Comparative Assessment of Antioxidant Status and Levels of Heavy Metals in occupants of Crude Oil Polluted Area (Imiringi-Bayelsa State) and Non-Crude Oil Polluted Environment (Ibadan-Oyo State) of Nigeria

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Abstract

Environmental pollution from crude oil exploration and associated activities has raised significant public health concerns, particularly in the Niger Delta region of Nigeria. This study presents a comparative assessment of antioxidant status and levels of selected heavy metals in individuals residing in a crude oil–polluted area (Imiringi, Bayelsa State) and those living in a non-polluted environment (Ibadan, Oyo State).A cross-sectional design was employed involving apparently healthy adult participants from both locations. Blood samples were collected and analyzed for heavy metals including lead (Pb), cadmium (Cd), nickel(Ni), Arsenic(As) and vanadium (V), using standard analytical techniques. Antioxidant status was evaluated by measuring key biomarkers such as superoxide dismutase (SOD), catalase (CAT), and total antioxidant status. Results indicated significantly elevated levels of heavy metals in individuals from Imiringi compared to those from Ibadan ($p < 0.05$). Correspondingly, antioxidant enzyme activities (SOD, CAT, and Total antioxidant status) were markedly reduced in the exposed population. In contrast, participants from Ibadan exhibited relatively lower heavy metal burden and better antioxidant defense profiles. The findings demonstrate a clear association between environmental exposure to crude oil pollution and increased oxidative stress, mediated by heavy metal accumulation. This imbalance may predispose affected individuals to various chronic diseases. The study underscores the need for continuous environmental monitoring, public health interventions, and policies aimed at mitigating pollution-related health risks in oil-producing communities.

Keywords: Antioxidant status, heavy metals, crude oil pollution, oxidative stress, Niger Delta, environmental health.

Introduction

Perturbation of the antioxidant defense system due to excessive generation of reactive oxygen species (ROS) have gained recognition as one of the mechanisms underpinning the pathogenesis of many diseases allied with exposure to heavy metals and environmental pollution. The relationships between ROS in humans and the morbidity on many diseases (cancers, rheumatoid arthritis, arteriosclerosis, diabetes, diseases of central nervous and alimentary system, hematids, reproductive and neuronal disorders etc.) have been reported (Nohl and Stolze, 1998; Olin´ ski and Jurgowiak, 1999; Schulz et al., 1999; Siems et al., 2000; Beisswenger et al., 2001; Buonocore et al., 2001; Darlington and Stone, 2001; Kawanishi et al., 2001; Sayre et al., 2001, etc). Studies focusing on the impact of chemical elements on human health and oxidant stress in humans have been of global concern in the past few decades. However, the researches include mostly individuals exposed to the impact of occupational factors, causing changes in the defense mechanisms of an organism against oxidant stress. Among examined elements especially toxic heavy metals are harmful for health (Monika et al., 2012).

Nigerian crude oil is classified as light crude oil with aromatic hydrocarbons accounting for up to 45% of the total hydrocarbons (Orisakwe et al., 2004a). It (Nigerian Light Crude Oil) has been reported to contain vanadium, nickel, Lead, Cadmium, Arsenic, asphaltenes, nitrogen, and low sulfur content, all of which have been shown to be toxic (Akintonwa and Ebere, 1990, Essiet et al., 2010). Anthropogenic activities have represented a growing environmental problem affecting food quality and human health in the Niger Delta region of Nigeria (Essiet et al., 2010). Nigeria as a major producer and exporter of crude petroleum oil continues to experience oil spills and this exposes the environment to hazards and its attendant effects on the biota of the polluted area (Agbogidi et al., 2005). Cases of oil pollution have been reported in the area; and notable areas of oil pollution menace include community which the biota were sampled (our previous study). Heavy metals may have significant toxic and hazardous effects on human health, especially cadmium, vanadium and lead which are contained in crude oil. Heavy metal contamination affects the bio-sphere in many places worldwide (Cunningham and Lee, 1997; Raskin and Ensley, 2000; Meagher, 2000). Certain plants do not only accumulate metals in the roots but also translocate from roots to the leaves or shoots (Baker et al., 2000) and eventually enters the food chain and many believe that this is one of the major ways by which constituents of crude oil can find their way into the human system.

