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Activity of Lactate Dehydrogenase and the Levels of Glucose and Starch in *Vigna unguiculata* Seedlings Exposed to Different Fractions of Crude Oil

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ABSTRACT: The study examined lactate dehydrogenase activity and the levels of glucose and starch in *Vigna unguiculata* seedlings exposed to Bonny Light whole crude oil (WC), its water soluble fraction (WSF) and water insoluble fraction (WIF). An *in situ* experiment was conducted with 500g sandy loam soil (0%) which served as control, and other bags of sandy loam soil which were mixed with different fractions of crude oil to give 2%, 5% and 10% contamination. Another sets of 500g filled bags of sandy loam soil were collected from a crude oil spill site at Ubeji community in Niger Delta. Three seeds of *V. unguiculata* were planted in each 500g soil bags and their roots recovered for analysis after 7, 14 and 21 days post germination. Lactate dehydrogenase (LDH) activity of the root had 46%, 57% and 60% increase in the 2%, 5% and 10% crude oil contaminated soil indicating a dose dependent increase when compared with the control. Glucose content also increased in the root of the contaminated soil from a range of 33% to 92% in the 2% contaminated soil and 33% to 94% in the 5% and 10% crude oil contaminated soil respectively. The WIF indicates the highest increase in both LDH activity and glucose content. However, starch content decreased significantly within the range of 13% to 67%. The WIF of 10% crude oil contamination having the highest decrease of 67% 21 days post germination (DPG) while the least decrease was observed in the root of 2% WC, 21 DPG. The result of the study suggests that the reduction in percentage germination and growth may be due to the decrease observed in starch content. The high LDH activity and high glucose content observed may indicate that high LDH activity is connected with increased energy demand to enable the seeds overcome the stress of germination and crude oil toxicity.

Key words: Crude oil, *Vigna unguiculata*, lactate dehydrogenase (LDH), glucose, starch.

Introduction

The Niger Delta area have been polluted over time from crude oil exploration and exploitation and the main stay of the population is agriculture and fishing and these water bodies provide drinking water for man and animals. There is the recent drive by the Federal government to clean up lands that have been polluted with crude oil over time. The cost of this cleanup of crude oil is quite enormous. The constituents of the contaminants are such that some of them can remain in soil for a long time. For example, trace metals are non-biodegradable. Repeated pollution of the land (soil/water) may cause an increase of some

of the non-biodegradable contaminants which will have adverse effect on plants, animals and humans as they can accumulate in the trophic chain (Azmat *et al*. 2013).

Several studies have shown that crude oil can affect crop plants because of some toxic metals like Cd, Ni, Zn and Pb (Singh and Agrawal, 2010) present as its constituents as well as its hydrophobic nature (Olubodun and Eriyamremu, 2013).

A major study in seedling development is the mobilization of complex polymers such as starch, proteins and lipids from storage tissues such as endosperm or cotyledons. These compounds, considered as seed reserves, are used as energy sources and building blocks for seedling growth during germination (Delouche, 2016; Pennsylvania Certified Organic, 2011).

Starch $(C_6H_{12}O_5)n$ is formed from α-D-gluco-pyranose with a high polymerization degree. It is present in all the vegetative plant organs in small quantity but high in granulated form in the grain caryopsis, seeds, tubers and in wooden stems endoderm (Lopez *et al*, 2014).

Soluble sugars are a universal component of most living organisms and a fundamental building block in biosynthesis. It is derived from carbon dioxide during the dark reaction of photosynthesis and represents transport and storage units of conserved energy. As such, it is utilized as precursors for developmental processes and numerous compounds required to maintain plant health and as signaling molecules for stress responses (Poschet *et al*., 2011).

