International Journal of Plant & Soil Science
2(1): 144-154, 2013; Article no. IJPSS.2013.012

SCIENCEDOMAIN *international*
www.sciencedomain.org

Effect of Different Crude Oil Fractions on Growth and Oxidative Stress Parameters of Maize Radicle

O. Stella Olubodun[1*] and E. George Eriyamremu[2]

[1]Department of Science Laboratory Technology, Edo State Institute of Technology and Management, Usen, Edo State, Nigeria.
[2]Department of Biochemistry, Faculty of Life Sciences, University of Benin, Nigeria.

Authors' contributions

Both the authors were involved in all aspects of this study.

Research Article

Received 26th March 2013
Accepted 27th June 2013
Published 18th July 2013

ABSTRACT

This study investigated the effect of different crude oil fractions (whole crude WC, water soluble fraction WSF, water insoluble fraction WIF and soil from a crude oil contaminated site, Ubeji) at different percentages of soil contamination (2, 5 and 10%) on growth (percentage seedling emergence, plant height, leaf number, leaf area, root length and radicle length) and oxidative stress (catalase (CAT), superoxide dismutase (SOD), peroxidase (POX) and malondialdehyde (MDA) concentrations) parameters in maize radicle. A total of 330 bags containing 500g of soil were used for this study. 30 bags containing sandy loam soil served as control, 270 bags of sandy loam soil were mixed with different crude oil fractions to give 2%, 5% and 10% contamination, while 30 bags containing 500g of soil were collected from a crude oil contaminated site in Ubeji, south-south, Nigeria. The maize grown in these soils was harvested after 7, 14 and 21 days of seedling emergence and assessed for growth and oxidative stress parameters. Descriptive statistics and analysis of variance were employed to statistically analyse data that were obtained. The growth parameters in maize, including percentage seedling emergence, leaf number, leaf area, root length etc, significantly (P<.01) reduced as percentage contamination increased. Maize grown in soil contaminated with 10% WIF and the Ubeji soil produced the greatest reduction in growth parameters. Oxidative stress assessed by measuring maize radicle CAT, SOD, POX and MDA levels, revealed a

**Corresponding author: E-mail: sabukadi@yahoo.com;*

percentage contamination dependent alteration of these parameters implying the presence of oxidative stress. The results of this study have again shown the deleterious effects of crude oil contamination on plant growth. Efforts to intensify effective cleanup of crude oil contaminated sites must be given top priority.

Keywords: Crude oil; maize; growth parameters; oxidative stress; contamination.

1. INTRODUCTION

Nigeria is an established crude oil exporting nation producing medium and light crude oil, such as Bonny light [1]. Crude oil exportation is the mainstay of Nigeria's economy. Crude oil is a colloidal mixture of different hydrocarbons (90%) and non-hydrocarbon (10%) components [2]. Various activities in crude oil exploration, exploitation, storage and transportation lead to spillage of oil to the environment [3]. Crude oil causes harmful effects on the environment, where it poses a serious threat to organisms and farmland that are linked in a complex food chain that includes humans [4]. The effects of crude oil on the growth and performance of plants have been reported in many studies [5,6,7,8]. Crude oil in soil makes the soil condition unsatisfactory for plant growth. It can reduce the level of available plant nutrient in contaminated soils [9] and can also raise the levels of certain elements such as iron and zinc to toxic amounts [10]. Water and oil are usually considered to be immiscible. However, crude oil contains a very small soluble portion referred to as the water soluble fraction (WSF). The soluble constituents are dispersed particulate oil, dissolved hydrocarbons and soluble contaminants such as metallic ions. The components of crude oil that go into solution make up the WSF. The lower the molecular weight of the constituent hydrocarbon of crude oil, the higher is its concentration in the water-soluble fraction [11]. The analysis of the water soluble fraction of South Louisiana and Kuwait crude oils revealed that the WSF contained 20 aromatic compounds ranging from benzene to dimethyl phenothrenes and up to 14 saturated hydrocarbons ranging from C14 to paraffin [12]. Adverse biological effects have been attributed to dissolved low molecular weight hydrocarbons particularly aromatics such as toluene. Some researchers consider naphthalene as a more important source of crude oil toxicity than low molecular weight aromatics. It is believed that the low boiling point unsaturated hydrocarbons such as benzene, toluene, xylene and naphthalene, are the most toxic components in crude oil [11,12].

