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Toxicology studies involving optimization of soil contaminated with spent engine oil using leaves of maize

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Abstract

The effect of spent engine oil-contaminated soil on morphological and biochemical properties of *Zea mays* (maize) 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 greenhouse in a factorial combination of five treatment levels (5 ml, 10 ml and 15 ml) of spent engine oil and additional three variants of each with pH adjustment to 5.5, 7 and 8.5 and were designated S0 for Control, S5 for soil containing 5 ml engine oil with no pH adjustment and S5A, S5B and S5C for pH adjustment to 5.5, 7.0 and 8.5 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). The maize planted in contaminated soil with pH adjustment recorded significantly higher height than their corresponding contaminated soil without pH adjustment ($p<0.05$). The girth of plant decreased with increased concentration of contaminant (spent engine oil). SPC of Control (S0) is significantly higher ($p<0.05$) than any of the maize planted in soil contaminated with spent engine oil. Generally, activities of antioxidant enzymes of Control were significantly lower ($p<0.05$) than those of maize planted in soil contaminated with spent engine oil. It could be inferred that spent engine oil in soil hindered the growth and development of maize, it created artificial water deficit to cellular system of maize and induced a condition of oxidative stress. However, maize planted in soil amended to pH 5.5 recorded favourable biochemical indices.

Keywords: Toxicology; Optimization; Soil; Spent engine oil; Maize

1 Introduction

In Nigeria today, peri-urban agriculture is rising due to increasing population and rising demand for arable foods such as cereals. A cereal like maize is cultivated on any available land in the urban area because of its ability to grow well on any type of soil. Maize, therefore, is cultivated both on contaminated soils and contaminated soils in the vicinity of industries including automobile workshops where hazardous wastes and other pollutants can contaminate the surrounding soil. Indiscriminate disposal of spent engine oils is common among automobile mechanics in some urban areas (Okpashi et al., 2020).

Engine-oil is a single petrochemical product that has been recognized over the years as a required emollient for motor engines (Abdulyekeen *et al.*, 2016). Spent engine oil refers to expended or used motor oils collected from mechanical or automobile workshops, garages, and industrial sources like hydraulic oil, turbine oils, engine oils, process oil and metal working fluids (Olugboji and Ogunwole, 2008). Spent engine oil is a mixture of several different chemicals including low and high molecular weight (C15-C20) aliphatic hydrocarbons, aromatic hydrocarbons, polychlorinated biphenyls,

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lubricant additives, chlorodibenzofurans, decomposition products, heavy metal contaminants such as aluminum, chromium, tin, lead, manganese, nickel and silicon that come from engine parts as they wear and tear down (Wang *et al*., 2000).

The disposal of spent oil into open vacant plots and farms, gutters and water drains is an environmental risk (Odjegba and Sadiq, 2002). Since it is liquid, it easily percolates into the soil and leaches into the environment from where it eventually pollutes either water or soil (Olugboji and Ogunwole, 2008). Pollution from waste engine oil has been reported to be more widespread than that of crude oil (Odjegba and Sadiq, 2002). This unregulated discharging of spent engine oil undesirably affects the flora, microbes and aquatic organisms (Nwoko *et al.,* 2007; Adenipekun *et al.,*2008) because of the large quantity of hydrocarbons and highly lethal polycyclic aromatic hydrocarbons contained in the oil (Wang *et al.,* 2000; Vwioko and Fashemi, 2005).

Investigations on the soil properties and growth of *Zea mays* have shown that spent engine oil had no effect on both the texture and pH of the soil. However, organic C, N and Mg as well as heavy metals (Fe, Cu, Zn, Cd, Mn, Ni, Cr and Pb) increased while P and yield of the plants grown on contaminated soil decreased. Also affected were the plant height, root number and root length (Okonokhua *et al*., 2007). Uhegbu *et al*. (2012) also reported a decrease in the moisture content of spent engine oil-polluted soil as well as delay in seed germination, leaf spread and rate of growth of *Zea mays* (Nwite and Alu,2015). Further studies on effects of spent engine oil on soil and *Zea mays* showed an increase in nitrogen, bulk density and organic carbon of the soil as well as reduced porosity, moisture content, hydraulic conductivity, Na, P, Mg, Ca and K (Udonne and Onwuma, 2014; Nwite and Alu, 2015).

