



## Research Article

# Simulated Microgravity, Indole-3-Acetyl Acid, and Cow Dung influence on Zea Mays Plant Growth in Contaminated Soil

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## Abstract

This study investigated the effects of simulated microgravity, indole-3-acetic acid (IAA), and cow dung on *Zea mays* L. plants growing in soil contaminated with crude oil. Crude oil contamination affects plants negatively in morphology and physiology. For simulated microgravity exposure, IAA introduced and non-IAA introduced seeds of *Zea mays* L. were grown in a clinostat for 4 days before taken to the nursery for planting. IAA was introduced to the seed by soaking in IAA solution for 90 minutes after which it was introduced to the microgravity stimulator (clinostat). After 14 days in the nursery, the seedlings were taken to the field with different concentration of crude oil contaminants and cowdung augmentation. Plant height and chlorophyll index were measured biweekly with a meter rule and chlorophyll meter respectively. Plant biomass (root and shoot) were measured at the termination of the experiment. The results showed that the addition of cow dung significantly improved plant height in treatment microgravity exposed in contaminated soil with IAA and cow dung (MGGHCDCS). The highest root-shoot biomass value was obtained in treatment non-microgravity exposed in contaminated soil with IAA and cow dung (NMGGHCD), closely followed by treatment non-microgravity exposed *Zea mays* with IAA growth hormone and cow dung augmentation (NMGHCD), and treatment MG had the lowest value. For 5% crude oil contamination, treatment NMGHCD had the highest root biomass and the lowest root biomass value obtained. This study suggests that combining the three treatments can improve plant morphology in contaminated soil. The improper germination of plants in crude oil contaminated soil will be adequately and successfully addressed by the employment of three combination treatments.

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## Introduction

The contamination of the soil by crude oil is the result of various oil exploration and exploitation activities [1]. They affect plant morphology by disturbing plant growth and biomass [2–3] as well as by negatively affecting plant chlorophyll content [4]. This has stimulated interest in research and has focused on studies

evaluating the physiological and morphological effects of crude oil contamination on plants.

Crude oil inhibits the roots of plants' pores, prevents or reduces the absorption rate of oxygen and water, and impedes plant nutrition absorption [4]. Furthermore, the dissolution and absorption of crude oil-associated chemical compounds alkalizes plant cell sap and affects plant physiological function, disrupts chlorophyll formation,

and reduces plant growth and overall plant biomass. Previous studies [3–5] reported the negative impacts of oil pollution on the height and chlorophyll content of various plant species. Although the importance of these studies on the negative effects of crude oil on plant development cannot be exaggerated, effective methods to help plants overcome the stress caused by the polluted soil of crude oil are research areas that need to be addressed.

In horticulture, plant growth regulators and organic manures provide various advantages in mediation of plant response and growth in a unique environment. Plant growth regulators (organic compounds that affect plant biological processes with low concentrations), such as Indole acetic acid (IAA), have a positive impact on plant height, chlorophyll content, and fresh and dry weight [6–7]. IAA is a phytohormone that improves plant growth by stimulating cell elongation, root-seeding, seed-germination, and seed-producing plants. The use of cow dung to improve plant growth in contaminated soils is also extensively researched [1, 3, 8–9]. They also help plants overcome the challenges of crude oil-contaminated soils. These techniques pose challenges because plant growth regulators depend on concentration, and cow dung can introduce organic metal toxins into plants [8]. New innovative ways of combating crude oil pollution through phytoremediation technologies must be explored. To overcome the challenges or drawbacks associated with IAA and cow dung, this study investigated a more efficient and environmentally friendly method known as enhanced phytoremediation, in which plants grown in a microgravity environment, in conjunction with the application of Indole-3-acetic acid (IAA) and cow dung, were used for the treatment of crude oil contaminated soil. In this method, the plant was first exposed to a simulated microgravity environment before being grown in crude oil contaminated soil. It is hypothesized that plant performance will be significantly improved in an anoxic environment in terms of plant height, chlorophyll index, and biomass.

Microgravity is defined as gravity-conforming acceleration of less than 1 g on Earth's surface [10]. Microgravity is stimulated on the earth's surface using a clinostat [10]. Exposing a plant to microgravity has an anatomical and physiological impact due to structural changes that occur at the cellular level. It has also been shown to increase plant enzymatic activity [11] Other researchers have previously reported on the

effects of simulated microgravity on plant growth, development, and metabolism [12–13]. In light of these developments, this study proposed to use a microgravity environment to grow a plant in crude oil-contaminated soil; and the effects of simulated microgravity, IAA, and cow dung treatments on *Zea mays* plants growing in soil contaminated with crude oil will be investigated. The use of *Zea mays* in this study was inspired by earlier research on the plant's growth and development using a horizontal clinostat to create a microgravity environment that controls the stem cells' osmotic and mechanical properties, both of which are necessary for growth in higher plants [14–15].

## Material and methods

### 1) Study area

The simulated microgravity exposure of *Zea mays* experiment took place at the University of Benin's Space-Earth Environment Research Laboratory, which is run by the National Space Research and Development Agency (NASRDA), and was followed by pot experiments in a screen house in the same University's botanical garden (6.3971492N, 5.6149716E).

### 2) Preparation of contaminated soil

The crude oil used to prepare the contaminated soil was Emreke-kokori light crude oil from Ughelli Delta State, Nigeria. Soils weighing approximately 150 kg (0–15 cm and 15–30 cm) were obtained from the agricultural department of the University of Benin in Benin, Nigeria. The soil was thoroughly mixed before being sieved and sun-dried before being transported to the laboratory for characterization. About 2,500 g of sun-dried and sieved soil were mixed with 5%, 3%, 1%, and 0% (control) crude oil, respectively.

