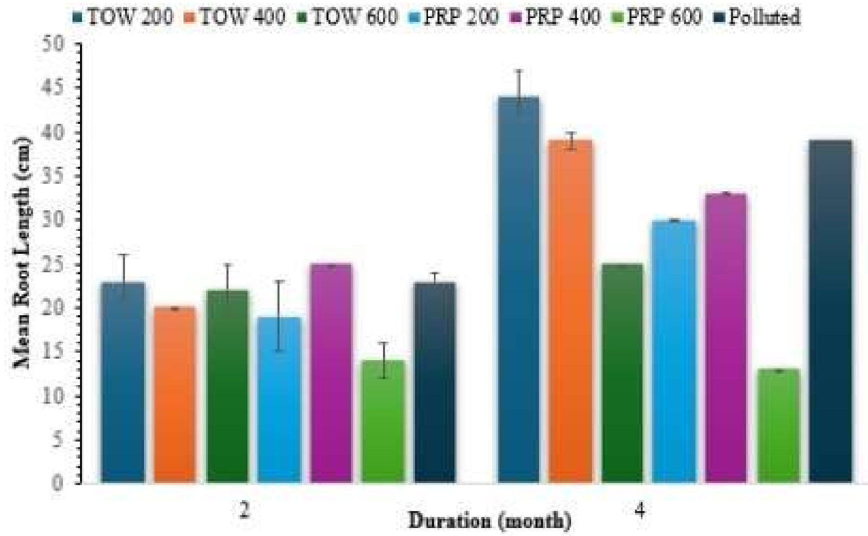

Figure 2: Effect of Different Levels of Organic and Synthetic Amendments on the Plant Root Length

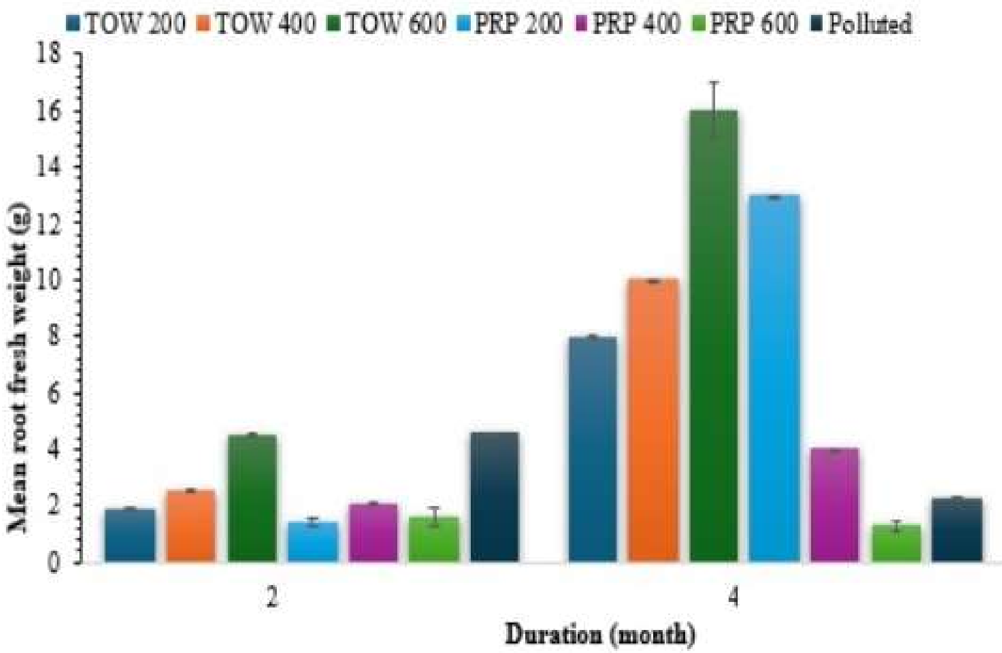

Figure 3: Effect of different levels of organic and synthetic amendments on root fresh weight

