Pollution, 4(1): 183-193, Winter 2018
DOI: 10.22059/poll.2017.240286.306
Print ISSN: 2383-451X Online ISSN: 2383-4501
Web Page: https://jpoll.ut.ac.ir, Email: jpoll@ut.ac.ir

Responses of Accessions of Zea Mays to Crude Oil Pollution Using Growth Indices and Enzyme Activities as Markers

Njoku, K. L., Okporuanefe, F. O. and Ude, E. O.

Department of Cell Biology and Genetics, Environmental Biology Laboratory, University of Lagos, Akoka Lagos, Nigeria

Received: 22.08.2017 Accepted:23.10.2017

ABSTRACT: The performance of every plant in an environment is an indicator on how the plant can withstand various environmental conditions. This study investigates the toxicity of crude oil on growth performance, chlorophyll contents, enzymatic activities, and oxidative stress biomarkers of eight accessions of *Zea mays*. It assays growth enzyme activities (for amylase and invertase) as well as oxidative stress biomarkers (malondialdehyde, peroxidase, superoxide dismutase, and catalase) in *Z. mays*, using spectrophotometric method. The maize accessions have been grown in experimental pots with crude oil treatments (2%, 4%, 6%, 8%, and 10%) and harvested after 14 days of seedling emergence. Results show that the percentage seedling emergence, leaf size, root length, stem girth, and shoot length of each accession have decreased significantly ($p<0.001$, $p<0.01$, and $p<0.05$) as pollution level has ascended. Significant differences in chlorophyll content have also been observed in the plants grown in soil samples, polluted by crude oil, compared to non-polluted soil ($p<0.0001$; $p<0.01$; $p<0.05$) with a decrease in growth enzymes activities as well as oxidative stress biomarkers at 10% pollution. TZE Comp 5 accession and BR-9928 DMR SR-Y have been the most resistant and the most sensitive accessions, respectively. Results suggest that parameters, activities, and expression levels of growth enzymes as well as oxidative stress responses can be used as biomarkers to evaluate the influence of crude oil on the growth of *Z. mays*. They also suggest that there are intraspecific differences in the responses of the accessions of *Z. mays* to crude oil pollution.

Keywords: Pollution, Plant performance, Phytotoxicity, Oxidative stress, Biomarkers

INTRODUCTION

Crude oil contributes a large quota to environmental pollution (Etiosa and Agho, 2007), causing critical environmental and health defections. Considered toxic to both plants and animals, leading even to their demise (Merkl *et al*., 2005), it also can contribute to groundwater pollution, loss of thousands of hectares of farm lands, soil infertility, low crop production, and loss of biodiversity (Shanky, 2013), altering species/population structures through appearance or disappearance of an indicator species (Shanky, 2013). It can result in the loss of biodiversity.

Crude oil in soil is devastating to plant communities (Gbadebo and Adenuga, 2012), causing some changes in community and ecosystem structure such as species richness/diversity, dominance, abundance, food chain length/complexities, biotic indices, and biomass (Shanky, 2013). Studies on crude oil exposure to plants have found what releases Reactive Oxygen Species (ROS) and free radicals, which induce oxidative stress

* Corresponding author, Email: kecynjoku@gmail.com, knjoku@unilag.edu.ng

and lipid peroxidation (Blokhina *et al.,* 1999; Olubodun and Eriyamremu, 2013). Thus, crude oil pollution may induce biochemical changes, manifested by physiological and morphological responses like emergence, growth, or mortality rates. The effects of crude oil on the growth and performance of plants have been reported in many studies (Ekundayo *et al.,* 2001; Njoku *et al.,* 2008; Omosun *et al.,* 2008; Olubodun and Eriyamremu, 2013) that have shown morphological aberrations and other abnormalities like yellowing, dropping, and complete shedding of leaves and even death in plants, exposed to heavy crude oil pollution.

The importance of using plants to study toxicity of chemicals has been reported by several researchers. According to Omosun, *et al*. (2008) and Njoku, *et al.* (2011), plant responses to pollutants provide a simple and cost-effective method of monitoring environmental pollutants. They also help choosing the crops that can be planted in such polluted areas for agricultural purposes or plants, capable of phytoremediation of such polluted area. The present study aims at determining the responses of eight accessions of *Zea maize* to crude oil pollution, using growth performances and biochemical activities as toxicity markers.

MATERIALS AND METHODS

The Bonny light crude oil, used for the study, was obtained from Warri Refinery and Petrochemical Company, Delta state, Nigeria. Loamy soil was obtained from an abandoned farm land at Ikorodu, Lagos State. Eight accessions of maize (*Zea mays*) seeds (ART/98-SW6-OB-W, BR-9928 DMR SR-Y, TZBR-ELD 3, DMR-ESR-Y, TZE Comp 5, TZPB-SR-W, ART/98-SW1-Y, and BR-9943-DMR-SR-W) were obtained from the Institute of Agricultural Research and Training, Ibadan, Oyo State, Nigeria.

In order to achieve crude oil pollution of 2%, 4%, 6%, 8%, and 10% v/w, 500g of sandy loamy soil was measured into each pot and treated with 10ml, 20ml, 30ml, 40ml, and 50ml of crude oil, respectively. The control samples did not receive any treatment. Each treatment was replicated three times, giving a total of 96 pots in all. The polluted soil was left for five days for stabilization.

Seed viability was done using floatation method (Abolfazi, *et al*., 2016). Three viable seeds of each accession were sown in each pot at a depth of about two centimeter as described in Olubodun and Eriyamremu, (2013). The percentage of seedling emergence was calculated, using the formula described in Njoku *et al.* (2008):

$$\text{Percentage emergence} = \frac{\text{Number of seedlings that emerged}}{\text{Number of seeds sown}} \times 100$$

The shoot length, leaf size, plant girth, and root length of each treatment were measured on the 14th day of the germination, using a metre rule (Ogbuehi, *et al.,* 2014), while the plant girth was measured using the micrometer screw gauge (Olubodun and Eriyamremu, 2013). The leaf size was estimated using the formula below:

Leaf size = Length of the longest part of leaf X Breath of the widest part of leaf X 0.75 (Ogbuehi, *et al.,* 2014).

