Toxicological Studies On Heavy Metal Bioaccumulation And Oxidative DNA Damage In Residents Of An Oil Producing Area

Ifenkwe John Chimerenka, Nwanjo Harrison Ugochukwu, Nwosu Dennis Chimezie, Ogbotobo Roland Fiezibe

Abstract: This study was carried out on blood samples collected from 200 residents each from Igbeta-Ewoama (Oil Producing Area) and Odi (Non-Oil Producing Area) both in Bayelsa State, Nigeria, to determine the blood levels of Cadmium, Chromium, Lead, Mercury and Selenium and also the oxidative DNA damage marker 8-Hydroxy-2-deoxyguanosine (8-OHdG). Blood samples were also analyzed for WBC, Hb, platelets, prothrombin time and serum concentrations of AST, ALT, albumin, ALP, bilirubin, gamma GT, total protein, sodium, potassium, chloride, bicarbonate, urea and creatinine. Residents in Igbeta-Ewoama had the highest blood levels of Mercury, Lead, Selenium, Cadmium, Chromium and 8-OHdG and they were all significantly different at $p<0.05$. Residents in Igbeta-Ewoama had the highest mean values of all the measured liver and kidney functions parameters with the exception of albumin, total protein, sodium, chloride and bicarbonate. These values were all statistically different at $P<0.05$. There was significant difference in the levels of Hb, WBC and Platelet while the length of bleeding time was prolonged in the residents of Igbeta-Ewoama community. The findings of this study suggest that the probability of occurrence of diseases associated with metal toxicity and oxidative stress might be higher among residents in the oil producing area.

Keywords: Heavy Metal, Bioaccumulation, Oxidative Stress, DNA Damage Contaminants Reactive Oxygen Species.

1 INTRODUCTION

Bayelsa State is located in the Niger Delta region of Southern Nigeria. It is a centre of oil and gas production activities. These activities include drilling, storage, refining and transportation of products. Large amount of petroleum products and its wastes are discharged into the surrounding environments in the course of these activities. Crude oil contains different proportions of 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 oils having relatively high concentrations of Fe, Zn, Cu, Pb and Hg [1]. Heavy metals represent a unique aspect of toxicology in that they do not undergo breakdown into other metals. Unlike organic contaminants, they are not degraded further and also cannot decompose into other chemicals with time [2]. This results to their bioaccumulation in the ecosystem, agriculture and the human body. Their absorption, disposition and excretion are largely dependent on physical factors such as solubility, ionization, particle size and chemical species(especially for metal salts).

- *Ifenkwe John Chimerenka Ph.D., is a Medical Laboratory Scientist currently working in the Chemical Pathology Department of Health Check Services, Yenagoa, Bayelsa State, Nigeria. E-Mail: healthcheck.hc@gmail.com*
- *Nwanjo Harrison Ugochukwu Ph.D., is a professor of Chemical Pathology and currently the Dean of Faculty of Health Sciences, Imo State University, Owerri, Nigeria*
- *Nwosu Dennis Chimezie Ph.D., is a professor of Chemical Pathology and currently the Head of the Department of Medical Laboratory Science, Imo State University, Owerri, Nigeria*
- *Ogboto Roland Fiezibe M.Sc., is a Medical Laboratory Scientist currently working in the Chemical Pathology Department of Federal Medical Centre, Yenagoa, Bayelsa State*

Heavy metals exhibit toxicity at low concentrations and the toxic effects can be long lasting due to their accumulation in the biota .A peculiar feature of these xenobiotics is the induction of oxidative stress [3]. Oxidative Stress is a condition characterized by 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), proteins, lipid membranes or by oxidatively inactivating specific enzymes by oxidation of Co-factors [4]. Some basic properties which have a bearing on the expression of toxicity by metals include the fact that metals seldom interact with biological systems in the elemental form but are usually active in the ionic form. [5]. Availability of metal ions to biological processes is often dependent on solubility. Soluble salts of metals readily dissociate in the aqueous environment of biological membranes, making transport into the body easy. On the other hand, insoluble salts are poorly absorbed (for example, reduction of chromium (VI) to the less soluble chromium (III) will decrease its absorption. [5]. Absorption of solute salts may be modified by the formation of insoluble compounds in biological materials. A typical example is the reduction of lead absorption by high dietary levels of phosphate due to the formation of insoluble lead phosphate. Some metals are produced as alkyl compounds. These are often very lipid soluble and readily pass across the lipid phase of biological membranes. Examples include methylmercury and organo-tin compounds. Strong attractions between metal ions and organic compounds will influence the deposition of metals and their rate of excretion. Most of the toxicologically important metals bind strongly to tissues, and are only excreted slowly and therefore tend to accumulate on continued exposure. Affinities for different tissues vary significantly. Elements such as lead are bound in the bones while mercury and cadmium localize in the kidneys [6]. Even though the Niger Delta area has grown to be fairly dependent on oil and has equally become the center of current industrial development and economic activities, considerations are rarely made on how oil exploration

processes create environmental, health, and social problems in local communities near oil producing fields. The necessity for this study is rooted on the fact that there is bioaccumulation of heavy metals in plants growing in crude oil contaminated soil [7]. These plants include food crops such as yam, cassava, vegetables and fruits. These plants are consumed by both man and animals living in these areas. As part of the food chain, the animals that feed on the plants are equally consumed by residents in these areas, thus doubling the effect. Some of these plants are equally used for medicinal preparations and thus in the bid to find curative solutions for medical problems, harmful agents may be inadvertently introduced into the body system. Heavy metals are equally bio-accumulated in aquatic species/animals living in crude oil polluted environments [8]. These aquatic species include fishes, crustaceans (crabs, crayfish, lobsters, shrimps prawns etc) and molluscs (periwinkles and water snails). These aquatic species are widely consumed in these areas as they serve as the major protein source for the residents. They are equally sold in different markets to unsuspecting consumers. There are high levels of heavy metals in crude oil impacted rivers above the WHO acceptable limits [9]. These rivers in most cases serve as the only source of water for drinking, cooking and all that water is needed for to the residents in these areas. From the foregoing, it becomes expedient to assess the levels of heavy metals and oxidative stress markers in residents of these communities who are exposed to these pollutants, drink from the polluted water, eat plants harvested from the surrounding soil and equally consume aquatic species found in these environments. Bearing in mind the cumulative health effects that may result from these, this study tried to explore the impact of crude oil contamination on human health to improve mitigation efforts and also to prevent adverse health effects on individuals living in affected areas.

