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Alterations in Glutathione Reductase, Superoxide Dismutase and Lipid Peroxidation of Tadpoles (*Xenopus laevis*) Exposed to Bonny Light Crude Oil and its Fractions

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Abstract: In this study the effect of sub-lethal levels of bonny light crude oil and its water soluble and insoluble fractions on stress enzymes was determined in tadpoles (*Xenopus laevis*) following two and four weeks exposure at different concentrations. Malondialdehyde (MDA) superoxide dismutase (SOD) and Glutathione Reductase (GR) were determined. It was observed that the treatment of tadpoles with whole crude or its fractions increased MDA. The head region contained a higher level SOD and GR compared with the tail region. We found that the Whole Crude (WC) had a less negative effect on lipid peroxidation and weight gain of tadpoles compared to the WSF. Presence of the WC, WSF or WIF was generally associated with increases in SOD and GR at lower concentration of exposure but decreases in the enzymes at higher doses of exposure was observed. However, longer exposure of tadpoles to WC or its fractions resulted in a negative effect on SOD and GR activities. Tadpole weights were negatively affected with WC and its fractions. The study shows that sub-lethal contaminations with WC, WSF or WIF induces membrane lipid peroxidation and reduce the ability of tadpoles to produce SOD and GR which may have metabolic costs.

Key words: Crude oil, malondialdehyde, superoxide dismutase, glutathione reductase, tadpoles

INTRODUCTION

Crude oil derived from crude petroleum has complex mixtures of chemicals, varying widely in composition of hydrocarbons and hydrocarbon-like chemicals (Miklosovicova and Trzilova, 1991; Albers, 1995). Crude oil also contains some trace elements like vanadium, nickel, aluminiun, copper and some heavy metals like lead and cadmium (National Research Council, 1985) and their exploration and exploitation impacts seriously on the environment. Nigeria is an established crude oil exporting nation producing medium and light crude oil, such as Bonny Light (Amund and Akangbou, 1993). Akpovire (1989) reported 2,000 oil spillages in Nigeria between 1976 and 1988; during which period more than two million barrels of crude oil were discharged into the environment. Spilled oil can not only harm the environment, it can poison exposed organisms (Suleiman, 1987) and pose serious threats to fresh water and marine environments that are linked in a complex food chain that includes human.

Tadpoles at contaminated sites may experience an alteration of biochemical events compared to amphibians in uncontaminated conditions. Exposure of animals to crude oil has been reported to

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produce Reactive Oxygen Species (ROS) and other free radicals which induce oxidative stress and cause lipid peroxidation (Khan *et al*., 2001; Downs *et al*., 2002). These ROS can cause cell death and tissue injury (Freeman and Crapo, 1982). Glutathione Reductase (GR), an enzyme that is widespread in tissues, maintains glutathione in the reduced form and this function in many reductive processes including the protection of cells against ROS and free radicals (Meister, 1988). Glutathione (GSH) also play an important role in the detoxification of xenobiotics and the inhibition of glutathione reductase may lead to low concentration of GSH and a concomitant high concentration of exogenous compounds in the tissues resulting in severe pathological conditions (Gul *et al*., 2000).

Crude oil has been generally shown to affect some enzymes. Tube feeding of crude oil to rats was shown to increase enzymes of energy metabolism like malate dehydrogenase and lactate dehydrogenase activities and decrease glucose 6-phosphatase and catalase activities (Haim and Kalir, 1986). Gagnon and Holdway (1999) showed that exposure of salmon fish to whole crude or its water accommodated fraction inhibited citrate synthase and lactate dehydrogenase in the gills, but did not affect cytochrome oxidase action. Studies on the effect of single and some interactive contaminants on superoxide dismutase (SOD) and GR activities have been reported (Regoli *et al*., 2005), but information on the effect of crude oil, which contains multiple interactive chemicals, on glutathione reductase activity is lacking. This study is thus aimed at establishing the effect of varying concentrations of crude oil and its fractions on SOD and GR activities of tadpoles.

