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The Effect of Crude Oil Impacted Soil on the Biochemical Properties of Guinea Corn

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Abstract
This study investigated the effect of crude oil impacted soil on physiomorphological and biochemical properties of guinea corn (Sorghum vulgare). Thirty polythene pots with drainage holes at the bottom, each containing 10 kg of surface soil, were randomly placed on a table in a screenhouse in a factorial combination of five treatment levels (0.4%, 0.3%, 0.2%, 0.1% and 0%w/w) of crude oil. The soil inside the pots was homogenized by stirring using a glass rod, wetted with distilled water and allowed to equilibrate for two weeks. Two weeks after the application of crude oil, three seeds of guinea corn per pot were planted. Result revealed a significant decrease ($p<0.05$) in girth length of Guinea corn planted in crude oil-impacted soil relative to the control. The relative water content RWC ranged between 80% and 87%. Generally, activities of the antioxidant enzymes of leaves and stems of Guinea corn planted in crude oil-impacted soil increased significantly relative to the control ($p<0.05$). The soluble protein content (SPC) of the leaf of Guinea corn in P0.4 was less than ½ that of the control. Conversely, the malondialdehyde (MDA) of the leaf of Guinea corn in P0.4 was about 1 ½ folds that of the control. The estimation of antioxidative enzymes and non-enzymatic antioxidative parameters after contamination with higher level of crude oil has shown that the defence mechanism of the plant against the toxic influence of the crude oil is overwhelmed and hence predisposed to oxidative stress. No spill should, therefore, be taken for granted. Crude oil-impacted soil induced a condition of oxidative stress in guinea corn, which could in turn be transferred to the consumer.

Keywords: Crude oil, soil, guinea corn, biochemical, oxidative stress

Introduction
Crude oil is a complex mixture of thousands of hydrocarbons and non-hydrocarbon compounds including heavy metals. It is the largest and most important source of hydrocarbons (1). Crude oil varies in appearance and composition from one oil kind to another (2). The varying compositions of one crude oil from the other have diverse effects on different organisms within the same environment (3). However, crude oil is not found naturally in every part of the world. It is transported from one place to another for refining. A seemingly inescapable consequence of these transport activities is the accidental spill of the oil into both land and water. Crude oil pollution has been reported to have deleterious effects on plant germination and seedling growth (4). Soils polluted with petroleum hydrocarbon (PHC) are low in fertility and hence, do not support adequate crop growth and development (5). Bioassays such as measurements of seed germination and early seedling growth have been used to monitor treatment effects of oil-contaminated sites (6). This has necessitated a similar investigation in Nigeria, where a lot of pollution of farmlands occurs.
Guinea corn is a cereal grain plant belonging to the family *Graminea* which has its origin from tropical African. It is also cultivated in the United States, India, Palestine, Southern America and Southern Europe (7). Guinea corn has several uses which include uses as food, feed for livestocks, and industrial raw materials. It stimulates respiration and improves digestion (8).
An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction involving the loss of electrons or an increase in oxidation state. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols (9). Substituted phenols and derivatives of phenylenediamine are common antioxidants used to inhibit gum formation in gasoline (petrol). Although oxidation reactions are crucial for life, they can also be damaging; plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, vitamin A, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Insufficient levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells. Oxidative stress is damage to

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cell structure and cell function by overly reactive oxygen-containing molecules and chronic excessive inflammation.

This study evaluated the toxicity response of guinea corn to different concentrations of crude oil using shoot growth, physiological and biochemical indicators including oxidative indices.

Material and Methods

Crude oil

The crude oil sample was collected from Warri Refinery and Petro-chemical Companies (WRPC) Warri Delta State, Nigeria.