The formula used to calculate the percentage of crude oil applied to the soil is as follows Eq. 1.

After being contaminated with crude oil, the soil was placed in experimental pots measuring approximately 25 cm in diameter and 15 cm in depth and labeled according to treatment. All treatments were performed in triplicate. Following the mixture, the contaminated soil was left for 7 d to acclimate [3].

### 3) Experimental design

The experimental design for the screen house was a completely randomized design where individual treatments were replicated thrice.

$$\% \text{ of crude oil in the soil} = \frac{\text{Weight of crude oil (g)}}{\text{Weight of soil (g)}} \times 100 \quad (\text{Eq. 1})$$

#### 4) Microgravity stimulator

Microgravity was created using a one-axis clinostat. The horizontal rotational axis of the clinostat is perpendicular to the gravity vector on the ground. *Zea mays* seeds were exposed around the axis of rotation. By equalizing the gravity vector, the clinostat provided the simulated microgravity condition. The rotational speed was set to 0.5 rpm.

#### 5) Preparation of agar-agar substrate

The agar was prepared by weighing 1.5 g in a beaker with 100 mL of distilled water. When the mixture was boiling, it was constantly stirred to ensure uniform dissolution. The prepared agar solution was allowed to cool for 10 min after being poured into 33.33 mL Petri dishes, followed by seed sowing. The seeds were half-implanted in the agar.

#### 6) Sowing

The seeds of *Zea mays* were obtained from seed banks at the Space-Earth Environment Research Laboratory in Benin City, Edo State, Nigeria. Seed viability testing was carried out by immersing seeds in water for 20 min [10]. Viable seeds were sterilized by immersing them in a 5% sodium hypochlorite solution for 5 min and then washing them three times with sterile distilled water [23]. Ten seeds were then inoculated into petri dishes with prepared agar media [38]. Sowing was carried out after the Agar had been allowed to cool by inoculating the Petri dishes with 12 seeds of *Zea mays*. Three Petri dishes were inoculated: one for microgravity experiments (clinorotation), one for earth gravity experiments, and one spare in case of damage.

#### 7) Preparation of plant growth regulator (IAA) and sowing

IAA at a concentration of 150 ppm was made by dissolving 0.15 g of powdered IAA in 1,000 mL of distilled water. By vigorous shaking, the mixture was thoroughly mixed. Following the viability test, a set of seeds was soaked in 150 ppm IAA for 90 min before being inoculated into the poured agar plates.

#### 8) Mounting in the clinostat

The petri dishes were covered with lids, and one was placed on the clinostat with a double-sided nano tape at R.M.P of 0.5. The other 2 petri dishes were subjected to gravity. The germinated seeds were transferred to the nursery and grown for 2 weeks under gravity before being transplanted to the contaminated soil in the screen house.

#### 9) Determination of shoot length

For 12 weeks, plant height was measured and recorded biweekly. A meter rule was used to measure from the top of the visible soil to the peak of the plant [31].

#### 10) Determination of chlorophyll content index

The chlorophyll content index (CCI) of the plants' leaves was estimated biweekly from 2 weeks to 12 weeks using a CCM-200 plus chlorophyll content meter. CCM-200 is a non-harmful chlorophyll content estimating meter that takes readings from any three random leaves of a plant [22].

#### 11) Determination of biomass

At the end of the experiment, whole plants were harvested; the roots and shoots were separated, washed with tap water, and repeatedly rinsed with distilled water to remove any soil debris. Afterwards, it was dried in an oven at 80°C for 2 d. The dried roots and shoots were weighed with an electronic balance and recorded [37].

#### 12) Determination of root-shoot biomass

The root-to-shoot biomass ratio was determined by taking the ratio of the dried mass of root divided by the dried mass of shoot of the plant in grams.

#### 13) Statistical analysis

To see how the treatments affected the measured variables of plant height, chlorophyll content, and plant biomass. Means, standard error, and analysis of variance at  $p < 0.05$  were used as statistical tools in Graph Pad Prism, while the bar chart was created in Microsoft Excel (10.0). Tukey posthoc analysis was performed on treatments with significant mean values.

The results presented are mean values; the error bars represent standard error ( $n=3$ ). 0% = no crude oil contamination, 1% = soil contaminated with 1% crude oil, 3% = soil contaminated with 3% crude oil, and 5% = soil contaminated with 5% crude oil. MG = microgravity exposed *Zea mays*, NMG = non-microgravity exposed *Zea mays*, MGCD = microgravity exposed *Zea mays* augmented with cow dung, NMGCD = non-microgravity exposed *Zea mays* augmented with cow dung, MGGH = microgravity exposed *Zea mays* with IAA growth hormone, NMGGH = non-microgravity exposed *Zea mays* with IAA growth hormone, MGGHCD = microgravity exposed *Zea mays* with IAA growth hormone and cow dung augmentation, NMGGHCD = non-microgravity exposed *Zea mays* with IAA growth hormone and cow dung augmentation. MGNCS = microgravity exposed in non-contaminated soil, NMGNCS = non-microgravity exposed in non-contaminated soil, MGCS =

microgravity exposed in contaminated soil, NMGCS = non- microgravity exposed in contaminated soil, MGCDCS = microgravity exposed in contaminated soil augmented with cow dung, NMGCDCS = non-microgravity exposed in contaminated soil augmented with cow dung, MGCDNCS = microgravity exposed in non-contaminated soil augmented with cow dung, NMGNCS = non-microgravity exposed in non-contaminated soil. MGGHCS= microgravity exposed in contaminated soil with IAA, NMGGHCS = non-microgravity exposed in contaminated soil with IAA, MGGHCDCS = microgravity exposed in contaminated soil with IAA and cow dung, NMGGHCDCS = non-microgravity exposed in contaminated soil with IAA and cow dung, MGGHNCS = microgravity exposed with IAA in non-contaminated soil, NMGGHNCS = non-microgravity exposed with IAA in non-contaminated soil.

