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Risk Assessment of Heavy Metal Mining on Some Oxidative Parameters in Artisan Miners of Enyigba, Ebonyi State, Nigeria

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Abstract Efforts to eliminate communicable diseases and other third world challenges have reduced public interest on the health hazards posed by heavy metals mining. As developing countries becomes industrialized and urbanized, heavy metal pollution is likely to reach disturbing levels. This study was designed to determine the health risk assessment and oxidative parameters as a result of heavy metal mining in inhabitants of the lead-zinc area of Enyigba community in Ebonyi State. A total of 120 subjects (89 male and 31 female) comprising 60 artisan miners resident in the mining area (37.40 ± 9.08 years) and 60 control subjects (35.30± 9.59yrs) were randomly recruited into the study. Plasma levels of lead, copper and cadmium were determined using flame atomic absorption spectrophotometry. Plasma levels of malondiahyde, glutathione peroxidase, total antioxidants capacity were determined using standard biochemical methods. The risk of non-carcinogenic health of exposure to heavy metals was assessed using Hazard Index (HI) and Total Hazard Index (THI). The result showed that the artisan miners had significantly higher levels of malondiahyde, lead, copper, and cadmium compared with the control (P<0.005). On the other hand, the levels of glutathione peroxidase and total antioxidants capacity were significantly lower in the artisan miners compared to the control (P<0.005). Plasma level of lead also correlated significantly with length of exposure as measured by the number of years residing in the community (p<0.005). The HQ and THI were less than 1 while Cd was the highest contributor to risk (40.43%). In summary, there was increased concentration of these heavy metals on the artisan miners with an attendant depletion in antioxidant status. The risk assessment indicates that the inhabitants would not have significant health risk as a result of mining activities. Artisanal miners should be enlightened about safety hazards and environmental conservation.

Keywords: *heavy metals, mining, antioxidants, Enyigba, risk assessment*

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1. Introduction

Environmental contamination and exposure to heavy metals is a serious growing problem throughout the world [1].The term “heavy metals” refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration and adversely affect the environment and living organisms [2,3]. Mining generates 2.7 billion tons of waste annually, an amount which far exceeds the world’s total accumulated municipal garbage [4]. Mining activities are responsible for an increase in certain heavy metals in soils, sediments and groundwater reserves occurring within the influence sphere of the mine [4].

Various sources of heavy metals include soil erosion, weathering of the earth's crust, mining, industrial effluents, urban runoff, sewage discharge, insect or disease control agents applied to crops etc [5]. Heavy metal pollution is not only toxic to plants and deteriorates soil optimal productivity but is a severe threat to human health especially at elevated level. They are non-biodegradable and they have the tendency to bioaccumulate and biomagnify from one trophic level to another [6].

The toxicological effects of heavy metals has been documented and include gastro-enteritis, inhibition of haemoglobin formation, sterility, miscarriage, growth retardation, central nervous system disorder, kidney dysfunction, hypertension, and mental retardation [7,8].These effects may range from simple ailment to serious changes in sensory perception, immune and nervous system impairment, damage to vital organs, reduced fertility, spontaneous abortion and fatality [9].

At a molecular level two general mechanisms are believed to be responsible for heavy metal toxicity: primary displacement of essential metals (e.g., ionic mimicry) [10], and a secondary effect of oxidative stress due to interference with enzyme that maintain reducing state in cells [11]. In many biological systems where reactive oxygen species production increases antioxidant reserve are depleted [12].

With the discovery and exploitation of metal-rich deposit in Enyigba since 1925, Pb-Zn was intermittently mined. Mining operations ceased due to low income return as well as the 1967-1970 civil war. After the civil war, mining activities have been ongoing till date. The area is inhabited mostly by agrarian people with artisan mining as next significant occupation and source of livelihood among the inhabitants [13]. Lead mining has affected the environment of the mining area negatively as heap of mining tails are scattered everywhere exposing the inhabitants of the community to potential health problems. The prevalent mining process further exacerbates the problem, throwing up thick clouds of dust.