Exposure of plants to crude oil and heavy metal poisoning has been reported to produce reactive oxygen species (ROS) and other free radicals which induce oxidative stress and cause lipid peroxidation [13]. Even at an early stage, it can cause a reduction in cell proliferation and growth. Various researchers have reported activation of lipid peroxidation in plants exposed to different pollutants [13,14]. Increase in superoxide dismutase (SOD) activity has also been reported in oat, wheat and *Arabidopsis thaliana* in soils contaminated with various pollutants [15, 16]. Several plant species have also been shown to have elevated peroxidase activities in response to increased pollutant concentrations [17,18]. Reactive oxygen species is thought to increase cellular damage through the oxidation of several macromolecules such as lipids and proteins [19].

Maize (*Zea mays*) ranks as one of the major staple food consumed in Nigeria. Maize is grown in most agro ecological areas especially in the Niger Delta region where oil industrial activities are predominant [3]. Most studies of the effects of crude oil in maize have

examined the effect of whole crude on physiological parameters such as percentage germination and growth parameters like plant height, root length etc. There is however, lack of information on the effects of crude oil and its fractions (WSF and WIF) on biochemical parameters such as oxidative stress parameters in maize. It was therefore the aim of the present study to investigate the effect of whole crude and different crude oil fractions, as well as *ex situ* crude oil contaminated soil from Ubeji, Delta State, Nigeria, on growth and oxidative stress parameters of *Zea mays*.

2. MATERIALS AND METHODS

2.1 Study Location

The *ex situ* study was carried out at the University of Benin, Benin City, Edo State, Nigeria from the month of March to May, 2009. The crude oil contaminated soil was collected from Ubeji, South-South Nigeria, the same geographic location as the study site. The region is made up of flat plains with sandy loam soil characterized as coastal plain sand. The region experiences moderate rainfall and humidity for most part of the year. Temperature and humidity remain relatively constant throughout the year. The climate is marked by two distinct seasons: the dry season and the rainy season. The area is characterized by tropical equatorial climate with mean annual temperature of 32.8ºC and annual rainfall of about 2673.8 mm. The natural vegetation is dense tropical rainforests with swamp forest in some areas.

2.2 Experimental Design

Sandy loam soil (0-20cm depth) of pH 6.15 was weighed into 300 polythene bags such that each bag contained 500g soil. 30 bags of 500g of soil collected from a Bonny light crude oil contaminated soil (Ubeji, pH 6.74, total hydrocarbon content (THC) = 74.88 mg/kg) were also obtained; they were grouped as shown below:

Table 1. Concentration of crude oil contamination in soil

Group	% Contamination	Number of bags
Control	-	30
2% Whole crude (WC)	2%	30
5% WC	5%	30
10% WC	10%	30
2% Water soluble fraction (WSF)	2%	30
5% WSF	5%	30
10% WSF	10%	30
2% Water insoluble fraction (WIF)	2%	30
5% WIF	5%	30
10% WIF	10%	30
Ubeji	THC (74.88 mg/kg)	30

2.3 Plant Materials

Maize (*Zea mays*) seeds, of the variety Dmr-Esr-w, were bought from a local market in Benin City, Edo State, Nigeria. Seed viability was assessed by floatation method. The seeds were

placed in a beaker containing tap water and stirred. The seeds that did not float were regarded as viable seeds.

2.4 Crude Oil and Fractionation

Crude oil (Bonny light, ^{o}API (American Petroleum Institute) gravity =37) was obtained from Warri Refinery and Petrochemical Company, Delta State, Nigeria. A portion of the crude oil was fractionated by the method of Anderson et al. [12] into water soluble fraction (WSF) and water insoluble fraction (WIF). For the fractionation, a 1:2 dilution of 200ml of crude oil was put in a 1 litre conical flask and constantly stirred with a magnetic stirrer for 48h. The WSF then separated from the WIF in a separating funnel.

2.5 Soil Treatment

Control soil contained only sandy loam soil. The soil in the bags contaminated with whole crude (WC), water soluble fraction (WSF) and water insoluble fractions (WIF) were mixed thoroughly in their respective polythene bags containing 500g sandy loam soil with the aid of a plastic spade. Soil of 500g was treated with 10 ml, 25ml and 50ml of crude oil to obtain 2, 5 and 10% v/w crude oil contamination.

2.6 Planting of Seeds and Seedling Emergence Studies

The seeds were planted by a modified version of Vavrek and Campbell [20]. Three viable maize seeds were sown in 500g sandy loam soil with a depth of about 1-2 cm. The time and number of seeds that emerged from each bag were noted and the percentage seedling emergence in each treatment was calculated using the formula:

$$Percentage\ seedling\ emergence\ = \frac{number\ of\ seedlings\ that\ emerged}{number\ of\ seeds\ sown}\ x\ \ 100$$

Equal amounts of seeds that emerged were harvested at day 7, day 14 and day 21. Growth parameters were assessed after each harvest.

