Crude oil induced oxidative stress in *Capsicum annum* L.

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Abstract: This study was conducted to evaluate the effects of Bonny light crude oil on the malondialdehyde (MDA) and antioxidant enzymes activity in *Capsicum annum*. Seedlings were grown for 5 weeks in nursery bags filled with sandy-loam soil amended with crude oil to achieve 0, 1, 3 and 5% v/w. Metabolic parameters representative of oxidative damage and antioxidant enzymes activity were evaluated after the treatments. The results showed that crude oil caused a significant decrease in the relative water content (RWC) of the plant and a significant increase in MDA level, and activities of catalase and ascorbate peroxidase. It was observed that the effects were concentration dependent. It was concluded that crude oil in soil made water absorption by plants difficult and also induced oxidative stress in plants.
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1. Introduction

Crude oil is a naturally occurring liquid found in the earth and it is a complex mixture of hydrocarbons and hydrocarbon-like chemicals (Albers 1995). Crude oil also contains some inorganic elements like sulphur, nitrogen, phosphorus; trace elements such as vanadium, nickel, iron, aluminum, copper, and some heavy metals like lead and cadmium (National Research Council, 1985), and oil exploration and exploitation can seriously impact on the environment. Nigeria is an oil producing and exporting nation producing medium and light crude oil, such as Bonny Light (Amund and Akangbou, 1993). Since commercial exploration of oil started in Nigeria in 1958 (Okoh, 2003), it has become the mainstay of the Nigerian economy, as the annual budgets are based on oil revenue. However, the exploration of petroleum has led to the pollution of both terrestrial and aquatic environments in Nigeria. The agricultural lands have become less productive and the creeks and the fishing waters have become more or less dead (Odokuma and Inor, 2002). Spill incidences are now common either by accident or deliberate actions via pipeline vandalism. Between 1958 and 2012, several spill incidences were recorded and large quantities of crude oil were discharged into the environment in each case there by polluting both terrestrial and aquatic ecosystem. Several civil unrests due to environmental degradation caused by oil pollution have also been witnessed in the Niger Delta region of Nigeria.

The largest contributor to the oil spill is corrosion of pipes and tanks, this made rupturing or leaking inevitable. As a result of the small size of the oilfields in the Niger Delta, there is an extensive network of pipelines between the fields, as well as numerous small networks of flow lines: - the narrow diameter pipes that carry oil from wellheads to flow stations—allowing many opportunities for leaks. In onshore areas, most pipelines and flow lines are laid above ground passing through farmlands. Plants can take in some of the spilled oil either through foliar penetration or absorption by roots and can cause injury as well as alterations in both physiological and biochemical processes in the plant. Most investigations conducted in the past here in Nigeria, mainly focused on germination, growth and morphological parameters at whole plant level (Omosun et al 2008), while data on biochemical parameters are very few and most times inaccessible . It has been reported that crude oil caused poor germination of seeds, reduction in plant biomass, height, number of leaves, stomatal index, pigment content, uptake of water and mineral nutrients (Kahle, 1993; Anoliefo and Vwioko, 1995; Sanita di Toppi and Gabbrielli 1999; Odjegba and Okunnu, 2012). In order to add to the existing data in Nigeria on crude oil effects on plants, we have examined the effects of Bonny light crude oil on some biochemical parameters that are representatives of oxidative stress using *Capsicum annum* as a model plant.

2. Materials and methods

2.1 Plant growth and treatments

Fresh *C. annum* were purchased from a local market at Obalende, Lagos. The seeds were extracted from the fruits and broadcasted on a nursery bed (1 x 1.5 m) in the Botanical garden of University of Lagos. The nursery bed was kept moist by regular watering. After germination, seedlings were allowed to grow for 3 weeks and thereafter, relatively equal height seedlings (10 cm) were selected for the study. Bonny

Light crude oil was collected from Warri Refinery and Petrochemical Company in Delta State, Nigeria, in a single batch and enough for the study. The physicochemical properties of the soil and oil were determined according to AOAC (2005). Soil treatment was carried out by manual mixing of weighed soil with known volume of crude oil to achieve the required concentrations of 1, 3 and 5 % v/w oil/soil (Odjegba and Sadiq, 2002).The mixing was gradually done to ensure thorough and even mixing. Soil devoid of oil served as the control. Two seedlings were planted in each nursery bag representing each treatment but later thinned out to one seedling per bag after 1wk of acclimation. The experimental set up was randomized and each treatment was replicated 6 times. Individual seedling was an observation. Water was added to all samples when necessary to keep the soil moist. The seedlings were allowed to grow in the treated soil for 5 weeks and thereafter, plants were harvested for analyses.

