Journal of
Plant Biochemistry & Physiology

Olalekan, J Plant Biochem Physiol 2014, 2:4
DOI: 10.4172/2329-9029.1000138

Research Article Open Access

The Effect of Palm Kernel Oil (PKO) Biodiesel-Contaminated Soil on Morphological and Biochemical Properties of *Zea mays*

Olalekan A*

Department of Environmental Science, Federal University of Petroleum Resources, PMB 1221, Effurun, Delta State, Nigeria

Abstract

The effect of palm kernel oil (PKO) biodiesel-contaminated soil on morphological and biochemical properties of *Zea mays* (corn) was investigated. 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 (4%, 3.0%, 2.0%, 1.0% and 0% w/w) of PKO biodiesel and were designated S4, S3, S2, S1 and S0 respectively. Three seeds of maize per pot were planted. Growth parameters (plant height, stem girth, relative water content (RWC), selected leaf properties (chlorophyll content (SPAD value) and soluble protein content (SPC)) and antioxidant indices were determined in the corn over a period of eight weeks after planting (WAP). Results showed that growth of corn planted in contaminated soil was significantly lower ($p<0.05$) than that of control. Corn planted in the contaminated soil also showed a significant increase ($p<0.05$) in superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) and glutathione reductase (GR) activities in leaves when compared with control plants. The results suggest that PKO biodiesel-contaminated soil hindered availability of water, air and nutrients to corn roots, creating a drought condition which could induce oxidative stress in the plant and consequently retarding growth and yield of corn plant.

Keywords: Palm kernel oil; *Zea mays*; Soluble protein biodiesel; Morphological; Biochemical

Introduction

Biofuel has gained considerable recognition in recent times as it is widely believed to be environmental friendly. Transportation of petroleum and petrochemicals is through pipelines that cut across land and water. Accidentals spills and pipeline damage result in pollution of land and water. Biodiesel, on the other hand, cannot be transported by pipelines except by truck, rail, boat or ship which pose greater risk of environmental pollution. Oil spills are most often caused by accidents involving tankers, barges, pipelines, refineries, and storage facilities during the transportation of the oil [1,2]. Like fossil fuel, spill of biofuel may have serious impacts. Tanker accidents contribute 5% and tanker operation account for 7% of oil spills [3]. Pollution of the soil with petroleum derivatives is often observed in Municipal Soils, around industrial plants and in areas where petroleum and natural gas are obtained [4,5].

Processing and distribution of petroleum hydrocarbon as well as the use of petroleum products are the main cause of soil contamination [6]. Changes in soil properties due to contamination with petroleum derived substances can lead to water and oxygen deficit as well as shortage of available forms of Phosphorous [7]. Contamination of the soil environment can also limit protective functions, upset metabolic activity, unfavourably affects its function and chemical characteristics, reduced fertility, negatively influence plant production and induce oxidative stress in plant [8,9].

Plant stress invariably leads to oxidative stress in the plant cell due to the higher leakage of electrons towards O_2 during photosynthetic and respiratory processes leading to enhancement in reactive oxygen species (ROS) generation [10]. The ROS such as O_2^-, H_2O_2 and OH radicals have potential to interact with many cellular components, causing significant damage to membrane and other cellular structures, and consequently growth inhibition [11]. Some of the ROS are highly toxic and must be detoxified by cellular responses if the plant survives and grows [12]. The ROS scavenging depends on the detoxification mechanism, which may occur as a result of sequential and simultaneous action of a number of antioxidant enzymes, including superoxide dismutase (SOD; EC1.15.1.1), catalase (CAT; EC 1.11.1.6), peroxidase (POD; EC 1.11.1.7), ascorbate peroxidase (APX; EC 1.11.1.11) and glutathione reductase (GR; EC 1.8.1.7).

