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Effects of crude oil contaminated-soil on the germination and growth of cowpea *Vigna unguiculata* (L.) Walp

Enajeria, G.,[1] **Raimi, I. O.**[2] and **Odiwe, A. I.**[1]
[1]Department of Botany, Obafemi Awolowo University, Ile Ife, Nigeria
[2]Institute of Ecology and Environmental Studies, Obafemi Awolowo University
Ile Ife, Nigeria
*Corresponding author: aiodiwe@oauife.edu.ng

Abstract

A greenhouse experiment was conducted to investigate the germination and growth response of cowpea (*Vigna unguiculata* (L.) Walp) to soils contaminated at different concentrations of crude oil. This was done with a view to access the impact of crude oil pollution on growth and nutrient content after harvest. The contaminated crude oil polluted soils were prepared at different concentrations of 0.0, 10.0, 20.0, 30.0, 40.0 and 50.0 (w/v) on 5 kg each of air-dried soil collected from the Obafemi Awolowo University Biological Garden rich in organic matter. Each treatment was replicated three times and arranged in complete randomized design. The following parameters, leaf length, breadth and leaf number, N and protein were determined. Results showed that leaf length, breadth and leaf number were significantly higher ($p<0.05$) recorded in the control pots. The least % N and protein were recorded in the pots with highest crude oil concentration (50.0 ml). The results also showed that increasing crude oil concentration, posed higher risk of edibility of the test-plant and no detectable amount of petroleum hydrocarbon was found in the soils at the end of the experiment. The study concluded that although cowpea germinated and grew well in crude oil-contaminated soils, its nutrient content was impaired and edibility not guaranteed.

Keywords: Crude oil; cowpea; germination; growth response; hydrocarbon.

Introduction

Crude oil exploration and exploitation in Nigeria have evolved through a long history [1]. Oil exploration and exploitation despite its economic importance has however, resulted in the contamination of a significant number of sites with crude oil and petroleum bye-products [2]. United Nations for Environmental Protection (UNEP) report on oil pollution in Ogoni shows that the Niger Delta Area of Nigeria has experienced several contaminated sites with petroleum hydrocarbon, metals pesticides, salts and other related contaminants [3]. Heavy human consumption of crude oil extract such as petroleum, kerosene, gasoline requires deep intense researches into some of accompanied crises which may arise during its exploration.

Petroleum is used in manufacturing a wide range of materials, and it is estimated that the world consumes about 90 million barrels each day [4]. Petroleum industry as an economic sector of Nigeria has created an economy boom for the nation; Nigeria ranks the Africa's largest producer of crude oil and the sixth largest oil producing country in the world with a maximum crude oil production of 2.5 million barrels per day [5]. The consequences and negative impacts caused by crude oil exploration and exploitation have resulted into a global environmental crisis like pollution of the environment and youth restiveness in such polluted area. Pollution is the process of contaminating the earth and atmosphere to such an extent that normal environmental processes are adversely affected [6]. The effects of crude oil on the growth and performance of plants have been reported in many researches. These effects have been observed to occur due to the interference of the plant uptake of nutrients by crude oil and the unfavorable soil conditions due to pollution with crude oil [7-9].

Despite the exploration of crude oil and its importance to the economy of many nations where they are found, the exploration has impacted negatively on the destruction of flora and fauna of such ecosystem. The destruction of food crops, farmlands, fish lakes and subsequent restiveness that follows the unsustainable processes of crude oil exploration in the Niger Delta region requires urgent attention by all and sundry. Various researchers have worked on testing different plants for crude oil or spent engine oil remediation processes [10-12]. However, little work have been done and reported on edible crops grown on or around crude-oil-polluted sites, hence, the need for this study. In Africa, cowpea is the most popular legume and the largest part of world production originates from the Africa continent. Cowpea has been reported by various researchers because of its adaptability to stressful environments where other crops either fail or do not perform well. Cowpea (*V. unguiculata* (L.) Walp.) was chosen as the test plant due to its nitrogen fixing ability, also, as a follow-up on some of the literature reviewed where several reports abound about its potential to surviving polluted soils.