Experimental design and agronomic details

The experiment was conducted in a screenhouse of the College of Science, Federal University of Petroleum Resources, Effurun, Nigeria. The method described by Adewole and Aboyeji (10) and slightly modified by Adeyemi (29) was used. Thirty polythene pots with drainage holes at the bottom, each containing 10 kg of surface soil, were randomly placed on a table in the screenhouse in a factorial combination of five treatment levels (0.4%, 0.3%, 0.2%, 0.1% and 0%w/w) of crude oil and designated P0.4, P0.3, P0.2, P0.1 and Control respectively. The soil inside the pots, homogenized by stirring using a glass rod, wetted with distilled water and allowed to equilibrate for two weeks. Two weeks after the application of crude oil, three seeds of guinea corn (obtained from Effurun market, Effurun, Nigeria) per pot were planted. The guinea corn stands were regularly watered throughout the growing stage. The Guinea corn plants were thinned to two stands per pot at two weeks after planting (WAP). The thinned stands were retained inside the pots from which they were removed so as to put back into the soil what might have been taken up by the plant within the first two weeks of growth. Fortnightly, growth parameters of Guinea corn such as plant height and stem girth were measured till 9 WAP when the experiment was terminated.

Relative Water Content (RWC) Determination

Relative water content (RWC) of leaves was determined at 9 WAP by the standard method (11).Two leaves from each plant were harvested and weighed to obtain the fresh weight.The leaves were then floated in a closed Petri dish containing distilled water for 4hours.After 4hours, the leaves were dried with filter paper and weighed immediately to obtain the turgid weight.The leaves were then oven dried at 80^{o}C for 24hrs, then cooled in a dessicator for 1hour. After cooling, the leaves were weighed and recorded as the dried weight.

The relative water content (RWC) was calculated as;

$$RWC\ \% = \frac{\text{(Fresh weight-dry weight)}\ \times 100}{\text{(Turgid weight-dry weight)}}$$

Soil analysis

The pH, temperature, moisture content, soil particle size, phosphorus, potassium, sodium, calcium, and magnesium content of the soils were analyzed using the conventional standard methods [12-14].

The soil pH was electrometrically determined (15). The pH was determined when the electrode of the standardized pH meter was inserted into the partly settled suspension of soil/distilled water in the ratio 1:1.

The carbon contents in the soil were determined using the standard method (16). This is a wet oxidation method when 1 N $K_2Cr_2O_7$ is titrated against the wet oxidized samples using concentrated H_2SO_4 and *o*-phenanthroline ferrous complex as an indicator.

The total nitrogen content of the soil was determined by the macro-Kjeldahl method of Bremner and Mulvaney (17

The available phosphorus concentrations in the soil were determined using the Bray P1 method (18). The extracting solutions for available P were 0.5 N HCl and 1.0 N NH4F.

Exchangeable cations ($K^+ + Na^+ + Ca^{2+} + Mg^{2+}$) were extracted with 1 M ammonium acetate buffered at pH 7.0 (19) and the concentrations of Ca^{2+} and Mg^{2+} in the soil extracts were read using a Buck Scientific 210/211 VGP Atomic Absorption Spectrophotometer (AAS) (East Norwalk, Connecticut, USA) while Na+ and K+ concentrations were read on a Gallenkamp flame photometer. Iron and Cu were extracted with 0.1 M HCl while Pb was extracted with 5 ml of the mixture (conc. HNO_3 and conc. $HClO_4$ in the ratio 2:1) with 5 ml of conc. H_2SO_4 (20) and their concentrations in the soil extracts were read using an AAS.

The exchangeable acidity ($H^+ + Al^{3+}$) in the soil were extracted with 1 M KCl according to Thomas (19) and the extract was titrated with 0.05 M NaOH using phenolphthalein as an indicator (21).

Lipid peroxidation

Lipid peroxidation was estimated from the level of malondialdehyde (MDA) production using thiobarbituric acid (TBA) according to Sairam and Srivastava [22].

Soluble protein content

Total soluble protein was extracted from 0.5 g leaf tissue in 5 ml 0.1 M Tris-HCl (pH 7.5) containing 50 mM ascorbic acid, 1% β-mercaptoethanol and 1 mM phenylmethylsulfonyl fluoride after centrifugation

(15000 g for 30 min) at 4°C. Protein content was determined by the procedure of Jiang and Huang [23] using bovine serum albumin as standard.

Assay of antioxidant enzymes

Fresh leaf tissue (0.5 g) was homogenized at 4°C in 5 ml of 0.05 M sodium phosphate buffer (pH 7.0) containing 0.1 mM ethylene diamine tetra acetic acid, 1 mM L-isoascorbic acid, 1% (w/v) polyvinylpyrrolidone and 0.5% (w/v) Triton X-100. Extracts were centrifuged at 15000 g for 30 min and the supernatants were used for the assays of enzyme activities. Superoxide dismutase (SOD) activity was determined according to Sarkar et al. [24] using the photochemical nitrobluetetrazolium (NBT).