Plants under stress exhibit some defense mechanisms to protect themselves from the damaging effect of oxidative stress. Plants with high constitutive and induced antioxidant levels have better resistance to damage [13]. The scavenging of ROS is one among the common defense responses against abiotic stresses [14]. The degree of damage by ROS depends on the balance between the product of ROS and its removal by these antioxidant scavenging systems [15]. When ROS increases, chain reactions start, in which superoxide dismutase (SOD) catalyzes the dismutation of O_2^- radicals to molecular O_2 and H_2O_2. CAT and POD catalyze the breakdown of H_2O_2. The H_2O_2 is then detoxified in the ascorbate-glutathione cycle which involves the oxidation and re-reduction of ascorbate and glutathione throughthe ascorbate peroxidase (APX) and glutathione reductase (GR) action [14]. The increased production of superoxide radicals and H_2O_2 was paralleled by malondialdehyde (MDA) accumulation under drought stress. MDA is a decomposition product of polyunsaturated fatty acids of bio membranes which is an important indicator of membrane damage [16].

The corn plant (*Zea mays*) requires a temperature of 16°C to 32°C for proper growth. It is the cereal plant that thrives well in the tropical regions of the world. It has various industrial uses and has

***Corresponding author:** Olalekan A, Department of Environmental Science, Federal University of Petroleum Resources, PMB 1221, Effurun, Delta State, Nigeria, Tel: +234(0)8037159452; E-mail: adeyemi.olalekan@fupre.edu.ng

Received November 01, 2014; **Accepted** November 1,4 2014; **Published** November 28, 2014

Citation: Olalekan A (2014) The Effect of Palm Kernel Oil (PKO) Biodiesel-Contaminated Soil on Morphological and Biochemical Properties of *Zea mays*. J Plant Biochem Physiol 2: 138. doi:10.4172/2329-9029.1000138

been found to be a major source of carbohydrate in the third world countries [17]. The research was aimed to clarify whether palm kernel oil (PKO) biodiesel-contaminated soil will not hinder growth of corn at the phyto-physiological level. The objective was to investigate the oxidative status of corn planted in PKO biodiesel-contaminated soil at the molecular level.

Materials and Methods

Reagents and solvents were of analytical grade and are products of British Drug House, Poole, England.

Perm Kernel Oil (PKO)

Palm kernel oil was purchased at the local market in Effurun, Nigeria. 100 g PKO was used for the transesterification process. The ethanol used (99% pure) is an analytical grade with boiling point of 78°C; while the NaOH used was also an analytical grade product of Aldrich Chemicals, England. The blender used was a Dry and Wet mill Blender with a clear glass (1,250 cc capacity) containers and stainless steel cutting blades. Other major materials used include scales, translucent white plastic container with bung and screw-on cap, funnels, PET bottles and thermometer.

Preparation of Bio-diesel from PKO

Biodiesel was prepared from PKO in accordance with the method described by Alamu et al. [18]. 20.0 g of ethanol was measured and poured into a plastic container after which 1.0 g of NaOH was carefully added. The container was swirled round thoroughly for about 2 min repeatedly about six times for complete dissolution of NaOH in the ethanol to form sodium ethoxide.100.0 g of PKO was measured out, pre-heated to 60°C in a beaker and poured into the blender. Sodium ethoxide from the plastic container was carefully poured into the PKO, the blender lid was secured tightly and the blender switched on while agitation in the blender was maintained for 90 min. The mixture was poured from the blender into a PET bottle for settling and the lid was screwed on tightly. The reaction mixture was allowed to stand overnight while phase separation occurred by gravity settling. The PKO biodiesel was carefully decanted into a PET bottle leaving the glycerol at the base. The biodiesel was washed with water. The procedure was replicated three times and average biodiesel yield as well as glycerol yield was measured on weight basis.

Characterization of PKO biodiesel

ASTM standard fuel tests were conducted on the PKO biodiesel. Specific gravity and viscosity measurements were made using the Thermal-Hydrometer apparatus and Viscometer (Canon Fenske Calibrated, 15cSt max. range), following ASTM standards D1298 and D445 respectively. The biodiesel was analyzed for cloud point and pour point using Baskeyl Setapoint cloud and pour point apparatus following ASTM standards D25100-8 and D97 respectively.

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 [19] though slightly modified was used. Bulk surface soil samples (0-15 cm) were collected from an area in the University, air-dried for seven days, sieved using 2 mm sieve and analysed using standard methods. 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 (4%, 3.0%, 2.0%, 1.0% and 0% w/w) of PKO biodiesel and designated S4, S3, S2, S1 and S0 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 PKO biodiesel, three seeds of maize (obtained from Effurun market, Effurun, Nigeria) per pot were planted.

