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BIOACCUMULATION OF HEAVY METALS AND ASSOCIATED INDUCTION OF OXIDATIVE STRESS INDUCED BY CRUDE OIL CONTAMINATION

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ABSTRACT

This research was carried out on 20 male albino rats. which were reared for a period of one year. They were divided into two groups of 10 rats in each group. Rats in group 1 were fed with normal diet (standard rodent laboratory chow -Coppens) and borehole water while rats in group 2 were fed with normal diet and water from the river in a crude oil impacted community (Igbeta-Ewoama). Levels of the following metals manganese, lead, cadmium, selenium, mercury and chromium as well as the oxidative stress biomarker 8 hydroxy -2-deoxyguanosine were measured in the two groups at the end of the experimental period.. Rats fed with water from the crtude oil impacted community (Group 2) had higher levels of the six metals that were measured than the control group. The differences in the measured values were statistically significant ($p < 0.05$).. Group 2 also had higher and significant levels of 8 hydroxy -2- deoxyguanosine. The results obtained show that there is bioaccumulation of the measured metals and also increase in the level of 8 hydroxy -2- deoxyguanosine in rats fed with water obtained from the river in the environment where crude oil drilling activities is carried out.

I. INTRODUCTION

Oil spillage and contamination of the surrounding environment is a common fallout of oil exploration and refining activities in the Niger delta region of Nigeria. An estimated total number of over 7000 oil spill incidents have been reported in over a 50-year period in this region [1]. There are no consistent records of the exact quantity of crude oil spilled in the Niger delta, but it has been estimated that over 13 million barrels (1.5 million tons) of crude oil have been spilled since 1958 from over 7000 oil spill incidents. This gives a yearly average of about 240,000 barrels [2].

Crude oil contains different proportions of different heavy metals namely Zinc (Zn), Lead (Pb) Manganese (Mn), Chromium (Cr), Cadmium (Cd), Iron (Fe), Nickel (Ni), Cobalt (Co), Vanadium (Vd), Mercury (Hg), Copper (Cu) ,Molybdenum (Mo) and Selenium (Se) with Nigeria crude oil having relatively high concentrations of Fe, Zn, Cu, Pb and Hg [2] [3]

The measured concentration of the heavy metals ranged from 2.20 – 3.5 ppm for Mn, 1.42-1.62 ppm for Ni, 1.04 – 1.44 ppm for Fe, 0.68 – 0.74 ppm for Cr, 0.48 – 0.54 ppm for Zn, 0.28 – 1.12 ppm for Co, 0.31-0.34 ppm for Cd, 017 – 0.19 ppm for Pb and 0.08-0.12 for Cu [3]. It is obvious from these studies that Nigerian crude oil have low metal content. However, the low concentrations could pose an intrinsic health hazard considering their cumulative effect in the environment [4]. These heavy metals unlike organic contaminants are not degraded further and also cannot decompose into other chemicals with time. This results to their bioaccumulation in the ecosystem and the body of aquatic animals.

Aquatic animals and plants in oil polluted environments have been shown to contain different amounts of these heavy metals. A study carried out to determine the metal contents of various fish and shellfishes from the Niger delta area of Nigeria showed that metal levels in the shellfishes were higher than those in the fish, which could be due to their being bottom feeders [5] . The levels in fish varied from <0.01– 0.10 µg/g for Cd, < 5–39 µg/g for Cu, 0.49–16.52 µg/g for Fe, and 0.08–6.90 µg/g for Zn [5].

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Heavy metals are known to cause oxidative stress which is an imbalance between the concentration of reactive oxygen species (ROS) and antioxidants. Excessive accumulation of ROS will lead to cell injury due to damage to nuclear materials (DNA/RNA). 8 Hyydroxy -2- Deoxyguanosine (8-OHdG) is one of the products of DNA damage and also a marker of oxidative stress for the assessment of DNA damage in cells. Its formation is enhanced by carcinogens [6]. Under normal conditions, ROS are essential to cell growth and survival. For example, by interacting with critical signaling molecules, ROS drive processes including proliferation, apoptosis, and iron homeostasis, while their release from macrophages and neutrophils represents an important defence mechanism against invading pathogens [7]. Under conditions of oxidative stress, excess ROS levels have been shown to cause approximately 100 different oxidative base lesions and 2-deoxyribose modifications [8]. Of the most widely studied types of ROS – namely, superoxide radicals (•O2−), hydrogen peroxide (H2O2), and the hydroxyl radical (•OH) – the hydroxyl radical is by far the most reactive [8]. Produced during the reaction between H2O2 and Fe2+, the hydroxyl radical causes DNA damage through its addition to the double bonds of DNA bases and its abstraction of hydrogen atoms from methyl groups and the C-H bonds of 2′-deoxyribose sugars [9].

Since water bodies contaminated with crude oil are known to contain heavy metals, and also heavy metals are known to induce oxidative stress, it becomes necessary to assess the possible accumulation of these metals in animals fed with crude oil contaminated water and also assess the possible induction of oxidative stress by these xenobiotics.

