

Growth Responses of Two Cultivated Okra Species (*Abelmoschus caillei* (A. Chev) Stevels and *Abelmoschus esculentus* (Linn.) Moench) in Crude Oil Contaminated Soil

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ABSTRACT: The morphological distinctiveness of two cultivated Okra species- *Abelmoschus caillei* and *Abelmoschus esculentus* was investigated using six accessions; three for each species in crude oil contaminated soil. The seeds were collected from home gardens in Benin City and NIHORT. Morpho-agronomic characters such as numbers of days from sowing to germination, plant height, stem base diameter, stem color and pubescence, leaf shape and color, number of leaves produced, growth habit, branching, fruit and fruiting characters were determined. The growth response of the different accessions varied significantly ($p < 0.05$). Soil chemical analysis revealed decreased levels of pH, Phosphorus and Potassium in the contaminated soil. Generally, all the quantitative characters including number of flower buds and flowers produced, fruit length, height of plant and stem girth were reduced in plants (Okra) grown in the contaminated soil while most of the qualitative characters such as pigmentation and shape of plant organs were less affected. Thus, it can be suggested from the study that crude oil contamination of soil may lead to reduction in growth characteristics.

Keywords: Crude oil, Soil, Morpho-agronomic characters, *Abelmoschus caillei*, *A. esculentus*.

INTRODUCTION

Understanding the importance of soil is essential for agriculture (Taylor *et al.*, 2007) as soil is one of the components of the biosphere that houses most plants and microbes. Humans are currently exacting pressure on this soil through mineral exploration, urbanization, desertification and deforestation (Anoliefo *et al.*, 2005). The environmental impact accruing from mineral exploration especially oil exploration and exploitation has been reported by Amadi *et al.* (1993). Anoliefo and Gill (1994), Anoliefo and Okoloko (2002), Anoliefo *et al.* (2003) and Vwioko *et al.* (2008a and b) described it as an inevitable consequence of economic development. Products and by products from these activities are deleterious to life especially plant life when nutrient elements for growth development are affected. (Anoliefo, 2003). The adverse effects of oil pollution on economic plants have been reported by Odu (1981), Isirumah *et al.* (1989) Amadi *et al.* (2003), Anoliefo and Okoloko (2003), Anoliefo *et al.* (2003). At high concentration of crude oil in soils, most plant species suffer depression in growth (Udo and Fayemi, 1975; Amakiri and Onofeghara, 1984). This has been attributed to poor soil conditions, dehydration and impaired nutrient uptake by the roots created by the presence of crude oil. Plants exposed to light oil pollution suffer from leaf yellowing, and leaf-drop whereas, complete shedding of entire leaves may follow heavy contamination. Stress responses in plants

may be attributed to genetically expressed variations (Vwioko *et al.*, 2008a)

The genus *Abelmoschus* belongs to the Mallow family (Malvaceae) and order Malvales where members are characterized by bark fibres and mucilage. The family is well represented in tropical regions by several cultivated species of economic importance like cotton (*Gossypium* sp.) and Jute (*Corchorus* sp.). The studies by Martins *et al.* (1981), Siemonsma (1982), Ariyo (1993) and Kehinde (1999) distinguished two major cultivated Okra in Africa – *A. esculentus* and *A. caillei*. Both species are widely cultivated vegetable in Nigeria as well as in tropical and subtropical regions where they are grown for their leaves, fruits, seeds, floral parts and stems. These parts are edible when young, succulent and dried in powdery form. They are cultivated in home gardens and distant farms in the oil rich Niger Delta region of Nigeria. They play important roles in food and income security. Both species are widely distributed between 12°N and 12°S and most commonly found between 5 °N and 12 °N.

The objective of this study is to determine growth responses of these *Abelmoschus* species in crude oil contaminated soil and assess if same could serve as pointers for variations between them.

MATERIALS AND METHODS

Study area

The study area lies within the humid tropical rainforest vegetation at the Experimental Plot of the University of Benin, Department of Plant Biology and Biotechnology (6.20 °N and 5.37 °E). Soil sample for the study were obtained from Auchu (7.04 °N and 6.16 °E), located at the Northern axis of Edo State, Nigeria. It lies within the derived savannah vegetation.

Soil Collection/Treatment

Two samples of about 100Kg each were collected at the premises of Nigeria National Petroleum Corporation (NNPC) sub-station measuring (2 X 2) Km² in Auchu. One of the soil samples was collected 30 meters away from a site that was reported to have been contaminated with crude oil in February, 2008 during a test run on newly fixed pipelines. The crude oil was reported to have overflowed from a pit of about (60 X 60 X 30) M³ into adjoining farms from a radius distance of 100 m² away from the terminal point of the spill. After seven days, 2.75 Kg of each soil was prepared, analysed and transferred into thirty polythene bags for field trials.