The maize stands were regularly watered throughout the growing stage. The maize 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 maize such as plant height and stem girth were measured till eight WAP when the experiment was terminated. Relative water content (RWC) of leaves was determined at 3 and 6 WAP by the standard method [20] according to the following equation:

RWC (%) = [(fresh weight – dry weight) / (turgid weight – dry weight)] × 100

At 8 WAP, when plants had attained the maximum vegetative growth stage, SPAD readings were taken using SPAD-502 chlorophyll meter. Readings were taken on about same location of each plant. For biochemical analysis, the samples were collected on 8 WAP.

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 [21-23]. The experimental soil is loamy sand (Figure 1) and other characteristics determined at the beginning of the experiment are presented in Table 1.

Lipid peroxidation

Lipid peroxidation was estimated from the level of malondialdehyde (MDA) production using thiobarbituric acid (TBA) according to Sairam and Srivastava [24]. Fresh leaf (0.5 g) was homogenized in 5 ml of 0.1% trichloroacetic acid (TCA) and centrifuged at 10000 g for 10 min. The mixture (containing 1 ml sample supernatant, 4 ml 20% TCA and 0.5% TBA) was heated at 95°C for 30 min, quickly cooled and centrifuged at 10000 g for 10 min. The resulting supernatant was used for spectrophotometric determination of MDA. Absorbance of +TBA was read at 532 and 600 nm using the corresponding TBA as the blank. The MDA concentrations were calculated as follows:

Property	Soil
pH (1:1 soil-water)	6.30 ± 0.27
Organic carbon (g kg^{-1})	13.4 ± 0.85
Total nitrogen (g kg^{-1})	91.05 ± 5.8
Available phosphorus (mg kg^{-1})	23.5 ± 1.25
K (mg kg^{-1})	80.3 ± 4.2
Na (mg kg^{-1})	78.3 ± 3.1
Ca (mg kg^{-1})	15.5 ± 1.03
Mg (mg kg^{-1})	4.5 ± 1.03
Exchangeable acidity (mg k^{-1})	0.7 ± 0.02
Fe (mg kg^{-1})	2.8 ± 0.44
Cu (mg kg^{-1})	6.4 ± 1.01
Pb (mg kg^{-1})	1.3 ± 0.01
Bulk density (g cm^{-3})	1.41 ± 0.02
Temperature (°C)	27 ± 0.03
Moisture (%)	13 ± 0.05

Values are means ± SEM of three determinations.

Table 1: Physicochemical characteristics of experimental soil.

MDA (µmol g-1fresh weight) = [(A532– A600) / 156] × 103 × dilution factor.

Soluble protein content

Total soluble protein was extracted from 0.5 g leaftissue 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 [25] 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 EDTA, 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. [26] using the photochemical nitrobluetetrazolium (NBT). One unit of SOD is defined as that which is present in the volume of extract that causes inhibition of the photo reduction of NBT by 50%. 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 [24]. 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 [27]. 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. [28]. 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. [28] in a reaction mixture containing 50 mM sodium phosphate buffer (pH 7.8), 0.2 mM NADPH, 0.5 mM glutathione, 2 mM EDTA 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 [29].

Results

The mean plant height of corn planted in different levels PKO biodiesel-contaminated soil over a period of eight weeks is presented in Figure 2. At 2WAP, the plant height of corn planted in 3% (S3) and 4% (S4) PKO biodiesel-contaminated soil were significantly lower than the control ($p<0.05$) while the height of corn planted in 1% (S1) and 2% (S) PKO biodiesel-contaminated soil were found not to be significantly different from that of control ($p>0.05$). From 4 WAP to the end of the experiment, the plant height of corn planted in S2 was observed to be significantly lower than control ($p<0.05$) but significantly higher than those of S3 and S4 ($p<0.05$). The height of the corn in S1 was significantly not different from the control ($p>0.05$) throughout the period of the experiment.

Generally, stem girth of experimental corn increased from 2 WAP through to 6 WAP and dropped at 8 WAP (Figure 3). The stem girth of corn planted in PKO biodiesel-contaminated soil was significantly lower than that of control ($p<0.05$). The stem girth of corn in S1 was significantly ($p<0.05$) higher than S2 which in turn was significantly higher than S3 and S4 whose stem girths were not significantly different ($p>0.05$).

