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Research paper

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Adenosine triphosphatase activities of *Zea mays* and *Vigna unguiculata* exposed to
different crude oil fractions

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Comment [ECS1]: Convert all title to Upper case.

ABSTRACT

Aim: To investigate the activities of adenosine triphosphatase in maize and beans exposed to crude oil.

Study Design: Sodium/potassium adenosine triphosphatase, calcium adenosine triphosphatase and magnesium adenosine triphosphatase activities, were evaluated in maize and beans grown in soil contaminated with different crude oil fractions.

Place and Duration of Study: Laboratories of Department of Biochemistry, Faculty of Life Sciences and Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria. From March to August, 2009

Materials and Methods: A total of 660 bags containing 500g of sandy loam soil, with no known crude oil contamination, was used for this study. 330 bags was used for planting maize while the other 330 was used for planting beans. 30 bags containing soil served as control, 270 bags of soil were mixed with varying 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, Delta State, Nigeria. The maize and bean seeds grown in these soils were harvested after 7, 14 and 21 days post germination and the adenosine triphosphatase activities measured. The data obtained were statistically analysed using descriptive statistics and analysis of variance.

Results: The activities of adenosine triphosphatases was significantly ($P < .01$) altered in the plants grown in contaminated soil compared with control. These effects of crude oil and its fractions were more pronounced as percentage contamination and days post germination increased. Higher adenosine triphosphatase activity was observed in the germinating maize than in beans.

Conclusion: The results of this study show that beans was relatively more sensitive than maize to crude oil-contaminated soil during the period of study. This may have great effects on the yield of these plants.

Keywords: Crude oil, Maize, Beans, Adenosine triphosphatase, Contamination

1. INTRODUCTION

Crude oil is a colloidal mixture of different hydrocarbons (90%) and non-hydrocarbon (10%) components [1]. Water and oil are usually considered to be non-miscible. However, crude oil contains a very small soluble portion referred to as the water soluble fraction (WSF). The components of crude oil that go into solution make up this WSF. WSF have been reported to contain 20 aromatic compounds ranging from benzene to dimethyl phenothrenes and up to 14 saturated hydrocarbons ranging from C14 to parafins [3]. Nigeria is an established crude oil exporting nation and it forms the mainstay of its economy [2]. Various activities in crude oil

Comment [ECS2]: Were planting of the grains and growth monitoring carried out inside a laboratory or field around the University. One should suppose that the Adenosine Triphosphate assay was conducted inside the laboratory not the planting of crop except if green house is also a component of your laboratory?

Comment [ECS3]: Consider stating the number of days weeks or month.

Comment [ECS4]: Avoid starting sentences with figures. Always begin your sentences with alphabets even in scientific literatures like this. State the quantities placed in each of the 660 bags. Your explanation is not specific. Your parameters need to be quantified to standard measurement for ease of replication of experiment elsewhere.

Comment [ECS5]: The language 'altered' is not appropriate even for statistical analysis let alone scientific explanations. Use significant increase or decrease as the case may be.

Comment [ECS6]: Could it be past tense ie 'showed'?

Comment [ECS7]: This conclusion does not capture the aim of the experiment. ATP is synonymous to energy utilisation. The explanation should be in terms of ATP measured as it relates to germination and growth of maize and beans. Rewrite the whole abstract with the listed corrections.

exploration, storage and transportation lead to spillage of oil to the environment [4] . This spillage or discharge of crude oil to the environment causes harmful effects, where it poses serious threat to organisms and farmland that are linked in a complex food chain that includes humans [5] . The effects of crude oil on the growth and performance of plants have been reported in many studies [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]. 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 [11]. Even at an early stage, it can cause a reduction in cell proliferation and growth. ROS is thought to increase cellular damage through the oxidation of several macromolecules such as lipids and proteins [12]. However, to cope with ROS activities, plants have developed antioxidative mechanisms, including superoxide dismutase, catalase, etc. which are induced whenever there is environmental stress.

Adenosine triphosphatases (ATPases) catalyse the decomposition of Adenosine triphosphate (ATP) into Adenosine diphosphate (ADP) and free phosphate ion (Pi). The enzyme is important in transport, signal transduction, protein biosynthesis and cell differentiation and are believed to mediate energy transfer from ATP to the ion transport system in plant roots [13,14]

Maize (*Zea mays*), a typical cereal crop of temperate and subtropical zones is one of the major sources of carbohydrate consumed in Nigeria. Beans (*Vigna unguiculata*), a typical legume on the other hand is one of the major source of protein consumed in Nigeria since meat protein is beyond the reach of the 'common man'. Both plants are grown in most agro ecological areas especially in the Niger Delta region where oil industrial activities are predominant [4]. As a result of the global melt-down, increasing economic crisis and high dependence on plant such as beans and maize to generate income and meet the protein and carbohydrate need of humans and animals, it become increasingly important to conserve the major sources of protein and carbohydrate.