2.0 Materials and Methods

2.1 Study Area

This study was carried out in two communities in Bayelsa state.

(a) Igbeta-Ewoama Community (Oil Producing Area). Igbeta-Ewoama is a community in Nembe Local Government Area of Bayelsa State. It has several oil wells operated by the Nigeria Agip Oil Company (NAOC). Oil drilling activities in Igneta-Ewoama has been on for over 40 years. Residents here are mainly fishermen due to the limited availability of land compromised by large expanse of water. This area is rich in aquatic splendours such as fishes, lobsters, shrimps, crabs oysters, periwinkles and other crustaceans.

(b) Odi Community (Non-Oil Producing Community).The community is located in Kolokuma/Opokuma Local Government Area of Bayelsa state. The two communities are located in Bayelsa State which lies within, latitude 04^0 15^1 North, 05^0 23^1 South and longitude 05^0 22^1 West and 06^0 45^1 East [10]. Igbeta-Ewoama is the test community while Odi community served as control.

2.2 Study Population

A total of 400 subjects were recruited for this study. This comprised:

a. 200 participants from Igbeta-Ewoama community (Oil Producing Area)
b. 200 participants from Odi (Non-oil producing community) as control.

Of the 200 subjects from Igbeta-Ewoama community, 77 were males aged between 2 and 77 years. 123 were females aged between 2 and 80 years. In Odi community, 106 were males aged between 2 and 80 years while 94 were females aged between 2 and 79 years.

2.3 Advocacy, Mobilization and Pre-survey Contacts

Ethical clearance was obtained from the Bayelsa State Ministry of Health. The traditional rulers as well as members and leaders of the community development committees of each community were met and informed about the study that was to be carried out in their various communities. Their co-operation and support were solicited in mobilizing their subjects. Meetings were held with the members of the communities and informed consent was obtained from the subjects recruited into the study.

2.4 Selection Criteria

Questionnaire was used to obtain the required information needed for inclusion or exclusion of participants.

2.4.1 Inclusion Criteria

Subjects two years and above that consented to the study were included. The sample population was classified according to sex and age groups.

2.4.2 Exclusion Criteria

Tobacco smokers and subjects with known illnesses such as cancer, diabetes mellitus and Parkinson's disease were excluded from this study. This is because cigarette smoke is an exogenous source of oxidative stress [11] while levels of oxidative stress biomarkers are known to be raised in the above mentioned disease conditions [12].

2.5 Sample Collection

Blood samples were collected by venipuncture using pyrogen free sterile disposable syringes. Samples for measurement of the oxidative stress biomarker (8-OHdG) and biochemical parameters were collected into plain serum separating tubes. These were allowed to stand for 10-20 minutes after which they were centrifuged at 3,000 rpm for 20 minutes and the serum separated using a Pasteur's pipette. Samples for measurement of haematological parameters (prothrombin time not inclusive) and heavy metals were collected into K_3 EDTA anti coagulated plastic bottles and mixed thoroughly by gentle repeated turning. Samples for prothrombin time were dispensed into containers containing 3.2% tri sodium citrate at a ratio of 9 parts of blood to 1 part of 3.2% tri sodium citrate. The samples were centrifuged for 15 minutes at 3000 rpm to obtain platelet poor plasma. The supernatant plasma was subsequently transferred into plain appendorf tubes.

2.6 Laboratory Procedures

All reagents were commercially purchased from Afro Famous Nigeria Limited, Abakpa Nike, Enugu, Enugu State and all manufacturers' SOPs were followed strictly.

(A) 8- Hydroxy -2- Deoxyguanosine (8-OHdG) [13].
The Elabscience oxidative DNA damage Elisa kit which is a competitive ELISA technique for the quantitative measurement of 8-OHdG was used

(B) Measurement of Cd, Se, Hg, Pb and Cr
Measurement of these metals was carried out on 240 FS AA Agilent Technologies flame atomic absorption spectrometer with deuterium lamp background correction.

(**C) Alanine Aminotransferase (ALT)**[14]
RANDOX ALT kit was used.

(D) Aspartate Aminotransferase (AST) [14]
RANDOX AST test kit was used.

(E) Albumin [15]
RANDOX Albumin test kit was used.

(F) Bilirubin [16]
RANDOX Bilirubin kit was used.

(G) Alkaline Phosphatase (ALP) [17].
TECO Diagnostics, California, USA direct colorimetric ALP reagent kit was used.

(H) Gamma-glutamyl Transferase (γ-GT)[17]
RANDOX colorimetric (Kinetic Method) test kit was used.

(I) Urea [16]
RANDOX Urease-Berthelot Colorimetric method kit was used.

(J) Creatinine [18]
RANDOX Creatinine kit was used.

(K) Electrolytes (Na^+, K^+, Cl^-, $HCO3^-$) [19]
EA-1000B ISE electrolyte analyzer from Perlong Medical Equipment Company was used to measure these parameters.

