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Heavy Metal Induced Protein Oxidation Toxicity in People Living in an Oil Producing Area

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ABSTRACT

Blood samples collected from 200 residents each from Igbeta-Ewoama (Oil producing area) and Odi (Non-oil producing community) all in Bayelsa State, Nigeria were analyzed to determine the levels of cadmium, chromium, lead, mercury, selenium and also the protein oxidation oxidative stress marker- protein radicals. Blood levels of hemoglobin (Hb), white blood cells (WBC), platelets, prothrombin time and serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, alkaline phosphatase (ALP), bilirubin, gamma glutamyl transferase, total protein, sodium, potassium, chloride, bicarbonate, urea and creatinine were equally measured. Significantly higher levels of cadmium, chromium mercury, lead, selenium and protein radicals were observed in residents from Igbeta-Ewoama community($P<0.05$). With the exception of albumin, total protein, sodium, chloride and bicarbonate, Igbeta-Ewoama residents also had the highest mean values of all the measured liver and kidney functions parameters and these values were all statistically different at $P<0.05$. Measured differences in blood levels of Hb, WBC and Platelets were equally significant while the length of bleeding time was significantly prolonged in the residents of Igbeta-Ewoama community. The findings of this study suggest that the risk of occurrence of diseases associated with metal toxicity and protein oxidation damage might be higher among residents in the oil producing area.

Keywords: Protein Oxidation, Bioaccumulation, Heavy Metal, Oxidative Stress, Reactive Oxygen Species and Oil Spillage.

1. INTRODUCTION

Pollution by petroleum and its products is a widespread and common problem that can arise either accidentally or operationally wherever oil is produced, transported, stored, processed, or used at sea or on land . Bayelsa State located in the Niger Delta region of Southern Nigeria is one place where these activities take place at very high levels daily and therefore residents in this region have been victims of these crude oil associated pollution for many years. Crude oil contains different proportions of heavy metals such as 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). Nigeria crude oils having relatively high concentrations of iron, zinc, copper, lead and mercury [1].

Exposure to crude oil or its complex chemical constituents such as heavy metals can cause toxic effects and also pose serious health problems in humans, livestock and other animal species. Crude-oil-induced bio-accumulation of heavy metals in water, soil, plants, fishes and other aquatic animals in the Niger-Delta is well documented [2] [3]. Cadmium, chromium and lead concentrations were found to be high within the topsoil of the soil profiles of a crude oil polluted environment [4] The levels of cadmium, chromium, copper, iron, nickel and lead analyzed in a river in an oil producing area were all above W.H.O. recommended standards for surface waters which is a very significant indication of pollution [5] [6].

Heavy metals are among the most abundant and persistent environmental inorganic pollutants because they cannot degrade readily [7]. They therefore bio accumulate through multiple trophic levels in food chains [8].

It has been shown that most metals exhibit the ability to produce reactive oxygen species (ROS) [9] and consequently induce oxidative stress. Oxidative stress is a situation that occurs when the production of reactive oxygen species or free radicals is greater than the body's ability to detoxify or remove the reactive intermediates. This imbalance leads to oxidative damage to proteins, molecules, and genes within the body. During this process some electrons escape from the metals and interact with oxygen to generate ROS such as superoxide anion ($O_2^{\bullet-}$), hydroxyl radical (•OH), singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2) [10]. Each of these ROS is highly reactive and unstable due to the fact that they contain an unpaired electron in their outer electron shell. This configuration promotes their ability to rapidly interact with cellular macromolecules such as proteins, lipids and nucleic acids [10]. Thus, when cells are unable to sufficiently regulate the levels of ROS, or are unable to adequately remove or replace oxidized macromolecules, cellular dysfunction can occur via oxidative stress. The formation of ROS in the cells induce lipid peroxidation and DNA damage, deplete sulfhydryl groups, as well as alter signal transduction pathways and calcium homeostasis [11], [12], [13]. ROS or free radicals being extremely reactive, when generated in the intracellular spaces, are able to attack and modify all main cellular constituents.

ROS have been implicated in protein radical formation through the removal of an electron or hydrogen atom. These protein radicals can lead to internal cross-linking of the polypeptide chain, cause protein backbone cleavage, and form protein peroxides as well as peroxyl radicals. The formation of protein radicals has been linked to various disorders including amyotrophic lateral sclerosis, aging, Huntington's Disease, and Alzheimer's disease.

