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Full Length Research.

Effects of crude petroleum oil on the germination, growth and yield of *Vigna unguiculata* Walp L.

Otitoloju KEKERE, *Beckley IKHAJIAGBE and Bunmi R. APELA
Dept. of Plant Sci & Biotech, Adekunle Ajasin University, Akungba-Akoko
*Dept. of Plant Biol. & Biotech, University of Benin, Benin City

*Corresponding author: ikhaj@yahoo.com

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The present study was carried out to determine the effects of crude petroleum oil pollution on the germination, growth and yield of *Vigna unguiculata*. Top soil (0-10cm) of known physiochemical parameters was collected randomly from a marked area measuring 50 x 50m on a farmland situated beside Faculty of Social and Management Sciences, Adekunle Ajasin University Akungba-Akoko, Ondo State, and sun-dried to constant weight. Thereafter, 5kg of the soil was each placed in experimental pots with 8 perforations made with 2 mm diameter nails at the bottom of each pot. Crude oil was added to soil in the pots and mixed thoroughly to obtain 4 different concentrations on weight basis: 2, 4, 8 and 16% w/w oil in soil. The unpolluted soil (0%) served as the control. Cowpea seeds were sown one week after exposure of soil to oil contamination. Germination percentage and germination rate were negatively affected by oil pollution. Oil pollution was also found to negatively affect leaf number, total leaf area, plant height, stem girth, leaf development. Significant decreases in total plant biomass as well as crop yield were observed. Crude oil pollution reduced the leaf chlorophyll content and nutritional composition of *Vigna unguiculata* fruit and caused an elevated level of heavy metal uptake in fruits.

Keywords: Petroleum, Pollution, Phytoassessment, *Vigna unguiculata.*

Introduction

In Nigeria, an increase in the demand for petroleum as a source of energy and national income brings increase in its exploration and exploitation. Adegeye *et al*. (1993) reported that oil is the life wire of the Nigerian economy. It is produced in the southern states, which are located in the Niger Delta including Edo, Rivers, Imo, Bayelsa, Akwa-Ibom, Cross-river and Ondo states. Petroleum supplies 43% of the energy consumed in the United States and have also been confirmed by (Nwilo, 1998) to account for over 90% of Nigeria's national income. Increasingly large amount of petroleum is entering the environment from damaged oil tankers, storage vehicles, leakages of oil pipelines, oil tankers overflow, rupture of loading hoses and flange connections (NAS, 1975; Ossai *et al.,* 1990).

Baek *et al.* (2005), reported that crude oil in soil makes it unsatisfactory for plant growth. This is due to insufficient aeration of the soil because of displacement of air from spaces between the soil particles by crude oil. Rowell (1997) reported that soil pollution with oil can result to severe physical and chemical changes, Russel (1960) reported that soil aggregates were broken down and the soil frequently appeared to be laminated. Water could not penetrate such soil from the surface but could enter easily from the side. Odu (1981) concluded that oil penetration of soil was limited to the top soil even in sandy soil. He observed that symptoms of oil pollution in soil were typical of extreme nutrient deficiency in plants. Lack of seed germination in oil polluted soil has been severely associated with induced loss of vitality or with unfavourable soil condition, probably resulting from insufficient aeration due to a decrease in air- filled pore space (De Jong, 1980). Udo and Fayemi (1975) discovered that plants growing in an oil- polluted environment are generally retarded with chlorosis of leaves, coupled with dehydration of the plant. Jernelov and Linden (1981) reported that mangrove swamps in Ecuador and Columbia were severely contaminated by oil and this caused defoliation of mangrove trees and epiphytic flora. Generally, the effects of oil spillage on vegetation result in the reduction of plant growth, and cowpea is one of such crops. However, some authors have shown the capability of cowpea to accumulate petroleum hydrocarbons (Ikhajiagbe and Anoliefo, 2010). Cowpea seed is a nutritious component in human diet as well as livestock feed. The fruit can be consumed when young and when matured. Asumugha (2002) reported that, cowpea are consumed in various ways in form of "Akara and Moinmoin", which are very popular breakfast and snacks. Cowpea are drought tolerant which can grow on poor soil, so they are good for use as green manure to enrich the soil with nitrogen and can also be used in crop rotation with cereals. It also provides high protein pastorage for pigs and cattle as well as hay and silage (usually mixed with corn or sorghum). The objective of this research is to examine germination and growth responses of *Vigna unguiculata* to crude petroleum oil pollution as well as the possibility for the use of *V. unguiculata* in phytoremediation.