Catalase (CAT) activity was estimated by monitoring the disappearance of H_2O_2 by recording the decline in absorbance at 240 nm according to the method of Sairam and Srivastava [14]. The reaction mixture contained 50 mM sodium phosphate buffer (pH 7.0), 15 mM H_2O_2 and crude enzyme extract.

Peroxidase (POD) activity was determined by recording the oxidation of guaiacol in the presence of H_2O_2. The increase in absorbance was recorded at 470 nm (25). The reaction mixture contained 100 µl crude enzyme, 500 µl H_2O_2, 500 µl guaiacol and 1900 µl potassium phosphate buffer (pH 6.1).

Ascorbate peroxidase (APX) activity was measured following the procedure described by Kuk et al. (26). The reaction mixture contained 50 mM sodium phosphate buffer (pH 7.0), 0.2 mM EDTA, 0.5 mM ascorbic acid and 0.25 mM H_2O_2. APX activity was determined by monitoring the decline in absorbance at 290 nm for 2 min as ascorbate was oxidized.

Glutathione reductase (GR) activity was assayed by monitoring the glutathione-dependent oxidation of NADPH at 340 nm by the method of Kuk et al. [26] in a reaction mixture containing 50 mM sodium phosphate buffer (pH 7.8), 0.2 mM NADPH, 0.5 mM glutathione, 2 mM ethylene diamine tetra acetic acid and enzyme extract.

Statistical analysis

Data collected were subjected to descriptive and one-way analysis of variance to test their treatment effects. The experimental precision achieved was reported by standard error at the probability level of 95% and mean values were separated by the DUNCAN Multiple Range Test (27).

Results

The experimental soil is loamy sand (Figure 1) and other characteristics determined at the beginning of the experiment are presented in Table 1.

Table 1: Physicochemical characteristics of experimental soil

Property	**Soil**
pH (1:1 soil-water)	6.50±0.22
Organic carbon (g kg^{-1})	12.8±0.35
Total nitrogen (g kg^{-1})	89.05±5.8
Available phosphorus (mg kg^{-1})	21.6±1.05
K (mg kg^{-1})	81.3±3.2
Na (mg kg^{-1}	68.23±3.2
Ca (mg kg^{-1})	14.9±1.00
Mg (mg kg^{-1})	3.8±1.01
Exchangeable acidity (mg k^{-1})	0.6±0.01
Fe (mg kg^{-1})	2.6±0.38
Cu (mg kg^{-1})	5.2±1.00
Pb (mg kg^{-1})	0.65±0.01
Bulk density (g cm^{-3})	1.41±0.02
Temperature (^oC)	26±0.03
Moisture (%)	20±0.05

Values are Means ± SEM of three determinations.

Figure 2 shows all the groups of Guinea corn increased in height from 1WAP through to 9WAP The height of control was significantly higher ($p<0.05$) than the Guinea corn planted in oil-impacted soil (P0.1, P0.2, P0.3, P0.4). Height of plant in groups P0.1 and P0.2 were not significantly different ($p>0.05$) by 9WAP. The height of Guinea corn in P0.4 was observed to be the lowest at 9WAP.

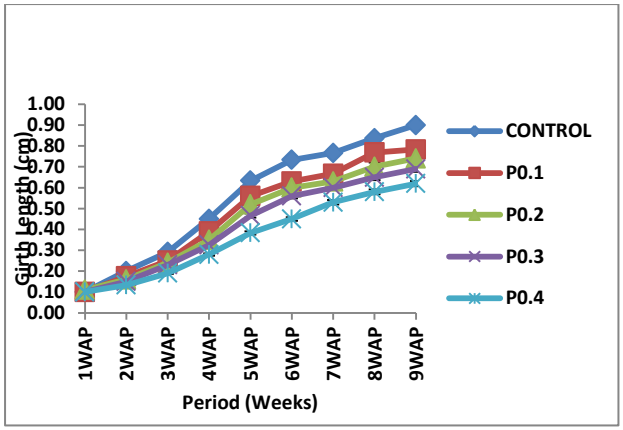

Figure 2: Effect of crude oil-imparted soil on height of Guinea corn Over a period of nine (9) weeks. Results are Means of 5 determinations ± SEM.
WAP: weeks after planting

