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Serum Heavy Metals and Lipid profile Analyses of Male Albino Rats Housed at Ugwuele Quarry site

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Abstract

Background:

An assay on the body mass, heavy metals and lipid profile of 60 male albino rats (*Rattus norvegicus*) of 8 weeks old which weighed between 115.5 to 128.6g, housed at different activity areas of Ugwuele quarry site was carried out using standard methods.

Objective:

This work intended to evaluate the risks of heavy metal toxicity and cardiovascular disease in quarry workers, using male albino rats as animal models.

Methods:

The rats were placed in six (6) groups containing five (5) rats per group. Group 1, which is the control was housed at a distance of 7.0km from the quarry site. Group 2 was housed at the crusher location of the quarry site. Groups 3,4,5,6 were housed at the administration block, gate, plant house and drilling pit positions of the quarry site respectively. The study was carried out in both dry and wet seasons for 90 days in each season.

Results:

From the results obtained, the final body mass of the albino rats exposed to the quarry decreased significantly ($p<0.05$) from 113.40±0.09 (at the plant house) to 105.70±0.13g (at the crusher) in the wet season and 120.45±0.09 (from the drilling pit) to 110.10±0.09g (from the crusher) in dry season, compared to the control. Serum heavy metals (Co, Zn, Cd, Cr, As and Pb) concentrations of the quarry exposed albino rats increased significantly ($p<0.05$) compared to the control. Serum total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and triacylglycerides (TG) values of the exposed albino rats were raised significantly ($p<0.05$) compared to the control while serum high density lipoprotein cholesterol (HDL-C) values of the exposed rats decreased significantly ($p<0.05$) compared to the control.

Conclusion:

These results imply that the quarry workers at Ugwuele are at greater risks of cardiovascular disease and toxicity by heavy metals, and workers at the crusher section of the quarry site are at the highest risks.

Keywords: Albino rats, heavy metals, cardiovascular disease, quarry workers, lipid profile.

INTRODUCTION

Stone quarrying is a source of pollutants, which include heavy metals and dusts in the form of particulate matter. Heavy metals are important group of elements of atomic density greater than $5g/cm^3$ most of which are toxic at low concentrations. Madu *et al*. [1] reported that soil samples of farm lands within Ugwuele quarry site are polluted by various heavy metals. These heavy metals and other pollutants from the quarry, adversely affect the quarry workers who are often seen working without appropriate safety equipment [2].

Long-term exposure to dust and particulate matter leads to risk of cardiovascular diseases [3]. Again, inhalation of heavy metal contaminated air or ingestion of heavy metal contaminated foods can cause cardiovascular diseases [4]. However, Orozco-Beltran *et al*. [5] agreed that lipid profile parameters like total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL) and triacylglyceride (TG) are used as biomarkers of the risk of cardiovascular disease. Andrzejak and Lepetow [6] found that there is a significant negative correlation between serum lead (Pb) and high density lipoprotein cholesterol (HDL-C) of industrial workers.

Mass is the quantity of matter in a body. Many researchers often use the term weight when in actual sense they mean mass. However, there are differences between mass and weight. While mass is the quantity of matter in a body, weight is the force of gravity on a body [7]. The international unit (S.I. unit) of mass is the kilogram (kg) whereas the S.I. unit of weight is the Newton (N) [8]. The mass of a body remains the same despite where it is measured on the surface of the earth while the weight of a body is not the same at all positions on the surface of the earth [9]. Thus, the term "body mass" of albino rats is used in this study instead of body weight since what was measured with the electronic weighing balance (which reads in gram and kilogram) is actually the mass of the albino rats used.

Albino rats have been in use as models in human research for the past eight decades. Rats are used as models for humans since both share similar fundamental body organs and physiology even though there are slight differences [10]. Their endocrine, cardiovascular and nervous systems are similar and they suffer the same types of diseases with humans. Rats also share about ninety percent of genome with humans [11]. Rat models are generally very important and useful in research but there should be caution when applying the results to humans [12]. The objective of this work was to evaluate the risks of heavy metal toxicity and cardiovascular disease in quarry workers, using male albino rats (*Rattus norvegicus*) as animal models.