(L) Platelets, Haemoglobin (Hb) [20].
SYSMEX pocH-100i automated haematology analyzer was used for the measurement of platelets in the study population.

(M) Prothrombin Time (PT) [21]
AGAPE Diagnostics Switzerland Prothrombin Time kit was used.

2.7 Statistical Analysis

Data was analyzed using Statistical Package for Social Sciences (SPSS) statistical software (Version 17 for windows) (SPSS Inc, Chicago, USA). Results were expressed as mean and standard deviation and were presented in tables. Test of significance was done using Z-test, Pearson correlation coefficient statistics and Tukey HSD post HOC test. Values above 95% confidence limit were considered statistically significant.

3.0 RESULTS

Table 1 shows blood heavy metal and serum 8-OHdG concentrations measured in the two study groups. Highest levels of all the measured heavy metals and 8-OHdG were observed in subjects residing in Igbeta-Ewoama community. Z-test of the means of the two groups showed statistically significant differences for all the measured metals and 8-OHdG ($p<0.05$)

Table 1: *Heavy Metal and 8-OHdG Levels in the Study Populations.*

Study Community	Cd(ppm)	Cr(ppm	Hg(ppm)	Pb(ppm)	Se(ppm)	8-OHdG
Igbeta-Ewoama (n = 200)	0.226 ± 0.406	0.055 ± 0.122	0.331 ± 0.62	1.256 ± 2.34	1.060 ± 0.51	122.57 ± 28.33
Odi (n = 200)	0.017± 0.01	0.004 ± 0.011	0.081 ± 0.098	0.929 ± 0.314	0.037 ± 0.064	25.17 ± 12.16
P Value	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.0001(S)

S=Significant

The effect of gender induced differences on the bioaccumulation of the measured metals and 8-OHdG in the two groups is as presented in table 2. It shows that males in Igbeta-Ewoama community had highest levels of Cadmium, Chromium and Mercury while females had the highest levels of Lead, Selenium and 8-OHdG. These observed variations in concentrations among the two sub-groups were statistically significant in all the parameters $P<0.05$ (Table 2). Results from Odi community show that females had higher mean values of Cadmium, Chromium, Lead and Selenium while males had higher mean values of Mercury and 8-OHdG than females. These observed differences were significant in all the measured metals except in Cadmium and Lead at $P<0.05$ (Table 2). The mean concentrations of the measured heavy metals and 8-OHdG in the different age groups of Igbeta-Ewoama community are presented in table 3. Test of significance of these observed age induced differences using Tukey Post HOC analysis are presented in table 4. The highest mean level of Cadmium was observed in residents 31- 40 years old (0.425 ± 0.75) while the least mean level (0.061± 0.03ppm) was observed in residents 51 – 60 years old. These values were not statistically different at $P<0.05$ (Table 4). The highest mean value of chromium (0.131 ± 0.193ppm) was recorded in the age group 31-40 years while the least value (0.003 ± 0.007ppm) was recorded among residents

Table 2: *Heavy Metals and 8-OHdG Levels in Males and Females.*

Igbeta-Ewoama					Odi			
Males n = 77		Females n =123	F value	P value	Males n = 106	Females n = 94	F value	P value
Cd(ppm)	0.271 ± 0.601	0.122 ± 0.18	6.629	0.011(S)	0.017 ±0.014	0.018 ± 0.006	0.412	0.522
Cr(ppm)	0.082 ± 0.161	0.004 ± 0.009	28.813	<0.0001(S)	0.04 ± 0.013	0.004 ± 0.009	505.75	<0.0001(S)
Hg(ppm)	0.403 ± 0.92	0.166 ± 0.20	7.61	0.006(S)	0.109 ± 0.122	0.052 ± 0.06	16.89	<0.0001(S)
Pb(ppm)	0.755 ± 0.18	1.126 ± 0.29	101.43	<0.001(S)	0.732 ± 0.212	0.741 ± 0.18	0.103	0.748
Se(ppm)	1.039 ± 0.59	1.216 ± 0.42	6.123	0.014(S)	0.051 ± 0.07	0.022 ± 0.05	10.56	0.001(S)
8-OHdG (pg/ml)	94.86 ± 25.14	104.5 ± 24.4	7.108	0.011(S)	28.13 ± 23.89	23.30 ± 27.89	1.742	0.1884

S = Significant

Table 3*: Heavy Metals and 8-OHdG Levels in Subjects in Relation to Age in Igbeta-Ewoama Community.*

Age Range	Cd(ppm)	Cr(ppm	Hg(ppm)	Pb(ppm)	Se(ppm)	8-OHdG (pg/ml)
2– 10(n=30)	0.249 ±0.234	0.022 ±0.042	0.286 ± 0.403	0.689 ± 0.12	0.474 ± 0.109	95.60 ± 9.12
11 –20(n=34)	0.283 ± 0.26	0.080 ± 0.14	0.422 ± 0.41	1.079 ± 0.433	0.897 ± 0.46	105.80 ± 5.12
21 – 30 (n=26)	0.388 ± 0.51	0.011 ± 0.034	0.542 ± 0.40	1.001 ± 0.193	0.900 ± 0.44	108.20 ± 15.26
31 – 40 (n=30)	0.425 ± 0.75	0.131 ± 0.193	0.643 ± 0.856	0.717 ± 0.17	0.907 ± 0.68	110 ± 21.21
41 – 50 (n=23)	0.125 ± 0.75	0.08 ± 0.11	0.341 ± 0.48	0.701 ± 0.15	1.00 ± 0.56	70.20 ± 20.26
51 – 60(n=25)	0.061± 0.03	0.05 ± 0.01	0.081 ± 0.068	0.719 ± 0.20	1.07 ± 0.34	97.40 ± 31.93
61 – 70(n=15)	0.094 ± 0.03	0.034 ± 0.01	0.092 ± 0.07	0.704 ± 0.13	1.14 ± 0.33	95.40 ± 31.13
71 – 80(n=17)	0.136 ± 0.23	0.003 ± 0.007	0.177 ± 0.09	0.876 ±	1.36 ± 0.308	93.60 ± 30.30

