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Bonny Light Crude Oil and its Fractions Alter Radicle Galactose Dehydrogenase Activity of Beans (*Phaseolus vulgaris* L.) and Maize (*Zea mays*)

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Abstract: The effect of contaminating soil with Bonny Light whole crude, or its fractions on germinating beans (*Phaseolus vulgaris* L) and maize (*Zea mays*) was studied. The percentage germination, level of malondialdehyde and galactose dehydrogenase activity was measured in the radicle of these plants after 7 and 14 days of germination. Soil was contaminated with either 0.1-0.3% Whole Crude (WC), its Water Soluble Fraction (WSF) or its Water Insoluble Fraction (WIF). There was dose dependent reduction in the number of bean or maize seeds that germinated in the contaminated soils compared with the control ($p<0.05$), with the least number recorded in the 0.3% contaminated soil. The WSF had the least effect on germination relative to the WC and WIF in all the contamination studies. There was also a dose dependent increase in lipid peroxidation ($p<0.05$) and a corresponding decrease in galactose dehydrogenase activity ($p<0.05$) in the crude oil treated plants compared with the control. These effects of crude oil or its fractions were more elaborated after 14 days of germination. Higher galactose dehydrogenase activity was observed in the radicle of germinating maize than in beans and there was also an increase in the percentage germination of maize relative to beans. This study reports that crude oil and its fractions increase the production of malondialdehyde and compromise the production of vitamin C occasioned by decreasing galactose dehydrogenase activity in the radicle of beans and maize, with an elaborated effect in beans. This may have profound effects on low yield of these plants.

Key words: Crude oil, beans, maize, radicle, malondialdehyde, galactose dehydrogenase

INTRODUCTION

Crude oil is a complex mixture of chemicals, which vary widely in composition though it is rich in hydrocarbons (aliphatic and aromatic) (Miklosovicova and Trzilova, 1991; Albers, 1995). Crude oil also contains some trace elements like vanadium, nickel, iron, aluminiun, copper and some heavy metals like lead and cadmium (National Research Council, 1985). Some of these metals may be beneficial to plants, others are deleterious. Whenever there is a spill or blowout, there is destruction of biodiversity, loss of fertile soil, pollution of air, degradation of farmland and damage to aquatic ecosystems (Suleiman, 1987) and plant seeds which would have sprouted after their normal dormancy period do not (Okoloko and Anoliefo, 2000).

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Crude oil spillage on soil makes it unsatisfactory for plant growth (De Jong, 1980) and is a major cause of plants poor growth and or death in oil spillage sites, while the risk of such effects is considerably low in regions far away from these sites (Odjegba and Sadiq, 2002). In the Niger-Delta of Nigeria, spillages arising from accidents or sabotage occur frequently in and around farmlands. Some of the contents of crude oil are readily taken up by the roots of many plant species and affect many biochemical processes in the plant which include reduction in photosynthesis, diminish water and nutrient uptake (Sanita di Toppi and Gabbrielli, 1999) and can result in plant injuries such as morphological aberrations, reduction in biomass, to stomatal abnormalities, chlorosis, growth inhibition, browning of root tips and death (Kahle, 1993).

It has been suggested that death that arise may be induced by some heavy metals present in crude oil which when taken up by plants can generate free radicals and can cause membrane damage (Zenk, 1996; Gallego *et al*., 1996). Ascorbic acid is involved in the detoxification of free radicals generated by xenobiotics (Noctor and Foyer, 1998). Different reports have shown varying effects of xenobiotics on ascorbate levels. Gallego *et al*. (1996) reported low levels of ascorbate in cadmium exposed *Helianthus annuus*, while Piqueras *et al*. (1999) reported elevated levels in *Phaseolus vulgaris*. Galactose dehydrogenase is a key enzyme in ascorbate synthesis and information on the effect of crude oil on its activity in the radicle of germinating plants is lacking. This study is thus aimed at assessing the effect of graded crude oil spills of soil on galactose dehydrogenase activity in the radicle of germinating beans (*Phaseolus vulgaris* L.) and maize (*Zea mays*) plants.

MATERIALS AND METHODS

Viable bean (*Phaseolus vulgaris* L.) seeds and maize (*Zea mays*) seeds from the botanical garden of the Department of Crop Science, University of Benin, Benin City, Nigeria, were each planted in 400 g soil obtained from a farm near Benin City. The soils were treated with either distilled water (control); Whole Crude (WC); the Water Soluble Fraction (WSF) of the crude oil; or with the Water Insoluble Fraction (WIF) of the crude oil in our laboratory. This soil treatment was done to mimic conditions on farmlands at site of spillage or farmlands close to spillage sites and represent 0.1, 0.2, or 0.3% contamination. With one seed planted per bag, one hundred viable seeds of each species were planted for each treatment. The viability of the plant seeds was determined by the floatation method. After 7 days of germination, half of the germinated plants were harvested and the radicle recovered for assays, the other half were harvested after 14 days post germination.

