

Research paper

ADENOSINE TRIPHOSPHATASE ACTIVITIES OF ZEA MAYS AND VIGNA UNGUICULATA EXPOSED TO DIFFERENT CRUDE OIL FRACTIONS

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ABSTRACT

This study was carried out to investigate the activities of adenosine triphosphatase in maize and cowpea exposed to crude oil contaminated soil. Total adenosine triphosphatase, Calcium adenosine triphosphatase, sodium/potassium adenosine triphosphatase and Magnesium adenosine triphosphatase activities were evaluated in various crude oil fractions. Cowpea and maize seeds were planted outside in polythene bags in the department of Biochemistry, Faculty of life sciences laboratory garden. Adenosine triphosphatase (ATPase) assay was carried out in the department of Biochemistry and School of basic medical sciences in university of Benin City, Nigeria. The experiment lasted for a period of five months. A total of 630 bags, each containing 500g of sandy loam soil, with no known history of crude oil contamination was used for this study. 300 bags were used for planting test samples of 2%, 5% 10% crude oil fraction while 30 bags served as control for planting cowpea and maize respectively. 30 bags containing 500g of soil were collected from a crude oil contaminated site in Ubeji, Delta State, Nigeria.

The seedlings were harvested after 7, 14, and 21 days post germination and the activities of adenosine triphosphatase determined. The data obtained were subjected to descriptive statistic of variance analysis.

The activities of total ATPase, Ca²⁺-ATPase, Mg²⁺-ATPase, and Na⁺/K⁺-ATPase had significant increase (P<.01) in cowpea compared to control in all the fractions of crude oil but total ATPase, Ca²⁺-ATPase, Mg²⁺-ATPase, and Na⁺/K⁺-ATPase in maize had some significant increase and some significant decrease (P<.01) when compared to control with increased percentage crude oil contamination. The study indicated that crude oil fraction affects the activities of adenosine triphosphatase.

Keywords: Crude oil, Maize, Growth parameters, Oxidative stress, Contamination

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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 phenanthrenes and up to 14 saturated hydrocarbons ranging from C14 to paraffins [2]. Nigeria is an established crude oil exporting nation and it forms the mainstay of its economy [3]. Various activities in crude oil 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, 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.

Cowpea (*Vigna unguiculata*), a typical legume is, on the other hand, 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 warming, increasing economic crisis and high dependence on plant such as cowpea and maize to generate income and meet the protein and carbohydrate need of humans and animals, it becomes 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, and number of leaves. Also, maize has been displayed as a variable stress tolerant plant under environmental extremities ranging from drought or heavy metals [15]. Recently, Olubodun and Eriyamremu [16] examined the effect of different crude oil fraction on superoxide dismutase and catalase activity and lipid peroxidation of maize radicle. A wide range of adenosine triphosphatases (ATPases) is localized in biological systems [17, 18] and there is lack of information on the effects of crude oil on such biochemical parameters (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 hydrocarbons, particularly aromatics such as toluene [19]. Also, other researchers considered naphthalene as a more important source of crude oil toxicity than low molecular weight aromatics [2].

According to another source, low boiling point unsaturated hydrocarbons such as benzene, toluene, xylene and naphthalene, are the most toxic components in crude oils [2,19]. This prompted the research to investigate the effects of crude oil and its fractions on the activities of adenosine triphosphatase in cowpea and maize radicle to make a comparison in both plants on crude oil contaminated 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 as well as soil where there was crude oil contamination was collected from Ubeji, Delta State, Nigeria (same geographic region as the study site).

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. Holes were dug at five different points within the land to a 20cm depth each using plastic spade. Also, soil from the same community where there was crude oil spillage from a Bonny light crude oil pipe line (referred to as Ubeji) was collected and used for planting. The soil samples were 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 contained 500g soil and another 30 bags containing the soil collected from crude oil spillage site as shown below in table 1:

Table 1: Concentration of Bonny light crude oil contamination in soil

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

2.3 Plant Materials

Maize (*Zea mays*) and cowpea (*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.

