

Assessment of Growth Performance of Two Cultivated Okra Species (*Abelmoschus esculentus* (L.) Moench and *Abelmoschus caillei* (A. Chev.) Stevels) Exposed to Crude Oil Contaminated Soil

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Abstract:

Six accessions of cultivated Okra (*Abelmoschus caillei* (A. Chev.) Stevels) and *Abelmoschus esculentus* (L.) Moench) were evaluated using growth parameters in crude oil contaminated soil. Seeds were collected from collected from Nigerian Institute of Horticulture (NIHORT), Ibadan and from home gardens in Benin City. Contaminated soil sample were collected from Nigerian National Petroleum Coperation (NNPC) substation in Auchi. The contaminated soil had higher concentrations of heavy metals than control soil obtained as top soil from the experimental garden of the Department of Plant Biology and Biotechnology, University of Benin. Morpho-agronomic characters such as numbers of days from sowing to germination, dry and fresh weight of the accessions in both soil sample was determined. Others were copper (Cu), Zinc (Zn), Manganese (Mn), Cadmium (Cd) and Lead (Pb) concentration in plant parts (leaves and fruits). The growth responses of the different accession varied considerably although *A. esculentus* accessions performed better. Soil chemical analysis revealed decreased levels of pH, Phosphorus and Potassium in the contaminated soil. The chemical analysis of plants grown in these soils showed that heavy metals like Cu, Zn, Mn, Cd and Pb were present in all the organs of the accession. These heavy metals accumulate in the tissue and make the plant unsafe for consumption. The study suggests that Okra diversity is vulnerable to crude oil contamination.

Key words: Crude oil, Soil, Growth parameters, *Abelmoschus esculentus*, *A. Caillei*, Heavy metals

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Introduction

Okra is a widely cultivated vegetable in tropical and subtropical regions. It is grown for its leaves, fruits, seeds, flora parts and stems. These are edible at young and succulent stage of the plant. The fresh unripe and tender pods (fruits) are sliced, grated, boiled or steamed or fried and beaten into soup. This soup facilitates the swallowing of relatively rough or coarse textured starchy foods (Schippers, 2000). It is also used in salads along with egg plants. Fruits can be conserved year-round for consumption. Seeds are removed from the matured pod and sun dried. These are made into powder and used for flavoring. The genus *Abelmoschus* is said to have originated from South East Asia (Hamon and Hamon, 1991). Siemonsma (1982) recognized nine species in *Abelmoschus* based on cytogenetic evidence. The morphological characters such as number, dimensions and persistency of the epicalyx segment, form and dimensions of the capsule (including pedicel) and characteristics of the indumentums are unique. In West Africa, the wide varieties of okra cultivars are of two distinct species, the common okra, *Abelmoschus esculentus* (L) Moench and West African okra *Abelmoschus. caillei* (A. chev.) Stevels (Charrier, 1984). *A. esculentus* adapted to the Sudano-sahelian zone and the *A. caillei* is referred to as Guinean type in relation to its zone of cultivation (Charrier, 1984; Chedda and Fatokun, 1991; Schippers,

2000). This study focuses on these two cultivated species. Osawaru and Dania-Ogbe (2010) reported that both species are wide spread between 12°N and 12°S and most commonly found between 5°N and 12°N. The common Okra (*A. esculentus*) is mainly grown for market gardening in areas with limited rainfall, especially under irrigation. West African Okra (*A. caillei*), is a cultigens of West Africa sub-region and liked for its ability to make better soup than common Okra. Both are grown mainly for subsistence economy. Cytologically, Siemonsma (1982) and Ariyo (1993) reported that West African Okra contain 194 diploid chromosomes as against 130 of the common okra thereby indicating that West African Okra is a separate species. There is also some overlapping characters in these features which have not been clearly or exclusively defined. *A. caillei* is highly polymorphic and unique in fruit and indumentum characters. The nutritional, industrial and cultural roles of these okra species is documented by Siemonsam and Hamon (2002), Schipper (2000), Grubken (1977), Obire (2002), Chevalier (1940) and Charrier (1984).

In this study, we investigated the growth performance of *A. esculentus* and *A. caillei* in crude oil contaminated soil to access the metal sink. Amakiri and Onofeghara (1984) and Udo and Fayemi (1975) reported that "at high concentrations of oil in soils, most plant species suffer serious depression in growth which 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". Wong *et al.* (2001) reported that acidic condition (pH) favors nutrients absorption and availability of some heavy metals. The bioavailability of components of the contaminants in the plant parts (fruit and leaves) was also determined. Crude oil in soil makes the soil condition unsatisfactory for plant growth.