Radical formation results in an unstable molecule that contains an atom with one unpaired electron in its outer orbital, giving the atom unique paramagnetic properties.

Metal ions can cause cellular damage indirectly by lowering the level of glutathione (GSH) [14] [15]. GSH, being the most abundant non-protein sulfhydryl in most cells, acts as a scavenger for various electropiles and free radicals, and as such plays an important role against oxidative damage. Reduced glutathione can react directly with ROS and can act as a substrate in the glutathione peroxidase (GPX) - mediated break down of Hydrogen peroxide (H_2O_2). GSH can bind with some heavy metals to form a Metal–GSH complex which results in the excretion of the toxic metals. This leads to the depletion of intracellular GSH [16]. Cellular defense against toxic onset can be impaired when GSH is depleted and this may eventually lead to cell injury and death.

There are many types of oxidative damage and increasing evidences suggest an important role for protein oxidation in aging and multiple diseases [17] [18] [19]. The importance of protein oxidation towards cellular homeostasis derives from the fact that proteins serve vital roles in regulating cell structure, cell signaling, and various enzymatic processes of the cell. ROS can induce the cleavage of peptide bonds in proteins via two separate pathways: the diamide pathway and α – amidation pathway [20]. Hydroxyl radical, generated from radiolysis of water or generated from H_2O_2 , reacts with proteins to form water and a carbon-centered radical (alkyl-radical). This radical can then cross-link with other alkyl-radicals and form protein aggregates or reacts with O_2 to generate an alkyl-peroxide radical. The cleavage of the peptide bond can also be obtained by the reaction of the free radical $^{\bullet}OH$ with the glutamyl, prolyl and aspartyl residues of the protein chain [21].

Several amino acids can be directly modified via side chain reactions with ROS. The most sensitive amino acids are those with aromatic side chain groups and those containing sulfhydryl groups. ROS-induced oxidation in this instance can occur through a variety of intermediates. For example, the oxidation of phenylalanine residues leads to the formation of mono- and di-hydroxy derivatives whereas tryptophan residues are converted to several hydroxy-derivatives, to formylkynurenine and to nitrotryptophan [19]. In contrast to aromatic amino acids, methionine and cysteine residues are oxidized via reactions at the site of sulfhydryl residues. Both cysteine and methionine residues can interact with ROS resulting in the production of sulfoxide, sulfenic acids, and disulfide bridges [19] [20] .

Mildly oxidized proteins may undergo several alterations which increase their susceptibility to proteolysis. One of the most important changes is the rearrangement of their secondary and tertiary structure. This unfolding process induces them to expose hydrophobic residues otherwise hidden in the normal conformation. Studies have shown that the exposure of these hydrophobic residues allows the proteasome to recognize and bind its substrates, and start the degradation process [22]. The result of proteasome activity is the production of short peptides which can be further hydrolyzed by other cellular peptidases.

Oxidative stress can contribute to cellular dysfunction by directly damaging the lysosomal membrane. This leads to the release of the lysosomal enzymes into the cytosol with serious consequences for cytosolic components and an increased risk in cell death. Nevertheless, there is also evidence for a protective role of lysosomes during stressful periods. They can contribute to the elimination of oxidized and damaged proteins, especially through the chaperone-mediated autophagy pathway. Removal of oxidized proteins is crucial for cell survival. If oxidized proteins are not eliminated either through proteasomal or lysosomal pathways, they will accumulate and potentially aggregate. These aggregates can alter cell functions and lead to necrosis or apoptosis. Furthermore, these aggregates are extremely resistant to proteolysis and can act as inhibitory compounds towards both the proteasome and lysosome degradation pathways [23]. For example, lysosome hydrolase activity has been shown to be impaired by protein aggregates resulting from prolonged exposure to free radicals.

