

Effect of oil palm leaf treatment of crude oil impinged soil on biochemical indices of cowpea (vigna unguiculata) seedlings

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Abstract

The possibility of oil palm leaf (OPL) being applied as ameliorative agent in unrefined petroleum oil tainted soil was examined in this study. Six treatment protocols, comprising group 1 (untainted soil), group 2 (untainted soil +50g of OPL), group 3 (untainted soil +100g of OPL), group 4 (petroleum oil tainted soil), group 5 (petroleum oil tainted soil +50g of OPL) and group 6 (petroleum tainted soil +100g of OPL) were used in this study. A decrease in photosynthetic pigments of cowpea seedlings in unrefined oil tainted soil had lower levels of antioxidants relative to the control. Conversely, lipid peroxidation was elevated in seedlings raised in unrefined oil tainted soil of OPL as compared to those of the control and all OPL protocols. The significant improvement in photosynthetic pigments, beta carotene as well as the antioxidant profile of cowpea seedlings in untainted soil treated with OPL when compared with those in control group, alongside the substantial ameliorative effect on chlorophyll, beta carotene and the antioxidant profile of cowpea seedlings.

Keywords: Enzymes, oil palm, petroleum, seedlings, soil, remediation

Introduction

Crude oil is a fossil fuel occurring underground and is composed of many thousands of organic compounds amongst which hydrocarbons predominate (Alkindi et al., 1996). Adulteration of soil by petroleum derivatives is a widespread ecological problem (Sivaraj et al., 2014). The soils of the Niger Delta region of Nigeria are heavily tainted with unrefined oil and its refined products because this region hosts oil related operations and industries (Nwaogu and Onyeze, 2014; Anochie and Mgbemena, 2015). The dominant route of pollution of soils include oil well drilling and production operations, oil spillage and haphazard dumping of petroleum-related substances into the environment by auto mobile mechanics (Anoliefo et al., 2001; Onwurah et al., 2007; Osuagwu et al., 2017). Petroleum in its various forms has a negative impact on all shades of animal and plant species (Achuba, 2008; Nwaogu and Onyeze, 2010). The pollution of the soil by crude oil and related chemicals undesirably disturbs both physical and chemical properties of the soil. It can lead to water and oxygen shortages, deficiency in nitrogen and phosphorous, reduced soil microorganisms and fertility (Samina and Adams, 2002; Achuba and Peretiemo-Clarke, 2008). Besides harming the environment, toxic chemicals in crude oil can pose serious threats to many living organisms (Adedoku and Ataga, 2007; Achuba and Okoh, 2015, Achuba, 2018a).

Crude oil affects plants in a variety of ways such as alterations in the anatomical, physiological and metabolic processes (Daniel-Kalio and Pepple, 2006; Adenipekun *et al.*, 2008; Achuba, 2006; Achuba, 2010). Plants raised in soils tainted with petroleum have been reported to exhibit retardation in growth parameters (Onwusiri *et al.*, 2017; Osuagwu *et al.*, 2017) and metabolic alterations (Achuba, 2006). Moreover, plants raised in crude oil tainted soil were reported to exhibit increased free radical generation, oxidative stress and lipid peroxidation (Achuba, 2014). Recently, spent engine oil was reported to adversely affect the germination and growth parameters of fruited pumpkin (*T. occidentalis*), *Zea mays, Arachis hypogea* and *Vigna unguiculata* (Onwusiri *et al.*, 2017; Osuagwu *et al.*, 2017)

Tropical African palm tree, *Elaeis guineensis* Jacq., is a highly profitable cash crop and is reputed economically, the most efficient oil crop worldwide (Dislich *et al.*, 2017). Oil palm fond (OPF) is the largest oil palm biomass generated and is a promising source of raw materials (Ooi *et al.*, 2017). Recently, the improvement in soil enzyme activities upon treatment of polluted soil with oil palm leaf was

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reported (Achuba, 2018b). However, no study has been carried out to ascertain whether oil palm leaf can remediate the adversative upshot of crude oil tainted soil on plants. This study therefore aimed at evaluating the preventive capacity of oil palm leaf on the anomalies in the metabolism of cowpea seedlings raised in crude oil tainted soil.