Molecular study of the effect of spent engine oil on enzymic and non-enzymic antioxidant of maize is scanty. There is also paucity of information on the effect of optimization, by pH adjustment, of spent engine oil contaminated soil on maize. The present study, however, investigated the activities of antioxidant enzymes of leaf of maize grown on pH optimized spent engine oil contaminated soil.

2 Material and methods

2.1 Reagents

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

2.2 Spent Engine-oil

The spent engine-oil sample was collected from automobile Mechanic workshop in Warri Delta State, Nigeria.

2.3 Experimental design and agronomic details

The experiment was conducted in a greenhouse of the College of Science, Federal University of Petroleum Resources, Effurun, Nigeria. The method described by Adewole and Aboyeji (2003) 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 greenhouse in a factorial combination of three treatment levels (5 ml, 10 ml and 15 ml) of spent engine oil and additional three variants of each with pH adjustment to 5.5, 7 and 8.5 and were designated S0 for Control, S5 for soil containing 5 ml engine oil with no pH adjustment and S5A, S5B and S5C for pH adjustment to 5.5, 7.0 and 8.5 respectively (Table 1).

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 spent engine oil, three seeds of maize (obtained from Ugbomro market, Ugbomro, 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.

Table 1 Plant Groups and Treatment

Group	Treatment	Period Of Treatment
S0	Control	8weeks
S5	Soil containing 5 ml engine oil with no pH adjustment	8weeks
S5A	Soil containing 5 ml engine oil with pH adjustment to 5.5	8weeks
S5B	Soil containing 5 ml engine oil with pH adjustment to 7.0	8weeks
S5C	Soil containing 5 ml engine oil with pH adjustment to 8.5	8weeks
S10	Soil containing 10 ml engine oil with no pH adjustment	8weeks
S10A	Soil containing 10 ml engine oil with pH adjustment to 5.5	8weeks
S10B	Soil containing 10 ml engine oil with pH adjustment to 7.0	8weeks
S10C	Soil containing 10 ml engine oil with pH adjustment to 8.5	8weeks
S15	Soil containing 15 ml engine oil with no pH adjustment	8weeks
S15A	Soil containing 15 ml engine oil with pH adjustment to 5.5	8weeks
S15B	Soil containing 15 ml engine oil with pH adjustment to 7.0	8weeks
S15C	Soil containing 15 ml engine oil with pH adjustment to 8.5	8weeks

2.4 Relative Water Content (RWC) Determination

Relative water content (RWC) of leaves was determined at 3 and 6 WAP by the standard method (Schonfeld, *et al*; 1988).

2.5 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 by Black, (1982); AOAC (1996); APHA/AWWA (1985).

2.6 Preparation of Homogenate and Biochemical Analysis

The fresh leaf and stem tissues were collected from each pot of the plant and 0.5 g was weighed and chopped into very small pieces and then homogenized using pre-cooled pestle and mortar in a bowl of ice cubes. The fresh leaf and stem tissue homogenates were diluted using normal/physiological saline of 9 g of NaCl in 1litre of distilled water. The diluted homogenates were stored at temperature of -8 °C until required for use.

The protein content in the tissue of experimental plants was determined following the method reported by Gornal *et al* (1949). The malondialdehyde (MDA) concentration in the serum and tissues of rats experimental was determined following the method described by Bird *et al.* (1982). The Reduced Glutathione (GSH) concentration in the tissues of experimental plants was determined following the method described by Jollow *et al,* (1974). The Superoxide Dismutase (SOD) activity of the tissues of experimental animals was determined following the method described by Misra and Fridovich (1972). The catalase activity of the tissue homogenate obtained from the experimental plant was determined following the method described by Sinha (1971). The activity of ascorbate peroxidase was assayed by the method of Nakano and Asada (1987). Peroxidase activity was determined specifically by the method of Putter, (1971).