Biochemical analysis was done using chlorophyll content and enzyme activity with the chlorophyll content of *Z. mays* leaves, determined by means of Olubodun and Eriyamremu, (2013) methodology. The amylase and invertase activities got assayed via Somagi (1944) method, while malondialdehyde levels (lipid peroxidation), superoxide dismutase activity, and catalase activities were assayed in accordance with the methods of Nahed, (2011) and peroxidase according to Olubodun and Eriyamremu, (2013) methodology.

Two-way Analysis of Variance (ANOVA) was employed to test the group

means' differences with Turkey's multiple comparison test used to determine the significant variations among the means. Statistical significance differences were tested at $p<0.001$, $p<0.01$ and $p<0.05$. All analyses were carried out, using Graphpad Prism 7.0 software.

RESULTS AND DISCUSSION

The impact of different crude oil levels on maize seedlings emergence varied (Figure 1) and influenced maize emergence significantly. The seedlings of BR-9928/DMR-SR-Y and BR-9943-DMR-SRW had the least percentage emergence (15% seed emergence at 10% treatment for each). In average, TZE-Comp 5 and TZPB-SR-W accessions had the best percentage emergence across all treatments (80% and 60% seed emergence at 10% treatment respectively). The emergence of the seedling in different treatments varied significantly from one another ($p<0.001$, $p<0.01$ and $p<0.05$).

Non-germination of some seeds along with the death of some accessions' seedlings after germination suggests the lethal nature of crude oil at high concentrations. Different response levels, demonstrated by the accessions and recorded in this study, suggest that the effect of crude oil on lethality and survival rate to different plants does indeed vary. According to Ogbuehi *et al.* (2014), the presence of oil and level of pollution in the soil affects germination and, subsequently, plants' growth in such soil. Similar effects were observed in this study, too. The effect could be due to the formation of polar compounds dissolved in water that can penetrate the seed coat, exerting polar narcosis (Adam and Duncan, 2002; Ogbuehi *et al.*, 2014). Reduction in percentage emergence of seedlings was also recorded for six agronomic plants by Issoufi *et al*. (2006). The results in this study also agreed with the works of Anoliefo and Vwioko (1995) as well as Anoliefo and Edegbai (2000), stating that hydrocarbons can inhibit plant growth. Atuanya (1997) reported that the seeds, sown in the soil which is polluted with crude oil, either fail to germinate at all or undergo retarded growth. These findings conform with the non total germination and death of some seedlings, observed in this study. The higher emergence percentage of TZE-Comp 5 and TZPB-SR-W in the contaminated soil suggests that these accessions have better resistance to crude oil impact or have better adaptive qualities such as invertase activities, noticed in this study; however, crude oil pollution can be detrimental to food security as a result of non-germination or non-survival of these crop plants.

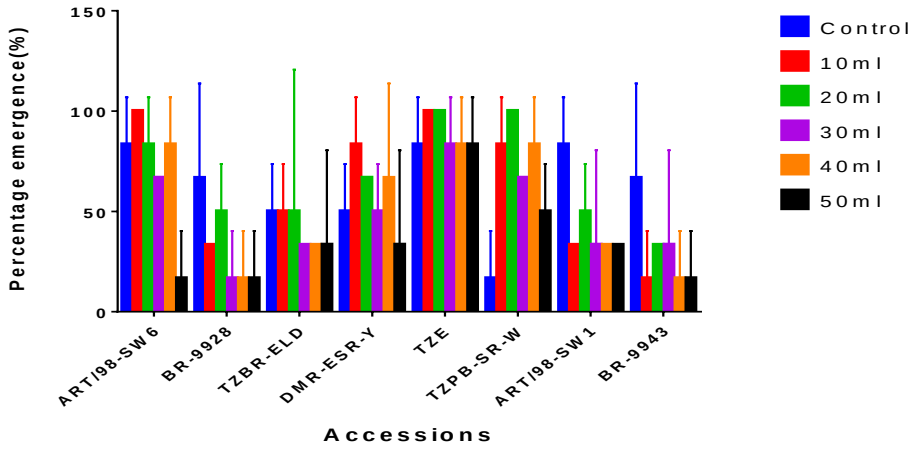

Fig. 1. Emergence percentage of different accessions of *Z. mays*, treated with various concentrations of crude oil. Values are mean ± standard error of double-replicate treatment

Table 1 shows the shoot length of *Z. mays* accessions, exposed to crude oil pollutionn. The shoot length of the seedlings of the accessions in the different treatments significantly differed from each other ($p<0.001$, $p<0.01$, and $p<0.05$). Generally, the seedlings in 0% treatment had the highest shoot length which lessened as the crude oil level increased with BR-9928-DMR-SR-Y and DMR-ERS-Y acting as exceptions, not following any particular order, whatsoever. In all, the TZE Comp 5 and TZPB-SR-W had better shoot lengths across all treatments while those of BR-9928 accession were the least.

Variations of plant height among the accessions could be largely because of intraspecific differences in plants' genetic activity since they are of the same species (Akinola and Njoku, 2007; Njoku *et al.*, 2011). The observed reduction in the height of maize plants, subjected to higher doses of oil, is similar to the findings of Agbogidi *et al*. (2007) which can be attributed to some reasons given in previous studies concerning the effects of oil pollution on soil and plants which include water disruption and disruption of nutrient uptake (Njoku *et al.* 2008) and depletion of nitrogen and phosphorus contents of the soil (Baran *et al.*, 2002), resulting in oxidative stress (Alscher, *et al.* 2002). Plants' stress from crude oil pollution may lead to yellowing of leaves, loss of photosynthetic ability, and general physiological weakening of the plants which cause their demise in the long run, as seen in some of the two-week-old plants, exposed to higher percentages of pollution. As the study showed, reduced growth can be a symptom of extreme nutrient deficiency in plants. Also, previous work on plants' exposure to crude oil and heavy metals reported that it led to reactive oxygen species and other free radical, which induce oxidative stress and cause lipid peroxidation (Bliokhina *et al.* 1999; Olubodun and Eriyamremu, 2013), thus inhibiting plant growth. This could also be the reason for reduced growth and death of some accessions, noticed in this study.

