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Starch and triglycerides in the root of maize (*Zea mays*) and cowpea (*Vigna unguiculata*) grown in crude oil polluted soil treated with ash from palm bunch

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ABSTRACT: The ash from burnt oil palm fruit bunch has been commercially known and used as natural fertilizer for neutralizing peaty and acidic soils. This study was conducted to evaluate the use of ash from palm bunch (APB) as a source of biological fertilizer and soil amendment to neutralize the acidic pH created by crude oil contamination on the starch and triglycerides contents in the roots of maize and cowpea seedlings. The physicochemical characteristics of the APB reveals it may be a good source of nutrients to improve soils to be used for agriculture. The results shows that application of APB significantly (P<0.05) increased soil pH, soil sodium, potassium, calcium and magnesium. The plant height were significantly increased and comparable to that of the control. This shows that APB contains nutrients important for plant growth and may contribute to bioremediation. There were significant changes in the starch and triglyceride contents of the maize and cowpea seedlings in contaminated soils when compared with control. The study reveals alterations comparable to control with the application of APB, however, the degree of response of the two plants to APB differed. The study suggests that APB could be used as fertiliser to increase the pH and the nutrient contents of acidic soils and thus improve the soil quality. Further studies are needed to elucidate the mechanism of alteration of starch and triglyceride contents in the plant species.

Keywords: APB, Cowpea, Crude Oil, Maize, Polluted Soil, Starch, Triglycerides

Introduction

Maize (*Zea mays*) belongs to the family *Poaceae*. Its cultivation started as a subsistence crop in the Nigerian diet but has gradually become a more important crop. Maize has risen to a commercial crop on which many agro-based industries depend as raw materials. It is a cereal crop of temperate and subtropical zones. It grows in most agro ecological areas especially in the Niger Delta region where crude oil industrial activities are predominant (1). Maize has also been shown to tolerate a variety of stressful conditions and environmental extremities ranging from drought to heavy metals contamination (2).

Cowpea (*Vigna unguiculata* L.) is a dicotyledonous crop in the subfamily *Faboideae* (3). It has been said to originate in southern Africa. It is well adapted to conditions in many parts of the world. The seeds are most often harvested and dried for storage and consumption either after cooking whole or milled as a flour product and used in various recipes, providing a major source of dietary protein that nutritionally complements low-protein cereal and tuber crop staples (3).

Soil contamination by crude oil is increasingly becoming a global menace not only to plants and animals but to the ecosystem in general. The dependence on crude oil and its refined products as major sources of energy continues to make it a "necessary evil". Increase in population, rapid industrialization and complete disregard for environmental health has continued to have impact on the soil. Crude oil spill due to human activities or through accident is the main cause of water and soil pollution. Crude oil constituents have been shown to belong to the family of carcinogens and neurotoxic organic pollutants (4).

The soils contaminated by crude oil have been reported to have moderately acidic pH and seeds planted have reduced percentage germination, reduced growth and development (5, 6). In severe conditions, the plant roots may die, and this would prevent uptake of water and other nutrients. It can also disrupt plant and water relationship in soil (7).

Since crude oil pollution is a threat to the environment, the remediation of oil-contaminated soils is a major challenge for environmental research. The use of plants to remove pollutants from the environment or to render them harmless (bio-remediation) becomes necessary because other chemical methods are cumbersome, produce toxic by-products and are very expensive (8, 9). Bio-remediation offers a cost effective remediation technique, compared to other remediation methods, because it is a natural process and does not usually produce toxic by-products. It also provides a permanent solution as a result of complete mineralization of the contaminants in the environment. The advantages of bio-remediation include: i) destruction rather than transfer of the contaminants to another medium, ii) minimal exposure of workers to the contaminants, iii) long-term protection of public health and possible reduction in the duration of the remediation (6, 11). Although different materials such as poultry waste, cow dung, etc, have been used for remediation of soil contaminated with crude oil, the use of palm bunch ash is relatively new.