Materials and Methods

The soil sample and oil palm leaves used were previously described (Achuba, 2018b). Similarly, cowpea seeds were obtained as earlier reported (Achuba and Okoh, 2015). Analytical grade reagents were used for determination of each parameter:

Parameters	Values
pH	7.01 ± 0.02
Organic matter (%)	0.47 ± 0.12
Total Nitrogen (%)	$0.16{\pm}0.01$
Phosphorus (ppm)	3.31±0.32
Cation exchange capacity (M	eq/100 g) 6.64±0.70
Source: Achuba	(2018b)

Experimental design

Six treatment protocols, comprising group 1(seedlings cultivated in 400g of untainted soil), group 2(seedlings cultivated in 400g of untainted soil +50g of OPL), group 3 (seedlings cultivated in 400g of untainted soil +100g of OPL), group 4 (seedlings cultivated in 400g of unrefined oil impacted soil), group 5 (seedlings cultivated in 400g of unrefined oil tainted soil +50g of OPL) and group 6 (seedlings cultivated in 400g of unrefined oil tainted soil +100g of OPL) were used in this study. Planting of viable seeds was done as described earlier (Achuba, 2006).

Determination of chlorophyll and carotene

Chlorophyll and carotene were determined as briefly described. The leaf (1.0 g) was weighed, homogenized and extracted with 80% acetone. This was followed by filtration and centrifugation at 2500 rpm for ten minutes. The absorbance of the supernant was read at 480, 510, 645and 663 nm. The amount of chl. a, chl.b and carotenoid were calculated using the formula of Duxbury and Yentsch (1956).

Preparation of homogenate biochemical assays

Homogenate for biochemical analysis was prepared thus. The leaf was removed from the stalk washed with icecold water (4^{0} C), 0.5g measured, added to 0.1g butylated hydroxy toluene (BTH) and homogenized with 10mL of 0.05M Phosphate buffer, pH 7.4 with a blender immersed kept inside ice. The mixture was filtered with cheese cloth and the filtrate was centrifuged at 7000g for 20 minutes $(4^{\circ}C)$ to generate the supernatant (S1) used for the determination of various biochemical indices

Determination of lipid peroxidation and nonenzymatic antioxidants

Lipid peroxidation was determined with the method of Gutteridge and Wilkins (1982). This method is used to evaluate Malondialdehyde (MDA), an index of lipid peroxidation with a molar extinction coefficient of 1.56 x10⁵ M/cm. The supernant (S1) prepared earlier was used to assay for reduced glutathione (EIIman, 1959) which is based on the development of chromophore that absorbs light maximally at 412 nm. A measured amount of the leaf, 2.0g, was added to 20mL of 0.05M phosphate buffer, pH 7.4 and acidified with 5% metaphosphoric acid (in a ratio of sample to acid 5: 1 v/v). Ascorbic acid was determined by titration using 2,6-dichlorophenol-indophenol (DCIP) as electron acceptor. (Achuba 2008). Uric acid in the plant extract was determined using commercial kits supplied by Teco Diagnostics, USA following the procedure in the user's manual (Caraway and Hard, 1963).

Determination of enzymatic antioxidants

Superoxide dismutase (SOD) activity was determined based on its ability to inhibit the oxidation of epinephrine by superoxide anion. One unit of superoxide dismutase activity is calculated as the amount of enzyme required for 50% inhibition of the oxidation of epinephrine to adrenochrome at 480 nm per min (Misra and Fridovich 1972). Manganese dependent SOD was assayed in the presence of 1 mM NaCN to inhibit Cu-ZnSOD activity, and the cytosolic Cu-ZnSOD activity was determined as the difference between total and cyanide -sensitive enzyme activity (Crapo et al., 1978). The method of Cohen et al. (1970) was used to determine catalase activity. This reaction is based on monitoring the disappearance of the purple colour of potassium permanganate at 480 nm at 30-60 intervals, was due to oxygen generated by the breakdown of hydrogen peroxide by catalase. Glutathione peroxidase was measured as described by Rani et al. (2004). This is based on the reduction of hydrogen peroxide and organic peroxides to the corresponding stable alcohols and water using cellular glutathione as the reducing reagent producing a chromophore that absorbs maximally at 412 nm.