The root lengths of the accessions did not follow any particular trend; however, there were some significant differences among the accessions at $p<0.001$, $p<0.01$, and $p<0.005$. The TZBR-ELD-3 in 10% treatment had the smallest roots (1.00±1.00cm). ART/98-SW6-OB-W, BR-9928-DMR-SR-Y, and DMR-ESR-Y accessions at 10% treatment did not survive up to the 14th day, hence having no roots. Similarly, at 8% treatment of BR-9928-DMR-SR-Y and TZBR-ELD-3 accessions did not survive and had no roots, as a result. The longest root belonged to 2% treatments of TZBR-ELD-3 and DMR-ESR-Y as well as 4% and 6% treatments of TZPB-SR-W accessions, being 30.15±3.85 cm, 26.50±0.50 cm, and 28.65±1.35 cm, respectively. Generally the root lengths of BR-9928-DMR-SR-Y accessions across all treatments showed the poorest root development, having the shortest roots.

Table 1. The Shoot Length of *Z. mays* Accessions, exposed to crude oil pollution (NS = no survivor). Values in same row with the same superscript do not differ from each other significantly

Accessions	Control (0.00ml)	2% (10ml)	4% (20ml)	6% (30ml)	8% (40ml)	10% (50ml)
ART/98-SW6	43.75^{a} ±3.25	37.00^{b} ±2.00	33.35^{c} ±0.65	33.25^{b} ±0.75	15.15^{d}±15.15	NS
BR-9928 DMR	17.85^{b} ±17.85	36.00^{a} ±2.00	12.75^{c}±12.75	11.50^{c} ±11.50	14.00^{c} ±14.00	NS
TZBR-ELD 3	41.00^{a} ±2.00	30.15^{b} ±3.85	12.75^{c} ±12.75	29.75^{b}±1.25	NS	3.50^{d} ±3.50
DMR-ESR-Y	46.00^{a} ±1.00	46.75^{a} ±1.75	36.25^{b} ±2.25	29.75^{c} ±0.75	13.50^{d} ±13.50	NS
TZE Comp 5	42.50^{a} ±3.50	38.50^{b} ±2.50	31.25^{c} ±1.25	39.25^{b} ±1.25	29.25^{c} ±0.75	30.50^{c}±3.50
TZPB-SR-W	24.50^{d} ±24.50	39.85^{a} ±1.15	36.15^{b} ±2.45	35.00^{b} ±1.50	26.75^{d} ±0.25	32.25^{c} ±0.25
ART/98-SW1	39.10^{a} ±3.40	38.50^{a} ±4.50	33.00^{b} ±0.00	10.50^{d} ±10.50	12.00^{d} ±12.00	14.50^{c}±14.50
BR-9943-DMR	44.30^{a}±6.70	20.00^{b} ±20.00	45.50^{a} ±0.50	16.50^{c} ±16.50	16.50^{c} ±16.50	8.00^{d} ±8.00

Table 2. The root lengths of *Z. mays* accession, exposed to crude oil pollution (NS = no survivor). Values in the same row with the same superscript do not differ from each other significantly

Accessions	Control (0.00ml)	2% (10ml)	4% (20ml)	6% (30ml)	8% (40ml)	10% (50ml)
ART/98-SW6	21.25^{a} ±0.25	22.00^{a}±0.00	19.15^{a} ±1.15	21.50^{a} ±0.50	9.00^{b} ±9.00	NS
BR-9928 DMR	9.75^{b} ±9.75	16.50^{a} ±1.50	9.75^{b} ±9.75	12.00^{b} ±12.00	NS	NS
TZBR-ELD 3	24.50^{b} ±2.50	30.15^{a} ±3.85	10.80^{d} ±10.80	18.00^{c} ±3.00	NS	1.00^{e}±1.00
DMR-ESR-Y	24.50^{a}±3.50	26.50^{a} ±0.50	21.25^{b} ±0.75	17.00^{c} ±1.00	6.00^{d} ±6.00	NS
TZE Comp 5	23.00^{a} ±1.00	21.75^{a} ±0.25	20.75^{a}±1.25	19.75^{a} ±0.25	19.75^{a}±2.25	22.25^{a}±0.7
TZPB-SR-W	9.00^{c} ±9.00	23.25^{b} ±7.25	28.65^{a} ±1.35	28.85^{a} ±3.15	20.25^{b}±2.75	23.25^{b}±0.75
ART/98-SW1	22.00^{a} ±2.00	22.50^{a} ±1.50	18.75^{b}±1.25	7.75^{c} ±7.75	5.00^{c} ±5.00	7.00^{c} ±7.00
BR-9943-DMR	20.00^{a} ±2.00	9.50 ±9.50	19.00^{a}±0.00	9.50^{b}±9.50	10.50^{b}± 10.50	9.00^{b} ±9.00

The leaf size in different treatments varied from one another significantly ($p<0.001$, $p<0.01$, and $p<0.05$) (Table 3). Generally, the leaf size of TZPB-SR-W accession at all treatments was the largest (22.97 ±0.05cm^2 at 10% treatment). The reduction in the size of the test plants' leaves may be attributed to the drought, caused by crude oil which creates some conditions that limit water supply to the plants (Ogbuehi *et al*., 2014), form a hydrophobic layer over the root limiting the absorption of both water and nutrients (Omosun *et al.*, 2008), and decrease the level of phytohormones such as auxins (Reem *et al*'., 2012). Leaf size gives understanding of the interaction between plant growth and environment while determining a crop's productivity (Ogbuehi, *et al.*, 2014). The larger the leaf size, the higher the amount of light energy absorbed by such plant and, invariably, the higher the amount of light, the greater the rate of photosynthesis and, consequently, the amount of food produced by the plants (Ogbuehi *et al*., 2014). Thus the reduced leaf size, observed in this study, implies reduction of photosynthesis as a result of crude oil contamination, hence less primary productivity.