MATERIALS AND METHODS

Bonny light crude oil obtained from Warri Refinery and Petrochemical Company in Delta State, Nigeria was fractionated by the method of Anderson *et al*. (1974). For the fractionation, 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. Thereafter, the Water Soluble Fraction (WSF) was separated from the Water Insoluble Fraction (WIF) in a separating funnel, sealed and frozen until required. The tadpoles used for this study were obtained from the zoological garden of the Department of Animal and Environmental Biology, University of Benin, Benin City, Nigeria. The tadpoles were housed individually in plastic containers which contained sand and water so as to create an aquatic/terrestrial environment (mini-island) for these organisms. The tadpoles were weighed and assigned to a control group of 20 animals and three other test classes of 60 animals each. Each test class was treated with either Whole Crude (WC), or WSF or WIF. The tadpoles in each test class were further subdivided into three groups of treatment of 20 tadpoles with each group treated with 10, 20, or 30 ppm. The groupings were done such that the weight difference between all the study groups was about 0.01 g. Prior to the commencement of the treatment the tadpoles were acclimatized for five days. The tadpoles were maintained on daily rations of 0.5 g lettuce that was boiled for fifteen minutes and chopped. The soil in each mini-pond group was treated with the WC, WSF, or WIF every two days. The study lasted for 4 weeks after which the tadpoles were weighed and then cut into head and tail regions. After two weeks into the study, half of the animals in each group (10 tadpoles) were sacrificed for mid-term studies. The tadpoles were housed separately (shortly after one week of hatching) in outdoor mesocosms (polyethylene tanks) with about one-third of each container packed with rich humus that simulated natural ponds and enhanced environmental realism relative to the laboratory. Water obtained from the original habitat of the tadpoles, was used to soak the soil in the containers to stabilize the humus. This water was tested for heavy metals like cadmium, lead, nickel and mercury using Absorption Spectroscopy and was found to contain non detectable levels of these metals. The crude oil was likewise tested for the presence of heavy metals.

Treatments

Each region (head or tail) was weighed and homogenized in ice-cold 0.9% normal saline to obtain 10% (W/V) homogenates. The homogenates were centrifuged at 5000 g for 10 min and the supernatant obtained were immediately used for analysis of lipid peroxidation, superoxide dismutase (SOD) and Glutathione Reductase (GR) activities.

Biochemical Analysis

The crude oil and its fractions were each diluted to 100 mL with distilled deionised water and analyzed for cadmium, nickel and lead with a Varian spectre AA10 Atomic absorption spectrophotometer. Estimation of membrane lipid peroxidation was by the method of Gutteridgre and Wilkins (1982). The procedure involved the determination of thiobarbituric acid reactive substances (TBARS), which are indicators of membrane lipid peroxidation. Values for TBARS are reported as malondialdehyde (MDA) and quantified using a Molar extinction coefficient of 1.5×10^5 M cm^{-1} and expressed as mmole MDA g^{-1} tissue weight. Superoxide dismutase activity was assayed according to the method described by Misra and Fridovich (1972) and was expressed as units g^{-1} tissue weight. One unit of the enzyme was defined as the amount of the enzyme required for 50% inhibition of oxidation of epinephrine to adrenochrome in one minute. Glutathione reductase enzyme activity was determined according to Mavis and Stellwagen (1968) method. The protein content of the samples was estimated by the method of Lowry *et al*. (1951).

Statistical Analysis

The results are expressed as means±SEM. Analysis of variance was used to test for differences in the groups. Duncan's multiple range test was employed to test for significant differences between the means (Sokal and Rohlf, 1969).

RESULTS

The crude oil does 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).

Exposure to WC or its fractions did not result in mortality, but affected the weights of the tadpoles (Table 2). The exposure of tadpoles to 10, 20 or the 30 ppm whole crude or its WSF induced weight loss after 2 weeks of treatment compared with the control. The WIF induced loss in weight in the tadpoles only when exposed at the 30 ppm level; however after 4 weeks of exposure to WC or its fractions, weight loss was observed in all the groups compared with the control. Increase in the concentration of treatment generally affected head weights. While head weights increased when the animals were given 20 ppm WC compared with when exposed to 10 ppm WC after 2 weeks, there was a steady increase in the WSF and the WIF exposed tadpoles. After 4 weeks of treatment, there was a steady decrease in the head weight of the WC and the WIF treated animals. The study reveals that weights of tadpoles are affected by crude oil and its fraction.