Plant Collection

Okra pods and seeds for the study were collected from two locations. Two accessions **47 – 4** and **LD -88** (*A. esculentus*) were collected from National Institute of Horticultural Research (NIHORT), Ibadan, (7.22 °N and 3.52 °E). Four accessions labelled as **OS/AC/001**, **OS/AC/002** (*A. caillei*), **OS/AE/006** and **OS/AE/007** (*A. esculentus*) were collected from home gardens around Benin City (6.20 °N and 5.37 °N). These latter collections were made and identified based on the identification given by the gardeners due to their high yield and superior quality. Further identification was done using IBPGR (1984) and Stevels (1988), (1990).

Determination of Soil Characteristics and Composition

The two soil samples collected for experimentation were subjected to soil analysis after treatment before use at the Soil Science Laboratory, Nigerian Institute

for Oil Palm Research (NIFOR). The method used for the analysis was as outlined by Ogunwale and Udoh (1990).

Planting and Plant Husbandry

The thirty bags from the two soil samples were transferred to the experimental plot. Two plots of (6 X 3) M³ one each for the contaminated and control were demarcated on the site with a distance of 3m apart. Spacing of bags was done 1m apart in each plot. These bags were left on the field for seven days before planting was done. Viable seeds were selected for field trials. Planting was done simultaneously on the two plots. Bags for each soil sample were arranged in a randomized block design and each accession with five replications. Crops were rain fed throughout the period of experimentation from April to June. After 2 weeks of sowing, each stand on both plots (where germination occurred) were thinned to a plant per stand. Weeding was done weekly while pest control was done using methods outlined by Osawaru and Dania-Ogbe (2010). There was no fertilizer application during trials.

Measurement of Agronomic characters:

Determination of germination and germination percentage

Daily visit was made to the plots for 14 days. Emerged seedlings were counted in each plot and from each bag for the accessions and percentage germination was determined using method outlined by Osawaru and Dania-Ogbe (2010).

Growth Parameters and Plant Characterization

Characterization of plant was carried out using IBPGR descriptor/Charrier (1984) and Hamon (1991). This was used to evaluate the growth response of the 2 cultivated Okra species in both soils.

Qualitative Characters

Main Stem (erect, medium or procumbent),
Branching (orthotropic, medium or strong)
Stem Pubescence (glabrous, slight or conspicuous),
Stem Color (green, green with red patches, purple)

Leaf Shape:

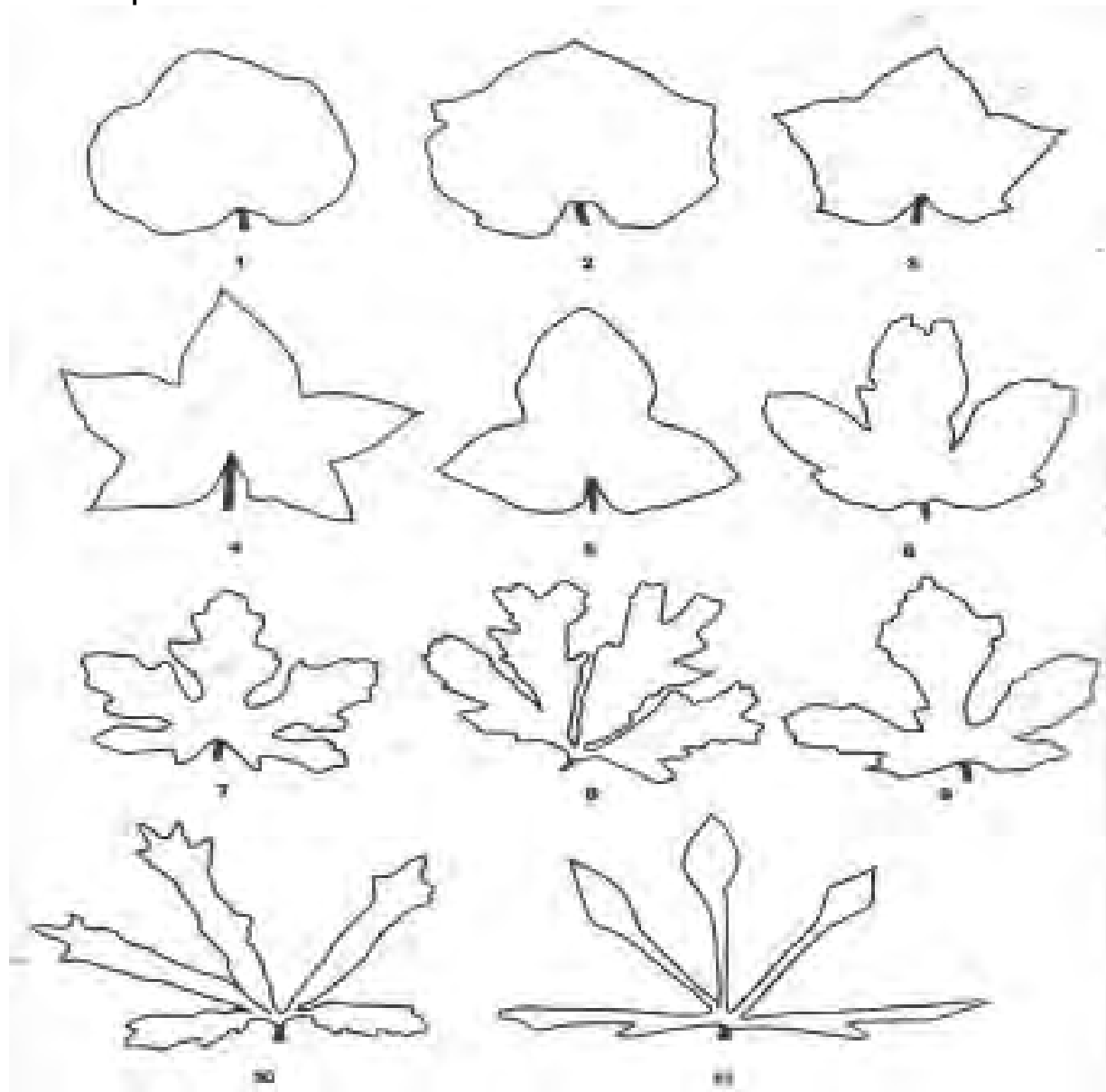


Figure 1: Leaf Shapes
Source: Charrier (1984)

Leaf color (green, green with red patches or red) as stated by Charrier (1984)

Fruiting and Fruit Characterization through Epicalyx Segment(number of segments): 5 to 7, 8 to 10, more than 10),

Shape of Epicalyx segment(linear, lanceolate or triangular), **Persistence of Epicalyx segment** (non persistent, partially persistent or persistent), **Petal Color** (cream, yellow or golden),

Petal Blotch (inside only, outside only or both side), **Position of fruit on main stem** (erect, horizontal or pendulous)

Fruit color(yellowish green, green, green with red patches and red),

Fruit shape and fruit pubescence (downy, slightly rough or prickly)

