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Adenosine Triphosphatase Activities of *Zea Mays* and *Vigna unguiculata* Exposed to Different Crude Oil Fractions

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

This study was carried out to investigate the activities of some adenosine triphosphatases (ATPases) in the radicle of maize and cowpea grown in soils contaminated with crude oil and its fractions. Total ATPase, Ca^{2+} ATPase, Na^{+}/K^{+} ATPase and Mg^{2+} ATPase activities were evaluated. Cowpea and maize seeds were planted in polythene bags containing 500g of sandy loam soil each. The soils used had no known history of crude oil contamination and the study was carried out in the Department of Biochemistry, University of Benin garden and laboratory. A total of 660 bags were used in the study. Of these, 60 bags were used for planting maize and cowpea (30 bags for each plant) in soils not contaminated, which served as controls for the plants and another 60 bags with soils from Ubeji (a crude oil contaminated site in, Delta State, Nigeria) was planted maize or cowpea (30 for each plant). The remaining 540 bags had soils that were contaminated with 2%, 5%, or 10% whole crude oil or its water soluble or water insoluble fraction, and in it were planted either maize or cowpea and they served as the test groups. The experiment lasted for a period of one month. Seedlings in 10 bags from each group of 30 bags were harvested after 7, 14, or 21 days post germination and the activities of ATPases were determined. The data obtained were subjected to descriptive statistic of variance analysis.

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In a general sense, contamination of soils with crude oil or its fractions or in Ubeji significantly increased (P<.01) the activities of total ATPase, Ca^{2+}-, Mg^{2+}-, and Na^{+}/K^{+}-ATPases of cowpea compared with the uncontaminated control. In maize the effect of crude oil was mixed however Mg^{2+}- ATPases was consistently reduced with crude oil contamination. The study indicated that crude oil and its fraction affects the activities of adenosine triphosphatases in ways which are species related.

Keywords: Crude oil; maize; growth parameters; oxidative stress; 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 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 [6-8]. 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 hydrolysis 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*) on the other hand is a typical legume, one of the major sources 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]. Most studies of the effects of crude oil in plants grown commonly in this area of Nigeria are mainly on physiological parameters ranging from percentage germination to plant height, and number of leaves. 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. MATERIALS 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 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 contained 500g soil and another 30 bags containing the soil collected from crude oil spillage site as shown below in Table 1.

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.

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

2.4 Crude oil and Fractionation

Bonny Light Crude Oil, oAPI (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 200 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.

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 cowpea 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.

2.6 Planting of Seeds and Germination Studies

The seeds were planted by a modified version of Vavrek and Campbell [20]. Three seeds of each plant were sown in each bag of soil with a depth of about 1-2cm. 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:

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

2.7 Biochemical Assays

2.7.1 Isolation of crude mitochondria

A modification of Douce et al. [21] was used for the isolation of mitochondria from the radicle of the germinating maize and cowpea seedling. The radicle was homogenized in ice-cold extraction medium (Sucrose 0.25 M, 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 10 min. 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 0.25 M, EGTA 30mM, in HEPES-TRIS 10mM, pH.7.4) and stored in ice. The samples obtained were subsequently used for the determination of ATPases.

2.7.2 Measurement of ATPase

ATPase was measured according to the method of Matsukawa and Takiguchi [22]. Activity of ATPase was determined 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 [23]. The reaction mixture (final volume 2ml, including 0:1ml enzyme extract) for the assay of total ATPase activity contained tris-HCl buffer, pH 7.4, 50mM; $MgCl_2$, 5mM; NaCl, 100mM; KCl, 20mM; ATP, 5mM. 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 Pi 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 (Pi) released min^{-1} (mg protein)$^{-1}$. The standard reaction mixture for Ca^{2+}-ATPase in a final volume of 2 ml contained 50mM tris-HCl buffer, pH 7.4; 3mM $CaCl_2$, 3mM 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 ± 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 Ca2+-ATPase activity will lead to increasing Ca2+ ion absorption or influx into the plant. Ca2+ 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 Ca2+ would help to cushion the damaging effect of the slight increase in lipid peroxidation observed during the study (result not shown) [16].

The increased Mg2+ ATPase may cause increased influx of Mg2+ ion into the plant. Magnesium ions are closely related to energy metabolism. Thus 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 [25,26].

The increased activity of Na+/K+ ATPase may have been a result of increased influx of Mg2+ 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 [17,18,26]. 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+) [27]. 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 [25].

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

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 (Ca2+ 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 above).