Most studies of the effects of crude oil in plants are mainly on physiological parameters ranging from percentage germination, to plant height, number of leaves, etc, and on crude petroleum (crude oil). Also, maize has been displayed as a variable stress tolerant plant under environmental extremities ranging from drought, heavy metals, etc [15]. There is however, lack of information on the effects of crude oil on biochemical parameters such as adenosine triphosphatase (ATPase) activities as well as the effects of crude oil fractions (WSF and WIF of crude oil) in plants since some researchers have attributed adverse biological effects to dissolved low molecular weight hydrocarbon particularly aromatics such as toluene. Also, other researchers considered naphthalene as a more important source of crude oil toxicity than low molecular weight aromatics. According to another source, the low boiling point unsaturated hydrocarbon such as benzene, toluene, xylene and naphthalene, are the most toxic components in crude oils [2,16]. This prompted us to investigate the effects of crude oil and its fractions on the activities of adenosine triphosphatase in beans and maize radicle to make a comparison in both plants as well as a crude oil spillage soil from Ubeji, Delta State, Nigeria.

2. MATERIAL 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 August, 2009. The soil from an uncultivated land with no known crude oil contamination and soil where there was crude oil contamination was collected from Ubeji, Delta State, Nigeria, and same geographic region as the study site.

Comment [ECS8]: delete

Comment [ECS9]: add 'and' after this.

Comment [ECS10]: Delete

Comment [ECS11]: Avoid the use of etc as much as possible. State the issues involved.

Comment [ECS12]: Remove and add 'the research so as to'

Comment [ECS13]: Delete and add 'on'

Comment [ECS14]: replace with 'contaminated'.

Comment [ECS15]: Delete and add 'as well as'

Comment [ECS16]: Delete

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2.2 Soil Sampling and Pre-treatment

Top soil (0 – 20cm depth) sample was collected from an uncultivated land without known crude oil contamination at Ubeji, Delta State. A hole was dug at five points within the land to a 20cm depth using plastic spade. Also, soil from a Bonny light crude oil spillage site from the same community was collected. The soil samples was collected into polythene bags and taken to the laboratory. A composite of all the samples was made by mixing thoroughly equal amounts of soil from each point. The composite soil was weighed into 300 polythene bags such that each bag contains 500g soil and another additional 30 bags of 500g of soil collected from a Bonny light crude oil contaminated soil as shown below in table 1:

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	Unknown	30

Comment [ECS18]: Replace with 'Holes were dug at five different points

Comment [ECS19]: Add 'each' after depth

Comment [ECS20]: And used for what. The understanding is that Ubeji soil was contaminated with Bonny light crude oil as you experimental design. Make this clear and simple for all to understand. Re-write.

Comment [ECS21]: Confusing. Is this a total of 500g of soil in 30bags or 30bags with each bag containing 500g? Rewrite please. Crude oil in polythene bags are normally perforated at some point below to allow for vertical leaching as a true situation when crude oil spillage occurs. Did you perforate your polythene bags?Otherwise there will be percolation of crude oil in polythene thus a faulty experimental design.

Comment [ECS22]: Is it Bonny light crude oil? Add crude oil source please. You did not tell about the significance of your experimental design using various crude oil types ie WC, WSFand WIF. Why do you have to use all these different crude oil fractions as well as whole crude?

2.3 Plant Materials

Maize (*Zea mays*) and beans (*Vigna unguiculata*) seeds were bought from a local market in Benin City, Edo State, Nigeria and identified as Dmr-Esr-w and ITA 189 - 288 cultivars respectively, 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.

Comment [ECS23]: Remove

2.4 Crude oil and fractionation

Crude oil (Bonny light, °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.* [2] 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

The composite soils were treated with either distilled water (control); whole crude (WC), water soluble fraction (WSF) of the crude oil; or with the water insoluble fractions (WIF) of the crude oil in the laboratory. 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 top soil with the aid of a plastic spade. Soil of 500g was treated with 10ml, 25ml and 50ml of crude oil to obtain 2, 5 and 10% v/w crude oil contamination.

