

3 **EFFECT OF CRUDE OIL CONCENTRATIONS ON THE VASCULAR BUNDLE SIZE**
4 **OF TWO MAIZE VARIETIES**

5 **Abstract**

6 The reliance of humanity on fossil fuels particularly petroleum hydrocarbons has been on the
7 increase and had led to increased pollution of agricultural lands. This study was aimed at
8 investigating the effect of crude oil concentrations on the vascular bundle sizes of two *Zea mays*
9 varieties – Oba Super Zaria (OSZM) and SMZ 37 Kaduna commonly cultivated in the South-
10 eastern Nigeria. The study was conducted in the green house in the Department of Biological
11 Sciences, Faculty of Natural Sciences, Kogi State University, Ayingba, Kogi State. The
12 experiment was setup as a 2×4 factorial laid out in a Completely Randomized Design replicated
13 three times with four treatment concentrations (0-control, 15, 30 and 45ml) of petroleum crude
14 and two maize varieties. Highly significant effects ($P < 0.001$) of the concentrations as well as
15 the varieties were observed on the vascular bundles. The results showed a significant ($P < 0.05$)
16 decrease in vascular bundle sizes with increasing concentration, with plants exposed to 45ml
17 crude oil concentration having the smallest vascular bundle sizes. The vascular bundle sizes of
18 Oba super Zaria decreased significantly ($p < 0.05$) with increasing concentrations of crude oil,
19 while significant differences were also observed with the increasing concentrations in SMZ 37
20 Kaduna as compared to the control. Based on the results obtained from this investigation, OSZM
21 appeared to be more susceptible to soils affected with crude oil.

22
23 Keywords: Crude; Oil; Pollution; Soil; Vascular Bundle; *Zea Mays*

24 **Introduction**

25 Maize is ranked as the third most essential cereal crop following wheat and rice in the world
26 production of cereal crops (FAO, 2002). Maize is one of the main staple consumed in Nigeria, as
27 it makes available an inexpensive nutritious food that is of great assistance in sustaining the
28 rapidly increasing population. It also serves as raw material in many industrial processes and in
29 the feeding of livestock (Olaniyan and Lucas, 2004). Maize is grown in most agro ecological

30 areas especially in the Niger Delta region where oil industrial activities are predominant
31 (Agbogidi, *et al.*, 2007). The growth and yield of crops are adversely affected by crude oil
32 pollution, and this has been reported by many workers to include poor and stunted growth, low
33 production and sometimes outright death of crops (Anoliefo, 1991; Anoliefo and Vwioko, 1994;
34 Agbogidi, *et al.*, 2007).

35 The reliance of humanity on fossil fuels particularly petroleum hydrocarbons had been on the
36 increase. This had led to the increased pollution of agricultural lands, as a result of spillage of
37 crude oil during the process of extraction and processing (Ayotamuno and Kogbara, 2007). In
38 Nigeria, reports have it that from 1976 to 1998, a sum of about 5,724 incidences of oil spills
39 which introduced crude oil to both the terrestrial and aquatic ecosystems to the tune of
40 2,571,113.90 barrels (Aroh *et al.*, 2010). Since then, crude oil contamination of farmlands has
41 become common experience in the country, and many of these farmlands have been abandoned
42 in the aftermath of pollution.

43 The presence of crude oil in the soil renders it inadequate for plant growth, because of the
44 reduced level of available plant nutrients as well as the rising toxic levels of certain micro
45 elements (Barua *et al.*, 2011). The susceptibility of plants to crude contamination is high, and
46 plants may end up dying in a matter of few weeks or months. Some plant species are able to
47 grow in hydrocarbon polluted soils and bring about their degradation via the rhizosphere part of
48 their roots. This is because the rhizosphere supports the growth of many microorganisms which
49 increases biomass and microbial activity, thereby accelerating degradation process (Quinones-
50 Aquilar *et al.*, 2003; Agbogidi, *et al.*, 2005).