These findings implicate oxidative stress as a contributing factor in the impairment of proteolytic systems responsible for the removal of oxidized and damaged macromolecules. This impairment, in turn, induces an increase in the cytoplasmic levels of

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oxidized proteins, leading to further elevations in protein oxidation. Oxidatively modified proteins that are not completely removed can potentially interact with other cellular components like lipids, carbohydrates and metals giving rise to an autofluor-escent, brown-yellow pigment, termed lipofuscin which is mainly generated in the lysosomal compartment [24]. It has been shown that lipofuscin production is increased by the presence of H_2O_2 derived from the mitochondrial respiration [25]
It is more likely that several metals exist together and their individual toxicities are exhibited simultaneously and interactively. Studies have shown that interactions that occur during exposure to heavy metal mixtures may result in additive, synergistic or antagonistic effects [26]. Exposure to metal mixtures may even lead to new effects that have not been shown in single chemical exposures. From these stated facts, it becomes expedient to assess the levels of heavy metals and protein oxidation marker (protein radicals) in people living in oil producing areas who are exposed to crude oil pollution, drink from the polluted water, eat plants harvested from the surrounding soil and equally consume aquatic species found in these environments. Taking into cognizance the cumulative health effects that may result from these exposures, 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. MATERIALS AND METHODS

2.1 Study Area

This study was done in two communities in Bayelsa state, Nigeria.

(i) Igbeta-Ewoama (Oil Producing Area). Igbeta-Ewoama is a community in Nembe Local Government Area of Bayelsa State. Oil drilling activities in this place have been on for over 40 years. Inhabitants in this area are mainly fishermen. Many aquatic species such as fishes, lobsters, shrimps, crabs oysters, periwinkles and other crustaceans are abundantly found in this area.

(ii) Odi (Non-Oil Producing Community).The community is located in Kolokuma/Opokuma Local Government Area of Bayelsa state.

These 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 was used as control.

2.2 Study Population

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

a. 200 residents of Igbeta-Ewoama community (Oil Producing Area)

b. 200 residents from Odi (Non-oil producing community) as control.

Out of the 200 subjects from Igbeta-Ewoama community, 77 were males between the ages of 2 and 77 years. 123 were females between the ages of 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 and Mobilization

The ethical clearance for this study was granted by Bayelsa State Ministry of Health. The traditional rulers 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 including or excluding 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

Subjects with known illnesses such as cancer, diabetes mellitus and Parkinson's disease as well as tobacco smokers 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 protein radicais 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 the reagents used for this study were commercially purchased from Afro Famous Nigeria Limited, Abakpa Nike, Enugu, Enugu State and all manufacturers' SOPs were followed strictly.

(A) Protein Radicals [29]. The Elabscience protein radicals ELISA technique kit was used for the quantitative measurement of protein radicals in the test subjects.

(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)[30]

RANDOX ALT kit was used.

(D) Aspartate Aminotransferase (AST) [30]

RANDOX AST test kit was used.

(E) Albumin [31]

RANDOX Albumin test kit was used.

(F) Bilirubin [32]

RANDOX Bilirubin kit was used.

(G) Alkaline Phosphatase (ALP) [33].

TECO Diagnostics, California, USA direct colorimetric ALP reagent kit was used.

(H) Gamma-glutamyl Transferase (γ-GT)[34]

RANDOX colorimetric (Kinetic Method) test kit was used.

(I) Urea [35]

RANDOX Urease-Berthelot Colorimetric method kit was used

(J) Creatinine [36]

RANDOX Creatinine kit was used.

(K) Electrolytes (Na^+, K^+, Cl^-, $HCO3^-$) [37]

EA-1000B ISE electrolyte analyzer from Perlong Medical Equipment Company was used to measure these parameters.

(L) Platelets, Haemoglobin (Hb) [38].

SYSMEX pocH-100i automated haematology analyzer was used for the measurement of platelets in the study population.

(M) Prothrombin Time (PT) [39]

AGAPE Diagnostics Switzerland Prothrombin Time kit was used.

2.7 Statistical Analysis

Data obtained 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.

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3. RESULTS

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

Table 1: Protein Radicals and Heavy Metal Levels in the Study Populations.

Study Community	Cd(ppm)	Cr(ppm	Hg(ppm)	Pb(ppm)	Se(ppm)	Protein Radicals (ng/ml)
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	20.44 ± 22.29
P Value	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.0001(S)

S=Significant

Table 2 shows the correlation between protein radicals and the five measured metals in Igbeta-Ewoama community. A positive and statistically significant correlation was observed only between protein radicals and lead.