Sucrose and glucose are the most prominent soluble sugars in many plants and function in nutrition, signaling and osmoregulation. To sustain developmental processes in non-green tissues, plants have to distribute these sugars through long-distance transport directly in the form of sucrose (Dinant and Lemoine, 2010) or as glucose and fructose. A localized change in quantity and form of carbohydrates is often associated with plant's responses to stress. These changes may be due to increased or decreased sugar biosynthesis, conversion of starch or other storage forms to soluble sugars, breakdown of cell wall polysaccharides, and/or changes in the rate of sugar transport. Depending on the speed of onset of stress, plant tissues can exhibit rapid and very site-specific shifts in their soluble carbohydrate pool (Xu *et al*., 2012).

When crude oil gets into the soil, the air dissolved in soil water is reduced because of the slow diffusion rate of oxygen in water and its limited solubility. This results in hypoxia. Under low oxygen conditions, sugar metabolism and fermentation pathway are triggered (Hagman and Piškur, 2015: Fadaka *et al*, 2017) as an adaptive phenomenon to maintain the energy of ATP and growing of the plant (Xu *et al*., 2012). The fermentation metabolism pathway produces enzymes one of which is lactate dehydrogenase (LDH, EC 1.1.1.27) (Xu *et al*., 2012).

Leaf L-lactate dehydrogenase was proposed as a constituent of the systems regulating the cellular pH and/or controlling the concentration of reducing equivalents in the cytoplasm of leaf cells (Line *et al*, 2010; An *et al*, 2017; Voon *et al*, 2018). Lactate dehydrogenase is an enzyme that catalyzes pyruvate and lactate with concomitant interconversion of NADH and NAD^+. At elevated concentrations of lactate, the enzyme exhibits feedback inhibition, and the rate of conversion of pyruvate to lactate is decreased.
Photosynthesis at the root level is affected by crude oil. This is because it increases the acidity of the soils and prevents the plants from taking up nutrients, thus slowing the process of photosynthesis and the overall growth of the plant (Olubodun and Eriyamremu, 2013).

The aim of the study was to assess the effect of different fractions of crude oil on lactate dehydrogenase (LDH) activity and the levels of glucose and starch in the root of *Vigna unguiculata* seedlings.

Material and Methods

Study location

The study location was the University of Benin, Benin City, Edo State, Nigeria. Aggregate soil from an uncultivated land in Edo State was used for the study. There has been no record of crude oil contamination in the uncultivated land where the soil was collected. The study location is characterized

by tropical equatorial climate with mean annual temperature and annual rainfall of about 2673.8 mm (Akpoborie *et al*, 2014; Iserhien-Emekeme *et al*, 2017).

Soil Sampling and treatment

Holes were dug with plastic spade at five different points to a depth of 0–15cm in an uncultivated land with no history of crude oil contamination in Edo State. The sandy-loam 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. 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 and mixed thoroughly in their respective polythene bags. Soil of 500g was treated with 10ml, 25ml and 50ml of crude oil to obtain 2, 5 and 10% v/w crude oil contamination (Table 1). The same procedure for the uncontaminated soil was use to collect the Ubeji soil that had crude oil contamination due to spillage and weighed into 500g.

In each bag, three (3) viable bean seeds were planted. Equal amounts of seeds that germinated were harvested at day7, day 14 and day 21 and their root taken for analysis.

Plant Materials

Bean (*Vigna unguiculata*) seeds were bought from a local market in Benin City, Edo State, Nigeria. The Department of Crop Science, University of Benin, identified it as ITA 189 - 288 cultivar.

Crude oil and fractionation

The crude oil known as 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.* (1979) 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 was then separated from the WIF in a separating funnel.

Biochemical Assays

Enzyme extraction

Lactate dehydrogenase (LDH) extraction was done on ice. LDH was extracted by homogenizing **(**0.5 g) of the root in 0.1 mol/L phosphate buffer (pH 7.0) containing 4 µmol/L NADH and 0.24 mmol/L pyruvate. All homogenates were centrifuged at 5,000 X g for 20 minutes and the supernatant was used to assay for the enzyme activity according to the method of Mustroph and Albrecht (2003). All experiments were performed in triplicates.