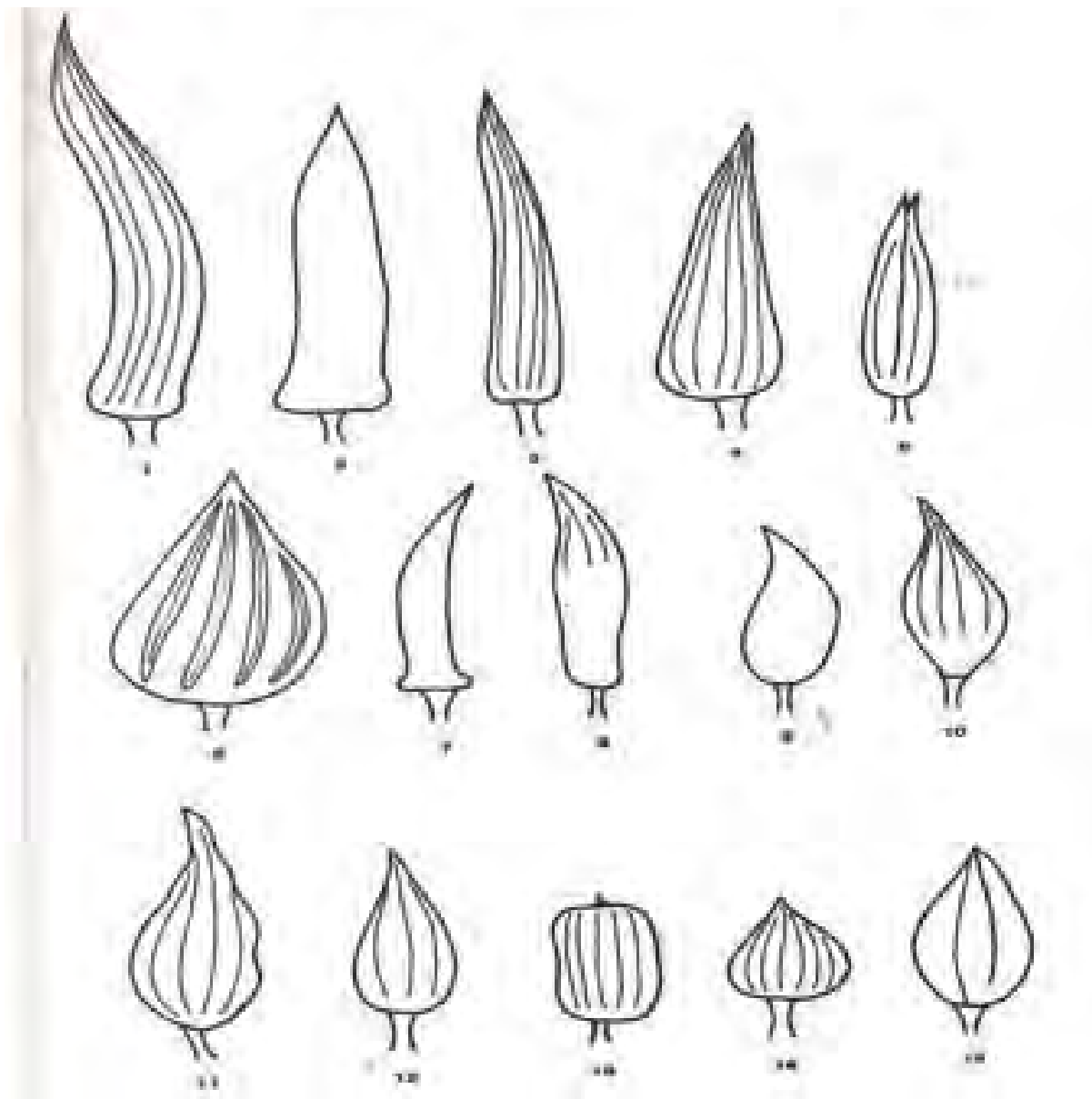


Figure 2: Fruit Shape at Maturity
Source: Charrier (1984)

Analysis of Data: Data obtained during the studies were subjected to statistical analysis using Microsoft office excel 2003 package.

RESULTS

Chemical analysis of soil

The result of the soil samples used for the study is presented in Table 1. The values obtained for C,N,I, Na, Ca, Mn and ECEC were higher in the crude oil contaminated soil. The values obtained for P and K were higher in the control. A1⁺ and H⁺ were undetected

in both soil samples. The pH value of the contaminated soil was close to that for the control soil. The particle size analysis (Table 1) shows that the contaminated soil had less clay and more silt in comparison with the control soil. The contaminated soil has higher values for all the selected heavy metals analyzed.

Table 1: The Physicochemical properties of the soil samples

Soil Sample	Control	Contaminated
pH	7.7	7.6
C (%)	0.93	1.38
N (%)	0.10	0.14
P (%)	11.25	6.41
Na (ppm)	0.34	0.35
K (ppm)	0.24	0.13
Ca (Meq/100soil)	9.60	9.84
Mg (Meq/100soil)	3.84	3.90
H ⁺ (Meq/100soil)	0.10	0.10
Al ³⁺ (Meq/100soil)	0.00	0.00
ECEC (Meq/100soil)	14.12	14.32
Cu (mg/Kg Soil)	2.79	7.26
Mn (mg/Kg Soil)	1.46	5.54
Pb (mg/Kg Soil)	3.56	8.44
Fe (mg/Kg Soil)	7.58	50.60
Zn (mg/Kg Soil)	2.69	5.58
Cr (mg/Kg Soil)	0.13	0.49
Ni (mg/Kg Soil)	-	-
Cd (mg/Kg Soil)	0.78	1.46
Clay (%)	4.10	3.10
Silt (%)	1.70	4.70
Sand (%)	94.20	92.20

Germination and Germination Percentage

The results from germination were expressed as percentages for two weeks from the day germination was initiated. The germination percentage for the control and contaminated soil is presented in Figures 3 and 4 respectively.

Growth Responses**Maximum Plant Height**

As at the time of this report, the maximum plant height of accessions **OS/AC/001** and **OS/AC/002** were 71.00 cm and 75.00 cm in control and 8.00 cm and 17.00 cm in contaminated respectively. **OS/AE/006** in the control was 28.00 cm and 8.00 cm in the contaminated soil. **OS/AE/007** had a maximum height of 51.00 cm in the control and 10.00 cm in the contaminated while **LD - 88** and **47 - 4** had 20.00 cm and 25.00 cm in the control soil while it was 15.00 cm and 12.00 cm respectively in the contaminated soil sample.

Main stem

OS/AC/001 and **OS/AC/002** are generally erect in both control and contaminated soil samples, **OS/AE/006** and **LD-88** were all medium while **47-4** is procumbent and **OS/AE/007** had an orthotropic main stem.

Stem diameter at base (cm)

In the control soil sample, the stem diameter at the base ranged from 2.00 cm to 4.20 cm in accession **OS/AC/001**, 1.50 cm to 3.60 cm in accession **OS/AC/002**, 1.40 cm to 2.40 cm in accessions **OS/AE/006** and **OS/AE/007**, 1.70 cm to 2.50 cm in accession **LD - 88** and 1.80 cm to 2.00 cm in accession **47 - 4**. In the contaminated soil sample, it ranged from 0.30 cm to 0.90 cm in accession **OS/AC/001**, 0.60 cm to 2.50 cm in accession **OS/AC/002**, 0.70 cm to 1.00 cm in accessions **OS/AE/006** and **OS/AE/007**, 0.70 cm to 1.10 cm in accession **LD - 88** and 0.50 cm to 1.00 cm in accession **47 - 4**.