MATERIALS AND METHODS

Area of Study

Ugwuele quarry site in Uturu, Abia State Nigeria is the study area. It lies within latitude $05^{O}50^{'}18''$ N of the equator and longitude $07^{O}25^{'}$ 17" E of the Greenwich Meridian.

Animal Sampling

A total of sixty male albino rats (*Rattus novergicus*) of 8 weeks old which weighed between 115.5 to 128.6g were used in this study. They were bought from the animal unit of the Physiology department of the University of Ibadan, Nigeria. The rats were randomly placed in six (6) groups containing five (5) rats per group. Group 1, which is the control, was housed at a distance of 7.0km from the quarry site. Group 2 was housed at the crusher location of the quarry site. Groups 3,4,5,6 were housed at the administration block, gate, plant house and drilling pit positions of the quarry site respectively. The rats were fed standard feed and drinking water ad libitum, housed in sterile wire cages for ninety (90) days and monitored each morning. This was done separately in two seasons – dry and wet seasons (December 19, 2018 - March 18, 2019 and July 1-September 28, 2019). The animals were anaesthetized in a desiccator which has a considerable amount of chloroform in a cotton wool before they were sacrificed.

Blood Sample Collection

After the sacrifice of the rats, blood samples were drawn from their hearts and put into sterile test tubes. The blood samples which clotted after about 15 minutes were spun in a centrifuge at 4000rpm for 10min. Pasteur pipette was used to draw serum into sterile sample tubes for the measurement of heavy metals and lipid profile. Ethical approval of protocol was obtained from the ethical committee for the use and care of animals, research and publications of Abia State University, Uturu.

Measurement of Body Mass of Albino Rats

The body mass of the rats was measured using an electronic weighing balance (JJIOOO, G & G). The mass of the conical flask was first measured then the rat was put in the conical flask and placed on the weighing balance. Mass of the rat and conical flask minus mass of the empty conical flask gave the mass of the rat.

Determination of Serum Heavy Metals

Serum heavy metals were determined using the method as described by Hoenig and De Kersabiec [13]. This was done using atomic absorption spectrophotometer (AAS),UNICAM model 939.

Determination of Total Cholesterol

The method described by Yuzo *et al*. [14] was used in the determination of total cholesterol. Three different test tubes were racked and labeled blank, standard and sample respectively. Exactly 10µl of distilled water was pipetted into the test tube labeled blank, 10µl of standard (from Agappe Cholestrol test kit) was introduced into the test tube labeled standard and 10µl was pipetted into the test tube labeled sample. Finally, 1µl of the reagent was pipetted into all the test tubes, mixed and incubated for 10 minutes at temperature of 25^{O}C. Sample absorbance (at 630nm) was taken against the reagent blank using a spectrophotometer (752s, England).

Calculation

$$\text{Concentration of Cholesterol (mg/dL)} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times \text{concentration of standard}$$

Determination of Serum High Density Lipoprotein Cholesterol (HDL-C)

The method described by Rifai and Warnick [15] was used in the determination of high density lipoprotein (HDL) cholesterol. Exactly 30µl of sample and 30µl of HDL reagent was mixed and allowed to stand for 10 minutes at a temperature of 27^{O}C, mixed the second time and spun in a centrifuge for 10 minutes at 4000rpm. The blank, standard and sample test tubes were racked and exactly 10µl of cholesterol reagent (from Agappe cholesterol kit) was introduced respectively in all the racked test tubes. Exactly 500µl of standard HDL was pipetted into test tube labeled sample. These were properly mixed and incubated for 5 minutes at 37^{O}C. Sample and standard absorbance were taken at 630nm using a spectrophotometer (752s, England).