In each bag, three (3) viable maize or bean seeds were planted. Equal amounts of seeds that emerged were harvested at day 7, day 14 and day 21. Growth parameters were assessed after each harvest.

Comment [ECS24]: Re-evaluate your crude oil contamination and see if it tallies with the percentage contaminant.

2.6 Planting of seeds and germination studies

The seeds were planted by a modified version of Vavrek and Campbell (2002) [17]. Three bean seeds were sown in 500g top soil with a depth of about 1-2 cm. The time and number of seeds that germinated from each bag were noted and the percentage germination in each treatment was calculated using the formula:

Percentage germination = $\frac{\text{number of seeds that germinated}}{\text{number of seeds sown}} \times 100$

Comment [ECS25]: Remove

Comment [ECS26]: Top soil in a bag or in the field?

2.7 Biochemical assays

2.7.1 Enzyme extraction

Enzyme extraction was done on ice.

2.7.2 Isolation of crude mitochondria

A modification of Douce *et. al.*, (1987) [18] was used for the isolation of mitochondria from the radicle of the germinating bean seedling. The radicle was homogenized in ice-cold extraction medium (Sucrose 0.25M, EDTA 5mM, EGTA 1mM, dithioerythritol 1mM, BSA 0.1%, polyclar-AT 0.6%, in HEPES-TRIS 10mM pH.7.4). The homogenate was filtered with a clean white cloth and the mitochondria immediately separated from the cytoplasmic fraction by centrifugation at 15,000g for 10mins. The resulting crude mitochondrial pellet was resuspended in a solution (Sucrose 0.25M, EDTA 5mM, EGTA 1mM, BSA 0.1% in HEPES-TRIS 10mM pH.7.4) and centrifuged at 600g for 5 minutes to remove nuclei and heavy cell debris. This washing procedure was repeated twice. The washed crude mitochondrial pellet were resuspended in another solution (Sucrose 0.25M, EGTA 30mM, in HEPES-TRIS 10mM pH.7.4) and stored in ice. The samples obtained were subsequently used for the determination of ATPases.

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2.7.3 Measurement of ATPase

ATPase was measured according to the method of Matsukama and Tagikuchi (1981) [19]. Activity of ATPase was assigned by measuring the amount of inorganic phosphate liberated following incubation with 25mM disodium ATP. The inorganic phosphate liberated was estimated according to the method of Fiske and Subarrow (1925) [20].

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Comment [ECS29]: Delete and replace with 'determined'.

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2.8 Quality control and assurance

In order to ensure the accuracy and reliability of the results obtained, all reagents used for the preparation of standard solutions and analysis was analytical grades (BDH or Sigma). All glasses and plastic was acid-washed.

Buck scientific standard solutions were used to calibrate the Atomic Absorption Spectrophotometer. Procedural blank samples was subjected to similar extraction method using the same amounts of reagents.

Comment [ECS31]:

Add 'plastics used'

2.9 Statistical analysis

The result of the study was expressed as mean $\pm$ 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 $P < 0.01$ was considered statistically significant

3. RESULTS AND DISCUSSION

3.1 Effect of crude oil and its fractions on adenosine triphosphatase (ATPase) activities of the radicle of beans

There was an increase in ATPase activity in the values obtained as the concentrations of the crude oil increased in beans and this was found to be significant ($p < 0.01$) between (2-10%) oil in soil contamination relative to the control value. As the plants grew, the ATPase activity also increased. The activated Ca^{2+} -ATPase activity will lead to increase Ca^{2+} ion absorption or influx into the plant. Ca^{2+} has been reported to maintain membrane integrity and protect the plant from the injurious effects of hydrogen ion (H^+), high salts and other potentially toxic ions present in the contaminated environment [21]. This increase influx of Ca^{2+} will help to cushion the damaging effect of the slight increase in lipid peroxidation observed during the study (result not shown) [22].

The increase in Mg^{2+} ATPase will increase influx of Mg^{2+} ion into the plant. The increase influx may also protect the plant against the damaging effect of lipid peroxidation by maintaining membrane integrity as well as stimulating Na^+/K^+ ATPase activity for carbohydrate metabolism, hence, providing energy for metabolic processes in the growing plant and facilitating osmotic adjustment [21].

The increased activity of Na^+/K^+ ATPase may have been as a result of increase influx of Mg^{2+} ion. Increase Na^+/K^+ ATPase activity in turn increases absorption of Na^+/K^+ .