Table 2. Effects of crude oil and its fractions on adenosine triphosphatase activities of cowpea radicle

	Control	Whole crude			Water soluble fraction			Water insoluble fraction			Ubeji THC (74.88mg/kg)
		2%	5%	10%	2%	5%	10%	2%	5%	10%	
Number of seedsplanted	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											
TotalATPase (µmole/mg/ min)	0.40±0.10a	0.89 ±0.09b	0.58±0.12b	3.25±0.18b	1.41±0 .23c	2.01±0.23c	0.70±0.13c	1.99±0.87d	2.06±0.07d	4. 08±0.15d	3.98±0.17e
Mg2+ATPase (µmole/mg/ min)	0.17±0.15a	0.59 ±0.15b	0.11±0.24b	0.53±0.15b	0.68±0 .24c	1.02±0.20c	0.21±0.15c	0.15±0.63a	0.28±0.10d	0. 32±0.10d	1.38±0.09d
Ca2+ATPase (µmole/mg/ min)	0.06±0.21a	0.05 ±0.18a	0.10±0.15b	0.95±0.30b	0.16±0 .09b	0.64±0.12c	0.37±0.12c	0.09±0.10c	0.08±0.10d	0. 26±0.10d	0.33±0.29d
Na+/K+ATPase (µmole/mg/ min)	0.17±0.12a	0.23 ±0.24b	0.37±0.09b	1.78±0.09b	0.55±0 .24c	0.35±0.21c	0.14±0.26c	1.75±0.06d	1.71±0.16d	3. 44±1.00d	2.27±0.35e
14 days after germination											
TotalATPase (µmole/mg/min)	0.45±0.23a	1.09±0.10b	0.63±0.31b	3.47±0.21b	1.41±0. 23c	2.01±0.23c	0.70±0.13c	1.99±0.87d	2.06±0.07d	4.0 8±0.15d	3.98±0.17e
Mg2+ATPase (µmole/mg/ min)	0.21±0.15a	0.66±0.40b	0.12±0.44b	1.06±0.12b	0.43±0. 40c	0.98±0.15c	0.48±0.35c	0.74±0.10d	2.56±0.10d	0.5 2±0.10d	1.61±0.31e
Ca2+ATPase (µmole/mg/min)	0.03±0.06a	0.06±0.10b	0.11±0.12b	1.02±0.35b	0.16±0. 20c	2.84±0.15c	0.52±0.31c	0.46±0.10d	0.22±0.10d	0.5 6±0.10d	1.50±0.29e
Na+/K+ ATPase (µmole/mg/min)	0.21±0.18a	0.37±0.1b	0.39±0.18b	1.39±0.10b	0.56±0.7 c	2.89±0.55c	0.62±0.15c	4.65±0.10d	3.59±0.10d	6.36± 0.52d	1.04±0.06e
21 days after germination											
TotalATPase (µmole/mg/ min)	0.48±0.35a	1.33±1.20b	0.72±0.17a	0.93±0.23b	3.32±0. 10c	3.80±0.10b	5.71±0.67c	1.70±0.12d	4.86±0.29c	7.7 1±1.10d	0.59±0.29e
Mg2+ATPase (µmole/mg/ min)	0.22±0.40a	0.78±0.42b	0.13±0.12b	1.12±0.10b	0.48±0. 37c	0.30±0.10c	1.15±0.2B	1.42±0.10d	2.64±0.26d	0.57 ±0.19c	0.48±0.36b
Ca2+ATPase (µmole/mg/ min)	0.02±0.17a	0.13±0.18b	0.12±0.09b	1.11±0.30b	0.19±0. 10c	2.33±0.10c	1.15±1.00c	0.27±0.10d	0.32±0.10d	0.6 1±0.47d	0.42±0.58e
Na+/K+ATPase (µmole/mg/ min)	0.24±0.19a	0.54±0.22b	0.47±0.30b	1.70±0.10b	2.64±0. 36c	1.18±0.10c	3.33±0.17c	0.01±0.12d	1.89±0.17d	6.6 4±0.17d	2.68±0.12e

Values are means of 4 determinations ± S.E.M. Means carrying different notations are statistically different at P<.01. ATPase (= Adenosine triphosphatase) activity is expressed as µmole of free phosphate (Pi) released min^{-1} $(mg\ protein)^{-1}$

Table 3. Effects of crude oil and its fractions on adenosine triphosphatase activities of maize radicle