Risk assessment is a quick mode used to assess the impact of a hazard on human health and also to determine the levels of response to solving environmental problems that arises daily in life [14]. The THI presently used to assess non-carcinogenic risk do not give quantitative estimate of the likelihood of experiencing non-carcinogenic effects from contaminant exposure. The THI due give an indication of the risk level associated with pollutant exposure, a valid and useful results [14,15,16] and was applied in this research..

Studies so far on the impact of mining activities in Enyigba lead-zinc mining area have focused mainly on water, soil, and plants metal load [9], their health and environmental impacts [17]. The impacts of lead-zinc mining on plants physiology, anatomy, and biochemistry are also available in local and international literature [18,19,20]. The aim of this study was to determine the impact of heavy metal mining on the antioxidant status of inhabitants of the mining community. By using the THI, the health risk associated with heavy metals as a result of mining was evaluated justifying the study.

2. Materials and Methods

2.1. Study Area

This study was carried out in Enyigba, Abakaliki, Ebonyi state which lies between latitude 6^0 09^IN to 6^0 14^IN and longitude $8^0 5^I$E to 8^0 10^IE and covers about 54.56km. The city is in the mid of the South Eastern Nigeria and lies within the mineralized zone of lead-zinc deposits of the river Benue trough which stretches for hundreds of kilometers North Easterly from Zurak [21].

2.1.1. Subjects and Sample Collection

This is a cross- sectional study. A total of one hundred and twenty (120) subjects comprising sixty (60) artisan miners that have been residing for 10 years in the mining community, Enyigba, Abakaliki, Ebonyi State, and sixty (60) control subjects who had no history of exposure to heavy metals were selected from Ndubia, a town that is about 145 km from Enyigba. The test subjects were recruited from five mining pits. The control subjects were age and sex matched. The community heads of both communities were approached and their support and approval obtained. Thereafter, adults in both communities were approached and the rationale for the study was explained to them. Thereafter, those who gave an informed verbal consent were recruited for the study. Participate less than 18 years and have not resided for more than 10 years were excluded in the study. Those with previous or present history of tumor or toxicity and any debilitating illness were also excluded. The same protocol and equipment were used for data collection which was performed in three batches. The researcher trained the field data collectors and supervised collection and collation. The questionnaires were specifically designed to ascertain indices such as age, sex, level of education, marital status, number of years of residence(duration of exposure), and use of safety gadgets among others. Ten (10) mls of venous blood were collected from each subject during morning hours by venipuncture technique from the cubital fossa into well labeled metal-free plain test tubes using standard method [22]. Ethical clearance was obtained from the Ethical Committee of the Faculty of Health Sciences and Technology Nnamdi Azikiwe University, Nnewi Campus.

2.2. Risk Assessment

The non-carcinogenic health risk of exposure to heavy metals was assessed using Hazard Quotient (HQ) and Total Hazard Index (THI) [14,23,24]. Hazard quotient is the ratio of determined exposure level/risk of a pollutant at a site to the reference dose while THI studies the potential risk of adverse health effects from a mixture of chemical substances/constituents. The risk assessment was calculated thus:

$$\text{Hazard quotient } (\text{HQ}) = \frac{\text{Ec } (\text{mg/kg/d})}{\text{ORD } (\text{mg/kg/d})} \qquad (1)$$

Ec =Exposure concentration,
ORD= Oral reference dose/concentration
HQ< 1(safe)
HQ>1 (unsafe).

$$\text{Total hazard index } (\text{THI}) = \text{HQ}_1 + \text{HQ}_2 + \ldots\ldots + \text{HQn} \qquad (2)$$

The oral reference dose for the metals in mg/kg/d were Pb= 0.004, Cu= 0.04, Cd=0.001 [22].