Determination of xenobiotic metabolizing enzymes

The activity of glutathione-s-transferase (GST) was determined with the method of Habig *et al.* (1974) based on



the reaction between 1-chloro-2, 4, dinitrobenzene (CDNB) and reduced glutathione to produce a dinitrophenyl thioether which absorbs maximally at 340 nm. The activity of xanthine oxidase was assayed by the method of Stirpe and Della Corte (1969) with xanthine as the substrate and oxygen as electron acceptor. The enzyme activity is expressed in units per gram tissue. Aldehyde oxidase (AO) was assayed following the method prescribed by Omarov *et al.* (1998) which is dependent on the conversion of benzaldehyde to benzoate using 2,6–dichlorophenol (DCIP) as the electron by the enzyme. Sulphite oxidase (SO) was assayed following the method prescribed by Macleod *et al.* (1961) which is dependent on the conversion of sulphite to sulphate using 2,6–dichlorophenol (DCIP) as the electron by the enzyme

Statistical analysis of data

Analysis of variance (ANOVA) was used to analyze the data for statistical difference and significance was set at p values < 0.05.

seedlings, meanwhile, addition of 100 g of OPL to crude oil tainted soil significantly increased the photosynthetic pigments and beta carotene contents of cowpea seedlings.

Treatment of untainted soil with 50g OPL caused a substantial increase in lipid peroxidation while addition of 100g OPL decreased in lipid peroxidation parallel to the control (group 1) (Table 2). Tainting soil with crude oil caused substantial elevation of lipid peroxidation as compared to the control group. However, addition of oil palm leaf to crude oil tainted soil caused a substantial (p < 0.05) decrease in lipid peroxidation whereas glutathione and vitamin C of cowpea seedlings were increased by treatment of untainted soil with OPL when compared to untreated soil (group 1). Cowpea seedlings cultivated in crude oil tainted soil had a substantial decrease in the levels of glutathione and vitamin C related to the control but the addition of oil palm leaf to the crude oil tainted soil meaningfully increased the levels of glutathione and vitamin C in cowpea seedlings as paralleled to those cultivated in crude oil tainted soil without OPL. Cowpea seedlings raised

Table 1: Oil palm leaf alters photosynthetic pigments in cowpea seedlings cultivated in crude oil tainted soil

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GROUPS	Total chlorophyll (mg/g)	Chlorophyll b (mg/g)	Chlorophyll b (mg/g)	Beta Carotene (mg/g)	
1	209.5±1.2ª	$124.0{\pm}1.4^{a}$	$80.75{\pm}1.7^{a}$	22.27±1.1ª	
2	210.0±6.0ª	129.0±2.5ª	67.0±2.1°	25.2 ± 1.0^{b}	
3	213.25±2.2ª	120.25±2.6 ^b	82.25±1.7 ^b	26.20±1.0 ^b	
4	153.25±7.5 ^b	$98.0{\pm}2.1^{d}$	57.25 ± 2.9^{d}	19.52±1.1°	
5	182.25 ± 8.1^{ab}	109.0±2.6°	71.25±2.2°	22.95±1.3ª	
6	204.75±2.2 ^a	119.5±1.2 ^b	$78.0{\pm}1.6^{a}$	22.6±1.8ª	
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Results are expressed as mean + SEM. Results with different letters in column are significantly different.