Calculation

$$\text{HDL-C concentration (mg/dL)} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times N \times 2$$

Where

N = Concentration of standard (50mg/dL)

2 = Sample dilution factor

Determination of Low Density Lipoprotein Cholesterol (LDL-C)

Low density lipoprotein (LDL) cholesterol was determined using the method as described by by Rifai and Warnick [15]. Exactly 400µl of sample and 200µl of reagent (A) cholesterol kit were pipetted into different test tubes. This was allowed to mix thoroughly and left to stand for 15 minutes at room 27^oC. Spun at 4000rpm for 15 minutes, the supernatant was carefully collected. Blank, standard and sample test tubes were racked and exactly 200µl of distilled water was pipetted in the test tube labeled blank, 200µl of cholesterol standard in test tube labeled standard, 200µl of sample supernatant was added into the test tube labeled sample and 1.0ml of reagent (from Agappe cholesterol kit) was pipetted in all the racked test tubes. The content was thoroughly mixed and incubated for 30 minutes at 27^OC. The absorbance of the standard and sample was read at 500nm against the blank.

Calculation

$$\text{Cholesterol concentration} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{conc. of standard} \times \text{dilution factor}$$

LDL cholesterol = Total cholesterol – cholesterol in supernatant

Determination of Triacylglycerides (TG)

The method of Rifai and Warnick [15] was also used to determine total triacylglycerides (TG). Blank, standard and sample test tubes were racked and exactly 10µl each of the sample and standard were added into the respective test tubes. Exactly 1µl of reagent was also added in each of the three test tubes, mixed and incubated for 10 minutes at 25^OC. Sample and standard absorbance at 630nm were read against the reagent blank within 60 minutes.

Calculation

$$\text{Triacylglyceride concentration} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