2.3. Biochemical Analysis

Ten (10) mls of venous blood were collected from each subject during morning hours by venipuncture technique from the cubital fossa into well labeled metal-free plain test tubes using standard method [22]. Plasma levels of lead, copper and cadmium were determined using flame atomic absorption spectrophotometry (Contra AA® 800F, Analytik Jena AG) [25]. Plasma level of malondiahyde was determined as described by Gutteridge and Wilkins [26]. The activity of glutathione peroxidase was as determined by Rotruck *et al.* [27]. Total antioxidant capacity was estimated by Ferric Reducing Ability of Plasma (FRAP) method, modified by Benzie and Strain, [28].

2.4. Statistics

Data analysis was done using SPSS statistical software version 19.0 while t-test was used to test the statistical significance of intergroup difference. Correlation coefficient was calculated by the Spearman method. All P- values ≤ 0.05 was considered statistically significant.

3. Results

The result in Table 1 shows that the mean plasma levels of lead, cadmium, and malondialdehyde were significantly higher in the artisan miners than the control group ($P<0.005$). The mean plasma level of copper was also significantly higher in the artisan miners compared to the control ($P<0.05$). The levels of heavy metals were generally higher ($P<0.05$) in the artisan miners than the control group. Similarly, the plasma levels of total antioxidant capacity and glutathione peroxidase were significantly lower ($p<0.001$) in the artisan miners than in the control.

The results in Table 2 show a non-significant increase in the mean total antioxidant capacity and glutathione peroxidase with the number of years of exposure ($P>0.05$), while the mean malondialdehyde concentration was reduced at the highest year of exposure. The mean serum level of cadmium also increased with increasing level of exposure but the difference was not significant whereas the level of copper decreased with increase in length of exposure as determined by the number of years of residing in the community. The results also indicated that there was a significant increase in the mean serum lead concentration as the years of residence increased ($P<0.05$).

The results in Table 3 shows that only plasma level of lead showed significant correlation with duration of exposure/years of residence ($R>0.5$, $P<0.05$), while other parameters showed no significant correlation ($R<0.5$, $P>0.05$).

The hazard quotients (HQ) of the studied metals through exposure to mining activities for the residents were determined and listed in Table 4. The estimated hazard quotients for the individual metal in the test group decreased in the following order Cd>Pb>Cu while for the control group it decreased in the following sequence Cu>Cd>Pb. The results show that the estimated hazard quotient and the total hazard index were less than 1. The result shows that cadmium was a major risk contributor for the exposed group accounting for 46.43% the THI. The results show that the concentration of the individual metals was less than the oral reference dose.

Table 1. SERUM LEVELS OF SOME HEAVY METALS AND OXIDATIVE PARAMETERS IN THE EXPOSED AND CONTROL GROUPS

PARAMETERS	Test group(n=60)	Control group(n=60)	t-test	p-value
Total Antioxidant Capacity(umol/l)	798.29±21.85	957.52±17.03	-3.810	<0.001*
Glutathione Peroxidase(U/ml)	0.627±0.16	0.823±0.14	-5.840	<0.001*
Malondialdehyde(nmol/ml)	3.69±1.30	2.45±0.72	4.991	<0.001*
Lead (mg/kg/dl)	0.00044±0.001	0.00019±0.0004	7.104	<0.001*
Copper (mg/kg/dl)	0.00152±0.03	0.00133±0.0017	2.119	0.036*
Cadmium (mg/kg/dl)	0.00013±0.001	0.00008±0.0002	4.135	<0.001*

Results are mean ± standard deviation of triplicate readings. Values with *are significant (P<0.05].

Table 2. OXIDATIVE PARAMETERS AND HEAVY METALS WITH DURATION OF EXPOSURE

PARAMETERS	10-14yrs (n=28)	15-19yrs (n=20)	≥20yrs (n=12)	p. value
Total Antioxidant Capacity (umol/l)	784.41±19.02	814.60±23.15	870.82±60.96	0.63
Glutathione Peroxidase (U/ml)	0.614±0.167	0.655±0.176	0.66±0.11	0.53
Malondialdehyde (nmol/l)	3.62±1.34	3.94±1.21	2.84±1.09	0.22
Lead(mg/kg/dl)	0.0004±0.0008	0.0005±0.0012	0.0006±0.0017	<0.001*
Copper(mg/kg/dl)	0.0016±0.0034	0.0015±0.0025	0.001±0.0031	0.1
Cadmium(mg/kg/dl)	0.0001±0.0034	0.0002±0.0006	0.0002±0.0007	0.55

Results are mean ± standard deviation of triplicate readings. Values with *are significant (P<0.05).