Hence the objectives of this study was to assess heavy metal levels in individuals living in crude oil polluted environment relative to those occupants of non-crude oil polluted environment and to correlate this effect to antioxidant status of the volunteers.

Study area

Studies were carried out in Ibadan (Latitude: 7° 24' 45" N Longitude: 3° 55' 45" E, Western Nigeria) non crude oil polluted area which serves as the control and Imiringi (Latitude: 4° 8' 53"N Longitude: 6° 3' 71"E Southern Nigeria) an environment which is frequently experiencing oil spill from oil pipe lines and the activities of oil exploring companies in the area.

Materials and Methods

Human Population

The study material included blood samples of volunteers from polluted Imiringi Community and unpolluted Ibadan area. The number of humans studied was 50 and 40 for Imiringi and Ibadan, respectively. The volunteers were at the age below 40 years because of depletion of antioxidants (Sulochana et al., 2002). The age structures (Table 1) of volunteers from polluted (Imiringi area) and control (Ibadan) were similar. The volunteers divisions depending on the nicotinic and alcoholic habits from polluted and the control were also

similar (Table 2). Blood samples were collected in tubes to receive blood serum for antioxidant enzymes activity, and total antioxidant status detection and to the tubes with heparin for elements concentration analysis.

Table 1

Age structure of volunteers from Imiringi neighborhood (polluted area) and Ibadan (unpolluted control area). There was no significant difference in age structure in examined environments (Student's t test $P<0.05$).

Environment	Age (years) 20-24	Age (years) 25-29	Age (years) 30-34	Age (years) 35-40
Polluted area	15	20	10	5
Control area	11	18	8	3

Source: ***Questionnaire administered to volunteers, for illustration purposes only***

Table 2

Volunteers depending on smoking cigarettes and/or alcohol in Imiringi (polluted area) and Ibadan (unpolluted area). There were no significant differences in numbers of smokers/alcoholics and non-smokers/alcoholics in examined environments ($p < 0.05$).

Environment	Smokers/ alcoholics	Non smokers/alcoholics	Only smokers	Only alcoholics
Polluted area	13	20	non	17
Control area	11	17	non	12

Source: ***Questionnaire administered to volunteers, for illustration purposes only***

Serum antioxidant enzymes activity

Determination of Superoxide Dismutase (SOD) activity

Superoxide dismutase activity was determined by measuring the inhibition of autoxidation of epinephrine at pH 10.2 at 30 °C by the method of Misra and Fridovich (1972). Briefly, 20µL of the serum sample was added to 2.5 mL of 0.05M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3 mL of freshly prepared 0.3 mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5 mL buffer, 0.3 mL adrenaline and 20µL of water. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds. Value was expressed in Units/mg protein.

Determination of Catalase activity

Catalase activity was assayed by the method of Claiborne (1995). Briefly, the assay mixture containing 2.40 mL of phosphate buffer (50 mM, pH 7.0), 10 µL of 19mM hydrogen peroxide and 50 µL serum sample was allowed to run for 3 min at 30 s intervals. The reaction was terminated by adding 2 mL of dichromate/acetic acid solution and subsequently heated for 10 min in a boiling water bath. The solution was cooled at room temperature and the decrease in absorbance measured with a spectrophotometer at 570 nm. The concentrations of the standard were plotted against absorbance. Value was expressed as µmole H_2O_2 consumed/min/mg protein.

Serum total antioxidant status

Serum total antioxidant status was measured spectrophotometrically by the modified Benzie and Strain (1996) method. Solution containing Fe^{3+} ($FeCl_6$) was added to portion of serum. Antioxidant being found in serum reduces Fe^{3+} to Fe^{2+}. A colored complex is created in the presence of 2, 4, 6 tripirydylo-S- triazine (TPTZ). The increase of absorbance of TPTZ- Fe^{2+} complex is proportional to the quantity of antioxidants present in the sample.