2.7 Statistical Analyses

All numerical results were obtained from the thirteen groups (control and treated). Data obtained were presented as mean±SEM and subjected to statistical analysis using a one way analysis of variance method of Steel and Torrie (1960). Significant difference between the treatment means was determined at 95% confidence level using Duncan's Multiple Range Test (1955).

3 Results

The height of maize planted in spent engine oil contaminated soil over a period of eight weeks is shown in Figure 1. Generally, height of plant progressed until the eighth week. Beginning from week two till the last week, the height of maize planted in Control soil was significantly higher than the maize planted in the contaminated soils (S5, S5A, S5B, S5C, S10, S10A, S10B, S10C, S15, S15A, S15B and S15C). The maize planted in contaminated soil with pH adjustment recorded significantly higher height than their corresponding contaminated soil without pH adjustment. Height of plant decreased significantly with increased concentration of spent engine oil. Plants in soil contaminated with 10 ml and 15 ml of spent engine oil did not germinate at 2 weeks after planting (WAP).

Figure 2 presents the girth of maize planted in soil contaminated with spent engine oil over a period of 8 weeks. Similar to Figure 1, stem girth of plant increased significantly over the eight weeks period of the experiment. Stem girth of maize planted in contaminated soil was significantly lower than Control ($p<0.05$). The girth of plant decreased with increased concentration of contaminant (spent engine oil).

The relative water content (RWC) of maize planted in soil contaminated with spent engine oil. At 3 WAP RWC of maize in groups S10A, S10B and S10C were not significantly different ($p>0.05$), similarly RWC of S15B and S15C were not significantly different. At 6WAP S15B and S15C were still not significantly different. However, RWC of Control was significantly higher than those of maize planted in soil contaminated with spent engine oil ($p<0.05$).

Table 2 shows the effect of spent engine oil on SPC, MDA and SPAD value of maize leaves. SPC of Control (S0) was significantly higher ($p<0.05$) than any of the maize planted in soil contaminated with spent engine oil. Particularly, SPC of Control is about 2 folds that of S5 and 3 ½ folds that of S15. MDA of S5, S10 and S15 is about 2, 3 and 5 folds respectively of the Control. SPAD values of maize in S5A, S10A, S15B and S15C were not significantly different.