Enzyme activity

The activity of lactate dehydrogenase (LDH) was determined with the Randox test kit (Randox Laboratories Ltd., United Kingdom). All experiments were performed in three replicates.

Determination of starch and glucose content

Starch determination was carried out after enzymatic hydrolysis into glucose. Starch and glucose were estimated by the RANDOX test kit according to the manufacturer's instructions. Bio-molecular activities, based on enzyme-linked formation of NADPH, were expressed as a specific activity measured at 570 nm. The corresponding concentration was determined against hydrogen peroxide standard curve prepared by using hydrogen peroxide solution. The amount of sugar was expressed as mg g^{-1} fresh weight and all experiments were performed in triplicates.

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 (Sokal and Rohlf, 1969) 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 $P<0.05$ was considered statistically significant.

Results

Lactate dehydrogenase (LDH) activity of bean seedlings exposed to crude oil are presented in Table 2. The results showed that LDH activity significantly increased when compared with control (root of 2% WC, WSF, WIF and Ubeji soil had 0.60 µmole/g, 0.68 µmole/g, 0.54 µmole/g and 0.67 µmole/g respectively) with the WSF presenting the highest activity at all percentages of contamination followed by Ubeji soil. However, the WIF had the highest percentage increase (46%) when compared with the control followed by WC (40%), Ubeji (33%) and WSF (32%) which showed the least percentage increase.

The results of the effects of different concentration of crude oil and its fractions on starch and glucose contents of the root of bean seedlings are presented in Tables 3 and 4. The results indicated reduction in starch content when compared with the control. The root of the 2% crude oil contaminated soil had 38% WC, 27% WSF and 42% WIF starch reduction respectively, 7 days post germination (DPG) while Ubeji soil presented a 49% decrease when compared with the control. The root of the 5% crude oil contaminated soil presented 41% WC, 37% WSF and 56% WIF starch reduction respectively, 7 DPG. The root of the 10% crude oil contaminated soil presented 47% WC, 38% WSF and 52% WIF starch reduction respectively, 7 days post germination (DPG). The highest starch reduction of the root when compared with the control was observed in the WIF of 5% crude oil contaminated soil.

Table 1: Percentage Contamination of soil with Crude Oil and its Fractions

Group	% Contamination	Number of bags
Control	0%	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 soil	THC (74.88 mg/kg)	30

THC = Total Hydrocarbon Content

Table 2: Effects of Different Concentrations of Crude Oil and its Fractions on Lactate Dehydrogenase Activities in the Root of Bean Seedlings

2% Contamination					
Days /Sample	**Control**	**WC**	**WSF**	**WIF**	**UBEJI**
7	0.46±0.02^{a}	0.60±0.04^{b}	0.68±0.03^{c}	0.54±0.03^{d}	0.67±0.03^{e}
14	0.77±0.03^{a}	0.96 ±0.02^{b}	1.02±0.03^{c}	0.87±0.02^{d}	0.99±0.02^{e}
21	0.96±0.02^{a}	1.05±0.03^{b}	1.29±0.02^{c}	1.13±0.02^{d}	1.46±0.02^{e}
5% Contamination					
Days /Sample	**Control**	**WC**	**WSF**	**WIF**	**UBEJI**
7	0.46±0.03^{a}	0.68±0.03^{b}	0.73 ±0.02^{c}	0.63±0.03^{d}	0.67±0.03^{e}
14	0.77±0.03^{a}	1.05 ±0.02^{b}	1.17±0.03 ^{c}	0.98±0.02^{d}	0.99±0.02^{e}
21	0.96±0.02^{a}	1.41±0.03^{b}	1.57±0.03^{c}	1.37±0.02^{d}	1.46±0.02^{e}
10% Contamination					
Days /Sample	**Control**	**WC**	**WSF**	**WIF**	**UBEJI**
7	0.46±0.02^{a}	0.77±0.03^{b}	0.80 ±0.04^{c}	0.68 ±0.02^{d}	0.67±0.03^{d}
14	0.77±0.03^{a}	1.19 ±0.03^{b}	1.31±0.02^{c}	1.12±0.02^{d}	0.99±0.02^{e}
21	0.96±0.02^{a}	1.44±0.02^{b}	1.60±0.02^{c}	1.40±0.03^{b}	1.46±0.02^{b}