Table 2: Correlation between Measured Heavy Metals and 8-OHdG in Igbeta-Ewoama Community

	Cadmium		Chromium		Mercury		Pb(ppm)		Se(ppm)	
	R	P	R	P	R	P	R	P	R	P
Protein Radicals	-0.178	0.012(S)	0.009	0.899	-0.173	0.014(S)	0.242	0.0001(S)	-0.056	0.431

Protein radicals and heavy metals levels measured in the different age groups of residents 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. Residents between the ages of 31- 40 years had the highest mean level of cadmium (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 (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 71- 80 years old. This difference was not statistically significant. Subjects 31-40 years old, had the highest mean concentration 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 equally 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 mean value of 0.474 ± 0.109ppm was observed in those 2 – 10 years old. These observed differences in lead and selenium were statistically significant at P <0.05 (Table 4). Residents within the ages of 51 – 60 years had the highest mean concentration of protein radicals (127.22 ± 28.33ng/ml) while the least mean level 102.17 ± 28.33 was observed in residents between the ages 2 – 10 years. These differences were statistically significant.

Table 3: Protein Radicals and Heavy Metals Levels in Relation to Age in Igbeta-Ewoama Community.

Age Range	Cd(ppm)	Cr(ppm	Hg(ppm)	Pb(ppm)	Se(ppm)	Protein Radicals (ng/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	102.17 ± 28.33
11 –20(n=34)	0.283 ± 0.26	0.080 ± 0.14	0.422 ± 0.41	1.079 ± 0.433	0.897 ± 0.46	106.60 ± 24.30
21 – 30 (n=26)	0.388 ± 0.51	0.011 ± 0.034	0.542 ± 0.40	1.001 ± 0.193	0.900 ± 0.44	117.27 ± 22.09
31 – 40 (n=30)	0.425 ± 0.75	0.131 ± 0.193	0.643 ± 0.856	0.717 ± 0.17	0.907 ± 0.68	121.58 ± 22.10
41 – 50 (n=23)	0.125 ± 0.75	0.08 ± 0.11	0.341 ± 0.48	0.701 ± 0.15	1.00 ± 0.56	124.71 ± 25.03
51 – 60(n=25)	0.061± 0.03	0.05 ± 0.01	0.081 ± 0.068	0.719 ± 0.20	1.07 ± 0.34	127.22 ± 28.33
61 – 70(n=15)	0.094 ± 0.03	0.034 ± 0.01	0.092 ± 0.07	0.704 ± 0.13	1.14 ± 0.33	126.21 ± 20.08

71 – 80(n=17)	0.136 ± 0.23	0.003 ± 0.007	0.177 ± 0.09	0.876 ±	1.36 ± 0.308	123.27 ± 27.32

Table 4: Comparison of Protein Radicals and Heavy Metals Levels in Subjects in Relation to Age in Igbeta-Ewoama Community.