Branching

OS/AC/001 and **OS/AC/002** produced strong branches. This was true for **OS/AC/002** in both control and contaminated soil sample while in **OS/AC/001**, branches were observed only in the control soil sample. **OS/AE/006**, **OS/AE/007** and **LD-88** had orthotropic stems and medium branches in both soil samples. **47 - 7** had an orthotropic stem only.

Stem pubescence

OS/AE/007, **OS/AC/002**, **OS/AE/006** and **OS/AE/007** are slight in control soil sample and glabrous in the contaminated soil sample. **LD - 88** and **47-4** were glabrous in both soil samples.

Stem colour

OS/AC/001, **OS/AC/002**, **OS/AE/006** and **OS/AE/007** are green with red patches in both soil samples while **LD - 88** is completely green in soil sample. **47 - 7** is green the control and green with red patches in the contaminated soil samples.

Leaf shape

OS/AC/001 and **OS/AC/002** are palmate – which was regular and slightly lobed. **OS/AE/006** and **OS/AE/007** are deeply lobed with **OS/AE/007** having deeper lobes. **LD - 88** and **47-4** were more deeply lobed in both soil samples. Accession **OS/AC/001** was unable to exhibit the leave dimorphism – that is from entire to crenate.

Leaf colour

In both contaminated and control soil samples, the leaves of accessions **OS/AC/001** and **OS/AC/002** are green with red venations extending to the leave petiole. Those of accessions **OS/AE/006** and **OS/AE/007** are green with red patches terminating at the leave base

while the leaves of accession **LD - 88** and **47 - 4** were completely green in control soil but accession **47 - 4** had red patches in the contaminated soil.

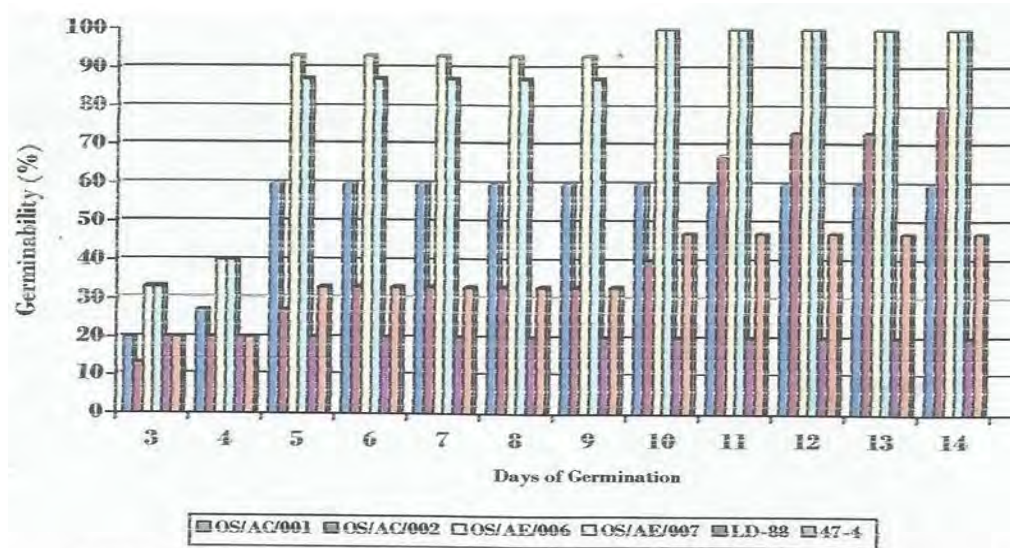


Figure 3: Percentage germination in the control soil

Germination was initiated on the third day. Accessions OS/AC/001, OS/AE/006 and OS/AE/007 had hi germination percentages than the other accessions