Table 1: Concentration of heavy metals (cadmium (Cd), Nickel (Ni) and Lead (Pb) in water

Heavy metal	WC(ppm)	WSF (ppm)	WIF (ppm)
Lead (Pb)	ND	ND	ND
Nickel (Ni)	0.06	0.01	0.03
Cadmium (Cd)	1.00	0.01	0.50

ND = Not Detected, WC = Whole Crude, WSF = Water soluble fraction of crude oil, WIS = Water insoluble fraction of crude oil

Table 2: Weight changes in tadpoles exposed to varying concentrations of crude oil and its fraction after two and weeks exposure

	Groups			
Parameters	Control	WC	WSF	WIF
2 weeks exposure				
10 ppm				
Weight change	1.79±0.76^{a}	-0.33±0.02^{b}	-0.38±0.73^{b}	2.44±0.01^{a}
Final head weight	0.29±0.03^{a}	0.19±0.02^{b}	0.16±0.02^{b}	0.15±0.01^{b}
20 ppm				
Weight change	1.79±0.76^{a}	-0.77±0.03^{b}	-0.95±0.18^{b}	1.89±0.03^{a}
Final head weight	0.29±0.03^{a}	0.38±0.10^{b}	0.22±0.03^{b}	0.26±0.03^{a}
30 ppm				
Weight change	1.79±0.76^{a}	-0.83±0.10^{b}	-1.09±0.10^{c}	-0.99±0.05^{b}
Final head weight	0.29±0.03^{a}	0.23±0.03^{b}	0.28±0.10^{a}	0.35±0.06^{c}
4 weeks exposure				
10 ppm				
Weight change	0.40±0.02^{a}	-1.05±0.01^{b}	-1.05±0.43^{b}	-1.83±0.09^{c}
Final head weight	0.23±0.007^{a}	0.48±0.02^{b}	0.20±0.01^{a}	0.16±0.01^{b}
20 ppm				
Weight change	0.40±0.02^{a}	-2.69±0.02^{b}	-2.78±0.70^{b}	-2.36±0.09^{c}
Final head weight	0.23±0.007^{a}	0.14±0.02^{b}	0.20±0.02^{a}	0.31±0.10^{c}
30 ppm				
Weight change	0.40±0.02^{a}	-3.42±0.02^{b}	-3.74±0.79^{b}	-3.97±0.03^{c}
Final head weight	0.23±0.007^{a}	0.17±0.009^{b}	0.16±0.02^{b}	0.16±0.02^{b}

Values expressed as mean±SEM weights are in grams, (n = 10), Means of the same row followed by different letter(s) differ significantly ($p<0.05$)

Table 3: Level of malondialdehyde (lipid peroxidation), superoxide dismutase and glutathione reductase activities of tadpoles' head region after 2 weeks of exposure to crude oil and its fractions

	Groups			
Parameters	Control	WC	WSF	WIF
10 ppm				
MDA	0.10±0.01^{a}	0.13±0.02^{ab}	0.15±0.02^{b}	0.12±0.01^{a}
SOD	36.64±3.01^{a}	37.38±2.94^{a}	43.22±3.01^{b}	34.78±2.42^{a}
GR	10.48±1.18^{a}	15.75±2.34^{b}	22.41±2.82^{c}	12.14±1.23^{a}
20 ppm				
MDA	0.10±0.01^{a}	0.16±0.02^{b}	0.21±0.02^{c}	0.14±0.02^{b}
SOD	36.64±3.01^{a}	45.48±3.27^{b}	52.34±2.02^{c}	43.04±1.84^{b}
GR	10.48±1.18^{a}	26.99±2.08^{b}	35.53±3.82^{c}	19.18±1.64^{d}
30 ppm				
MDA	0.10±0.01^{a}	0.21±0.01^{b}	0.28±0.02^{c}	0.16±0.02^{d}
SOD	36.64±3.01^{a}	46.42±3.13^{bc}	50.22±3.01^{c}	42.88±3.47^{b}
Glutathione reductase	10.48±1.18^{a}	27.61±3.70^{b}	35.39±3.73^{c}	20.14±2.00^{d}