Oil palm is very important in Nigeria, for its economic value. When the oil in the fruit is extracted from the nuts, the empty bunches are thrown away, constituting nuisance in the environment. Palm bunch is the solid waste that remains after processing of oil palm fruits. Palm fruit bunches are removed after ripening and the fruits processed to express edible industrial mesocarp and seed oils. Palm bunch ash is obtained by burning the solid waste (palm bunch), generated during the processing of oil (7). In its natural state, plant ash has been applied as an amendment to soils and as a substitute for fertilizer. Palm bunch ash is reported to be alkaline (pH > 10) in nature and contains relatively high potassium and sodium contents. Palm bunch ash is an effective fertilizer and liming material for increasing soil fertility, pH and nutrient uptake because of its rich content in nitrogen, phosphorus, potassium, calcium and magnesium. Since palm bunch ash is a good source of sodium and potassium (12, 13), it can be exploited in remediation of contaminated soil (14). It has been shown that the effect of palm bunch ash on crops is due to its possession of vital mineral elements needed for growth and development. Palm bunch ash contributes varying amount of calcium, phosphorus, potassium and magnesium which affect the yield of crop (7, 15).

In recent years studies have shown the usefulness of ash from palm fruit bunch in soil fertility restoration by providing essential constituents needed for plants growth and protection (4, 16). The aim of this study was to assess the qualities of ash from palm bunches in protecting plants against crude oil pollution. The main objectives were to analyse the composition of ash made from oil palm bunches, it's effects in soil exposed to crude oil and its effects on the starch and triglyceride contents of growing cowpea and maize seedlings.

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Materials and Methods

The study was carried out at the University of Benin, Benin City, Edo State, Nigeria. The soil was collected from an uncultivated land with no history of crude oil contamination in Edo State, Nigeria. Top soil samples were collected from an uncultivated land with no history of crude oil contamination by digging holes with a plastic spade at five different locations within the land to a depth of about 15cm. The soil samples was collected into polythene bags and taken to the laboratory. All the soil samples were made into a composite soil by mixing equal amounts from each location, thoroughly. Four hundred grams (400g) of the composite soil was weighed into 120 polythene bags. The soils were air-dried at room temperature (28-31°C), crushed in a porcelain mortar and sieved through a 2mm sieve. The air-dried < 2mm samples were stored in polythene bags and labeled. Ash from oil palm bunch was applied by incorporating appropriate quantities into the soils and properly mixed to ensure even distribution within the soil. All treatments were transferred into evenly perforated planting bags and incubated.

Maize (*Zea mays*) and cowpea (*Vigna unguiculata L*.) seeds were bought from a local market in Benin City, Edo State, Nigeria and identified, in the Department of Crop Science, University of Benin, Benin City, 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.

Bonny Light Crude Oil was obtained from Warri Refinery and Petrochemical Company Delta State, Nigeria. A portion of the crude oil was fractionated by a modified method of (17) into water soluble fraction (WSF) and water insoluble fraction (WIF). For the fractions, a 1:1 dilution of 100 ml 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.

A pilot study was conducted in an earlier experiment with 0.1%, 0.2% and 0.3% crude oil. In the experiment, 0.3% crude oil contamination was found to have the highest stress level and adverse effect on the plants. The composite soils were treated with distilled water (control) and 0.3% whole crude (WC), or with the water insoluble fractions (WIF) of the crude oil in the laboratory. The soil in the bags contaminated with whole crude (WC) and water insoluble fractions (WIF) were mixed thoroughly in their respective polythene bags containing 400g top soil with the aid of a plastic spade.

The seeds were planted by a modified version of (18). Three viable maize or cowpea seeds were sown in 500g sandy loam soil with a depth of about 1 cm and watered daily with distilled water. The time and number of seeds that sprouted from each bag were noted and the germination percentage seedling in each treatment was calculated using the formula:

Germination Percentage	=	<u>Number of seeds that sprouted</u> x	<u>100</u>
		Number of seeds planted	1

In each bag, three (3) viable cowpea and maize seeds were planted. Equal amounts of seeds that sprouted were harvested on the 7th day and Starch and Triglycerides contents were assessed in the roots after thorough washing with tap water.