K^+ ion has been reported to increase carbohydrate metabolism by translocating sugars, help to increase stomata opening, regulate the water in plant cell by preventing loss of water that may physiologically dry the plant [21].

The significant increase in ATPase activity in the values obtained as the concentrations of the crude oil increased in beans relative to the control value may be as a result of increasing concentrations of Mg^{2+} and ATP complex (Mg-ATP) and decreasing concentrations of ATP, or activation by Mg^{2+} and monovalent cations ($\text{K}^+ > \text{Rb}^+ > \text{Cs}^+ > \text{Na}^+ > \text{Li}^+$) [22]. The non-significant decrease in ATPase activity observed at some concentrations, for example beans on 7DPG at 2% concentration, magnesium ATPase (Mg^{2+} ATPase) activity of WIF (0.15) and calcium ATPase (Ca^{2+} ATPase) activity of WC (0.05) relative to the control (0.17) (Table 2) may be due to the presence of divalent cation other than Mg^{2+} which could either partially substitute for Mg^{2+} (i.e Mn^{2+} and Co^{2+}), is ineffective or inhibitory (i.e Ni^{2+} , Zn^{2+} , and Ca^{2+}) to the enzyme and thus prevents maximum stimulation by monovalent cations ($\text{K}^+ > \text{Rb}^+ > \text{Cs}^+ > \text{Na}^+ > \text{Li}^+$) or a decrease/increase in optimum conditions of pH and temperature for maximum activity or the presence

Comment [ECS32]: Replace with 'increased'

Comment [ECS33]: Replace with 'helped'

Comment [ECS34]: Recaste sentence in past tense otherwise quote literature to support your claim

Comment [ECS35]: You reported a cited literature with uncertainty of claim of Mg^{2+} ATPase effect in protection of plant. Rewrite and be certain of fact. Consult other literatures otherwise delete.

Comment [ECS36]: use past tense in your report.

Comment [ECS37]: see comment above

of substrates other than ATP (i.e mono-, di-, and triphosphatases) since the enzyme exhibited a clear preference for ATP as the substrate over other nucleoside [23].

The significant inhibition of sodium/potassium ATPase (Na^+/K^+ ATPase) activity of WIF (0.01) on 21DPG (Table 2), may be as a result of inhibition by Ca^{2+} . The inhibition by Ca^{2+} of K^+ -ATPase of oat roots suggested by Balke *et al.*, 1974 [24] to be uncompetitive, was an interference with the ATP- Mg-enzyme complex which would require action on the inside of the cell. However, it may be possible that Ca^{2+} competes at K^+ -sites on the ATPase to inhibit K^+ influx. The inhibitory effects of Ca^{2+} on K^+ -ATPase at relatively low concentration as observed by Leonard and Hotchkiss (1976) [23] suggested that levels of free Ca^{2+} in cells were low, and that Ca^{2+} concentrations in the cytoplasm could have a regulatory effect on ion transport rates [25, 26] Ca^{2+} may inhibit ATPase by interfering with the ATP Mg-enzyme complex which in turn prevents Mg^{2+} translocation, which may lead to its deficiency in the plant. At 5% contamination, magnesium ATPase (Mg^{2+} ATPase) activity of WC (0.11, 0.12 and 0.13 respectively) was significantly inhibited on 7, 14 and 21DPG relative to control (0.17, 0.21 and 0.22 respectively) (Table 2). This inhibition may be as a result of the effect of inorganic ions on the plant ATPase which is reported to either slightly stimulate or inhibit its activity and/or the possibility that all fractions contain a variety of membrane structures and the existence of more than one ATPase associated with different membrane structures in each fraction. It can be inferred that the cell wall fraction of ATPase is most expressed hence the relative inhibition and there won't be sufficient Mg^{2+} stimulated ATPase to account for the Mg^{2+} that needs to be absorbed in the plant roots [24]. There will be reduced absorption of Mg^{2+} and the protective potential of the cations will be reduced. This may lead to damage of membrane and deactivation of Na^+/K^+ ATPase.

At 10% contamination, sodium/potassium ATPase (Na^+/K^+ ATPase) activity of WSF (0.14) was significantly inhibited on 7DPG relative to control (0.17) (Table 2). The result of this study is similar to the findings of Senthil-Nathan *et. al.*, (2005) [27] who observed significant inhibition of ATPase activity in different animal species exposed to different contaminants.