112 2.4 Crude oil and fractionation

113 Crude oil (Bonny light, ⁰API (American Petroleum Institute) gravity =37) was obtained
114 from Warri Refinery and Petrochemical Company Delta State, Nigeria. A portion of the
115 crude oil was fractionated by the method of Anderson *et al.* [2] into water soluble fraction
116 (WSF) and water insoluble fraction (WIF). For the fractionation, a 1:2 dilution of 200 ml of
117 crude oil was put in a 1 litre conical flask and constantly stirred with a magnetic stirrer for
118 48h. The WSF then separated from the WIF in a separating funnel.

119 2.5 Soil treatment

120 The composite soils were treated with either distilled water (control); whole crude (WC),
121 water soluble fraction (WSF) of the crude oil; or with the water insoluble fractions (WIF) of
122 the crude oil in the laboratory. The soil in the bags contaminated with whole crude (WC),
123 water soluble fraction (WSF), and water insoluble fractions (WIF) were mixed
124 thoroughly in their respective polythene bags containing 500g top soil with the aid of a
125 plastic spade. Soil of 500g was treated with 10ml, 25ml and 50ml of crude oil to obtain 2, 5
126 and 10% v/w crude oil contamination.

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

130 2.6 Planting of seeds and germination studies

131 The seeds were planted by a modified version of Vavrek and Campbell [20]. Three seeds
132 of each plant were sown in each bag of soil with a depth of about 1-2 cm. The time and
133 number of seeds that germinated from each bag were noted and the percentage
134 germination in each treatment was calculated using the formula:

135 Percentage germination = $\frac{\text{number of seeds that germinated}}{\text{number of seeds sown}} \times 100$
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137 2.7 Biochemical assays

138 2.7.1 Isolation of crude mitochondria

139 A modification of Douce *et al.* [21] was used for the isolation of mitochondria from the
140 radicle of the germinating maize and cowpea seedling. The radicle was homogenized in
141 ice-cold extraction medium (Sucrose 0.25 M, EDTA 5 mM, EGTA 1 mM, dithioerythritol
142 1 mM, BSA 0.1%, polyclar-AT 0.6%, in HEPES-TRIS 10 mM pH.7.4). The homogenate
143 was filtered with a clean white cloth and the mitochondria immediately separated from
144 the cytoplasmic fraction by centrifugation at 15,000g for 10 min. The resulting crude
145 mitochondrial pellet was resuspended in a solution (Sucrose 0.25 M, EDTA 5 mM, EGTA
146 1 mM, BSA 0.1% in HEPES-TRIS 10 mM, pH.7.4) and centrifuged at 600g for 5 minutes to
147 remove nuclei and heavy cell debris. This washing procedure was repeated twice. The
148 washed crude mitochondrial pellet were resuspended in another solution (Sucrose 0.25 M,
149 EGTA 30 mM, in HEPES-TRIS 10 mM, pH.7.4) and stored in ice. The samples obtained
150 were subsequently used for the determination of ATPases.

151 2.7.2 Measurement of ATPase

152 ATPase was measured according to the method of Matsukawa and Takiguchi [22]. Activity
153 of ATPase was determined by measuring the amount of inorganic phosphate liberated
154 following incubation with 25 mM disodium ATP. The inorganic phosphate liberated was
155 estimated according to the method of Fiske and Subarrow [23]. The reaction mixture (final