Values are mean of three (n=3) replicates, ± standard error of mean (±SEM), M = Matured plant. WC = Whole Crude. WSF = Water Soluble Fraction of crude oil, WIF = Water Insoluble Fraction of crude oil, lactate dehydrogenase activities = µmole of NADH utilized/g wet tissue, Means of the same row carrying different notations are statistically different at P<0.05 using Instatgraphpad.

Table 3: Effects of Different Concentrations of Crude Oil and its Fractions on Starch Content in the Roots of Bean (mg/g)

2% Contamination					
Days /Sample	**Control**	**WC**	**WSF**	**WIF**	**UBEJI**
7	10.29±0.06^{a}	6.36±0.02^{b}	7.50 ±0.02^{c}	5.92±0.03^{d}	5.25±0.02^{d}
14	6.54±0.03^{a}	5.71 ±0.03^{b}	5.49±0.01^{c}	4.36±0.02^{d}	4.46±0.05^{d}
21	4.46±0.04^{a}	3.42±0.04^{b}	3.83±0.04^{c}	3.44±0.02^{bd}	3.19±0.02^{e}
5% Contamination					
Days /Sample	**Control**	**WC**	**WSF**	**WIF**	**UBEJI**
7	10.29±0.06^{a}	6.09±0.02^{b}	6.50 ±0.02^{c}	4.49±0.03^{d}	5.25±0.02^{e}
14	6.54±0.03^{a}	4.24 ±0.02^{b}	4.72±0.03^{c}	3.82±0.04^{d}	4.46±0.05^{e}
21	4.46±0.04^{a}	3.02±0.03^{b}	3.34±0.03^{c}	3.00±0.02^{b}	3.19±0.02^{d}
10% Contamination					
Days /Sample	**Control**	**WC**	**WSF**	**WIF**	**UBEJI**
7	10.29±0.06^{a}	5.44±0.04^{b}	6.42 ±0.02^{c}	4.92±0.03^{d}	5.25±0.02^{e}
14	6.54±0.03^{a}	4.37 ±0.01^{b}	4.64±0.01^{c}	2.46±0.02^{d}	4.46±0.05^{b}
21	4.46±0.04^{a}	2.24±0.03^{b}	2.86±0.04^{c}	1.48±0.02^{d}	3.19±0.02^{e}

Values are mean of three (n=3) replicates, ± standard error of mean (±SEM), M = Matured plant. WC = Whole Crude. WSF = Water Soluble Fraction of crude oil, WIF = Water Insoluble Fraction of crude oil, starch contents = mg/g wet weight, Means of the same row carrying different notations are statistically different at P<0.05 using Instatgraphpad.

Table 4: Effects of Different Concentrations of Crude Oil and its Fractions on Glucose Content in the Roots of Bean (mg/g)

2% Contamination					
Days /Sample	**Control**	**WC**	**WSF**	**WIF**	**UBEJI**
7	0.03±0.01[a]	0.04±0.01[a]	0.04±0.01[a]	0.05±0.01[a]	0.06±0.01[a]
14	0.07±0.01[a]	0.05±0.01[a]	0.05±0.01[a]	0.07±0.01[a]	0.08±0.01[a]
21	0.04±0.01[a]	0.07±0.01[a]	0.07±0.01[a]	0.08±0.01[a]	0.09±0.01[b]
5% Contamination					
Days /Sample	**Control**	**WC**	**WSF**	**WIF**	**UBEJI**
7	0.03±0.01[a]	0.05±0.01[a]	0.05±0.01[a]	0.06±0.01[a]	0.06±0.01[a]
14	0.07±0.01[a]	0.06 ±0.01[a]	0.07 ±0.01[a]	0.08 ±0.01[a]	0.08 ±0.01[a]
21	0.04±0.01[a]	0.08±0.01[a]	0.08±0.01[a]	0.09±0.01[b]	0.09±0.01[b]
10% Contamination					
Days /Sample	**Control**	**WC**	**WSF**	**WIF**	**UBEJI**
7	0.03±0.01[a]	0.06±0.01[a]	0.06±0.01[a]	0.06±0.01[a]	0.06±0.01[a]
14	0.07±0.01[a]	0.08±0.01[a]	0.08±0.01[a]	0.08±0.01[a]	0.08±0.01[a]
21	0.04±0.01[a]	0.09±0.01[b]	0.09±0.01[b]	0.10±0.01[b]	0.09±0.01[b]