71-80 years. This difference was not statistically significant. Subjects 31-40 years old, had the highest mean concentrations of mercury (0.643 ± 0.856ppm), while those 2 – 10 years old had the least values (0.286 ± 0.403). This age induced difference was not statistically significant. Residents between the ages of 11 – 20 years recorded the highest levels of Lead (1.079 ± 0.433ppm) while those 41 – 50 years had the least values (0.701 ± 0.15ppm). Selenium (1.36 ± 0.308ppm) was found to be highest in residents between 71 – 80 years old while the least value of 0.474 ± 0.109ppm was observed in those 2 – 10 years old. This observed difference was statistically significant at P <0.05 (Table 4). Residents within the ages of 31 – 40 years had the highest mean concentration of 8-OHdG (110 ± 21.21pg/ml) while least levels of 8-OHdG (93.60 ± 30.30) was observed in residents between the ages of 71 – 80 years. These differences were not statistically significant at P<0.05. The mean concentrations of the measured heavy metals and 8-OHdG in the different age groups of Odi community are presented in table 5. Test of significance of these observed age induced differences using Tukey HSD post HOC test are presented in table 6.The highest mean value of Cadmium (0.022 ± 0.01ppm), Mercury (0.097 ± 0.08ppm) and Selenium (0.098 ± 0.08ppm) were recorded in residents between the ages of 71 – 80 years. Highest mean values of Chromium (0.038 ± 0.07ppm) and Lead (0.919 ± 0.25ppm) were recorded in residents 11 – 20 and 31 – 40 years respectively. Children 2 – 10 years old had the least mean values of Cadmium (0.012 ± 0.008ppm), Lead (0.502 ± 0.101ppm) and Selenium (0.045 ± 0.024ppm). Least mean values of Chromium (0.001 ± 0.001ppm) and Mercury (0.001 ± 0.001ppm) were observed in residents 71 – 80 and 21 – 30 years respectively. These observed differences in these age groups were statistically different at P<0.05. The highest mean concentration of 8-OHdG (52.6 ± 11.59pg/ml) was recorded in residents 71 -- 80 years old while residents that were between 2-10 years old recorded the least mean values. This measured difference was statistically significant at P<0.05. Table 7 shows the correlation between measured metals and 8-OHdG in Igbeta-Ewoama community. A strong and positive correlation was observed between Mercury and 8-OHdG (r = 0.523). 8-OHdG correlated weakly but positively with Lead (r = 0.321). A weak and negative correlation was however observed between selenium and 8-OHdG (r = -0.340). These observed correlations were statistically significant at P<0.005. There was no significant correlation between 8-OHdG and the other measured heavy metals. Table 8 shows the mean values of measured liver function parameters in the study groups. It shows that residents in

Igbeta-Ewoama community had the highest mean values of all the measured liver function parameters with the exception of albumin and total protein which were highest in the residents of Odi community. Results of the kidney function parameters measured in the study populations are presented in table 9. The table shows that highest levels of sodium (141 ± 2.2mmol/l), chloride (103 ± 3.6) and bicarbonate (29.8 ± 4.4) were recorded in residents in Odi. Residents in Igbrta-Ewoama had the highest levels of potassium (4.6 ± 0.5mmol/l), creatinine (104 ± 3.0µmol/l) and urea (6.1 ± 1.0mmol/l). These differences were statistically significant at $p < 0.05$.

Table 4: *Comparison of Heavy Metals and 8-OHdG Levels in Subjects in Relation to Age in Igbeta-Ewoama Community.*

Age Range	Cd (P Value)	Cr (P Value)	Hg (P Value)	Pb(P Value)	Se (P Value)	8-OHdG (P Value)
2-10 vs 11-20	1.0000	0.5595	0.9736	0.0000	0.0331	0.7160
2-10 vs 21-30	0.9702	1.0000	0.5781	0.0002	0.0349	0.4720
2-10 vs 31-40	0.8812	0.0085	0.1323	0.9999	0.0210	0.2363
2-10 vs 41-50	0.9902	0.6714	1.0000	1.0000	0.0083	0.0054(S)
2-10 vs 51-60	0.8898	0.9892	0.8460	0.9999	0.0008	1.0000
2-10 vs 61-70	0.9627	0.0000	0.8907	1.0000	0.0002	1.0000
2-10 vs 71-80	0.9968	0.9996	0.9976	0.2606	0.0000	1.0000
11-20 vs 21-30	0.9876	0.1926	0.9751	0.8862	1.0000	0.9999
11-20 vs 31-40	0.9176	0.5186	0.5453	0.0000	1.0000	0.9906
11-20 vs 41-50	0.9319	1.0000	0.9988	<0.0000	0.9933	<0.001(S)
11-20 vs 51-60	0.6547	0.9690	0.1440	0.0000	0.8635	0.8381
11-20 vs 61-70	0.8314	0.0000	0.2005	0.0000	0.5556	0.6632
11-20 vs 71-80	0.9740	0.3070	0.7225	0.0980	0.0341	0.6187
21-30 vs 31-40	1.0000	0.0003	0.9892	0.0001	1.0000	0.9999
21-30 vs 41-50	0.5069	0.3256	0.8152	0.0002	0.9949	<0.001(S)
21-30 vs 51-60	0.1849	0.8893	0.0117	0.0004	0.8819	0.6046
21-30 vs 61-70	0.3393	0.0000	0.0202	0.0002	0.5886	0.4128
21-30 vs 71-80	0.6900	1.0000	0.2356	0.6785	0.0399	0.3962
31-40 vs 41-50	0.2856	0.6708	0.2935	1.0000	0.9959	<0.001(S)
31-40 vs 51-60	0.0704	0.0879	0.0003	1.0000	0.8838	0.3281
31-40 vs 61-70	0.1613	0.0000	0.0008	1.0000	0.5798	0.1888
31-40 vs 71-80	0.4838	0.0037	0.0353	0.3312	0.0353	0.2018
41-50 vs 51-60	0.9998	0.9825	0.6020	1.0000	0.9996	0.001(S)
41-50 vs 61-70	1.0000	0.0000	0.6779	1.0000	0.9752	0.0044(S)
41-50 vs 71-80	1.0000	0.4087	0.9711	0.3305	0.2986	0.0336(S)
51-60 vs 61-70	1.0000	0.0000	1.0000	1.0000	0.9996	1.0000
51-60 vs 71-80	0.9997	0.8879	0.9987	0.4396	0.5501	0.9995
61-70 vs 71-80	1.0000	0.0000	0.9995	0.3406	0.8473	1.0000