Materials and methods

Seed viability test was done on the seeds to determine the seed viability efficiency which was ascertained to be 90% viable. The procedure for seed viability test followed [13]. This was done by soaking ten (10) viable seeds of cowpea in distilled water containing cotton wool. After 48 hours, nine (9) seeds germinated out of 10 placed in the petri-dish. The experiment was carried out in the greenhouse of the Faculty of Agriculture, Obafemi Awolowo University (OAU), Ile Ife, Nigeria. Surface soil from the OAU Biological Garden was collected and air-dried for 7 days and then sieved using a 2 mm mesh to remove debris. Air-dried and sieved soil (5 kg) was weighed into each of 18 plastic bowls measuring 30 cm by 18 cm (diameter by depth) and perforated at the base to drain water and increase soil aeration [11]. Crude oil obtained from the Nigerian National Petroleum Corporation (NNPC), Eleme, Rivers State, Nigeria with a concentration of 155.88 ppm/L was used as the contaminant.

There were six treatments and each was replicated 3 times. The treatments included: 0.0, 10.0, 20.0, 30.0, 40.0 and 50.0 ml of crude oil and were randomly assigned to bowls arranged in a completely randomized design [14]. The contaminated-soil was watered regularly for a week to allow penetration of the crude oil. Three viable seeds of cowpea (04k-339-1) collected from the seed bank of International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria, were planted in each of the eighteen bowls of the 5 kg soil already polluted with known concentration of crude oil. The experiment was left for one week to establish, thereafter, pruned to two seedlings per pot before the commencement of data collection. Germination results were taken one week after planting (WAP) the seeds, the number of seeds that germinated from each pot was summed up after seven (7) days. Plants were watered regularly and the pots were maintained weed-free.

Plate 1. Experimental pots at one week after planting.

Measurement of germination and growth performance

Germination results were taken one week after planting the seeds, the number of seeds that germinated from each pot was summed up after seven (7) days. The percentage of germination in each treatment was calculated using the formula below [15-17].

Percentage germination:

$$= \frac{\text{Number of seeds that germinated}}{\text{Number of seeds sown}} \times 100$$

Growth performance such as plant height (cm) was measured from the base of the plant to the terminal bud; leaf length (cm) was measured from the base of the leaf to the leaf apex; leaf breadth (cm) was measured at its widest, and number of leaves were measured starting from the second week after planting (2WAP) on a weekly basis using non-destructive sampling method. Also, the number and weight (g) of seeds harvested were also measured by counting and using weighing balance respectively. Thinning of the plants to two plants per pot was done 1WAP to enable limited competition for nutrient by the plants [18]. The plastic pots were perforated at the bottom to allow for aeration. Watering of the plants was done evenly and at regular intervals and also leaching of soil nutrients was avoided by adding water to carrying capacity of the polluted soil. All removed-weeds were returned to the pot for nutrient return. All measurement and readings starts from the lowest pollution level to highest level to avoid contamination.

Proximate composition and heavy metals analysis

The proximate chemical compositions of the seeds were analyzed after drying at 70°C in an oven to constant weight according to standard [19] methods, while heavy metals were analyzed using Atomic Spectrophotometer (Buck 2.0) for the seeds in International Institute of Tropical Agriculture (IITA), Ibadan. Analyses done on the dried harvested seeds include the moisture, nitrogen and ash contents, crude fiber, protein, fat and carbohydrate. Heavy metals such as iron, zinc, copper, lead and nickel were determined in the harvested seeds.

Statistical analysis

A statistical comparison of means of different treatments was carried out using one way analysis of variance and treatment means were separated using the Duncan Multiple Range Test. Significance level was set at $p<0.05$. The data analysis was done using SPSS version 13.0 for Windows.

Results

Percentage germination of seeds

The percentage germination of seeds one week after planting were; 100%, 83.33%, 66.67%, 58.33%, 58.33% and 58.33% in the 0.0 ml, 10.0 ml, 20.0 ml, 30.0 ml, 40.0 ml and 50.0 ml respectively. There was a significant difference ($p<0.05$) in the germination rate of the cowpea seeds compared to the control. The control (0.0 ml) had the highest number of germinated seedlings while 30.0 ml, 40.0 ml and 50.0 ml had equal number of germinated seedlings (Figure 1).