II. METHODOLOGY

Twenty male albino rats with an average weight of 52.40 ± 4.40g that were obtained from the small animal holding unit of the Department of Pharmacy, Niger Delta University, Wilberforce Island, Amassoma, Bayelsa State, Nigeria were used for this study.

The rats were acclimated to environmental conditions for one week before the commencement of the experimental procedures. They were divided into two groups of ten rats each and all reared for a period of one year. Group 1 served as the control while Group 2 was the experimental group.

Group 1 (Control) was fed with normal diet (standard rodent laboratory chow -Coppens) and borehole water as they were reared for one year.

INDUCTION OF OXIDATIVE STRESS

The rats in group 2 were fed with normal diet and water from the river from the crude oil impacted community (Igbeta-Ewoama) for one year.

LABORATORY PROCEDURES

At the end of the rearing period, the rats were anaesthetized using chloroform and blood samples were collected from the jugular vein. Blood samples for measurement of 8-OHdG were collected into plain serum separating tubes. They were allowed to stand for 10 – 20 minutes after which the serum was separated by centrifuging at 3,000 rpm for 20 minutes. Samples for the measurement of heavy metals were collected into K_3EDTA anti coagulated plastic bottles and mixed thoroughly by repeated gentle turning.

EVALUATION OF HEAVY METAL BIOACCUMULATION

The levels of the following heavy metals; Mn, Pd, Cd, Se Hg and Cr were determined in the two groups at the end of the experimental period. Measurement of these metals was carried out using 240 FS AA Agilent Technologies flame atomic absorption spectrometer.

ASSESSMENT OF OXIDATIVE STRESS BIOMARKER

The concentrations of 8-OHdG were determined in the two groups at the end of the experimental period. The Elabscience oxidative DNA damage competitive ELISA technique for the quantitative measurement of 8-OHdG was used.

III. RESULTS

Heavy metal levels measured in the two groups is presented in figure 1. Rats fed with crude oil contaminated water (Group B) had higher levels of the six metals that were measured than the control group. The differences

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in the measured values were statistically significant in cadmium, chromium, mercury, lead and selenium ($p < 0.05$) . The differences in the levels of manganese in the two groups were not significant.

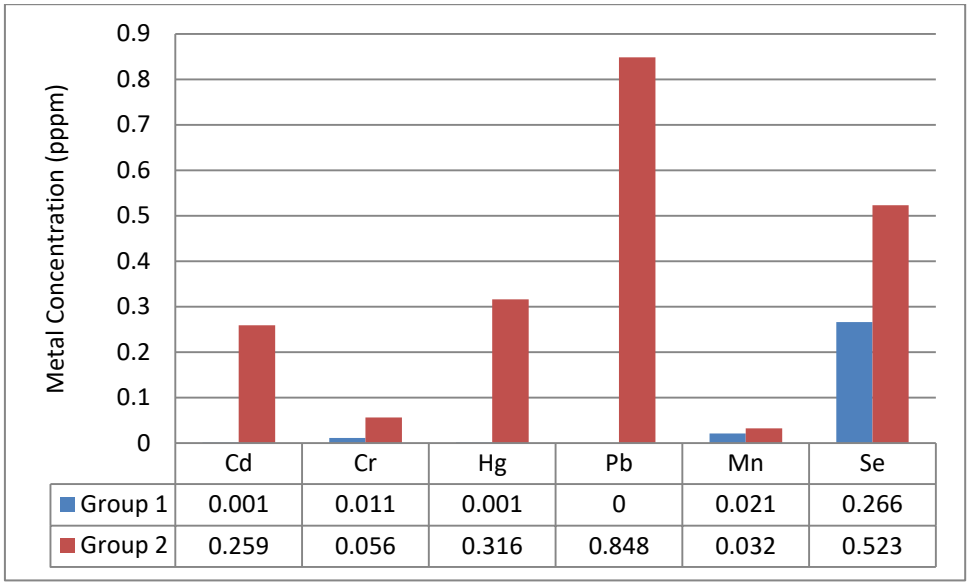

Figure 1: Mean Values of Heavy Metal Levels in the Animal Groups.

Figure 2 shows 8-OHdG concentrations that were measured in the two groups. The concentration of this oxidative stress biomarker was higher in animals in group 1. This difference in concentration was statistically significant ($p < 0.05$)