Table 10 shows the mean levels of the different measured haematological parameters in the study communities. There was statistically significant lower levels of Hb, WBC and Platelet in the test community (Igbeta-Ewoama) ($p<0.05$) compared to the control community (Odi). Subjects in Odi community recorded the highest levels of Hb (12.02 ± 1.53g/dl), WBC (6.6 ± 0.63 $\times 10^9$/l) and Platelets (208.5 ± 7.52 $\times 10^9$/l). On the other hand, the length of bleeding time was prolonged in the residents of Igbeta-Ewoama community (13.16 ± 1.3 seconds) as compared to subjects from Odi community (12.74 ± 1.09). These differences in prothrombin time in the two populations were statistically significant.

Table 5: *Heavy Metals and 8-OHdG Levels in Subjects in Relation to Age in Odi Community.*

Age Range	Cd(ppm)	Cr(ppm	Hg(ppm)	Pb(ppm)	Se(ppm)	8-OHdG (ng/ml)
2-10(n=27)	0.012 ± 0.008	0.021 ± 0.004	0.016 ± 0.014	0.502 ± 0.101	0.045 ±0.024	27.70 ± 11.22
11- 20(n=36)	0.018 ± 0.004	0.038 ± 0.07	0.021 ± 0.02	0.612 ± 0.16	0.081 ± 0.011	30.80 ± 9.96
21-30(n=26)	0.016 ± 0.004	0.010 ± 0.002	0.001 ± 0.001	0.666 ± 0.17	0.056 ± 0.063	33.50 ± 10.06
31 – 40(n=32)	0.013 ± 0.003	0.012 ± 0.002	0.002 ± 0.002	0.919 ± 0.25	0.055 ± 0.05	30.80 ± 10.06
41- 50(n=20)	0.014 ± 0.002	0.009 ± 0.001	0.06 ± 0.04	0.900 ± 0.09	0.057 ± 0.004	38.40 ± 11.06
51- 60(n=21)	0.016 ± 0.02	0.006 ± 0.001	0.06 ± 0.04	0.90 ± 0.09	0.060 ± 0.04	43.80 ± 17.06
61- 70(n=20)	0.017 ± 0.01	0.004 ± 0.001	0.07 ± 0.08	0.864 ± 0.08	0.065 ± 0.07	48.30 ± 12.06
71- 80(n=18)	0.022 ± 0.01	0.001 ± 0.001	0.097 ± 0.08	0.818 ± 0.08	0.098 ± 0.08	52.67 ± 13.59

Table 6: *Comparison of Heavy Metals and 8-OhdG Levels in Different Age Groups in Odi Community*

Age Range	Cd (P Value)	Cr (P Value)	Hg (P Value)	Pb (P Value)	Se(P Value)	8OHdG (P Value)
2-10 vs 11-20	0.1445	0.2479	0.9999	0.0567	0.1371	0.9681
2-10 vs 21-30	0.7280	0.8390	0.9458	0.0011(S)	0.9950	0.6222
2-10 vs 31-40	0.9999	0.9202	0.9509	<0.0001(S)	0.9962	0.9724
2-10 vs 41-50	0.9948	0.8275	0.0408(S)	<0.0001(S)	0.9945	0.0466(S)
2-10 vs 51-60	0.7821	0.5861	0.0359(S)	<0.0001(S)	0.9776	0.0001(S)
2-10 vs 61-70	0.5493	0.4385	0.0039(S)	<0.0001(S)	0.9052	0.0001(S)
2-10 vs 71-80	0.0001(S)	0.0616	<0.0001(S)	<0.0001(S)	0.0011(S)	0.0001(S)
11-20 vs 21-30	0.9881	0.0031	0.7311	0.8256	0.5970	0.9863
11-20 vs 31-40	0.2914	0.0039	0.7247	<0.0001(S)	0.4698	1.0000
11-20 vs 41-50	0.7428	0.0058	0.0706	<0.0001(S)	0.7344	0.2875
11-20 vs 51-60	0.9919	0.0011	0.0623	<0.0001(S)	0.8353	0.0020(S)
11-20 vs 61-70	0.9999	0.0005	0.0068(S)	<0.0001(S)	0.9596	0.0001(S)
11-20 vs 71-80	0.4583	.0001(S)	<0.0001(S)	<0.0001(S)	0.8294	0.0001(S)
21-30 vs 31-40	0.9066	1.0000	1.0000	<0.0001(S)	1.0000	0.9882
21-30 vs 41-50	0.9950	1.0000	0.0011(S)	<0.0001(S)	1.0000	0.8549
21-30 vs 51-60	1.0000	0.9997	0.0009(S)	<0.0001(S)	1.0000	0.0614
21-30 vs 61-70	0.9999	0.9962	<0.0001(S)	0.0002(S)	0.9992	0.0009(S)
21-30 vs 71-80	0.1082	0.8878	<0.0001(S)	0.0005(S)	.0278(S)	0.0001(S)
31-40 vs 41-50	0.9999	0.9999	0.0007(S)	0.9998	1.0000	0.3150
31-40 vs 51-60	0.9311	0.9946	0.0006(S)	0.9998	1.0000	0.0028(S)
31-40 vs 61-70	0.7636	0.9729	<0.0001(S)	0.8799	0.9978	0.0001(S)
31-40 vs 71-80	0.0004(S)	0.6674	<0.0001(S)	0.0461(S)	0.0105(S	0.0001(S)
41-50 vs 51-60	0.9963	1.0000	1.0000	1.0000	1.0000	0.8213
41-50 vs 61-70	0.9630	0.9992	0.9978	0.9933	0.9997	0.1394
41-50 vs 71-80	0.0191(S)	0.9605	0.0739	0.3865	0.0754	0.0058(S)
51-60 vs 61-70	1.0000	1.0000	0.9976	0.9927	1.0000	0.9230
51-60 vs 71-80	0.1699	0.9973	0.0645	0.3637	0.1152	0.2713
61-70 vs 71-80	0.4110	0.9999	0.3996	0.9295	0.2757	0.9457