Materials and Methods

Study area: The study area lies within the humid tropical rainforest vegetation at the Experimental Garden of the University of Benin, Department of Plant Biology and Biotechnology (6.20 °N and 5.37 °E). A Completely Randomized Block experimental design was set up in the study area. More so, top soil from the garden was used as control. The contaminated soil sample for the study were obtained from Auchi (7.04 °N and 6.16 °E), located at the Northern axis of Edo State, Nigeria. The study area lies within the derived savannah vegetation.

Plant Collections: Three of the accessions used in the study were obtained from traditional agriculture system (home gardens) in Benin City, Edo state. Two were collected from Nigerian Institute of Horticulture (NIHORT), Ibadan. Further identification was done using IBPGR/Charrier (1984) and Stevels (1988, 1990).

Table 1: *Abelmoschus* accessions used in the study, their origin and location

S/N	Accession	Origin	Latitude and Longitude
1	47-4	NIHORT	07° 22' N, 03° 52' E
2	LD-88	NIHORT	07° 22' N, 03° 52' E
3	OS/AC/001	Benin	06° 20' N, 05° 37' N
4	OS/AC/002	Benin	06° 20' N, 05° 37' N
5	OS/AE/006	Benin	06° 20' N, 05° 37' N
6	OS/AE/007	Benin	06° 20' N, 05° 37' N

After cultivation the accessions will be determined to be either *Abelmoschuscaillei* or *A. esculentus* using the characteristics outlined in Table 2.

Soil Collection and Analysis: Soil samples of about 100 kg each were collected at the premises of Nigeria National Petroleum Corporation (NNPC) sub-station measuring 2 X 2 km² in Auchi. The soil samples were 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 overflow from a pit of about (60 X 60 X 30) M³ into adjoining farms from a radius distance of 100m² away from the terminal point of the spill. The second soil sample (not contaminated) was collected from a pool of top soil at the experimental plot. After collection, 2.75 kg of each soil was prepared and transferred into thirty polythene bags for field trials. The two soil samples collected for experimentation were subjected to soil analysis at the Soil Science laboratory, Nigerian Institute for Oil Palm Research (NIFOR). The method used for the analysis is as outlined by Ogunwale and Udoh (1990). Total elemental content, organic nutrient and presence of heavy metals were determined.

Table 2: Main Characteristics of two cultivated Abelmoschusspp

Features	<i>A. caillei</i>	<i>A. esculentus</i>
Epicalyx segment		
Number	5-10	7-18
Length (mm)	8-35	5-25
Width (mm)	4-13	0.5-3
Persistence	Drops at young fruit stage	Throughout flowering only
Pedicle of Fruit		
Length	1-13 (often curved)	0.35 (mainly upright)
Capsule	7-20	15-25
Length (cm)	7-20	15-25
Width (cm)	4-10	3-8
Shape	Ovoid and triangular	Long and Fusiform

Sources: Siemonsma (1982), Schipper (2000), Osawaru (2008).

Crop Management: Seeds of the six accessions collected were subjected to floatation test and viable seeds were selected for field trials. The thirty bags (fifteen each for contaminated and control soil) were transferred to the experimental plot. Two plots of (6X 3) M³ one each for the contaminated and control were demarcated on the site with a distance of 7 m apart. Spacing of bags was done 3 m in each plot. 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. There was no fertilizer application during trials. After two weeks of sowing, each stand on both plots (where germination occurred) were thinned to a plant per stand. Weeding was done weekly. Pest control was done using methods outlined by Osawaru and Dania-Ogbe (2010).

Data Collection: Plant parts (leaves and fruits) from the six accessions grown in the control and contaminated soil samples were harvested. Leaves from the third to fifth nodes and the first fruits of the accessions were harvested and weighed fresh. The parts were oven dried for three days. After which the dry weight of the samples were taken. The oven dried materials were ground using an electric grinding machine. The ground samples were then subjected to heavy metal analysis using dry ash method as outlined by Ogunwale and Udoh (1999). All the data obtained in the study was subjected to analysis using Microsoft Excel 2007 for windows.

Results:

Soil: The result of the soil samples used for the study is presented in Table 3, 4 and 5. The values obtained for C, N, I, Na, Ca, Ma and ECEC were higher in the contaminated soil (Table 3). The values obtained for P and K were higher in the control soil. A1⁺ was undetected in both soil samples H⁺. The pH value of the contaminated soil (7.6) was slightly lower than the control soil (7.7). The particle size analysis (table 5) shows that the contaminated soil had less clay (3.10) and more silt (4.70) in comparison with the control soil which is 4.10 and 1.70 respectively. The contaminated soil had higher values for all heavy metal assessed (Table 4)

Table 3: The chemical properties of the two Soil Samples

Soil Sample	%		(ppm)				Meq/100soil		H+	Al+	ECEC
	pH	C	N	P	Na	K	Ca	Mg			
Control	7.7	0.93	0.10	11.25	0.34	0.24	9.60	3.84	0.10	0.00	14.12
Contaminated	7.6	1.38	0.14	6.41	0.35	0.13	9.84	3.90	0.10	0.00	14.32