Table 3. CORRELATION OF DURATION OF EXPOSURE TO HEAVY METAL CONCENTRATIONS, RENAL, AND LIVER PARAMETERS.

PARAMETERS	n	R	p-value
Duration of exposure vs TAC	60	0.160	0.132
Duration of exposure vsGPx	60	0.179	0.091
Duration of exposure vs MDA	60	-0.086	0.418
Duration of exposure vsPb	60	0.533	<0.001*
Duration of exposure vs Cu	60	-0.141	0.185
Duration of exposure vs Cd	60	0.104	0.330

Legend: TAC = Total antioxidant capacity; GPx = Glutathione peroxidase; MDA = Malondialdehyde; Pb = Lead; Cu = Copper; Cd = Cadmium; AST = Aspartate transaminase; ALT = Alanine transaminase; ALP = Alkaline phosphatase.

Table 4. HAZARD QUOTIENT(HQ) AND TOTAL HAZARD INDEX(THI) OF Pb, Cu AND Cd IN THE EXPOSED AND CONTROL GROUP

Element	Hazard quotient exposed	Hazard quotient control	Percentage risk contribution
lead	0.11	0.05	39.29
Copper	0.04	0.32	14.29
Cadmium	0.13	0.08	46.43
THI=	0.28	0.46	

4. Discussion

In countries without proper mining legislations, artisan mining flourishes with huge revenue lose to the government and increased risk of environmental pollution. Industrial operations have the potential to affect the health of the work force and the general environment thereby impacting negatively on the health of nearby populations [7]. The worry is the routine release of contaminants into the environment with severe environmental costs.

Several studies have reported high pollution index of heavy metals in arable crops and drinking water especially in the mining areas [17,29]. In the present study the plasma level of lead was shown to be significantly higher on inhabitants of mining area. It also increased with the duration of exposure of the subjects. Likewise, its level also showed a significant correlation with the duration of exposure of the subjects. This is in accord with the findings of Nnabo *et al*. [29] and Taylor *et al*. [30]. This is possibly the resultant effect of uncontrolled mining activities in the area over a long period of time, which led to the generation of heavy metal pollutants with negative influence on the environment [30]. Obviously, mining and other related operations are the most important anthropogenic sources of heavy metals to the environment. Lead like other heavy metal therefore bioaccumulate, leading to the significant increase with the duration of exposure.

In this study the levels of cadmium also increased with duration of exposure. The levels of cadmium not only increased significantly in the exposed group, but also increased with increased duration of exposure. The present finding is in agreement with the findings of Chakraborty *et al.* [32] were long-term cadmium occupational exposure led to chronic cadmium toxicity. Cadmium is highly toxic to the kidney and it accumulates in the proximal tubular cells in very high concentrations. It can cause renal dysfunction. Earlier studies have shown that cadmium causes renal stone formation and hypercalciuria [33].

Similarly, the plasma levels of copper showed significant increase in the artisan miners than the control group. This is, also in agreement with the results of Arinola *et al.* [34]. However, the plasma levels of copper decreased with increases in duration of exposure. This may be due to chemical interactions with other metals such as cadmium and lead that are capable of displacing copper from its binding sites. Also, free copper is known to reduce oxidative stress as it is involved in the metabolic elimination of reactive oxygen species, such as with the superoxide radical through copper-zinc dependent superoxide dismutase. Moreover, excessive free copper impairs antioxidant enzyme function thus increasing oxidative stress [35]. Though copper act as an antioxidant reducing oxidative stress, chronic exposure to copper can lead to kidney and liver damages [35].