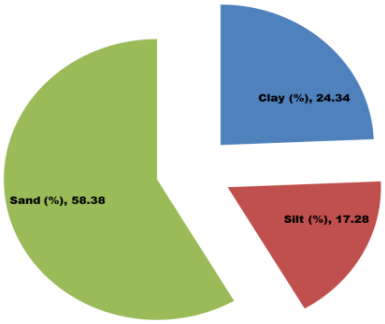

Figure 1: Particle size analysis of experimental soil

Figure 3 shows the girth length of Guinea corn planted in crude oil-impacted soil. Result revealed a significant decrease ($p<0.05$) in girth length of Guinea corn planted in crude oil-impacted soil (P0.1, P0.2. P0.3, P0.4) relative to the control.

The relative water content (RWC) of Guinea corn planted in crude oil-impacted soil is presented in Figure 4. The RWC ranged between 80% and 87%. RWC of Guinea corn in groups P0.1 and P0.2 were not significantly different ($p>0.05$), similarly RWC of Guinea corn in groups P0.3 and P0.4 were not significantly different ($p>0.05$). Conversely, the RWC of Guinea corn planted in crude oil-impacted soil was significantly lower ($p<0.05$) than the control.

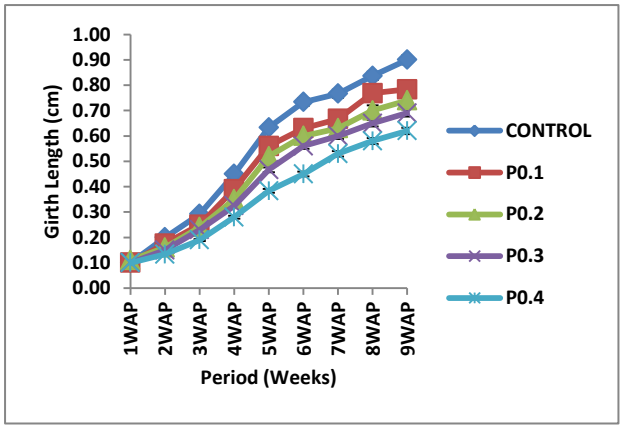

Figure 3: Effect of crude oil-imparted soil on stem girth of Guinea corn over a period of nine (9) weeks. Results are means of 5 determinations ± SEM.
WAP: weeks after planting

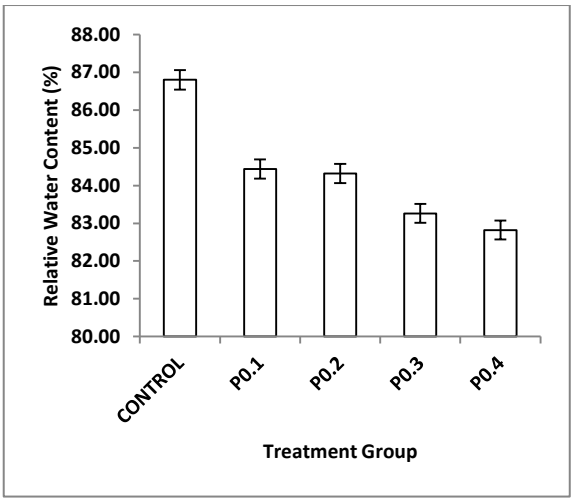

Figure 4: Effect of crude oil-imparted soil o Relative Water Content of leaves of Guinea corn over a period of nine (9) weeks.
Results are means of 5 determinations ± SEM.

Effect of crude oil-impacted soil of selected antioxidant enzymes of leaves of Guinea corn is shown in Table 2. Generally, activities of the antioxidant enzymes of leaves of Guinea corn planted in crude oil-impacted soil increased significantly relative to the control ($p<0.05$). However, enzyme activity, except Catalase (CAT), of Guinea corn in P0.1 group was not significantly different from control ($p>0.05$).