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Table 2: Effects of crude oil and its fractions on adenosine triphosphatase activities of beans radicle

	Control	Whole crude			Water soluble fraction			Water insoluble fraction			Ubeji
		2%	5%	10%	2%	5%	10%	2%	5%	10%	

Number of seeds planted	100	100	100	100	100	100	100	100	100	100	100
% germination	100	60	47	37	72	59	42	56	40	27	37
7 days after germination											
Total ATPase ($\mu\text{mole/mg/min}$)	0.40 $\pm$ 0.10 ^a	0.89 $\pm$ 0.09 ^b	0.58 $\pm$ 0.12 ^b	3.25 $\pm$ 0.18 ^b	1.41 $\pm$ 0.23 ^c	2.01 $\pm$ 0.23 ^c	0.70 $\pm$ 0.13 ^c	1.99 $\pm$ 0.87 ^d	2.06 $\pm$ 0.07 ^d	4.08 $\pm$ 0.15 ^d	3.98 $\pm$ 0.17 ^e
Mg ²⁺ ATPase ($\mu\text{mole/mg/min}$)	0.17 $\pm$ 0.15 ^a	0.59 $\pm$ 0.15 ^b	0.11 $\pm$ 0.24 ^b	0.53 $\pm$ 0.15 ^b	0.68 $\pm$ 0.24 ^c	1.02 $\pm$ 0.20 ^c	0.21 $\pm$ 0.15 ^c	0.15 $\pm$ 0.63 ^a	0.28 $\pm$ 0.10 ^d	0.32 $\pm$ 0.10 ^d	1.38 $\pm$ 0.09 ^d
Ca ²⁺ ATPase ($\mu\text{mole/mg/min}$)	0.06 $\pm$ 0.21 ^a	0.05 $\pm$ 0.18 ^a	0.10 $\pm$ 0.15 ^b	0.95 $\pm$ 0.30 ^b	0.16 $\pm$ 0.09 ^b	0.64 $\pm$ 0.12 ^c	0.37 $\pm$ 0.12 ^c	0.09 $\pm$ 0.10 ^c	0.08 $\pm$ 0.10 ^d	0.26 $\pm$ 0.10 ^d	0.33 $\pm$ 0.29 ^d
Na ⁺ /K ⁺ ATPase ($\mu\text{mole/mg/min}$)	0.17 $\pm$ 0.12 ^a	0.23 $\pm$ 0.24 ^b	0.37 $\pm$ 0.09 ^b	1.78 $\pm$ 0.09 ^b	0.55 $\pm$ 0.24 ^c	0.35 $\pm$ 0.21 ^c	0.14 $\pm$ 0.26 ^c	1.75 $\pm$ 0.06 ^d	1.71 $\pm$ 0.16 ^d	3.44 $\pm$ 1.00 ^d	2.27 $\pm$ 0.35 ^e
14 days after germination											
Total ATPase ($\mu\text{mole/mg/min}$)	0.45 $\pm$ 0.23 ^a	1.09 $\pm$ 0.10 ^b	0.63 $\pm$ 0.31 ^b	3.47 $\pm$ 0.21 ^b	1.14 $\pm$ 0.20 ^c	6.52 $\pm$ 0.09 ^c	1.62 $\pm$ 0.27 ^c	5.86 $\pm$ 0.10 ^d	4.37 $\pm$ 0.10 ^d	7.50 $\pm$ 0.10 ^d	4.16 $\pm$ 0.57 ^e
Mg ²⁺ ATPase ($\mu\text{mole/mg/min}$)	0.21 $\pm$ 0.15 ^a	0.66 $\pm$ 0.40 ^b	0.12 $\pm$ 0.44 ^b	1.06 $\pm$ 0.12 ^b	0.43 $\pm$ 0.40 ^c	0.98 $\pm$ 0.15 ^c	0.48 $\pm$ 0.35 ^c	0.74 $\pm$ 0.10 ^d	2.56 $\pm$ 0.10 ^d	0.52 $\pm$ 0.10 ^d	1.61 $\pm$ 0.31 ^e
Ca ²⁺ ATPase	0.03 $\pm$ 0.06 ^a	0.06 $\pm$ 0.10 ^b	0.11 $\pm$ 0.12 ^b	1.02 $\pm$ 0.35 ^b	0.16 $\pm$ 0.20 ^c	2.84 $\pm$ 0.15 ^c	0.52 $\pm$ 0.31 ^c	0.46 $\pm$ 0.10 ^d	0.22 $\pm$ 0.10 ^d	0.56 $\pm$ 0.10 ^d	1.50 $\pm$ 0.29 ^e