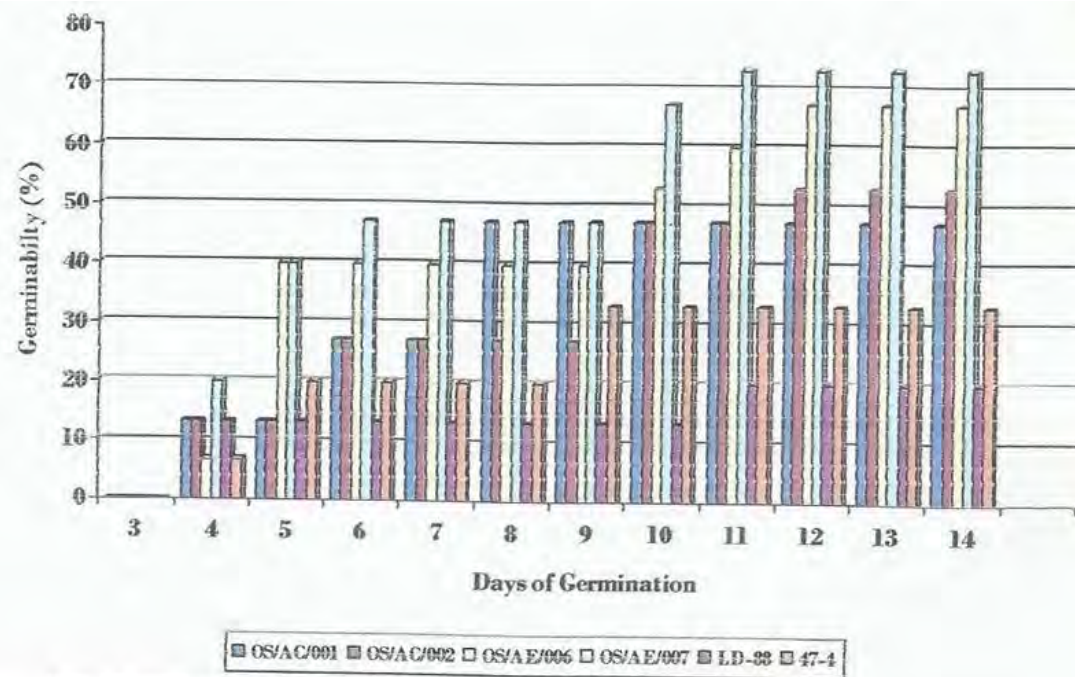


Figure 4: Percentage germination of accessions in the crude oil contaminated soil.

Germination was initiated on the fourth day. Accessions LD-88 and 47-4 never attained 50 % germination.

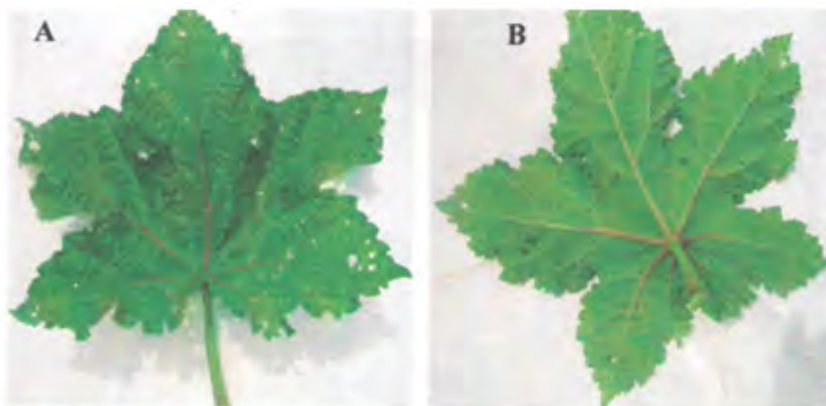


Plate 1 A and B: The leaf shape and color of the adaxial and abaxial surfaces of accessions OS/AC/001 and OS/AC/002 in control soil



Plate 2 A and B: The leaf shape and color of the abaxial and adaxial leaf surface of accession OS/AE/006



Plate 3 A and B: Leaf shape and color of the abaxial and adaxial surfaces of accession OS/AE/007



Plate 4 A and B: Leaf shape and color of the abaxial and adaxial surfaces of accession LD-88

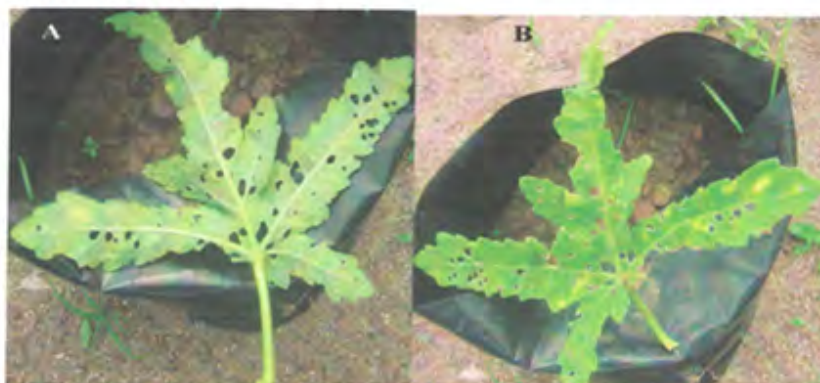


Plate 5 A and B: Leaf shape and color of the abaxial and adaxial surface of accession 47-7

Reproductive (Fruit and Fruiting characters)

There are no results for **OS/AC/001** and **OS/AC/002** as the plants are still awaiting flowering. Therefore, the data below only accounts for accessions **OS/AE/006**, **OS/AE/007**, **LD – 88** and **47 – 4**.

Days from Sowing to first flowering

In the control soil sample, the number of days from sowing to first open flower of accessions **LD – 88** and **47 – 4** was 40 days while for accession **OS/AE/006** and **OS/AE/007** it was 49 days. In the contaminated soil, in accession **LD – 88** and **47 – 4** were 62 days and 91 in **OS/AE/006** and **OS/AE/007** were 95 days.