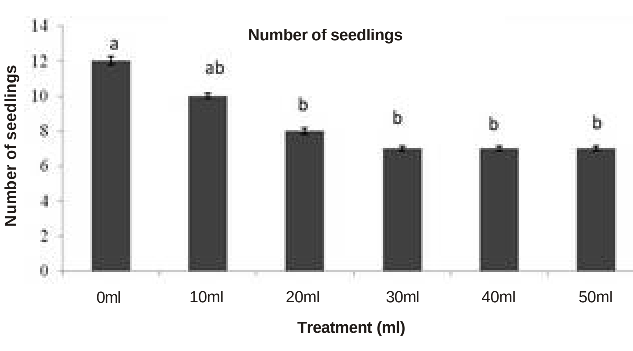

Figure 1. Effect of crude oil-treated soil on the germination of cowpea seeds. Means with the same superscript are not significantly different ($p<0.05$). Each value is a mean of three readings ± standard error.

Leaf length

The result on leaf lengths showed that there was significant difference ($p<0.05$) between the various treatments when compared with the control. The result for the leaf lengths indicated that there was a progressive increase from 1WAP to 9WAP, the leaf length ranged from 3.24 ± 0.34 cm to 10.27 ± 0.05 cm. A sharp decrease in the leaf length occurred from 9WAP to 11WAP, with the leaf length ranging from 10.27 ± 0.05 to 6.32 ± 0.06. The result also showed that there were significant difference ($p<0.05$) between the polluted plants and the control plants as influenced by the crude oil treatments except in the 6WAP and 11WAP with 50 ml having the highest negative effect on the plants' leaf length. No significant differences ($p<0.05$) were observed between 20.0 ml and 30.0 ml at 11WAP as well as 30.0 ml and 40.0 ml at 11WAP (Figure 2).

Plate 2. Experimental pots at ninth week after planting.

Plate 3(a&b). Germination of cowpea in 40 ml polluted soil at three and seven weeks after planting.

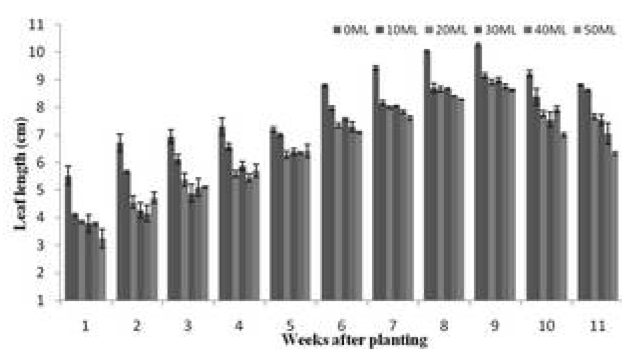

Figure 2. Effect of crude oil-treated soil on the leaf length of cowpea over the study period. Each value is a mean of readings ± standard error.

Leaf breadth

The results show that there was a significant difference ($p<0.05$) in the leaf breadths among the various concentrations of crude oil used compared to the control plants. It should however be noted that the differences were not ($p<0.05$) between 30.0 ml, 40.0 ml and 50.0 ml concentrations except at 10WAP and 11WAP. There was a rapid increase in the leaf breadth from the 1WAP to 9WAP and a significant reduction was observed from 9WAP to 11WAP as shown (Table 1).

Number of leaves

The number of leaves show that there was a significant difference ($p<0.05$) between the various concentrations compared to the control (Figure 3). There was a significant difference ($p<0.05$) at 5WAP to 8WAP. A rapid increase was observed in the number of leaves from 5WAP week to the 10WAP and this was followed by a reduction during the last week of the study, where the most reduced leaves were observed in the pots with 50.0 ml concentration. The effect was less pronounced from 1WAP to 4WAP (Figure 3).