MATERIALS AND METHODS

The plant material used was seeds of *Vigna unguiculata*, obtained from Federal Ministry of Agriculture Iwaro, Oka-Akoko, Ondo State. Crude oil (Bonny Light) was obtained from Delta State of Nigeria.

Top soil (0-10cm) was collected randomly from a marked area measuring 50 x 50m on a farmland situated beside Faculty of Social and Management Sciences, Adekunle Ajasin University Akungba-Akoko, Ondo State. The physiochemical parameters of the soil include, 5.60 pH, 6.190 clay, 4.290 silt, 89.7% S and 2.89% C, 0.14% N, 9.02Mg/kg P, 6.24Mg/100g Ca, 1.84Mg/100 Mg, 0.34Mg/100g Na, 0.23Mg/100 K, 0.20Mg/100 H and 8.86Mg/100 CEC.

Thereafter, 5kg sun-dried soil was each placed into large perforated pots with 8 perforations made with 2 mm diameter nails at the bottom of each pot. Crude oil was added to soil in the pots and mixed thoroughly to obtain 4 different concentrations on weight basis: 2, 4, 8 and 16% w/w oil in soil. The unpolluted soil (0%) served as the control.

After one week of exposure of soil, 10 seeds were sown at a depth of 3cm in each pot containing topsoil. The plants were adequately watered and weeded. There were 8 replicates per treatment and the plants were arranged in a completely randomized experimental design.

Parameters under investigation

Percentage seedling emergence was calculated as the percentage of seeds that sprouted above soil level of the 10 seeds originally sown per bucket. Morphological changes in cowpea plant were observed and recorded. Other measurable growth parameters including leaf number, height, total leaf area, stem girth, fresh and dry weight of plant parts, and biomass were evaluated. Plant yield was also determined as pod length, seeds/pod, fresh and dry weight of fruit. Nutrition composition of dry cowpea seeds were determined by using the methods of SSSA (1971) and IITA (1979).

Statistical analyses/ Data analyses

Data were subjected to one-way ANOVA, while treatment means were separated using Least Significant Difference test at $p<0.05$ level of significance.

RESULTS

The figure 1 below shows that increase in oil intensity resulted in decrease in seedling emergence when compared with the control level, control treatment has the highest level while 8% has the lowest level, and hence, there was no germination in the 16% treatment.

