



Bioremediation: Assessment of Growth Attributes of Maize (*ZEA MAYS*) on Crude oil-Polluted Soils

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ABSTRACT

Environmental pollution has posed a major threat to terrestrial, aquatic, and marine ecosystems, thereby affecting microflora and micro-fauna populations. This study assessed the growth attributes of maize plants on crude oil-polluted soils amended with agro-wastes. Six kilograms each of composite soil sample was weighed and transferred into one hundred and fifty labeled plastic buckets with drainage holes for soil aeration and spiked with 300mls each of crude oil, allowing for 14 days of soil acclimatization. Soil amendments such as groundnut husks, cassava peels, empty fruit bunch of oil palm, and maize cob powder were applied and allowed for 90 days. Maize seeds were sowed, while periodic data were collected and subjected to a three-way ANOVA. The result obtained revealed that maize seeds grown on agro-wastes treated and pristine control soils show early seed germination than the crude oil-polluted control soil. The plant height obtained for $GnH_{14}P + MaC_{14}P$ at 10% was the highest with a mean (of 152.81cm^2), and the leaf area of the maize from soil treated with $GnH_{14}P + EFBOP_{14}P$ at 10% had the highest mean (756cm^2), the leaf length of maize from soil treated with $GnH_{14}P + CasP_{14}P$ at 3%, 6%, and 10% was the highest with mean ranging ($54-97\text{cm}^2$) with no significant difference in mean values obtained. The stem girth, number of leaves, and leaf width were generally improved in the bio-remediated soils. The result for the yield performance of maize shows that the days to flowering were shortened in the bio-remediated soil compared to the prolonged flowering days observed in the crude-oil polluted control. The number of seeds per cob was high in the bio-remediated soils while no seed was obtained in the crude-oil-polluted control soils. It can be concluded that the ameliorated treatment with the agro-wastes improves the performance of maize plants in crude oil-polluted soils.

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INTRODUCTION

The sustainability of the human environment or ecosystem depends on the judicious management of natural resources. The maintenance of this natural resource constitutes the platform upon which our civilization is based. Petroleum products have been widely and commercially explored since the middle of the 19th century. It has been used for many decades for illumination and, on a smaller scale, as a lubricant. The invention of the internal combustion engine and its adoption in all transport forms enlarged the application of this natural resource, thus increasing its production, transport, storage, and distribution. All these activities bring about

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inadvertent or deliberate pollution risks that can be minimized, but not eliminated resulting in environmental problems. The environmental consequences of oil pollution on most agricultural lands have turned hitherto productive areas into wastelands, with increasing soil infertility due to the destruction of soil microorganisms, and dwindling agricultural productivity thus resulting in the abandonment of this land by farmers. The growth performance of crops on soils depends on the right amount and proportion of nutrients, water, oxygen, and organisms. The alteration in the fertility status of soils reduces the survivability and development of crops in soil (Abii and Nwosu, 2009; Elkhously *et al.*, 2021). Oil spills have been known to cause acute and long-term damage to plants. These impacts include abnormal growth and re-growth after an initial impact. Mangroves are generally more vulnerable to oil spills than salt marshes because oil on the partially submerged roots of mangroves interferes with respiratory activity (Duke *et al.*, 1997; Evans 1985). The degree of oil impact also depends on various factors, such as the type and amount of oil, the extent of oil coverage, the plant species, the season of the spill, the soil composition, and the flushing rate. Oiled shoots of crops like pepper, okra, cocoyam, yams, and tomatoes may wilt and die off due to blockage of stomata thereby inhibiting photosynthesis, transpiration, and respiration. Germination, growth performance, and yield of these crops are stifled by oil spillage (Anoliefo *et al.*, 1994; Eigbuluese *et al.*, 2021). Earthworms play a significant role in soil fertility by converting organic materials into humus – the fraction of soil that is rich in nutrients needed for crop growth. The greater the humus contents of soil, the greater its ability to produce quality crops in large quantities. Earthworms are aerobic organisms; thus they perform better in well-aerated soils. Hence, in hydrocarbon-polluted soils where there is reduced or total absence of oxygen, earthworms' activity is adversely affected resulting in poor crop development (Owaid *et al.*, 2022., Agbor *et al.*, 2023).

However, heavy contamination by light oil can lead to widespread mortality, and plants may require a decade or more to recover. Different crops respond differently to oil effects. Lin and Mendelssohn (1996) examined the effects of South Louisiana crude oil on three different types of coastal marshes and found that the sensitivity of these marshes to the crude oil increased in the order of *Spartina lancifolia* (salt marsh), and *S. patens* (brackish). Plants are more sensitive to oil during the growing season than during other periods (Pezeshki *et al.*, 2000). Crop growth is generally improved under slight contamination of the soil of the order of 1% or less. Rahbar *et al.*, (2012) reported a reduction in leaf area and root length in sunflower (*Helianthus annuus*) growing on hydrocarbon-polluted soil. The authors attributed this reduction to the water deficit induced by the presence of hydrocarbon in this soil. It is important to note that the deeper the roots of crops, the greater their tolerance to hydrocarbon pollution especially when the pollution is within the topsoil. Hydrocarbons may also have a positive effect on crops, as they are known to stimulate crop growth when present in low concentrations (McGill *et al.*, 1981). Furthermore, Nicolotti and Eghi (1998) are of the view that these pollutants become lethal to crops only when they come into direct contact with crop tissues. The study aimed to examine the growth performance of maize on soil enhanced with agricultural wastes.

MATERIALS AND METHODS

Study location and source of materials

The research was conducted in the Environmental Biotechnology Unit, Department of Genetics & Biotechnology, University of Calabar. Crude oil (Bonny light) was obtained from Nigerian Agip Oil Company (NAOC), Port Harcourt, Rivers State, Nigeria, while the groundnut husks (GnH), maize cobs (MC), empty fruit bunch of oil palm (EFBOP) and cassava peels (CP) were collected from local farmers and processing industries. The collected agro-wastes (**GnH**, MC, EFBOP, and CP) were sun-dried for 10 days, then pulverized into powder using an electric blender (Model 4250, Braun, Germany). The powdered substances were sieved to pass through

a 2 mm sieve, labeled, and stored in containers.

Calculation of treatment in percentage

$$\text{Percentage of treatment} = \frac{\text{Quantity of organic wastes}}{\text{Quantity of soil}} \times 100$$

OR

$$PT = \frac{QOW}{QS} \times 100$$

$$0\% \text{ treatment} = \frac{0g}{6000g} \times 100$$

$$3.33\% \text{ treatment} = \frac{200g}{6000g} \times 100$$

$$6.67\% \text{ treatment} = \frac{400g}{6000g} \times 100$$

$$10\% \text{ treatment} = \frac{600g}{6000g} \times 100$$

(Adopted from the report of Agbor et al. 2019)

Soil sample collection

Topsoils (0-25cm depth) were randomly collected from four points, using a Dutch auger, bulked to form a composite soil sample, and six kilograms (6kg) each of the composite soil samples were weighed and transferred into hundred and fifty (150) labeled plastic buckets (PB) with drainage holes at the base. The plastic buckets were arranged in triplicate in a completely randomized design (CRD).

Artificial pollution of soil and Experimental Design

The soil contained in each PB, except the pristine control groups was polluted with 300mls of crude oil. The PB containing the polluted soils was mixed thoroughly and allowed to stand for 14 days. A 5x6 factorial in a Completely Randomized Design (CRD) was adopted as the experimental design. However, agro-wastes were at six levels (plantain stem powder (PSP), bush mango peels powder (BMPP), fruited pumpkin husk powder (FPHP), PSSP + BMPP, PSSP + FPHP, and BMPP+ FPHP). While, the concentrations of the agro-wastes were at five (5) levels (Pristine soil (unpolluted, 0 gram), crude oil polluted control (Polluted, 0 gram), 150g, 250g, and 350g of the amendments). The polluted soils were treated with the various agro-wastes in single and combined forms. After the application of the Agro-wastes, remediation was allowed for 90 days before the planting of maize seeds.

Baseline physicochemical and microbial analysis of the soil

The physicochemical properties of the soil was determined based on the following parameters: pH, organic carbon, total nitrogen, potassium, sodium, aluminum, hydrogen, ECEC (Effective cation exchange capacity), base saturation, available phosphorus, calcium, magnesium and TPH using the procedures as described by Agbor et al., (2021).