In brief, to 20 µl of samples, 580 µl of solution containing acetate buffer, $FeCl_6$ X $6H_2O$ and 2,4,6-tris(2-pyridyl)-1,3,5- triazine was added. After 20 min etc incubation at room temperature the absorbance was read at 593 nm. The results were interpreted comparatively with Trolox standard well known concentration. The results were given in Trolox- equivalents (Bartosz, 2006).

Determination of heavy metals in the blood

Lead, cadmium, Ni, As and V concentrations were measured by the AAS method. A volume of 2 ml of blood was placed in glass tubes calibrated to the capacity 25 ml were digested according to the

method described by Hoenig and de Kersabiec (1996). The levels of Cd, Ni, Pb, As and V in the filtrates from each digested sample were then determined with the aid of Buck Scientific 205 Atomic Absorption Spectrophotometer. Heavy metal analysis of the crude oil was also determined in our previous study. An acetylene air mixture was used as the flame. The working standard for each of the metals were aspired into the flame in the order of 0.0, 0.2, 0.4, 0.8 and 1.6 ppm before the tissues were aspirated into the flame.

Statistical analysis

By the central terminal statement for the number of cases above 50, to find whether essential differences in Pb, Cd, Ni, As and V concentrations, and SOD and CAT activities and TAS exist, the ''z'' test was used, and a value of $p< 0.05$ was considered to be significant. To show what kinds of interactions between studied parameters exist, the multiple regression analysis was used (Stanisz, 2006). Arithmetic means with standard deviations, minimum and maximum values of analyzed parameters and ''p'' value for ''z'' test for comparison were described in the tables while the correlations were demonstrated on scattering graphs The results were presented as arithmetic means ± SD for volunteers from studied environments.

Results

Lead, cadmium, Nickel, and Arsenic concentrations in blood, and lipid peroxidation, antioxidant enzymes activity, reduced glutathion and total antioxidant status in serum were found to be poles apart in volunteers from studied regions. We observed a significant higher lead, cadmium, Nickel and Arsenic concentrations in blood samples of volunteers from polluted area as compared with those from the control (0.170mg/l vs. 0.008mg/l, 0.107mg/l vs. 0.004mg/l, 0.218mg/l vs 0.007mg/l and 0.009mg/l vs. 0.003mg/l, respectively), but a non significant higher concentration of vanadium (0.018mg/l vs. 0.015mg/l; (Table 3).

Table 3

Levels of Lead (Pb), cadmium (Cd), nickel (Ni), Arsenic (As) and vanadium (V) in whole blood of volunteers from Imiringi (polluted area)and Ibadan (unpolluted control area); p for ''z'' test of comparisons, N—number, mean —arithmetic mean, SD—standard deviation, min. and max.—minimum and maximum values, respectively.

Parameter	Polluted area	Control	Polluted vs Control
Pb (µg/dl)			
N	50	40	P=0.000
Mean ± SD	0.170 ± 0.003	0.008 ± 0.002	
Min. – Max.	0.013 – 0.021	0.005 – 0.010	
Cd (µg/dl)			
N	50	40	P=0.000
Mean ± SD	0.107 ± 0.002	0.004 ± 0.001	
Min. – Max.	0.008 – 0.013	0.003 – 0.004	
Ni (µg/dl)			
N	50	40	P=0.000
Mean ± SD	0.218 ± 0.002	0.007 ± 0.001	
Min. – max.	0.019 – 0.025	0.005 -0.008	
As (µg/dl)			
N	50	40	P=0.000
Mean ± SD	0.009 ± 0.001	0.003 ± 0.001	
Min. – max.	0.002 – 0.009	0.001 – 0.005	
V (µg/dl)			
N	50	40	P=0.356
Mean ± SD	0.018 ± 0.002	0.015 ± 0.022	
Min. – max.	0.015 – 0.021	0.008 - 0.11	

The levels of LPO increased significantly followed by a significant decrease in the activity of SOD and CAT in serum of volunteers from polluted area compared with the serum from the control area (5.75 nmol/MDA/mg protein vs 4.50 nmol/MDA/mg protein, 0.637 unit/mg protein vs 1.030 unit/mg protein and 2.666 nmol H_2O_2/min/mg protein vs 11.250 nmol H_2O_2/min/mg protein respectively). Simultaneously, we have demonstrated significant lower GSH and TAS in serum of volunteers from polluted area compared with those from the control (2.55 unit/mg protein vs 4.84 unit/mg protein, 0.603 Trolox-equivalents vs. 1.038 Trolox-equivalents, respectively), (Table 4).