## Results and discussion

### 1) Plant height

Plant height is influenced by crude oil through shoot retardation [5]. The mechanism at work is that crude oil creates an anoxic environment for the plant, depriving it of water, while heavy metal toxicity sometimes destroys the plant's metabolism. When a plant is deprived of water, its physiological activities suffer because water is required for the plant's physiology to function properly. Table 1 shows the biweekly height measurements of *Zea mays* in the experimental pot. All treatments show an increase in plant height over time. The highest data for plant height was recorded in MGNPS ( $64.67 \pm 6.6$ ), followed by NMGNPS ( $56.33 \pm 5.3$ ), and the lowest data for plant height was documented in NMGPS ( $31.73 \pm 3.3$ ). This finding is consistent with the findings of Aarouf et al. [13] and Orukpe et al. [10], who found that increasing plant height leads to a significant increase in the shoot of the clinostated plant. The current study's observation was confirmed by the work of Nhut et al. [30] on the significance of

clinorotation of the plant to a significant increase in the plant's shoot. Plant growth is hampered by crude oil contaminants, which cause stunting or reduce plant height. The plant's anatomy [39] and physiology [10] were altered by microgravity before being introduced to the contaminated soil, which was required for the plant to overcome the stress imposed by the crude oil contaminant. Nakajima et al. [29] demonstrated that microgravity-exposed plants have an enhanced ability to take up water from the substrate via clinostat, implying that stem elongation is caused by higher water content. The stunting observed in MGCS and NMGCS in week 12 (Table 1) was compensated for by the addition of cow dung, resulting in a significant increase in MGCDCS and NMGCDCS in week 12. Various researchers have reported that crude oil pollution has a negative impact on plant growth [5, 21]. The positive changes observed in cow dung-augmented treatments support the findings of Njoku et al. [3] and Essien et al. [9]. They both reported an increase in plant height in crude oil-contaminated soil after adding cow dung to the contaminated soil. Cow dung introduces petroleum hydrocarbon degrading microbes into crude oil-contaminated soil, thereby breaking the surface barrier created by the crude oil. Microorganisms attack the carbon chain compound and sometimes break it down into smaller chain compounds, allowing water to pass into the soil and then to the plant roots. Water enters the plant through the roots and travels to the xylem tissue, where it is used by the plant for a variety of physiological activities. Cow dung also improves plant growth (height) by balancing the carbon to nitrogen (C-N) ratio in the soil.

Table 2 clearly shows how IAA affected the plant's height. With time, a gradual height gain was noticed. At week 12, the influence of micro-gravity exposure was counter felt by IAA. The obtained values of MGGHCDCS ( $80.5 \pm 2.04$ ) and NMGGHCDCS ( $80.1 \pm 2.23$ ) demonstrate this.

**Table 1** Mean of biweekly measurement of the height (cm) of microgravity exposed and unexposed *Zea mays* and cow dung augmentation grown in the experimental pot

Treatments	Weeks after planting (WAP)					
	2WAP	4WAP	6WAP	8WAP	10WAP	12WAP
MGCS	16.2±1.78	23.3±2.79	27.5±4.08	30.4±2.12	31.2±2.07	33.2±1.09
NMGCS	18.6±0.75	25±0.51	28.6±0.77	27.4±0.73	28.7±1.19	31.7±3.32
MGCDCS	17.0±1.44	26.3±2.11	36.2±4.83	36.9±3.75	42.4±3.69	43.1±3.84
NMGCDCS	20.2±1.13	27.4±0.62	29.9±0.87	34.3±1.40	34.5±1.52	35.5±1.99
MGNCS	15.0±2.52	33.7±2.96	35.0±4.04	38.7±6.17	60.3±6.89	64.7±6.64
MGCDNCS	17.6±0.94	36.6±1.81	38.3±1.22	42.0±1.95	43.9±1.84	46.8±1.83
NMGNCS (Control)	15.5±0.29	24.6±1.26	28.0±2.65	36.0±3.21	48.0±8.88	56.3±5.29