($\mu\text{mole/mg/min}$)											
Na ⁺ /K ⁺ ATPase ($\mu\text{mole/mg/min}$)	0.21 $\pm 0.18^a$	0.37 $\pm 0.1^b$	0.39 $\pm 0.18^b$	1.39 $\pm 0.10^b$	0.56 $\pm 0.7^c$	2.89 $\pm 0.55^c$	0.62 $\pm 0.15^c$	4.65 $\pm 0.10^d$	3.59 $\pm 0.10^d$	6.36 $\pm 0.52^d$	1.04 $\pm 0.06^e$
21 days after germination											
Total ATPase ($\mu\text{mole/mg/min}$)	0.48 $\pm 0.35^a$	1.33 $\pm 1.20^b$	0.72 $\pm 0.17^a$	0.93 $\pm 0.23^b$	3.32 $\pm 0.10^c$	3.80 $\pm 0.10^b$	5.71 $\pm 0.67^c$	1.70 $\pm 0.12^d$	4.86 $\pm 0.29^c$	7.71 $\pm 1.10^d$	0.59 $\pm 0.29^e$
Mg ²⁺ ATPase ($\mu\text{mole/mg/min}$)	0.22 $\pm 0.40^a$	0.78 $\pm 0.42^b$	0.13 $\pm 0.12^b$	1.12 $\pm 0.10^b$	0.48 $\pm 0.37^c$	0.30 $\pm 0.10^c$	1.15 $\pm 0.2^B$	1.42 $\pm 0.10^d$	2.64 $\pm 0.26^d$	0.57 $\pm 0.19^c$	0.48 $\pm 0.36^b$
Ca ²⁺ ATPase ($\mu\text{mole/mg/min}$)	0.02 $\pm 0.17^a$	0.13 $\pm 0.18^b$	0.12 $\pm 0.09^b$	1.11 $\pm 0.30^b$	0.19 $\pm 0.10^c$	2.33 $\pm 0.10^c$	1.15 $\pm 1.00^c$	0.27 $\pm 0.10^d$	0.32 $\pm 0.10^d$	0.61 $\pm 0.47^d$	0.42 $\pm 0.58^e$
Na ⁺ /K ⁺ ATPase ($\mu\text{mole/mg/min}$)	0.24 $\pm 0.19^a$	0.54 $\pm 0.22^b$	0.47 $\pm 0.30^b$	1.70 $\pm 0.10^b$	2.64 $\pm 0.36^c$	1.18 $\pm 0.10^c$	3.33 $\pm 0.17^c$	0.01 $\pm 0.12^d$	1.89 $\pm 0.17^d$	6.64 $\pm 0.17^d$	2.68 $\pm 0.12^e$

Values are means of 4 determinations $\pm$ S.E.M. Means carrying different notations are statistically different at $P < 0.01$.

ATPase = Adenosine triphosphatase activity is expressed as μmole of free phosphate (Pi) released/min/mg protein.

3.2 Effect of crude oil and its fractions on adenosine triphosphatase (ATPase) activities of the radicle of maize

In maize radicle, the effect of the contaminants on ATPase activity decreased as the concentration of the crude oil increased relative to control values, evidence that the plant may be undergoing oxidative stress. There was significant ($p < .01$) difference in the activity of the enzyme in soil contaminated with 2-10% of crude oil in the maize radicle except in some instances, where significant increase was evident. For example, calcium ATPase (Ca^{2+} ATPase) activity on 7, 14 and 21 days post germination (DPG, 0.49, 0.97 and 1.05 respectively) for 2% WC increased significantly ($p < .01$) relative to the control (0.21, 0.73 and 0.73 respectively) (Table 3). The increased or activated Ca^{2+} -ATPase activity may lead to increase Ca^{2+} ion absorption or influx into the plant since Ca^{2+} has been reported to maintain membrane integrity and protect the plant from the injurious effects of hydrogen ion (H^+), high salts and other potentially toxic ions present in the contaminated environment [21]. This increased influx of Ca^{2+} may help to cushion the damaging effect of the slight increase in lipid peroxidation (result not shown) observed during the study. Sodium/potassium ATPase (Na^+/K^+ ATPase) activity of Ubeji on 7, 14 and 21 DPG (2.39, 4.83, 2.68 respectively) increased significantly ($p < 0.01$) relative to the control (1.32, 2.46, 2.10 respectively) (Table 3). Sodium/potassium ATPase (Na^+/K^+ ATPase) activity of Ubeji on 7, 14 and 21 DPG (2.39, 4.83, 2.68 respectively) increased significantly ($p < 0.01$) relative to the control (1.32, 2.46, 2.10 respectively) Table (3). K^+ ion has been reported to increase carbohydrate metabolism by translocating sugars, help to increase stomata opening, regulate the water in plant cell by preventing loss of water that may physiologically dry the plant [21]. Increased Na^+/K^+ ATPase activity means increased

Comment [ECS41]: Replace with either increase or decrease. Significant difference alone gives no known distinction in quantity analysed except when its increase or decrease is stated.