The crude oil was of the Bonny light grade and was obtained from Warri Refinery and Petrochemicals. For the fractionation of the crude oil, a 1:2 dilution of 200 mL of the crude oil was put in a 1 L conical flask and constantly stirred with a magnetic stirrer for 48 h. After which, the WSF was separated from the WIF in a sealed separating funnel, the sealed fractions were then frozen until required (Anderson *et al*., 1974). Each planting bag (30 cm in diameter) was packed with 400 g of soil and dampened with 100 mL distilled water, varying concentrations of WC, WSF, or the WIF thereafter a viable seed is planted on pre-damp soil. Watering of the planted seeds was done each day with 25 mL deionized water in the morning and evening. The heavy metal content of the crude and its fractions was determined by atomic absorption spectroscopy. Galactose dehydrogenase activity was determined by the method described by Mapes *et al*. (1970). NAD utilized from galactose per minute per mg protein was used to assign activity to the enzyme.

RESULTS

Bonny light crude oil used in this study do not contain detectable levels of lead, it however contains lower quantities of nickel than cadmium. The WIF contain higher levels of nickel and cadmium than the WSF (Table 1).

Table 1: Concentration of some heavy metals in the crude oil and its fractions

Heavy metal	WC (ppm)	WSF (ppm)	WIF (ppm)
Lead (Pb)	ND	ND	ND
Nickel (Ni)	0.06	0.01	0.03
Cadmium (Cd)	1.0	0.01	0.5

ND = Not Detected. WC = Whole Crude. WSF = Water Soluble Fraction of crude oil, WIS = Water Insoluble Fraction of crude oil

Table 2: Lipid peroxidation and galactose dehydrogenase activity of beans (*Phaseolus vulgaris* L.) radicle after germination in soil contaminated with crude oil

	Groups			
Parameters	Control	WC	WSF	WIF
0.1% contamination				
No. of seeds planted	100	100	100	100
% germination	100	56	68	49
7 days after germination				
Lipid peroxidation	2.4±0.5^{a}	9.3±0.7^{b}	4.9±0.6^{c}	9.6±1.0^{b}
Gal.DH	6.9±0.4^{a}	4.9±0.6^{b}	5.8±0.7^{b}	3.7±0.5^{c}
14 days after germination				
Lipid peroxidation	2.7±0.6^{a}	13.2±0.8^{b}	7.6±0.9^{c}	15.4±0.9^{d}
Gal.DH	5.3±0.3^{a}	4.3±0.3^{b}	5.0±0.4^{a}	3.0±0.3^{c}
0.2% Contamination				
No. of seeds planted	100	100	100	100
% germination	100	43	52	37
7 days after germination				
Lipid peroxidation	2.4±0.5^{a}	12.1±1.2^{b}	6.0±0.8^{c}	14.3±1.1^{b}
Gal.DH	6.9±0.4^{a}	3.2±0.6^{b}	5.0±0.5^{c}	3.4±0.5^{b}
14 days after germination				
Lipid peroxidation	2.7±0.6^{a}	17.1±2.3^{b}	9.7±1.1^{c}	19.7±2.6^{b}
Gal.DH	5.3±0.3^{a}	3.4±0.2^{b}	4.8±0.3^{a}	2.9±0.4^{b}
0.3% Contamination				
No. of seeds planted	100	100	100	100
% germination	100	29	38	20
7 days after germination				
Lipid peroxidation	2.4±0.5^{a}	14.6±1.1^{b}	9.4±1.3^{c}	17.8±1.4^{d}
Gal.DH	6.9±0.4^{a}	2.9±0.3^{b}	3.7±0.6^{c}	1.7±0.3^{d}
14 days after germination				
Lipid peroxidation	2.7±0.6^{a}	22.5±2.6^{b}	15.7±1.9^{c}	27.8±2.2^{d}
Gal.DH	5.3±0.3^{a}	2.1±0.4^{b}	3.7±0.4^{c}	1.3±0.3^{d}

Values are Means±SEM. For 0.1% contamination, n = 24; 0.2% contamination, n = 16 and 0.3% contamination, n = 10. Means of the same row followed by different superscripts differ significantly ($p<0.05$). Lipid peroxidation is presented in µmole MDA/g tissue. Gal.DH = Galactose dehydrogenase. Galactose dehydrogenase activity is expressed as µmole of NAD utilized/min/mg protein