The total heterotrophic bacteria and fungi count were determined using the spread plate method on nutrient and Sabouraud dextrose agar respectively. Soil suspensions was prepared by 10- fold serial dilutions with 1 gram of soil, 0.1 ml of 10^6 and 10^7 dilutions was spread on

the plates in triplicates. The colony forming units of the bacteria and fungi were counted after incubation at 28°C for 24 hours and 37°C and 72 hours respectively.

Planting of maize (Zea mays)

Three seeds of maize each were sown at different levels of amendments after a bioremediation study.

Morphological attributes of the plants

Data were collected at seeds germination, 30, 60, and 90 days for the following growth parameters: plant height, leaf area, number of leaves, leaf width, and leaf length while data for days to flowering and number of seeds per cob were collected at plant maturity (Plate 1).

Statistical analysis

Data generated were subjected to a three way-analysis of variance (ANOVA). The Data were logged in micro-excel and transferred to SPSS (Special package for Social Science), Version 8. Significant means were separated using the least significant difference (LSD) test at a 5% and 1% probability level.

RESULTS AND DISCUSSION

Baseline soil physicochemical properties

The result obtained for the soil baseline indicates that the moisture content of the soil before



Plate 1. Maize Plant Grown on crude oil bio-remediated soil

pollution had a mean value of $12.24 \pm 0.10\%$, higher than the mean obtained for the moisture content after pollution ($9.89 \pm 0.06\%$). The soil before pollution had a pH of 5.30 ± 0.10 while after pollution the pH of the soil was 5.10 ± 0.10 . The organic carbon content of the soil after pollution was $5.25 \pm 0.10\%$ higher than the soil before pollution with a mean of $1.24 \pm 0.02\%$ (Table 1). The results also show that the nitrogen content in the soil before pollution was higher than the nitrogen content in the soil after pollution with mean values of $0.11 \pm 0.01\%$ and $0.08 \pm 0.01\%$ respectively. Reduced phosphorus level was obtained in the soil after pollution while soil before pollution had high available phosphorus content with means of $20.09 \pm 0.03 \text{ mg/kg}$ and $34.37 \pm 0.26 \text{ mg/kg}$ respectively. An increase in the TPH content of the soils after pollution was observed compared to the low value obtained in the soil before pollution (Table 5). The bacterial counts in the soil before pollution were observed to be higher than the counts obtained in the soil after pollution which had mean counts of $2.45 \pm 0.03 \times 10^6 \text{ CFU/g}$ and $5.44 \pm 0.02 \times 10^6 \text{ CFU/g}$ respectively. The fungal counts in soil before pollution were higher with a mean of $1.30 \pm 0.03 \times 10^4 \text{ CFU/g}$, than the mean counts obtained for soil after pollution $1.32 \pm 0.04 \times 10^4 \text{ CFU/g}$ (Table 1).

Microbial and total petroleum hydrocarbon (TPH) content of the soil

Soil amelioration with agro-wastes achieved a significant high microbial proliferation than the crude oil control. Agro-wastes are biological materials with essential nutrients that enhance microbial growth in soil environment. The activity of microbial community was mostly enhanced with the combined forms of the agro-wastes than the single amendments groups. The increased in the bacteria and fungi counts in the polluted soils treated with the agro-waste, resulted in the reduction of the soil TPH (Table 3).

Growth and yield attributes of maize in post-remediated soils

The results obtained for the germination of maize in remediated soils showed that the soil amended with 6% GnH_{14}P , $\text{GnH}_{14}\text{P} + \text{MaC}_{14}\text{P}$, 6%, 10% MaC_{14}P , $\text{GnH}_{14}\text{P} + \text{CasP}_{14}\text{P}$, 10% $\text{MaC}_{14}\text{P} + \text{EFBOP}_{14}\text{P}$, and $\text{MaC}_{14}\text{P} + \text{CasP}_{14}\text{P}$ showed early germination rate in the soils than

Table 1. Baseline Soil physicochemical properties before and after pollution with crude oil

| Elements | Before pollution | After pollution | LSD |
|---|-------------------------------|-------------------------------|------|
| Moisture (%) | $12.24^a \pm 0.10$ | $9.89^b \pm 0.06$ | 0.36 |
| Sand (%) | $77.30^b \pm 0.76$ | $78.3^a \pm 0.40$ | 0.15 |
| Silt (%) | $9.70^a \pm 0.06$ | $9.70^a \pm 0.32$ | NS |
| Clay (%) | $13.0^a \pm 8.0$ | $12.0^a \pm 0.17$ | NS |
| pH | $5.30^a \pm 0.10$ | $5.10^b \pm 0.10$ | 0.08 |
| Org. C (%) | $1.24^b \pm 0.02$ | $5.25^a \pm 0.03$ | 0.29 |
| Total N (%) | $0.11^a \pm 0.01$ | $0.08^a \pm 0.01$ | 0.02 |
| Avail. P (mgkg^{-1}) | $34.37^a \pm 0.26$ | $20.09^b \pm 0.03$ | 0.45 |
| Ca (Cmolkg^{-1}) | $4.60^a \pm 0.15$ | $3.60^b \pm 0.10$ | 0.12 |
| Mg (Cmolkg^{-1}) | $2.00^a \pm 0.10$ | $0.80^b \pm 0.05$ | 0.09 |
| K (Cmolkg^{-1})s | $0.10^b \pm 0.01$ | $0.14^a \pm 0.01$ | 0.01 |
| Na (Cmolkg^{-1}) | $0.08^a \pm 0.01$ | $0.10^a \pm 0.01$ | NS |
| Al^{3+} (Cmolkg^{-1}) | $0.85^b \pm 0.03$ | $1.20^a \pm 0.06$ | 0.19 |
| H^+ (Cmolkg^{-1}) | $1.20^a \pm 0.06$ | $1.04^b \pm 0.06$ | 0.05 |
| ECEC (Cmolkg^{-1}) | $7.82^a \pm 0.05$ | $5.84^b \pm 0.25$ | 0.16 |
| BS (%) | $87.0^a \pm 0.75$ | $79.0^b \pm 1.3$ | 0.25 |
| TPH (mgkg^{-1}) | $36.2^b \pm 1.03$ | $1703.2^a \pm 5.03$ | 2.98 |
| Bacterial (CFUg^{-1}) | $2.45^b \pm 0.03 \times 10^6$ | $5.44^a \pm 0.02 \times 10^6$ | 0.15 |
| Fungal (CFUg^{-1}) | $1.30^a \pm 0.03 \times 10^4$ | $1.32^a \pm 0.04 \times 10^4$ | NS |
| Texture | Sandy loam | Sandy loam | |

Mean with the same superscript along the horizontal arrays indicates no variations

Table 2. Microbial and Total petroleum hydrocarbon content in soil after remediation