The physicochemical analysis of the soil and APB were assayed according to the methods described by A.O.A.C, (19) using Atomic Absorption Spectrophotometer (AAS), Bulk Scientific. Potassium (K) was determined by aspirating directly into flame photometer (PFP7) while calcium (Ca) and Sodium in the extract was determined using the Atomic Absorption Spectrometer (AAS). The pH, Particle Size Analysis, Organic Carbon, Organic matter, Potassium, Sodium and Calcium were assayed and the parameters determined were expressed in percentages and centimole per kilogram (Cmol/kg).

The roots were recovered for the analysis. Weighed quantity of the roots was oven dried at 60°C for 48h to constant weight. After drying, the tissue was immediately placed in a desiccator before the final weighing. A portion of the root tissue was crushed and boiled in a few ml of isopropanol to activate phospholipases, then homogenized in 20 volumes (w/v) of chloroform and methanol (2:1 v/v) and stored at

9-4°C until ready for use. A portion of the tissue was put into 80% ethanol and stored. The extraction of lipids was done by the methods of Folch *et al* (20). A suitable aliquot of lipid extract was evapourated to dryness to determine the lipid content by weighing. The methods used for the estimation of different lipid classes were according to Munshi *et al* (21). The extraction and estimation of starch, total soluble sugars and reducing sugars were done by earlier described methods (21).

The data collected were subjected to analysis of variance (ANOVA), using Instat-Graphpad statistical package (version 6), and where significant differences were observed, Duncan's multiple comparisons test at 5% probability level was used to compare the treatment means. The results of the study were expressed as mean \pm standard error of mean (SEM).

Results and Discussion

The results of the physicochemical properties of the soil and ash from palm bunch (APB) are shown in Table 1. The results obtained using the universal soil classification method show that the soil used in the experiment were sandy loam soil. The results obtained for total organic carbon and total organic matter were significantly higher (P < 0.05) in the crude oil contaminated soil when compared to control soil but higher in the APB. The total organic carbon (TOC) and Total Organic Matter (TOM) values obtained in the results are comparable to those earlier reported (6, 22). The levels of organic matter in soils affect the soil chemical and physical processes and acts as an indicator of the soils ability to hold plants (22).

Parameter/Sample	Control	0.3% WC	APB
pH (H ₂ O)	6.28±0.03 ^a	5.94±0.03 ^a	8.50
Total Organic Carbon (%)	2.60 ± 0.01^{a}	3.90±0.01 ^b	6.82
Total Organic Matter (%)	$2.34{\pm}0.02^{a}$	4.68 ± 0.02^{b}	8.26
Mg^{2+} (Cmol/kg)	2.50 ± 0.02^{a}	2.90 ± 0.02^{a}	18.98
K^+ (Cmol/kg)	0.42 ± 0.02^{a}	0.20 ± 0.02^{b}	421.28
Na ⁺ (Cmol/kg)	0.50 ± 0.02^{a}	0.39 ± 0.04^{a}	19.42
Ca^{2+} (Cmol/kg)	3.00 ± 0.02^{a}	4.14 ± 0.05^{b}	73.48
Clay (%)	15.34	16.28	
Silt (%)	10.30	10.70	
Sand (%)	74.36	73.02	

Table 1: Physicochemical characteristics of the soils and ash from palm bunch (dry weight)

Values are mean of three (n=3) replicates \pm standard error of mean, Cmol/kg (centimoles of charge per kg soil = meq/100 g). Means of the same row carrying different notations are statistically different at P<0.05

However, pH was significantly lower (P < 0.05) in the crude oil contaminated soil when compared to control soil but higher in APB. The low pH indicates that the control and crude oil contaminated soils were slightly acidic. Similar pH values have been reported for soils with cassava processing effluents (23), soils in the Niger Delta and some soils in other parts of Nigeria (6). However, it is at variance with pH values reported for dumpsites (24) which is similar to the pH observed in APB. The observed pH values in the soils may have altered the physicochemical compositions of the soil as well as the chemical fractionation (22). The soil pH which was originally acidic (pH: 6.28) and made more acidic by 0.3% crude oil contamination (pH: 5.94) may have increased to near/above neutral by addition of the ash from palm bunch whose pH (8.50) is alkaline (14). The increase in pH may have been involved in the remediation process of the soil by the ash from palm bunch which may have led to the increase in plant height.