Values are mean of three (n=3) replicates ± standard error of mean (±SEM), M = Matured plant. WC = Whole Crude. WSF = Water Soluble Fraction of crude oil, WIF = Water Insoluble Fraction of crude oil, glucose contents = mg/g wet weight, Means of the same row carrying different notations are statistically different at $P<0.05$ using Instatgraphpad.

Discussion

Soils contaminated with crude oils have been reported to alter the physiochemical properties of the soil (Olubodun and Eriyamremu, 2015) and adversely affect organisms (plants) (Olubodun and Eriyamremu, 2013) and (animals) (Okoye and Okwute, 2014) by causing oxidative stress.

The results of this study showed higher activity of LDH at higher contamination levels (Table 2). An increase in LDH activity in seedlings of *Lepidium latifolium* when exposed to 7 days of anoxia was reported by Chen and Quallis (2003). However, Kulkarni and Chavan (2013) reported a decrease in LDH activity in the root of finger millet and rice plant subjected to waterlogging stress.

This result is consistent with previous reports (Amora-Lazcano *et. al*., 2010; Alves *et al*., 2012; Xu *et. al*., 2012). However, it is at variance with the report of Kulkarni and Chavan (2013) and the Davis-Roberts pH-stat hypothesis.

The high LDH activity and high glucose contents of the bean root when compared with the control may indicate elevated energy demand to overcome the stress for germination of seeds as well as crude oil stress. The limitation or absence of oxygen as a result of the crude oil may have induced conversion of aerobic respiration to anaerobic respiration (fermentation) hence increase in the lactate dehydrogenase activity (Amora-Lazcano *et. al*., 2010; Alves *et al*., 2012; Xu *et. al*., 2012; Azmat *et al*, 2013; Huang *et al*, 2018).

Plants exposed to crude oil normally are exposed to lower oxygen supply, reducing ATP generation, and using the fermentation process as a secondary route in plant metabolism for energy production (Alves, *et al* 2012).

One of the most studied processes on seedling development is the mobilization of complex polymers from storage tissues such as cotyledons. These polymers which serve as seed reserves are used as energy sources and building blocks for seedling growth during germination (Delouche, 2016; Pennsylvania Certified Organic, 2011; Lopez *et al*, 2014). The reduction in starch content of the bean 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 (Cha-um *et. al*., 2009). The increase in glucose content of the bean root observed in the study compares favourably with decrease in lipid and increase in glucose contents previously reported (Amora-Lazcano *et al*, 2010; Azmat *et al*, 2013). This may indicate that starch was the main source of energy in the plant during crude oil exposure. Thus starch can be converted into glucose under stress condition as starch (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 helps the plant in providing energy in time of emergency for survival. The starch could be used by the growing plants to produce glucose as energy source to sustain the metabolic activities occurring in the plant. The result of the study suggests that the reduction in percentage germination and growth may be due to the decrease observed in starch content. The high LDH activity and high glucose level observed may indicate that high LDH activity is connected with increased energy demand to enable the seeds overcome the stress of germination and crude oil toxicity.

Conclusion

The increase in glucose level in the root of the bean seedlings, may be due to the breakdown of starch and increased LDH activity may have resulted from a switch from aerobic respiration to anaerobic respiration in crude oil stress.

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