There was a significant reduction of stem girth as crude oil pollution increased (Table 4). The stem girth of 0% treatment was the highest in almost all accessions. For TZBR-ELD 3, DMR-ESR-Y, and BR-9943-DMR, the stem girth was the lowest at 10% treatment, yet in other accessions it was the lowest in 8%. BR-9928-DMR-SR-Y had the least stem girth in all treatments while TZE-Comp 5 had the highest, with TZPB-SR-W accession following closely.

Table 3. The leaf size of *Z. mays* accession, exposed to crude oil pollution (NS = no survivor). Values in the same row with the same superscript do not differ from each other significantly

Accessions	Control (0.00ml)	2% (10ml)	4% (20ml)	6% (30ml)	8% (40ml)	10% (50ml)
ART/98-SW6	37.79^{a} ±0.05	29.82^{b} ±0.05	25.35^{b} ±0.10	24.66^{b} ±0.10	6.58^{c} ±0.65	NS
BR-9928 DMR	7.31^{b} ±0.65	25.31^{a} ±0.05	4.13^{b} ±0.55	3.71^{b} ±0.55	4.33^{b} ±0.55	NS
TZBR-ELD 3	27.68^{a} ±0.10	9.38^{b} ±0.05	4.84^{b} ±0.60	22.95^{a} ±0.10	NS	0.28^{c} ±0.25
DMR-ESR-Y	34.13^{a} ±0.00	34.43^{a} ±0.05	23.93^{b} ±0.05	18.36^{b} ±0.00	1.69^{c} ±0.25	NS
TZE Comp 5	39.11^{a} ±0.10	25.65^{b} ±0.00	21.83^{b} ±0.00	27.38^{b} ±0.05	15.32^{c} ±0.05	15.32^{c} ±0.05
TZPB-SR-W	8.33^{c} ±0.60	27.89^{a} ±0.00	27.44^{a} ±0.00	27.54^{a} ±0.00	20.48^{b} ±0.00	22.97^{a} ±0.05
ART/98-SW1	27.42^{a}±0.05	27.00^{a} ±0.00	21.99^{a} ±0.05	2.81^{b} ±0.50	2.55^{b} ±0.40	3.75^{b}±0.50
BR-9943-DMR	37.90^{a} ±0.05	7.31^{b}±0.65	33.28^{a} ±0.05	5.51^{b} ±0.60	4.69^{b}±0.50	0.68^{c} ±0.20

Table 4. Stem Girth of maize accessions, exposed to crude oil pollution (NS = no survivor). Means with the same superscript along the horizontal array indicate no significant difference (P ≤ 0.05)

	Control (0.00ml)	2% (10ml)	4% (20ml)	6% (30ml)	8% (40ml)	10% (50ml)
ART/98-SW6	3.25^{a} ±0.15	2.56^{a} ±0.26	2.37^{a} ±0.21	2.65^{a} ±0.05	1.04^{a} ±1.04	NS
BR-9928 DMR	1.31^{a} ±1.31	2.67^{a} ±0.22	1.41^{a} ±1.41	1.15^{a} ±1.15	0.90^{a} ±0.90	NS
TZBR-ELD 3	2.87^{a} ±0.13	2.20^{a} ±0.20	1.05^{a} ±1.05	2.01^{a} ±0.20	NS	0.84^{a} ±0.84
DMR-ESR-Y	3.53^{a} ± 0.37	2.77^{a} ±0.13	2.62^{a} ±0.24	2.63^{a} ±0.17	1.35^{a} ±1.35	NS
TZE Comp 5	2.99^{a} ±0.33	2.58^{a} ±0.06	2.34^{a} ±0.06	2.43^{a} ±0.13	2.19^{a} ±0.15	2.23^{a} ±0.06
TZPB-SR-W	1.20^{a} ±1.20	2.81^{a} ±0.07	2.73^{a} ±0.03	2.50^{a} ±0.19	2.42^{a} ±0.065	2.53^{a} ±0.10
ART/98-SW1	3.09^{a} ±0.29	2.84^{a} ±0.21	2.51^{a} ±0.06	1.03^{a} ±1.03	1.00^{a} ±1.00	1.10^{a} ±1.10
BR-9943-DMR	3.61^{a} ±0.06	1.40^{a} ±1.40	2.97^{a} ±0.25	1.12^{b} ±1.12	1.13^{b} ±1.13	0.95^{b} ±0.95

Generally, the chlorophyll contents of the accessions did not follow any particular order (figure 2). TZE-Comp 5 and TZPB-SR-W accessions had the highest chlorophyll content in all treatments while BR-9928-DMR-SR-Y and BR-9943-DMR-SR-W accessions had the lowest. As for ART/98-SW6-OB-W, its chlorophyll content at 10% treatment was considerably lower than that of the control ($p<0.05$ and $p<0.01$). The variations in the chlorophyll content of different accessions can be attributed to the differences in their genetic activities. The reduced chlorophyll content of the plants in crude-oil-polluted soil may also cause leaves' yellowing, loss of photosynthetic ability of the plants, and –hence—reduced productivity and plant yields

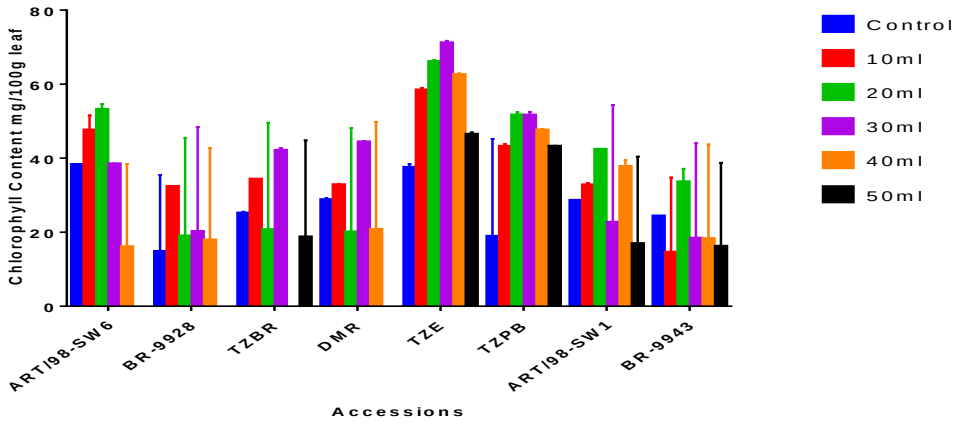

Fig. 2. The Chlorophyll content of different *Z. mays* accession treated with varied concentrations of crude oil. Values are mean ± standard error of two replicates.