2.7 Biochemical Assays

Antioxidant enzymes; catalase (CAT), superoxide dismutase (SOD) and peroxidase (POX), as well as lipid peroxidation were also assessed in the radicle of the maize plant after each harvest.

2.7.1 Enzyme extraction

All enzyme extraction was done on ice. SOD and POX were extracted by homogenizing the samples in 0.1M phosphate buffer (pH 7.5) containing 1% polyvinylpyrrolidone (PVP), 1mM EDTA and 10µM β-mercaptoethanol. CAT was extracted with 0.05M phosphate buffer (pH 7.5) containing 1% PVP. All homogenates were centrifuged at 5,000 X g for 20 minutes and the supernatants used to assay the enzymes [21].

2.7.2 Thiobarbituric acid reactive substance (TBARS) assay

Thiobarbituric acid reactive substance (TBARS) assay was carried out to determine malondialdehyde levels (lipid peroxidation) according to the method of Gutteridge and

Wilkins [22]. The radical was homogenized under cold conditions with Tris-HCl buffer (pH 7.4) in the presence of 1.5% Polyclar-AT (w/v) to eliminate polyphenols, which interferes with the assay. It was centrifuged at 3,000 X g for 30 minutes. Thiobarbituric acid (1% [w/v] in glacial acetic acid was added to an aliquot of the supernatant in a test tube which was placed in a boiling water bath for 30 minutes. After cooling and centrifugation, the absorbance of the supernatant was measured at 532 nm.

2.7.3 Superoxide dismutase (SOD)

Superoxide dismutase (SOD) activity determination was carried out according to the method of Misra and Fridovich [23] and was expressed as units/mg tissue weight.

2.7.4 Catalase (CAT)

Catalase activity determination was carried out according to the method of Sinha [24] and was expressed as units/g tissue weight.

2.7.5 Peroxidase (POX)

Peroxidase was assayed according to the method of Chance and Maehly [25]. This method is based on the oxidation of pyrogallol to purpurogallin by peroxidase at 20^0C. The amount of purpurogallin formed was taken as an activity unit and expressed as unit/mg protein.

2.8 Statistical Analysis

The result of the study was expressed as mean ± standard error of mean (SEM). Analysis of variance was used to test for differences in the groups while Duncan's multiple comparisons test was used to determine significant differences between means. The Instat-Graphpad software, San Diego, California, USA, was used for this analysis. A $P<.01$ was considered statistically significant

3. RESULTS AND DISCUSSION

3.1 Effect of Crude oil Contamination on Growth Parameters of Maize

The percentage seedling emergence of the maize seeds for each of the fraction used decreased as the percentage contamination increased. The seeds grown on 10% water insoluble fraction (WIF) contaminated soil exhibited the lowest seedling emergence rate of 27% while the highest seedling emergence rate was seen in the soil contaminated with 2% water soluble fraction (Table 2). After 7 days post germination, the leave number (LN) of maize grown in the different treatment groups did not significantly differ from control. However after 14 and 21 days post germination, the LN of maize in test groups significantly reduced ($P<.01$) with 10% WIF showing the greatest reduction. Plant height (PH), leaf area (LA), root length (RL) and radical length (RaL) reduced for the various fractions used in a nearly percentage contamination dependent manner. These parameters also reduced in maize grown in the contaminated soil from Ubeji (Table 2). For all the growth parameters assessed, 2% whole crude (WC) and water soluble fraction (WSF) appeared to exert the least negative effects, whereas 10% WIF and soil from Ubeji contaminated site exerted the most negative effects.