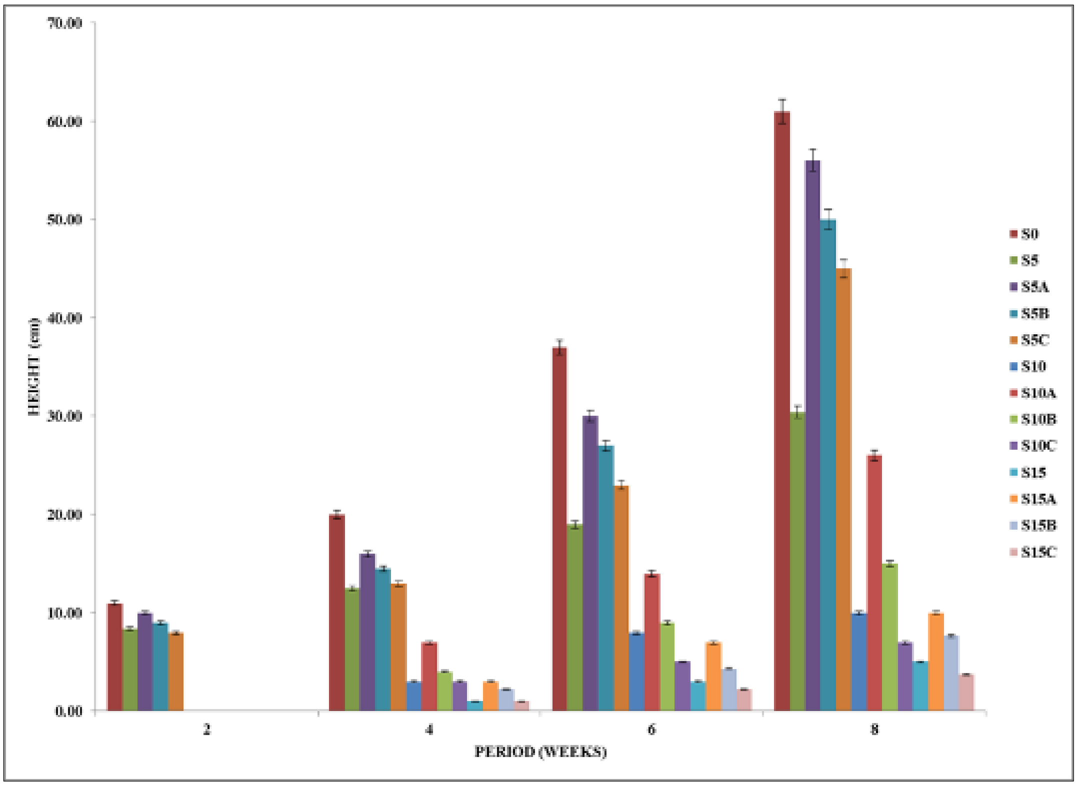

Figure 1 Effect of spent engine oil on the height of *Zea mays.* Plotted values are means ± SEM of six determinations

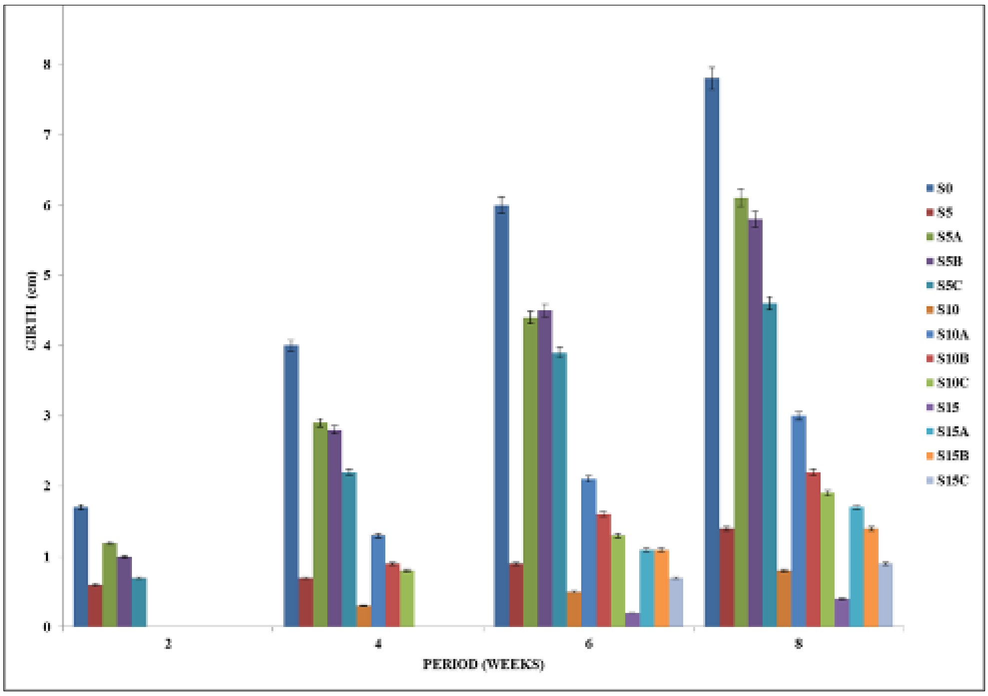

Figure 2 Effect of spent engine oil on the girth of *Zea mays.* Plotted values are means ± SEM of six determinations