Table 5 shows the enzyme activities of maize plants, exposed to crude oil pollution. The amylase activity in dissimilar treatments differed significantly from one another ($p<0.001$, $p<0.01$, and $p<0.05$) in case of each plant. The amylase activity was highest in TZE-Comp 5 (13.39 ±0.15*U/mg*) at 8% pollution followed by TZPB-SR-W (12.88 ±0.04 *U/mg*) at 8% pollution, while it was the lowest in BR-9928-DMR-SR-Y accessions in all treatments. Generally, amylase activity was significantly increased by crude oil pollution, except for ART/98-SW6 (at 10%), BR-9928 DMR (at 10%), TZBR-ELD 3 (at 8%) and DMR-ESR-Y (at 10%) which did not survive.

The invertase activity in TZE-Comp 5 (3.97 ±0.01 *U/mg*) at 4% treatment was higher than the other accessions in all treatments, while it was the lowest in BR-9928-DMR-SR-Y accession (0.49 ±0.49 *U/mg*) at 8% level of pollution. Invertase activities in the different treatments differed significantly from each other along with

from the treatments at $p<0.001$, $p<0.01$, and $p<0.05$ level of confidence. Invertase activity depended on the dose: the higher the concentration, the lower the activity.

The significant amount of amylase and invertase present in these plants may be due to the role they play in supporting plants' growth by converting sucrose and starch into monosaccharide in order to provide energy for respiration (Anigboro and Tonukari, 2008). Reduced enzymes in plants, treated with high levels of crude oil, suggest that it could act as an inhibitor that inhibited/interfered with the activities of these enzymes, perhaps the reason behind the observed reduced growth. Anigboro and Tonukari (2008) reported decreased amylase and invertase activities in cowpea, due to crude oil pollution. They suggested that reduced amylase and invertase activities may have been accompanied by nutrient mobilization such as sucrose and that mobilization of sucrose for metabolic activities was essential to support growth in germinating seeds. Denniston, *et al* (2001) asserted that amylase and invertase hydrolyses of starch gave rise to diverse products such as dextrins and glucose, needed for growth and development of plants in maintaining adequate water level. Therefore, the reduced amylase and invertase activities might have decreased stem girth and leaf size, observed in this study, which can result in general physiological weakening of the plants. The influence of the amylase and invertase enzymes on plant growth could be inferred in this study, using TZE Comp 5 accession which recorded the highest enzyme activities and had better growth performance, compared to other accession.

The malondiadehyde (MDA) level was the highest in ART/98-SW6 accession (0.82±0.01 *U/mg*) at 6% treatment and the lowest in TZPB-SR-W accession (0.08±0.08 *U/mg*) in the control. For most treatments of various accession, malondiadehyde activity decreased as the pollution increased. Malondiadehyde activity in the treatments did not show any considerable difference within the treatments, themselves, but stood out greatly within the accessions ($p<0.001$, $p<0.01$, and $p<0.05$). Increase in malondialdehyde activity, as observed in our results, suggests that negative biological effects like oxidative stress ascended as the level of crude oil contamination rose, since malondialdehyde is a reliable indicator of free radical formation, itself indicating damages on the tissues due to free radicals (Halliwall and Chirico, 1993). It is also an indicator of lipid peroxidation (Su *et al*., 2015) and that crude oil induces oxidative stress on the accessions.

The peroxidase enzyme activity increased from the control to the 2% treatment in all accessions of *Z. mays* where it initially stood at its zenith, then to decline steadily as pollution percentage increased, with an exception of BR-9943-DMR-SR-W accession. All the accessions (except BR-9943-DMR-SR-W) showed significant variations between the control and the treatments of various accessions at various levels of pollution ($p<0.001$, $p<0.01$, and $p<0.05$). TZPB-SR-W had the highest peroxidase activity (18.1 ±0.09 *U/mg*), while BR-9943-DMR had the lowest (0.01 ±0.01 *U/mg*) at similar levels of pollution.

Superoxide Dismutase (SOD) activity for most of the accessions decreased as the crude oil increased. There was a significant difference in the treatments of various accessions ($p<0.001$, $p<0.01$, and $p<0.05$). BR-9943-DMR had the highest activities (10.26 ±0.02 *U/mg*) while BR-9928 DMR and TZBR-ELD 3 had the lowest (1.37 ±1.37 *U/mg*) in the treatment. Peroxidases are known to be the main oxidative enzyme in plants (Gramss, 2000). Decreases in peroxidase activity as the concentration of crude oil increased after 2% contamination portends that crude oil mediated reduction in peroxidase activity in maize seedlings could contribute to oxidative stress through

its decreased ability to metabolize aromatic hydrocarbon. This could also be the reason for the observed reduced growth arising from the toxicity of crude oil.