Age Range	Cd (PValue)	Cr (P Value)	Hg (P Value)	Pb(P Value)	Se (P Value)	Protein Radicals (P Value)
2-10 vs 11-20	1.0000	0.5595	0.9736	0.0000	0.0331	0.9993
2-10 vs 21-30	0.9702	1.0000	0.5781	0.0002	0.0349	0.4415
2-10 vs 31-40	0.8812	0.0085	0.1323	0.9999	0.0210	0.1089
2-10 vs 41-50	0.9902	0.6714	1.0000	1.0000	0.0083	0.0819
2-10 vs 51-60	0.8898	0.9892	0.8460	0.9999	0.0008	0.0239(S)
2-10 vs 61-70	0.9627	0.0000	0.8907	1.0000	0.0002	0.0435(S)
2-10 vs 71-80	0.9968	0.9996	0.9976	0.2606	0.0000	0.2175
11-20 vs 21-30	0.9876	0.1926	0.9751	0.8862	1.0000	0.6757
11-20 vs 31-40	0.9176	0.5186	0.5453	0.0000	1.0000	0.1923
11-20 vs 41-50	0.9319	1.0000	0.9988	<0.0000	0.9933	0.1488
11-20 vs 51-60	0.6547	0.9690	0.1440	0.0000	0.8635	0.0421(S)
11-20 vs 61-70	0.8314	0.0000	0.2005	0.0000	0.5556	0.0788
11-20 vs 71-80	0.9740	0.3070	0.7225	0.0980	0.0341	0.3688
21-30 vs 31-40	1.0000	0.0003	0.9892	0.0001	1.0000	0.9965
21-30 vs 41-50	0.5069	0.3256	0.8152	0.0002	0.9949	0.9622
21-30 vs 51-60	0.1849	0.8893	0.0117	0.0004	0.8819	0.8139
21-30 vs 61-70	0.3393	0.0000	0.0202	0.0002	0.5886	0.8968
21-30 vs 71-80	0.6900	1.0000	0.2356	0.6785	0.0399	0.9941
31-40 vs 41-50	0.2856	0.6708	0.2935	1.0000	0.9959	0.9998
31-40 vs 51-60	0.0704	0.0879	0.0003	1.0000	0.8838	0.9879
31-40 vs 61-70	0.1613	0.0000	0.0008	1.0000	0.5798	0.9969
31-40 vs 71-80	0.4838	0.0037	0.0353	0.3312	0.0353	1.0000
41-50 vs 51-60	0.9998	0.9825	0.6020	1.0000	0.9996	1.0000
41-50 vs 61-70	1.0000	0.0000	0.6779	1.0000	0.9752	1.0000
41-50 vs 71-80	1.0000	0.4087	0.9711	0.3305	0.2986	1.0000
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	0.9997
61-70 vs 71-80	1.0000	0.0000	0.9995	0.3406	0.8473	1.0000

The mean concentrations of the measured heavy metals and protein radicals 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

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± 0.001ppm) were observed in residents 71 – 80 and 21 – 30 years respectively. These observed differences in these age groups were statistically significant at $P < 0.05$. The highest mean concentration of protein radicals (23.74 ± 19.20 ng/ml) was recorded in residents 51 -- 60 years old while residents between the ages of 2-10 years recorded the least mean value. This measured difference was not statistically significant.

The effect of gender induced differences on the bioaccumulation of the measured metals and protein radicals in the two groups is as presented in table 7. 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 protein radicals. These observed variations in concentrations among the two sub-groups were statistically significant in all the parameters. 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 protein radicals than females. These observed differences were significant in chromium, mercury and selenium only.

Table 5: Protein Radicals and Heavy Metals Levels in Subjects in Relation to Age in Odi Community.

Age Range	Cd(ppm)	Cr(ppm	Hg(ppm)	Pb(ppm)	Se(ppm)	Protein Radicals (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	19.32 ± 22.29
11- 20(n=36)	0.018 ± 0.004	0.038 ± 0.07	0.021 ± 0.02	0.612 ± 0.16	0.081 ± 0.011	21.34 ± 12.29
21-30(n=26)	0.016 ± 0.004	0.010 ± 0.002	0.001 ± 0.001	0.666 ± 0.17	0.056 ± 0.063	21.69 ± 18.20
31 - 40(n=32)	0.013 ± 0.003	0.012 ± 0.002	0.002 ± 0.002	0.919 ± 0.25	0.055 ± 0.05	21.56 ± 21.09
41- 50(n=20)	0.014 ± 0.002	0.009 ± 0.001	0.06 ± 0.04	0.900 ± 0.09	0.057 ± 0.004	20.28 ± 19.20
51- 60(n=21)	0.016 ± 0.02	0.006 ± 0.001	0.06 ± 0.04	0.90 ± 0.09	0.060 ± 0.04	23.74 ± 19.20
61- 70(n=20)	0.017 ± 0.01	0.004 ± 0.001	0.07 ± 0.08	0.864 ± 0.08	0.065 ± 0.07	22.10 ± 23.51
71- 80(n=18)	0.022 ± 0.01	0.001 ± 0.001	0.097 ± 0.08	0.818 ± 0.08	0.098 ± 0.08	21.24 ± 24.2