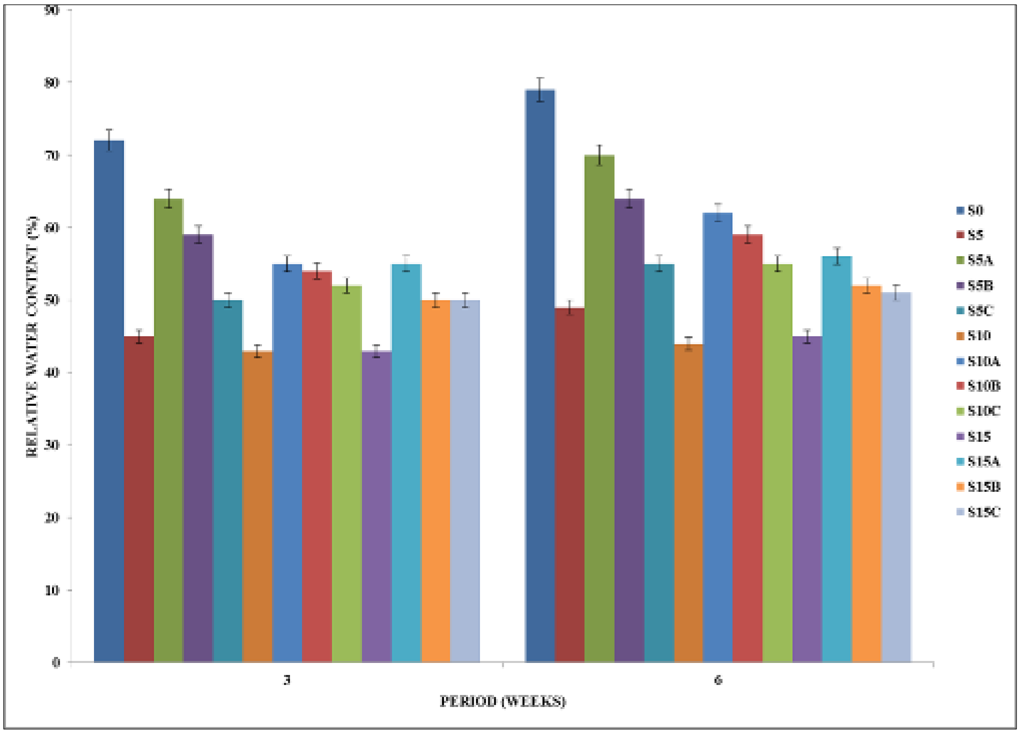

Figure 3 Effect of spent engine oil on the relative water content of *Zea mays.* Plotted values are means ± SEM of six determinations

Specific activities of selected enzymes of maize planted in soil contaminated with spent engine oil are presented in Table 3. Generally, activities of enzymes of Control were significantly lower ($p<0.05$) than those of maize planted in soil contaminated with spent engine oil. Activity of SOD of S5 was 50% higher than Control, while that of S5A was just 15%

higher than Control. SOD activity of S10 was about 90% that of Control whereas that of S10A was about 26% that of Control.

Table 2 Effect of spent engine oil on soluble protein content (SPC), malondialdehyde (MDA) and SPAD value of maize leaves

GROUPS	SPC (mg g^{-1}FW)	MDA (µmol g^{-1}FW)	SPAD value
S0	3.25±0.50^{a}	5.50±0.25^{a}	30.25±2.00^{a}
S5	1.78±0.03^{b}	13.00±1.30^{b}	18.20±1.44^{b}
S5A	2.90±0.10^{c}	7.64±0.36^{c}	28.10±1.50^{b}
S5B	2.51±0.09^{d}	9.00±0.50^{d}	23.20±1.40^{c}
S5C	2.47±0.11^{d}	10.55±0.85^{d}	20.50±1.45^{d}
S10	1.25±0.02^{e}	17.60±1.40^{e}	15.50±1.20^{e}
S10A	2.20±0.08^{f}	8.20±0.82^{f}	25.48±1.50^{b}
S10B	1.78±0.05^{b}	10.00±1.04^{d}	20.70±1.53^{d}
S10C	1.80±0.05^{b}	11.80±1.50^{d}	19.64±1.45^{d}
S15	0.89±0.03^{g}	25.20±1.89^{f}	13.89±0.90^{e}
S15A	2.25±0.10^{f}	14.70±1.35^{b}	23.20±1.30^{c}
S15B	2.00±0.14^{g}	18.25±1.45^{e}	19.00±1.20^{b}
S15C	2.00±0.10^{g}	20.00±1.25^{e}	17.70±0.88^{b}