Low concentrations of crude oil (2%) raised the activity of Superoxide Dismutases (SOD) enzyme. The maize's response to the stress was an increase in SOD activity at 2% treatments and the subsequent decrease in all accessions, suggesting that these changes were a result of oxidative stress induced by crude oil. This is in accordance with the observations of Alscher *et al.,* (2002) and Gomez *et al* (1999) who reported an increase in SOD in response to UV irradiation and severe salt stress, respectively. Within a cell, the Superoxide Dismutases (SODs) constitute the first line of defense against ROS (Alscher *et al.,* 2002) hence SODs usually increase with stress. However, Olubodun and Enyamremu (2013) reported that maize plants from contaminated soil, the SOD decreased with an increase in crude oil contamination, which confirms the significantly reduced activity of SOD at high crude oil concentrations, observed in this study. Oxidative stress induced by free radicals due to crude oil could have been the reason for the observed reduced growth parameters by overpowering the activities of this enzyme.

Catalase enzyme activity increased as levels of pollution increased in case of most accessions. Catalase activity was least generally in BR-9943-DMR-SR-W accession across all treatments, compared to other accessions, while it was the highest in TZPB-SR-W accession at all treatments. The catalase activity of accessions in the different treatments differed significantly from each other ($p<0.001$, $p<0.01$, and $p<0.05$). TZE Comp 5 had the highest catalase activities (93.14 ±0.330 *U/mg*) while BR-9943-DMR had the lowest (19.82 ±19.82 *U/mg*) in all the treatment. This, too, was reported in previous works by Olubodun and Eriyamremu (2013) and Odjegba and Badejo (2013). Catalase is a very important enzyme that protects the cell from oxidative damage. Increasing its activity could be an adaptive response to increase reactive oxygen species, produced due to crude oil pollution. This also conforms to a previous study by Saborni, *et al.* (2012) who suggested that increasing catalase activities could be an adaptive response to the increase in reactive oxygen species production due to crude oil pollution.

Table 5. Enzyme Activities (*U/mg)* of eight accessions of maize, exposed to crude oil pollution

Enzymes	Accessions	TREATMENT					
		Control (0.00ml)	2% (10ml)	4% (20ml)	6% (30ml)	8% (40ml)	10% (50ml)
AMYLASE (*U/mg*)	ART/98-SW6	2.39 ±0.03	3.91 ±0.03	4.19 ±0.17	5.95 ±0.03	3.71 ±3.71	NS
	BR-9928 DMR	0.84 ±0.85	2.79 ±0.50	1.58 ±1.58	1.62 ±1.62	2.31 ±2.31	NS
	TZBR-ELD 3	1.82 ±0.02	2.97 ±0.02	2.02 ±2.02	5.04 ±0.08	NS	3.12 ±3.12
	DMR-ESR-Y	1.50 ±0.06	2.68 ±0.04	1.97 ±1.97	5.08 ±0.04	2.12 ±2.12	NS
	TZE Comp 5	2.70 ±0.04	4.92 ±0.04	8.70 ±0.04	12.40 ±0.01	13.39 ±0.15	10.36 ±0.03
	TZPB-SR-W	1.18 ±1.18	4.51 ±0.03	9.38 ±0.05	11.3 7±0.01	12.88 ±0.04	10.08 ±0.04
	ART/98-SW1	1.83 ±0.04	3.405 ±0.02	5.18 ±0.02	3.24 ±3.24	8.45 ±0.09	3.61 ±3.61
	BR-9943-DMR	4.00 ±0.35	2.70 ±2.71	6.30 ±0.04	3.83 ±3.83	2.58 ±2.58	2.14 ±2.14
INVERTASE (*U/mg*)	ART/98-SW6	2.86 ±0.21	1.69 ±0.03	1.33 ±0.10	1.04 ±0.01	0.47 ±0.47	NS
	BR-9928 DMR	1.38 ±1.38	2.60 ±0.06	0.96 ±0.96	0.78 ±0.78	0.63 ±0.63	NS
	TZBR-ELD 3	2.45 ± 0.05	2.14 ±0.02	0.93 ±0.93	1.41 ±0.03	NS	0.46 ±0.46
	DMR-ESR-Y	2.41 ±0.05	2.18 ±0.04	1.82 ±0.00	1.575 ±0.15	0.49 ±0.49	NS