Various activities of crude oil exploration and exploitation results in the spillage of oil on land, including farmlands. Ekundayo et al. [26] reported delayed germination and reduction in grain yield of maize in crude oil polluted soil. Beyond 3% concentration, crude oil has been reported to be increasingly deleterious to soil biota and crop growth [27,28,29]. Most people in the Niger Delta region of Nigeria still depend on subsistent farming for their livelihood, large scale farming also provides for the region and other parts of Nigeria. Maize is particularly important to this region since it can be grown almost all year round due to the riverine nature of this region. This study revealed that several growth parameters of maize, including percentage of seedling emergence, were adversely affected by crude oil contamination and these negative effects increased as the percentage contamination of the soil increased. Several studies have continuously reported that crude oil spillage results in decreased crop production and the more the concentration of the spillage the greater the damage done. Omosun et al. [7] reported that growth parameters in *Amaranthus hybridus* decreased as the concentration of crude oil contamination increased. In fact, Agbogidi et al. [3] reported that crude oil levels have a significant effect on the growth of maize. The findings of this study thus add to the growing overwhelming evidence of the deleterious effects of crude oil spillage on plant crops. Reduction in the general growth parameters of plants grown on contaminated sites have been attributed to insufficient aeration of crude oil contaminated soils [5] and limited water supply to the plants [3]. Air and water, which contains dissolved nutrients, are necessary for plant growth. The reduction in root length observed in this study, limits the quantity of nutrients the plant is able to absorb. Also, the reduction in leaf number and leaf area reduces the rate of photosynthesis, these effects together culminates in the reduction of plant growth. The water insoluble fractions, which may have further limited the availability of water soluble nutrients in the soil, could account for the greater reduction in maize growth parameters observed in maize grown in soil contaminated with WIF especially at the highest concentration used (10%). This agrees with the observation of Eriyamremu et al. [30].

3.2 Effect of Crude Oil Contamination on Lipid Peroxidation and Enzymatic Antioxidant Activities of Maize Radicle

Evaluation of catalase activities at day 7, 14 and 21, showed significant (P<.01) increases in enzyme activities in the radicle of maize grown in different fractions of crude oil contaminated soil and Ubeji soil (Table 3), the rate of increase reduced as percentage contamination increased. The maize radicle grown in the WIF contaminated soil exhibited the lowest rate of increase in CAT activity whereas the highest increases were seen in the WSF group. Generally, a percentage contamination dependent decrease was observed in the radicle SOD activities of maize grown in crude oil contaminated soils (Table 3) with the WIF and Ubeji groups recording the lowest values. Maize radicle peroxidase activities 7 days post germination increased in the lowest percentage contamination for all fractions whereas the high percentage contaminated soils drastically decreased in peroxidase activities. The peroxidase activities of radicle of maize grown in Ubeji contaminated soil were similar to control 7 days post germination. For the various fractions used, the same trend as in day 7 was observed for day 14; the only exception was that the radicle peroxidase activities of Ubeji soil increased slightly.

Table 2. Effect of different fractions of crude oil contamination on growth parameters of maize