Table 7: *Correlation between Measured Heavy Metals and 8-OHdG in Igbeta-Ewoama Community*

8OHdG	Cadmium		Chromium		Mercury		Pb(ppm)		Se(ppm)	
	R	P	R	P	R	P	R	P	R	P
	0.067	0.346	0.016	0.822	0.523	<0.0001(S)	0.321	<0.0001(S)	-0.340	<0.0001(S)

Table 8: *Mean and SD Values of Liver Function Parameters in the Study Populations*

STUDY COMMUNITY n = 200	ALT (U/l)	AST (U/l)	G-GT (U/l)	ALP (U/l)	Albumin (g/l)	TP (g/l)	TB (µmol/l)	CB (µmol/l)
Igbeta-Ewoama	8.4 ± 1.1	11.8 ± 2	38.7 ± 3.6	26 ± 2	37.8 ± 2	63.3 ± 1	8.7 ± 0.06	2.4 ± 0.20
Odi	5.6 ± 1.3	9.5 ± 1.8	25.5 ± 4.2	22.3 ± 1	38.5 ± 2	63.8 ± 1.3	5.7 ± 0.1	2.0 ± 0.11
P Value	<0.0001(S)	<0.0001(S)	<0.001(S)	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.001(S)

S = Significant

Table 9: *Mean and SD Values of Kidney Function Parameters in the Study Populations.*

Study Community	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)	HCO_3^- (mm0l/l)	Creatinine (µmol/l)	Urea (mmol/l)
Igbeta-Ewoama	139 ± 3.5	4.6 ± 0.5	101 ± 4.1	26.4 ± 3.8	104± 3.0	6.1 ± 1.0
Odi	141 ± 2.2	4.0 ± 0.5	103 ± 3.6	29.8 ± 4.4	97.7± 4.0	6.0 ± 0.7
P Value	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.0001(S)

S = Significant

Table 10: *Mean and SD Values of Haematological Parameters in the Study Populations.*

STUDY COMMUNITY	Hb (g/dl)	WBC ($\times 10^9$/l)	Platelet ($\times 10^9$/l)	PT (Seconds)
Igbeta-Ewoama	11.8 ± 1.4	5.4 ± 0.47	191.6 ± 5.8	13.16 ± 1.3
Odi	12.02 ± 1.5	6.6 ± 0.63	208.5 ± 7.5	12.74 ± 1.1
P Value	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.001(S)

S = Significant

4.0 DISCUSSION

The results of this study have shown high levels of bioaccumulation of metals in the blood samples of residents in the area where oil producing activities are carried out in relation to the control community. The differences in the levels of these measured metals in the blood samples of these residents were equally found to be statistically significant ($P<0.05$). All the measured levels of these metals in the two communities were above the recommended reference ranges except for selenium which was found to be within the reference range in the control community. These findings, point to widespread pollution which varies significantly depending on the predominant activities in the area. The observed metal levels in the oil producing area may have resulted from spilled oil which contaminates both vegetation and aquatic life. Contaminated vegetation, aquatic animals and water are consumed by these residents causing an accumulation of metals in their bodies over time as the food chain progresses (22) as revealed by the results obtained. Males in the oil producing community (Igbeta-Ewoama) had the highest levels of all the metals and all the measured differences were significantly different at $P<0.05$. Some authors also reported significantly higher blood levels of lead and cadmium in males than in females [23], cadmium [24], lead [25], and nickel [26]. Conversely, some other authors [27] and [24] reported no difference between heavy metals blood concentrations in men and women. Evidently, gender is only one of many factors influencing blood heavy metal concentrations. An assessment of the effect of age differences on heavy metal bioaccumulation in the subjects show that highest levels of cadmium, chromium and mercury were recorded in residents 31 – 40 years while highest levels of lead and selenium were recorded in residents between 11 – 20 years. The presence of high amounts of these metals in these younger ages may indicate serious medical concerns. This is as a result of the known toxic natures of these metals which could lead to eventual mortality of the residents with high concentrations of these metals before they get to older ages. In this study, the serum level of the oxidative stress biomarker and indicator of DNA oxidative damage (8-OHdG) was higher in Igbeta-Ewoama residents than in the control community just as observed in the measured metals. The results suggest that the metals influenced the concentrations of the oxidative stress biomarker as can be seen in the positive correlations between them. This suggests strongly that Mercury, Lead and Selenium are strong oxidative factors eliciting high levels of 8-OHdG which is an indicator of oxidative DNA damage. Humans are exposed to these metals from