Table 4

Superoxide dismutase (SOD), catalase (CAT) activities, Lipid peroxidation (LPO) and Reduced glutathione (GSH) Levels and total antioxidant status (TAS) in serum of volunteers from Imiringi, (polluted area) and Ibadan (unpolluted control area); p for ‘‘z’’ test of comparisons, N—number, arithmetic mean, SD— standard deviation, min. and max. — minimum and maximum values, respectively

Parameter	Polluted area	Control	Polluted vs. Control
SOD (unit/mg protein)			
N	50	40	P=0.000
Mean ± SD	0.64 ± 0.29	1.03 ±0.33	
Min.-max.	0.18 – 1.09	0.36 -1.91	
CAT (nmol H_2O_2/min/mg protein)			
N	50	40	P=0.000
Mean ± SD	2.67 ± 1.27	11.25± 5.78	
Min.-max.	1.40 – 4.30	4.30 – 29.30	
LPO (nmol MDA/mg protein)			
N	50	40	P=0.000
Mean ± SD	5.75 ± 0.74	4.50 ± 0.38	
Min. – max.	4.70- 7.10	4.00 – 5.30	
GSH (Unit/mg protein)	50	40	P=0.000
N	2.55 ± 0.001	4.84 ± 0.13	
Mean ± SD	1.05 – 4.55	1.10 – 6.05	
Min.- max.			
TAS (Trolox- equivalent)			
N	50	40	P=0.000
Mean ± SD	0.60 ± 0.29	1.04 ± 0.26	
Min.- max.	0.30 – 1.24	0.46 – 1.24	

The interactions between analyzed parameters were examined and we observed positive correlations between lead, cadmium, Nickel, Arsenic and vanadium ((partial correlation: r = 0.181 vs 0.283; r = 0.266 vs 0.250; r = 0.363 vs 0.261; r = 0.305 vs 0.284 respectively) concentrations in blood LPO levels, SOD and CAT activity and GSH levels in serum of volunteers from polluted area as well as unpolluted area. Moreso, blood was also positively correlated metals with TAS (partial correlation r = 0.083 vs r = 0.280) in volunteers from polluted and unpolluted area.

Discussion

Our research allows corroboration of the effect of lead, cadmium, nickel, arsenic and vanadium exposure as constituents of Nigerian light crude oil pollution and marked by these elements level in blood, upon defense antioxidant mechanisms in humans (Table 3 and 4). Environmental factors are essential either for human health or for the condition of any living organism, because the increase of concentration of toxic metals plays significant role in disorders involving macro- and microelements’ homeostasis in the organism (Monika et al., 2012). Disorders involving alteration of the homeostasis of elements has been linked to various pathologic conditions including cancer and weakening of the immune system via the mechanism of induction of oxidative stress (Nohl and Stolze, 1998; Olin´ ski and Jurgowiak, 1999; Schulz et al., 1999; Siems et al., 2000; Beisswenger et al., 2001; Buonocore et al., 2001; Darlington and Stone, 2001; Kawanishi et al., 2001; Sayre et al., 2001).

Antioxidant system of the organism includes also non-enzymatic antioxidants, such asuric acid, α-tocopherol, ascorbic acid, bilirubin, albumin and many other active substances. The methods assessing the total antioxidant status in human serum have been developed because of the difficulties of measuring particular antioxidant components. Among these the ferric reducing ability of plasma or serum (FRAP) assay by the method of Benzie and Strain (1996) is significant. The FRAP assay is simple and relatively inexpensive, because it measures the ferric-to-ferrous iron reduction in the presence of antioxidants, whilst TAS is expressed as one-electron equivalent of Trolox (6-hydroxy- 2,5,7,8-tetramethylchroman-2-carboxylic acid).There are many antioxidant mechanisms targeting to control physiological level of reactive oxygen species and to protect human organism against oxidant damage. We tried to estimate the impact of environmental stress, which is determined by the magnitude of heavy metal concentrations in blood, on the makers of oxidative stress (LPO), antioxidant enzymes activity (CAT and SOD), non enzymatic antioxidant (GSH) and total antioxidant status (TAS) in serum (this study). On the other hand, many publications concerning occupational exposure on heavy metals and its influence on the antioxidant enzymes activity exist. They found the impact of metals upon