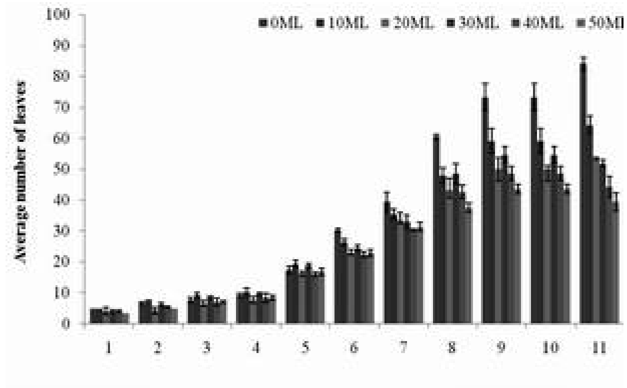

Figure 3. Effect of crude oil-treated soil on the number of leaves of cowpea over the study-period. Each value is a mean of three readings ± standard error.

Plant height

There was no significant difference ($p<0.05$) in the plant heights recorded across the various treatment levels when compared with the control plots in this study (Table 2). Although, there was rapid increase in the plants' heights from the 4WAP to the 9WAP as recorded in the 0.0 ml, 10.0 ml and 20.0 ml treatments.

Table 1. Effect of crude oil treated soil on the leaf breadth of cowpea over the study-period. Each value is a mean of mean three readings ± standard error.

Concentration	Week										
	1	2	3	4	5	6	7	8	9	10	11
0 ml	5.52± 0.34	6.71± 0.32	6.94± 0.24	7.30± 0.31	7.20± 0.09	8.78± 0.05	9.43÷ 0.06	10.03± +0.04	10.27 ÷0.05	9.22± 0.12	8.82± 0.03
10 ml	4.10± 0.04	5.65± 0.04	6.13± 0.17	6.57± 0.13	6.99± 0.05	7.97± 0.07	8.16÷ 0.09	8.70± 0.16	9.15÷ 0.09	8.38± 0.29	8.61± 0.03
20 ml	3.84± 0.05	4.55± 0.23	5.37± 0.23	5.57± 0.16	6.28± 0.14	7.34± 0.08	7.99÷ 0.04	8.65± 0.09	8.91÷ 0.10	7.76± 0.10	7.66± 0.10
30 ml	3.79± 0.32	4.26± 0.28	4.89± 0.29	5.87± 0.16	6.38± 0.11	7.57± 0.06	8.04÷ 0.03	8.66± 0.03	8.99÷ 0.10	7.55± 0.28	7.54± 0.19
40 ml	3.79± 0.05	4.15± 0.29	5.09± 0.32	5.45± 0.15	6.34± 0.05	7.30± 0.18	7.84÷ 0.06	8.40± 0.01	8.76÷ 0.07	7.93± 0.10	7.04± 0.38
50 ml	3.24± 0.34	4.72± 0.21	5.09± 0.03	5.71± 0.24	6.41± 0.24	7.08± 0.04	7.62÷ 0.07	8.29± 0.01	8.62÷ 0.04	6.99± 0.07	6.32± 0.06

Table 2. Effect of crude oil treated soil on the plant height of cowpea over the study-period. Each value is a mean of three readings ± standard error.

Duration	**Treatment**					
	0 ml	10 ml	20 ml	30 ml	40 ml	50 ml
Week 1	20.30±0.75	16.28±2.22	11.07±3.13	14.20±2.67	15.28±1.83	11.10±6.52
Week 2	23.42±1.05	20.63±1.70	12.15±3.02	15.05±2.72	16.37±4.80	13.40±5.63
Week 3	24.38±2.05	23.32±5.28	13.42±4.35	18.45±6.12	17.53±5.57	16.92±7.75
Week 4	26.80±1.30	24.67±5.32	14.48±4.15	19.95±6.49	19.00±5.83	18.33±8.11
Week 5	33.04±0.08	28.38±0.25	18.32±0.35	23.52±0.46	22.03±0.45	21.73±0.56
Week 6	34.28±0.08	30.20±0.35	20.95±0.64	25.60±1.07	22.77±0.79	23.72±0.52
Week 7	35.80±0.22	31.87±0.50	23.43±0.73	27.13±0.85	26.13±0.93	25.72±0.75
Week 8	38.65±0.27	34.33±0.61	25.45±0.53	30.18±0.70	27.47±0.93	27.42±0.92
Week 9	41.22±0.17	36.72±0.43	28.17±o0.64	31.90±0.82	28.75±0.84	29.80±0.96
Week 10	42.78±0.38	38.60±0.45	29.72±0.83	33.33±0.86	30.67±0.84	31.17±0.92
Week 11	43.02±0.52	39.08±0.74	30.37±0.95	33.62±0.93	30.95±0.91	31.87±0.63