		TREATMENT					
	TZE Comp 5	3.48 ±0.04	3.78 ±0.04	3.97 ±0.01	3.68 ±0.13	2.86 ±0.02	2.23 ±0.02
	TZPB-SR-W	1.40 ±1.40	2.87 ±0.02	1.94 ±0.06	1.47 ±0.01	1.24 ±0.02	0.86 ±0.01
	ART/98-SW1	2.43 ±0.02	1.97 ±1.01	1.24 ±0.20	0.52 ±0.52	0.82 ±0.06	0.25 ±0.25
	BR-9943-DMR	3.06 ±0.02	1.26 ±1.26	1.61 ±0.07	0.64 ±0.64	0.53 ±0.53	0.37 ±0.37
Malondiadehyde (*U/mg*)	ART/98-SW6	0.28 ±0.01	0.57 ±0.01	0.75 ±0.01	0.82 ±0.01	0.31 ±0.31	NS
	BR-9928 DMR	0.08 ±0.08	0.43 ±0.01	0.26 ±0.26	0.18 ±0.18	0.16 ±0.16	NS
	TZBR-ELD 3	0.15 ±0.03	0.38 ±0.01	0.24 ±0.24	0.59 ±0.01	NS	0.18 ±0.18
	DMR-ESR-Y	0.23 ±0.01	0.47 ±0.01	0.31 ±0.31	0.72 ±0.01	0.28 ±0.26	NS
	TZE Comp 5	0.16 ±0.01	0.41 ±0.01	0.56 ±0.01	0.64 ±0.01	0.49 ±0.01	0.35 ±0.02
	TZPB-SR-W	0.08 ±0.08	0.38 ±0.02	0.57 ±0.02	0.46 ±0.01	0.39 ±0.01	0.36 ±0.01
	ART/98-SW1	0.23 ±0.01	0.38 ±0.01	0.50 ±0.01	0.28 ±0.28	0.43 ±0.39	0.19 ±0.19
	BR-9943-DMR	0.22 ±0.00	0.21 ±0.21	0.56 ±0.01	0.32 ±0.32	0.39 ±0.39	0.35 ±0.35
Peroxidase (*U/mg*)	ART/98-SW6	5.17 ±0.28	9.43 ±0.21	6.70 ±0.13	3.16. ±0.04	0.67 ±0.67	NS
	BR-9928 DMR	2.92 ±2.92	10.6 ±0.05	2.11 ±2.11	0.81 ±0.81	0.46 ±0.46	NS
	TZBR-ELD 3	6.69 ±0.24	13.8 ±0.39	6.08 ±6.08	8.16 ± 0.24	NS	0.82 ±0.82
	DMR-ESR-Y	7.83 ±0.17	14.5 ±0.26	5.13 ±5.13	4.59 ±0.07	0.98 ±0.98	NS
	TZE Comp 5	8.64 ±0.10	16.3 ±0.15	7.41 ±0.07	2.61 ±0.07	0.94 ± 0.03	0.06 ±0.01
	TZPB-SR-W	4.72 ±4.72	18.1 ±0.09	12.35 ±0.38	3.61 ±0.13	1.38 ±0.02	0.66 ±0.03
	ART/98-SW1	6.56 ±0.22	8.82 ±0.22	4.40 ±0.06	0.94 ±0.94	0.72 ±0.02	0.06 ±0.06
	BR-9943-DMR	4.76 ±0.09	0.88 ±0.88	0.615 ±0.03	0.09 ±0.09	0.04 ±0.04	0.01 ±0.01
SUPEROXIDE DISMUTASE (*U/mg*)	ART/98-SW6	8.55 ±0.11	9.37 ±0.07	6.41 ±0.03	5.39 ±0.02	1.65 ±1.65	NS
	BR-9928 DMR	4.46 ±4.46	7.06 ±0.02	3.12 ±3.12	2.21 ±2.21	1.37 ±1.37	NS
	TZBR-ELD 3	8.05 ±0.01	6.43 ±0.03	2.60 ±2.60	3.51 ±0.05	NS	1.37 ±1.37
	DMR-ESR-Y	7.89 ±0.05	5.77 ±0.01	2.11 ±2.11	3.81 ±0.03	1.81 ±1.81	NS
	TZE Comp 5	6.52 ±0.06	6.11 ±0.02	5.38 ±0.02	5.24 ±0.06	3.91 ±0.02	2.79 ±0.05
	TZPB-SR-W	2.87 ±2.87	4.98 ±0.03	5.41 ±0.03	5.70 ±0.05	4.57 ±0.03	4.28 ±0.02
	ART/98-SW1	6.80 ±0.04	5.92 ±0.02	6.84 ±0.05	2.61 ±2.61	5.25 ±0.53	1.94 ±1.94
	BR-9943-DMR	9.23 ±0.13	4.84 ±4.84	10.26 ±0.02	4.42 ±4.42	4.11 ±4.11	3.85 ±3.85
CATALASE (*U/mg*)	ART/98-SW6	25.60 ±0.945	40.05 ±0.190	34.07 ±0.455	79.04 ±0.410	43.27 ±43.27	NS
	BR-9928 DMR	12.88 ±12.88	44.20 ±0.040	29.72 ±29.72	37.27 ±37.27	46.17 ±46.17	NS
	TZBR-ELD 3	26.91 ±0.065	38.17 ±0.260	32.73 ±32.73	83.82 ±0.400	NS	49.32 ±49.32
	DMR-ESR-Y	27.35 ±0.390	47.25 ±0.360	33.94 ±33.94	86.82 ±0.590	47.60 ±47.60	NS
	TZE Comp 5	28.36 ±0.065	18.43 ±0.215	39.47 ±0.015	89.00 ±0.210	2.700 ±0.540	93.14 ±0.330
	TZPB-SR-W	13.80 ±0.085	55.55 ±0.070	73.82 ±0.555	93.17 ±0.095	2.550 ±0.190	21.85 ±0.220
	ART/98-SW1	26.81 ±0.085	42.76 ±0.405	70.15 ±0.235	44.21 ±44.21	14.01 ±0.295	61.81 ±61.81
	BR-9943-DMR	22.52 ±0.220	19.82 ±19.82	46.02 ±0.375	34.36 ±34.36	39.79 ±39.79	45.61 ±45.61

NS = NO SURVIVOR

CONCLUSION

This study demonstrated that high concentrations of crude oil can bring about adverse effects on maize, including inhibition of growth parameters (such as seed emergence, shoot length, leaf size, root length, stem girth, etc.) as well as chlorophyll and oxidative stress enzyme (such as superoxide dismutase, peroxidase, and malondialdehyde), not to mention induction of catalase activities. The differences in maize response to crude oil could have arisen largely because of intraspecific differences in their genetic make up. The present results

suggest that the growth parameters, activities, and expression levels of oxidative stress enzymes can be used as biomarkers to evaluate the influence of crude oil as well as potentials in phytoremediation, especially TZE Comp 5 and TZPB-SR-W accession.

REFERENCES

Abolfazi, D., Mulualem, T., Assaddollar, K. and Per-Christer, O. (2016). Floatation techniques to improve viability of *Juniperus polycarpos*. *Journal for Resource*, 28b(2):231-238

Adam, G. and Duncan H. (2002). Influence of Diesel on Seed Germination. *Environmental Pollution*, 120:363-370.

Agbogidi, O.M., Eruotor, P.G. and Akparobi, S.O. (2007). Effects of Crude Oil Levels on the Growth of Maize. *American Journal of Food Technology*. 2:526 – 535.

Akinola, M.O. and Njoku, K.L. (2007). Mutagenic effect of crude oil on accessions of *Glycine max* L. (Merril). *Pakistan Journal of Scientific and Industrial Research*,50: 330 -334.

Alscher R.G., Erturk, N. and Heath, L.S. (2002). Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *Journal of Experimental Botany*, 53:1331– 1341.

Anigboro, A. A. and Tonukari, N. (2008). Effect of crude oil on invertase and amylase activities in cassava leaf extract and germinating cowpea seedlings. *Asian Journal of Biological Science*, 1:50-60.