Values expressed as mean±SEM, (n = 10), Malondialdehyde (MDA) units are in mmole MDA g^{-1} tissue, superoxide dismutase (SOD) is in units g^{-1} tissue, Glutathione Reductase (GR) activity is in µmole NADPH utilized min^{-1} mg^{-1} protein. Means of the same row followed by different letter(s) differ significantly ($p<0.05$)

Statistical analysis reveal that there was a significant ($p<0.05$) increase in the level of MDA in the head region of tadpoles treated with 20 or 30 ppm crude oil or its fractions compared with the control (Table 3). The WSF resulted in the highest increase in MDA compared with the WC and WIF. The MDA levels also increased with increase in the levels of contamination and this translated to an increase of about 23 and 61% in the 20 and 30 ppm, respectively over the 10 ppm WC exposed tadpoles. The percentage increase in the WSF treated tadpoles was 40 and 87% in the 20 and 30 ppm, respectively compared with the 10 ppm ones. The increase in MDA occasioned by WC, WSF and WIF treatment resulted in a corresponding significant ($p<0.05$) increase in the activity of SOD in the 20 and 30 ppm exposed tadpoles. In the 10 ppm treated animals, there was increase in the SOD

activity, but the increase reached a significant level only in the WSF treated tadpoles compared with the other test groups and control. Glutathione reductase activity was significantly ($p<0.05$) increased in all the test groups compared with the control. A further increased in GR activity was observed in the WSF group compared with the other test groups in all the treatments. The study shows that low doses of crude oil and its fractions alter MDA and GR activity in the head region of tadpoles.

MDA levels were significantly increased in the test groups exposed to WC or its fractions compared with the control (Table 4). Increase in the dose of exposure also led to increase in MDA levels. The effect of the crude and its fractions on SOD activity in the tail regions was not consistent. When the tadpoles were treated with 10 ppm WC or its fractions, there was no observed significant change ($p>0.05$) in the activity of SOD. In the 20 ppm treatment, SOD was fairly raised and the increase reached a significant level only in the WSF treated tadpoles compared with others. In the 30 ppm treatment, there was an observed significant ($p<0.05$) decrease in SOD activity in the test groups compared with the control. In all the treatments, GR activity was consistently elevated in the test groups compared with the control with the highest activity recorded in the WSF exposed tadpoles.

Whole crude or its fractions significantly elevated the level of MDA in this region of the tadpoles compared with the control (Table 5). The highest level of lipid peroxidation was observed in the WSF exposed tadpoles. Increase in the dose of exposure from 10 to 20 ppm and then 30 ppm also translated

Table 4: Level of malondialdehyde (lipid peroxidation), superoxide dismutase and glutathione reductase activities of the tadpoles' tail region after 2 weeks of exposure to crude oil and its fractions

	Groups			
Parameters	Control	WC	WSF	WIF
10 ppm				
MDA	0.09±0.01^{a}	0.14±0.02^{b}	0.15±0.02^{b}	0.13±0.02^{b}
SOD	28.22±2.31^{a}	28.07±2.35^{a}	30.40±2.41^{a}	26.81±2.23^{a}
GR	6.81±0.61^{a}	12.52±1.72^{b}	19.41±2.52^{c}	8.22±1.04^{a}
20 ppm				
MDA	0.09±0.01^{a}	0.17±0.02^{b}	0.21±0.03^{c}	0.15±0.02^{b}
SOD	28.22±2.31^{a}	30.32±2.29^{a}	37.94±2.22^{b}	29.90±2.31^{a}
GR	6.81±0.61^{a}	20.08±3.31^{b}	29.53±3.33^{c}	17.11±2.59^{b}
30 ppm				
MDA	0.09±0.01^{a}	0.26±0.03^{b}	0.34±0.03^{c}	0.20±0.02^{d}
SOD	28.22±2.31^{a}	19.82±2.54^{b}	9.62±0.81^{c}	20.23±2.11^{b}
GR	6.81±0.61^{a}	22.64±3.56^{b}	30.59±3.43^{c}	14.96±2.39^{d}