Table 2: oil palm leaf alters lipid peroxidation	and non-enzymatic antioxidants in	cowpea seedlings cultivated in
crude oil tainted soil		

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GROUPS	Lipid peroxidation	Reduced glutathione	Vitamin C	Uric Acid
	(nMol cm ⁻³)	$(\mathbf{ug} \mathbf{g}^{-1})$	(ug g ⁻¹)	(ug g ⁻¹)
1	1.09±0.01 ^b	$0.40{\pm}0.09^{e}$	$3.64{\pm}0.15^{d}$	3.52±0.09°
2	$1.19{\pm}0.14^{\circ}$	$0.48{\pm}0.09^{d}$	3.67 ± 0.59^{d}	3.00±0.18°
3	$1.05{\pm}0.01^{a}$	$0.49{\pm}0.13^{d}$	4.22±0.38°	3.06±0.26°
4	$1.39{\pm}0.18^{\rm f}$	0.25±0.01ª	2.37±0.52ª	$1.84{\pm}0.08^{a}$
5	$1.34{\pm}0.10^{e}$	$0.27{\pm}0.03^{b}$	$3.04{\pm}0.48^{b}$	$2.38{\pm}0.07^{b}$
6	1.21 ± 0.08^{d}	0.29±0.02°	3.37±0.09°	3.01±0.43°

Results are expressed as mean + SEM. Results with different letters in column are significantly different.

Results

The results in Table 1 show that treatment of untainted soil with oil palm leaf (OPL) improved the photosynthetic pigments and beta carotene content of cowpea seedlings compared to the control (group 1). On the contrary, adulteration of soil with crude oil significantly (p<0.05) decreased the photosynthetic pigments and beta carotene level (group 4). However, addition of 50 g of OPL to crude oil tainted soil did not significantly increase the photosynthetic pigments and beta carotene level of cowpea in untainted soil treated with OPL exhibited decrease in uric acid content comparative to the control. Cowpea seedlings cultivated in crude oil tainted soil had a substantial decrease in uric acid levels as paralleled to the control. Nevertheless, cowpea seedlings cultivated in soil tainted with crude oil and modified with OPL had a substantial increase in uric acid as paralleled to those cultivated in crude oil adulterated soil.

In Table 3, treatment of untainted soil with OPL significantly increased the levels of antioxidant enzymes in



cowpea seedlings paralleled to the control. Conversely, adulteration of soil with crude oil decreased the activities of the enzymes relative to the control (group 1). But addition of OPL to the crude oil tainted soil instigated a substantial (p<0.05) increase in the activities of these enzymes while no difference was observed in the activities of Cu/ZnSOD in all the groups. Treatment of untainted soil with OPL meaningfully reduced the level of MnSOD as paralleled to

processes in plants (Achuba, 2006; Achuba and Asagba 2015). The observed marked decrease in the levels of photosynthetic pigments and beta carotene in cowpea seedlings raised in crude oil tainted soil (Table 1) correlates with previous studies which found carotenoids and photosynthetic products decreased by crude oil. (Achuba, 2006; Agbogidi and Ilondu, 2013). Onwusiri *et al.* (2017) also reported marked reduction in growth parameters in

Table 3: Oil palm	leaf alters enzy	matic antioxidants of c	owpea seedlings	cultivated in crude oil tainted soil
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Groups	Total SOD (Unit g ⁻¹ fw)	Cu/ZnSOD (Unit g ⁻¹ fw)	MnSOD (Unit g ⁻¹ fw)	Catalase (nMol min ⁻¹ gfw)	Glutathione peroxidase (nMol min ⁻¹ gfw)
1	2.31±0.12°	$1.78{\pm}0.04^{a}$	0.93±0.04 ^e	$1.23{\pm}0.09^{d}$	6.91±0.14 ^b
2	2.33±0.06 ^e	$1.82{\pm}0.04^{a}$	$0.92{\pm}0.05^{d}$	$1.24{\pm}0.08^{d}$	7.98±0.03°
3	$2.39{\pm}0.09^{f}$	$1.84{\pm}0.03^{a}$	0.91±0.02°	1.34±0.05 ^e	8.50±0.16°
4	$1.96{\pm}0.04^{a}$	$1.61{\pm}0.16^{a}$	$0.64{\pm}0.09^{a}$	$1.03{\pm}0.05^{a}$	5.23±0.02ª
5	$2.32{\pm}0.10^{d}$	$1.67{\pm}0.09^{a}$	$0.75 {\pm} 0.08^{b}$	1.12 ± 0.06^{b}	$5.88{\pm}0.08^{\rm ab}$
6	2.26±0.03 ^b	$1.84{\pm}0.05^{a}$	0.93±0.04 ^e	1.18±0.03°	$6.08 {\pm} 0.04^{b}$
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Results are expressed as mean + SEM. Results with different letters in column are significantly different.