Table 2: Effect of crude oil-imparted soil on activities of superoxide dismutase (SOD), Catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX)and glutathione reductase (GR) activities of Guinea corn leaves

GROUPS	SOD (units mg^{-1} protein)	CAT (µmol H_2O_2 min^{-1} mg^{-1} protein)	POD (Units mg^{-1} protein)	APX (µ mol ascorbate min^{-1} mg^{-1} protein)	GR (µ mol NADPH min^{-1} mg^{-1} protein)
Control	98.55.56±2.36^{a}	113.30±4.22^{a}	397.12±7.42^{a}	423.77±6.68^{a}	82.11±3.22^{a}
P0.1	119.42±3.63^{b}	119.37±3.19^{a}	425.60±7.56^{b}	437.30±6.24^{ab}	87.50±3.67^{a}
P0.2	125.19±3.78^{bc}	129.82±2.89^{b}	457.52±5.76^{c}	442.52±7.21^{b}	102.20±4.12^{b}
P0.3	131.38±4.16^{cd}	136.49±3.15^{c}	484.20±8.50^{d}	465.50±6.31^{c}	118.40±3.89^{c}
P0.4	139.15±3.88^{d}	158.10±4.36^{d}	503.22±7.68^{e}	498.33±5.47^{d}	124.30±4.47^{d}

Values are means ± SEM of six determinations. ^{a,b,c} Column values with different superscripts are significantly different ($p<0.05$).

Table 3 : Effect of crude oil-imparted soil on activities of superoxide dismutase (SOD), Catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) and glutathione reductase (GR) activities of Guinea corn stems

GROUPS	SOD (units mg^{-1} protein)	CAT (µmol H_2O_2 min^{-1} mg^{-1} protein)	POD (Units mg^{-1} protein)	APX (µ mol ascorbate min^{-1} mg^{-1} protein)	GR (µ mol NADPH min^{-1} mg^{-1} protein)
Control	18.30±1.12^{a}	23.30±0.56^{a}	2.75±0.52^{a}	3.23±0.25^{a}	26.20±1.06^{a}
P0.1	19.45±0.98^{a}	23.94±1.11^{a}	3.02±0.48^{a}	3.90±0.18^{a}	28.92±1.11^{b}
P0.2	20.52±1.25^{ab}	26.82±0.78^{b}	4.18±0.45^{b}	4.70±0.33^{b}	33.45±1.00^{c}
P0.3	22.36±1.13^{b}	29.50±1.09^{c}	5.22±0.62^{c}	6.35±0.29^{c}	39.62±1.13^{d}
P0.4	22.78±1.01^{b}	31.33±1.05^{c}	5.68±0.58^{c}	7.28±0.33^{d}	41.05±1.22^{d}

Values are means ± SEM of six determinations. ^{a,b,c} Column values with different superscripts are significantly different ($p<0.05$).

Table 3 presents the effect of crude oil-impacted soil on the activities of selected enzymic antioxidants of Guinea corn stems. It was generally observed that the activity of enzymic antioxidants increased significantly in stems of Guinea corn planted in crude oil-impacted soil relative to the control ($p<0.05$). Except the activity of glutathione reductase (GR), activity of all other antioxidant enzymes of guinea corn in P0.1 were not significantly different from control ($p>0.05$). Similarly, activities of enzymic antioxidants, except ascorbate peroxidase (APX) and GR, of the stem of Guinea corn in P0.3 and P0.4 were found not to be significantly different ($p>0.05$).

Table 4 shows the effect of crude oil-impacted soil on the soluble protein content (SPC) and malondialdehyde (MDA) concentration of Guinea corn leaves. The SPC of the leaf of Guinea corn in P0.4 was less than ½ that of the control. Conversely, the MDA of the leaf of Guinea corn in P0.4 was about 1 ½ folds that of the control. Both SPC and MDA of leaves of Guinea corn in P0.3 and P0.4 were not significantly different ($p>0.05$).

Generally, Table 5 revealed a significant decrease ($p<0.05$) of SPC of stem of Guinea corn planted in crude oil-impacted soil relative to the control. The SPC of stem of Guinea corn in P0.4 was 1/3 that of control. MDA content of stem of Guinea corn planted in crude oil-impacted soil was significantly higher than the control ($p<0.05$).