Similarly, the exchangeable potassium (K) and sodium (Na) of the soil which were reduced respectively after contamination, may have been remediated by addition of the APB with high nutrient contents (K and Na). Treatment with APB may have increased the original Ca and Mg concentration which may have led to the increase in nutrient contents in the soil. Since K, Na, Mg and Ca are essential plant

nutrients, the use of APB for soil remediation may have resulted in the enrichment of the soil with vital nutrients.

The high total organic carbon (TOC) content of APB may also increase the control and 0.3% contaminated soil because Adjei and Boahen (25) reported that TOC of soils increased with the application of APB. Similar increase may be observed for the soil total organic matter (TOM), and other nutrients an indication of soil fertility improvement by applying the APB (4, 14).

The effects of different crude oil fractions and APB on germination percentage, starch and triglyceride contents in maize is shown in Table 2. The result shows that treatment of crude oil polluted soils with ash from oil palm bunch resulted in remediation of the soil leading to enhanced sprouting of maize seeds (Table 2 and Figure 7). Several researches have observed that the presence of crude oil in soils hinders seed germination and attributed these situation to the hydrophobic nature of the soils which leads to unavailability of water and oxygen essential for seed germination (6, 15 & 26). In this study APB enhanced germination percentage. This indicates that the hydrophobic nature of the soil may have been reversed by the APB which may have resulted to water and oxygen being available for germination enhancement thus improving the soil fertility. This result agrees with the study of other researchers for enhancement of soil fertility with palm fruit ash (4, 7, 14 & 15).

Table 2: Ash from Palm bunch on the effect of 0.3% contamination of various fractions of crude oil on percentage germination, total protein, starch and triglyceride contents in the root of maize 7 days post germination

Maize 7 days	Germination %	Total protein	Starch	Triglyceride
Control	100	0.337 ± 0.043^{a}	$0.078\pm0.006^{\mathrm{a}}$	$0.640\pm0.033^{\rm a}$
RMD	100	$0.371\pm0.021^{\text{b}}$	$0.173\pm0.004^{\text{b}}$	0.680 ± 0.122^{ab}
WC	70	0.054 ± 0.025^{c}	0.200 ± 0.004^{bc}	0.579 ± 0.035^{ac}
WC+R	94	0.138 ± 0.050^{d}	0.201 ± 0.005^{bcd}	$0.722\pm0.143^{\text{d}}$
WIF	56	0.039 ± 0.026^{e}	$0.069\pm0.008^{\text{ae}}$	0.593±0.029ace
WIF+R	86	$0.083\pm0.002^{\rm f}$	0.070 ± 0.006^{aef}	0.621 ± 0.054^{acef}

Values are represented as mean \pm SEM (n=3). Total protein unit is in mg/ml, Starch content is in mg g⁻¹, while triglyceride unit is in g 100 g⁻¹

The results also show significantly higher concentrations of total protein (P<0.05) in the treated groups against that of control, but WIR was slightly lower. The various remediated groups shows an improvement in the total protein concentrations as against their respective non-remediated groups. That is, remediated group only was higher than control group in protein content; while WC+R showed higher protein content than WC. Similarly, WIF+R showed higher protein content than WIF (Figure 1).

The starch content was highest in WC group than in control and lowest on WIF (P<0.05). Their various remediated groups showed no significant changes in starch content (Figure 3).