Researchers have shown that oxidative stress in living cells is caused by the imbalance between the production of free radicals and the generation of antioxidants to detoxify the reactive intermediates or to repair the resulting damage [11,36]. Heavy metal toxicity leads to free radicals damage via two separate, although related pathways: the generation of reactive oxygen species (ROS), (including hydroperoxide, singlet oxygen and hydrogen peroxide) and the direct depletion of anti-oxidants reserve [12].

In this study the levels of glutathione peroxidase differs significantly between the artisan miners and the control groups. However, there was non-significant correlation with the duration of exposure to heavy metals. This result is in line with the earlier reports of Ahmad *et al. [37]* and Ding *et al. [38]*.

Our data indicated that malonylaldehyde was significantly higher in the artisan miners than the control. While, plasma total antioxidant capacity and glutathione peroxidase were significantly lower in the artisan miners there was no significant correlation of malonylaldehyde with duration of exposure. Earlier studies have linked induction of lipid peroxidation and alteration in the antioxidant enzymes with heavy metal exposure [7,37]. The apparent increase in lipid peroxidation may be attributed to the accumulation of the heavy metals in the organs. Metals catalyze formation of reactive oxygen species (ROS) which is capable of damaging cells and tissues such as DNA and proteins.

Another bio-indicator of oxidative stress is the total antioxidant capacity. The level of total antioxidant capacity from our finding shows an apparent decrease with increase in duration of exposure. Similarly, its levels differ significantly between the artisan miners and the control groups. Again, this is in agreement to the findings of Ahamed *et al.* [37]. Cells, tissues, and body fluids are equipped with powerful defense systems that help counteract oxidative challenge to maintain a steady-state of metabolites and functional integrity in the aerobic environment [39]. This antioxidants defense are altered and depleted during oxidative stress leading to an increase in free radicals and oxidative insults. Studies have also linked heavy metals with oxidative stress and its associated organ injuries [39].

The HQ is recognized as a useful tool to evaluate the risk of non-carcinogenic effects or from the consumption of metal contaminated food crops [14,24]. The HQ ratio values are valuable with values less than one generally indicative of acceptable hazard. In reverse order, an exposed population will be at health risk if the dose is equal to or greater than the oral reference dose/concentration or more than one. In this study, the THI was calculated as provided in the United States EPA Region III risk-based concentration table [14,41].

The risk assessment results showed that the HQ and THI were less than 1 in both the test and control groups.

We also reported exposure concentrations to be less than the oral reference doses. This implies that the level of exposure is lesser than the ORD though a daily exposure at this level may cause adverse effects during a person's lifetime [14]. Previous reports have shown that exposure to two or more chemicals/pollutants could result in additive and /or interactive effect [14,41]. For this report, the THI was derived as the arithmetic sum of the individual metals HQ. This study found that Cd could be a major risk contributor for the residence of the mining community accounting for 46.43% of the THI while Pb was the next higher risk contributor at 38.29%. This study demonstrates the relatively lesser risk from copper and major contribution from Cd for the inhabitants of Enyigba mining community in Ebonyi State of Nigeria. The HQ of each metal evaluated in this study is generally less than 1, meaning that the inhabitants of this mining community may not experience significant health risk from exposure to individual metal as a result of mining. We observed traces of these metals in the control subjects selected from communities and individuals far away from the miming sites. Although the study was to evaluate the concentration of heavy metals as a result of mining activity, humans can be exposed to heavy metals through various pathways including consumption of vegetables, fruits, marine foods, water, milk etc [24].

Conclusion: It can be inferred from the results that there are increased concentrations of heavy metals on the artisan miners with attendant depletion in antioxidant status leading to oxidative stress. The THI from exposure to the heavy metals was less than 1 indicating that the inhabitants would not have significant health risk as a result of mining activity.

Conflict of Interest

The authors have no conflict of interest with regards to this publication.

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