Discussion

The present study is an attempt to elucidate the underlying biochemical implications of crude oil impacted soil on guinea corn with a view to interpolating the results to similar crops. This understanding will expose new opportunities to salvage these categories of crops in case of accidental oil spill to avoid the colossal loss to farmers and to the oil companies to reduce damages/compensation.

Results from this study revealed that accidental spills at about 0.2%v/w may not affect the height of guinea corn (Figure 2). However, girth of guinea corn was significantly reduced even at a crude oil level of 0.1v/w (Figure 3). The possible explanation for this observation is abnormal growth of the plant as a result of the crude oil. The height is not proportional to the stem girth, consequently the plant is susceptible to breakage at the slightest wind. It could be drawn that the yield may be reduced since a higher yield may result into plant breakage. This result agrees with several similar experiments (10, 28-29)). Another physiological imbalance also observed in the guinea corn planted

Table 4: Effect of crude oil-imparted soil on soluble protein content (SPC) and malondialdehyde (MDA) of Guinea corn leaves

GROUPS	SPC (mg g^{-1}FW)	MDA (µmol g^{-1}FW)
Control	2.18 ± 0.22^{a}	4.82 ± 0.32^{a}
P0.1	1.97 ± 0.10^{b}	5.15 ± 0.52^{b}
P0.2	1.43 ± 0.11^{c}	6.71 ± 0.56^{c}
P0.3	1.02 ± 0.09^{d}	7.14 ± 0.48^{cd}
P0.4	0.96 ± 0.08^{d}	7.44 ± 0.43^{d}

Values are means ± SEM of six determinations. ^{a,b,c} Column values with different superscripts are significantly different ($p<0.05$).

Table 5: Effect of crude oil-imparted soil on soluble protein content (SPC) and malondialdehyde (MDA) of Guinea corn stems

GROUPS	SPC (mg g^{-1}FW)	MDA (µmol g^{-1}FW)
Control	0.99 ± 0.02^{a}	3.24 ± 0.13^{a}
P0.1	0.87 ± 0.01^{b}	4.18 ± 0.12^{b}
P0.2	0.53 ± 0.01^{c}	4.98 ± 0.10^{c}
P0.3	0.32 ± 0.01^{d}	5.13 ± 0.18^{d}
P0.4	0.26 ± 0.01^{e}	5.78 ± 0.13^{e}

Values are means ± SEM of six determinations. ^{a,b,c} Column values with different superscripts are significantly different ($p<0.05$).

in crude oil-impacted soil is reduced relative water content (RWC) (Figure 4). Uptake and transportation of nutrients by plants, generally, depends on efficient water conduction through the vascular tissues. Reduced RWC is suggestive of impaired water conduction, and subsequently may lead to poor plant growth. It follows from these observations that the plant is under phytophysiological stress condition.

Plant stress may lead to stomata closure, thereby reducing CO_2 availability in the leaves and inhibiting carbon fixation. This exposes the chloroplast to excessive excitation energy, which could in turn increase the generation of free radicals and induce oxidative stress (30). The guinea corn plant which is considered moderately drought tolerant (31) might have inadequate reactive oxygen species (ROS) scavenging system in addition to other tolerance mechanisms to cope with stress. The stress indices determined in leaves and steams of plant in this study include Catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), Ascorbate peroxidase (APX), malondialdehyde (MDA), etc. (Tables 2-5).

CAT and GPx play a significant role in the elimination of hydrogen peroxide. Catalase is the most efficient enzyme known. It is so efficient that it cannot be saturated by H_2O_2 at any concentration. Because of differences between catalase and peroxidase in the Michaelis constant to H_2O_2, their contribution to hydrogen peroxide detoxification is also different. It has been suggested that GPx is responsible for the detoxification of H_2O_2, when

it is present in low concentration, whereas CAT plays its role when GPx pathway reaches saturation with substrate. The GSH-dependent antioxidative system consists of two enzymes: GPx and GR. GR catalyses the reduction of GSSG to GSH. By the contrast to GPx, this enzyme is involved in the maintenance of glutathione inreduced form and owing to this, GSH plays its antioxidant functions. The complicated reactions between the antioxidant enzymes make interpretation of the results difficult. The estimation of antioxidative enzymes and non-enzymatic antioxidative parameters after contamination with higher level of crude oil has shown that the defence reaction of the plant against toxic influence of the crude oil is sometimes greater after contamination with lower concentration.