Table 4: The particle size analysis in percentage of the two Soil Samples

Soil Samples	Clay %	Silt %	Sand %
Control	4.10	1.70	94.20
Contaminated	3.10	4.70	92.20

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 determined using method outlined by Osawaru and Dania-Ogbe (2010). Germination was initiated in the third day by the six accessions in the control soil sample with a gradual increase of percentage germination recorded day by day. Accessions OS/AC/001, OS/AE/006, OS/AE/007 had 60 and above germination percentage on the fifth day which is in accordance with FAO (1998) recommended seed standard for agronomic practice. The reverse was observed for accessions OS/AC/002 which had above 50 % on the eleventh day LD-88 and 47-4 which did not have up to 50% germination percentage eve on day 14. In the contaminated soil, 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 that was needed for them to attain 50% germination in the contaminated soil. OS/AC/002 had above 50% on the fourteenth day, OS/AC/001 was unable to reach 50% germination on the fourteenth day while accessions LD-88 and 47-4 never up to 50% germination, the same percentage germination was maintained with that of the control soil sample. The relatively low germination percentage of LD-88 and 47-4 in both soil samples may be due to the revival of the seeds from the storage bank of NIHORT two months before planting. During this period the seeds were stored at a room temperature which may have induced shock on them. This may have resulted in low percentage germination. The positive germination value obtained from the land races shows suggest *in situ* method of conservation practice promotes seed viability and readily available against the seeds obtained *ex situ* from NIHORT. This also suggest the need for frequent cultivation of seeds held under *ex situ* conservation.

Plant Analysis: Figure 3 and 4 shows the concentration of Copper in the leaves and fruits of Okra accessions. In the control and contaminated soil OS/AC/001 and OS/AC/002 had no Copper in their fruits. Accession OS/AC/002 had the highest amount of Copper concentration in the control soil with accession OS/AE/006 having highest value in the contaminated soil. Accession LD-88 and OS/AE/006 had the highest concentration of copper in there fruits in the contaminated and control soils respectively.

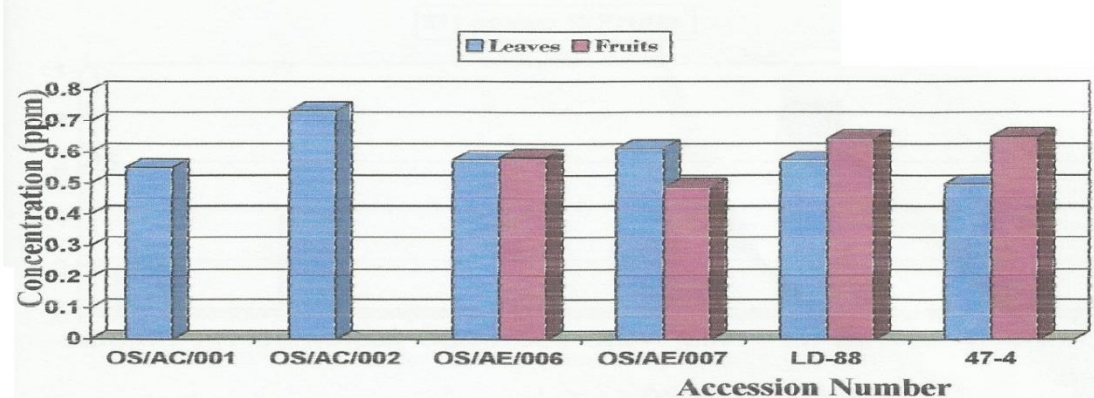


Figure 3: Concentration of Copper in the leaves and fruits of the six accessions grown in control soil sample

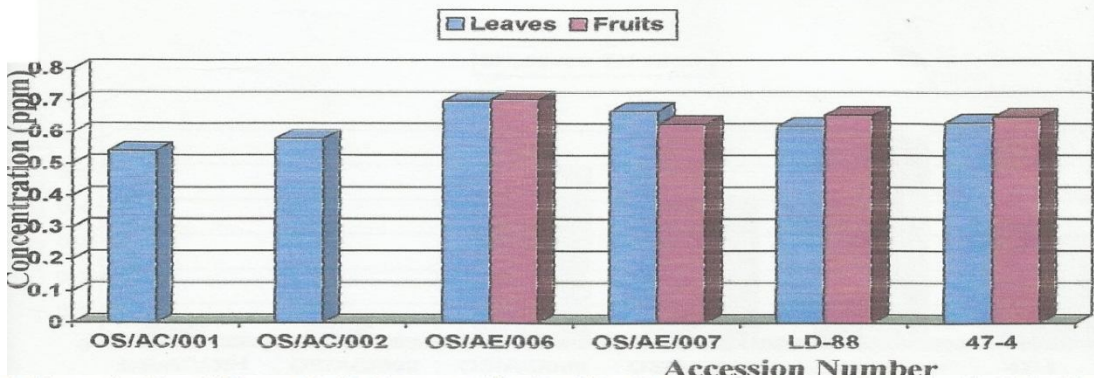


Figure 4: Concentration of Copper in the leaves and fruits of the six accessions grown in contaminated soil sample