| Agro-wastes | Treatment levels | Bacteria (CFU/g) | Fungi (CFU/g) | TPH (mg/kg) |
|--|------------------|-------------------------------|-------------------------------|---------------------------|
| GnH ₁₄ P | PC | 6.71 x 10 ^{6h} ±0.00 | 3.92x10 ^{6d} ±0.00 | 48.08 ⁱ ±0.53 |
| | COC | 4.48 x 10 ⁶ⁱ ±0.58 | 3.43 x 10 ^{6d} ±0.58 | 1894.5±2.45 |
| | 3% | 1.01x10 ^{7g} ±0.23 | 4.48x10 ^{6d} ±0.03 | 580.89 ^c ±4.15 |
| | 6% | 1.38x10 ^{7c} ±0.10 | 5.57x10 ^{6c} ±0.09 | 428.89 ^c ±3.46 |
| | 9% | 1.84x10 ^{7c} ±0.13 | 5.79x10 ^{6c} ±0.01 | 298.7 ^e ±3.46 |
| MaC ₁₄ P | 3% | 9.69x10 ^{6g} ±0.03 | 5.17x10 ^{6c} ±0.13 | 480.56 ^c ±1.94 |
| | 6% | 1.27x10 ^{7f} ±0.06 | 6.78x10 ^{6c} ±0.10 | 267.03 ^d ±2.28 |
| | 9% | 1.58x10 ^{7d} ±0.07 | 7.72x10 ^{6c} ±0.12 | 197.04 ^f ±4.09 |
| GnH ₁₄ P+MaC ₁₄ P | 3% | 1.97x10 ^{7bc} ±0.03 | 6.29x10 ^{6c} ±0.09 | 650.23 ^d ±5.00 |
| | 6% | 2.12x10 ^{7b} ±0.06 | 7.03x10 ^{6c} ±0.04 | 459.11 ^g ±2.82 |
| | 9% | 2.27x10 ^{7b} ±0.08 | 8.18x10 ^{6c} ±0.13 | 308.4 ^h ±0.67 |
| Cas ₁₄ P | 3% | 1.72x10 ^{7 d} ±0.12 | 5.01x10 ^{6c} ±0.03 | 723.22 ^b ±0.81 |
| | 6% | 1.92x10 ^{7c} ±0.08 | 5.89x10 ^{6c} ±0.09 | 461.44 ^d ±1.72 |
| | 9% | 2.24x10 ^{7b} ±0.12 | 6.39x10 ^{6c} ±0.05 | 245.89 ^f ±1.52 |
| EFBOP ₁₄ P | 3% | 2.18x10 ^{7b} ±0.04 | 6.0x10 ^{6c} ±0.03 | 656.0 ^b ±4.06 |
| | 6% | 2.57x10 ^{7a} ±0.05 | 9.38x10 ^{6a} ±0.18 | 568.22 ^a ±1.32 |
| | 9% | 2.64x10 ^{7a} ±0.07 | 1.04x10 ^{7a} ±0.06 | 376.34 ^a ±2.76 |
| CasP ₁₄ P+ EFBOP ₁₄ P (1:1) | 3% | 2.57x10 ^{7a} ±0.16 | 7.14x10 ^{6c} ±0.09 | 592.78 ^a ±1.92 |
| | 6% | 2.66x10 ^{7a} ±0.01 | 8.43x10 ^{6c} ±0.05 | 409.22 ^a ±2.81 |
| | 9% | 2.73x10 ^{7a} ±0.09 | 1.09x10 ^{7a} ±0.33 | 236.21 ^a ±0.72 |
| GnH ₁₄ P+ EFBOP ₁₄ P (1:1) | 3% | 1.97x10 ^{7bc} ±0.06 | 5.79x10 ^{6c} ±0.06 | 443.67 ^a ±1.82 |
| | 6% | 2.21x10 ^{7b} ±0.07 | 8.08x10 ^{6c} ±0.08 | 359.33 ^a ±1.77 |
| | 9% | 2.37x10 ^{7b} ±0.07 | 9.78x10 ^{6a} ±0.09 | 202.89 ^a ±2.51 |
| GnH ₁₄ P+CasP ₁₄ P (1:1) | 3% | 1.70x10 ^{7c} ±0.06 | 5.01x10 ^{6c} ±0.01 | 534.8 ^a ±1.31 |
| | 6% | 1.88x10 ^{7d} ±0.07 | 5.89x10 ^{6c} ±0.05 | 424.33 ^a ±3.53 |
| | 9% | 2.07x10 ^{7b} ±0.09 | 6.39x10 ^{6c} ±0.04 | 256.89 ^a ±2.45 |
| MaC ₁₄ P+ EFBOP ₁₄ P (1:1) | 3% | 1.43x10 ^{7c} ±0.12 | 5.20x10 ^{6c} ±0.06 | 547.78 ^a ±0.92 |
| | 6% | 1.66x10 ^{7d} ±0.07 | 6.53x10 ^{6c} ±0.09 | 364.33 ^a ±0.45 |
| | 9% | 1.84x10 ^{7c} ±0.07 | 7.12x10 ^{6c} ±0.08 | 185.34 ^a ±1.03 |
| MaC ₁₄ P+Cas ₁₄ P (1:1) | 3% | 1.88x10 ^{7c} ±0.06 | 7.97x10 ^{6c} ±0.02 | 388.00 ^a ±0.06 |
| | 6% | 2.19x10 ^{7b} ±0.03 | 9.02x10 ^{6a} ±0.03 | 196 ^a ±1.28 |
| | 9% | 2.37x10 ^{7b} ±0.09 | 1.04x10 ^{7a} ±0.06 | 176.01 ^a ±0.76 |

Legend: NSPC (Number of seeds per cob), MaC₁₄P (Maize cob 2014 powder), EFBOP₁₄P (Empty fruit bunch of oil palm 2014 powder), CasP₁₄P (Cassava peels 2014 powder), PC (Pristine control), COC (Crude oil control)

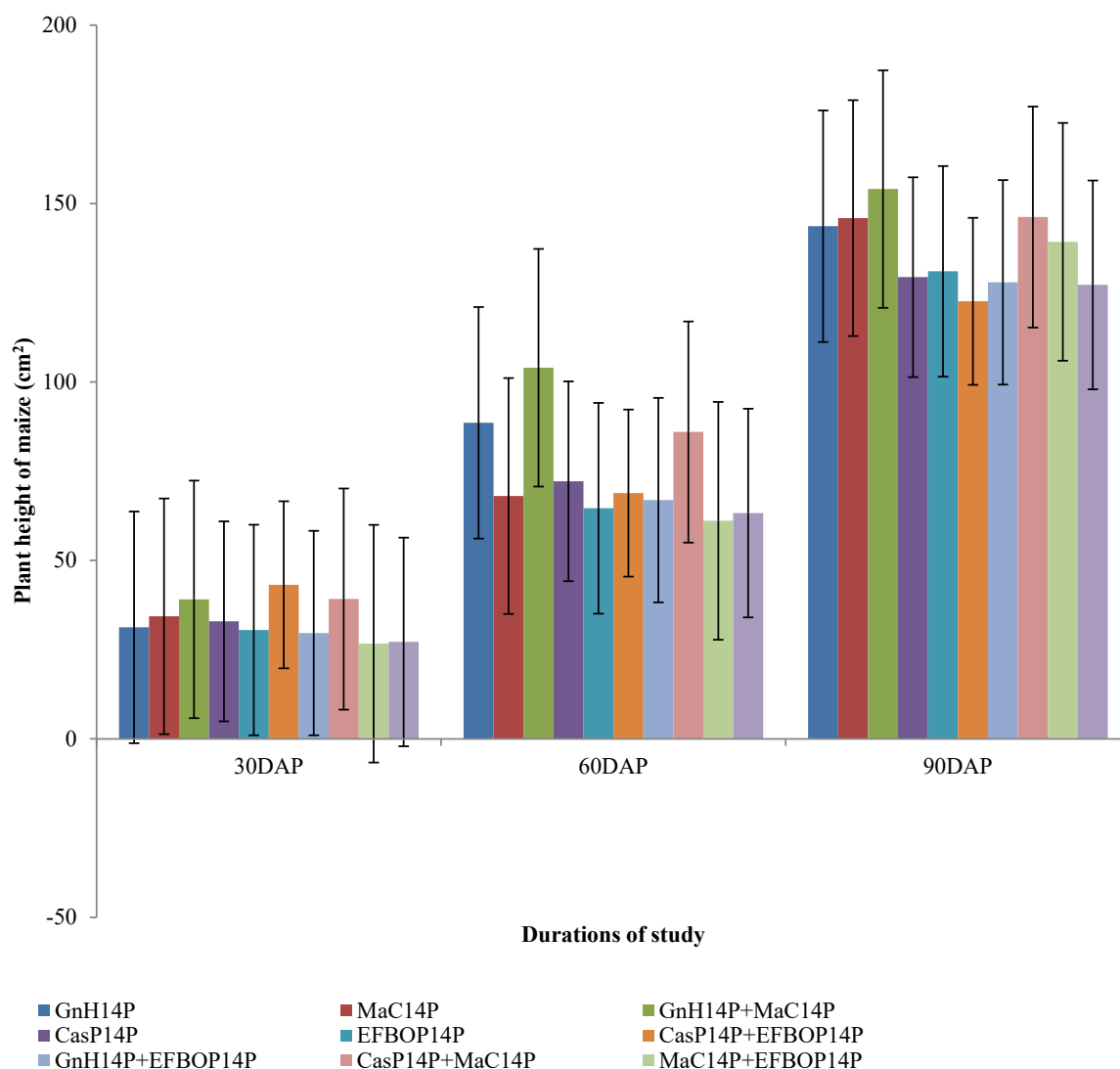
the pristine soil and crude oil-polluted soils that germinated late (Table 3). The plant height of maize in soil amended with 10% GnH₁₄P+ MaC₁₄P shows a significantly higher ($P<0.05$) mean value (Fig. 1). These were followed by soils amended with 3%, 6% and 10% GnH₁₄P, MaC₁₄P, CasP₁₄P, EFBOP₁₄P, CasP₁₄P+ EFBOP₁₄P, GnH₁₄P + EFBOP₁₄P, MaC₁₄P+ CasP₁₄P, MaC₁₄P+EFBOP₁₄P, and 6%, 10% GnH₁₄P+ CasP₁₄P, with no variation in the mean plant height.