However, since activities of the antioxidant enzymes studied increased in the tissues of plant in crude-oil impacted soil, it could be inferred that a condition of oxidative stress is imminent. To buttress this submission was the elevated MDA level and the reduced SPC level (Tables 4 and 5). Oxidative stress affects cellular integrity only when antioxidants are no longer capable of coping with ROS. ROS reacts with the unsaturated fatty acid of cellular or subcellular membranes. Therefore, they lead to peroxidation of membrane lipids. The oxidative stress caused by different xenobiotics is often estimated by the level of MDA.

This report is the first to document the effect of crude oil-impacted soil on a arsenal of plant antioxidants and the experimental evidence revealed that the effect of crude oil on the enzymes depends on the concentration of the crude oil. Further examination showed that lower crude oil concentration could present asymptomatic damage which may later show up at fruiting stage as poor yield.

Conclusion

In conclusion, therefore, no spill should be taken for granted. Crude oil-impacted soil placed guinea corn under oxidative stress, which could in turn be transferred to man, the consumer. Whether the effect of guinea corn planted in crude oil-impacted could be transferred to the consumer becomes an hypothesis to be unraveled in further studies.

References

1. Hunt JM: Petroleum Geochemistry and Geology; 2nd Edition. 1996.
2. Craig CF: Occupational safety and health administration. Washington D.C. 2003.
3. Overton EB, Sharp WD, Roberts P: Toxicity of petroleum. In Basic Environ. Toxicol. CRC Press: Boca Raton, FL. Pp. 133-156. 1994.
4. Kyung-Hwa B, Hee-Sik K, Hee-Mock O, Byung-Dae Y, Jaisoo K, In- Sook L: Effect of crude oil, oil components, and bioremediation on plant growth, J. Environ.Sci. health. (39)9: 2465-2472. 2004.
5. Abii, TA, Nwosu PC: The effect of oil-spillage on the soil of Eleme in Rivers State of the Niger Delta area of Nigeria, Research journal of environmental sciences, 3(3), pp 316-320. 2009.
6. Sverdrup GD, Krogh CM, Nielsen PH, Kjaer T, Sternersen C: Toxicity of eight polyclic aromatic compounds to red clover (Trifolium pretense), ryegrass (Lolium perenne), and mustard (Sinapsis alba). Chemosphere 53: 993-1003. 2003.
7. Doherty C, Faubion JM, Rooney LW: Semi-automated determination of phytate in sorghum and sorghum products.cer. chem.59:373-377. 1982.
8. Eggum BOL, Monowar KE, Bochknusden L, Axtell L: Nutrition quality of sorghum foods for sudan .J. of Cer. Sci. 1:127-137. 1983.
9. Sies H: Oxidative stress: oxidants and antioxidants. Experimental Physiology 82 (2): 291–5. 1997.
10. Adewole MB, Aboyeji AO: Yield and quality of maize from spent engine oil contaminated soils amended with compost under screenhouse conditions. J Agrobiol 30: 9-19. 2013.
11. Schonfeld MA, Johnson RC, Carver BF, Mornhinweg DW: Water relations in winter wheat as drought resistance indicator. Crop Sci., 28: 526-531. 1988.
12. AOAC: Official Methods of Analysis. Association of Official Analytical Chemists. Washington DC P14. 1996.
13. APHA/AWWA: Standard methods for the examination of water and waste water quality. J. Environ. Toxicol. Water qual. 11, 72-82. 1985.
14. Sairam RK, Srivastava GC: Water stress tolerance of wheat (*Triticum aestivumL.*): Variations in hydrogen peroxide accumulation and antioxidant activity in tolerant and susceptible genotypes. J. Agron. Crop Sci., 186: 63–70. 2001.
15. McLean EO: Soil pH and lime requirement.In Page AL, Miller RH, Keeney DR (eds.): Methods of soil analysis. 2nd ed., Part 2 ed., Agronomy monograph No. 9. Madison, WI: American Society of Agronomy, pp. 595–624. 1982.
16. Nelson DW, Sommers LE: Total carbon, organic carbon and organic matter. In Page AL, Miller RH, Keeney DR (eds.): Methods of soil analysis. 2nd ed., Part 2 ed., Agronomy monograph No. 9. Madison, WI: American Society of Agronomy, pp. 539–579. 1982.
17. Bremner JM, Mulvaney CS: Nitrogen– Total. In Page AL, Miller RH, Keeney DR (eds.): Methods of soil analysis. 2nd ed., Part 2ed., Agronomy monograph No. 9. Madison, WI: American Society of Agronomy, pp. 295–324. 1982.
18. Olsen SR, Sommers LE: Phosphorus. In Page AL, Miller RH, Keeney DR (eds.): Methods of Soil Analysis. 2nd ed., Part 2 ed., Agronomy Monograph No. 9. Madison, WI: American Society of Agronomy, pp. 403–430. 1982.
19. Thomas GW: Exchangeable cations. In Page AL, Miller RH, Keeney DR (eds.): Methods of soil analysis. 2nd ed., Part 2 ed., Agronomy monograph No. 9. Madison, WI: American Society of Agronomy, pp. 159–165. 1982.
20. Juo ASR: Selected methods for soil and plant analysis. Manual series No. 1. Published and printed by International Institute of Tropical Agriculture, Ibadan, Nigeria. 1979.
21. Odu CTI, Babalola O, Udo EJ, Ogunkunle AO, Bakare TA, Adeoye GO: Laboratory manual for agronomic studies in soil, plant and microbiology. Department of Agronomy, University of Ibadan, Ibadan, Nigeria. 1986.