The relative water content (RWC) of leaf of corn planted in PKO biodiesel-contaminated soil was observed to be significantly lower than control ($p<0.05$). However, at 3 WAP that of S1 was not significantly ($p>0.05$) different from control. At 6 WAP, RWC of corn in S2, S3 and S4 was not significantly ($p>0.05$) different from one another, however, that in S1 was significantly higher than those of S2, S3 and S4. These observations are shown in Figure 4.

The chlorophyll concentration (SPAD values), SPC and the MDA content of leaves of experimental corn are shown in Table 2. Generally, it was observed that the SPC and SPAD values of leaf of corn planted in PKO biodiesel-contaminated soil was significantly ($p<0.05$) lower than that of control while their MDA was significantly higher than the control ($p<0.05$).

The activities of antioxidant enzymes of the leaf of experimental corn studied followed a definite pattern in which the activities increased as the percentage concentration of PKO biodiesel increased in the soil (Table 3). Although, activities of enzymes of leaf of corn in S1, S2, S3 and S4 were significantly ($p<0.05$) higher than that of control (S0), those of S2, S3 and S4 were not significantly different from one another ($p>0.05$).

GROUPS	SPC (mg g^{-1}FW)	MDA (µmol g^{-1}FW)	SPAD value
S0	3.00 ± 0.12^{a}	6.37 ± 0.23^{a}	28.4 ± 1.73^{a}
S1	2.64 ± 0.13^{b}	7.25 ± 0.42^{b}	25.3 ± 1.56^{b}
S2	2.21 ± 0.11^{c}	8.64 ± 0.36^{c}	22.7 ± 1.63^{c}
S3	2.11 ± 0.12^{c}	8.67 ± 0.38^{c}	22.3 ± 1.43^{c}
S4	2.10 ± 0.11^{c}	8.70 ± 0.33^{c}	22.1 ± 1.44^{c}

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

Table 2: Effect of PKO biodiesel on soluble protein content (SPC), malondialdehyde (MDA) and SPAD value of maize leaves.

Discussion

The present study elucidates the mechanism by which PKO biodiesel affects the biochemistry and physiology underlying the growth of *Zea mays*. The study is the first documented report on the biochemical and morphological response of corn to PKO biodiesel-contaminated soil. The particle size analysis of the experimental soil indicated that it is loamy sand (Figure 1) while the physicochemical analysis suggested that the soil is good to support the growth of corn (Table 1).

Soil nutrients had been reported to be less mobile in contaminated soils [30] and this condition had also been reported to adversely alter the growth pattern of plants [31]. The reduced plant height (Figure 2) and stem girth (Figure 3) of maize in PKO biodiesel-contaminated soil may be attributed to the stress imposed by artificially created drought conditions as well as the non-availability of nutrients suffered by plant roots due to the roots' inability to get sufficient water and plant nutrients from the soil [32]. Experimental data showed that the applied PKO biodiesel had a remarkable effect on corn growth. Previous research under both field and laboratory conditions also showed similar results [33-35].

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)
S0	110.56 ± 3.76^{a}	130.90 ± 3.71^{a}	430.03 ± 4.12^{a}	500.45 ± 4.72^{a}	74.09 ± 1.24^{a}
S1	121.67 ± 3.31^{b}	139.13 ± 2.89^{b}	452.24 ± 3.78^{b}	533.08 ± 5.01^{b}	83.11 ± 1.35^{b}
S2	129.13 ± 2.89^{c}	145.56 ± 3.02^{c}	460.10 ± 3.55^{c}	549.37 ± 4.89^{c}	87.23 ± 1.65^{c}
S3	133.24 ± 3.52^{c}	147.82 ± 2.57^{c}	467.21 ± 4.01^{c}	555.48 ± 5.11^{c}	89.71 ± 1.40^{cd}
S4	134.15 ± 3.74^{c}	148.16 ± 3.12^{c}	470.49 ± 4.04^{c}	558.19 ± 4.77^{c}	90.68 ± 1.29^{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 PKO biodiesel on activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX)and glutathione reductase (GR) activities of maize leaves.