## 2) Chlorophyll index

The current study supports Ogwu's [7] claim that plants exposed to various IAA concentrations grew taller. It has been noted that auxin growth hormone promoted seedling growth [19]. Although the height of the plant was increased by the combined effects of MG, cow dung, and IAA. The combined effects of MG, cow dung, and IAA for higher plant height with a direct 0.5 rpm, 5 0g cow dung for 5,000 g of soil, and plant seeds submerged in IAA for 90 min are recommended based on the results. As a result of their concentration, amount, and specificity, variations in these can have an impact on the process. IAA increases the differentiation of the meristematic apical cells and the root cell tips, acidifies the cell wall, and promotes plant development [19, 34]. The total chlorophyll index also increased with time, following the similar pattern. Week 12 had the greatest value across all treatments (Tables 3 and 4), which indicated a long-term impact on plant biomass. When compared to the control (NMGNCs =  $3.9 \pm 0.58$ ), contamination significantly lowers the chlorophyll index on microgravity exposed (MGCS =  $2.23 \pm 0.37$ ) and non-microgravity exposed (NMGCS =  $1.5 \pm 0.21$ ). This finding is consistent with Xu et al. [12] finding that plant chlorophyll content decreases with crude oil concentration in contaminated soil. The presence of crude oil also inhibits enzyme synthesis in plants [4]. Reduced chlorophyll

content weakens plant photosynthetic functioning, which is an important component of non-stomatal limitations [12]. However, in the presence of the contaminant, the addition of cow dung significantly improved the chlorophyll index value of MGCDCS ( $5.13 \pm 0.42$ ). This result of increased chlorophyll content in plant leaves in contaminated soil supplemented with cow dung corresponds to the findings of Njoku et al. [1] and Okafor et al. [33], who acknowledge the role of cow dung in increasing chlorophyll content in plants in crude oil contaminated soil. They discovered a higher total chlorophyll content in *Glycine max* leaves in contaminated soil supplemented with cow dung. The increase in chlorophyll content observed in plant leaves following cow dung addition suggests that the cow dung improved the soil's condition [1, 33]. Furthermore, the chlorophyll index of all microgravity-exposed *Zea mays* was significantly higher than those that were not exposed. Jagtap et al. [24] previously reported that microgravity-exposed plants undergo changes in their biological systems related to chlorophyll, resulting in an increase in the plant's total chlorophyll content. Musgrave et al. [28] found higher chlorophyll concentrations in *Arabidopsis* during space flight (microgravity) compared to the earth control (1 g), despite the fact that the environmental matrix used was not contaminated.

**Table 2** Mean biweekly measurement of microgravity height (cm) exposed and unexposed *Zea mays* treated with the plant growth regulator (IAA) grown in the experimental pot

IAA treatments	Weeks after planting (WAP)					
	2WAP	4WAP	6WAP	8WAP	10WAP	12WAP
MGGHCS	20±1.15	39.0±2.31	53.4±5.10	59.0±8.5	56.4±8.43	58.4±8.43
NMGHCS	18.0±1.51	35.1±2.98	41.5±4.39	46.0±4.51	51.6±5.59	51.3±5.61
MGGHCDCS	21.1±2.47	42.6±5.26	60.6±2.89	79.8±2.51	76.4±2.73	80.5±2.04
NMGHCDCS	19.6±0.94	38.3±1.25	58.3±2.03	76.6±0.58	78.1±2.22	80.1±2.23
MGGHNCS	26.3±0.44	53.9±1.54	57.5±6.86	72.2±2.66	67.2±4.95	70.4±5.72
NMGHCS	25.2±1.01	49.8±2.18	62.8±2.15	63.7±1.86	60.8±1.18	63.3±0.33
NMGNCs (Control)	15.5±0.29	24.6±1.26	28.0±2.65	36.0±3.21	48.0±8.88	56.3±5.29

**Table 3** Mean biweekly measurement of microgravity's chlorophyll index exposed and unexposed *Zea mays* grown in the experimental pot

Treatments	Weeks after planting (WAP)					
	2WAP	4WAP	6WAP	8WAP	10WAP	12WAP
NMGNCs (Control)	3.6±0.46	3.8±1.02	3.93±0.74	4.03±0.64	3.77±0.58	3.9±0.58
MGCS	1.97±0.24	1.97±0.20	1.67±0.22	1.27±0.12	2.06±0.642	2.23±0.37
NMGCS	2.27±0.07	1.97±0.27	1.87±0.19	1.27±0.09	1.26±0.12	1.50±0.21
MGCDCS	2.63±0.38	3.23±0.44	3.10±0.26	2.37±0.20	4.87±0.44	5.13±0.42
NMGCDCS	2.83±0.33	2.93±0.26	2.70±0.00	1.83±0.30	2.60±0.10	2.83±0.03
MGNCS	4.70±0.31	4.90±0.25	4.37±0.23	5.53±0.07	5.93±0.09	6.03±0.13
MGCDCS	2.63±0.38	3.23±0.44	3.89±0.31	7.31±0.29	8.50±0.12	8.20±0.14

**Table 4** Mean biweekly measurement of the chlorophyll index of microgravity exposed and unexposed *Zea mays* treated with the plant growth regulator (IAA) grown in the experimental pot