22. Sairam RK, Srivastava GC: Water stress tolerance of wheat (*Triticum aestivumL.*): Variations in hydrogen peroxide accumulation and antioxidant activity in tolerant and susceptible genotypes. J. Agron. Crop Sci., 186: 63–70. 2001.
23. Jiang MY, Zhang JH: Effect of abscisic acid on active oxygen species, antioxidative defence system and oxidative damage in leaves of maize seedlings. Plant Cell. Physiol., 42: 1265–1273. 2001.
24. Sarkar RK, Das S, Ravi I: Changes in certain antioxidative enzymes and growth parameters as a result of complete submergence and subsequent re-aeration of rice cultivars differing in submergence tolerance. J. Agron. Crop Sci., 187: 69–74. 2001.
25. Hernandez JA, Jimenez A, Mullineaux P, Sevilla F: Tolerance of pea (*Pisum sativum L.*) to long-term salt stress is associated with induction of antioxidant defenses. Plant Cell. Environ., 23: 853-862. 2000.
26. Kuk YI, Shin JS, Burgos NR, Hwang TE, Han O, Cho BH, Jung S, Guh JO: Antioxidative enzymes offer protection from chilling damage in rice plants. Crop Sci., 43: 2109–2117. 2003.
27. Adeyemi O, Osubor CC, Adeyemi O: Toxicological evaluation of the effect of Clarias gariepinus (African catfish) cultivated in water contaminated with phthalate, benzene and cyclohexane on liver of albino rats. *Afri. J. Fd. Sci.* 4(1), 026-031. 2010.
28. Okon IE, Udofot EE: Response of Telfairia occidentalis (Hook) to arbuscular mycorrhizal fungi and Gliricidia sepium leaves manure in spent engine oil contaminated soil. World J Agric Sci 8: 20–25. 2012.
29. Adeyemi, O: The Effect of Palm Kernel Oil (PKO) Biodiesel-Contaminated Soil on Morphological and Biochemical Properties of *Zeamays*. J Plant Biochem Physiol 138 (2): 1-6. 2014.
30. Islam MR, Hu Y, Mao S, Jia P, Eneji AE, Xue X: Effects of water-saving superabsorbent polymer on antioxidant enzyme activities and lipid peroxidation in corn (Zea maysL.) under drought stress. J. Sci. Food. Agric., 91: 813-819. 2011a.
31. Islam MR, Xue X, Mao S, Ren C, Eneji AE, Hu Y: Effects of water-saving superabsorbent polymer on antioxidant enzyme activities and lipid peroxidation in oat (Avena sativaL.) under drought stress. J. Sci. Food. Agric., 91: 680-686. 2011b.