However, significant reduction occurred from the 9th week to the end of the study duration with 20.0 ml having the highest negative effect on the plant height followed by 50.0 ml, whereas the highest plant height was recorded in the control followed by the 10.0 ml. There were no significant difference (p<0.05) between 30.0 ml and 40.0 ml as well as between 40.0 ml and 50.0 ml (Table 2).

Number and weight of seeds harvested as influenced by crude oil

Results show that a significant difference (p<0.05) exists between the various treatments compared with the control, where the 50 ml treatment had the lowest number of harvested seeds as shown in the (Figure 4). The 50.0 ml concentration also recorded the least number of harvested seeds. However, there is no significant difference between the 20.0 ml and 40.0 ml treatments at (p<0.05) (Figure 5). The result of weight of harvested seeds as influenced by the different levels of crude oil treatments show that there was significant difference (p<0.05) between the various treatments and the control (Figure 5).

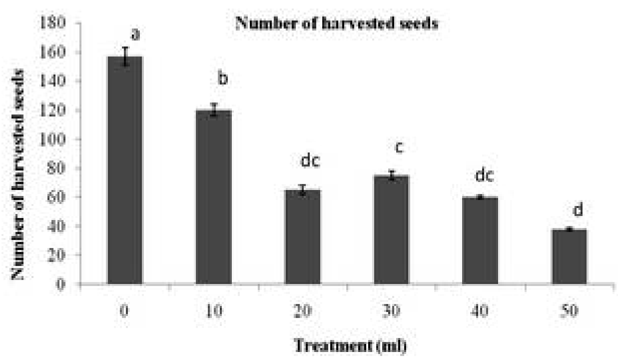

Figure 4. Effect of crude oil-treated soil on the number of harvested seeds of cowpea. Means with the same superscript are not significantly different (p<0.05), Each value is a mean of three readings ± standard error.

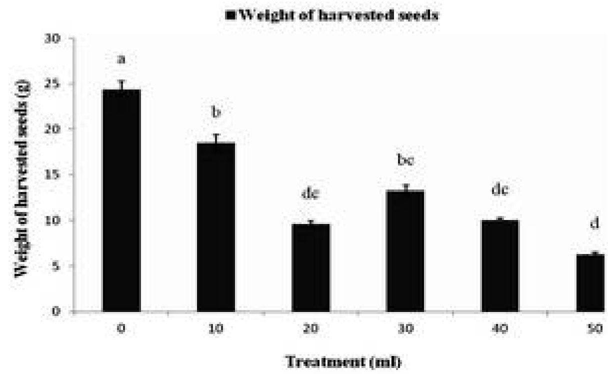

Figure 5. Effect of crude oil-treated soil on the weight of harvested seeds of cowpea Means with the same superscript are not significantly different (p<0.05). Each value is a mean of three readings ± standard error.

Percentage composition of nutrients in the cowpea seeds

The percentage N and protein stored in the cowpea seeds are found to be significantly different (p<0.05) between the various treatments and the control, where the lowest amount of nitrogen and protein contents stored in the seed was lowest in the 50.0 ml concentration. However, there were no significant differences (p<0.05) in the percentage moisture content % M.C, % Ash, % Fat, % Crude fiber and Carbohydrate (% CHO) between the treatments compared with the control, the highest percentage of moisture content % M.C and % Crude fiber were recorded in the 20.0 ml concentration (Table 3). The lowest % M.C, % Ash, % Fat and the highest % crude fiber and % were observed in 30.0 ml concentration treatment, while 20.0 ml had the lowest % CHO (Table 3). It was also observed that the highest amount of stored nitrogen, protein, moisture, ash and CHO were observed in the lowest treatment levels (10.0 ml and 20.0 ml) (Table 3).