Triglyceride was significantly higher in WC than control and WIF and lowest in WIF. The remediated only was higher than control in triglyceride content and the remediation of the treated groups was also in triglyceride content than the untreated groups (Figure 5).

The mobilization of complex polymers such as starch, proteins and lipids from storage tissues such as cotyledons is one of the most studied processes on seedling development. These polymers or seed reserves, are used as energy sources and building blocks for seedling growth during germination (27). The reduction in starch and triglyceride contents in the maize root observed in the study are consistent with earlier reports that a reduction in oxygen supply may lead to reduction in adenosine triphosphate (ATP) production which invariably leads to accelerated sugar metabolism and glycolysis (28, 29 & 30). The decrease in starch and

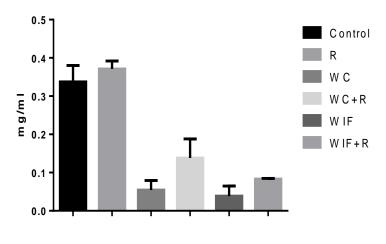
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lipid contents of the cowpea root observed in the study compares favourably with increase in glucose contents previously reported (31). This may indicate that starch and triglycerides were the main source of energy in the plant during crude oil exposure. Thus starch and lipids can be converted into glucose under stress condition as starch and lipids (storage carbohydrate) are dissociated as primary metabolites in stress condition to overcome the energy demand by the plant for growth and to overcome oxygen deprivation. The decrease in starch content may be related to the toxicity of crude oil because starch and/or lipids helps the plant in providing energy in time of emergency for survival. The results suggests that starch and/or lipids could be used by the growing plants to produce glucose as energy source to sustain the metabolic activities occurring in the plant (5).

Table 3: Ash from Palm bunch on the effect of 0.3% contamination of various fractions of crude oil on percentage germination, total protein, starch and triglyceride contents in the root of cowpea 7 days post germination

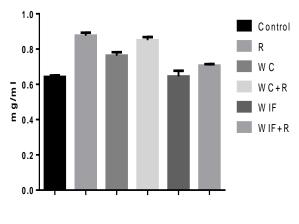
Cowpea 7 days	Germination%	Total protein	Starch	Triglyceride
Control	100	$0.642\pm0.01^{\rm c}$	0.077 ± 0.004^{a}	$0.667 \pm 0.015^{\rm a}$
Remediated	100	$0.876\pm0.02^{\text{b}}$	0.095 ± 0.002^{ab}	$0.690\pm0.023^{\text{b}}$
WC	60	$0.851\pm0.02^{\rm a}$	0.103 ± 0.004^{ac}	0.251 ± 0.012^{ac}
WC+R	90	0.763 ± 0.02^{abc}	0.133±0.009 ^{abc}	0.507 ± 0.020^{bd}
WIF	52	0.644 ± 0.03^{abc}	0.098±0.003 ^{ade}	0.387 ± 0.041^{ef}
WIF+R	86	0.706 ± 0.01^{abc}	0.099 ± 0.004^{bcd}	0.585 ± 0.028^{abc}

Values are represented as mean \pm SEM (n=3). Total protein unit is in mg/ml, Starch content is in mg g⁻¹ and Triglyceride unit is in g 100 g⁻¹.



Total Protein

Fig 1: Effect of Crude oil and palm fruit bunch ash on Total Protein of maize seedlings



Total Protein

Fig 2: Effect of Crude oil and palm fruit bunch ash on bean seedlings

The increased starch content observed in the WC fraction when compared to control maize may be a switch to triglyceride degradation as lipids are those compounds which dissociated as primary metabolites in stress condition to overcome the energy demand by the plant for growth (32). While the increased starch content observed in the remediated fraction may be a compensatory rise by the nutrients present in APB.