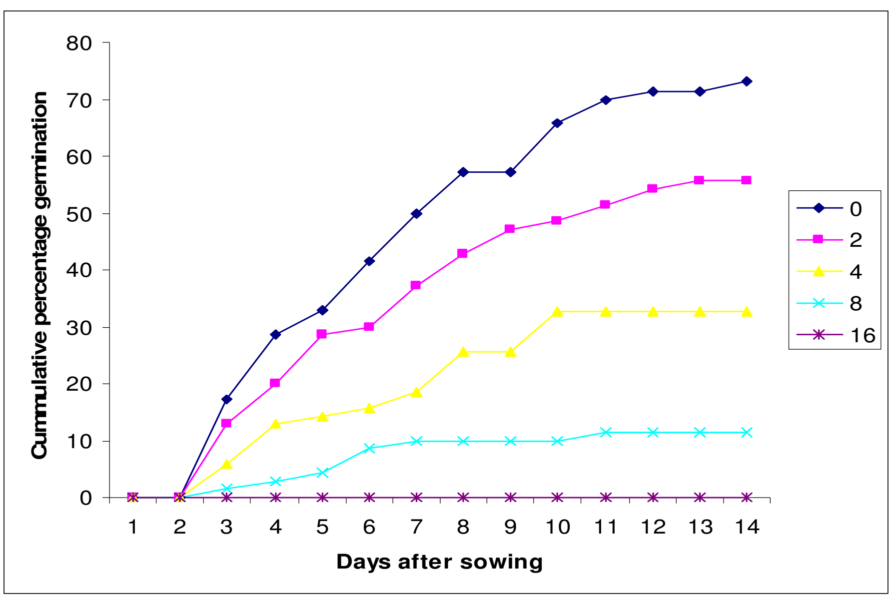

Fig. 1. Effects of crude oil pollution on the cumulative percentage germination of *V. unguiculata*.

Table 1 shows the effects of crude oil pollution on the morphological responses of *Vigna unguiculata*. Morphological changes were observed in *Vigna unguiculata* polluted with crude oil throughout the duration of the experiment. In the 2% oil-in-soil treatment, leaves were initially wettable after oil application, basal stem were slimy and brownish in colour, leaves became yellow in colour with defoliation of leaves. Plants dried up gradually from shoot to the apex and later looked burnt. In the 16% concentration however, leaves also became chlorotic and slimy after 4days of crude oil application, withered with defoliation. Basal stem became brown in colour and slimy. Plant shoots remained wetable for more than one week (i.e *Vigna unguiculata* shoots absorbed crude oil to the middle of the plant), leaves turned yellow, withered with defoliation of leaves. The dropping and eventually collapse of leaves stretched over a period of 1 - 2weeks after pollution, decreasing accordingly with increase in intensity of crude oil. At the end of the experiment, plant mortality occurred at all levels of crude oil pollution of *Vigna unguiculata* except in 2% and control treatments. Plants polluted with crude oil were greatly affected when compared with the control.

Table1: Morphological changes observed in *Vigna unguiculata* polluted with crude oil.

Intensity of crude oil(w/w)	Observed effects
0% (control treatment)	Luxuriant growth
2% treatment	Leaves initially wettable after oil application, basal stem were slimy and brownish in colour, leaves became yellow in colour with defoliation of leaves. Plants dried up gradually from shoot to the apex and later looked burnt.
4% treatment	Leaves remained unchanged for 2weeks after pollution, leaves became yellowish, withered with defoliation of leaves, slimy basal stem segment turned brownish and dead, 75% mortality was recorded, they became dead gradually from shoot to apex of the plant, and later looked burnt.
8%treatment.	Leaves became yellow after a week of pollution, withered with defoliation, slimy basal stem segment turned brownish. All the plants dead from shoot to apex and later looked burnt.
16% treatment.	Leaves became yellow and slimy after 4days of crude oil application, withered with defoliation. Basal stem became brown in colour and slimy.

Significant decreases in number of leaves of per plant were recorded (Table 2). The greater the intensity of pollution the greater the effect on plant. At 4 weeks, there were 10.88 leaves in the 2% treatment compared to 40 leaves in the control experiment. No leaves were obtained inn 4 – 16% oil-in-soil treatments at 4 weeks. Crude oil pollution at all intensities resulted in a significant reduction in leaf number when compared with the control treatment. Reduction in leaf number increased with increase in crude oil intensity.

Table 2: Effect of crude oil on the leaf number of *Vigna unguiculata.* Leaf number was obtained for only those leaves borne on the plant at that time.