	Control	Whole crude			Water soluble fraction			Water insoluble fraction			Ubeji THC (74.88mg/kg
		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 (µmole/mg/ min)	1.30 ±0.02a	2.30±0.01b	2.05±0.01b	2.34±0.01b	1.49±0.01c	1.75±0.01c	1.48±0 .04c	1.81±0.07d	1.52±0.03d	1.25±0.03d	3.19±0.04e
Mg2+ATPase (µmole/mg/ min)	0.77 ±0.10a	0.26±0.04b	0.55±0.01b	0.54±0.02b	0.30±0.09b	0.05±0.02c	0.06±0.0 1c	0.28±0.01c	0.05±0.03c	0.01±0.01d	1.00±0.01d
Ca2+ATPase (µmole/mg/ min)	0.21±0.02[a]	0.49±0.10[b]	1.03±0.01[b]	0.59±0.01[b]	0.13±0.01[c]	1.26±0.01[c]	0.36±0.02[c]	0.08±0.01[d]	0.08±0.01[d]	0.03±0.01[d]	0.39±0.01[e]
Na+/K+ATPase (µmole/mg/ min)	0.32 ±0.60a	1.54±0.06b	0.47±0.01b	1.21±0.01a	1.06±0.02c	0.45±0.02b	1.06±0 .02a	1.46±0.02b	0.43 ±1.9b	1.21±0.03a	1.80±0.10 d
14 days after germination											
Total ATPase (µmole/mg/min)	2.34±0.70a	2.39±0.01a	2.12±0.01b	2.81±0.01b	1.91±0.01b	3.16±0.01c	1.84±0.0 6c	1.89±0.01b	3.21±0.01c	3.06±0.01d	3.24±0.60 c
Mg2+ATPase (µmole/mg/ min)	0.30±0.75a	0.25±0.01a	0.31±0.01a	1.05±0.01b	0.35±0.0a	0.34±0.01a	0.25±0.01a	0.20±.30ab	0.05±0.01b	0.20±0.01a	0.35±.02 ac
Ca2+ATPase (µmole/mg/ min)	0.73±0.01a	0.97±0.01b	1.07±0.06b	1.04±0.06b	0.21±0.01c	1.05±0.01c	0.39±0.01 c	0.03±0.01d	2.73±0.01d	0.03±0.01d	0.56±0.05 e
Na+/K+ATPase(µmole/mg/min)	1.31±0.03a	1.17±0.01b	0.74±0.01b	0.72±0.01b	1.35±0.10 b	1.77±2.00c	1.21±0.0 2c	1.76±0.07bc	0.45±0.10b	2.83±0.01d	2.33±0.01d
21 days after germination											
Total ATPase (µmole/mg/min)	2.25±0.06a	2.54±0.01b	2.20±0.01b	1.92±0.01b	2.01±0.01c	2.58±0.01c	2.07±0.6 7c	3.92±0.01d	2.31±0.01d	2.12±0.02d	3.58±0.29e
Mg2+ATPase (µmole/mg/ min)	0.42±0.01a	0.16±0.01b	0.20±0.01b	0.60±0.01b	0.22±0.02c	0.34±0.03c	0.34±0.03 c	0.11±0.01d	0.23±0.01d	0.50±0.03d	0.48±0.36e
Ca2+ATPase (µmole/mg/ min)	0.73±0.01a	1.05±0.07b	1.22±0.01b	0.72±0.01a	0.30±0.01c	0.58±0.01c	0.29±0.01 b	0.12±0.01d	0.40±0.01d	0.40±0.04c	0.42±0.58e
Na+/K+ATPase (µmole/mg/min)	2.10±0.01a	1.32±0.07b	0.79±0.01b	0.59±0.01b	1.76±0.01 c	1.60±0.02c	0.45±0. 02c	3.66±0.30d	1.68±0.02d	1.22±0.01d	2.68±0.12e

Values are means of 4 determinations ± S.E.M. Means carrying different notations are statistically different at P<.01. ATPase (= Adenosine triphosphatase) activity is expressed as µmole of free phosphate (Pi) released min^{-1} $(mg\ protein)^{-1}$

The increased or activated Ca2+-ATPase activity may lead to an increased Ca2+ ion absorption or influx into the plant since Ca2+ 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 Ca2+ 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 above). 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].

4. CONCLUSION

The study showed increased ATPase activities ($P<.01$) (total ATPase, Ca^{2+}-ATPase, Mg^{2+}-ATPase, and Na^{+}/K^{+}-ATPase) in cowpea compared to control in almost all the fractions of crude oil as percentage contamination increased. However, there was significant decrease ($P<.01$) in Mg^{2+}-ATPase activities of cowpea radicle in WC fraction of crude oil contaminated soil. In maize radicle, however, ATPase activities (total ATPase, Ca^{2+}-ATPase, Mg^{2+}-ATPase, and Na^{+}/K^{+}-ATPase) did not follow a regular trend as there was significant increase ($P<.01$) as well as significant decrease when compared to control in all the fractions of crude oil as percentage contamination increased. The study, therefore, indicated that the activities of adenosine triphosphatase was affected by the various crude oil fractions, an indication that mitochondria energy production and viability may be affected and that crude oil and its fraction affects the activities of adenosine triphosphatases in ways which are species related.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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