Table 6: Comparison of Protein Radicals and Heavy Metals 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)	Protein Radicals (P Value)
2-10 vs 11-20	0.1445	0.2479	0.9999	0.0567	0.1371	0.9999
2-10 vs 21-30	0.7280	0.8390	0.9458	0.0011(S)	0.9950	0.9999
2-10 vs 31-40	0.9999	0.9202	0.9509	<0.0001(S)	0.9962	0.9999
2-10 vs 41-50	0.9948	0.8275	0.0408(S)	<0.0001(S)	0.9945	1.0000
2-10 vs 51-60	0.7821	0.5861	0.0359(S)	<0.0001(S)	0.9776	0.9945
2-10 vs 61-70	0.5493	0.4385	0.0039(S)	<0.0001(S)	0.9052	0.9997
2-10 vs 71-80	0.0001(S)	0.0616	<0.0001(S)	<0.0001(S)	0.0011(S)	1.0000
11-20 vs 21-30	0.9881	0.0031	0.7311	0.8256	0.5970	1.0000
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	1.0000
11-20 vs 51-60	0.9919	0.0011	0.0623	<0.0001(S)	0.8353	0.9998
11-20 vs 61-70	0.9999	0.0005	0.0068(S)	<0.0001(S)	0.9596	1.0000
11-20 vs 71-80	0.4583	.0001(S)	<0.0001(S)	<0.0001(S)	0.8294	1.0000
21-30 vs 31-40	0.9066	1.0000	1.0000	<0.0001(S)	1.0000	1.0000
21-30 vs 41-50	0.9950	1.0000	0.0011(S)	<0.0001(S)	1.0000	1.0000
21-30 vs 51-60	1.0000	0.9997	0.0009(S)	<0.0001(S)	1.0000	1.0000
21-30 vs 61-70	0.9999	0.9962	<0.0001(S)	0.0002(S)	0.9992	1.0000
21-30 vs 71-80	0.1082	0.8878	<0.0001(S)	0.0005(S)	.0278(S)	1.0000
31-40 vs 41-50	0.9999	0.9999	0.0007(S)	0.9998	1.0000	1.0000
31-40 vs 51-60	0.9311	0.9946	0.0006(S)	0.9998	1.0000	0.9999
31-40 vs 61-70	0.7636	0.9729	<0.0001(S)	0.8799	0.9978	1.0000
31-40 vs 71-80	0.0004(S)	0.6674	<0.0001(S)	0.0461(S)	0.0105(S	1.0000
41-50 vs 51-60	0.9963	1.0000	1.0000	1.0000	1.0000	0.9993
41-50 vs 61-70	0.9630	0.9992	0.9978	0.9933	0.9997	1.0000
41-50 vs 71-80	0.0191(S)	0.9605	0.0739	0.3865	0.0754	1.0000
51-60 vs 61-70	1.0000	1.0000	0.9976	0.9927	1.0000	1.0000

51-60 vs 71-80	0.1699	0.9973	0.0645	0.3637	0.1152	0.9999
61-70 vs 71-80	0.4110	0.9999	0.3996	0.9295	0.2757	1.0000

Table 7: 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)
Protein Radicals (ng/ml)	120.50 ± 25.47	127.3 ± 27.60	7.108	0.011(S)	21.60 ± 19.20	18.40 ± 16.22	1.742	0.1884

S = Significant

Table 8 shows the mean values of measured liver function parameters in the study groups. It shows that residents in Igbeta-Ewoama community recorded 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. 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 8: Mean and SD Values of Liver Function Parameters in the Study Populations

STUDY COMMUNITY n = 200	ALT (U/I)	AST (U/I)	G-GT (U/I)	ALP (U/I)	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

The mean levels of the different measured haematological parameters in the study communities are presented in table 10. There were statistically significant lower levels of Hb, WBC and Platelets in the test community (Igbeta-Ewoama)

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($p<0.05$) 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 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

Significantly elevated levels of heavy metals in the blood samples of residents in the oil producing area were observed in this study as compared to the control community. This may have resulted from crude petroleum oil spilled into the surrounding environment 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 (38). This finding is in line with a similar observation made in people living in a community where gas flaring activities are carried out [39].
Males in the oil producing community (Igbeta-Ewoama) had significantly higher levels of all the measured metals than females. Some authors also reported significantly higher blood levels of lead and cadmium in males than in females [40], cadmium [41], lead [42], and nickel [43]. Some other authors reported no difference between heavy metals blood concentrations in males and females [39] [44] [45]. This shows that gender is only one of many factors influencing blood heavy metal concentrations in humans. Highest levels of cadmium, chromium and mercury were recorded in Igbeta-Ewoama residents that were between the ages of 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 death of the residents with high concentrations of these metals before they get to older ages.