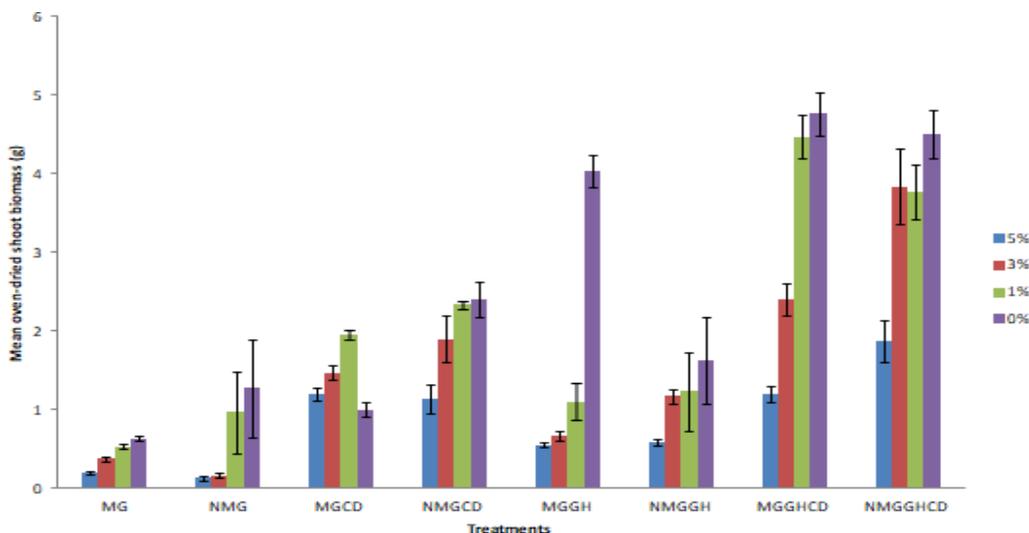
IAA Treatment	Weeks after planting (WAP)					
	2WAP	4WAP	6WAP	8WAP	10WAP	12WAP
NMGNCS (Control)	3.6±0.46	3.8±1.02	3.93±0.74	4.03±0.64	3.77±0.58	3.9±0.58
MGGHCS	3.67±0.22	5.48±0.36	7.13±0.62	6.3±0.49	8.22±1.39	8.43±1.39
NMGGHCS	3.71±0.54	4.92±0.58	6.23±0.55	5.61±0.77	5.33±0.81	5.30±0.96
MGGHCDCS	3.89±0.31	7.31±0.29	8.56±0.17	8.20±0.14	6.36±0.91	8.03±0.28
NMGGHCDCS	4.61±0.55	8.42±1.15	8.43±0.88	9.25±0.55	6.90±0.95	7.10±0.15
MGGHNCS	4.32±0.55	9.12±0.28	9.19±0.39	8.53±0.77	10.5±2.03	10.6±1.99
NMGGHNCS	4.74±0.39	11.27±2.11	9.37±0.47	6.84±0.41	7.69±0.29	9.03±0.96

IAA supplementation improved the values recorded in all treatments. In comparison to the control, IAA treatments in contaminated soil resulted in an exponential increase in chlorophyll content (Table 3). IAA treatment boosts chlorophyll content in plant leaves under stressful conditions such as contaminated soil by using antioxidant enzymes to protect chlorophyll pigments from oxidative stress [20]. The influence of IAA was evident in all treatments for both contaminated and uncontaminated soil. MGGHNCS had the highest chlorophyll value (10.6±1.99). This therapy combines the effects of microgravity and growth hormones. Another reason for the high value could be that the substrate (soil) was uncontaminated, so there was no negative impact on the plants. At week 12, treatments MGGHCDCS (8.03±0.25) and MGGHCS (8.43±1.39) performed relatively well and were not statistically different, implying that the effect of the plant growth regulator (IAA) suppresses the influence of cow dung on chlorophyll content. Table 2 shows that cow dung has a positive effect on chlorophyll content, but Table 3 shows that IAA has a greater impact on the plant's chlorophyll

index than cow dung. This could be attributed to the antioxidant protection enzymes that IAA provides to plants [20]. At week 12 (8.03±0.25), the combination of MG, cow dung, and IAA had no discernible effect on the chlorophyll content of *Zea mays* in the contaminated soil. It could be attributed to the plant's chlorophyll index reaching a peak in the presence of contamination. Although the addition of IAA and cow dung increased the chlorophyll content in MGGHCDCS (8.03±0.28), combining all three treatment processes (microgravity exposure, IAA, and cow dung) will have a significant impact on the chlorophyll index. Ogwu [7, 32] confirmed that using manure (organic or inorganic) in combination with IAA or IAA alone on plants increases chlorophyll content, which correlates positively with plant height increase.

### 3) Shoot biomass

Figure 1 shows the mean shoot biomass at the end of 12 weeks; the result shows increased shoot biomass and high tolerance to the different contaminant concentrations in treatments MGGHCD and NMGGHCD.



**Figure 1** Mean oven-dried shoot biomass production per planted treatment after 12 weeks in co-contaminated soil.

In 5% contamination, NMG had the lowest shoot biomass, while NMGGHCD had the highest. NMGGHCD also had the highest value in 3% contamination, but in 1% contamination and control, the MGGHCD treatment had the highest shoot biomass value. The treatments differed significantly ( $p < 0.05$ ). Except for MGCD, all treatments showed a decrease in shoot biomass as contamination concentration increased. The oven-dried shoot biomass decreases significantly as the percentage of crude oil increases (Figure 1). IAA and cow dung application increased shoot biomass at all concentrations. The application of IAA and cow dung improves shoot and root development [34]. IAA promotes shoot development by promoting continuous cell division and apical meristem elongation, resulting in increased shoot length and, later, shoot dried biomass [7].