	Control	Whole crude			Water soluble fraction			Water insoluble fraction			Ubeji
		2%	5%	10%	2%	5%	10%	2%	5%	10%	
Number of seeds planted/bag	3	3	3	3	3	3	3	3	3	3	3
% seedling emergence	100	70	54	37	86	65	42	63	47	27	46
7 days after germination											
LN (cm)	3^{a}	3^{a}	3^{a}	3^{a}	4^{ab}	4^{a}	3^{a}	3^{a}	3^{a}	3^{a}	3^{a}
PH (cm)	30.7 ± 2.4^{a}	19.5 ± 2.6^{b}	17.9 ± 1.9^{b}	15.8 ± 0.5^{b}	20.3 ± 0.7^{b}	26.3 ± 0.7^{ab}	27.3 ± 0.3^{a}	16.2 ± 0.2^{b}	20.3 ± 0.7^{b}	14.0 ± 1.0^{b}	17.5 ± 0.8^{b}
LA (cm^2)	41.6 ± 0.9^{a}	21.5 ± 0.3^{c}	19.6 ± 0.3^{c}	17.3 ± 0.2^{c}	20.3 ± 0.1^{c}	26.4 ± 0.06^{b}	32.9 ± 0.4^{b}	17.1 ± 1.1^{c}	21.2 ± 1.6^{c}	18.1 ± 0.8^{c}	23.7 ± 0.4^{c}
RL (cm)	30.0 ± 1.7^{a}	21.3 ± 0.4^{ab}	15.7 ± 0.7^{b}	14.7 ± 1.1^{c}	25.2 ± 1.1^{a}	22.0 ± 1.0^{b}	16.7 ± 0.3^{b}	20.2 ± 1.6^{ab}	18.8 ± 1.3^{b}	12.7 ± 0.4^{c}	14.3 ± 0.7^{c}
RaL (cm)	3.1 ± 0.9^{a}	3.0 ± 0.2^{a}	2.8 ± 0.1^{a}	2.3 ± 1.0^{b}	2.0 ± 0.2^{b}	1.8 ± 1.8^{b}	1.5 ± 0.7^{c}	2.2 ± 1.8^{b}	1.9 ± 0.7^{b}	1.7 ± 0.6^{c}	0.7 ± 0.7^{c}
14 days after germination											
LN (cm)	5.3^{a}	5^{ab}	5^{a}	4^{b}	4^{ab}	4^{ab}	4^{b}	4^{ab}	4^{ab}	3.7^{b}	3.7^{b}
PH (cm)	54.7 ± 2.0^{a}	38.7 ± 1.5^{b}	39.2 ± 3.3^{a}	29.3 ± 1.8^{b}	39.7 ± 0.7^{b}	32.3 ± 1.5^{b}	36.3 ± 1.5^{b}	30.0 ± 0.1^{b}	24.2 ± 0.4^{c}	22.3 ± 0.9^{c}	32.1 ± 0.5^{b}
LA(cm^2)	61.2 ± 1.9^{a}	52.4 ± 1.3^{a}	43.9 ± 1.5^{b}	34.2 ± 1.4^{b}	46.9 ± 0.5^{a}	36.2 ± 0.4^{b}	33.2 ± 1.0^{b}	36.7 ± 1.7^{b}	26.3 ± 0.2^{c}	21.9 ± 1.2^{c}	40.6 ± 1.4^{b}
RL (cm)	36.0 ± 1.1^{a}	30.3 ± 0.7^{a}	23.7 ± 1.7^{b}	21.5 ± 0.1^{b}	31.4 ± 1.4^{a}	24.3 ± 0.4^{b}	18.5 ± 0.1^{b}	23.0 ± 0.1^{b}	20.3 ± 1.5^{b}	13.8 ± 0.2^{b}	22.1 ± 0.9^{b}
RaL (cm)	3.9 ± 0.7^{a}	3.6 ± 0.1^{a}	3.3 ± 0.3^{b}	2.8 ± 0.1^{b}	3.6 ± 0.3^{a}	2.8 ± 0.1^{b}	1.8 ± 1.2^{c}	3.4 ± 0.7^{ab}	2.3 ± 0.3^{c}	2.2 ± 0.9^{c}	1.3 ± 0.3^{c}
21 days after germination											
LN (cm)	6.7^{a}	5^{a}	5.7^{a}	4.7^{b}	5.7^{a}	4^{b}	4^{b}	4^{b}	4^{b}	3.7^{b}	4^{b}
PH (cm)	79.7 ± 0.3^{a}	49.7 ± 1.2^{b}	54.5 ± 2.6^{b}	43.3 ± 1.5^{b}	53.6 ± 0.6^{b}	41.8 ± 1.0^{c}	37.0 ± 1.5^{d}	47.3 ± 2.2^{c}	28.8 ± 0.2^{d}	28.0 ± 1.5^{d}	46.7 ± 2.3^{b}
LA (cm^2)	84.2 ± 1.8^{a}	61.0 ± 1.1^{b}	50.7 ± 1.8^{b}	51.4 ± 1.9^{b}	58.5 ± 1.4^{b}	42.8 ± 1.3^{b}	35.5 ± 0.5^{bc}	29.8 ± 1.6^{c}	23.8 ± 1.2^{c}	22.8 ± 1.2^{c}	59.8 ± 0.6^{b}
RL (cm)	52 ± 1.2^{a}	41 ± 0.6^{b}	28.3 ± 0.9^{c}	24.2 ± 0.6^{c}	42 ± 0.9^{b}	26.5 ± 2.8^{c}	20.8 ± 1.6^{c}	27.3 ± 1.5^{c}	22.7 ± 1.3^{c}	17.5 ± 0.3^{c}	27 ± 2.8^{c}
RaL (cm)	7.3 ± 0.1^{a}	4.4 ± 0.3^{b}	3.9 ± 0.1^{b}	3.1 ± 1.0^{b}	4.1 ± 0.6^{b}	3.8 ± 0.1^{b}	2.3 ± 0.9^{c}	3.4 ± 0.7b	2.5 ± 0.6^{c}	2.2 ± 0.9^{c}	1.6 ± 0.7^{d}

PH = Plant height; RL= length of root; RaL= length of radicle; LN = number of leaves; LA = area of leaves.

Values are means of 4 to 8 determinations ± S.E.M. Means carrying different notations are statistically different at P<.01.