First Flowering node

In the control soil sample, the first flowering node in accession **OS/AE/006** was the thirteenth node and in accession **OS/AE/007** it was the eleventh node. In **LD – 88** and **47 – 4**, the first flowering node was the eighth. For these two accessions it was the same in the

contaminated soil while it was the ninth and eighth node respectively for accessions **OS/AE/006** and **OS/AE/007**.

First fruit producing node:

Shape of Epicalyx segment

For accessions **OS/AE/006**, **OS/AE/007**, **LD – 88** and **47 – 7** the shape of the epicalyx segments were lanceolate in both soil samples.

Persistence of epicalyx segment

Epicalyx segments were non persistent up to seven days in accessions **OS/AE/006**, **OS/AE/007**, **LD – 88** and **47 – 4** in both soil samples.

Petal colour

Accessions **OS/AE/006**, **OS/AE/007**, **LD – 88** and **47 -4** had yellow petals in both soil samples

Fruit colour

In both soil samples, accessions **OS/AE/006** and **OS/AE/007** had green fruits with red patches at the tip while the fruits of accessions **LD-88** and **47 - 4** were completely green.

Petal blotch

In both soil samples, accessions **OS/AE/006** and **OS/AE/007** had red coloration of petal base in both sides while accessions **LD - 88** and **47 - 4** had red coloration of petal base on the inside only.

Position of fruit on main stem

In all the accessions that produced fruits, the position of fruits on the main stem was erect in both soil samples.

Fruit pubescence

In the control soil sample, the fruits of accessions **OS/AE/006** and **OS/AE/007** were slightly rough and accessions **LD - 88** and **47-4** were downy. While in the contaminated soil sample, all the accessions had downy fruits.

Fruit shape

In both soil, the fruits of accessions **OS/AE/006**, **OS/AE/007** and **LD - 88** were triangular. Those of accessions **OS/AC/001**, **OS/AC/002** and **47 - 4** were ovoid.



Plate 6 A, B, C and D: Position of fruits on main stem, fruit pubescence, fruit shape and fruit color (A=47-7, B= OS/AE/006, C=OS/AE/007 and D=LD-88

DISCUSSION

Overton *et al.* (1994) (1994) reported that, all crude oils are mixture of the same compounds, but with the different quantities of the individual components present in crude oils from different locations. Therefore, the quantities of some compounds can be zero in a given mixture of component constituting crude oil from a specific location. Chemical analysis of the soil samples used shows difference between the elemental (organic and inorganic) content of the soils. The difference in the pH values can be attributed to the crude oil contamination. This is in agreement with Vwioko *et al.* (2008) that the contamination of soil increases the pH of the soil from acidic to neutral similarly, the particle size analysis of the two soil samples show that crude oil contamination of soils may also reduce the coarse texture of the soil to form salt.

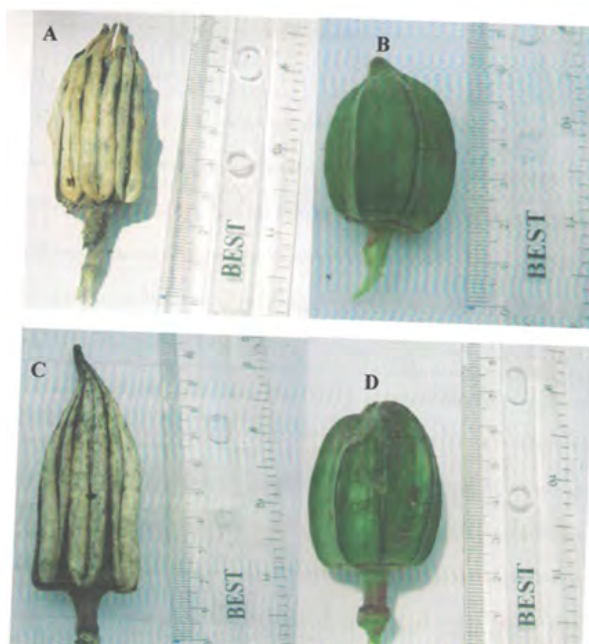


Plate 7 A, B, C and D: Fruit length at maturity of the accessions (A=LD-88, B=47-7, C= OS/AE/007 and D=OS/AE/006

Germination was initiated on the third day by the six accessions in the control soil with gradual increase of percentage germination daily. Accessions **OS/AC/001**, **OS/AE/006** and **OS/AE/007** had above 60 % germination on the fifth day which is in accordance with FAO (1993) and Sreeramulu (1983) recommended seed standard for agronomic practice (Figures 3 and 4). The reverse was the case for accessions **OS/AC/002** which had 50 % germination and 73 % on the eleventh day. Accessions of **LD-88** and **47-4** did not have up to 50 % germination even on day 14. In the crude oil contaminated soil sample germination was initiated on the fourth day in the six accessions. It took accessions **OS/AE/006** and **OS/AE/007** twice the number of days needed to attain 50 % germination in the control to do the same. Accession **LD-88** and **47-4** never had up to 50 % germination even on the fourteenth day. The low percentage germination of the two latter accessions in both soils samples may be due to the removal of the seeds from seed bank of NIHORT two months before planting. During this period, the seeds were stored at room temperature which may have induced shock on them resulting in low germination.