volume 2 ml, including 0.1 ml enzyme extract) for the assay of total ATPase activity contained tris-HCl buffer, pH 7.4, 50 mM; MgCl_2 , 5 mM; NaCl, 100 mM; KCl, 20 mM; ATP, 5 mM. For Mg^{2+} -ATPase assay the mixture was as above, but without NaCl and KCl. The reaction was initiated by the addition of the enzyme extract. The mixture was incubated for 10 min, and terminated by addition of 2 ml cold 10% trichloroacetic acid (TCA). After centrifugation at 1000 rpm for 10 min, the P_i liberated from ATP was determined. Na^+/K^+ -ATPase activity, was calculated by subtracting Mg^{2+} -ATPase activity from total ATPase activity, and expressed as micromole of free phosphate (P_i) released min^{-1} (mg protein^{-1}). The standard reaction mixture for Ca^{2+} -ATPase in a final volume of 2 ml contained 50 mM tris-HCl buffer, pH 7.4; 3 mM CaCl_2 , 3 mM ATP; and 0.1 ml of the appropriate enzyme solution. The experimental protocol was as reported for Na^+/K^+ -ATPase and Mg^{2+} -ATPase activities and was expressed.

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 were analytical grades (BDH or Sigma). All glasses and plastics used were acid-washed. Buck Scientific standard solutions (Buck Scientific Inc.) were used to calibrate the Spectrophotometer. Procedural blank samples were subjected to similar extraction method using the same amounts of reagents.

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 $P < .01$ 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 cowpea

There were increased ATPase activities in almost all the values obtained (table 2) as the percentage contamination of the crude oil and its fractions increased in cowpea, and this was found to be significant ($p < .01$) between (2-10%) relative to the control value. As the plants grew, the ATPase activity also increased. The activated Ca^{2+} -ATPase activity will lead to increasing 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 [24]. This increased influx of Ca^{2+} helped to cushion the damaging effect of the slight increase in lipid peroxidation observed during the study (result not shown) [16]. The increased Mg^{2+} ATPase may cause increased influx of Mg^{2+} ion into the plant. The increased 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 [26,27].

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

coupled to ATP hydrolysis, and generates electrochemical gradients across the cell membrane [17, 18, 27].

K⁺ ion has been reported to increase carbohydrate metabolism by translocating sugars, help to increase stomata opening, regulate the water in the plant cell by preventing loss of water that may physiologically dry the plant [24].

The significant increase in ATPase activity in the values obtained as the concentrations of the crude oil increased in cowpea relative to the control value may be a result of increasing concentrations of Mg²⁺ and ATP complex (Mg-ATP) and decreasing concentrations of ATP, or activation by Mg²⁺ and monovalent cations (K⁺ > Rb⁺ > Cs⁺ > Na⁺ > Li⁺) [25]. The non-significant decrease in ATPase activity observed at some concentrations, for example cowpea on 7DPG at 2% concentration, magnesium ATPase (Mg²⁺ ATPase) activity of WIF(0.15) and calcium ATPase (Ca²⁺ ATPase) activity of WC (0.05) relative to the control (0.17) (Table 2) may be due to the presence of divalent cation(s) other than Mg²⁺ which could either partially substitute for Mg²⁺ (i.e Mn²⁺ and Co²⁺), be ineffective or inhibitory (i.e Ni²⁺, Zn²⁺, and Ca²⁺) to the enzyme and thus prevent maximum stimulation by monovalent cations (K⁺ > Rb⁺ > Cs⁺ > Na⁺ > Li⁺) or a decrease/increase in optimum conditions of pH and temperature for maximum activity or the presence 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 [26].

The significant inhibition of sodium/potassium ATPase (Na⁺/K⁺ ATPase) activity of WIF (0.01) on 21DPG (Table 2), may be a result of inhibition by Ca²⁺. The inhibition by Ca²⁺ of K⁺-ATPase of oat roots suggested by Balke *et al.* [28] 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 Ca²⁺ competes at K⁺-sites on the ATPase to inhibit K⁺ influx. The inhibitory effects of Ca²⁺ on K⁺-ATPase at relatively low concentration as observed by Leonard and Hotchkiss [26] suggested that levels of free Ca²⁺ in cells were low, and that Ca²⁺ concentrations in the cytoplasm could have a regulatory effect on ion transport rates [29, 30] Ca²⁺ may inhibit ATPase by interfering with the ATP Mg-enzyme complex which in turn prevents Mg²⁺ translocation, which may lead to its deficiency in the plant.