Anoliefo, G.O. and Edgbai, B.O. (2000). Effect of spent engine oil as a soil contaminant on th growth of two egg plant species, *Solanum melongna* (L) and *S. incanum* (L). *Journal of Agriculture*, 1:21-25

Anoliefo, G.O. and Vwioko D.E. (1995). Effect of spent lubricating oil on the growth of *Caspium annum* and *Lycopersion esculentum*. *Environmental Pollution*, 88:306-314

Atuanya, E. I., (1997). Effect of waste engine oil pollution on physical and chemical properties: A case study waste oil contaminated soil in Bendel state, Nigeria.*Journal of Applied Science*, 5, 151-155.

Baran, S., Bielinska, E.J. and Wojcikowska-Kapusta, A. (2002). The formation of enzymatic activity of soil contaminated by petroleum products. *Acta Agrophisica*., 70: 9-19.

Blokhina, O.B., Fagerstedt K.V. and Chirkova, T.V. (1999). Lipid peroxidation and antioxidant systems under anoxia in plants differing in their tolerance to oxygen deficiency. *Russian Journal of Plant Physiology*, 45:55 – 62.

Denniston, K. J., Caret, R. L., and Toppig, J. J. (2001). *General, Organic and Biochemistry*. 3rd Ed. McGraw-Hill, New York. pp: 819.

Ekundayo, E. O., Emede, T.O., and Osayande, D.I. (2001). Effects of crude oil spillage on growth and yield of maize (*Zea mays* L.) in soils of mid-western Nigeria. *Plant Foods for Human Nutrition*, 56 (4): 313-324.

Etiosa U. and Agho, M. (2007). *Coping with Climate Change and Environmental Degradation in the Niger Delta of Southern Nigeria*: Community Research and Development Centre (CREDC) Nigeria. 30pp

Gbadebo, A.M. and Adenuga, M.D. (2012). Effects of crude oil on the emergence and growth of cowpea in two contrasting soil types from Abeokuta, Southwestern Nigeria. *Asian Journal of Applied Science*, 5:232 – 239.

Gomez J. M, Hernandez J. A, Jimenez A., del Rio L. A. and Sevilla F. (1999). Differential response of antioxidative enzymes of chloroplasts and mitochondria to long-term NaCl stress of pea plants. *Free Radical Research*, 31:11–18.

Gramss, G., (2000). Degradation of aromatic xenobiotics in aerated soils by enzyme systems of microorganisms and plants. In: Wise, D.L., Trantolo, D.J., Cichon, E.J., Inyang, H.I., Stottmeister, U. (Eds.), *Bioremediation of Contaminated Soils*, Marcel Dekker Inc., New York, Basel, pp. 489–535.

Halliwall, B.and Chirico, S. (1993). Lipid peroxidation: its mechanism, measurement and significance. *American Journal of Clinical Nutrition*, 57: 715-725.

Issoufi, I, Rhykerd, R.L. and Smiciklas, K. D. (2006). Seedling growth of agronomic crops in crude oil contaminated soil. *Journal of Agronomy and Crop Science*, 192: 310-317.

Merkl, N., Schultze-Kraft R. and Infinite C. (2005). Assessment of tropical grasses and legumes for phytoremediation of petroleum contamination. *Soil, Water and Air Pollution*,165 (114):195-209.

Nahed S. G. (2011). Oxidative stress and antioxidant enzymes in *Oreochromis niloticus* as biomarkers of exposure to crude oil pollution. *International Journal of Environmental Science and Engineering* (IJESE), 1: 49-58.

Njoku, K.L., Akinola M.O. and Oboh, B.O. (2008). Growth performance of *Glycine max* L. (Merrill) grown in crude oil contaminated soil

augmented with cow dung. *Nature and Science*, 6(1):48 – 56.

Njoku, K.L., Akinola, M.O. and Oshodin, O.R. (2011). Phytotoxicity Assay of Crude Oil Using Different Accessions of *Sorghum bicolor*. *World Applied Sciences Journal*, 15(1): 38 – 46.

Odjegba, V. J. and Badejo, J. O. (2013). Crude oil induce oxidative stress in *Capsicum annum. Nature and Science*,11(2): 46-50.

Ogbuehi, H. C, Ogbonanya, C. I, Ezeibekwe I. O, and Ukaoma, A. A., (2014). Dose-Response study of Plants (*Gycine max L., Vigna subteranea L.* and *Zea mays L.)* to Diesel oil pollution. *Global Journal of Agriculture, Biotechnology and Health sciences*, 3(1):294-303.

Olubodun, O.S. and Eriyamremu, E.G (2013). Effect of different crude fractions on growth and oxidative stress parameters of maize radicle. *International Journal of Plant and Soil Science*, 2:144 – 154.

Omosun, G., Markson, A. A., and Mbanasor O. (2008). Growth and anatomy of *Amaranthus hybridus* as affected by different crude oil concentrations. *American Journal of Science Research*, 3:70 – 74.

Reem M. S., W. M. Shukry, M. M. Azzoz and G.H.S. Al-Hawas (2012). Effect of crude oil on germination, growth and seed protein profile of Jojoba (*Simmodsia chinensis*). *Plant Science Journal*, 1(1): 20-35

Saborni, M. Nirmalia, G., Chiragib, M., Ringusk Das, N.D. and Malay, K.A. (2012). Response of the maize plant to chromium stress with reference to antioxidation activity. *Brazilian Society of Plant Physiology*, 24:203 – 212.

Shanky B. (2013). Ecotoxicology and Impact on Biodiversity. *Journal of Pharmacognosy and Phytochemistry*. 2:1-19

Somagi, N. (1944). A photometric adaptation of the Somagi method for the determination of glucose. *Journal of Biology and Chemistry*, 153:375 – 380.

Su R. N., Hae-Kwan C. , Mina ., Sang-Yong E., Heon K., Young-Hyun C. and Domyung P. (2015). Oxidative stress biomarkers in long-term participants in clean-up work after the Hebei Spirit oil spill. *Science of the Total Environment*, 515(516) 207–214.