Table 3. Germination and yield attributes of maize in crude oil bio-remediated soils

| Agrowastes | Treatment levels | Days to germination | Days to flowering | NSPC |
|--|------------------|-------------------------|--------------------------|---------------------------|
| GnH ₁₄ P | PC | 4.00 ^c ±0.00 | 68.00 ^b ±0.58 | 84.67 ^a ±3.53 |
| | COC | 7.00 ^a ±0.58 | 91.33 ^a ±0.88 | 0.00 ^b ±0.00 |
| | 3% | 4.33 ^d ±0.33 | 66.67 ^b ±1.20 | 460.00 ^a ±7.15 |
| | 6% | 4.00 ^c ±0.00 | 66.00 ^b ±0.58 | 486.00 ^a ±3.46 |
| | 9% | 4.33 ^d ±0.33 | 68.67 ^b ±0.67 | 492.00 ^a ±3.46 |
| MaC ₁₄ P | 3% | 4.33 ^d ±0.33 | 68.00 ^b ±1.53 | 320.67 ^a ±7.94 |
| | 6% | 4.00 ^c ±0.00 | 69.67 ^b ±0.88 | 350.00 ^a ±5.28 |
| | 9% | 4.00 ^c ±0.00 | 71.67 ^b ±0.88 | 355.00 ^a ±7.09 |
| GnH ₁₄ P+MaC ₁₄ P | 3% | 4.33 ^d ±0.33 | 68.00 ^b ±1.16 | 468.00 ^a ±8.00 |
| | 6% | 4.00 ^c ±0.00 | 72.00 ^b ±1.73 | 476.67 ^a ±8.82 |
| | 9% | 4.33 ^d ±0.33 | 74.00 ^b ±0.58 | 465.33 ^a ±8.67 |
| Cas ₁₄ P | 3% | 4.33 ^d ±0.33 | 75.67 ^b ±2.85 | 285.33 ^a ±5.81 |
| | 6% | 4.00 ^c ±0.00 | 76.67 ^b ±1.20 | 361.33 ^a ±2.72 |
| | 9% | 4.00 ^c ±0.00 | 79.67 ^b ±0.88 | 366.67 ^a ±3.52 |
| EFBOP ₁₄ P | 3% | 4.67 ^c ±0.33 | 69.00 ^b ±0.58 | 226.00 ^a ±9.06 |
| | 6% | 5.00 ^b ±0.58 | 68.33 ^b ±2.40 | 262.00 ^a ±3.32 |
| | 9% | 5.00 ^b ±0.58 | 70.00 ^b ±0.58 | 303.33 ^a ±1.76 |
| CasP ₁₄ P+ EFBOP ₁₄ P (1:1) | 3% | 4.67 ^c ±0.33 | 71.33 ^b ±1.20 | 359.33 ^a ±4.92 |
| | 6% | 4.67 ^c ±0.33 | 72.67 ^b ±1.45 | 387.33 ^a ±6.81 |
| | 9% | 4.67 ^c ±0.33 | 70.00 ^b ±0.58 | 409.33 ^a ±2.72 |
| GnH ₁₄ P+ EFBOP ₁₄ P (1:1) | 3% | 4.67 ^c ±0.33 | 69.00 ^b ±0.58 | 319.33 ^a ±6.82 |
| | 6% | 5.00 ^b ±0.58 | 66.00 ^b ±0.58 | 340.67 ^a ±4.77 |
| | 9% | 4.67 ^c ±0.33 | 69.00 ^b ±0.58 | 343.00 ^a ±6.51 |
| GnH ₁₄ P+CasP ₁₄ P (1:1) | 3% | 4.33 ^d ±0.33 | 73.00 ^b ±1.53 | 422.33 ^a ±7.31 |
| | 6% | 4.00 ^c ±0.00 | 70.67 ^b ±0.88 | 434.67 ^a ±3.53 |
| | 9% | 4.00 ^c ±0.00 | 69.00 ^b ±0.58 | 446.00 ^a ±9.45 |
| MaC ₁₄ P+ EFBOP ₁₄ P (1:1) | 3% | 4.67 ^c ±0.33 | 69.00 ^b ±0.58 | 402.00 ^a ±3.92 |
| | 6% | 5.00 ^b ±0.58 | 68.67 ^b ±2.40 | 405.00 ^a ±3.45 |
| | 9% | 4.00 ^c ±0.00 | 68.00 ^b ±0.58 | 439.67 ^a ±2.03 |
| MaC ₁₄ P+Cas ₁₄ P (1:1) | 3% | 4.00 ^c ±0.00 | 69.33 ^b ±0.33 | 388.00 ^a ±3.06 |
| | 6% | 4.33 ^d ±0.33 | 71.67 ^b ±0.88 | 404.67 ^a ±3.28 |
| | 9% | 5.00 ^b ±0.58 | 68.67 ^b ±0.33 | 408.67 ^a ±1.76 |
| | LSD | 0.25 | 3.50 | 20.6 |

Legend:

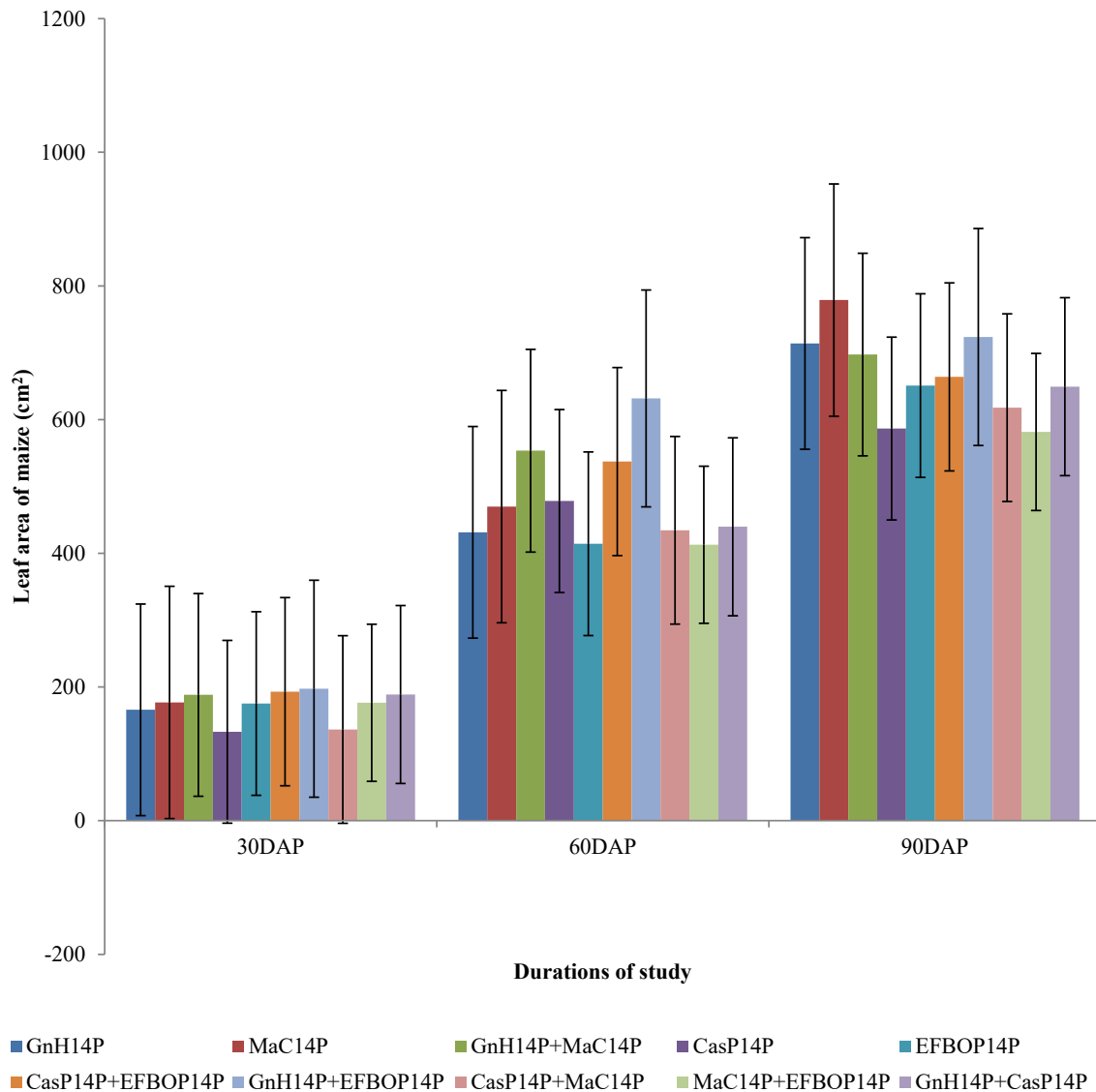
| | |
|-----------------------|---|
| NSPC | Number of seeds per cob |
| MaC ₁₄ P | Maize cob 2014 powder |
| EFBOP ₁₄ P | Empty fruit bunch of oil palm 2014 powder |
| CasP ₁₄ P | Cassava peels 2014 powder |
| PC | Pristine control |
| COC | Crude oil control |