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 spent engine oil on activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX)and glutathione reductase (GR) activities of maize 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)
S0	100.20±7.50^{a}	120.60±7.80^{a}	415.50±12.20^{a}	470.40±3.40^{a}	68.50±4.30^{a}
S5	154.60±9.80^{b}	189.70±8.40^{b}	600.90±9.10^{b}	565.20±5.50^{b}	88.10±2.30^{b}
S5A	115.40±6.80^{c}	129.50±7.40^{ae}	434.50±10.20^{a}	490.60±4.50^{c}	73.30±3.60^{c}
S5B	130.10±7.70^{d}	144.20±6.50^{c}	489.10±11.40^{c}	512.40±3.90^{d}	77.10±2.40^{c}
S5C	139.80±8.70^{d}	150.30±7.00^{cf}	500.00±10.80^{c}	518.30±4.00^{d}	80.20±3.20^{c}
S10	190.80±9.90^{e}	190.50±7.60^{d}	688.20±9.70^{d}	594.70±6.10^{e}	101.50±4.40^{d}
S10A	126.40±8.60^{cd}	139.40±8.20^{ce}	456.80±13.00^{e}	512.50±5.30^{d}	80.40±2.50^{c}
S10B	159.70±8.50^{b}	154.30±7.50^{cf}	489.40±9.70^{c}	545.70±5.10^{f}	88.50±2.40^{b}
S10C	160.50±7.90^{b}	166.20±8.60^{f}	492.50±8.20^{c}	552.70±4.70^{f}	90.60±3.30^{b}
S15	198.30±10.00^{e}	195.60±6.50^{b}	695.30±12.10^{d}	600.00±5.20^{g}	124.50±3.50^{e}
S15A	149.80±8.70^{d}	148.70±7.30^{c}	487.30±9.80^{c}	530.60±6.40^{h}	83.70±2.60^{bc}
S15B	161.60±7.80^{b}	159.50±6.30^{cf}	498.40±8.10^{c}	550.20±5.10^{f}	89.50±4.20^{b}
S15C	167.50±9.40^{b}	158.10±7.20^{cf}	502.10±7.60^{c}	562.10±7.70^{f}	90.70±3.20^{b}

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

4 Discussion

The indiscriminate disposal of spent engine oil is a growing concern, maize is a cereal planted by most families in Nigeria because it grows well on anywhere and ready for harvest within three months. The plant is grown even in the automobile mechanic yard to provide fast food for the workers. The practice of disposing spent engine oil on soil where maize is and / or will be planted has necessitated the present study. Here, the morphology and biochemistry of maize was examined in response to spent engine oil.

The delayed germination, reduced growth and decreased stem girth observed in this study (Figures 1-2) is a pointer to the fact that spent engine is a hindrance to normal growth and development of the maize plant. Soil nutrients had been reported to be less mobile in contaminated soils [Okon and Udofot, 2012] and this condition had also been reported to adversely alter the growth pattern of plants [Uhegbu et al., 2012].

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 [Islam et al., 2011a, 2011b]. In this study, reduced RWC in maize planted in soil contaminated with spent engine oil (Figure 3) portends a condition of cellular water deficit.

Low level of SPC and SPAD (Table 2) could be due to difficulty in getting appropriate nutrient. Reduced soil aeration due to thin film layer formation on the topsoil by the applied spent engine oil could have reduced the air passage through the soil pores, thereby leading to the inadequate air supply to the maize plants [Adeyemi, 2014] and hence, reduction in the SPAD value and SPC. The artificial drought condition created by the engine oil may also be responsible for the increased lipid peroxidation products, MDA, of leaves of experimental corn.

Maize planted in soil contaminated with spent engine oil showed a significant increase in SOD, CAT, POD, APX and GR activity (Table 3) in the leaves. The results suggest that soil contaminated with spent engine oil 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 [Yazdani et al., 2007]. The maize plant which is considered moderately drought tolerant [Mao et al., 2011] might have inadequate ROS scavenging system in addition to other tolerance mechanisms to cope with stress.

5 Conclusion

The present study revealed as follows;

- Spent engine oil in soil hindered the growth and development of maize
- It created artificial water deficit to cellular system of maize
- Induced a condition of oxidative stress

Experimental evidence from this study further underscore the importance of caution when generating and disposing spent engine oil to avoid contamination of the environment. Further research of soil contaminated with spent engine oil should consider superabsorbent polymer (SAP) and various fertilizer regimens.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare no conflict of interest.

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