Comment [ECS42]: Ubeji what? If maize cultivated on Ubeji crude oil contaminated soil please state same to avoid ambiguity.

Comment [ECS43]: See preceding comment above and act accordingly.

ion absorption, hence increased protection, increased adaptation, and/or increased compensation for the peroxidation process or oxidative damage that may have occurred because of the crude oil contamination [13, 14, 21].

Table 3: Effect of crude oil and its fractions on adenosine triphosphatase (ATPase) activities of maize radicle

	Control	Whole crude			Water soluble fraction			Water insoluble fraction			Ubeji
		2%	5%	10%	2%	5%	10%	2%	5%	10%	
Number of seeds planted	100	100	100	100	100	100	100	100	100	100	100
% germination	100	70	54	37	86	65	42	63	47	27	46
7 days after germination											
Total ATPase ($\mu\text{mole/mg/min}$)	1.30 $\pm$ 0.02 ^a	2.30 $\pm$ 0.01 ^b	2.05 $\pm$ 0.01 ^b	2.34 $\pm$ 0.01 ^b	1.49 $\pm$ 0.01 ^c	1.75 $\pm$ 0.01 ^c	1.48 $\pm$ 0.04 ^c	1.81 $\pm$ 0.07 ^d	1.52 $\pm$ 0.03 ^d	1.25 $\pm$ 0.03 ^d	3.19 $\pm$ 0.04 ^e
Mg ²⁺ ATPase ($\mu\text{mole/mg/min}$)	0.77 $\pm$ 0.10 ^a	0.26 $\pm$ 0.04 ^b	0.55 $\pm$ 0.01 ^b	0.54 $\pm$ 0.02 ^b	0.30 $\pm$ 0.09 ^b	0.05 $\pm$ 0.02 ^c	0.06 $\pm$ 0.01 ^c	0.28 $\pm$ 0.01 ^c	0.05 $\pm$ 0.03 ^c	0.01 $\pm$ 0.01 ^d	1.00 $\pm$ 0.01 ^d
Ca ²⁺	0.21 $\pm$	0.49	1.03	0.59	0.13 $\pm$ 0.	1.26	0.36	0.08	0.08	0.03	0.39