Values expressed as mean±SEM, (n = 10), Malondialdehyde (MDA) units are in mmole MDA g^{-1} tissue, superoxide dismutase (SOD) is in units g^{-1} tissue, Glutathione Reductase (GR) activity is in µmole NADPH utilized min^{-1} mg^{-1} protein. Means of the same row followed by different letter(s) differ significantly ($p<0.05$)

Table 5: Level of malondialdehyde (lipid peroxidation), superoxide dismutase and glutathione reductase activities of tadpoles' head region after 4 weeks of exposure to crude oil and its fractions

	Groups			
Parameters	Control	WC	WSF	WIF
10 ppm				
MDA	0.11±0.02^{a}	0.16±0.02^{bc}	0.21±0.03^{c}	0.13±0.02^{ab}
SOD	42.60±3.05^{a}	34.67±3.05^{b}	27.15±3.12^{c}	38.37±3.09^{ab}
GR	18.68±1.20^{a}	12.00±1.45^{b}	7.73±1.03^{c}	14.63±1.27^{b}
20 ppm				
MDA	0.11±0.02^{a}	0.23±0.02^{b}	0.29±0.02^{c}	0.16±0.02^{d}
SOD	42.60±3.05^{a}	27.17±3.03^{b}	20.18±2.85^{c}	33.09±2.01^{d}
GR	18.68±1.20^{a}	11.90±1.07^{b}	6.10±1.50^{c}	14.50±1.40^{d}
30 ppm				
MDA	0.11±0.02^{a}	0.28±0.02^{b}	0.35±0.03^{c}	0.21±0.02^{d}
SOD	42.60±3.05^{a}	19.11±2.92^{b}	13.62±2.53^{c}	28.08±1.03^{d}
GR	18.68±1.20^{a}	8.70±1.30^{b}	4.17±1.41^{c}	12.30±1.10^{d}

Values expressed as mean±SEM, (n = 10), Malondialdehyde (MDA) units are in mmole MDA g^{-1} tissue, superoxide dismutase (SOD) is in units g^{-1} tissue, Glutathione Reductase (GR) activity is in µmole NADPH utilized min^{-1} mg^{-1} protein. Means of the same row followed by different letter(s) differ significantly ($p<0.05$)

Table 6: Level of malondialdehyde (lipid peroxidation), superoxide dismutase and glutathione reductase activities of tadpoles' tail region after 4 weeks of exposure to crude oil and its fractions

	Groups			
Parameters	Control	WC	WSF	WIF
10 ppm				
MDA	0.11±0.02^{a}	0.25±0.03^{b}	0.34±0.04^{c}	0.16±0.02^{d}
SOD	38.60±3.05^{a}	28.67±3.05^{bc}	22.15±3.92^{b}	30.37±3.09^{c}
GR	14.68±1.20^{a}	10.00±1.05^{b}	7.73±0.83^{c}	12.63±1.07^{ab}
20 ppm				
MDA	0.11±0.02^{a}	0.31±0.03^{b}	0.59±0.03^{c}	0.18±0.02^{d}
SOD	38.60±3.05^{a}	20.15±2.62^{b}	14.00±2.18^{c}	27.20±3.07^{d}
GR	14.68±1.20^{a}	8.05±0.72^{b}	5.50±0.47^{c}	11.70±0.62^{d}
30 ppm				
MDA	0.11±0.02^{a}	0.48±0.04^{b}	0.67±0.04^{c}	0.27±0.03^{d}
SOD	38.60±3.05^{a}	17.15±3.15^{b}	9.14±2.08^{c}	24.95±2.29^{d}
GR	14.68±1.20^{a}	5.65±0.60^{b}	3.61±0.53^{c}	9.19±0.70^{d}

Values expressed as mean±SEM, (n = 10), Malondialdehyde (MDA) units are in mmole MDA g^{-1} tissue, superoxide dismutase (SOD) is in units g^{-1} tissue, Glutathione Reductase (GR) activity is in µmole NADPH utilized min^{-1} mg^{-1} protein. Means of the same row followed by different letter(s) differ significantly ($p<0.05$)

into increase in MDA levels. Unlike the 2 weeks study, the increase in MDA consistently resulted in an increase in the SOD activity in all the test animals. Like in the 2 weeks study, GR activity was significantly ($p<0.05$) decreased with increase in the level of MDA and dose of exposure. The data presented infer that the longer the treatment of tadpoles with crude oil or its fraction, the lower is the activity of SOD and GR.