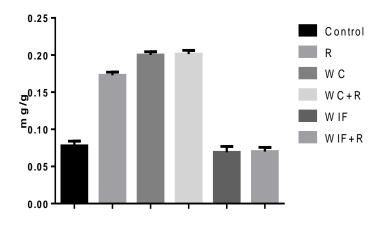
The results suggests that crude oil may have inhibited the growth of the plant possibly because of the decrease in starch and triglyceride content (5). However, the compensatory non-significant increase in starch content in the APB added group, may be attributed to the favorable soil condition established by the APB (7).

The effects of different crude oil fractions and APB on germination percentage, starch and triglyceride contents in cowpea is shown in Table 3. The result shows that treatment of crude oil polluted soils with ash from oil palm bunch resulted in remediation of the soil leading to enhanced germination percentage of cowpea seeds (Table 3 and Figure 8). Several researches have observed that the presence of crude oil in soils hinders germination of seeds and attributed this condition to the hydrophobic nature of the soils which leads to unavailability of water and oxygen essential for seed germination (6, 15 & 33). In this study APB enhanced germination percentage. This indicates that the hydrophobic nature of the soil may have been reversed by the APB which may have resulted to water and oxygen being available for germination enhancement thus improving the soil fertility. This result agrees with the study of other researchers for enhancement of soil fertility with palm fruit ash (4, 7, 14 & 15).

The results shows that total protein content of cowpea 7 days post germination was significantly improved in WC when compared with control and was considerably reduced in WIF than in the control. The remediation of the various treated soils as well as the remediation of the control was progressively increased compared to their non-remediated groups (P<0.05) (Figure 2).

The starch content in the treated groups WC and WIF were increased significantly as against the control. The remediation of WC and WIF was able to increase significantly their starch content (P>0.05) as well as the remediation only also increasing the starch level of the control (P<0.05) (Figure 4).

The triglyceride level of WC and WIF reduced considerably (P>0.05). The WC+R increased with respect to WC as well as remediated only with respect to control. WIF+R also increased reasonably to WIF (P<0.05) (Figure 6).



Starch Content



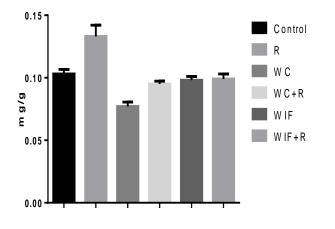
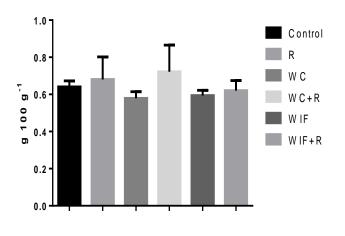


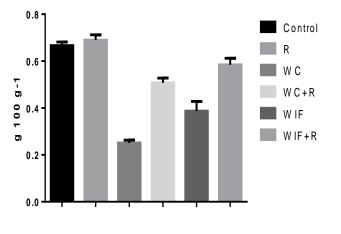


Fig 4: Effect of Crude oil and palm fruit bunch ash on bean seedlings



Triglyceride Content

Fig 5: Effect of Crude oil and palm fruit bunch ash on maize seedlings



Triglyceride Content

Fig 6: Effect of Crude oil and palm fruit bunch ash on bean seedlings

Plants exposed to crude oil normally are exposed to lower oxygen supply, reduced ATP generation, and uses the fermentation process as a secondary route in plant metabolism for energy production (34). The non-significant and significant increase in starch contents in the cowpea root of R, WC, WC-R, WIF and WIF-R observed in the study are not in agreement with earlier reports that a reduction in oxygen supply may lead to reduction in adenosine triphosphate (ATP) production which invariably leads to accelerated sugar metabolism and glycolysis (28, 29 & 30). The decrease in lipid content of the cowpea root observed in the study compares favourably with increase in glucose contents previously reported (31). This may indicate that triglycerides were the main source of energy in the plant during crude oil exposure and not starch in the case of cowpea seedling. Thus lipid was converted into glucose under stress condition as lipid (storage carbohydrate) are dissociated as primary metabolites in stress condition to overcome the energy demand by the plant for growth and to overcome oxygen deprivation. The decrease in lipid content may be related to the toxicity of crude oil because starch helps the plant in providing energy in time of emergency for survival that is if the energy supplied by carbohydrates is not enough or carbohydrate fails to supply enough energy for survival.