numerous sources including contaminated air, water, soil and food. Lead is known to affect the kidneys, heart and male gonads [28]. Serum 8-OHdG was found to be significantly higher in patients with colorectal cancer compared with patients without colorectal cancer [29]. Exposure to Cadmium can cause a variety of pathological alterations in several organs and tissues as well as induce diabetic complications, hypertension and osteoporosis [30]. Lead, cadmium and mercury deplete cells' major anti-oxidants and enzymes. Either redox-active or redox-inactive metals may cause an increase in production of reactive oxygen species such as the hydroxyl radical (HO-), superoxide radical (O_2^-) or hydrogen peroxide (H_2O_2). Enhanced generation of reactive oxygen species can overwhelm cells' intrinsic antioxidant defences and result in oxidative stress. The results of this study suggest a higher possibility of occurrence of renal impairment in residents of the oil producing area. This assertion is supported by the significantly increased levels of serum urea and serum creatinine found in this community compared to those in the control community. This is in line with the findings by Egwurugwu *et al* [31] who also reported significantly increased serum concentrations of urea, creatinine, potassium, uric acid and inorganic phosphate in subjects exposed to oil and gas pollutants. Crude oil has also been reported to cause destruction of the renal reserve capacity and also induced several pathological changes in the form of tubular necrosis in laboratory animals (32). The results also suggest a higher possibility of liver impairment in residents of Igbeta-Ewoama communities due to significantly higher levels of AST, ALT, ALP, Gamma-GT, total bilirubin and conjugated bilirubin measured in the subjects of this community as compared to the control community. Exposure to crude oil is known to cause some adverse changes in haematological parameters. These changes affect blood and blood-forming cells negatively and could give rise to anaemia (aplastic), pancytopenia and leukaemia [33]. The results of the measured haematological indices equally show that the residents in the oil producing area may be prone to anaemia due to significantly lower levels of haemoglobin measured among them. They may also be prone to suppressed immunity as well as bleeding disorders as evidenced by the significantly lower levels of WBC, platelets and longer prothrombin time measured in their blood samples as compared to the control community.

5.0 CONCLUSION

The findings of this study reveal bioaccumulation of Cadmium, Chromium, Lead, Mercury and Selenium in blood samples of subjects in Igbeta-Ewoama community. Higher levels of the oxidative DNA damage biomarker: 8-Hydroxy-2-deoxyguanosine was equally observed. The following measured biochemical indices: AST, ALT, ALP, Gamma-GT, total bilirubin, conjugated bilirubin, potassium urea and creatinine were found to be elevated in subjects in the test community. The levels of these haematological indices: Hb, WBC and Platelets were significantly lower in the test community than in the control community. Prothrombin time was significantly increased in the test community. The findings therefore suggest that chronic exposure to Cadmium, Chromium, Lead, Mercury and Selenium may result in oxidative stress which is an indicator for likely risk for occurrence of diseases associated with oxidative damage such as atherosclerosis, respiratory disorders, neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases, cancer, diabetes mellitus and inflammatory diseases among residents in Igbeta-Ewoama community.

9. RECOMMENDATIONS

In view of the higher risk of exposure of people living in areas where oil producing activities are taking place and the poor regulation of oil producing activities in these places, it is strongly recommended that all safety standards and guidelines regarding safe oil exploration and production must be adhered to strictly in order to reduce and if possible avoid contamination of the environment with pollutants. A comprehensive investigation of the heavy metal content of regularly consumed foods and water should be performed. The fact that the oxidative stress marker (8-OHdG) correlated with some heavy metal accumulation makes a case for their use in monitoring environmental pollution. Since oxidative stress is scientifically proven to be an important mechanism for heavy metal toxicity, then the inclusion of antioxidants in the treatment of metal induced toxicity deserves further consideration.

REFERENCES

[1]. Kakulu, S.E. (1985). Heavy Metals in the Niger Delta: Impact of Petroleum Industry on the Baseline Levels. Ph.D Thesis University of Ibadan.

[2]. Akporhonor, E.E., Iwegbue, C.M.A., Egwaikikhide, P.A and Emua, S.A. (2007). Levels of Cadmium, Lead and Mercury in Organs of some Fish Species from Warri River, Nigeria. J. Chem. Soc. Nigeria, 32 (1) 221-226.

[3]. Szymańska-Chabowska, A. , Beck, A. , Poręba, R., Andrzejak, A. and Antonowicz- Juchniewicz, J.(2009). Evaluation of DNA Damage in People Occupationally Exposed to Arsenic and Some Heavy Metals. Polish Journal Of Environmental Studies. (18): 1131-1139.

[4]. Durackova, Z. (2010). Some Current Insights into Oxidative S Stress Physiology Research. (59): 459 – 468.

[5]. Winder, C. (2004). Toxicity of Metals, in: Occupational Toxicology. Stacey , N. H. Ed., Taylor and Francis , London: 294.

[6]. Duane, M.E. and James, Y.K. (1997) Cellular and Molecular Mechanisms of Metal Toxicities, CRC Press, New York: 189-219.

[7]. Essiet, U.A., Effiong, G.S., Ogbemudia, F.A. and Bruno, E.J. (2010) Heavy Metal Concentration in Plants Growing in Crude Oil Contaminated Soil in Akwa Ibom State, South Eastern Nigeria African Journal of Pharmcology and Physiology (7): 165 – 470.