The data in Table 6 shows that there is a significant ($p<0.05$) increase in the MDA level in the test tadpoles compared with the control in all the treatments, while SOD and GR activities were consistently reduced.

DISCUSSION

Many habitats may be exposed to crude oil chemical contaminants, particularly in areas of oil exploration or sites where spillages are common, however, the effects of crude oil contamination is still undergoing investigation. The objective of present study was to examine the effects of sublethal levels of bonny light crude oil and its water soluble and insoluble fractions on stress enzymes in tadpoles (*Xenopus laevis*).

Changes in body weight have often been used as indices of toxicity of chemicals (Timbrell, 1991). The weight loss in the tadpoles induced by exposure to bonny light Whole Crude (WC) or its fractions (Table 2) is therefore not surprising and it corroborates the findings of Snodgrass *et al*. (2005) which also reported that tadpoles exposed to mine sediments of coal combustion waste suffered sublethal effects in the form of reduced growth. Monoaromatic hydrocarbons (benzene, toluene, xylene) and other chemicals of high water solubility usually contribute about 95% of the water-soluble fraction of crude oil and have been shown to have high acute toxicity (Capuzzo and McDowell, 1987), it is not surprising that the reduced weight was pronounced in the tadpoles exposed to the WSF. This study observed that the WIF has less effect on weight compared with the WSF or WC. This again is to be expected since an earlier study had reported that the Water-Insoluble Fraction (WIF) is mostly composed of alkanes and cycloalkanes, which have relatively low solubility and are less toxic compared with the WSF (Toomey and Epel, 1993). We also observed that the weight reduction was more elaborated in the tadpoles exposed for 4 weeks (Table 2) and agrees with earlier studies which show that the length of the period of exposure influence the sensitivity of amphibians to environmental pollutants (Snodgrass *et al*., 2005). Increase in the dose of exposure was directly correlated with reduction in weights of the tadpoles (Table 2). This disagrees with the findings of Karasov *et al*. (2005)

which reported that the level of contamination did not affect the weights of tadpoles beyond a particular level, though their study used a single contaminant. Studies by Boone *et al*. (2005) reported that the combination of carbaryl and nitrate (interactive effect of multiple contaminants) had a negative effect on development and mass of tadpoles compared to the positive effect that either contaminant had alone. It should be appreciated that since crude oil contains multiple chemicals, which are potential contaminants the present observation of the effect of WC, WSF and WIF on weights of tadpoles may be the result of the interactive effects of these contaminants.

It is becoming accepted that cadmium and other heavy metals can generate free radicals that are known to induce the peroxidation of polyunsaturated lipids in cell membranes and cause degradation of structural proteins, enzymes and ribonucleic acids and tissue damage (Fridovich and Freeman, 1986; Gupta *et al*., 1991). It was observed that the WC and its fractions increased the level of malondialdehyde, with the WSF producing the most effect (Table 3-6). A high MDA level has been known to serve as an index of membrane lipid peroxidation and thus tissue damage. The attribution of the increase in MDA to the presence of cadmium in the crude oil cannot be overemphasized as the WSF which contains the least level of cadmium (Table 1) resulted in the most increase in the MDA of the tadpoles. So the lipid peroxidation level observed in these tadpoles would have mostly arisen from other components which may be just as toxic.