#### 4) Root biomass

Figure 2 depicts the root biomass results. There was a significant difference ( $p < 0.05$ ) in root biomass between the contaminated soil and the control. For 5% crude oil contamination, treatment NMGGHCD had the highest root biomass, closely followed by NMGGH, and treatment MG had the lowest value. However, at 5% contamination, there was no significant difference ( $p > 0.05$ ). MGGHCD had the highest root biomass for 3% contamination when compared to MGGH and MGCD. All treatments showed a statistically significant difference at  $p < 0.05$ . Treatment MGGHCD had the highest root biomass value at 1% concentration, while treatment MG had the lowest. The control followed the pattern of having the highest shoot value and being significantly different from all treatments. The results showed a decrease in root biomass with an increase in crude oil percentage concentration in all treatments except NMGCD, where the treatment value was higher than the control with no contamination at 1% concentration. Homeostasis, or plant growth simulation at lower contaminant concentrations, could best explain this outlier [26]. Salanitro et al. [35] found that maize plants grown in high and medium crude oil contamination performed better than controls by more than 40%. Kirk et al. [26] found that lower diesel contamination resulted in higher root biomass in alfalfa and crown vetch compared to the control. After 12 weeks, the root biomass increased as the crude oil concentration

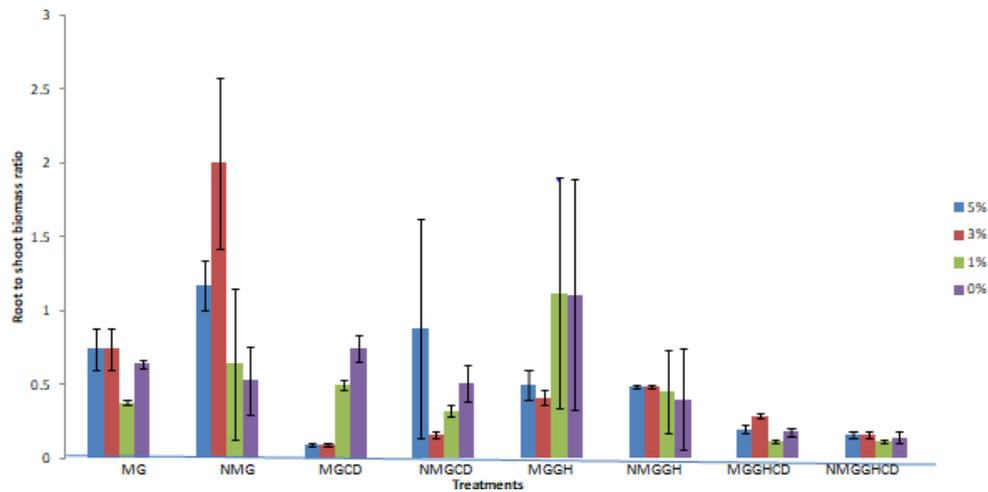
decreased. NMGGHCD had the highest value of about 1.99 g and 3% (3.02 g) for 5% crude oil concentration, but the MG value was higher for 1% and 0% contamination. This could be explained by the findings of previous studies [10, 18] which found the increased production of stress enzymes like ethylene, catalase, and malondialdehyde in MG-exposed plants. This finding indicates that cow dung supplementation and IAA application can improve plant ability in contaminated soil.

#### 5) Root to shoot ratio of biomass

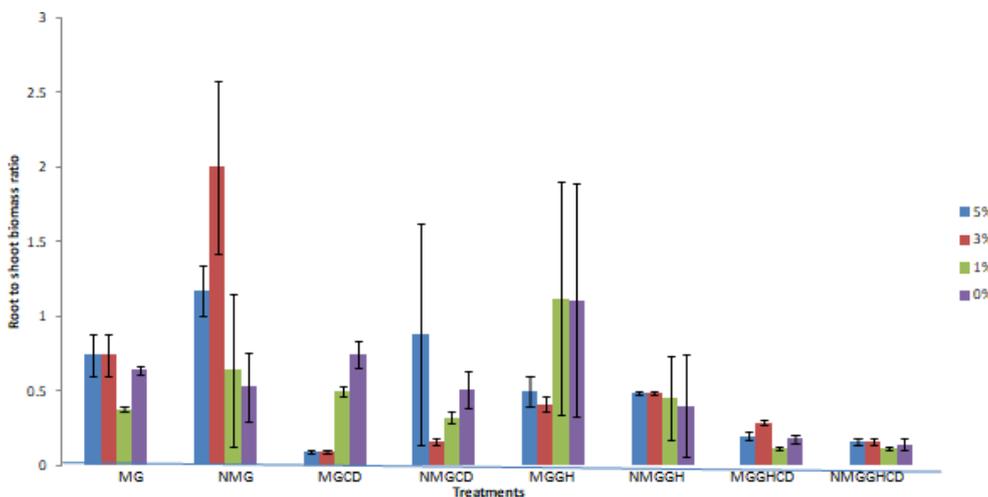
The increased root-to-shoot ratio was observed in 5% and 3% of MG and NMG, respectively, while MGGHCD and NMGGHCD had the lowest ratio value. When the root-to-shoot biomass ratio increases, it indicates that the plant is in a low nutrient condition [25]. To obtain nutrients, plants may allocate photo-synthates to their lower roots rather than the upper vegetative part. The increased root-to-shoot ratio observed in the different MG and NMG concentrations is due to the crude oil contaminant creating a stressful and nutrient-deficient environment for the plant. However, the unfavorable condition was alleviated by the use of cow dung and IAA treatment. This is why MGGHCD and NMGGHCD have the lowest value ratio. Merkl et al. [27] found a higher root-to-shoot ratio in grass species exposed to 5% crude oil contamination compared to the control. This finding is consistent with the current study, which found a significant increase in the root-shoot biomass of *Zea mays* treatment NMG. The highest root-shoot biomass value obtained in NMG and the lowest value obtained in MGGHAU support the significant importance of combining the three treatments in improving plant morphology in contaminated soil.

#### Conclusion

This study examines the relationship between *Zea mays* biomass, IAA and cow dung height, and micro-gravity exposure. The chlorophyll content, plant height, and biomass of contaminated plants are significantly impacted, which hinders normal plant establishment. The improper germination of plants in soil contaminated with crude oil will be adequately and successfully addressed by the employment of three combinations.