ATPase ($\mu\text{mole/mg/min}$)	0.02 ^a	$\pm 0.10^b$	$\pm 0.01^b$	$\pm 0.01^b$	01 ^c	$\pm 0.01^c$	$\pm 0.02^c$	$\pm 0.01^d$	$\pm 0.01^d$	$\pm 0.01^d$	$\pm 0.04^e$
Na ⁺ /K ⁺ ATPase ($\mu\text{mole/mg/min}$)	0.32 $\pm$ 0.60 ^a	1.54 $\pm$ 0.06 ^b	0.47 $\pm 0.01^b$	1.21 $\pm 0.01^a$	1.06 $\pm 0.02^c$	0.45 $\pm 0.02^b$	1.06 $\pm 0.02^a$	1.46 $\pm 0.02^b$	0.43 $\pm 1.9^b$	1.21 $\pm 0.03^a$	1.80 $\pm 0.10^d$
14 days after germination											
Total ATPase ($\mu\text{mole/mg/min}$)	2.34 $\pm 0.70^a$	2.39 $\pm 0.01^a$	2.12 $\pm 0.01^b$	2.81 $\pm 0.01^b$	1.91 $\pm 0.01^b$	3.16 $\pm 0.01^c$	1.84 $\pm 0.06^c$	1.89 $\pm 0.01^b$	3.21 $\pm 0.01^c$	3.06 $\pm 0.01^d$	3.24 $\pm 0.60^c$
Mg ²⁺ ATPase ($\mu\text{mole/mg/min}$)	0.30 $\pm 0.75^a$	0.25 $\pm 0.01^a$	0.31 $\pm 0.01^a$	1.05 $\pm 0.01^b$	0.35 $\pm 0.10^a$	0.34 $\pm 0.01^a$	0.25 $\pm 0.01^a$	0.20 $\pm 0.30^{ab}$	0.05 $\pm 0.01^b$	0.20 $\pm 0.01^a$	0.35 $\pm 0.02^{ac}$
Ca ²⁺ ATPase ($\mu\text{mole/mg/min}$)	0.73 $\pm 0.01^a$	0.97 $\pm 0.01^b$	1.07 $\pm 0.06^b$	1.04 $\pm 0.06^b$	0.21 $\pm 0.01^c$	1.05 $\pm 0.01^c$	0.39 $\pm 0.01^c$	0.03 $\pm 0.01^d$	2.73 $\pm 0.01^d$	0.03 $\pm 0.01^d$	0.56 $\pm 0.05^e$
Na ⁺ /K ⁺ ATPase ($\mu\text{mole/mg/min}$)	1.31 $\pm 0.03^a$	1.17 $\pm 0.01^b$	0.74 $\pm 0.01^b$	0.72 $\pm 0.01^b$	1.35 $\pm 0.10^b$	1.77 $\pm 2.00^c$	1.21 $\pm 0.02^c$	1.76 $\pm 0.07^{bc}$	0.45 $\pm 0.10^b$	2.83 $\pm 0.01^d$	2.33 $\pm 0.01^d$
21 days after germination											
Total ATPase ($\mu\text{mole/mg/min}$)	2.25 $\pm 0.06^a$	2.54 $\pm 0.01^b$	2.20 $\pm 0.01^b$	1.92 $\pm 0.01^b$	2.01 $\pm 0.01^c$	2.58 $\pm 0.01^c$	2.07 $\pm 0.67^c$	3.92 $\pm 0.01^d$	2.31 $\pm 0.01^d$	2.12 $\pm 0.02^d$	3.58 $\pm 0.29^e$
Mg ²⁺ ATPase ($\mu\text{mole/mg/min}$)	0.42 $\pm 0.01^a$	0.16 $\pm 0.01^b$	0.20 $\pm 0.01^b$	0.60 $\pm 0.01^b$	0.22 $\pm 0.02^c$	0.34 $\pm 0.03^c$	0.34 $\pm 0.03^c$	0.11 $\pm 0.01^d$	0.23 $\pm 0.01^d$	0.50 $\pm 0.03^d$	0.48 $\pm 0.36^e$
Ca ²⁺ ATPase	0.73 $\pm 0.01^a$	1.05 $\pm 0.07^b$	1.22 $\pm 0.01^b$	0.72 $\pm 0.01^a$	0.30 $\pm 0.01^c$	0.58 $\pm 0.01^c$	0.29 $\pm 0.01^b$	0.12 $\pm 0.01^d$	0.40 $\pm 0.01^d$	0.40 $\pm 0.04^c$	0.42 $\pm 0.58^e$

(μ mole/mg/ min)											
Na ⁺ /K ⁺ ATPase (μ mole/mg/ min)	2.10 $\pm 0.01^a$	1.32 $\pm 0.07^b$	0.79 $\pm 0.01^b$	0.59 $\pm 0.01^b$	1.76 $\pm 0.01^c$	1.60 $\pm 0.02^c$	0.45 $\pm 0.02^c$	3.66 $\pm 0.30^d$	1.68 $\pm 0.02^d$	1.22 $\pm 0.01^d$	2.68 $\pm 0.12^e$

Values are means of 4 determinations $\pm$ S.E.M. Means carrying different notations are statistically different at $P < 0.01$.

ATPase = Adenosine triphosphatase activity is expressed as μ mole of free phosphate (Pi) released/min/mg protein.

4. CONCLUSION

Changes in some physical and biochemical parameters of plant growth occurred in crude oil contamination with the changes dependent on the degree and duration of contamination. The contamination employed in this study evoked deleterious changes compared with a spilled site in Ubeji and maize was less susceptible to crude oil and its fractions than beans.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

The authors were involved in all aspects of this study.

CONSENT (WHERE EVER APPLICABLE)

Not applicable

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

Not applicable

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Comment [ECS44]: Give summary/comparison of effect of the different crude oil contaminated soil on ATPase activities on radicle of maize and beans here. Your explanation is vague and defeats an otherwise nice experiment due to poor presentation of research finding.

Comment [ECS45]: Replace with Researchers

Comment [ECS46]: Refer to author's instruction and make sure all your references are in accord with the standards stated by this journal.

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DEFINITIONS, ACRONYMS, ABBREVIATIONS

APPENDIX

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