**Legend:**

MaC₁₄P Maize cob 2014 powder
 EFBOP₁₄P Empty fruit bunch of oil palm 2014 powder
 CasP₁₄P Cassava peels 2014 powder

Fig. 1. Plant height of maize in polluted soils amended with different agro-wastes

The leaf area obtained in soil amended with 10% GnH₁₄P + EFBOP₁₄P had significantly higher ($P < 0.05$) mean values than other amended soils and controls (Fig. 2). The number of leaves obtained from soil amended with 3%, 6%, 10% GnH₁₄P, GnH₁₄P + MaC₁₄P, CasP₁₄P, CasP₁₄P + EFBOP₁₄P, GnH₁₄P + EFBOP₁₄P, CasP₁₄P + MaC₁₄P, MaC₁₄P + EFBOP₁₄P, and GnH₁₄P + CasP₁₄P had an increase in the number of leaves, with no variation in the number of leaves obtained (Table 4 and Fig. 3). The stem girth obtained in soils amended with 6%, 10% of GnH₁₄P, EFBOP₁₄P, CasP₁₄P + EFBOP₁₄P, GnH₁₄P + EFBOP₁₄P, 10% of CasP₁₄P + MaC₁₄P, MaC₁₄P + EFBOP₁₄P and GnH₁₄P + MaC₁₄P had high mean values than other amended soils and controls (Fig 4). The leaf lengths of the plant indicated that the soils amended with 3%, 6%, and 10% of GnH₁₄P + CasP₁₄P had the longest leaf lengths (Fig. 5). The mean values for the soil amended with the different agro-wastes had no variation in the days to flowering compared to the pristine control mean



Legend:

MaC₁₄P Maize cob 2014 powder
 EFBOP₁₄P Empty fruit bunch of oil palm 2014 powder
 CasP₁₄P Cassava peels 2014 powder
 DAP Days after planting

Fig. 2. Leaf area of maize in polluted soils amended with different agro-wastes

values, while the crude-oil polluted soil without the amendments had higher mean values. This shows that the presence of crude oil in soil could delay the flowering of biological plants. The number of seeds obtained per plant indicated that the mean values for the amended soils and pristine control were not significantly different ($P > 0.05$) while no seeds were obtained for the crude oil-polluted soils.

Petroleum products are known to be injurious to terrestrial and aquatic environments. Fertile soil is known for the quality of physical and chemical properties it possessed. The bioavailability of pollutants in an ecosystem alters the functionality and performance of the microbiota that inhabit the environment. However, over the years research had shown that the presence of crude oil in soil alters the productivity of plants (Ekpo *et al.*, 2012 and Choa *et al.*, 2020). The availability of hydrocarbon in

Table 4. Effects of agro-wastes on the morphological attributes of maize after remediation

| Parameters | Trt. levels | Plant height (cm ²) | Leaf area (cm ²) | No. of Leaves | Stem girth (cm ²) | Leaf length (cm ²) | Leaf width (cm ²) |
|---|-------------|---------------------------------|------------------------------|--------------------------|-------------------------------|--------------------------------|-------------------------------|
| GnH ₁₄ P | PC | 53.57 ^a ±11.83 | 324.22 ^a ±5.20 | 9.11 ^a ±0.48 | 5.80 ^b ±0.64 | 42.91 ^a ±4.87 | 6.71 ^a ±0.63 |
| | COC | 13.99 ^b ±1.60 | 113 ^a ±8.69 | 6.44 ^a ±0.56 | 4.50 ^a ±0.48 | 9.05 ^a ±4.97 | 5.24 ^a ±0.67 |
| | 3% | 113.48 ^b ±22.30 | 520.78 ^c ±11.12 | 9.89 ^a ±0.77 | 5.73 ^b ±0.50 | 85.71 ^b ±2.90 | 6.69 ^a ±0.56 |
| | 6% | 123.67 ^b ±22.56 | 593.56 ^c ±11.20 | 10.44 ^a ±1.02 | 6.68 ^a ±0.50 | 89.08 ^b ±3.09 | 7.41 ^a ±0.48 |
| | 10% | 134.33 ^b ±23.30 | 634.0 ^b ±11.8 | 11.89 ^a ±0.99 | 7.00 ^a ±0.47 | 89.91 ^b ±3.19 | 7.60 ^a ±0.54 |
| MaC ₁₄ P | PC | 51.78 ^c ±9.95 | 385.11 ^c ±7.79 | 7.44 ^b ±0.56 | 5.07 ^a ±0.56 | 40.30 ^a ±6.05 | 5.62 ^a ±0.60 |
| | COC | 11.53 ^b ±1.25 | 135.11 ^a ±6.81 | 5.78 ^c ±0.40 | 3.37 ^b ±0.45 | 10.67 ^a ±5.86 | 3.99 ^a ±0.35 |
| | 3% | 103.66 ^b ±23.23 | 524.11 ^c ±9.93 | 8.00 ^b ±0.29 | 5.14 ^a ±0.49 | 81.34 ^b ±4.78 | 5.80 ^a ±0.59 |
| | 6% | 120.66 ^b ±24.93 | 624.0 ^b ±12.3 | 9.44 ^a ±0.48 | 6.06 ^b ±0.65 | 85.39 ^b ±5.12 | 6.87 ^a ±0.77 |
| | 10% | 126.22 ^b ±23.96 | 707.67 ^b ±11.8 | 10.44 ^a ±0.48 | 6.44 ^b ±0.60 | 85.93 ^b ±4.93 | 7.18 ^a ±0.67 |
| GnH ₁₄ P+MaC ₁₄ P | PC | 70.86 ^c ±14.39 | 371.89 ^c ±7.64 | 7.33 ^b ±0.53 | 5.77 ^b ±0.47 | 50.34 ^c ±6.06 | 7.13 ^a ±0.70 |
| | COC | 19.73 ^b ±2.66 | 164.56 ^a ±2.68 | 5.44 ^c ±0.53 | 3.62 ^a ±0.45 | 14.90 ^f ±5.12 | 4.17 ^a ±0.50 |
| | 3% | 122.54 ^b ±23.45 | 538.78 ^c ±8.90 | 8.69 ^a ±0.53 | 5.92 ^b ±0.74 | 84.77 ^b ±6.11 | 7.33 ^a ±0.67 |
| | 6% | 129.27 ^b ±22.70 | 643.44 ^b ±9.38 | 9.44 ^a ±0.50 | 6.22 ^b ±0.72 | 87.73 ^b ±5.72 | 7.53 ^a ±0.60 |
| | 10% | 152.81 ^a ±23.45 | 680 ^b ±10.31 | 10.33 ^a ±0.58 | 6.70 ^a ±0.78 | 88.04 ^b ±3.98 | 7.90 ^a ±0.54 |
| CasP ₁₄ P | PC | 61.97 ^c ±8.97 | 316.22 ^d ±5.74 | 7.00 ^c ±0.47 | 4.87 ^a ±0.80 | 45.93 ^c ±6.36 | 4.83 ^a ±0.53 |
| | COC | 19.22 ^b ±2.13 | 150.22 ^a ±2.62 | 6.11 ^d ±0.59 | 3.83 ^a ±0.60 | 18.01 ^f ±5.06 | 4.16 ^a ±0.48 |
| | 3% | 96.86 ^b ±19.57 | 436.67 ^d ±8.32 | 8.78 ^a ±0.76 | 5.68 ^a ±0.53 | 73.16 ^b ±9.08 | 5.70 ^a ±0.55 |
| | 6% | 102.63 ^b ±19.3 | 514.22 ^c ±9.16 | 9.78 ^a ±0.62 | 5.92 ^b ±0.52 | 76.98 ^b ±8.38 | 6.46 ^a ±0.51 |
| | 10% | 110.07 ^b ±20.28 | 579.33 ^c ±8.48 | 10.44 ^a ±0.73 | 6.42 ^b ±0.54 | 80.40 ^b ±6.30 | 6.92 ^a ±0.38 |