Table 3. Effect of crude oil-treated soil on percentage nutrient composition of cowpea seeds over the study-period. Each value is a mean of mean three readings ± standard error.

Treatment	% N	% Protein	% M.C	% Ash	% Fat	% Crude fiber	% CHO
Pre-seed sample	$4.9^{a} \pm 0.1$	$30.5^{a} \pm 0.6$	$7.9^{a} \pm 0.3$	$9.1^{b} \pm 0.4$	$1.9^{b} \pm 0.2$	$3.6^{a} \pm 0.4$	$47.4^{a} \pm 1.1$
0 ml	$4.9^{a} \pm 0.2$	$30.6^{a} \pm 1.1$	$8.0^{a} \pm 0.5$	$10.4^{c} \pm 0.5$	$1.5^{c} \pm 0.1$	$3.7^{a} \pm 0.3$	$46.0^{a} \pm 1.3$
10 ml	$4.9^{a} \pm 0.2$	$30.1^{b} \pm 1.4$	$7.3^{a} \pm 0.4$	$9.9^{a} \pm 0.2$	$1.5^{a} \pm 0.1$	$4.3^{a} \pm 0.2$	$45.0^{a} \pm 2.8$
20 ml	$5.7^{b} \pm 0.2$	$35.2^{b} \pm 0.5$	$8.4^{b} \pm 0.5$	$9.6^{a} \pm 0.5$	$1.5^{a} \pm 0.3$	$4.7^{a} \pm 0.4$	$40.1^{b} \pm 1.1$
30 ml	$5.0^{a} \pm 0.1$	$31.2^{a} \pm 0.4$	$7.1^{c} \pm 0.2$	$8.5^{c} \pm 0.6$	$1.0^{c} \pm 0.1$	$4.7^{a} \pm 0.8$	$46.8^{a} \pm 0.3$
40 ml	$4.9^{a} \pm 0.1$	$30.2^{a} \pm 0.8$	$8.0^{a} \pm 0.2$	$9.3^{b} \pm 0.2$	$1.2^{c} \pm 0.1$	$4.6^{a} \pm 1.1$	$45.4^{a} \pm 1.5$
50 ml	$4.7^{a} \pm 0.4$	29.6 $^{a} \pm 0.9$	$7.4^{a} \pm 0.6$	$9.5^{a} \pm 0.3$	$1.7^{b} \pm 0.1$	$4.5^{a} \pm 0.8$	$45.6^{a} \pm 1.4$
p-value	**0.017**	**0.010**	**0.217**	**0.071**	**0.079**	**0.685**	**0.097**

Means in the same column having the same letter(s) are not significantly different (p< 0.05). Each value is a mean of three readings ± standard error. N = Nitrogen, M.C = Moisture Content and CHO = Carbohydrate.

Discussion

Effect of crude oil treated-soil on the germination of cowpea

The pattern of higher percentage emergence of cowpea at one week after planting (WAP) in the lower concentration treatments indicated that crude oil had negative effect on the germination of the cowpea seeds. The negative effect observed on the cowpea seed may possibly be as a result of blockage of the cotyledon by the crude oil thereby inhibiting its growth. Also, the interaction of the crude oil with the soil may result into making the soil so compact that air may not freely diffuse through the soil. This finding is in line with that of [20-22] who all have reported low germination rate caused by crude oil contaminants in the soil and blockage of soil air pores by crude oil with their different test crop. Clarkson and Hanson [20] attributed the low emergence rate to insufficient aeration which results from the decrease in air-filled space and an increase in demand for oxygen by oil decomposing microorganism. Adams and Duncan [23] have also observed that the low germination rate of seeds in crude oil polluted soil could be due to the physical barrier caused by the crude oil enveloping the seed, preventing oxygen and moisture availability to the seeds.