Figure 5 and 6 shows the concentration of Zinc in the leaves and fruits of Okra accessions. In the control soil LD-88 had the highest concentration of Zinc in leaves and fruits. In the contaminated soil OS/AE/007 had highest amount Zinc in the leaves while accession LD-88 had the highest concentration of Zinc in the fruits.

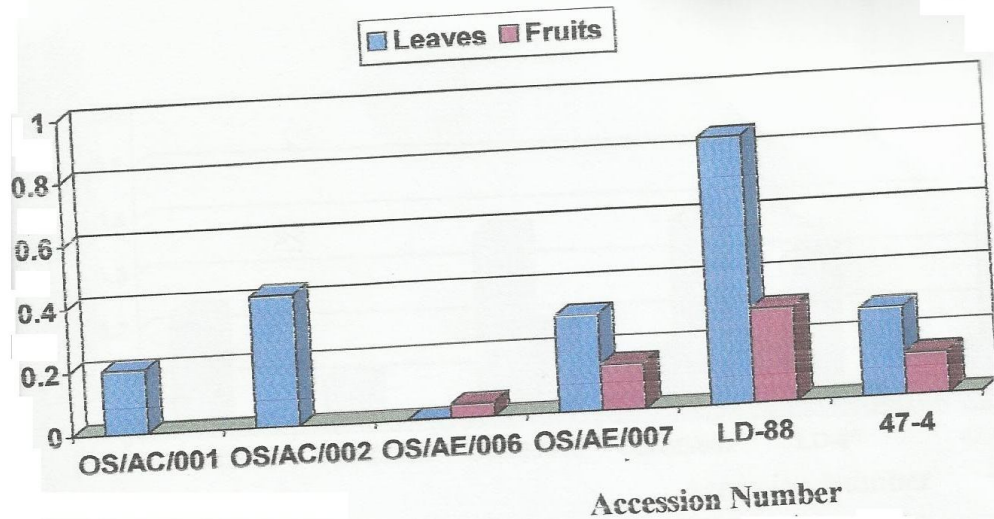


Figure 5: Concentration of Zinc in the leaves and fruits of the Six Accessions grown in the control soil sample

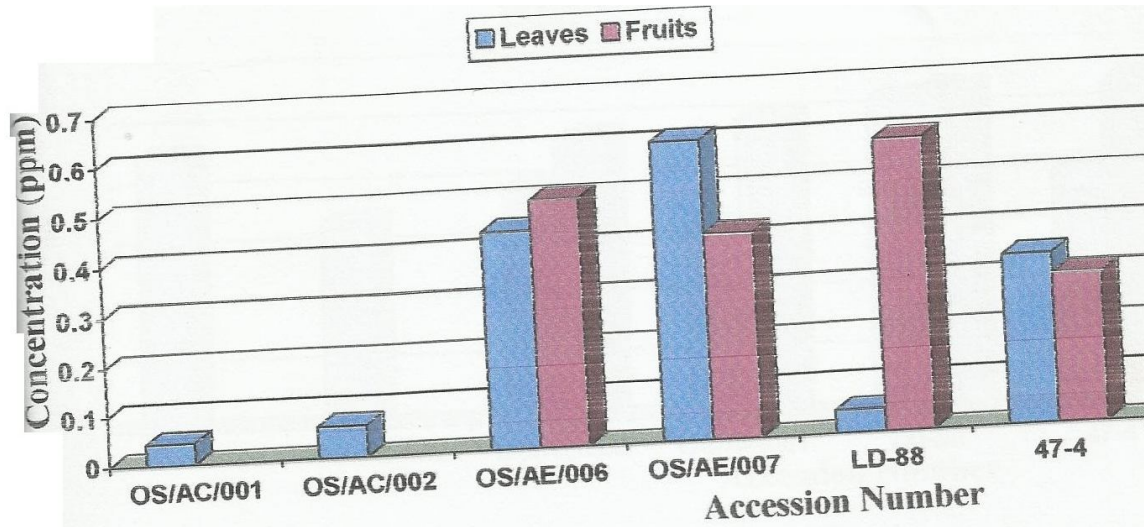


Figure 6: Concentration of Zinc in the leaves and fruits of the Six Accessions grown in the contaminated soil sample

Figure 7 and 8 shows the concentration of Lead in the leaves and fruits of Okra accessions. In the control soil OS/AE/006 had the highest concentration of Zinc in leaves while 47-4 had the highest concentration in the fruits. In the contaminated soil LD-88 had highest amount of Lead in the leaves while accession 47-4 had the highest concentration of Lead in the fruits.