(WAA)	Crude oil intensity (%w/w)				
Weeks	**0**	**2**	**4**	**8**	**16**
1	9.75^a	7.00^a	5.62^b	3.13^c	2.25^c
2	18.25^a	10.63^b	4.25^c	4.00^c	2.90^c
3	30.13^a	17.25^b	0.88^c	0.00^c	0.00^c
4	40.00a	10.88^b	0.00^c	0.00^c	0.00^c

Means on the same row with similar alphabetic superscripts do not differ from each other (p>0.05)
WAA – Weeks after application of crude oil

Plant height was significantly reduced (P<0.05) in *Vigna unguiculata* (Table 3). Plant height at 4 WAA was 23.88 cm in the 2%w/w treatment as against 48 cm in the control. Plants in the 4, 8, and 16 % oil-in-soil concentrations died from the 3rd week.

Table 3: Effect of crude oil the height (cm) of *Vigna unguiculata*. Plant height was recorded for only standing plants.

(WAA)	Crude oil intensity (%w/w)				
Weeks	0	2	4	8	16
1	13.75^{a}	10.00^{ab}	8.62^{b}	6.13^{b}	6.25^{b}
2	25.25^{a}	13.63^{b}	4.25^{c}	4.00^{c}	2.90^{c}
3	32.13^{a}	17.25^{b}	0.88^{c}	0.00^{c}	0.00^{c}
4	48.00^{a}	23.88^{b}	0.00^{c}	0.00^{c}	0.00^{c}

Means on the same row with similar alphabetic superscripts do not differ from each other (p>0.05)
WAA – Weeks after application of crude oil

Crude oil pollution at all intensities resulted in a significant reduction in leaf area when compared with control treatment (Table 4). Although plants in 4 – 16% soil concentrations did not survive up to the 4th week, total leaf area was 22.66 cm^2 in the 2% treatment compared to 32.88 cm^2 in the control.

Table 4: Effect of crude oil on the total leaf area (cm^2) of *Vigna unguiculata*.

WAA	Crude oil intensity (%w/w).				
Weeks	0	2	4	8	16
1	27.84^{a}	20.46^{ab}	20.45^{ab}	18.02^{b}	16.04^{b}
2	30.40^{a}	19.89^{b}	18.72^{b}	12.34^{bc}	6.82^{c}
3	30.68^{a}	22.45^{b}	11.69^{c}	0.00^{d}	0.00^{d}
4	32.88^{a}	22.66^{b}	0.00^{c}	0.00^{c}	0.00^{c}

Means on the same row with similar alphabetic superscripts do not differ from each other (p>0.05)
WAA – Weeks after application of crude oil

Stem girth increased from 0.80 cm at 1 WAA to 1.31 cm in the control (Table 8). Stem girth in the 2 %w/w treatment ranged from 0.61 at 1 WAA to 0.68 cm at 4 WAA. Crude oil pollution at all concentrations resulted in a significant reduction in stem girth of *Vigna unguiculata* when compared with the control treatment (Table 5). At the end of the experiment the highest reduction was recorded at 16% of crude oil pollution.

Table 5: Effect of crude oil on the stem girth (cm) of *Vigna unguiculata*. Stem girth was measured for both existing and dead plants.

Values are means ± standard error for 8 replicates.

WAA	Crude oil intensity(%w/w)				
Weeks	0	2	4	8	16
1	0.80^{a}	0.61^{b}	0.56^{b}	0.50^{c}	0.50^{b}
2	0.75^{a}	0.65^{a}	0.54^{ab}	0.44^{b}	0.40^{b}
3	0.90^{d}	0.72^{b}	0.51^{b}	0.38^{c}	0.38^{c}
4	1.31^{d}	0.80^{b}	0.41^{c}	0.34^{c}	0.31^{c}

Means on the same row with similar alphabetic superscripts do not differ from each other (p>0.05)
WAA – Weeks after application of crude oil

The concentrations of elements in the seeds of cowpea sown in polluted soils were significantly lower in unpolluted plants (Table 6). There was general decrease in elemtal composition of the cowpea seeds.. heavy metals present included Fe, Pb and Zn.

Table 6: Effect of crude oil on elemental analysis of fruit of *Vigna unguiculata*.