2.2 Whole plant dry weight determination

Plants were uprooted carefully and washed thoroughly in a running tap water to remove all attached soil particles. After rinsing with distilled water, they were placed in labeled paper bags and oven dried at 70 ^{o}C until constant weight was achieved.

2.3 Relative water content (RWC) of leaves

The fourth leaves were selected for the determination of relative water content (RWC). The RWC of each leaf was determined according to the method of Turner (1981) by using the formula RWC (%) = [(fresh weight - dry weight) / (turgid weight - dry weight)] x 100.

2.4 Malondiadehyde (MDA) quantification

The quantification of malondialdehyde (MDA) as an index of lipid peroxidation was conducted following a modified procedure of Wang and Jin (2005). Fresh leaves (0.3 g) were homogenized in 5 ml 20% trichloroacetic acid (TCA). The homogenate was centrifuged at 10000g for 5 min. The supernatant (1ml) was mixed with equal volume of 0.6% (w/v) thiobarbituric acid solution comprising 10% TCA. The mixture was incubated for 30 min in a boiling water bath and cooled quickly on ice bath. The absorbance of the mixture was read at 450, 532 and 600 nm. The concentration of MDA was calculated as 6.45 (A_{532}-A_{600})-0.56 A_{450}.

2.5 Enzyme determination

For enzyme analysis, fresh samples of leaves (300 mg each) were ground in a ceramic mortar and extracted with 10 ml of 100 mM potassium phosphate buffer pH 7.5, with 1% (w/v) polyvinylpyrrolidone. The homogenate was centrifuged at 10,000 rpm for 5 min. The supernatant was used for the estimation of antioxidant enzyme activities.

Catalase (CAT) activity was determined according to Aebi (1984). The assay mixture (3.0 ml) consisted of 100µl enzyme extract, 100µl H_2O_2 (300mM) and 2.8 ml 50mM phosphate buffer with 2mM EDTA (pH 7.0). CAT activity was assayed by monitoring the decrease in the absorbance at 240nm as a consequence of H_2O_2 disappearance. Ascorbate peroxidase (APX) activity was assayed according to the method of (Nakano and Asada 1981). The reaction mixture consisted of 100µl enzyme extract, 100µl ascorbate (7.5 mM), 100µl H_2O_2 (300mM) and 2.7 ml 25mM potassium phosphate buffer with 2mM EDTA (pH 7.0). The oxidation of ascorbate was determined by the change in absorbance at 290nm.

2.6 Statistical analysis

Means of three replicates as well as their standard errors (SE) were determined. The test of significance between the treatments was done using a one way analysis of variance (ANOVA).

3. Results

The results of the physicochemical analyses of the soil and the crude oil are depicted in Table 1. There were no detectable levels of cadmium, lead and nickel in the soil and the crude oil; however, the crude oil contained low level sulphur while the total hydrocarbon and density were 89.26% and 0.61cm^3 respectively.

Table 1. Physicochemical properties of the garden soil and the crude oil

Parameters	Level detected
Garden soil	
pH	7.12
EC (uScm^{-1})	28.2
Total organic matter	3.39 %
Cadmium	ND
Lead	ND
Nickel	ND
Crude oil	
Density	0.61 cm^3
THC	89.26 %
Ash	0.112 %
Sulphur	0.006 %
Cadmium	ND
Lead	ND
Nickel	ND

ND, not detected; EC, electrical conductivity; THC, total hydrocarbon.

Exposure of *C. annum* to crude oil consistently reduced the biomass as indexed by whole plant dry weight of the plant. The effect was concentration dependent. The control had a mean dry weight of 9.33 ± 0.33g while plants exposed to 1, 3 and 5% crude oil respectively had 8.05 ± 0.45, 5.02 ± 0.31 and 4.61 ± 0.33 g (Figure 1).