51 The essential elements of vascular systems are the xylem vessels, concerned with the transport of
52 water and dissolved salts, and the phloem, which translocates synthesized but soluble materials
53 around the plant to places of active growth or regions of use or storage (Cutler *et al.*, 2007).
54 Xylem and phloem are normally associated and together form the vascular bundle which is often
55 enclosed in a sheath of fibres. In addition, contains an outer sheath of parenchyma cells (the
56 bundle sheaths) in some instances. Vascular bundles make up the 'plumbing system' of primary
57 tissues, and organs without secondary growth in thickness (Cutler *et al.*, 2007). This study was
58 aimed at investigating the effect of crude oil concentrations on the vascular bundle sizes of two
59 *Zea mays* varieties – Oba Super Zaria (OSZM) and SMZ 37 Kaduna commonly cultivated in the
60 South-eastern Nigeria.

61 MATERIALS AND METHODS

62 The soil used in this study was obtained from the top soil collected from a farm land. The soil
63 was treated by sieving and autoclaving at 82⁰C for 30 minutes to remove roots that may serve as
64 an alternative host to pathogens that may affect the growth of the plant. Two maize varieties
65 (Oba Super Zaria maize and SMZ 37 Kaduna) were used in this study. Unrefined crude oil was
66 obtained from the Nigeria National Petroleum Corporation, (NNPC). The crude oil was mixed
67 with water to vary the concentration as 15, 30, and 45ml of the crude oil were mixed up into
68 500ml of distilled water.

69 Five hundred and fifty grams of treated top soil was weighed and put into 24 pots and each pot
70 was planted with three seeds of maize. The planting pots were perforated at the bottom and sides
71 to allow for aeration and drainage of excess water. After germination, the soil in the pots were all
72 polluted with crude oil at the different concentrations (15, 30 and 45ml), except the control, at
73 two weeks after germination. The experiment was observed for two week with continuous
74 watering. The experiment was setup as a 2 × 4 factorial laid out in a Completely Randomized
75 Design replicated three times. The experimental study was conducted in a green house in the
76 Department of Biological Sciences, Faculty of Natural Sciences, Kogi State University, Ayingba,
77 Kogi State.

78 The stems were harvested and cross-sections were made using a Reichert sledge microtome. The
79 cross-sections were stained, using the counter staining procedures (Jane 1962), as modified by
80 Ajuziogu *et al.* (2018). The counter staining procedures stained the lignified tissues of the
81 vascular bundles red, and contrasted them from the unlignified tissues of the grand meristem.
82 Fifteen measurements of vascular bundle diameter were made for each of the various treatment
83 samples and the control at × 100 magnifications using a calibrated microscope.

84 Data collected were subjected to a two-way analysis of variance via a univariate analysis using
85 IBM statistical package for social sciences (SPSS) version 20. Significant means were separated
86 using least significant difference test generated from GenStat Discovery Edition 4 statistical
87 package.

88

89 RESULTS

90 The analysis of variance on the effect of crude oil concentrations and the varietal effect on the
91 vascular bundle sizes were highly significant ($P < 0.001$) across the effect of the varieties,