The observed significantly higher levels of protein radicals in residents of Igbeta-Ewoama than in the control community as equally observed in the measured metals evidently suggests that the metals influenced the induction of the production of protein radicals which is an essential oxidative stress biomarker. This suggests strongly that Mercury and Lead are strong oxidative factors inducing the production of high levels of protein radicals which is an indicator of oxidative stress. Lead is known to cause toxic effects in the kidneys, heart and male gonads [45]. Exposure to Cadmium can cause a variety of pathological alterations in several organs and tissues including inducement of diabetic complications, hypertension and osteoporosis [46]. Lead, cadmium and mercury deplete cells' major anti-oxidants and enzymes in the body leading to oxidative stress and pathological alterations.

Both redox-active and 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). Increased generation of reactive oxygen species can overwhelm cells' intrinsic antioxidant defences and result in oxidative stress. Significantly increased levels of serum urea and creatinine found in residents in the oil producing area as compared to those in the control community evidently suggests a higher risk of occurrence of renal impairment in residents of the oil producing area. This is in line with the findings by Egwurugwu *et al* [47] 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 [48].
The results of this study also suggest a possible higher incidence of liver impairment in residents of Igbeta-Ewoama community due to significantly higher levels of AST, ALT, ALP, Gamma-GT, total bilirubin and conjugated bilirubin measured in their blood samples.
Some adverse alterations in haematological parameters are known to be caused by crude oil exposure. 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 shown by the significantly lower levels of WBC, platelets and longer prothrombin time measured in their blood samples as compared to the control community.

5. CONCLUSION

This study has revealed bioaccumulation of Cadmium, Chromium, Lead, Mercury and Selenium as well as increased level of protein radicals in the blood samples of subjects in Igbeta-Ewoama community. This supports the influence of heavy metals in the elicitation of reactive oxygen species and inducement of oxidative stress. Since proteins are major targets for oxidation, it is not surprising that an increased number of pathologies have been linked to changes in protein structure and function arising from oxidation.

These findings therefore suggest that prolonged 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.

6. RECOMMENDATIONS

Bearing in mind 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 followed strictly in order to reduce and if possible avoid contamination of the environment with hazardous pollutants. A comprehensive investigation of the heavy metal content of all foods and water consumed in oil producing areas should be performed.

It will be valuable to employ the measurement of protein radicals in the assessment of exposure status of residents in environmentally impacted areas. The inclusion of antioxidants in the treatment of metal induced toxicity deserves further consideration since oxidative stress is scientifically proven to be an important mechanism for heavy metal toxicity.

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] 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. [4] Ogboi, E, 2012 Heavy Metal Concentration Effect on Soil of Oil Producing Communities in Delta State, Nigeria. Frontier J., 2: 18-21`

[4] 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.

[5] John, K. N., Orish, E. O. and Linus, O. E. (2008) Some Physiochemical Parameters of Potable Water Supply in Warri, Niger Delta Area of Nigeria. *Scientific Research and Essay.* 3 (11): 547 – 551.

[6] Egwurugwu J.N., Nwafor A., Nwankpa P., Olorufemi O.J., Okwara J.E. (2013). Prolonged Gas Flaring and Water Quality in Obiakpu, Egbema Imo State Nigeria, *Inter. Res. J. Med. Sci...* 2(4): 1-5 (107): 39 - 44.

[7] Castro-Gonzalez, M. and Mendez-Armenta M. (2008). Heavy Metals: Implications associated to Fish Consumption. *Environ Toxicol. Pharmacol.* 10: 1016

[8] Seebaugh, D.R., Goto, D. and Wallace, W.G. (2005). Bio enhancement of Cadmium Transfer Along a Multi-Level Food Chain. *Marine Environmental Research.* 59(5): 473-491.

[9] Galaris, D. and Evangelou, A. (2002). The Role of Oxidative Stress in Mechanisms of Metal-Induced Carcinogenesis. *Crit. Rev. Oncol. Hematol.*42(1):93-103 .

[10] Halliwell, B, and Gutteridge, J.M (1984) . Oxygen Toxicity, Oxygen Radicals, Transition Metals and Disease, *Biochem. J.* 219: 1–14.