[8]. Kpobari, W.N., Matthew, O.W. and Eka, B. E. (2013). Heavy Metals Concentrations in Four

Selected Sea Foods from Crude Oil Polluted Waters of Ogoniland, Rivers State,Nigeria. Archives of Applied Science Research 5 (4):99-104.

[9]. Uzoekwe, S.A., and Achudume, A.C. (2011) Pollution Status and Effect of Crude Oil Spillage in Ughoton Stream Ecosystem in Niger Delta. Journal of Ecology and the Natural Environment.3(15):469-473.

[10]. www.bayelsa.gov.ng Assessed July, 2017.

[11]. Block, C., Freyermuth, S., Beyersmann, D. and Malviya, A.N. (1992). Role of Cadmium in Activating Nuclear Protein Kinase C and the Enzyme Binding to Nuclear Protein. Journal o Journal of Biology and Chemistry (28):19824–19828.

[12]. Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur, M., Telser, J. (2007). Free Radicals and Antioxidants in Normal Physiological Functions and Human Disease. International Journal of Biochemistry and Cell Biology (23): 209-213.

[13]. Saito, S., Yamauchi, H., Hasul., Y., Kurashige, J., Ochi and Yoshida, K. (2000). Quantitative Determination of 8-Hydroxydeoxyguanosine by Using ELISA, Research Communications in Molecular Pathology and Pharmacology (107): 39-44.

[14]. Reitman, S and Frankel, S (1957) Alanine Aminotransferase American Journal of Clinical Pathology (28): 56.

[15]. Doumas, B. T., Watson, W.A, and Briggs, H.G. (1971). Albumin Clinica Chimica Acta (31): 87-96.

[16]. Jendrassik, L. and Grof, P. (1938). Bilirubin, Biochemistry (81): 297.

[17]. Kochmar, J.F. and Moss, D.W. (1976). Fundamentals of Clinical Chemistry Tietz Edition. W.B. Saunders and Company, Philadelphia: 604.

[18]. Slot, C. (1965). Scandinevia Journal of Clinical Laboratory Investigations. (17): 381– 387.

[19]. Vanencia, A., Arulselvi, S., Kanchana, R. And Ravindra, M.P. (2011). Agreement of Two Different Laboratory Methods Used to Measure Electrolytes. Journal of Laboratory Physicians (2): 104-109.

[20]. Whisler, S and Dahlgren, C. (2005). Performance Evaluation of the Sysmex poch-100i Automated Haematology Analyzer Laboratory Haematology (2): 101-117.

[21]. Dacie, J.V and Lewis, S.M. (1984).Practical Hematology: 120.

[22]. Idodo-Umeh G. and Ogbeibu A.E.(2010) Bioaccumulation of the Heavy Metals in Cassava Tubers and Plantain Fruits Grown in Soils Impacted with Petroleum and Non-Petroleum Activities, Research Journal of Environmental Science. (4): 33-41

[23]. Nwankwo C.N. and Ogagarue D.O. (2011). Effects of Gas Glaring on Surface and Ground Waters in Delta State Nigeria, Journal of Geology and Mining Research, 3(5): 131-136.

[24]. Sirivarasai, J., Kaojaren, S., Wananukul, W., Srisomerang, P. (2002). Non-Occupational Determinants of Cadmium and Lead in Blood and Urine Among a General Population in Thailand. Southeast Asian Journal of Tropical Medicine and Public Health, 33(1): 180-187.

[25]. Khassouani, C.E., Soulaymani, R., Mauras, Y., Allain, P. (2000). Blood Cadmium Concentration in the Population of the Rabat Area, Morocco. Clinica Chimica Acta, 302(1-2): 155-160.

[26]. Bushnik, T., Haines, D., Levallois, P., Levesque, J., Van Oostdam, J., Viau, C.(2010). Lead and bisphenol A concentrations in the Canadian population. Health Reports, 21(3): 1-12.

[27]. Ibeto, C.N., Okoye, C.O.B. (2010). High levels of Heavy Metalsin Blood of the Urban Population of Nigeria. Research Journal of Environmental Science (4): 371-382.

[28]. Olsson, I.M., Bensryd, I., Lundh, T., Ottosson, H., Skerfving, S., Oskarsson, A.(2002). Cadmium in Blood and Urine - Impact of Sex, Age, Dietary Intake, Iron Status, and Former Smoking - Association of Renal Effects. Environmental Health Perspective, 110(12): 1185–1190.

[29]. Adedara, I.A , Ebokaiwe, A.P and Farombi, E.O (2011). Tissues Distribution of Heavy Metals and Erythrocytes Antioxidant Status in Rats Exposed to Nigerian Bonny Light Crude Oil. Toxicology and Industrial Health: 1–7.

[30]. Yang, Y., Tian Y., Yan C., Jin, X., Tang, J. and Shen, X. (2008). Determinants of Urinary 8-Hydnoxyl-2-Deoxyguanosine in Chinese Children with Acute Leukemia, Environmental Toxicology. (16): 128 – 133.

[31]. Egwurugwu J.N., Nwafor A. , Oluronfemi O.J.. Iwuji S.C. and Alagwu E.A.(2013). Impact of Prolonged Exposure to Oil and Gas Flares on Human Renal Functions. International Research Journal of Medical Science.. 1(11): 9-16.

[32]. Orisakwe, O. E., Njan, A. A, Afonne, O. J., and Akumba, D. D. (2004). Investigation into the Nephrotoxicity of Nigerian Bonny Light Crude Oil.

International Journal of Environmental Research and Public Health 1(2): 106-110.

[33]. Achparaki, M., Thessalonikeos, E., Tsoukali, H., Mastrogianni, O., Zaggelidou E., Chatzinikolaou, F., Vasilliades, N and Raikos, N (2012). Heavy Metals Toxicity. Aristotle University Medical Journal 39(1): 29 -34.