Crude oil intensity(%w/w)	Elemental concentration (ppm)								
	N	P	K	Ca	Mg	Na	Fe	Zn	Pb
0	1.57	0.10	1.38	0.71	0.18	0.03	3.42	12.55	0.16
2	1.25	0.08	0.74	0.58	0.09	0.03	3.76	16.74	0.37
4	1.02	0.06	0.61	0.52	0.05	0.02	2.91	10.98	0.11

At the end of the study, there was gradual decrease in biomass of *Vigna unguiculata* subjected to crude oil at all levels of pollution (Fig. 2). The highest reduction was recorded at 16%, while control carried the highest level. Hence there was significant difference between the control and the other treatments.

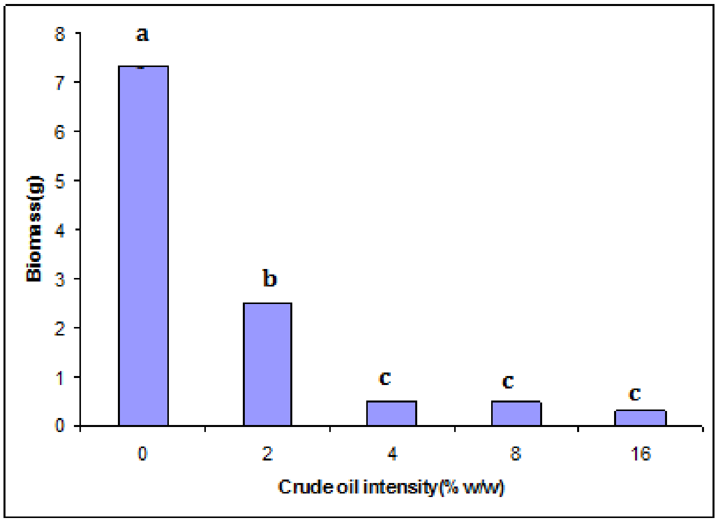

Fig. 2. Effect of crude oil pollution on the total biomass of *V. unguiculata.*
Bars with similar alphabets do not differ significantly (p>0.05)

There was significant decrease in yield parameters of cowpea with increasing concentrations of oil in soil (Table 7). There was no production in 8 – 16 % w/w oil-in-soil concentrations because the plants were entirely dead. Although plants in 4% w/w treatment were more than 80% dead, a few surviving replicates produced very abnormally 5.25 cm long pods with 3 seeds in each pod. Only 3.07 pods were produced per plant. However, pod length was 14.63 cm in the control experiment. Number of seeds/pod was 39.50 in the control and 16.38 in the 2% w/w treatment.

Table 7: Effect of crude oil on the fruit of *V. unguiculata*.

Fruits of *V.unguiculata*	**Crude oil intensity (%w/w)**				
	0	**2**	**4**	**8**	**16**
Pod length (cm)	14.63^{a}	10.44^{a}	5.25^{bc}	0.00^{c}	0.00^{c}
Seeds/pod	39.50^{a}	16.38^{b}	3.00^{c}	0.00^{d}	0.00^{d}
Fruit fresh wt. (g)	15.34^{a}	2.48^{bc}	0.42^{bc}	0.00^{c}	0.00^{c}
Pods/plant	13.11^{a}	7.23^{b}	3.76^{bc}	0.00^{c}	0.00^{c}
Fruit dry wt. (g)	1.42^{a}	1.68^{a}	0.30^{b}	0.00^{b}	0.00^{b}

Means on the same row with similar alphabetic superscripts do not differ from each other (p>0.05)
WAA – Weeks after application of crude oil