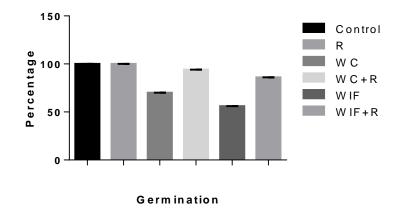


Fig 7: Effect of Crude oil and ash from palm bunch on Germination % of maize seedlings

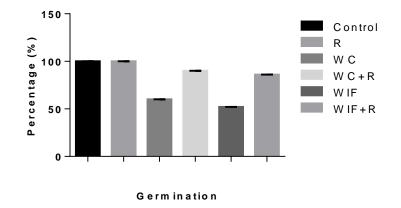


Fig 8: Effect of Crude oil and ash from palm bunch on germination % of Cowpea seedlings

The results suggested that lipid could be used by the growing plants to produce glucose as energy source to sustain the metabolic activities occurring in the plant (32). The increased starch contents observed in the fractions when compared to control cowpea may be a switch to triglyceride degradation as lipids are those compounds which dissociated as primary metabolites in stress condition to overcome the energy demand by the plant for growth (32). This may indicate that there was a positive relation between lipids and growth in early stage of development of cowpea seedlings.

The inter-relationships between starch and triglycerides (TAG) biosynthesis are not clear. It has been postulated that some species which accumulate both starch and TAG in developing embryos, starch precedes TAG biosynthesis and in some species the level of starch decreases in parallel with TAG accumulation, suggesting that the TAG may be synthesized from degradation of the starch (35, 36). Research has shown that environmental stress induces degradation of carbohydrate that seems to be temporally coordinated with biosynthesis of neutral lipids. The authors suggests that the findings represent a carbon flow from the starch to the lipids (36). A recent study shows that whereas most starch is made from assimilated CO₂, most TAG are produced from acetate (36 & 37). These studies are consistent with two parallel biosynthetic mechanisms, one for starch and another for TAG. However, none of these studies referred to inter-conversions between starch and lipids or between polar and neutral lipids and their potential contributions to TAG formation.

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The results presented in this study suggests that the plants may have responded to crude oil pollution in two distinct metabolic phases as reported (36). Either the cells continue to divide slowly, photosynthetic CO_2 assimilation activity drops, or the cells accumulate massive levels of starch in the chloroplast, accounting for over two-thirds of the total assimilated carbon or cell division almost stops, photosynthesis drops, the starch level reaches a steady state, and TAG is produced, mostly by recycling of starch carbon. So the general carbon metabolism switched from photosynthetic carbon assimilation to starch degradation and the carbon reserves gradually change from all starch to progressively increasing TAG levels (36 & 38).

The variations observed between the two plants with or without the presence of APB amendment implies that these crops has natural variations in their ecological and biological characteristics. Cowpea as a legume for example, have bacteria in its root nodules which may aid in the degradation of crude oil thus protecting the seedling (39) while maize as a cereal, may have the ability like other grasses, to be more efficient for their fibrous root system with extensive root surface area for microbial colonization and dense rhizosphere (40). This is consistent with previous reports of interspecies differences in sensitivity to crude oil, and may be related to differences in systemic uptake of oil compounds, nutrient availability, and cell wall structural differences (41).

The study, therefore, indicates that the starch and triglyceride contents were affected by the various crude oil fractions, an indication that membrane integrity, energy production and viability may be affected and that crude oil and its fraction affects starch and triglyceride contents in ways which are species related. However, APB has the potentials for protecting and maintaining starch and triglyceride contents for the seedlings in a crude oil polluted environment. The study suggests that APB could be used as fertiliser to increase the pH and the nutrient contents of acidic soils and thus improve the soil fertility. However, further studies is needed to elucidate the mechanism of alteration of starch and triglyceride contents in the plant species.

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