Table 3. Effect of different fractions of crude oil contamination on lipid peroxidation and antioxidant enzymes of maize radical

	Control	Whole crude			Water soluble fraction			Water insoluble fraction			Ubeji
		2%	5%	10%	2%	5%	10%	2%	5%	10%	
7 days after germination											
Catalase unit/g	1.38± 0.3^{a}	2.42 ± 0.2^{b}	4.15 ± 0.2^{c}	4.94 ± 0.1^{d}	2.68 ± 0.2^{b}	4.59 ± 0.2^{d}	5.07 ± 0.8^{d}	1.93 ± 0.6^{b}	3.62 ± 0.3^{c}	4.15 ± 0.5^{c}	2.63 ± 0.1^{b}
SOD unit/mg	3.13 ± 0.6^{a}	3.31 ± 1.2^{a}	2.03 ± 0.3^{b}	2.26 ± 1.4^{b}	4.02 ± 0.5^{d}	3.29 ± 0.4^{a}	2.25 ± 1.9^{b}	2.68± 0.7^{ab}	1.99 ± 1.1^{b}	1.42 ± 0.2^{c}	1.60± 0.09^{c}
Peroxidase unit/mg	2.2 ± 0.01^{a}	5.3 ± 0.01^{b}	0.083± 0.03^{c}	0.032 ± 0.01^{c}	6.0 ± 0.01^{b}	0.087 ± 0.01^{c}	0.096 ± 0.04^{c}	4.0 ± 0.03^{b}	0.074± 0.01^{c}	0.092 ± 0.01^{c}	2.0 ± 0.01^{a}
Malonedialdehyde levels(μmole/g)	0.1± 0.01^{a}	0.2± 0.01^{b}	0.2±0.01^{b}	0.3±0.01^{b}	0.1± 0.01^{a}	0.1±0.01^{a}	0.2 ± 0.01^{b}	0.1± 0.01^{a}	0.2±0.01^{b}	0.2 ± 0.01^{b}	0.2±0.01^{b}
14 days after germination											
Catalase unit/g	1.62 ± 0.5^{a}	3.68 ± 1.0^{b}	4.56 ± 0.7^{c}	5.68 ± 1.9^{c}	4.09± 0.2^{b}	4.98±0.2^{c}	5.71±0.8^{c}	3.10 ± 0.7^{b}	4.08 ± 0.3^{b}	4.62 ± 0.4^{c}	3.53 ± 1.0 ^{b}
SOD unit/mg	1.94 ± 0.02 ^{a}	2.44 ± 1.3 ^{b}	1.79 ± 0.6^{a}	1.43± 0.3^{a}	2.84 ± 1.2^{b}	2.63 ± 1.4^{b}	2.02± 0.6^{ab}	1.89 ± 0.7 ^{a}	1.61 ±0.9^{ac}	1.29±0.5^{c}	1.55 ±0.1^{ac}
Peroxidase unit/mg	2.6 ± 0.3^{a}	4.2±0.1^{b}	0.063±0.6^{c}	0.082± 0.5^{c}	5.5±0.6^{b}	0.051± 0.8^{c}	0.073± 0.2^{c}	3.6± 0.2 ^{b}	0.046± 0.2^{c}	0.067± 0.3^{c}	3.1±0.6^{a}
Malonedialdehyde levels(μmole/g)	0.1± 0.01^{a}	0.2±0.01^{b}	0.3±0.01^{b}	0.4±0.01^{b}	0.1± 0.01^{a}	0.1±0.01^{a}	0.2±0.01^{b}	0.2±0.01^{b}	0.2±0.01^{b}	0.2±0.01^{b}	0.2±0.01^{b}
21 days after germination											
Catalase unit/g	2.46 ± 1.9^{a}	4.39 ± 2.2^{b}	5.02 ± 1.2^{c}	7.01 ± 0.2^{d}	4.94 ± 0.2^{bc}	5.35 ± 0.6^{c}	7.21 ± 1.3^{d}	3.82 ± 0.3^{b}	4.81 ± 0.9^{bc}	4.93 ± 0.3^{c}	5.3 ± 1.4^{c}
SOD unit/mg	1.02± 0.04^{a}	1.90 ± 0.1^{c}	1.37 ±0.4^{b}	1.11± 0.6^{a}	2.03 ± 0.6^{b}	1.95 ± 0.30^{c}	1.84±0.4^{c}	1.37 ± 0.9^{b}	1.25 ± 0.30^{b}	0.89± 0.02^{a}	1.11 ± 0.5^{a}
Peroxidase unit/mg	7.0± 0.01^{a}	1.5±0.2^{b}	0.028±0.3^{c}	0.10±0.3^{c}	4.6± 0.01^{ab}	0.037± 0.2^{c}	0.089± 0.2^{c}	1.3±0.6^{b}	0.021± 0.6^{c}	0.061± 0.3^{c}	10.0± 0.01^{d}
Malonedialdehyde levels(μmole/g)	0.2± 0.01^{a}	0.3± 0.01^{b}	0.4± 0.01 ^{b}	0.4± 0.01^{b}	0.2± 0.01^{a}	0.2± 0.01^{a}	0.3± 0.01^{b}	0.2± 0.01^{a}	0.2± 0.01^{a}	0.3± 0.01^{b}	0.3± 0.01^{b}

Values are means of 4 to 8 determinations ± S.E.M. Means carrying different notations are statistically different at P<.01.