**Figure 2** Mean oven-dry root biomass production per planted treatment after 12 weeks in crude oil contaminated soil.



**Figure 3** Root-to-shoot ratio of biomass of the different treatments in the crude oil contaminated soil and the control.

## References

- [1] Njoku, K.L., Akinola, M.O., Oboh, B.O. Phyto-remediation of crude oil contaminated soil: The effect of growth of *Glycine max* on the physico-chemistry and crude oil contaminated soil. *Nature and Science*, 2009, 7(10), 79–87.
- [2] Okonohua, B.O., Ikhajagbe, B., Anoliefo, G.O., Emede, T.O. The effect of spent engine oil on soil properties and growth of maize (*Zea mays* L.). *Journal of Applied Science and Environmental Management*, 2007, 11(3), 147–152.
- [3] Njoku, K.L., Akinola, M.O., Oboh, B.O. Phyto-remediation of crude oil polluted soil: Effect of cow dung augmentation on the remediation of crude oil polluted soil by *Glycine max*. *Journal of Applied Sciences Research*, 2012, 8(1), 277–282.
- [4] Plabita, B., Saikia, R.R., Baruah, P.P., Deka, S. Effect of crude oil contamination on the chlorophyll content and morphoanatomy of *Cyperus brevifolius* (Rottb.) Hassk. *Environmental Science Pollution Research*, 2014, 21, 12530–12538.
- [5] Kekere, O., Ikhajagbe, B., Apela, B.R. Effect of crude petroleum on the germination, growth and yield of *Vigna unguiculata* Walp L. *Journal of Agriculture and Biological Sciences*, 2011, 2(6), 158–165.
- [6] Rostami, S., Azhdarpoor, A. The application of plant growth regulators to improve phytoremediation of contaminated soil: A review. *Chemosphere*, 2019, 220, 818–827.
- [7] Ogwu, M.C. Effect of Indole-3-acetic acid on the growth parameters of *Citrullus lanatus* (Thunberg) Matsum and Nakai. *Momona Ethiopian Journal of Science*, 2018, 10(1), 109–125.
- [8] Jidere, C.M., Akamigbo, F.O.R., Ugwuanyi, J.O. Phytoremediation potentials of cowpea (*Vigna unguiculata*) and Maize (*Zea mays*) for hydrocarbon degradation in organic and inorganic manure-amended tropical typical paleustults. *International Journal of Phytoremediation*, 2012, 14, 362–372.
- [9] Essien, E.B., Ifeanchio, M.O., Nwachukwu, G.A. The impact of cow dung augmentation for the remediation of crude oil polluted soil by *Elueusine indica*. *Journal of Applied Science and Environmental Management*, 2015, 19(1), 103–107.
- [10] Orukpe, A.O., Aneliefo, G.O., Ikhajagbe, B. Effect of clinorotation on the enzyme activities