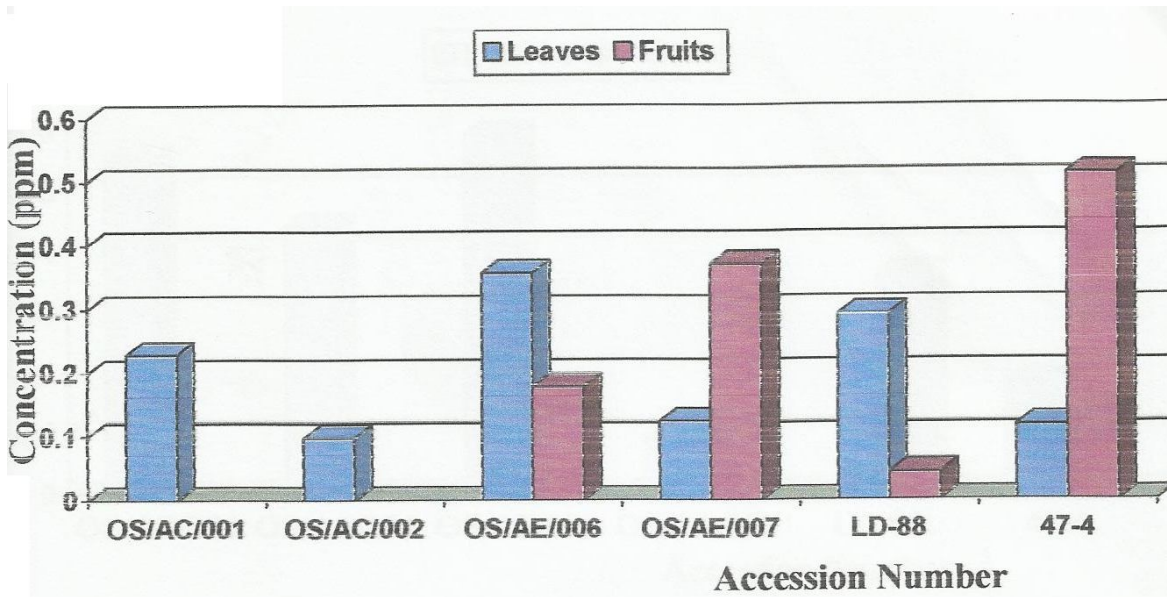


Figure 7: Concentration of Lead in the leaves and fruits of the Six Accessions grown in the control soil sample.

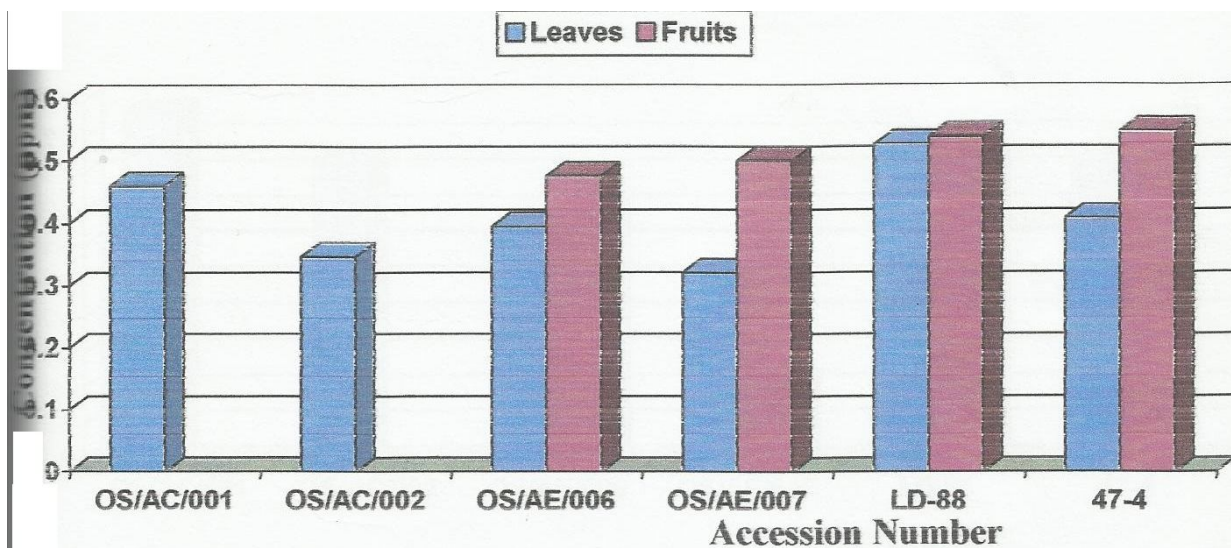


Figure 8: Concentration of Lead in the leaves and fruits of the six accessions grown in contaminated soil sample

Figure 9 and 10 shows the concentration of Cadmium in the leaves and fruits of Okra accessions. In the control soil OS/AE/006 had the highest concentration of Cadmium in leaves while LD-88 had amount amount in the fruits. In the contaminated soil OS/AC/001 had highest amount Cadmium in the leaves while accession 47-4 had the highest concentration of Cadmium in the fruits.