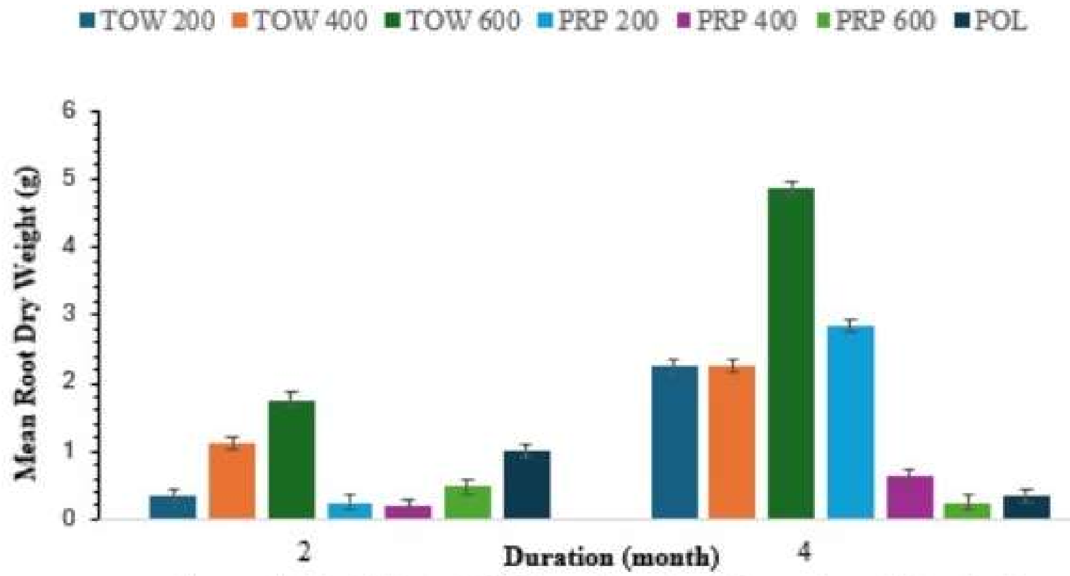

Figure 4: The Effect of Different Levels of Organic and Synthetic Amendments on the Root Dry Weight

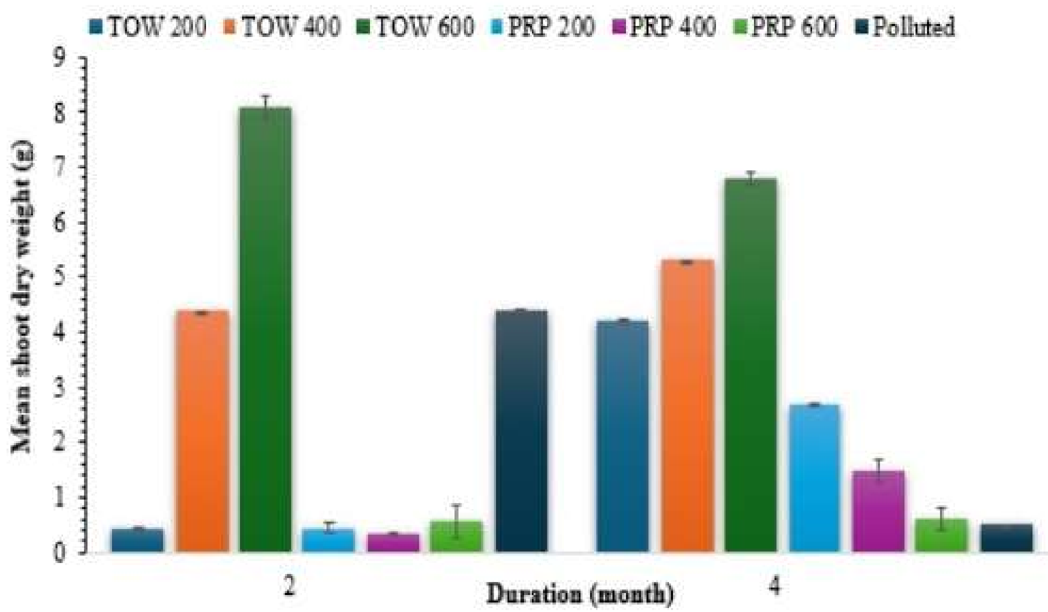

Figure 5: The Effect of Different Levels of Organic and Synthetic Amendments on the Plant Shoot Dry Weight

Effect of Different Levels of Treatments on the Morphological Properties of *A. esculentus*