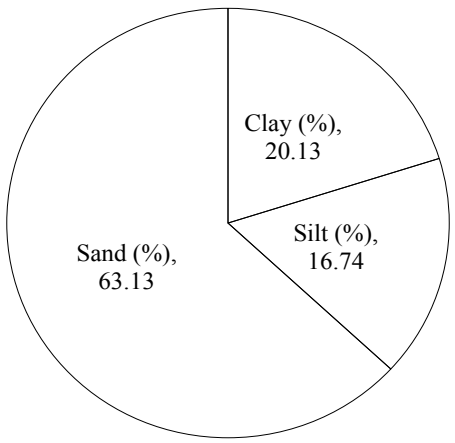

Figure 1: Particle size analysis of experimental soil.

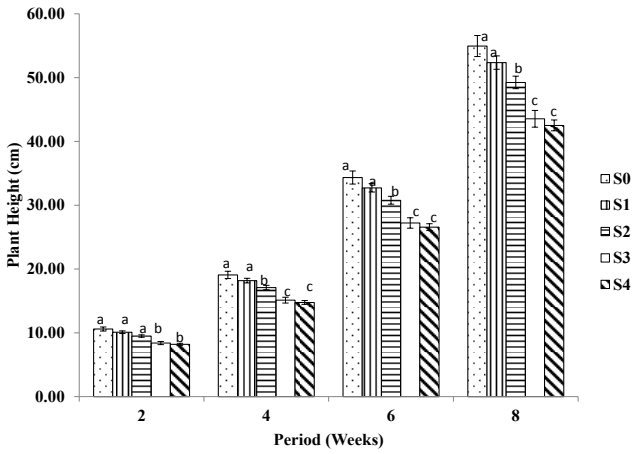

Figure 2: Effect of PKO biodiesel on the height of *Zea mays*. Plotted values are means ± SEM of six determinations. ^{a,b,c} Values with different superscripts are significantly different ($p<0.05$).

RWC is the appropriate measure of plant water status in terms of the physiological consequence of cellular water deficit, while water potential is an estimate of plant water status and it is useful in dealing with water transport in the soil-plant-atmosphere continuum [36]. In this study, it was observed that PKO biodiesel-contaminated soil substantially decreased the RWC at both growth stages (Figure 4). This result lends credence to non-availability of water adduced to the reason for decreased plant height and stem girth of corn planted in PKO biodiesel-contaminated soil.

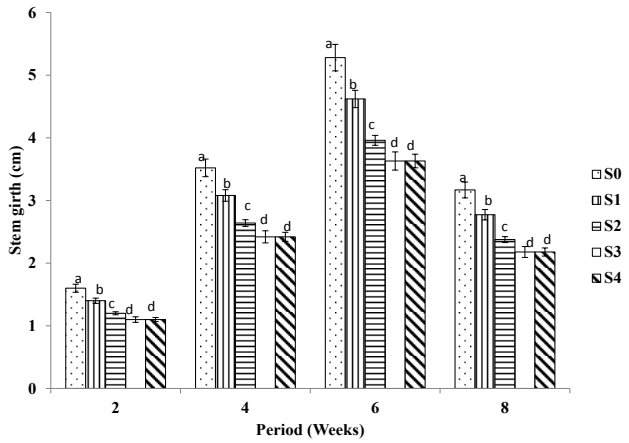

Figure 3: Effect of PKO biodiesel on the stem girth of *Zea mays*. Plotted values are means ± SEM of six determinations. ^{a,b,c} Values with different superscripts are significantly different ($p<0.05$).

The chlorophyll concentration (SPAD value) and SPC of the leave of corn reduced substantially following soil contamination with PKO biodiesel (Table 2). This could be due to difficulty in getting appropriate nutrient by the root of corn created by the biodiesel contamination. Reduced soil aeration due to thin film layer formation on the topsoil by the applied PKO biodiesel could have reduced the air passage through the soil pores, thereby leading to the inadequate air supply to the maize plants [19] and hence, reduction in the SPAD value and SPC. The artificial drought condition created by the PKO biodiesel may also be responsible for the increased lipid peroxidation products, MDA, of leaves of experimental corn.

Corn planted in PKO biodiesel showed a significant increase in SOD, CAT, POD, APX and GR activity (Table 3) in the leaves. The results suggest that soil contaminated with PKO biodiesel is directly associated with production of oxygen radicals which resulted in increased lipid peroxidation and oxidative stress in the plant (Table 2). Plant stress may also 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 [37]. The corn plant which is considered moderately drought tolerant [33] might have inadequate ROS scavenging system in addition to other tolerance mechanisms to cope with stress.