Table 4: Oil pal	lm leaf alters d	ug metabolizing	g enz	ymes in cow	pea seedling	gs cultivated	l in crude oil	tainted soil
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Groups	Glutathione-s- transferase (Unit g ⁻¹ fw)	Xanthine oxidase (Unit g ⁻¹ fw)	Aldehyde oxidase (Unit g ⁻¹ fw)	Sulphite oxidase (Unit g ⁻¹ fw)
1	1.77 ± 0.07^{d}	3.18 ± 0.41^{d}	1.58±0.06°	2.40 ± 0.08^{b}
2	2.05±0.10 ^e	$3.20{\pm}0.38^{d}$	1.58±0.07°	3.52 ± 0.18^{d}
3	2.18 ± 0.10^{f}	3.93±0.22°	1.73±0.12 ^d	$3.54{\pm}0.20^{d}$
4	1.46±0.09ª	$1.94{\pm}0.17^{a}$	$1.07{\pm}0.10^{a}$	1.95±0.14 ^a
5	1.60 ± 0.02^{b}	2.13±0.15°	1.36±0.09 ^b	2.53±0.43°
6	1.64±0.04°	2.86±0.16°	1.38±0.06 ^b	2.66±0.12°

Results are expressed as mean + SEM. Results with different in column letters are significantly different.

the control group while addition of OPL to the unrefined petroleum tainted soil occasioned in substantial elevation of MnSOD of cowpea seedlings.

Table 4 depicts a significant (<0.05) increase in the levels of glutathione s-transferase and the oxidases in cowpea seedlings raised in untainted soil treated with 100g OPL compared to the control. The addition of 50g OPL to the soil did not cause significant increase in xanthine oxidase and aldehyde oxidase. The levels of these enzymes significantly reduced in cowpea seedlings cultivated in soil tainted with crude oil without addition of OPL. However, adding OPL to soil tainted with crude oil caused a substantial increase in the levels of these enzymes compared with the group raised in soil tainted with crude oil without adding OPL.

Discussion

Several studies have established petroleum-derived substances as phytotoxic (Achuba, 2010; Bona *et al.*, 2011; Al-Hawas *et al.*, 2012). These substances are known to negatively influence both physiological and biochemical

fluted pumpkin raised in soil adulterated with used petroleum oil. The decreased levels of photosynthetic pigments and beta carotene may be centered on crude oilstimulated alteration of soil chemistry (Achuba and Peretiemo-Clarke, 2008). Crude oil in the soil had been implicated to disrupt C-N balance, limits soil nitrogen and phosphorus, and causes insufficient aeration and physiological drought (Nwaogu et al., 2008; Das and Chandran, 2011), which accounts for the reduction in the amount of photosynthetic pigments and beta carotene. Moreover, Agbogidi and Ilondu (2013) reported that spent engine oil caused reduction in the stomata indices of the leaf with eventual decrease in the photosynthetic products. Notwithstanding, the photosynthetic pigments and beta carotene content of cowpea seedlings improved by adding oil palm leaf (OPL) to the crude oil tainted soil which became significant at 100g of OPL while cowpea seedlings raised in untainted soil treated with OPL had higher contents of photosynthetic pigments and beta carotene. The positive effects of OPL on the cowpea seedlings may be attributed to its ability to enrich the soil with nutrients and its richness in