Figure 11 and 12 shows the concentration of Manganese in the leaves and fruits of Okra accessions. In the control soil OS/AC/001 had the highest concentration of Maganese in leaves while 47-4 had amount in the fruits. In the contaminated soil OS/AC/001 had highest amount Manganese in the leaves while accession LD-88 had the highest concentration of Manganese in the fruits.

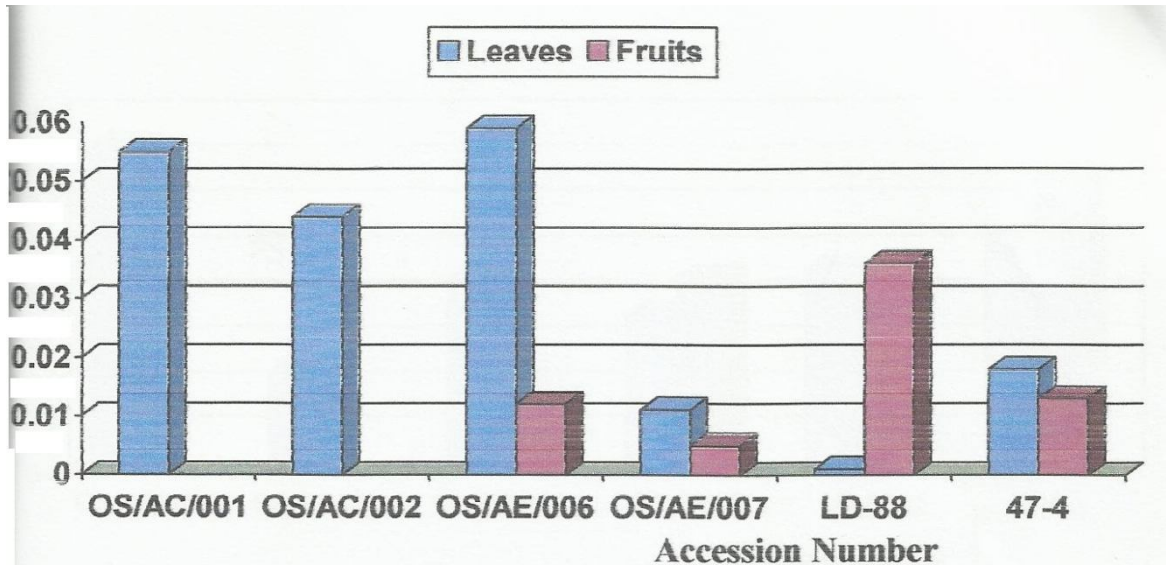


Figure 9: Concentration of Cadmium in the leaves and fruits of the Six Accessions grown in control soil sample.