DISCUSSION

Many authors have studied the effects of oil pollution on seed germination of crop plants. Oil-contaminated soil generally causes delayed seed emergence and that of WEO-contaminated soil is not different (Anoliefo and Vwioko, 1995; Odjegba and Sadiq, 2002; Akinola *et al.*, 2004; Merkl *et al.*, 2004; Agbogidi *et al.,* 2007). Terge (1984) reported that germination of seeds in oil-polluted soil varied with different plant species. Germination and seedling emergence of *Solanum melongena* and *S. incarnum* were inhibited by WEO. Germination of *Ricinus communis* in WEO-pollutedsoil was also inhibited (Vwioko and Fashemi, 2005). Udo and Fayemi (1975) reported that maize germination was adversely affected by the pollution of the soil, the effect being proportional to the level of crude oil pollution. Similar effect was observed in the present study. Germination was suppressed at higher levels of soil pollution. This can be attributed to disruption of water and nutrient uptake owing to the effects of oil in soil, and the depletion of soil nitrogen and phosphorus content (Baran *et al.,* 2002). The embryo of the seed could be killed or injured if it comes in contact with crude oil. Penetration of crude oil through seed micropylar end, scar, crack or injury would certainly endanger the life and growth of embryo, which are vital to germination. Obviously, the integrity and hardness of the seed coat affect the rate of penetration. Inhibition of plant growth can be caused by toxic compounds of petroleum hydrocarbon (Bossert and Bartha, 1985). Small molecules like aromatics that can enter and get across cell membranes could cause a reduction in membrane integrity and/or death of the cell. Therefore, the high content of aromatics in the oil might explain the considerate growth inhibition and subsequent death of seedlings.

Oil contamination is known to alter the physical, chemical and biological properties of soil. For instance, Merkl *et al*. (2005) reported that oil contamination alters soil moisture condition and can lead to non-homogenous distribution of water in soil due to the hydrophobic nature of oil. In addition, Terge (1984) suggested that oil contamination leads to poor wetness and aeration of soil. This leads to water and air deficiency in such soil and low availability of water to plants. Oil contamination also reduces the soil fertility by causing immobilization of nutrients by microbes (Agbogidi *et al*., 2007). Such immobilization of nutrients leads to difficulty in the uptake of nutrients in oil contaminated soil. Pollution of soil with crude oil affects plant growth at different rates. It also shows that crude oil are phytotoxic, their toxicity is not same. This is similar to the findings of Nwankwo and Ifeadi (1983) who reported differential effects of petrol and diesel oil on the content of organic carbon and mineral components of soil. Adedokun and Ataga (2007) reported differential effect of crude oil, automotive gasoline oil and spent engine oil on germination and growth of cowpea.

Morphological changes were observed in *Vigna unguiculata* polluted with crude oil throughout the duration of the experiment. Plant shoots remained wetable for more than one week (i.e *Vigna unguiculata* shoots absorbed crude oil to the middle of the plant), leaves turned yellow, withered with defoliation of leaves. The dropping and eventually collapse of leaves stretched over a period of 1 - 2weeks after pollution, decreasing accordingly with increase in intensity of crude oil. Polluted stems became brownish and slimy, showing the physical absorption of crude oil throughout the stem. Then defoliation occurred, this continued over a period of time until plant gradually dried up from shoot to the apex, and later died completely and looked burnt. At the end of the experiment, plant mortality occurred at all levels of crude oil pollution of *Vigna unguiculata* except in 2% and control treatments. Leaf chlorosis, as observed in the present study may be due phytotoxicity imposed by the presence of certain heavy metals like Pb and Zn (Miles *et al., 1*972; Ikhajiagbe and Anoliefo, 2010, 2011).

Crude oil pollution at all intensities resulted in a significant reduction in leaf number when compared with the control treatment. Reduction in leaf number followed increase in crude oil intensity. A decrease in leaf number can be attributed to a host of factors including blockage of conducting tissues thereby preventing water and nutrients into the leaf. Ikphemhosimeh (1996) showed that there was a decrease in number and size of xylem vessels in *Colocasia esculenta* owing to oil pollution. This limits the uptake of water by plant. Okoloko and Berley (1982) also reported that oil pollution impairs membrane integrity, enzymes system especially membrane bound enzymes and affects the metabolic system of the plant. Decrease in leaf number can also be attributed to shedding of leaves as observed in this study.