Continued Table 4. Effects of agro-wastes on the morphological attributes of maize after remediation

| Parameters | Trt. levels | Plant height (cm ²) | Leaf Area (cm ²) | No. of Leaves | Stem girth (cm ²) | Leaf length (cm ²) | Leaf width (cm ²) |
|--|-------------|---------------------------------|------------------------------|--------------------------|-------------------------------|--------------------------------|-------------------------------|
| EFBOP ₁₄ P | PC | 62.13 ^c ±7.94 | 372.0 ^c ±6.53 | 6.33 ^a ±0.60 | 3.99 ^d ±0.58 | 40.52 [±] 7.44 | 5.47 [±] 0.44 |
| | COC | 19.01 ^b ±3.78 | 157.56 [±] 2.15 | 4.44 [±] 0.29 | 3.31 ^b ±0.45 | 17.59 [±] 5.12 | 4.27 [±] 0.42 |
| | 3% | 90.03 ^b ±19.98 | 442.56 ^a ±8.64 | 7.78 [±] 0.47 | 6.10 ^b ±0.29 | 66.26 [±] 3.82 | 5.81 [±] 0.25 |
| | 6% | 98.61 ^b ±20.51 | 509.22 [±] 8.06 | 9.44 ^a ±0.78 | 6.93 ^a ±0.29 | 73.89 [±] 5.06 | 6.31 [±] 0.19 |
| | 10% | 107.03 ^b ±21.94 | 586.22 [±] 9.80 | 9.78 [±] 0.40 | 7.20 [±] 0.20 | 74.98 [±] 5.39 | 6.96 [±] 0.27 |
| CasP ₁₄ P+ EFBOP ₁₄ P | PC | 44.28 [±] 4.68 | 320.67 [±] 6.40 | 7.67 [±] 0.73 | 4.06 [±] 0.60 | 35.30 [±] 4.16 | 5.42 [±] 0.72 |
| | COC | 19.63 [±] 1.46 | 166.78 [±] 2.39 | 5.56 [±] 0.50 | 3.01 [±] 0.37 | 17.53 [±] 4.76 | 4.79 [±] 0.65 |
| | 3% | 99.89 [±] 17.46 | 554.0 [±] 8.94 | 9.00 [±] 0.50 | 5.79 ^b ±0.50 | 73.04 ^b ±6.37 | 7.54 [±] 0.50 |
| | 6% | 110.98 [±] 18.54 | 629.11 ^b ±9.37 | 9.78 [±] 0.66 | 6.61 ^a ±0.59 | 77.46 ^b ±4.99 | 7.87 [±] 0.59 |
| | 10% | 116.31 ^b ±18.94 | 653.22 ^b ±9.05 | 11.22 ^a ±0.76 | 7.10 [±] 0.56 | 77.92 ^b ±5.43 | 8.27 [±] 0.40 |
| GnH ₁₄ P+ EFBOP ₁₄ P | PC | 44.28 [±] 8.53 | 432.78 [±] 8.16 | 7.67 [±] 0.29 | 4.90 ^d ±0.60 | 36.80 [±] 7.29 | 5.32 [±] 0.61 |
| | COC | 16.0 ^b ±2.26 | 160.86 [±] 2.66 | 6.22 [±] 0.49 | 3.80 [±] 0.48 | 18.42 [±] 3.04 | 3.73 [±] 0.37 |
| | 3% | 100.17 [±] 20.03 | 536.33 [±] 7.43 | 9.67 [±] 0.47 | 6.29 ^b ±0.64 | 85.83 ^b ±5.89 | 7.33 [±] 0.67 |
| | 6% | 104.61 ^b ±20.17 | 702.11 ^b ±1.06 | 9.89 [±] 0.51 | 6.84 [±] 0.67 | 87.11 ^b ±5.18 | 7.53 [±] 0.60 |
| | 10% | 109.0 ^b ±20.9 | 756.00 [±] 11.8 | 10.56 [±] 0.50 | 7.11 [±] 0.66 | 87.74 ^b ±4.94 | 7.90 [±] 0.54 |
| CasP ₁₄ P+MacC ₁₄ P | PC | 43.93 [±] 4.55 | 301.89 [±] 2.94 | 7.56 [±] 0.98 | 5.10 [±] 0.84 | 38.72 [±] 3.58 | 5.71 [±] 0.68 |
| | COC | 27.06 [±] 1.49 | 169.89 [±] 2.94 | 6.89 [±] 0.98 | 3.32 ^b ±0.53 | 41.93 [±] 2.90 | 4.33 [±] 0.58 |
| | 3% | 118.56 [±] 23.49 | 440.78 [±] 7.78 | 9.33 [±] 0.94 | 5.88 ^b ±0.54 | 70.27 [±] 5.40 | 6.67 [±] 0.71 |
| | 6% | 129.67 [±] 24.33 | 507.89 [±] 8.89 | 9.78 [±] 0.80 | 6.13 ^b ±0.51 | 77.89 [±] 5.02 | 7.28 [±] 0.46 |
| | 10% | 133.07 ^b ±24.0 | 560.89 [±] 10.2 | 10.11 [±] 0.81 | 6.74 ^a ±0.55 | 84.02 ^b ±4.90 | 7.92 [±] 0.43 |

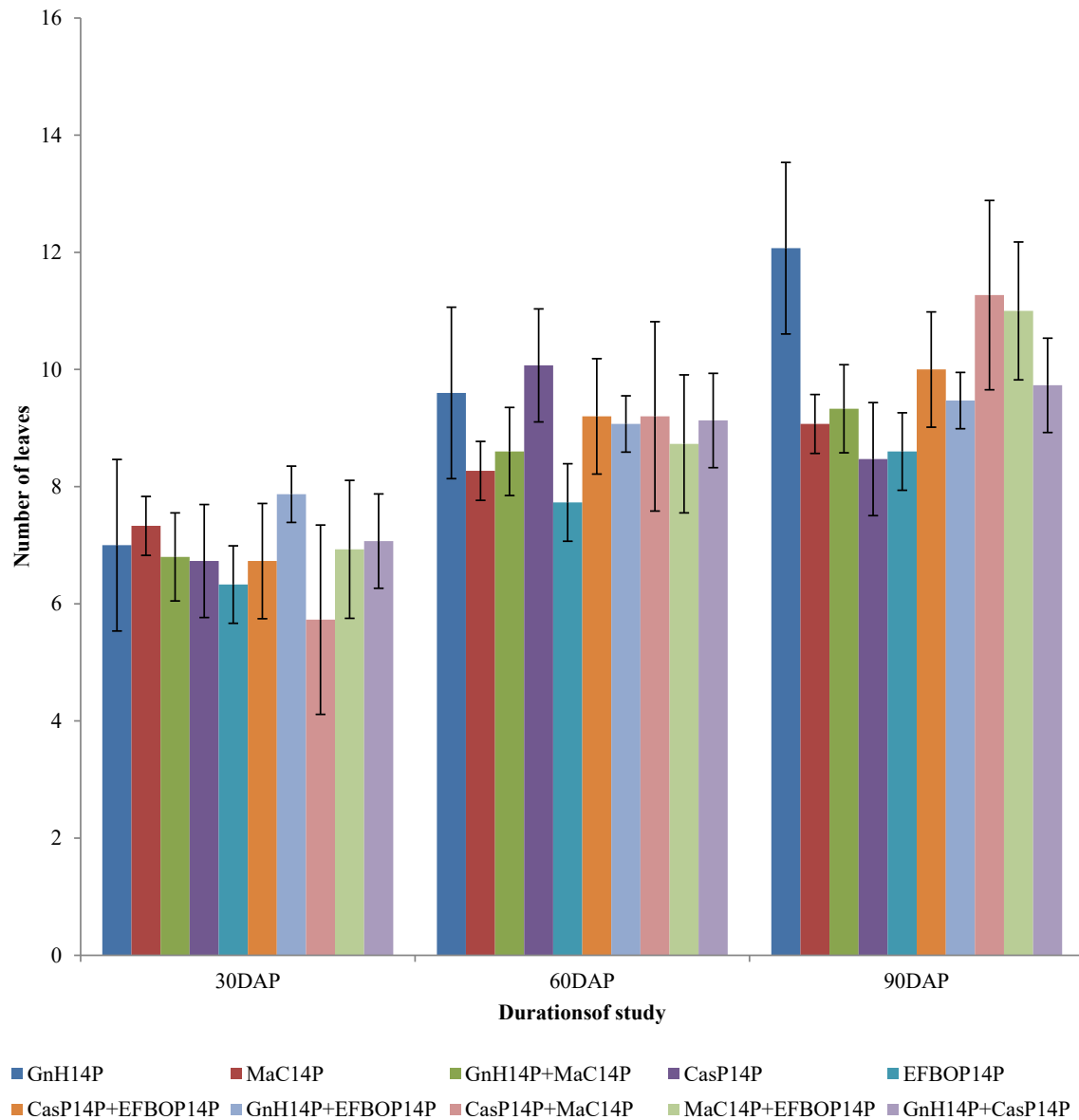
Continued Table 4. Effects of agro-wastes on the morphological attributes of maize after remediation