After 21 days post germination, the radicle peroxidase of all fraction decreased significantly ($P<.01$) while the peroxidase activity of maize radicle grown in Ubeji soil increased slightly (Table 3). After 7 days post germination, the level of lipid peroxidation (measured as MDA) in maize radicle grown in all contaminated soils increased insignificantly in a percentage contamination dependent manner. After 14 and 21 days post germination, the level of lipid peroxidation was significantly ($P<.01$) increased in the radicle of maize grown in crude oil contaminated soil in a percentage contamination dependent manner.

For plants, increase in reactive oxygen species (ROS) production may arise from biotic and abiotic factors [31]. Factors such as limited water [32] and oxygen [30] concentration in crude oil contaminated soils may increase the production of ROS. To counter the effects of ROS, plants possess antioxidative mechanisms including SOD, CAT and peroxidase (POX) [32]. The general increases seen the catalase activities of the maize radicle grown in contaminated soil could be an adaptive response of the plant to the increased ROS production. It can also be concluded that at higher concentrations, especially of the WIF group and the Ubeji group, the rate of enzyme increase reduced, probably due the production of ROS at levels that were high enough to affect the synthesis of catalase or facilitate its loss of activity. The oxidative stress conditions of maize grown in contaminated soil was also seen in the decrease in SOD activities in test plants especially the WIF and Ubeji groups, supporting the earlier claim that these two groups exhibited greater oxidative stress. The very drastic decrease observed in POX activities of the plants exposed to higher percentage of crude oil contamination, as well as the increases in MDA concentration, buttresses the results of several studies that negative biological effects, including oxidative stress, increases as the level of contamination of crude oil increases.

4. CONCLUSION

For people who already live in underprivileged conditions, the continuous incidence of crude oil contamination and the neglect of government and multinationals in carrying out appropriate clean up exercises further impoverish the people in oil producing areas. This study has shown that varying degrees of crude oil contamination significantly reduced several growth parameters of maize and induced oxidative stress in the maize radicle which may ultimately affect plant yield.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

ACKNOWLEDGEMENTS

We are grateful to the laboratory staff of the Department of Biochemistry, Faculty of Life Sciences and Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria. The incalculable advice and contributions of Dr. A. A. Omonkhua, Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin, is highly appreciated.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Amund OO, Akangou TS. Microbial degradation of four Nigerian crude oils in an estuarine microcosm. Lett Appl Microbiol. 1993;16:118–121.
2. Cadwellaer S. Encyclopaedia of Environmental Science and Engineering. 4th ed; 1993.
3. Agbogidi OM, Ernotor PG, Akparobi SO. Effects of crude oil levels on the growth of maize (*Zea mays* L.). Am J Food Tech. 2007;2:529-535.
4. Lundstedt S. Analysis of PHAs and their transformation products in contaminated soil and remedial processes. Solfjodern Offset AB, Umea, Sweden. 2003;55
5. Njoku KL, Akinola MO, Oboh BO. Growth and performance of *Glycine max* L. (Merrill) grown in crude oil contaminated soil augmented with cow dung. Nature and Science. 2008;6:48-56
6. Okolo JC, Amadi EN, Odu CTI. Effects of soil treatments containing poultry manure on crude oil degradation in sandy loam soil. Applied Ecology and Environmental Research. 2005;3:47-53
7. Omosun G, Markson AA, Mbanasor O. Growth and anatomy of *Amaranthus hybridus* as affected by different crude oil concentrations. Am- Euras. J. Sci. Res. 2008;3:70-74.
8. Ekpo IA, Agbor RB, Okpako EC, Ekanem EB. (2012). Effect of crude oil polluted soil on germination and growth of soybean (*Glycine max).* Annals of Biological Research. 2012;6:3049-3054
9. De Jong E. The effect of crude oil spill on cereals. Environ Pollut Ser Ecol Biol 1980;22:187-196.
10. Udo EJ, Fayemi AA. The effect of oil pollution of soil on germination, growth and nutrient uptake of corn. J Environ Qual. 1975;4:537-540.
11. Edema, N. Effects of Crude Oil Contaminated Water on the Environment. In: Crude Oil Emulsions- Composition Stability and Characterization. [Manar El-Sayed Abdul-Raouf (Ed.)]. InTech — Open Access Company 2012;9:170-180.
12. Anderson JW, Neff JM, Cox BA, Tatem HE, Hightower GM. Characteristics of dispersions and water-soluble extracts of crude oils and their toxicity to estuarine crustaceans and fish. Mar Biol. 1974;27:75 – 88.
13. Blokhina OB, Fagerstedt KV, Chirkova TV. Relationship between lipid peroxidation and anoxia tolerance in a range of species during post-anoxic re-aeration. Physiol Plant. 1999;105(4):626 – 632.
14. Chirkova TV, Novitskaya LO, Blokhina OB. Lipid peroxidation and antioxidant systems under anoxia in plants differing in their tolerance to oxygen deficiency. Russian J. Plant Physiol. 1998;45:55-62.
15. Alscher RG, Erturk N, Heath LS. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. J. Exp. Bot. 2002;53:1331–1341.
16. Draobzkiewicz M, Skórzyńska-Polit E, Krupa Z. Copper-induced oxidative stress and antioxidant defence in *Arabidopsis thaliana*. BioMetals. 2004;17:379–387.
17. Jouili H, Ezzedine EF. Changes in antioxidant and lignifying enzyme activities in sunflower roots (*Helianthus annuus* L.) stressed with copper excess. C. R. Biol. 2003;326:639–644.