DOI: 10.31695/IJASRE.2018.32851

[11] Cerutti, P.A. (1994) Oxy-radicals and cancer. *Lancet* 334:862–3.

[12] Stohs, S.J. and Bagchi D. (1995) Oxidative mechanisms in the toxicity of metal ions. *Free RadicBiol Med.* 18:321–36.

[13] Valko, M., Morris, H. and Cronin, M.T.D. (2005) Metals, toxicity and oxidative stress. *Current Medicinal Chemistry*. 2005;12(10):1161–1208.

[14] Hartwig, A (1998). Carcinogenicity of metalcompounds: Possible role of DNA repair inhibition. *ToxicolLett*.;102:235–9.

[15] Kasprzak, K.S, Jagura, P. and Zastawny, T. H. (1997). Oxidative DNA damage and its Repair in Kidneys and Livers of Nickel(II)-treated Male F344 Rats. *Carcinogenesis*.;18:271–7.

[16] Quig, D.(1998). Cysteine Metabolism and Metal Toxicity. *Altern Med.Rev* ;3:262–270.

[17] Grune, T., Jung, T., Merker, K. and Davies, K. J. (2004) Decreased proteolysis caused by protein aggregates, inclusion bodies, plaques, lipofuscin, ceroid, and 'aggresomes' during oxidative stress, aging, and disease, *Int. J. Biochem*. *Cell Biol*. 36 :2519–2530.

[18] Sohal, R.S. (2002). Role of oxidative stress and protein oxidation in the aging process, *Free Radic. Biol*. Med. 33 37–44.

[19] Stadtman, E. R. and Levine, R. L. (2003) Free radical-mediated oxidation of free amino acids and amino acid residues in proteins, Amino Acids 25 207–218.

[20] Garrison, W. M. (1989). Comparative free radical chemistry in the radiolytic deamination and dephosphorylation of bio-organic molecules: II. Oxygenated solutions, *Free Radic. Biol. Med*. 7 171–175

[21] Schuessler, H. and Schilling, K. (1984) Oxygen effect in the radiolysis of proteins:Part 2. Bovine serum albumin, Int. J. *Radiat. Biol. Relat. Stud. Phys.Chem. Med*. 45 (1984) 267–281.

[22] Davies, K. J. (2001). Degradation of oxidized proteins by the 20S proteasome, *Biochimie 83* 301–310.

[23] Szweda, P. A., Friguet, B. and Szweda, L. I. (e002). Proteolysis, free radicals, and aging, *Free Radic. Biol. Med*. 33 (2002) 29–36.

[24] Terman, A. and Brunk, U.T. (2004). Lipofuscin, Int. J. Biochem. Cell Biol. 36 :1400–1404.

[25] Terman, A. and Brunk, U.T. (1998)Lipofuscin: mechanisms of formation and increase with age, *Apmis* 106 265–276

[26] Ishaque, A. B., Johnson, L. Gerald, T., Boucaud , D., Okoh, J. and Tchounwou, P. B. (2006). Assessment of individual and combined toxicities of four non-essential metals (As, Cd, Hg and Pb) in the microtox assay. *Int. J. Environ. Res. and Publ. Health.* 3(1):118-120.

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

[28] 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.

[29] Lardinois, O. M., Detweiler, C.D., Tomer, K.B., Mason, R.P. and Deterding, I.J. (2008). Free Radic Biol. Med. 44: 893-906

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

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

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

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

[34] Teitz, N. N. (1987)Fundamentals of Clinical Chemistry ed. 3Philadelphia, W. B. Saunders Co., 391.

[35] Fawcett, J. K. and Scott, J.E (1960). *Clin. Path*.; 13: 156.

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

[37] 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.

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

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

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

[41]. Ifenkwe, J. C., Nwanjo, H. U., Nwosu D. C. and Ofor, I. B. (2017). Toxicological Studies on Heavy Metal Bioaccumulation and Oxidative DNA Damage in Residents of a Gas Flaring Community. *Int. J. Sci. Res*., 6 (12) 1868 – 1875

[42] 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.

[43] 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.

[44] 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.

[45] 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.

[46] 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.

[47] 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.

[48] 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

[49] 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.

[50] 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.

[51] 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.

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