- and morphology of *Zea mays* seedlings. *American Journal of Life Sciences*, 2021, 9(1), 11–18.
- [11] Xu, P., Chen, H., Jin, J., Cai, W. Single-base resolution methylome analysis shows epigenetic changes in *Arabidopsis* seedlings exposed to microgravity spaceflight conditions on board the SJ-10 recoverable satellite. *npj Microgravity* 2018, 4, 1–11.
- [12] Brown, C.S., Hilaire, E.M., Guikema, J.A., Piastuch, W.C., Johnson, C.F., Stryjewsky, E.C., ..., Vodermark, D.S. Metabolism, ultrastructure and growth of soybean seedling in microgravity. *ASGSB Bulletin*, 1995, 9, 93–99.
- [13] Aarouf, J., Darbelley, N., Demandre, C., Razafindramboa, N. Perbal, G. Effect of horizontal clinorotation on the root system development and lipid breakdown in rapeseed (*Brassica napus*) seedlings. *Plant Cell Physiology*, 1999, 40(4), 396–405.
- [14] Hafida B., Aparicio, J.D., Acuca, A., El Hadj-khelil, A.O., Sanchez, L., Polti, M. A., Alvarez, A. Effectiveness of the *Zea mays*-*Streptomyces* association for the phytoremediation of petroleum hydrocarbons impacted soils, *Ecotoxicology and Environmental Safety*, 2019, 184, 109591.
- [15] Grifoni, M., Rosellini, I., Angelini, P., Petruzzelli, G., Pezzarossa, B. The effect of residual hydrocarbons in soil following oil spillages on the growth of *Zea mays* plants, *Environmental Pollution*, 2020, 265, 114950.
- [16] Bishnu M.K.C., Gauchan, D.P., Khanal, S.N. Chimouriya, S., Lamichhane, J. Extraction of indole-3-acetic acid from plant growth promoting rhizobacteria of bamboo rhizosphere and its effect on biosynthesis of chlorophyll in bamboo seedlings. *Indian Journal of Agricultural Research*, 2020, 54 (6), 781–786.
- [17] Carman, E.P., Crossman, T.L., Gatliff, E.G. Phytoremediation of no.2 fuel oil-contaminated soil. *Journal of Soil Contamination*, 1998, 7(4), 455–466.
- [18] Carman, J.G., Hole, P., Salisbury, F.B., Bingham, G.E. Developmental, nutritional and hormonal anomalies of weightlessness-grown wheat. *Life Sciences in Space Research*, 2015, 6, 59–68.
- [19] Chouychai, W. Effect of some plant growth regulators on lindane and alpha-endosulfan toxicity to *Brassica Chinensis*. *Journal of Environmental Biology*, 2011, 33, 811–816.
- [20] Esan, A.M., Olaiya, C.O., Omolekan, T.O., Aremu, K.A., Adeyemi, H.R.Y. Comparative effect of indole acetic acid on photosynthetic pigments and mineral contents of two genotype okra under salinity stress. *European Journal of Agriculture and Food Sciences*, 2020, 2(6), 136.
- [21] Ikhajagbe, B., Anoliefo, G.O. Impact of soil amendment of phytotoxicity of a 5-month old waste engine oil polluted soil. *Journal of Ecology and the Natural Environment*, 2010, 2(6), 112–122.
- [22] Ikhajagbe, B., Omoregie, U. E. Growth, yield, genetic parameters and random amplified polymorphic DNA (RAPD) of five rice varieties treated with sodium azide and sown under different saline conditions. *Bulletin of the National Research Centre*, 2020, 4, 44–48.
- [23] Ishii, Y., Hosen, T., Kamisaka, S., Miyamoto, K., Ueda, J., Mantani, S., ..., Yamamoto, R. Plant growth processed in *Arabidopsis* under microgravity conditions simulated by clinostat. *Biological Science Space*, 1996, 10(1), 3–7.
- [24] Jagtap, S.S., Awhad, R.B., Santosh, B. Vidyasagar, P.B. Effect of clinorotation on growth and chlorophyll content of rice seedlings. *Microgravity Science and Technology*, 2011, 23, 41–48.
- [25] Kaimi, E., Mukaidami, T., Tamaki, M. Screening of twelve plant species for phytoremediation of petroleum hydrocarbon-contaminated soil. *Plant Production Science*, 2007, 10(2), 211–218.
- [26] Kirk, J.L., Klirnomos, J.N., Lee, H. Trevors, J.T. Phytotoxicity assay to assess plant species for phytoremediation of petroleum-contaminated soil. *Bioremediation Journal*, 2010, 6(1), 57–63.
- [27] Merkl, N., Schultze-Kraft, R. Infante, C. Assessment of tropical grasses and legumes for phytoremediation of petroleum-contaminated soil. *Water, Air and Soil Pollution*, 2005, 165, 195–209.
- [28] Musgrave, M.E., Kuang, A., Brown, C.S., Matthews, S.W. Changes in *Arabidopsis* leaf ultrastructure, chlorophyll and carbohydrate content during space flight depend on ventilation. *Annals of Botany*, 1998, 81, 503–512.
- [29] Nakajima, S., Nagata, M., Ikehata, A. Mechanism for enhancing the growth of mung bean seedlings under simulated microgravity. *Microgravity*, 2021, 7(26).
- [30] Nhut, D.T., Khai, H.D., Tuan, N.X., Bien, L., Tung, H.T. In vitro growth and development of plants under simulated microgravity conditions In: *Biomedical and Life Sciences*. Springer, Singapore, 2022.
- [31] Nwaichi, E.O., Chukwuere, C.O., Abosi, P.J., Onukwuru, G.I. Phytoremediation of crude oil impacted soil using purple nutsedge. *Journal of Applied Science and Environmental Management*, 2021, 25(3), 475–479

- [32] Ogwu, M.C., Aiwansoba, R.O. Osawaru, M.E. Effect of indole-3-acetic acid on germination in lead polluted petri dish of *Citrullus lanatus* (Thunberg) Matsumura and Nakai, Cucurbitaceae. *Aceh International Journal of Science and Technology*, 2015, 4(3), 107–113
- [33] Okafor, H.K., Achebe, M.K., Ezeugo, J.N.O., Anadebe, V.C. Cow dung augmentation remediates crude oil contaminated soil planted with maize seedlings. *International Journal of Scientific and Engineering Research* 2016, 7(12), 195–217.
- [34] Rostami, S., Azhdarpoor, A., Majid, R., Mohammad, R.S. The effect of simultaneous application of plant growth regulators and bio-augmentation on improvement of phytoremediation pyrene contaminated soils. *Chemosphere*, 2016, 161, 219–223.
- [35] Salanitro, J.P., Dorn, P.B., Huesemann, M.H., Moore, K.O., Rhodes, I.A., Jackson, L.M.R., ..., Wisniewski, H.L. Crude oil hydrocarbon bioremediation and soil ecotoxicity assessment. *Environmental Science and Technology*, 1995, 31, 1769–1776.
- [36] Shimazu T, Yuda T, Miyamoto K, Yamashita M, Ueda J. Growth and development in higher plants under simulated microgravity conditions on a 3-dimensional clinostat. *Advance Space Research*, 2001, 27(5), 995–1000.
- [37] Shu, J., He, Q., Zheng, H., Liao, M., Kuang, N., Huang, Z. Abscisic acid improves the ability of accumulator plants *Perilla frutescens* to perform phytoremediation in cadmium-contaminated soil. *International Journal of Environmental Analytical Chemistry*, 2023, 103(9), 2182–2191.
- [38] United Nations Office for Outer Space Affairs (UNOOSA). *Teacher's guide to plant experiment in microgravity*. Vienna, Austria, 2013, p. 63.
- [39] Zaidi, M.A., Murase, H. Growth and development of seedlings and stem cell under microgravity conditions. ICAME 2000, In the 3<sup>rd</sup> international Conference on Agricultural Machinery Engineering, Seoul, Korea, 2000.
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