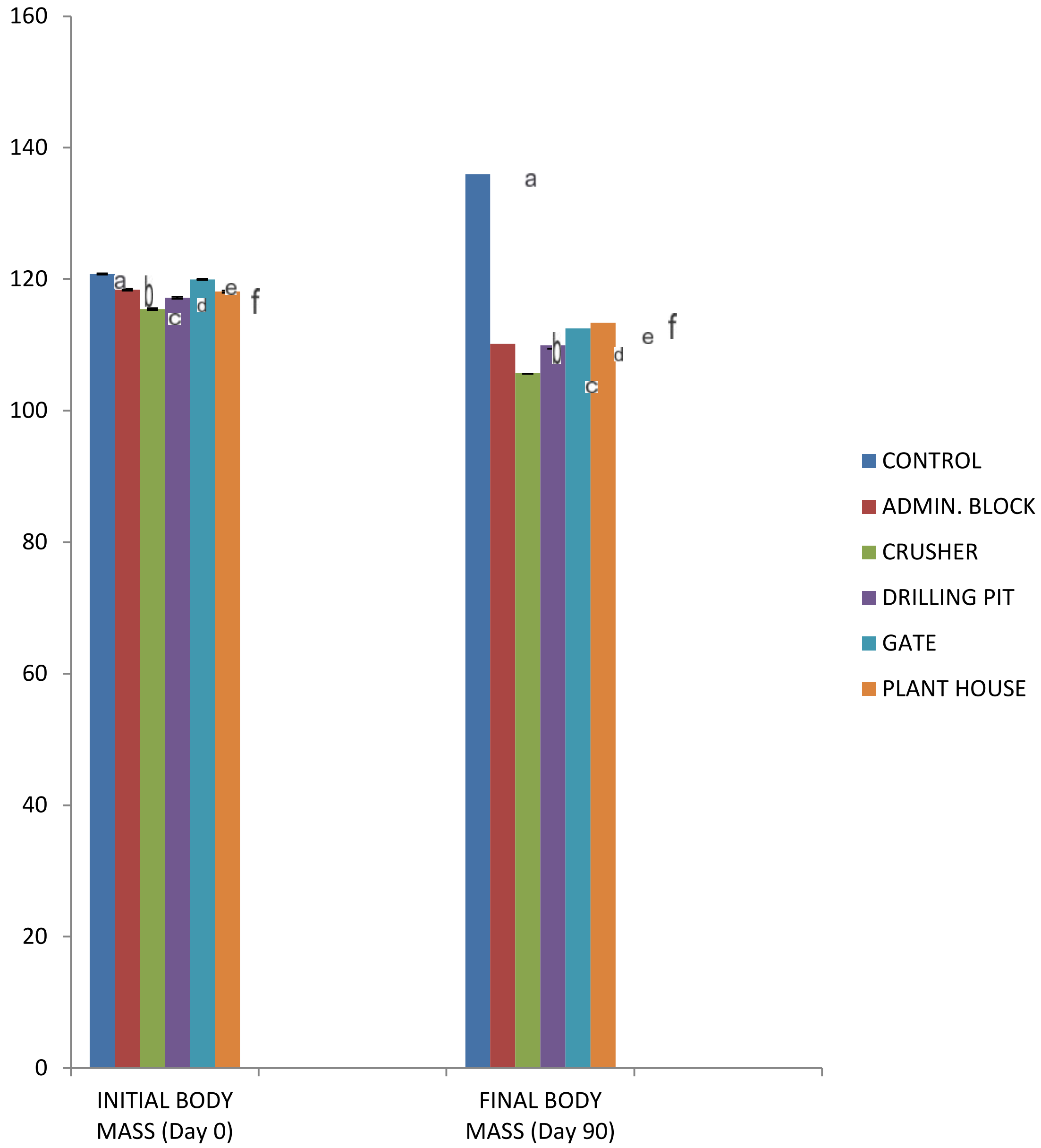
Statistical Analyses

Results obtained were analyzed with Analysis of Variance (ANOVA) and standard T-distribution test. Group means were compared for significance at $p<0.05$ using 95% level of confidence. These were done with the statistical package for social sciences (SPSS) version 20. Two way ANOVA was used for body mass while one way ANOVA was used for results of heavy metals and lipid profile.

RESULTS

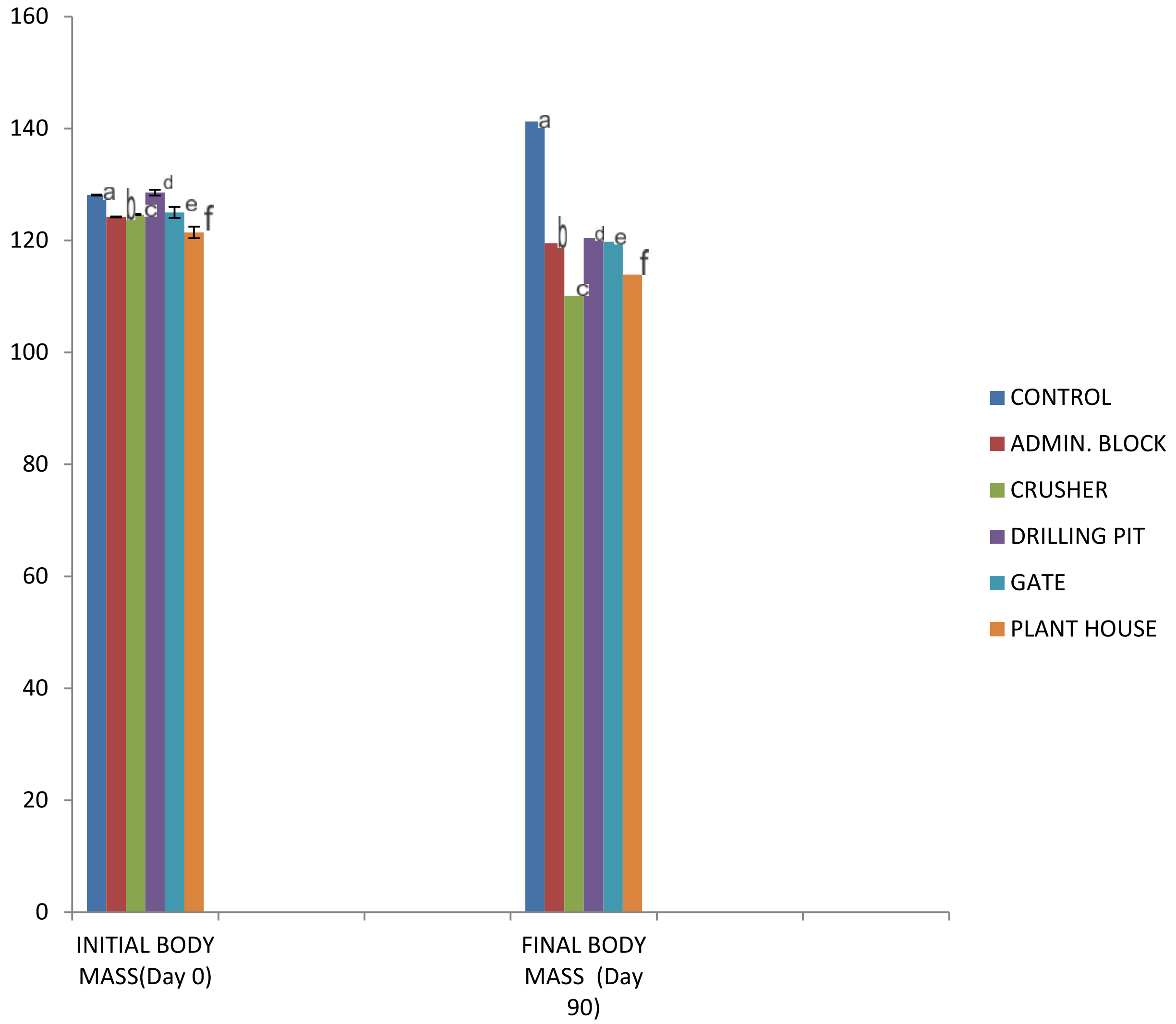
The results of the body mass of the albino rats are presented in figures 1 and 2 below.