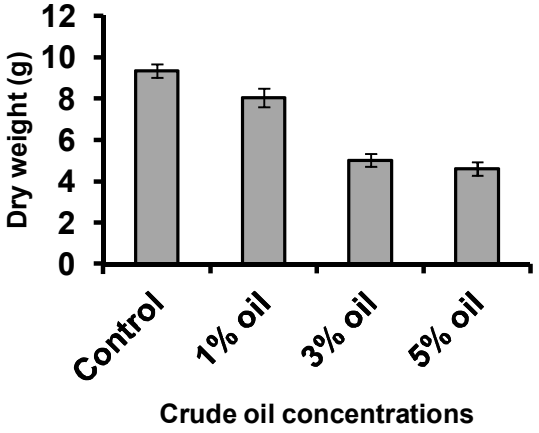

Figure 1. Whole plant dry weight of *C. annum* exposed to crude oil. Means and SE of 3 replicates are presented.

Data showing the effects of crude oil on the relative water content of *C. annum* is represented in figure 2. It was observed that crude oil treatment significantly ($p < 0.05$) reduced the relative water content of the plant. The severity of the effect was concentration dependent. While the control plants had a mean RWC of 74.33±1.20 %, plants that were treated with 1, 3 and 5 % crude oil had mean values of 47.67 ± 3.85, 35.33 ± 3.76 and 22.33 ± 2.81 % respectively.

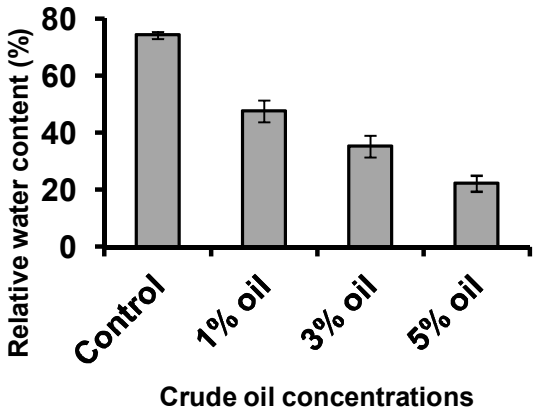

Figure 2. Relative water content of *C. annum* as affected by crude oil. Means and SE of 3 replicates are presented.

To evaluate the impact of crude oil on lipid peroxidation, malondialdehyde (MDA) in the leaves of *C. annum* was measured. It was observed that crude oil treatment caused lipid peroxidation, as it led to more than 100% increase in MDA when plants were exposed to crude oil (figure 3).

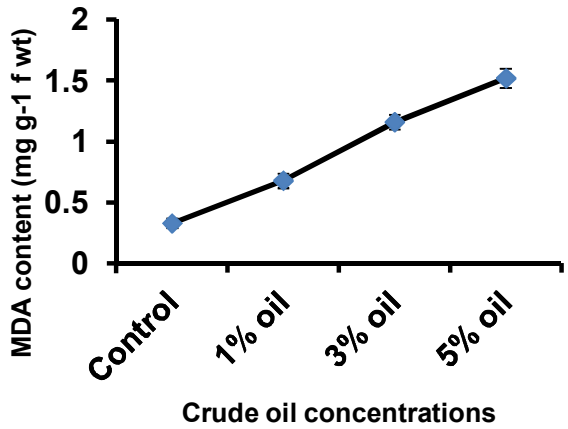

Figure 3. Malondialdehyde (MDA) content of *C. annum* exposed to crude oil. Means and SE of 3 replicates are presented.

In this study, we measured the activities of catalase (CAT) and ascorbate peroxidase (APX) as representative enzymes involved in antioxidant metabolism. The activities of the enzymes followed the same pattern when *C. annum* were grown in soil polluted with crude oil. It was observed that crude oil treatment led to a significant increase in the activities of the two enzymes, an indication that crude oil induced oxidative stress in *C. annum*. The control plants had a mean value of 17.61 ± 0.41 µmol min^{-1} g^{-1} f wt as CAT activity, while plants treated with 5% crude had a mean value of 30.12 ± 0.41 µmol min^{-1} g^{-1} f wt (figure 4). In the same vein, the control plants had the least ascorbate peroxidase activity of 0.0617 ± 0.0025 µmol ascorbate/mg protein/min, while plants exposed to 3 and 5% crude oil respectively had APX activities of 0.0878 ± 0.0023 and 0.0943 ± 0.0016 µmol ascorbate/mg protein/min (figure 5).