Conclusion

PKO biodiesel-contaminated soil could hinder availability of water, air and nutrients to corn roots, creating a drought condition which could induce oxidative stress in the leaves and consequently limiting

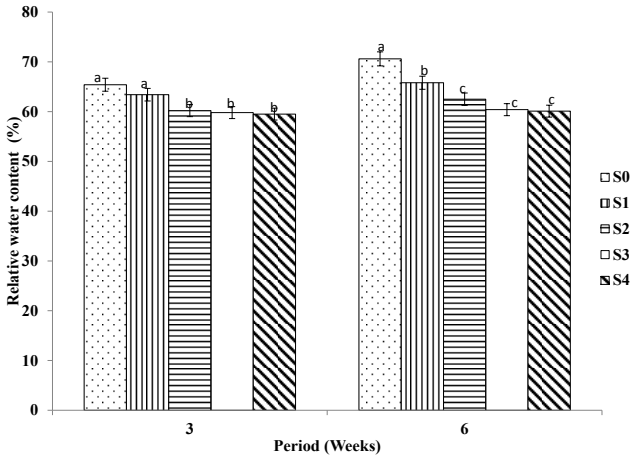

Figure 4: Effect of PKO biodiesel on the relative water content (RWC) of *Zea mays* leaf. Plotted values are means ± SEM of six determinations. [a,b,c] Values with different superscripts are significantly different ($p<0.05$).

growth and yield of corn plant. This is the first report on corn planted in PKO biodiesel-contaminated soil, most of the previous studies focused on soil contaminated with fuel diesel and spent engine oil. The data in this study is similar to the reality in the Niger-Delta region of Nigeria, where farm produce are destroyed by fuel diesel from oil spillage.

Experimental evidence from this study further underscore the importance of caution when producing and transporting biodiesel and other biofuel to avoid accidental spills and a rapid containment mechanism should be put in place in case of such spills. Further research of soil contaminated with biodiesel should consider superabsorbent polymer (SAP) and various fertilizer regimens. Toxicological evaluation of corn planted in soil contaminated with biodiesel should also be evaluated.