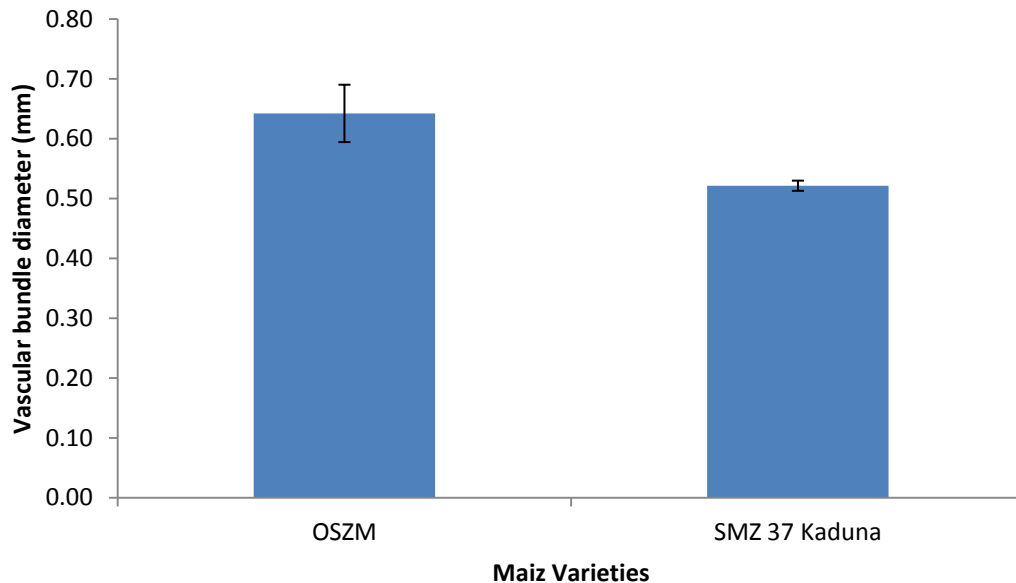
92 concentration and the combined effect of maize variety interaction with crude oil concentration
 93 (Table 1).

94 **Table 1: Analysis of Variance (ANOVA) showing the effect of crude oil concentration on**
 95 **two varieties of maize**

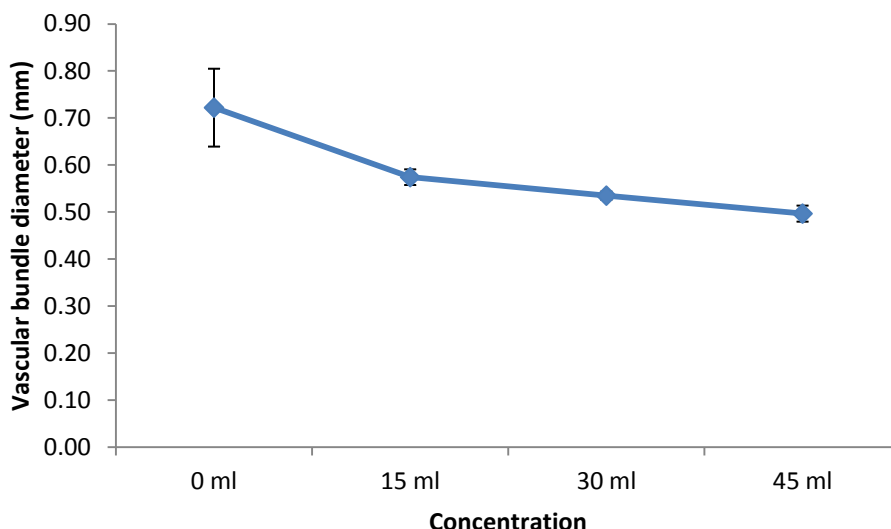
Source of Variation	Sum of Squares	Degree of freedom	Mean Square	F
Variety	0.09	1	0.09	139.67***
Concentration	0.18	3	0.06	93.06***
Variety × Concentration	0.13	3	0.04	67.69***
Error	0.01	16	0.001	
Total	0.40	23		

96 ***- Significant at $P < 0.001$

97 The mean differences of the vascular bundle diameter across the varieties as present on Fig. 1
 98 shows that Oba Super Zaria Maize (OSZM) had significantly ($P < 0.05$) wider vessels as
 99 compared to SMZ 37 Kaduna. As recorded in Fig 2, there was a crude oil dose dependent effect
 100 on the vascular bundle. As compared to the control which had an average vascular bundle of 0.72
 101 $\pm 0.08\text{mm}$, a significant ($P < 0.05$) decrease in vascular bundle with increasing concentration was
 102 observed with 45ml concentration having the smallest vessels (Fig 2).