In the wet season, the final body mass of the test albino rats ranged from 105.70±0.13g (at the crusher) to 113.40±0.09g (at the plant house). The highest recorded mass (135.95±0.10g) was from the control rats. During the dry season, the body mass of the test albino rats ranged from 110.10±0.09g (from the crusher) to 120.45±0.09g (from the drilling pit). The highest recorded value of 141.25±0.97g was from the control albino rats.



Results are mean ± standard deviation of triplicate results obtained. Bars of the same color and period having different letters of alphabet are statistically different ($p < 0.05$) using Least Significant Difference (LSD). **Legend:** Wet S. = Wet season, Dry S. = Dry season.

Figure 1: Body mass (g) of the albino rats housed at Ugwuele quarry site, Uturu (Wet season)



Results are mean ± standard deviation of triplicate results obtained. Bars of the same color and period having different letters of alphabet are statistically different ($p < 0.05$) using Least Significant Difference (LSD).

Figure 2: Body mass (g) of the albino rats housed at Ugwuele quarry site, Uturu (Dry season)

Concentrations (mg/L) of heavy metals in the serum samples of the albino rats are presented in figures 3 and 4 below.

Cobalt (Co): During the wet season, the level of cobalt in the test samples varied from 1.02±0.01 (from the plant house) to 2.14±0.04 (from the crusher). The least recorded value for the season was 1.00±0.00 (from the control). During the dry season, the values ranged from 1.09±0.02 (from the plant house) to 3.09±0.02 (from the crusher). The least recorded value (1.11±0.02) was from the control sample.