As a defense against these reactive free radicals, the body produces antioxidant enzymes and SOD and GR are some of the main antioxidant enzymes. So the observed increase in SOD and GR activities of the head and tail regions after 2 weeks of exposure to WC and its fractions (Table 3 and 4) may be an improved attempt by the tadpoles to mop up MDA produced and may be an adaptive feature. The interesting observation of a higher SOD and GR activities in the head region compared with the tail region after 2 and 4 weeks exposure (comparing Table 3 and 4; as well as Table 5 and 6) indicate that the adaptive mechanism is elaborated in the head region than the tail. It would be expected that majority of the metabolic processes for the survival of the tadpole would occur in the head region and thus would not be easily compromised as the tail region which is mostly adapted to movement of the animal. A decrease in SOD activity in the tail region of the tadpoles exposed to 30 ppm of WC, WSF or WIF (Table 4) would diminish the ability of this region to scavenge free radicals, which indicate that the tail will be more susceptible to damage by high MDA produced by the contaminants in the crude oil. SOD is a metalloenzyme requiring zinc for its structural stability and copper for its enzymatic activity (Gotz *et al*., 1994). The WC contains cadmium and it has been reported that cadmium can induce inhibition of this enzyme by binding to and displacing these metals from the active site of the enzymes (Gotz *et al*., 1994). The decrease in the activity of SOD at high doses of the crude oil may in part be the result of the effect of cadmium in the crude.

The chemicals present in crude oil are electrophilic and their metabolism involves two distinct stages, commonly referred to as phases I and II reactions. Phase II metabolism generally adds hydrophilic moieties to products of phase I reactions, thereby making them more water soluble and less bioactive (Capuzzo and McDowell, 1987; Toomey and Epel, 1993). At some point the phase II reactions may require reduced glutathione which is constantly produced from oxidized glutathione by glutathione reductase (Capuzzo and McDowell, 1987). This study reports an increase in the activity of GR after 2 weeks exposure of the tadpoles to (Table 3), with the tail region displaying relatively lower activity (Table 4) compared with the head region. This also may imply an attempt by the animals to not only scavenge free radicals but also improve the rate of detoxifying some of the toxic materials in the crude oil and its fractions. This argument become even more plausible as the WSF which had been shown to be more toxic (Capuzzo and McDowell, 1987) displayed the highest activity of GR in all levels of contamination in the 2 weeks study (Table 3 and 4).

As present study observed increased production of MDA in the tadpoles with increased time of exposure (Table 5 and 6), it indicates the increased chances of membrane lipid peroxidation and thus

tissue damage and its attendant loss in enzyme activity. This may have accounted for the decrease in GR activity in both the head and tail regions of the tadpoles in the 4 week treatment (Table 5 and 6). This would have serious implication for the tadpoles as it would limit the biotransformation capability of polycyclic (halogenated) hydrocarbons, thus influencing the bioaccumulation and biological effects of these chemicals in the crude oil and coupled with the low SOD activity would in turn diminished the ability of the animals to mop up free radicals. The combined results of these effects would further increase propensity for tissue damage and would have contributed immensely to the observed weight losses in these tadpoles. Such compromise in the health of animals have also been demonstrated in the antarctic rock cod (Trematomus bernacchii) which was exposed to model chemicals, including polycyclic aromatic hydrocarbons (benzo[a]pyrene), persistent organic pollutants (2,3,7,8-tetrachlorodibenzo-p-dioxin [TCDD]), cadmium and a combination of cadmium and TCDD (Regoli *et al*., 2005). They found that there was chemical bioaccumulation in the fish and compromise in the levels of the antioxidant system measured as decreases in catalase, SOD, glutathione, glutathione reductase, glutathione S-transferases and glutathione peroxidases (Regoli *et al*., 2005). Studies by Boone *et al*. (2005) also show that metabolic processes of amphibians can be compromised by the combination of the interactive effect of sublethal contaminants. The present study show that some of the metabolic processes that may be compromised are those of free radical scarvenging and detoxification processes and as earlier mentioned, the crude effect was lesser in the head region compared with the tail region.

In conclusion, whole crude or its fractions result in increase in MDA production and at early stages of the contamination, tadpoles improve the activities of SOD and GR in an attempt to handle the toxic chemicals in this crude oil. In prolonged period of exposure to crude oil, the ability of the animals to produce SOD and GR is compromised and may have serious consequence for the animals since the ability of tadpoles to scavenge and detoxify chemicals in crude oil or its fractions is compromised. So in countries such as Nigeria where sabotage accounts for most of the oil spills and clean up process is slow, the reduction in SOD and GR can reduce the chances of the survival of tadpoles.

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