References

1. Ginsbury P, Sterling S, Gotheherer S (1993) the citizens' advisory council as a means of mitigating environmental impacts of terminal and Tanker operations. Marine Policy 17:404-411.
2. Okoko KAB, Ibaba IS (1999) Oil spillages and community disturbances: The SPDC and Niger Delta experience. J.Oil Politic 2: 6-69.
3. Wade R (1990) The Exxon Valdez oil spill. The Environmental Health response to manmade disasters. J Environ Health 6: 34-40.
4. Adam G, Gamoh K, Morris DG, Duncan H (2002) Effect of alcohol addition on the movement of petroleum hydrocarbon fuels in soil. Sci Total Environ 286: 15-25.
5. Clark CS (2003) Field detector evaluation of organic clay soils contaminated with diesel Environ Florescence. 4: 167-173.
6. Ayotamuno MS, Kogbara RB, Egwuenum PN (2006) Acomparison of corn and elegant grass in the phytoremediation of a petroleum hydrocarbon polluted agricultural soil in Port Harcourt. Nigeria S food Agric Environ 4: 216-222.
7. Wyszkowska J, Kucharski S (2000) Biochemical Properties of Soil contaminated by petrol. Polish Journal Environ Studies 9: 479-485.
8. Gong P, Sun TH, Reudert G, Hahn HH (1996) Ecological Effects of combined organic and inorganic pollution on soil microbial activities. Water, Air and Soil Pollut 96: 133-143.
9. Wyszkowski M, Wyszkokwska S, Ziochokwska A (2004) Effect of soil contamination with diesel oil on yellow lupine, field and macro elements content. Plant Soil Environment 50: 218-226.
10. Asada K (1999) THE WATER-WATER CYCLE IN CHLOROPLASTS: Scavenging of Active Oxygens and Dissipation of Excess Photons. Annu Rev Plant Physiol Plant Mol Biol 50: 601-639.
11. Blum A (1996) Crop responses to drought and the interpretation of adaptation. Plant Growth Regul 20: 135-148.
12. Dramé KN, Clavel D, Repellin A, Passaquet C, Zuily-Fodil Y (2007) Water deficit induces variation in expression of stress-responsive genes in two peanut (Arachis hypogaea L.) cultivars with different tolerance to drought. Plant Physiol Biochem 45: 236-243.
13. Gigon A, Matos AR, Laffray D, Zuily-Fodil Y, Pham-Thi AT (2004) Effect of drought stress on lipid metabolism in the leaves of Arabidopsis thaliana (ecotype Columbia). Ann Bot 94: 345-351.
14. Gossett DR, Millhollon EP, Lucas MC (1994) Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. Crop Sci 34: 706-714.
15. Demiral T, Turkan I (2005) Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. Environ Exp Bot 53: 247-257.
16. Porcel R, Ruiz-Lozano JM (2004) Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress. J Exp Bot 55: 1743-1750.
17. Uhegbu FO, Akubugwo EI, Iweala EJ, Uhegbu OC (2012) Impact of spent engine oil on soil and the growth of Zea maysseeds. J Environ Sci 1: 1-8.
18. Alamu OJ, Akintola TA, Enweremadu CC, Adeleke AE (2008) Characterization of palm-kernel oil biodiesel produced through NaOH-catalysed transesterification process. Sci Res and Essay 3: 308-311.
19. Adewole MB, Aboyeji AO (2013) Yield and quality of maize from spent engine oil contaminated soils amended with compost under screenhouse conditions. J Agrobiol 30: 9-19.
20. Schonfeld MA, Johnson RC, Carver BF, Mornhinweg DW (1988) Water relations in winter wheat as drought resistance indicator. Crop Sci 28: 526-531.
21. Black CA (1982) Methods of soil analysis. Amer Soc Agron Madison Wisconsin. Agronomy Part 2 No: 9.
22. AOAC (1996) Official Methods of Analysis. Association of Official Analytical Chemists. Washington DC P14.
23. APHA/AWWA (1985) Standard methods for the examination of water and waste water quality. J Environ Toxicol Water qual 11: 72-82.
24. Sairam RK, Srivastava GC (2001) 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.
25. Jiang Y, Huang B (2002) Protein Alterations in Tall Fescue in Response to Drought Stress and Abscisic Acid. Crop Sci 42: 202-207.
26. Sarkar RK, Das S, Ravi I (2001) 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.
27. Hernandez JA, Jimenez A, Mullineaux P, Sevilla F (2000) Tolerance of pea (Pisum sativum L) to long-term salt stress is associated with induction of antioxidant defenses. Plant Cell. Environ 23: 853-862.
28. Kuk YI, Shin JS, Burgos NR, Hwang TE, Han O, et al. (2003) Antioxidative enzymes offer protection from chilling damage in rice plants. Crop Sci 43: 2109-2117.
29. Adeyemi O, Osubor CC, Adeyemi O (2010) 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: 26-31.
30. Atuanya EJ (1987) Effect of oil pollution on physical and chemical properties of soil, A case study of waste oil contaminated delta soil in Bendel State. Nigeria J Appl Sci 55: 155-176.
31. Okon IE, Udofot EE (2012) 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.
32. Uhegbu FO, Akubugwo EI, Iweala EJ, Uhegbu OC (2012) Impact of spent engine oil on soil and the growth of Zea mays seeds. J Environ Sci 1: 1-8.
33. Islam MR, Hu Y, Mao S, Jia P, Eneji AE, et al. (2011b) 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.

34. Islam MR, Xue X, Mao S, Ren C, Eneji AE, et al. (2011a) 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.

35. Yazdani F, Allahdadi I, Akbari GA (2007) Impact of superabsorbent polymer on yield and growth analysis of soybean (Glycine max L.) under drought stress condition. Pak J Biol Sci 10: 4190-4196.

36. Kramer PJ (1988) Measurement of plant water status Historical perspectives and current concerns. Irrig Sci 9: 275-287.

37. Mao S, Ilsam MR, Hu Y, Qian X, Chen F, et al. (2011) Antioxidant enzyme activities and lipid peroxidation in corn (Zea mays L.) following soil application of superabsorbent polymer at different fertilizer regimes. African Journal of Biotechnology 10: 10000-10008.