| Parameters | Trt. levels | Plant height (cm ²) | Leaf Area (cm ²) | No. of Leaves | Stem girth (cm ²) | Leaf length (cm ²) | Leaf width (cm ²) |
|---|-------------|---------------------------------|------------------------------|--------------------------|-------------------------------|--------------------------------|-------------------------------|
| MaC ₁₄ P+ EFBOP ₁₄ P | PC | 57.56 ^a ±11.11 | 298.44 ^f ±5.87 | 8.22 ^b ±0.85 | 5.18 ^e ±0.46 | 43.25 ^c ±1.66 | 6.12 ^a ±0.42 |
| | COC | 17.87 ^b ±2.84 | 133.89 ^g ±2.09 | 6.78 ^c ±0.55 | 3.26 ^h ±0.42 | 20.64 ^d ±0.23 | 4.11 ^a ±0.39 |
| | 3% | 95.46 ^b ±22.06 | 442.89 ^d ±8.03 | 9.22 ^a ±0.78 | 5.58 ^b ±0.30 | 80.73 ^b ±4.35 | 5.87 ^a ±0.42 |
| | 6% | 100.51 ^b ±23.02 | 508.78 ^e ±7.16 | 9.78 ^a ±0.62 | 6.16 ^b ±0.35 | 86.71 ^b ±2.79 | 6.51 ^a ±0.29 |
| | 10% | 105.37 ^b ±24.37 | 567.44 ^e ±6.48 | 10.44 ^a ±0.58 | 6.71 ^a ±0.21 | 88.80 ^b ±2.19 | 7.26 ^a ±0.47 |
| GnH ₁₄ P+CasP ₁₄ P | PC | 54.74 ^c ±7.94 | 319.33 ^f ±5.63 | 7.44 ^b ±0.53 | 4.32 ^f ±0.56 | 39.80 ^e ±1.03 | 6.58 ^a ±0.35 |
| | COC | 18.93 ^b ±2.33 | 136.33 ^g ±9.51 | 6.33 ^d ±0.53 | 2.94 ^g ±0.35 | 63.23 ^{cd} ±4.95 | 4.20 ^a ±0.22 |
| | 3% | 85.17 ^c ±19.38 | 489.33 ^e ±8.48 | 9.11 ^a ±0.48 | 5.09 ^e ±0.68 | 94.42 ^a ±1.58 | 6.70 ^a ±0.28 |
| | 6% | 97.11 ^b ±20.63 | 547.56 ^e ±7.96 | 9.78 ^a ±0.72 | 5.54 ^b ±0.63 | 95.60 ^a ±0.95 | 7.21 ^a ±0.29 |
| | 10% | 106.17 ^b ±23.45 | 637.44 ^b ±9.42 | 10.56 ^a ±0.56 | 6.04 ^b ±0.53 | 97.11 ^a ±0.65 | 7.77 ^a ±0.33 |
| LSD | | 4.28 | 25.6 | 0.25 | 0.15 | 2.80 | NS |

Mean with the same superscript along the vertical arrays indicate no significant difference ($P>0.05$) in mean values

Legend:

MaC₁₄P Maize cob 2014 powder
 EFBOP₁₄P Empty fruit bunch of oil palm 2014 powder
 CasP₁₄P Cassava peels 2014 powder
 PC Pristine control
 COC Crude oil control

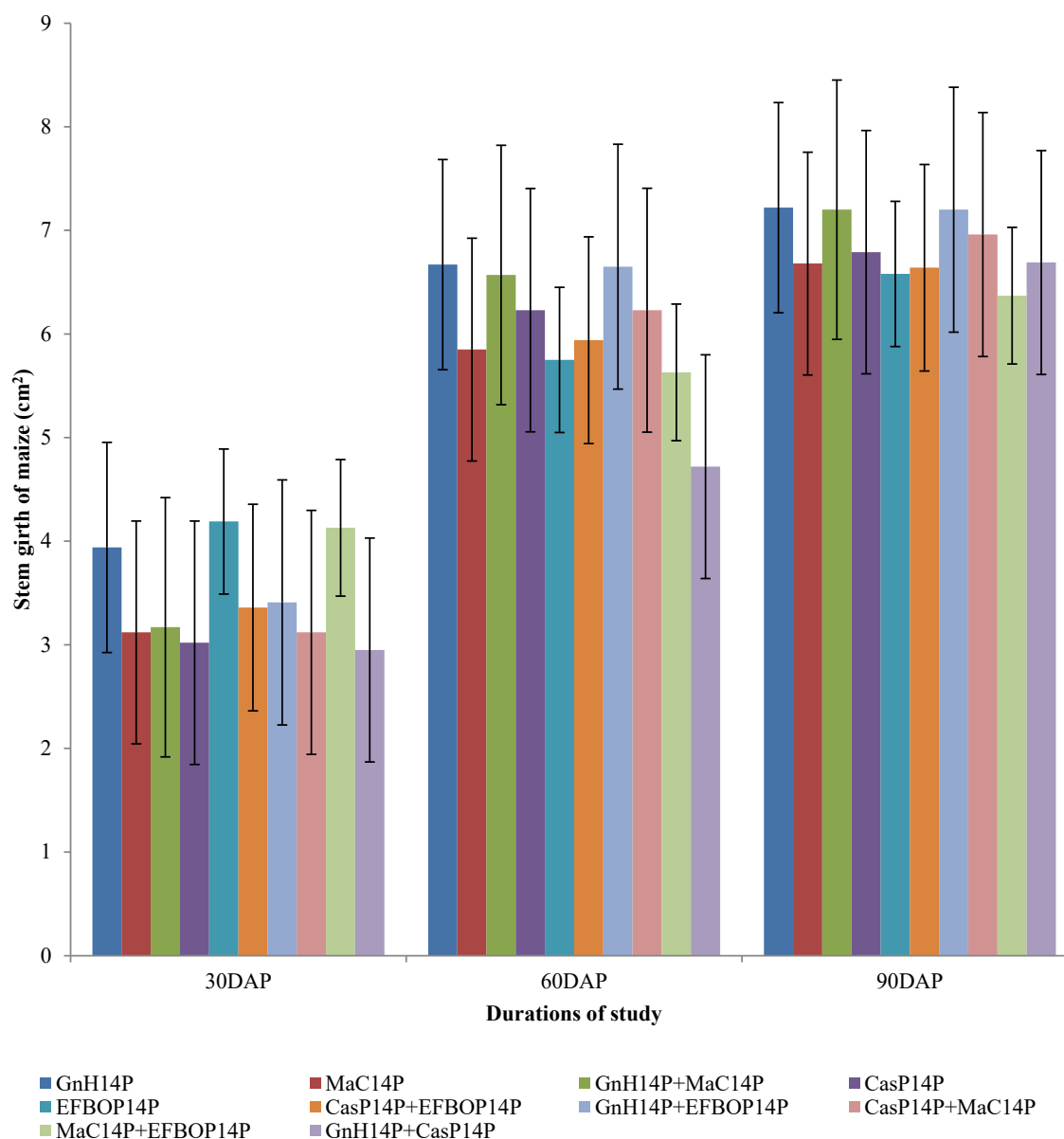


Legend:

MaC₁₄P Maize cob 2014 powder
 EFBOP₁₄P Empty fruit bunch of oil palm 2014 powder
 CasP₁₄P Cassava peels 2014 powder
 DAP Days after planting

Fig. 3. Number of leaves of maize in polluted soils amended with different agro-wastes

the soil leads to poor aeration by blocking air spaces between soil particles, these create a condition of aerobiosis which causes root stress in plants and reduces leaf growth. In nature, certain plants exist with special adaptive mechanisms that make them tolerant to hydrocarbon pollution. These mechanisms could be in the abilities of the plants to adapt, extract and sequester the oil in their roots, stems, or leaves. The inability of other plants to develop these adaptive features makes them prone to oil attacks, which invariably leads to poor germination and growth of plants. Thereby, resulting in the biodiversity loss of many economically important crop species. The investigation had shown that oil-polluted soils significantly reduce the presence of phosphorus, nitrogen, oxygen, and potassium