18. Tanyolac D, Ekmekci Y, Unalan S. Changes in photochemical and antioxidant enzyme activities in maize (*Zea mays* L.) leaves exposed to excess copper. Chemosphere. 2007;67:89–98.
19. Ortega-Villasante C, Rellán-Álvarez R, del Campo FF, Carpena-Ruiz RO, Hernández LE. Cellular damage induced by cadmium and mercury in *Medicago sativa*. J Expt Bot. 2005;56:2239–2251.
20. Vavrek MC, Campbell WJ. Identification of plant traits that enhance biodegradation of oil. 2002. Accessed 13 March 2013.
Available: http://ipec.utulsa.edu/Ipec/conf/2002/vavrek_campbell_20.pdf.
21. Kaur H, Gupta AK, Kaur N, Sandhu JS. Differential response of the antioxidant system in wild and cultivated genotype of chickpea. Plant growth Regul. 2009;57:109-114.
22. Gutteridge JMC, Wilkins C. Copper dependent hydroxyl radical damage ascorbic and formation of a thiobarbituric acid reactive products. FEBS Lett. 1982;137:327–340.
23. Misra HP, Fridovich I. The role of superoxide ion in the antioxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972;247:3170–3175.
24. Sinha KA. Calorimetric assay of catalase. Anal. Biochem. 1972;47:389-394.
25. Chance B, Maehly AC. Methods in Enzymology II. 1955;773-775.
26. Ekundayo EO, Emede TO, Osayande DI. Effects of crude oil spillage on growth and yield of maize (*Zea mays* L.) in soils of Midwestern Nigeria. Plant Foods Hum Nutr. 2001;56(4):313-324.
27. Baker JM (Ed.). Marine ecology and oil pollution. Applied Science Publishers Ltd., Essex England. 1976;483–536.
28. Amadi A, Dickson A, Maate GO. Remediation of oil polluted soils: Effect of organic and inorganic nutrient supplements on the performance of maize (*Zea mays* L.). Water Air Soil Pollut. 1993;66:59–76.
29. Osuji LC, Egbuson EJG, Ojinnak CM. Chemical reclamation of crude-oil- inundated soils from Niger Delta, Nigeria. Chem Ecol. 2005;21(1):1-10.
30. Eriyamremu GE, Asagba SO, Onyeneke EC, Aguebor-Ogie B. Bonny light crude oil and its fractions alter radicle galactose dehydrogenase activity of beans (*Phaseolus vulgaris* L.) and maize (*Zea mays*). Trends in Appl Sci Res. 2007;2:433-438.
31. Kaur N, Gupta AK. Signal transduction pathways under abiotic stresses in plants. Curr. Sci. 2005;88:1771-1780
32. Chugh V, Kaur N, Gupta AK. Evaluation of oxidative stress tolerance in maize (*Zea mays* L.) seedlings in response to drought. Indian J Biochem Biophys. 2011;48: 47.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=187&id=24&aid=1712