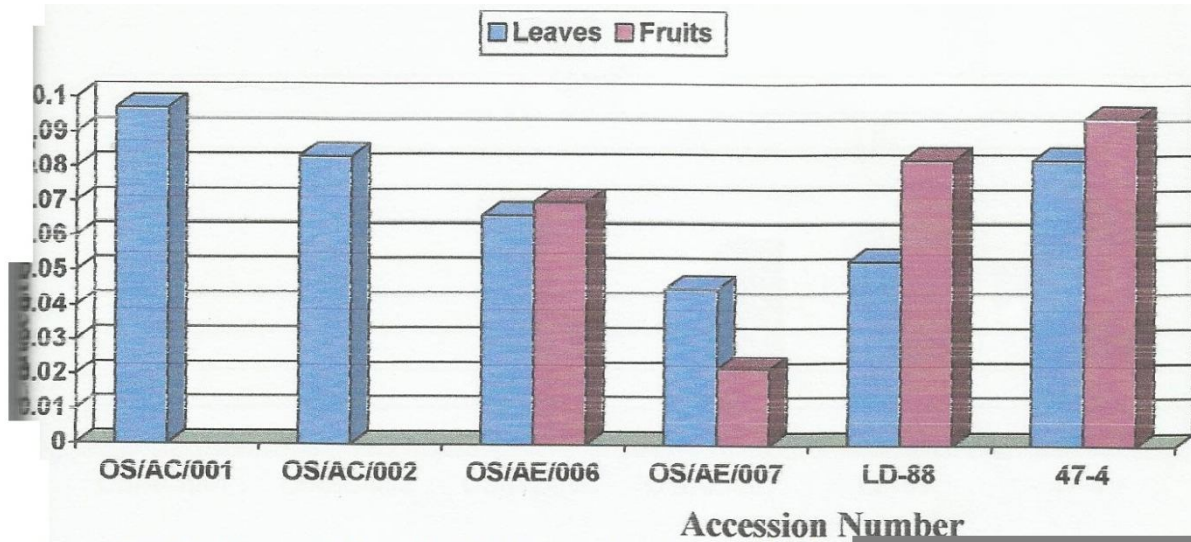


Figure 10: Concentration of cadmium in the leaves and fruits of Six Accessions samples grown in contaminated soil samples

Table 6 showed the fresh and dry weight of harvested organs of the Okra accessions in the control and contaminated soil. The highest fresh weight of fresh fruit and leaves was obtained from OS/AE/006.

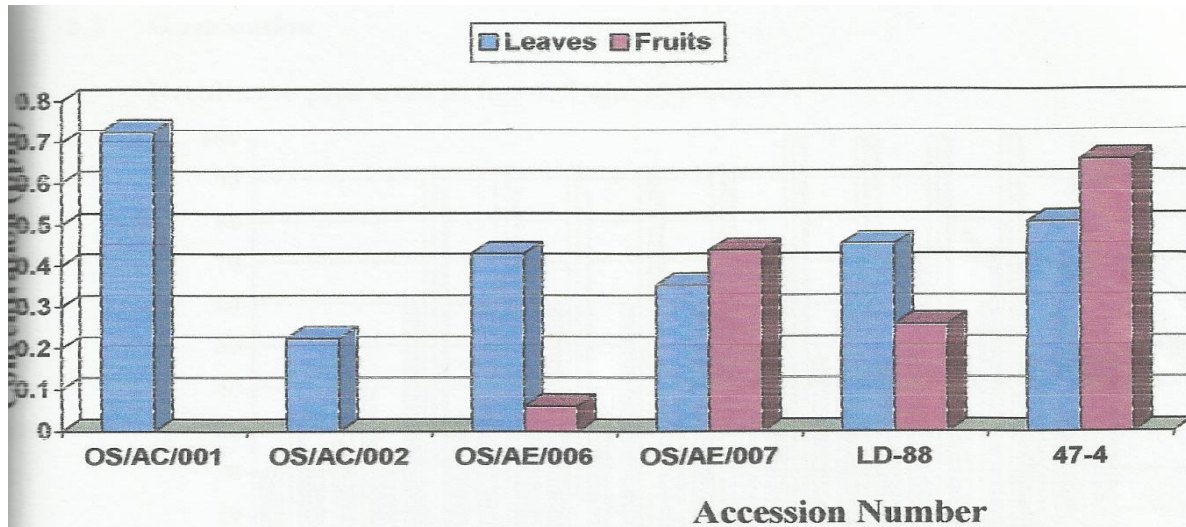


Figure 11: Concentration of Manganese in the leaves and fruits of the six accessions grown in control soil sample

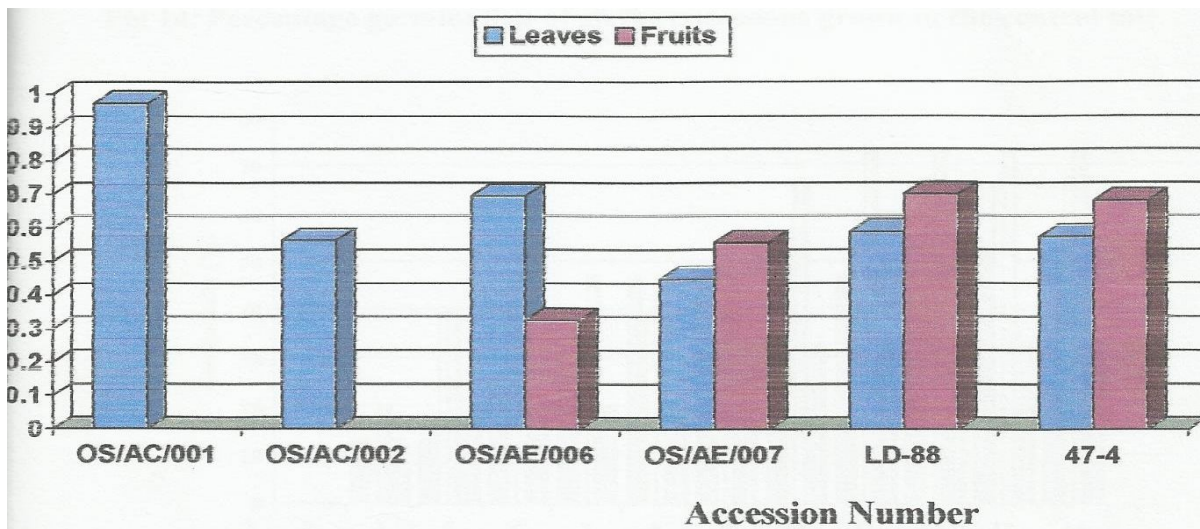


Figure 12: Concentration of Manganese in the leaves and fruits of the six accession grown in contaminated soil sample