Growth in plants is generally adversely affected when exposed to crude oil (Baker, 1970 and De Jong, 1980). The degree to which the plant growth is affected depends greatly on the level of contamination (Anoliefo, 1991). Similarly, the growth of the six accessions especially those of the land races where generally mostly affected in the crude oil contaminated soil. Affected characters include main stem, branching, stem pubescence, stem color, leaf shape, leaf color, petal blotch, fruit position on the main stem, fruit color, fruit shape and fruit pubescence This is supported by Amakiri and Onofeghara (1984) which states that at high CRC-s of oil in soil, most plant species suffer depression of growth and that this is attributed to poor soil conditions, dehydration and unpaired nutrient uptake by the roots even when they are present as compounds. Crude oil contamination may also be as a result of the stress produced by the contamination which may have resulted in low cellular division (Sabastiani, 2004).

In the local accession of the common okra, **OS/AE/006** had a maximum plant height of 28.00 cm in the control and 8.00 cm in the contaminated soil samples. **OS/AE/007** had 51.00cm and 10.00 cm in control and contaminated soil samples respectively. Amakiri and Onofeghara (1984) and Udo and Fayemi (1975)

reported that "at high concentrations of oil in soils, most plant species suffers serious depression in growth this has been attributed to poor soil conditions, dehydration and impaired nutrient uptake by the roots even when they are present, they are not usually in the absorbable form (ions) rather they are present as compounds. This is created by the presence of crude oil". Also, this may be due to the fact that the soil pH was alkaline. Wong *et al.* (2001) reported that acidic condition (pH) favours nutrients absorption and availability of some heavy metals. Furthermore, the total number of leaf production in these two accessions was drastically reduced in the contaminated soil and the plants never had more than three leaves at a time. This may be an adaptation to drought created by the presence of crude oil. Amadi *et al.* (1993) reported that "the presence of crude oil in the soil results in dehydration of the soil". When this occurs, the plant tends to minimize the small available water by shedding their leaves to reduce the rate of "transpiration"- the major source of losing water in plants. In these two accessions, there was also a delay in the number of days from sowing to first flowering in the contaminated soil by more than two times the number of day needed in the control to attain first flowering. This was not in agreement with Vwioko and Fashemi (2005). They reported that oil contamination reduces the number of days from sowing to flowering in plants. The first flowering node and the fruit production nodes of these two accessions were reduced in the contaminated soil when compared to the plants of the same accessions in the control soil sample. This reduction may be due to the impaired nutrient uptake by the roots (Amakiri and Onofeghara, 1984) which reduced the rate of cell division and ultimately results in poor or growth in plants. The length of fruits at full maturity was also reduced in the contaminated soil. The average number of flower buds and flowers produced per plant grown in contaminated soil in four accessions of common okra were less than those of plants grown in contaminated soil. From the data obtained, not all the flower buds produced developed into flowers in the control soil. Interestingly, all the flower buds produced by plants grown in the contaminated soil developed into flowers in all the accessions. In both soils, not all flowers produced developed into fruits. The percentage of flowers that developed into fruits are 75%, 67%, 88% and 95% in control and 80%, 67%, 89% and 94% in contaminated for accessions **OS/AE/006**, **OS/AE/007**, **LD-88** and **47-4** respectively. This may be as a result of the delay in germination of these accessions in the contamination

soil. It may also be as a result of stress produced by the contamination which may have resulted in low cellular division (Sebastiani *et al.*, 2004). Other characters like main stem, branching, stem pubescence, stem colour, leaf colour, number of epicalyx segment, shape of epicalyx segments, petal colour, petal blotch, fruits position on the main stem, fruits colour, fruit shape and fruit pubescence were not affected. This can be inferred as they remain the same in plants of these two accessions in both soil samples. On the other hand, in accessions **LD-88** and **47-4**, all characters remained the same in both soil samples. But there was chlorosis which resulted in yellowing of the leaves of accession **47-4**. In the two accessions of *A. caillei* (**OS/AC/001** and **OS/AC/002**), accessions **OS/AC/001** was observed to show more depression in growth of all the characters studies than **OS/AC/002** in the contaminated soil.

CONCLUSION

Result suggests that the morpho-agronomic characters investigated can be used to distinguish the two Okra species (*A. caillei* and *A. esculentus*). The varied growth response of the different accessions of these Okra species implicated the need for further investigations. Soil contamination was observed to show more effect on quantitative characters than on qualitative characters. Most anthropogenic activities associated with crude oil exploration result in soil contamination with potential impact on biological diversity. Okra has been implicated to be vulnerable to crude oil by this study.

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