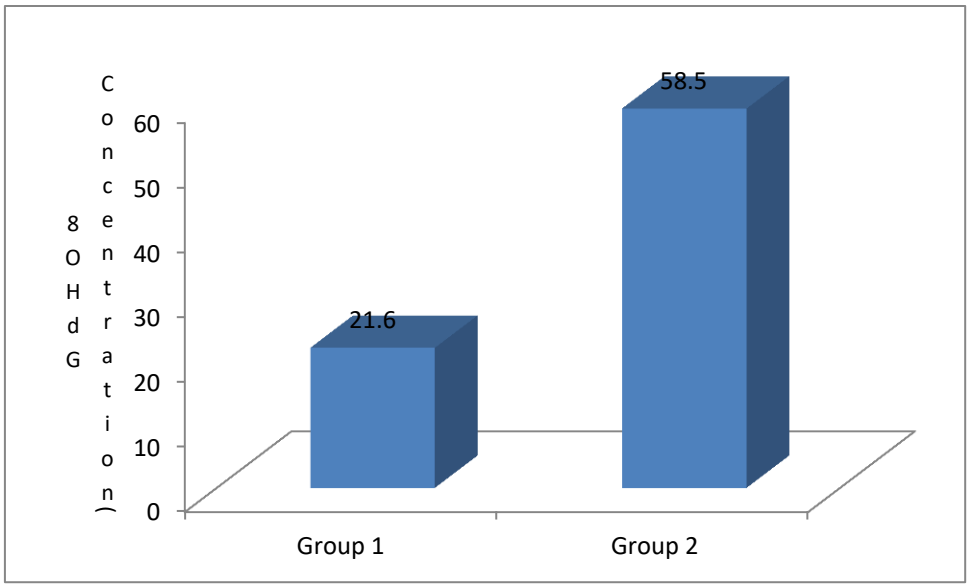

Figure 2: Mean Values of 8-0HdG Concentration in the Two Groups.

The relationship between the measured metals and oxidative stress biomarkers in animals in Group 1 was assessed using Pearson's correlation statistics. A positive and moderate correlation was observed between Chromium and 8-OHdG ($r = 0.346$). A moderate and positive correlation was also observed between Manganese and 8-OHdG ($r = 0.440$). Cadmium, Lead and Mercury had no correlations with the oxidative stress biomarker.

Table 1: Correlation Between the Measured Metals and 8-OHdG in Group 1

8-OHdG	Cadmium		Chromium		Mercury		Lead		Selenium		Manganese	
	R	P	R	P	R	P	R	P	R	P	R	P
	0.000	1.000	0.346	0.327	0.000	1.000	0.000	1.000	0.228	0.526	0.440	0.203

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The correlation between 8-OHdG and the metals that were measured in rats in experimental group 2 is presented in table 2. As shown in the table, 8-OHdG correlated strongly and positively with Cadmium (r = 0.603) and manganese (r = 0.861). Moderate and positive correlations were observed between 8OHdG and chromium (r = 0.309), mercury (r = 0.457) and lead (r = 0.407). A weak but positive correlation was observed between it and selenium (r = 0.210). However, only the correlation with manganese was significant statistically.

Table 2: Correlation Between the Measured Metals and and 8-OHdG in Group 2

8-OHdG	Cadmium		Chromium		Mercury		Lead		Selenium		Manganese	
	R	P	R	p	R	P	R	P	R	P	R	P
	0.603	0.065	0.309	0.385	0.457	0.184	0.407	0.243	0.210	0.560	0.861	0.001

IV. DISCUSSION

The results obtained as presented above have shown high levels of metals in the blood samples of rats fed with river water from a crude oil impacted environment as compared to the control group. The observed differences in the levels of these metals in the two study groups were equally found to be statistically significant ($P<0.05$). This observation evidently points to the fact that different amounts of these heavy metals are contained in crude oil and it has been shown that water bodies around areas where crude oil drilling and refining activities take place contain different significant amounts of heavy metals and other bio hazardous materials [3] [10] [11] [12]. The corresponding high level of 8-OHdG observed in rats fed with water from the oil producing environment suggest that the metals elicited the production of the oxidative stress biomarker (8-OHdG) in the rats. This supports the results of previous studies carried out in humans [13] [14]and rats [15]. This is further substantiated by the positive correlations the metals had with 8-OHdG.This finding suggests that the metals present in the blood of these rats fed with water from the crude oil impacted community elicited an increase in the level of 8-OHdG which is an indicator of oxidative DNA damage. These heavy metals are known to be capable of causing different adverse effects in living systems. Most of these toxic effects are mediated through the process of oxidative stress due to accumulation of reactive oxygen species in the living systems. Oxidative DNA damage has been linked to a broad range of diseases and is widely recognized for its contributory role in the initiation and progression of various types of cancers [16] [17]. Notably, 8-hydroxy-2′-deoxyguanosine (8-OHdG) is a predominant oxidative base lesion that is used as a biomarker of oxidative stress and cancer, and is frequently used to estimate the extent of DNA damage in patients following exposure to carcinogens such as tobacco smoke, asbestos fibres, or heavy metals [18]. Other conditions associated with oxidative DNA damage include neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease, autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus (SLE); as well as diabetes, cardiovascular disease, and cystic fibrosis [19].

V. CONCLUSION

There is significant bioaccumulation of heavy metals in rats fed with water from the river in Igbeta Ewoama community where impact of oil producing activities is evident. The Level of 8- OHdG was equally higher and also statistically significant as compared to the control group. These findings bring to limelight the health and environmental hazard faced by people living in these areas as they consume water, fish and other aquatic animals from this river.

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