At 5% contamination, magnesium ATPase (Mg²⁺ ATPase) activity of WC (0.11, 0.12 and 0.13 respectively) was significantly inhibited on 7, 14 and 21 DPG relative to control (0.17, 0.21 and 0.22 respectively) (Table 2). This inhibition may be 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 Mg²⁺ stimulated ATPase to account for the Mg²⁺ that needs to be absorbed in the plant roots [28]. There will be reduced absorption of Mg²⁺ 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 7 DPG relative to control (0.17) (Table 2). The result of this study is similar to the findings of Senthil-Nathan *et al.* [31] who observed significant inhibition of ATPase activity in different animal species exposed to different contaminants. Muzzanti *et al.* [32] reported that reduction in Na⁺ +K⁺-ATPase is a reflection of the

250 qualitative alterations of the phospholipid components of cells. Rauchova *et al.* [33]
251 established that changes in membrane microviscosity can affect kinetic characteristics of
252 membrane-bound enzymes. It is possible that the decreased ATPase activities may be
253 responsible for the slight increase in lipid peroxidation observed in the study (results not
254 shown) [16].

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303 **Table 2: Effects of crude oil and its fractions on adenosine triphosphatase activities of cowpea radicle**

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	Control	Whole crude			Water soluble fraction			Water insol	
		2%	5%	10%	2%	5%	10%	2%	5%
Number of seeds planted	100	100	100	100	100	100	100	100	100
% germination	100	60	47	37	72	59	42	56	40
7 days after germination									
Total ATPase (μmole/mg/min)	0.40 ±0.10 ^a	0.89 ±0.09 ^b	0.58 ±0.12 ^b	3.25 ±0.18 ^b	1.41 ±0.23 ^c	2.01 ±0.23 ^c	0.70 ±0.13 ^c	1.99 ±0.87 ^d	2.01 ±0.13 ^c
Mg ²⁺ ATPase (μmole/mg/min)	0.17 ±0.15 ^a	0.59 ±0.15 ^b	0.11 ±0.24 ^b	0.53 ±0.15 ^b	0.68 ±0.24 ^c	1.02 ±0.20 ^c	0.21 ±0.15 ^c	0.15 ±0.63 ^a	0.15 ±0.15 ^a
Ca ²⁺ ATPase (μmole/mg/min)	0.06 ±0.21 ^a	0.05 ±0.18 ^a	0.10 ±0.15 ^b	0.95 ±0.30 ^b	0.16 ±0.09 ^b	0.64 ±0.12 ^c	0.37 ±0.12 ^c	0.09 ±0.10 ^c	0.09 ±0.10 ^c
Na ⁺ /K ⁺ ATPase (μmole/mg/min)	0.17 ±0.12 ^a	0.23 ±0.24 ^b	0.37 ±0.09 ^b	1.78 ±0.09 ^b	0.55 ±0.24 ^c	0.35 ±0.21 ^c	0.14 ±0.26 ^c	1.75 ±0.06 ^d	1.75 ±0.06 ^d
14 days after germination									
Total ATPase (μmole/mg/min)	0.45 ±0.23 ^a	1.09 ±0.10 ^b	0.63 ±0.31 ^b	3.47 ±0.21 ^b	1.14 ±0.20 ^c	6.52 ±0.09 ^c	1.62 ±0.27 ^c	5.86 ±0.10 ^d	4.86 ±0.10 ^d
Mg ²⁺ ATPase (μmole/mg/min)	0.21 ±0.15 ^a	0.66 ±0.40 ^b	0.12 ±0.44 ^b	1.06 ±0.12 ^b	0.43 ±0.40 ^c	0.98 ±0.15 ^c	0.48 ±0.35 ^c	0.74 ±0.10 ^d	2.14 ±0.10 ^d
Ca ²⁺ ATPase	0.03 ±0.06 ^a	0.06 ±0.10 ^b	0.11 ±0.12 ^b	1.02 ±0.35 ^b	0.16 ±0.20 ^c	2.84 ±0.15 ^c	0.52 ±0.31 ^c	0.46 ±0.10 ^d	0.46 ±0.10 ^d
(μmole/mg/min)									
Na ⁺ /K ⁺ ATPase (μmole/mg/min)	0.21 ±0.18 ^a	0.37 ±0.1 ^b	0.39 ±0.18 ^b	1.39 ±0.10 ^b	0.56 ±0.7 ^c	2.89 ±0.55 ^c	0.62 ±0.15 ^c	4.65 ±0.10 ^d	3.89 ±0.10 ^d