Discussion and Conclusion

The growth characteristics exhibited by the accessions under cultivation suggests that OS/AC/001, OS/AC/002, OS/AE/006 and OS/AE/007 are *Abelmoschus esculentus* while accessions LD-88 and 47-4 are *A. caillei* (Table 2). Plant growth can be affected when exposed to crude oil. The degree to which the plant growth is affected depends greatly on the level of contamination (Anoliefo, 1991). Similarly, the growth of six accessions especially those of the land races (OS/AC/001, OS/AC/002, OS/AE/006 and OS/AE/007) were mostly affected in the crude oil contaminated soil. The substantial damage on the soil by the crude oil was observable in the cultivated accessions of Common Okra (*A. esculentus*) and West African Okra (*A. caillei*). In the control soil, OS/AC/001, OS/AC/002, OS/AE/006, OS/AE/007 showed variation in growth and development from the growth in the contaminated soil. It can be suggested that the depression in growth is as a result of the poor soil condition occasioned by the crude oil.

However, accession LD-88 and 47-4 had little or no variation in germination, growth and development in both soil samples. These *A. caillei* accessions were collected from the seed banks of NIHORT hence, their poor performance may be attributed to seed bank conditions among other reasons.

The analysis of the soil sample used in this study showed that the chemical elements Na, N and the ion H^+ in both control and the contaminated soil samples were almost the same. The concentration of C, P and K was observed to be higher in the control soil than in the contaminated soil. Ca, Mg, Mn, Pb, Fe, Zn, Cr, Cd and Cu concentrations were higher in the contaminated soil compared to the control (uncontaminated) soil. Ni and Al^{3+} were not detected in both soils (Table 3). The soil pH was alkaline, 7.7 in control and 7.6 in contaminated soil. It can be suggested that contamination of alkaline soils tends to reduce the soil pH to a less alkaline or a neutral one. This is in agreement with the report of Vwiokoet. *al.* (2008) which states that crude oil contamination of soil increases the pH of the soil from acidic to neutral. Similarly, the particle size analysis of the two soil samples showed that crude oil contamination of soils may be responsible for the difference in the soil silt content

Table 6: Fresh and dry Weight of harvested plant organs from six accessions of Okra Grown in Crude oil Contaminated and control soil.

Accession Number	Treatment	Fruits		Leaves	
		Fresh (g)	Dry (g)	Fresh (g)	Dry (g)
OS/AC/001	Control	-	-	2.70	0.77
	Contaminated	-	-	2.95	0.45
OS/AC/002	Control	-	-	3.00	1.00
	Contaminated	-	-	2.10	0.58
OS/AE/006	Control	6.54	1.00	3.20	0.82
	Contaminated	4.90	0.29	3.00	0.22
OS/AE/007	Control	6.50	0.89	3.00	0.54
	Contaminated	4.73	0.41	3.22	0.39
LD-88	Control	6.52	1.00	2.00	0.89
	Contaminated	5.55	1.00	2.00	0.65
47-4	Control	6.00	1.00	2.00	0.41
	Contaminated	5.00	0.87	2.06	0.40

The fresh weights of fruits and leaves of the four accessions of *A. esculentus* exhibited a strong response to the crude oil contaminations. The fruits and leaves of OS/AE/006 and OS/AE/007 grown in control soil gave weight values that were considerably higher than those cultivated in the contaminated soil. Accessions LD-88 and 47-4 showed little variation in weight for parts grown in both soil samples. Higher moisture content of parts was recorded for the control plants than in the contamination soil. The contaminants in the soil may be responsible for the water content of plant parts hamster plant grown in the contaminated soil. There were observable differences on the dry weights of harvested plant parts in both soils are plants harvested from the control soil gave higher values. The leaves of both *A. caillei* accessions had higher dry weight values when compared with the four accessions of *A. esculentus* growth in both soils; highest values were recorded with the accessions in the control soil.

In Conclusion, the present investigation suggest that Okra diversity is vulnerable to crude oil contamination. The heavy metals that accumulate in their tissues makes it unsafe for human and animal consumption. There is need for improved attitude and knowledge regarding the dangers of cultivating crude oil polluted soil especially in Niger-Delta region of Nigerian. Furthermore *A. esculentus* accessions performed better than *A. caillei* in the contaminated and control soil.

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