Of the 100 bean seeds planted in each treatment group, only 56, 68 and 49 seeds germinated in the WC, WSF and the WIF treated soils respectively with 0.1% contamination (Table 2). This compares with the control where all the 100 planted seeds germinated. Amongst the test groups, the WSF had the least effect on percentage germination and the germinated seeds represent an improvement of as much as 12 and 19% compared with the WC and WIF respectively in the 0.1% contamination. In the highest level of contamination (0.3%), the values obtained for the WSF treatment compared with the WC and WIF were 9 and 18%, respectively. After 7 days of germination in these 0.1% treated soils, lipid peroxidation significantly ($p<0.05$) increased in the test groups compared with the control with the highest level recorded in the WIF treated group. Galactose dehydrogenase activity was somewhat inversely related to the level of lipid peroxidation, where an increase in lipid peroxidation resulted in a corresponding decrease in the enzyme action. Consequently, the lowest activity of galactose dehydrogenase was recorded in the radicle of beans grown on the WIF treated soils where the highest lipid peroxidation was recorded and was followed by the WSF and then the WC treated soil protocols. When the beans were allowed to grow in the treated soils until the 14th day when they where harvested, the galactose dehydrogenase action was further reduced with a

Table 3: Lipid peroxidation and galactose dehydrogenase activity of maize (*Zea mays*) radicle after germination in soil contaminated with crude oil

	Groups			
Parameters	Control	WC	WSF	WIF
0.1% Contamination				
No. of seeds planted	100	100	100	100
% germination	100	67	78	59
7 days after germination				
Lipid peroxidation	1.4±0.5^{a}	9.7±1.0^{b}	6.6±0.9^{c}	12.4±1.3^{d}
Galactose dehydrogenase	12.1±1.7^{a}	8.2±1.2^{b}	11.4±1.8^{a}	6.0±0.6^{c}
14 days after germination				
Lipid peroxidation	1.7±0.4^{a}	10.3±1.6^{b}	5.6±1.3^{c}	12.4±1.3^{d}
Gal.DH	17.1±1.7^{a}	7.6±0.9^{b}	10.3±1.4^{c}	4.5±0.9^{d}
0.2% Contamination				
No. of seeds planted	100	100	100	100
% germination	100	53	62	47
7 days after germination				
Lipid peroxidation	1.4±0.5^{a}	13.1±1.9^{b}	8.7±1.4^{c}	16.2±2.1^{b}
Gal.DH	12.1±0.7^{a}	6.4±0.5^{b}	9.7±0.7^{c}	4.9±0.5^{c}
14 days after germination				
Lipid peroxidation	1.7±0.4^{a}	16.1±2.1^{b}	8.7±1.4^{c}	18.1±2.4^{b}
Gal.DH	17.1±0.7^{a}	4.4±0.8^{b}	9.7±0.7^{c}	3.1±0.8^{b}
0.3% Contamination				
No. of seeds planted	100	100	100	100
% germination	100	44	49	32
7 days after germination				
Lipid peroxidation	1.4±0.5^{a}	16.1±2.2^{b}	11.6±1.8^{c}	18.8±2.4^{b}
Gal.DH	12.1±0.7^{a}	4.1±0.6^{b}	6.2±0.5^{c}	3.3±0.5^{b}
14 days after germination				
Lipid peroxidation	1.7±0.4^{a}	23.1±3.2^{b}	11.6±1.8^{c}	25.8±2.6^{b}
Gal.DH	17.1±0.7^{a}	2.4±0.7^{b}	6.2±0.5^{c}	2.3±0.5^{b}

Values are Means±SEM. For 0.1% contamination, n = 29; 0.2% contamination, n = 23 and 0.3% contamination, n = 16. Means of the same row followed by different superscripts differ significantly ($p<0.05$). Lipid peroxidation is presented in µmole MDA/g tissue. Gal.DH = Galactose dehydrogenase. Galactose dehydrogenase activity is expressed as µmole of NAD utilized/min/mg protein

corresponding increase in lipid peroxidation compared with the data recorded after 7 days of germination. A similar trend was seen in the bean seeds grown on the 0.2 and 0.3% contaminated soils, only that lipid peroxidation levels increased and galactose dehydrogenase activity decreased with severity of contamination. The study shows that lipid peroxidation and galactose dehydrogenase activity are altered in the radicle of beans when grown in soils contaminated with Bonny Light crude oil or its fractions and that their effect is dose dependent.

The observed effect of Bonny Light crude oil or its fractions on beans is also true for maize only that the germinated seeds increased to 67, 78 and 59 in the WC, WSF and WIF treated soils, respectively (Table 3). The crude oil and its fractions also increased lipid peroxidation compared with the control; however the values recorded were higher than the values recorded for beans. In maize, the observed galactose dehydrogenase activity was elevated compared to the observed level of action in beans, but in general terms they followed the trend in beans with percentage germination and galactose dehydrogenase decreasing with increasing lipid peroxidation occasioned by increasing contamination.