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21 days after germination									
Total ATPase (μmole/mg/min)	0.48 ±0.35 ^a	1.33 ±1.20 ^b	0.72 ±0.17 ^a	0.93 ±0.23 ^b	3.32 ±0.10 ^c	3.80 ±0.10 ^b	5.71 ±0.67 ^c	1.70 ±0.12 ^d	4.0 ±0.1 ^c
Mg ²⁺ ATPase (μmole/mg/min)	0.22 ±0.40 ^a	0.78 ±0.42 ^b	0.13 ±0.12 ^b	1.12 ±0.10 ^b	0.48±0.37 ^c	0.30 ±0.10 ^c	1.15±0.25 ^B	1.42 ±0.10 ^d	2.0 ±0.1 ^c
Ca ²⁺ ATPase (μmole/mg/min)	0.02 ±0.17 ^a	0.13 ±0.18 ^b	0.12 ±0.09 ^b	1.11 ±0.30 ^b	0.19 ±0.10 ^c	2.33 ±0.10 ^c	1.15 ±1.00 ^c	0.27 ±0.10 ^d	0.3 ±0.1 ^c
Na ⁺ /K ⁺ ATPase (μmole/mg/min)	0.24 ±0.19 ^a	0.54 ±0.22 ^b	0.47 ±0.30 ^b	1.70 ±0.10 ^b	2.64±0.36 ^c	1.18 ±0.10 ^c	3.33±0.17 ^c	0.01 ±0.12 ^d	1.0 ±0.1 ^c

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308 Values are means of 4 determinations ± S.E.M. Means carrying different notations are
309 statistically different at P<.01. ATPase (= Adenosine triphosphatase) activity is expressed as
310 μmole of free phosphate (Pi) released min⁻¹(mg protein)⁻¹.

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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, which decreased as the concentration of the crude oil increased relative to control values, evidences that the plant may be undergoing oxidative stress. There was significant ($p < .01$) decrease 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 an increased 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 [24]. 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 maize radicle cultivated on Ubeji crude oil spillage soil on 7, 14 and 21 DPG (2.39, 4.83, 2.68 respectively) increased significantly ($p < .01$) 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 [24]. Increased Na^+/K^+ ATPase activity means increased 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, 24].

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387 **Table 3: Effects of crude oil and its fractions on adenosine triphosphatase activities of**
388 **maize radicle**

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	Control	Whole crude			Water soluble fraction			Water insol	
		2%	5%	10%	2%	5%	10%	2%	5%
Number of seeds planted	100	100	100	100	100	100	100	100	100
% germination	100	70	54	37	86	65	42	63	47
7 days after germination									
Total ATPase (μ 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	1.5 $\pm$ 0.01 ^d
Mg ²⁺ ATPase (μ 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	0.0 $\pm$ 0.01 ^c
Ca ²⁺ ATPase (μ mole/mg/min)	0.21 $\pm$ 0.02 ^a	0.49 $\pm$ 0.10 ^b	1.03 $\pm$ 0.01 ^b	0.59 $\pm$ 0.01 ^b	0.13 $\pm$ 0.01 ^c	1.26 $\pm$ 0.01 ^c	0.36 $\pm$ 0.02 ^c	0.08 $\pm$ 0.01 ^d	0.0 $\pm$ 0.01 ^d
Na ⁺ /K ⁺ ATPase (μ 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	0.4 $\pm$ 0.01 ^b
14 days after germination									
Total ATPase (μ 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	3.2 $\pm$ 0.01 ^b
Mg ²⁺ ATPase (μ 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}	0.0 $\pm$ 0.01 ^c
Ca ²⁺ ATPase (μ 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	2.7 $\pm$ 0.01 ^d