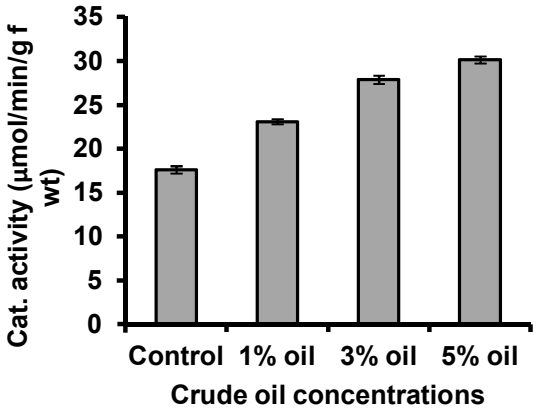

Figure 4. Catalase (CAT) activity of *C. annum* as affected by crude oil treatment. Means and SE of 3 replicates are presented.

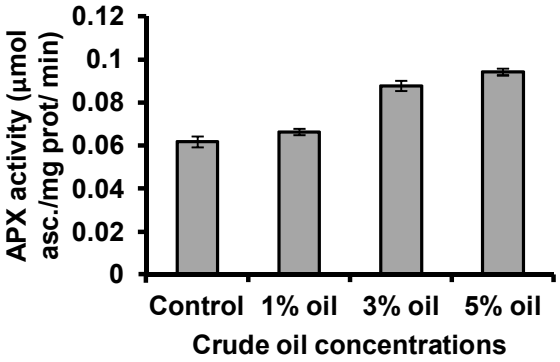

Figure 5. Ascorbate peroxidase (APX) activity of *C. annum* exposed to crude oil. Means and SE of 3 replicates are presented.

4. Discussion

The present study was conducted to assess the physiological and biochemical disorders that could be associated with simulated crude oil pollution in *Capsicum annum* seedlings. The low biomass accumulation observed in this study for plants grown in crude oil contaminated soil could be due to the unhealthy nature of the soil caused by crude oil application. Oily soil repels water which make absorption of mineral nutrients and water by plant roots difficult. This observation is in conformity with previous findings that crude oil inhibit growth in cereals (De Jong,(1980), *Rhizophora mangle* (Proffitt et al., 1995), *Manihot esculenta* (Odjegba and Okunnu, 2012) and *Glycine max* (Ekpo et al., 2012).

It was not a surprise that oil treatment resulted in low relative water content (RWC) in this study. Oil application to soil usually disrupts the normal plant-water relationship of the roots within the soil which consequently affect the amount of water absorbed by the plant.

In plants, oil pollution as well as other environmental stresses can induce oxidative stress by generating reactive oxygen species (ROS) which can rapidly attack and damage bio-molecules including proteins, lipids and nucleic acids (Acworth and Bailey, 1997). The malondialdehyde (MDA) assay, popularly used by researchers to evaluate peroxidation of lipids in membrane and biological systems, is a reliable indicator of free radical formation in tissues and is a good measure on damage due to free radicals (Halliwell and Chirico, 1993). In this study, positive correlations were found between the MDA content and crude oil concentrations that *C. annum* were exposed to, indicating that damage caused by crude oil to plants were mainly due to oxidative stress. Similar result was reported by Eriyamremu and Asagba (2007) that crude oil treatment increased the MDA content in *Phaseolus vulgaris* and *Zea mays*.

It is now known that higher plants resist free radicals by increasing the activities of antioxidant enzymes after exposure to pollutants (Halliwell and Chirico, 1993), and such response reflects an adaptation of a plant to its environment (Yordanova et al., 2004). In the present study, it was observed that the activities of catalase and ascorbate peroxidase increased significantly when *C. annum* seedlings were treated with crude oil. The increase in activity of these antioxidant enzymes as well as MDA content, underscored the fact that crude oil pollution caused oxidative stress in *C. annum*.

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