antioxidants (Ng and Choo, 2010; Comte et al., 2012). The observed significantly (p < 0.05) higher levels of photosynthetic pigments and beta carotene of cowpea seedlings raised in untainted soils treated with OPL especially at 100g of OPL compared to those raised in untainted soil (controls) suggests that OPL can be used as an organic fertilizer. Improvement in crop performance on application of organic materials to the soil had been reported (Arif et al., 2018; Billah et al., 2018; Ali et al., 2018; Achuba and Oshiokpu,2019). This gives credence to the assertion that OPL can be a potential organic nutrient. Moreover, the levels of photosynthetic pigments and beta carotene of cowpea seedlings raised in crude oil tainted soils improved upon addition of OPL compared to seedlings raised in crude oil tainted soil without treatment, thus suggests that OPL can be potentially used to remediate crude oil tainted soil.

Crops raised in soils tainted with crude oil and its byproducts have been associated with increased oxidative stress, as evidenced by increased lipid peroxidation and reduction in antioxidant enzymes (Ortega-Villasante, 2005; Achuba, 2010; Dietz, 2010; Al-Hawas *et al.*, 2012). This agrees with the present investigation that reported elevated lipid peroxidation and decreased glutathione and vitamin C and uric acid, though these indices were reversed by OPL addition to crude oil tainted soil (Table 2). Attenuation of crude oil toxicity in cowpea seedlings by organic substances is in literature (Achuba and Erhijivwo, 2017; Achuba and Iserhienrhien, 2018).

Antioxidant enzymes constitute mutually supportive team of defense against damaging effects of oxyradicals in plants (Asada, 1994; Achuba 2010). While reports on the effects of contaminants on physiological processes of plants have always been an increase in SOD, the present result showed that addition of crude oil to soil caused reduction of various SOD activities (Table 3). This agrees with the information in literature (Achuba, 2014). Achuba (2014) observed an increased SOD in cowpea seedlings at higher doses of petroleum and a decreased level of SOD at lower doses. According to Sgherri et al (2000), different levels of SOD activity might occur depending on stress intensity, species of plant and growth conditions. Moreover, organic materials- stimulated increase in yield and SOD activity in plants have recently been observed (AlAbdallah et al., 2017; El-Batanony, 2017). The decreased total SOD suggests increase in superoxide ion production and insufficient adaptive mechanism.

The addition of oil palm leaf to the crude oil tainted soil improved the activities of xenobiotic metabolizing enzymes (Table 4). These enzymes have been reported to play a vital role in the inactivation of xenobiotics in animals (Asagba *et al.*, 2017). Thus, suggesting that oil palm leaf in soil may

either reduce the absorption of crude oil from the soil or stimulate microbial biodegradation of crude oil. This observation is akin to former study where addition of saw dust improved the performance of *Abelmoschus esculentus* L (Tanee *et al.*, 2017). This may be the basis for the enhancement of the drug metabolizing enzymes in cowpea seedlings on adding OPL to the soil polluted with crude oil. This indicates that adding OPL to crude oil tainted soil improved the ability of cowpea seedlings to deal with noxiousness of crude oil when present in soil.

Conclusion

This study shows that treatment of untainted soil with OPL significantly improved photosynthetic parameters as well as the antioxidant profile of cowpea seedlings when compared with those in control group. This suggests that OPL can be used as soil conditioner. The study also shows that adulteration of soil with crude oil has adverse effects on the photosynthetic pigments, enzymatic and non-enzymatic antioxidant systems as well as detoxifying capacity of cowpea seedlings. However, amendment of crude oil tainted soil with OPL produced substantial ameliorative effects on the photosynthetic pigments, antioxidant profile and detoxifying capacity of cowpea seedlings, which indicates the reconditioning potential of OPL on crude oil tainted soil.

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