Na ⁺ /K ⁺ ATPase (μmole/mg/ min)	1.31 ±0.03 ^a	1.17 ±0.01 ^b	0.74 ±0.01 ^b	0.72 ±0.01 ^b	1.35 ±0.10 ^b	1.77 ±2.00 ^c	1.21 ±0.02 ^c	1.76 ±0.07 ^{bc}	0.4 ±0.
21 days after germination									
Total ATPase (μmole/mg/ min)	2.25 ±0.06 ^a	2.54 ±0.01 ^b	2.20 ±0.01 ^b	1.92 ±0.01 ^b	2.01±0. 01 ^c	2.58 ±0.01 ^c	2.07 ±0.67 ^c	3.92 ±0.01 ^d	2.3 ±0.
Mg ²⁺ ATPase (μmole/mg/ min)	0.42 ±0.01 ^a	0.16 ±0.01 ^b	0.20 ±0.01 ^b	0.60 ±0.01 ^b	0.22±0. 02 ^c	0.34 ±0.03 ^c	0.34 ±0.03 ^c	0.11 ±0.01 ^d	0.2 ±0.
Ca ²⁺ ATPase (μmole/mg/ min)	0.73 ±0.01 ^a	1.05 ±0.07 ^b	1.22 ±0.01 ^b	0.72 ±0.01 ^a	0.30±0. 01 ^c	0.58 ±0.01 ^c	0.29 ±0.01 ^b	0.12 ±0.01 ^d	0.4 ±0.
Na ⁺ /K ⁺ ATPase (μmole/mg/ min)	2.10 ±0.01 ^a	1.32 ±0.07 ^b	0.79 ±0.01 ^b	0.59 ±0.01 ^b	1.76±0. 01 ^c	1.60 ±0.02 ^c	0.45 ±0.02 ^c	3.66 ±0.30 ^d	1.6 ±0.

391
392 Values are means of 4 determinations ± S.E.M. Means carrying different notations are
393 statistically different at P<.01. ATPase (= Adenosine triphosphatase) activity is expressed as
394 μmole of free phosphate (Pi) released min⁻¹ (mg protein)⁻¹.

395 4. CONCLUSION

396 The study showed increased ATPase activities (P<.01) (total ATPase, Ca²⁺-ATPase, Mg²⁺-
397 ATPase, and Na⁺/K⁺-ATPase) in cowpea compared to control in almost all the fractions of
398 crude oil as percentage contamination increased. However, there was significant decrease
399 (P<.01) in Mg²⁺-ATPase activities of cowpea radicle in WC fraction of crude oil contaminated
400 soil. In maize radicle, however, ATPase activities (total ATPase, Ca²⁺-ATPase, Mg²⁺-
401 ATPase, and Na⁺/K⁺-ATPase) did not follow a regular trend as there was significant
402 increase (P<.01) as well as significant decrease when compared to control in all the fractions
403 of crude oil as percentage contamination increased. The study, therefore, indicated that the
404 activities of adenosine triphosphatase was affected by the various crude oil fractions, an
405 indication that mitochondria energy production and viability may be reduced.

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COMPETING INTERESTS

Authors declare that no competing interests exist.

AUTHORS' CONTRIBUTIONS

The authors were involved in all aspects of this study.

CONSENT (WHEREVER APPLICABLE)

Not applicable

ETHICAL APPROVAL (WHEREVER APPLICABLE)

Not applicable

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