Plant height was significantly reduced ($P<0.05$) in *Vigna unguiculata* subjected to crude oil pollution. This result agrees with previous findings of Amad *et al*. (1996) who reported that immediately after there is an oil spill, there is usually a horizontal migrating of oil into soil horizons. Oily scum on soil surface would impede oxygen and water. It may also cause some toxic elements to be more available to plants thereby causing reduction in plant growth. Therefore the general depression in growth is due to the adverse effect of crude oil. Ikhajiagbe and Anoliefo (2010; 2011) also reported significantly reduced plant growth occasioned by oil pollution.

A reduction in leaf area was recorded in the present study. This decrease in leaf area of *Vigna unguiculata* with increasing intensity of crude oil can be attributed to conductance (Smith *et. al*. 1989).

Significant reduction in stem girth of *Vigna unguiculata* was recorded with increased concentration of oil in soil, compared with the control treatment. This agrees with the previous findings of Gill and Sandata (1976) on the foliar application of oil on *Phaseolus aureus.* Udo and Fayemi (1975) also reported in their studies that germination in *Zea mays* sown in soil pre-soaked in water soluble fraction of crude oil was reduce even when they were sown in unpolluted soil. Atuanya (1987) reported that soil contaminated with petroleum hydrocarbon products developed shrinking physical characteristics.

Significant decreases in biomass of *Vigna unguiculata* subjected to crude oil at all levels of pollution were observed in the present study. Decrease in biomass is attributed a corresponding reduction in leaf area, leaf number and plant height of cowpea. Leaf is a site of photosynthetic activities. The reduction in leaf area brings about a reduction in surface area available for photosynthesis. Photosynthesis can also be reduced by reduction in leaf number which decrease the number of leaves available for photosynthesis and stomata density reduction which has been reported to be caused by oil pollution (Gill and Sandata,1976) This reduction in photosynthesis as a result of crude oil application was responsible for the reductions in the biomass observe in this study.

Adverse effects of crude oil pollution on the yield parameters of cowpea have been presented in Table 7. Due to plant mortality in 8% and 16%w/w treatments, there was no crop yield recorded in these treatments. There were significantly lower yields in higher levels of polluted soils compared to those in the control. Okonokhua *et al*. (2007) and Anoliefo *et al*. (2010) recorded similar yield decreases in maize and cowpea sown in oil polluted soils respectively.

Crude oil reduced seed quality and caused an elevated level of heavy metals in seeds which can cause health hazard when consumed by man and livestock. Iron, lead and Zinc found in the seeds of cowpea is an indication that they had been accumulated from the soil owing to oil pollution (Ikhajiagbe and Anoliefo, 2011). Iron is the key metal required for energy transformations needed for cellular functioning, but when in excess amounts (Foy *et al., 1*978). In the plant, lead may exist in naturally chelated form, or in pyro- or orthophosphate forms. The phytotoxicity of lead is relatively low compared with other trace elements. It affects mitochondrial respiration and photosynthesis by disturbing electron transfer reactions (Miles *et al., 1*972). Significant reduction in plant growth and yield owing to lead toxicity has been reported by Hassett *et al*. (1976) in corn, Khan and Frankland (1983) in radish, and Wierzbicka and Antosiewicz (1993) in mung bean. Similarly, Zinc plays an important role in disease protection and metabolism of carbohydrates and proteins. It is actively taken up by roots in ionic form and, to a lesser extent, in organically chelated form (Collins, 1981). However, as a toxicant, it acts to inhibit CO fixation, phloem transport of carbohydrates, and alter membrane permeability (Collins, 1981). Symptoms of Zn toxicity include chlorosis and depressed plant growth. These were observed in cowpea plants sown in higher concentrations of oil.

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