Effect of crude oil treated-soil on the growth performance

The highest growth performance, leaf length, leaf breadth, and number of leaves of cowpea seeds recorded in the control compared to other treatments in this experiment might be as a result of the activity of the crude oil in the soil which may likely hinder the uptake of nutrients by the plants in the crude oil treated soils. This finding is consistent with the results of [24], who observed a significant reduction in the leaf length and number of leaves for all levels of crude oil treatment relative to the control. Ogbuchi *et al* [25], Ekpo *et al* [26] reported a significant reduction in the plant growth (number of leaves) of soybeans cultivated on crude oil. Akpan *et al* [22] has pointed out that plant nutrition is not only based on the presence of mineral elements in the soil but their availability for uptake by the plant. It has also been postulated that the presence of hydrocarbons in the soil can change the fertility of the soil, thereby leading to a nutritional imbalance which may ultimately cause lower growth and biomass production [27]. Adenipekun *et al* [28] who worked on *C. olitorius* in soil contaminated with oil also reported a lower number of leaves and reduced leaf area compared to those in the uncontaminated-soil.

The results of a sharp increase in the leaf length, breadth, and number of leaves across the treatments from 1WAP to 9WAP could be due to the tolerance and adaptive ability of cowpea to extreme conditions and its ability to fix atmospheric nitrogen through the interaction with nitrogen fixing bacteria in its root nodules. This finding is supported by [29] who suggested that, plants may tolerate sites polluted with petroleum by creating a soil environment rich in microbial activity that can change the availability of organic contaminants or enhance their degradation.

However, the reduction in leaf length, leaf breadth and leaf area observed across all the treatment levels including the control from 9WAP to 11WAP for number of leaves could be as a result of flowering where nutrients stored in the leaves are been translocated to the buds, flowers and subsequent fruiting. [30] has also pointed out that aging tissues (especially senescing leaves) being triggered by ethylene is associated with fruit ripening, flower wilting and leaf fall.

The lack of significant difference in the plant height, recorded between the various treatments relative to the control throughout this study period could be attributed to the fact that as the plant continued to grow on the crude oil treated soil, the activeness or toxicity level of the crude oil in the soil continue to decrease while the tolerance and adaptive ability of plants in the treated soils increases, thus enhancing the height of the plants. Also, cowpea being a leguminous plant is able to fix atmospheric nitrogen through the interaction with nitrogen fixing bacteria in its root nodules as a substantial number of nodules were found in the root region after harvest. [31] has reported that activities of nitrogen fixing microorganisms may enhance the soil condition and thereby improving productivity.

Effect of crude oil-treated soil on the nutritive content and weight of harvested seeds

The higher amount of stored nitrogen, protein, moisture, ash and carbohydrate in the cowpea seeds after harvest at lower treatment levels (10.0 ml and 20.0 ml) could be due to the ability of cowpea to tolerate or resist the effect of the crude oil at minimal level. This result is in agreement with the reports of [31], who opined that plants may tolerate sites polluted with petroleum by creating a soil environment rich in microbial activity that can change the availability of organic contaminants or enhance their degradation.

However, reduced stored nutrients in the harvested seeds of cowpea at higher treatment levels could be attributed to the fact that crude oil has inhibitory effect on nutrient mobilization or uptake by the plants and as the concentration increases tolerance ability reduces. The reduced number and weight of harvested seeds recorded in this study across the treatments could also be attributed to the reduced availability or absorption of nutrients and water from the soil due to the inhibitory conditions caused by crude oil in the treated soils. This finding is in line with the observation of [32], who suggested that oil level seem to exert the greatest influence on plant growth and yield.

Conclusion

The study concludes that cowpea can adapt minimally to crude oil polluted sites but as crude oil concentration increases, the tolerance ability reduces. Although, the plant reached flowering stage and fruiting even at high concentration but the harvested weight of cowpea was adversely affected. Furthermore, the nutrient content in the harvested seeds in relation to the control was also high in that cowpea was able to produce more protein content at lower treatment levels than in the control as observed in the nutrient composition table. However, it is necessary to note that cowpea can take up heavy metal elements such as Fe, Zn, Cu, Pb and Ni from crude oil polluted soils leading to heavy metal accumulation in the seeds of the plant thereby posing threat to lives of humans and other livestock that feed on it.

Finally, it is strongly advised that cowpea should not be consumed if planted in crude oil polluted soil because the excessive uptake of metals by the plant may produce toxicity in human nutrition.

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