104 **Fig 1: Mean vascular bundle size of two maize varieties exposed to varied concentration of**
 105 **crude oil**



106
 107 **Fig 2: Mean crude oil concentration effect on the vascular bundle of two maize variety**

108 At 0 ml of crude oil, Oba Super Zaria maize, (OSZM) had a mean diameter of 0.91 ± 0.00 mm
 109 which was significantly higher ($p < 0.05$) than every other treatment combinations. This was
 110 followed by the vascular bundle of the same variety exposed to 15 ml of crude oil with an
 111 average diameter of 0.61 ± 0.01 mm which was also significantly wider as compared to higher
 112 concentrations (Table 2).

113 The smallest vascular bundle of 0.49 ± 0.03 mm was observed from SMZ 37 Kaduna variety
 114 exposed to 45ml concentration of crude oil. This was however not significantly different from
 115 the vascular bundle observed from OSZM variety treated with the same concentration (45ml)
 116 and SMZ 37 Kaduna variety exposed to 30ml (Table 2).

117
 118 **Table 2: Interactive effect of crude oil concentration on the vascular bundle size (mm) of**
 119 **two maize varieties**

Concentration	Super Zaria Maize	SMZ 37 Kaduna
0 ml	0.91 ± 0.00^a	0.54 ± 0.00^{cd}
15 ml	0.61 ± 0.01^b	0.54 ± 0.00^{cd}
30 ml	0.55 ± 0.01^c	0.52 ± 0.01^{cde}
45 ml	0.50 ± 0.02^{ce}	0.49 ± 0.03^e

120 *Means with different alphabets on the table are significantly different using Least Significant Difference (LSD) at
 121 $P < 0.05$

122 **DISCUSSION**

123 The study showed that an increase in crude oil concentration in the soil decrease the diameter of
124 the vascular bundle in maize. This might possibly be attributed to the differences in polarity
125 between water and crude oil. Water is a high polar solvent while hydrocarbon oil (crude oil) is a
126 non polar solvent, therefore a mixture of water and hydrocarbon oil will result in formation of
127 two layers, with the hydrocarbon oil layer above and the water below. However, pollution of soil
128 with this mixture of water and hydrocarbon oil would possibly result in the masking or shielding
129 of water molecules and other minerals in the soil by the hydrocarbon oil. As a result of this, there
130 would be no or less uptake of water and other nutrient contained in the soil by plant. Thus in turn
131 would lead to shrinking of the vascular bundles and retardation of plants growth within polluted
132 environment due to impose draught by hydrocarbon oil. Increasing the concentration of crude oil
133 led to a proportional increase in the rate of shrinking of the vascular bundles and hence,
134 reduction in the size of the plant. This reduction of vascular bundle will also lead to slow growth
135 and low yield of grains. These negative effects of crude oil pollution on crops had been earlier
136 highlighted by Odu (1983), Bello *et al.* (1999), Ekundayo *et al.* (2001) and Dung *et al.* (2008).
137 Therefore, this study supported the findings of the above named authors and confirmed that the
138 results were similar from the point of view of output reduction on crude oil pollution. Similarly
139 Ubochi *et al.* (2019) had reported decrease in nutrients uptake by plants as a result of the oil
140 content in coconut oil effluent.

141 In view of the dose-dependent decrease in vascular bundle of maize, Anoliefo and Edegbai
142 (2001) reported that low level of oil in pollution could be easily be degraded by natural
143 rehabilitation in soils, increase organic matter in soil and improve the fertility, physical and
144 chemical properties of the soil. The differences in the size of vascular bundles of maize varieties
145 in response to crude oil levels corroborates the reports of Baker (1970) and Naegele (1974) that
146 the effect of crude oil on plants is dependent on the variety amongst other factors. The
147 differences in plants reaction to pollution are due to an innate genetic response of the plant
148 system as modified by environmental influences (Baker, 1970; Naegele, 1974). Based on the
149 results obtained from this investigation, OSZM appeared to be susceptible to soils affected with
150 crude oil than the SMZ 37 Kaduna variety.

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