antioxidant enzymatic mechanisms in relation to dose and time of exposure (Monteiro et al., 1985; Sugawara et al., 1991; Aydinn et al., 2004; Han et al., 2005; Patil et al., 2006).

Taking into account the results of our studies (this work) and data from abovementioned authors, we can assume that the increase in the level of LPO could elicit changes of SOD and CAT activity depending on the condition of an organism and environmental determinations. Thus our studies (this paper) show a decrease in SOD and CAT activity, as an important mechanism of antioxidant toxicity as a result of environmental stress. Our environmental research analyzes the changes in the antioxidant enzymes activity in humans living under conditions of environmental stress, which considers the magnitude of Pb, Cd, Ni, As and V concentrations in blood. On the basis of our studies we can thus conclude that significant decrease of SOD and CAT activity in serum of volunteers from polluted areas can be a confirmation of an impact of organism against oxidative stress caused by environmental factors. It is also supported by positive correlation between the analyzed metal concentrations in blood and SOD/CAT activity in serum of volunteers from polluted area, which can be interpreted as antioxidant activity decrease together with toxic metals concentrations. On the other hand, we indicated that SOD/CAT activity was significantly higher in serum of volunteers from unpolluted area. We could thus conclude that, as it is connected from our research (this paper), SOD and CAT might be very sensitive upon environmental impact. Simultaneously, our environmental investigations showed the decrease of GSH and TAS and positive correlation between heavy metal concentration in blood GSH and TAS in serum of volunteers from polluted areas. This can be interpreted as saying that together with heavy metal height concentration, GSH and TAS in serum also decreases. These results suggest that non- enzymatic antioxidants, marked by GSH levels and total antioxidant status in serum, play significant role in the impact of heavy metal blood increase in volunteers from polluted areas. Reports have shown that major mechanism for heavy metal-induced oxidative stress is on the antioxidant defense systems of cells. Several studies have shown that heavy metals alter the activity of antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT)(Ito et al., 1985; Sugawara et al., 1991; Chiba et al 1996).The antioxidant enzymes SOD and CAT are potential targets of heavy metals. Overall, these inhibitory effects of heavy metals on various enzymes would probably result in impaired antioxidant defenses by cells and render cells more susceptible to oxidative attacks.

It can be suggested from our research that ongoing crude oil pollution in the polluted region can consent to an antioxidant mechanism deficiency. The inhabitants of polluted regions are at risk against oxidative stress based on heightened LPO level and the attendant decrease in SOD-CAT activity, whereas antioxidant defense in relation with heavy metals concentration in humans of polluted areas is also based on the non-enzymatic mechanisms marked by GSH levels and total antioxidant status.

Conclusions

1. Environmental pollution by crude oil makes for increase of actual heavy metal exposure, which manifests with lead, cadmium, Nickel, Arsenic and Vanadium blood concentrations increase.
2. Super oxide dismutase and catalase are susceptible to the influence of environmental factors. Thus decrease of SOD/CAT activity in serum of inhabitants from polluted areas is one of the mechanisms of toxicity in an organism against oxidative stress caused by environmental lead and cadmium exposure.
3. It is postulated from the higher serum MDA content associated with decrease activity of SOD and CAT in crude oil polluted area group exposed to heavy metals may be a part in the increased membrane lipid peroxidation. Hence increase serum MDA could be used as a

marker for free radical mediated destruction of liver parenchymal cells and other mechanisms that can lead to impaired reproductive function.

4. Non-enzymatic mechanisms marked by a higher GSH and total antioxidant status (TAS) are the main antioxidant defense in relation with lower metal concentration in humans of unpolluted areas.

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