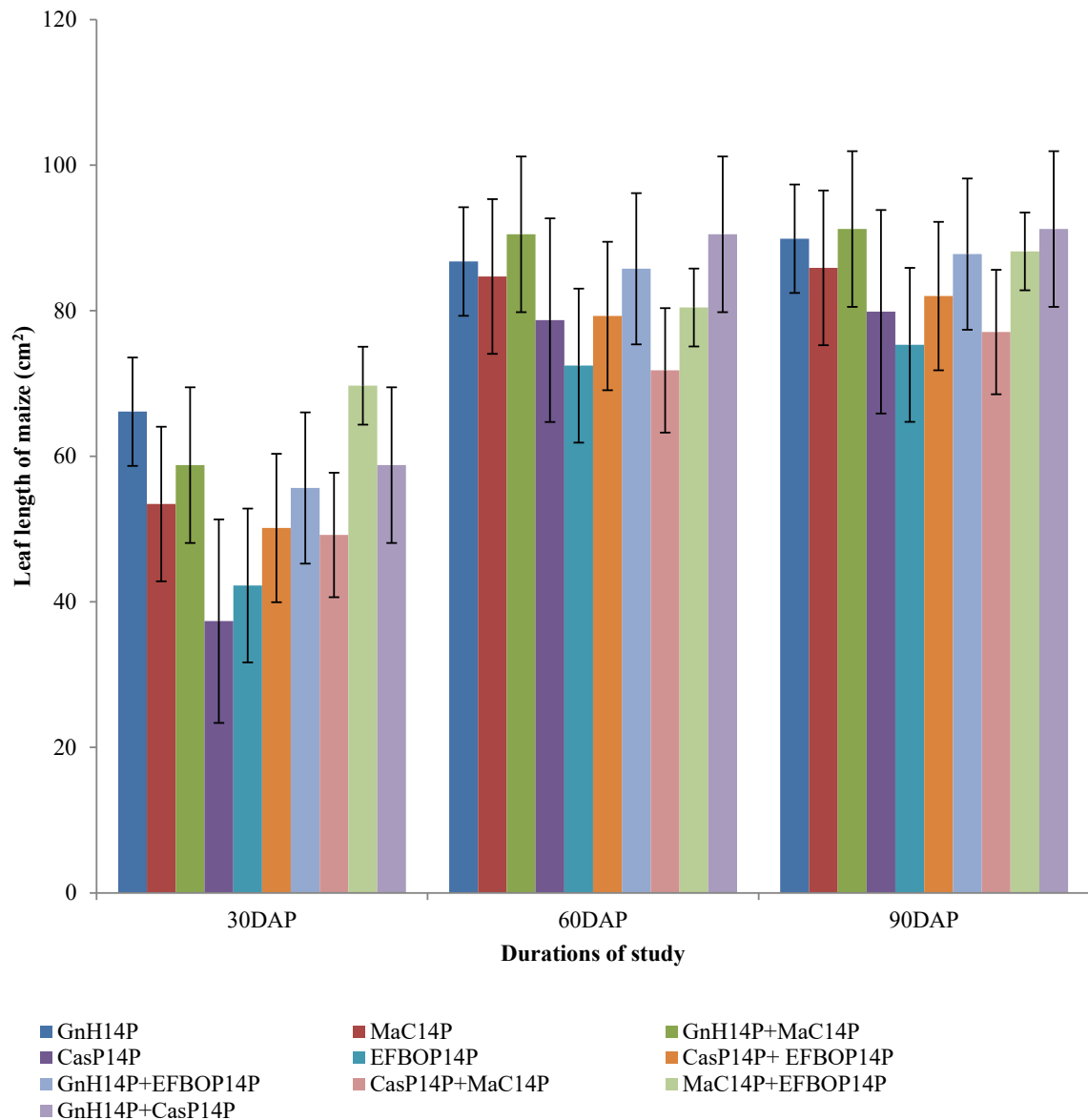


Legend:

MaC₁₄P Maize cob 2014 powder
 EFBOP₁₄P Empty fruit bunch of oil palm 2014 powder
 CasP₁₄P Cassava peels 2014 powder
 DAP Days after planting

Fig. 4. Stem girth of maize in polluted soils amended with different agro-wastes

in plants. These nutrients are essential to plant growth and development (Dimitrov and Markow, 2000). Bioremediation via activities of microorganisms has helped in eliminating contaminants in soil and has also proven effective in combating the advance and deleterious activities of spilled crude oil in soils. Bioremediation is mostly achieved by the mobilization of microorganisms that are sole-dependent on hydrocarbons as a source of carbon and energy. However, to be emphatic not all microorganisms are hydrocarbon degraders, and most times the percentages of hydrocarbon-degrading microbial species may be low, thus the process of bio-stimulation may be applicable to engineer the activities of the few hydrocarbon degraders to sequester the oil. Biostimulation deals



Legend:

MaC₁₄P Maize cob 2014 powder
 EFBOP₁₄P Empty fruit bunch of oil palm 2014 powder
 CasP₁₄P Cassava peels 2014 powder
 DAP Days after planting

Fig. 5. Leaf length of maize in polluted soil amended with agro-wastes

specifically with the addition of nutrients such as phosphorus, nitrogen, and oxygen in contaminated sites. This study enhances the process of microbial degradation of crude oil through the application of nutrients sourced from groundnut husks, maize cobs, oil palm fruit husks, and cassava peels as stated in the result. After the remediation process, which lasted for 90 days maize seeds were grown on the soil. The performance of maize in the remediated soils proves the effectiveness of the remediating materials used. However, maize is known as a C₄ plant which uses less water than C₃ plants for metabolism. Maize could be tolerant to crude oil pollution at a low level in the soil. Majdah (2010) reported that petroleum had a significant inhibitory effect on the germination of

maize, sunflower, acacia, wheat, and alfalfa seeds. They also observed that maize a C₄ plant can be tolerant to hydrocarbon-polluted soil at low concentrations, high concentrations of crude oil at a level of 10.6% (w/w) affects the germination of maize. It was observed in this study that the negative control without amendment significantly prolonged the days to germination of maize. Adedokun and Ataga (2007) noted that the availability of crude oil spent engine oil, and automotive gasoline oil had adverse effects on time to germination, leaf production, plant height, and biomass of *Vigna unguiculata*. Ekunndayo *et al.*, (2001) observed that crude oil pollution significantly reduces the percentage germination and growth performance of maize compared to the non-polluted soils and attributed the effect to the soil's hydrophobicity (Li *et al.* 1996). Okon and Mbong (2013) reported that nutrient amendment could remedy the effect of spent oil on the growth performance of plants. In the same view Okechalu *et al.*, (2014) noted that maize seeds grown on negative control without soil amendment did not germinate, but observed a better germination level in the amended soils. The significant increase in the plant height, leaf area, leaf length, stem girth and number of leaves of maize is an indication that the amendments used were effective in restoring the nutrients level of the soil, especially the nitrogen and phosphorus content. The increase in the growth parameters assessed was duration dependent. Christo *et al.* (2008) suggested that the improved yield of plants treated with poultry manure could be attributed to the manures which acts as biostimulant in enhancing microbial degradation of diesel as well as providing nutrient for the plant. The result of this study is in line with the result obtained for the microbial, total hydrocarbon content, and physicochemical properties of the soil, where it was observed that the combined amendments increase the performance of the soil more than the single amendments. Offor *et al.* (2013) noted that the amendment of crude oil-polluted soils with water hyacinth mulch significantly enhanced okra seed germination and improved the plant height and the number of leaves. The increase in the growth attributes of the maize plant in the amended soil was treatment dependent. The higher the treatment levels the higher the growth of the plants in the soils. Agbogidi *et al.* (2011) noted that amended soil with African breadfruit significantly increases the plant height, number of leaves, leaf area, and collar girth. Kanimozhi (2004) has shown that organic manure influence plant growth by modifying the physiology of plants and by improving the physical, chemical, and biological properties of soil (Amakiri and Onofeghara, 1994).

CONCLUSION

Bioremediation with agro-wastes has proven to be effective in the amelioration of polluted ecosystems. The enhancement of the soils with single and combined forms of the wastes increases the soil microbial activities. The increase in the bacteria and fungi growth could be the result of the reduction in the soil TPH. However, high soil-TPH hinders the growth of plants due to poor soil fertility affected by the excess hydrocarbon content. The cultivation of maize plant on the bioremediated soils shows a significant improvement in the morphological attributes of the plant compared to the crude oil control plants. It can be concluded that the agro-wastes used during the study are of great essence in bioremediation research.

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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