The result shows that crude oil pollution significantly reduced the height of *A. esculentus* as observed in the control polluted soil (0g amendment), however overtime the height increased in polluted soil due to natural attenuation taking place. *A. esculentus* treated with TOW amendment increased in height overtime as the concentration of TOW progressed, with the highest observed in 600g, PRP on the other hand showed less increase in height at high concentration (PRP 600ml). This could be attributed to the nutrient composition of TOW which stimulated the growth of *A. esculentus.* Hossain *et al.,* (2017), reported that organic amendment improved the physical, chemical, biological properties of soil, stimulates growth and increase yield of plant. Bhatia & Sindhu (2024) gave similar report that organic waste improves soil organic matter, nutrient availability and stimulates microbial growth, thus supporting this result finding. Additionally, Egobueze *et al.,* (2019), reported that organic amendment improved the height of plants grown in crude oil polluted environment overtime. Correspondingly, Chuku *et al.,*(2022), reported that African yam bean grown in treatment with $CaCl_2$ caused reduction in the height of the test plant whereas treatment with charcoal showed highest in height overtime. Contrary to this research report, Eremrena & Akonye (2015), reported that overtime, at high level of crude oil pollution, inorganic fertilizer increased the height of the test plant than organic amendment. Similarly, Okafor & Chidozie (2016), reported that inorganic and organic amendment were effective in increasing plant height, but the former showed more increase in the height of the test plant than the later. In both treatments at high concentration, the root length of *A. esculentus* reduced, but the reduction was more in treatment with PRP than treatment with TOW. Whereas at low concentration of the amendments, there was increase in the root length of *A. esculentus* with TOW showing more increase than PRP. This reduction observed at high level of the amendments could be attributed to toxicity threshold, meaning that at low concentration of organic amendments can help mitigate the toxic effect of crude oil on plant, allowing them to grow longer, However, at high concentration, the amendments may not be able to counteract the toxicity of crude oil, hindering the growth of the root. Several studies have reported on the use of amendments for remediation, however to the best of my knowledge, little have been reported on the effect of high concentration of these amendment on plant root length. On the other hand, some studies have reported that organic amendment are effective in improving root growth of plant grown in a crude oil polluted soil (Farooqi, et al., 2023). The increase observed in the Root fresh and dry biomass of *A. esculentus* grown in soil treated with *T. occidentalis* waste at high concentration (TOW 600g) could be attributed to the high level of potassium in the organic amendment which when added to the soil, contributed to the root growth of *A. esculentus*. Potassium is known to support plant root growth enabling them to access water in deeper and wider soil layers and increase the density and surface area to expand surface contact with the surrounding, it also helps plants to efficiently take up water and mitigate drought (Xu *et al.,* 2021). Ali *et al.,* (2021), observed that high potassium level increases the root growth and development, supporting this research finding. Amadi *et al.,* (2023) reported that application of organic amendments caused increase in the root fresh and dry weight due to the good amount of nutrient which they contain, and this helps to caution nutrient losses due to pollution, this corresponds to this research finding. Similarly, Law-Ogbomo *et al.,* (2012), reported improvement in the dry weight of the test plants with the application of organic stimulant at increasing concentration, this gives credence to this research result. Similarly, Oosten *et al.* (2017), observed improvement in the root dry weight with the application of organic stimulant at increasing concentration. Akhtar *et al.,* (2019) reported that inorganic amendment applied at high concentration reduced the root dry weight of the test plant, attributing this effect to the toxicity level of the inorganic amendment, thus supports this research finding. Contrary, to this report, Odoh *et al.,* observed increase in the root fresh weight of carrot at high concentration of the inorganic amendments. The highest increase in the shoot dry weight (SDW) of *A. esculentus* with the application of organic amendment at high concentration (TOW 600g) is attributed to high phosphorus content in the stimulant, which when applied to the soil increased the phosphorus level of the soil and resulted in shoot proliferation. Haque *et al.,* (2023) confirmed that soil treated with organic manure at elevated level had the highest shoot dry weight than those stimulated at low concentration. Adding that organic manure can improve the nutrient content (N, P, K) of the soil at increasing concentration, supporting this research findings. Similarly, Sistani *et al.,* (2010), reported that high levels of organic stimulant application significantly enhanced dry matter of test plant. Abeed *et al.,* (2021) also confirmed a similar increase in the plant dry biomass due to organic stimulant, thus complimenting this studys' result.

Conclusion

This study shows that crude oil pollution is harmful to soil and plants, leading to extinction of economic plants. Natural attenuation is a slow process and can cause prolonged damage. Although, organic (TOW) and inorganic (PRP) amendments can stimulate plant growth and reduce pollution. However, organic amendment particularly at high concentrations (TOW 600g) is more effective in promoting plant growth due to their nutrient content. In contrast, inorganic amendments work best at lower concentrations (PRP 200ml) due to toxicity threshold.

Recommendations

The implication of this study to Agricultural extension is that it will educate farmers on the detrimental effects of crude oil pollution and how plant wastes can be utilized to mitigate these effects to improve soil health and farm productivity. This approach will reduce waste generation and promote sustainable practices. Further research is needed to explore the optimal use of organic and inorganic amendments for plant growth in polluted environments.

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