DISCUSSION

Crude oil spillage issue is important because it destroys the environment and the productive capacity of the population. Low crop yield has long been associated with crude oil spillages in or around farm sites but the biochemical changes that result in low yield is still under investigation. This study reports changes in radicle membrane lipid peroxidation and galactose dehydrogenase activity of beans and maize in crude oil contamination.

Different types of crude oil contain varying levels of chemicals (Banks *et al*., 2003; Miklosovicova and Trzilova, 1991), however the low heavy metal content of the Bonny Light crude oil used in this study (Table 1) is not surprising as this blend of crude oil rank about one of the best in the world because of its low sulphur and heavy metal content. The lower content of the metals detected in the WSF compared with the WIF is also not surprising as most heavy metals have poor solubility in water.

The observation in a reduction in the number of seeds that germinated in the crude oil contaminated soils compared with the control (Table 2 and 3) is not surprising as similar reports have been presented by Odjegba and Sadiq (2002). Some earlier reports have attributed low growth to reduced uptake and leakage of nutrients by plants which, basically is responsible for the proper germination and growth of plants (Udo and Fayemi, 1975). De Jong (1980) reported that oil in soil discourages plant growth probably due to insufficient aeration of the soil caused by displacement of air from pore spaces and thus increase the demand for oxygen and suffocation of the plants.

A possible displacement of air from pore spaces of soils may result in oxidative stress in plants which could contribute to the observed increase in lipid peroxidation in the crude oil treated plants (Table 2 and 3). This is not surprising since studies show that under stress, the balance between the production and quenching of reactive oxygen species as superoxide radical, singlet oxygen and peroxide is disturbed (Okuda *et al*., 1991; Gallego *et al*., 1996; Noctor and Foyer, 1998). This results in the generation of $HO^{\cdot}$ radicals causing extensive damages of membranes by peroxidation of their constituent lipids and reducing growth and germination. Also as crude oil displaces air from air spaces in soil; it may result in anoxia in the radicle. Under anoxia, there is increased lipid peroxidation (Chirvoka *et al*., 1998; Blokhina *et al*., 1999). Thus the stress on the plant radicle brought on by the crude oil treatment and the possible anoxia may be the cause of the increased level of lipid peroxidation observed in this study. The WC and WIF would contain more greasy hydrocarbons which would likely coat soils more and reduce aeration and may have accounted for the increase in lipid peroxidation of the radicle of the plants grown in the soils treated with these oils (Table 2 and 3).

Vitamin C production is one of the important mechanisms by which plants scavenge free radical that cause lipid peroxidation. The study also shows that there is compromise in the production of vitamin C; a situation which offer little protection to the radicle. Galactose dehydrogenase is an important key enzyme in vitamin C production, thus the inhibition of this enzyme in the contaminated plants may lead to lower production of the vitamin and a decrease in the protection of the radicle against free radicals. So the low activity of galactose dehydrogenase in the crude oil treatment would mean a compromise of the ability of the radicle to scavenge free radical which would in turn damage tissues and lead to tissue damage and even death.

Higher galactose dehydrogenase activity was observed in the radicle of germinating maize (Table 3) than in beans (Table 2) which may indicate that maize had a higher ability to produce vitamin C than beans. However there are two pathways for vitamin C production; one requires galactose and the other mannose. This study assessed vitamin C production through galactose. It is conceivable that beans may have elaborated the production of vitamin C via mannose. If however the decrease in galactose dehydrogenase observed in beans compared with maize reflects the overall ability of beans to produce vitamin C, then the vitamin may not be available to offer protection to the radicle of beans against free radicals. This may account for the observed increase in the percentage of germination of maize seeds relative to beans seeds. The quantification of vitamin C in the radicle of both plants would have helped to confirm the ability of the plants to adapt to crude oil contamination.

The WSF of crude oil had the least effect in all the parameters studied in both plant species. This is a likely indication that this fraction contains components which are less toxic. Our view is that the WSF; apart from accommodating more oxygen in soil pore spaces (which would likely reduce oxidative stress) and improving the synthesis of ascorbate in the radicle of plants compared with WC and WIF, it may be rich in compounds that have antioxidant activity like phenolic compounds which are easily absorbed by the radicle of plants and help ameliorate the effect(s) of crude oil. If the low galactose

dehydrogenase activity occasioned by crude oil and its fractions observed in this study also manifest in the plant shoot then it may contribute to the low yield in plants particularly root plants.

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