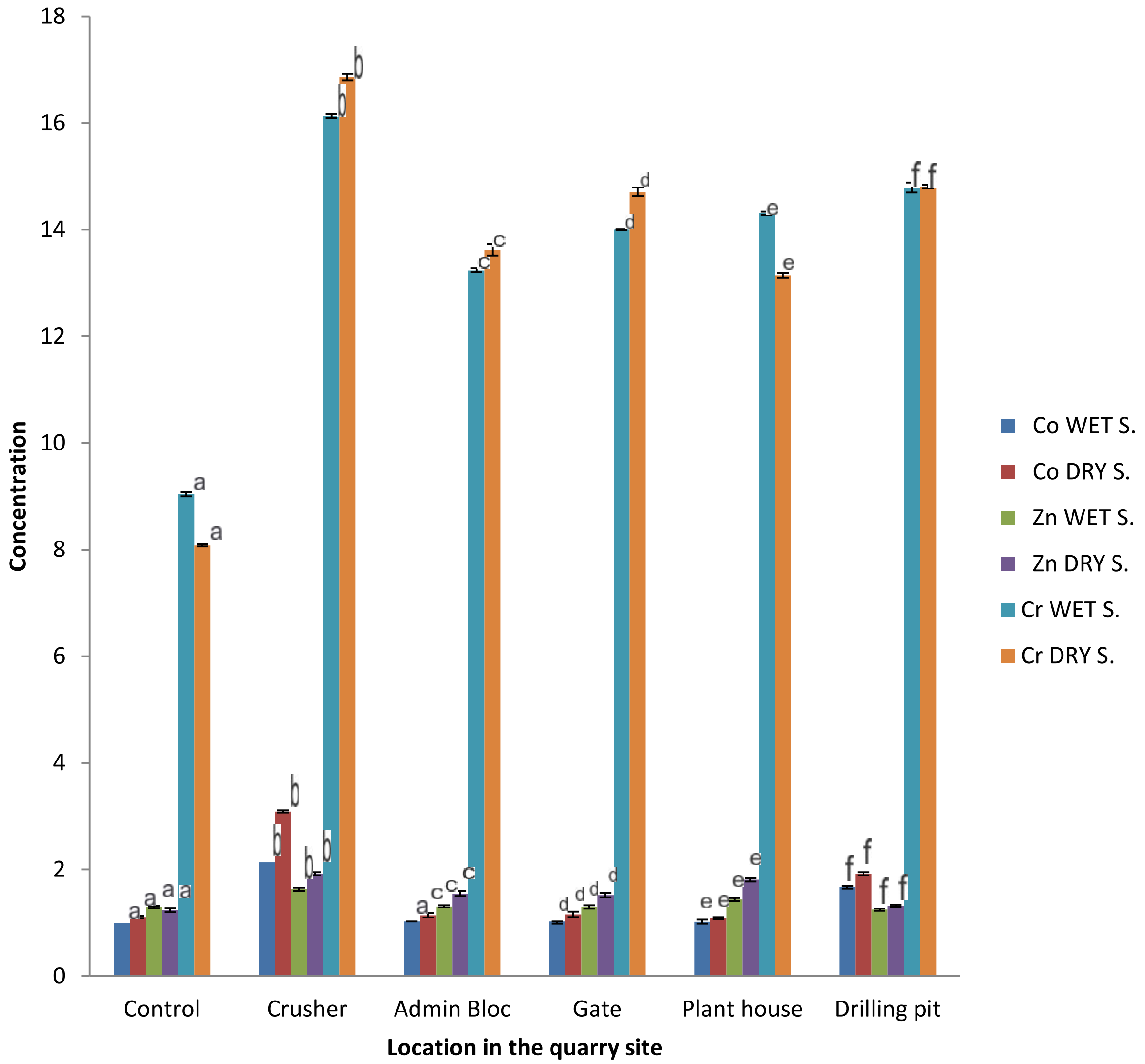
Zinc (Zn): In the wet season, the values of zinc in the test samples ranged from 1.25±0.02 (from the drilling pit) to 1.63±0.03 (from the crusher). The control sample recorded a value of 1.30±0.03. In the dry season, the concentration varied from 1.32±0.02 (from the drilling pit) to 1.92±0.03 (from the crusher). The least value (1.24±0.04) for the season was from the control.

Chromium (Cr): During the wet season, the level of chromium ranged from 13.24±0.04 (from the admin. block) to 16.13±0.04 (from the crusher). The least recorded value (9.04±0.04) was from the control sample. In the dry season, the level ranged from 13.14±0.04 (from the plant house) to 16.86±0.06 (from the crusher). The least recorded value of 8.08±0.02 was from the control sample.

Cadmium (Cd): In the wet season, the concentration of cadmium in the test samples was 0.1 in both plant house and drilling section. No cadmium was detected in other test samples and control. In the dry season, the concentration varied from 0.02 (in both admin. block and the gate) to 0.06±0.03 (from the plant house). The least recorded value (0.01±0.01) was from the control.

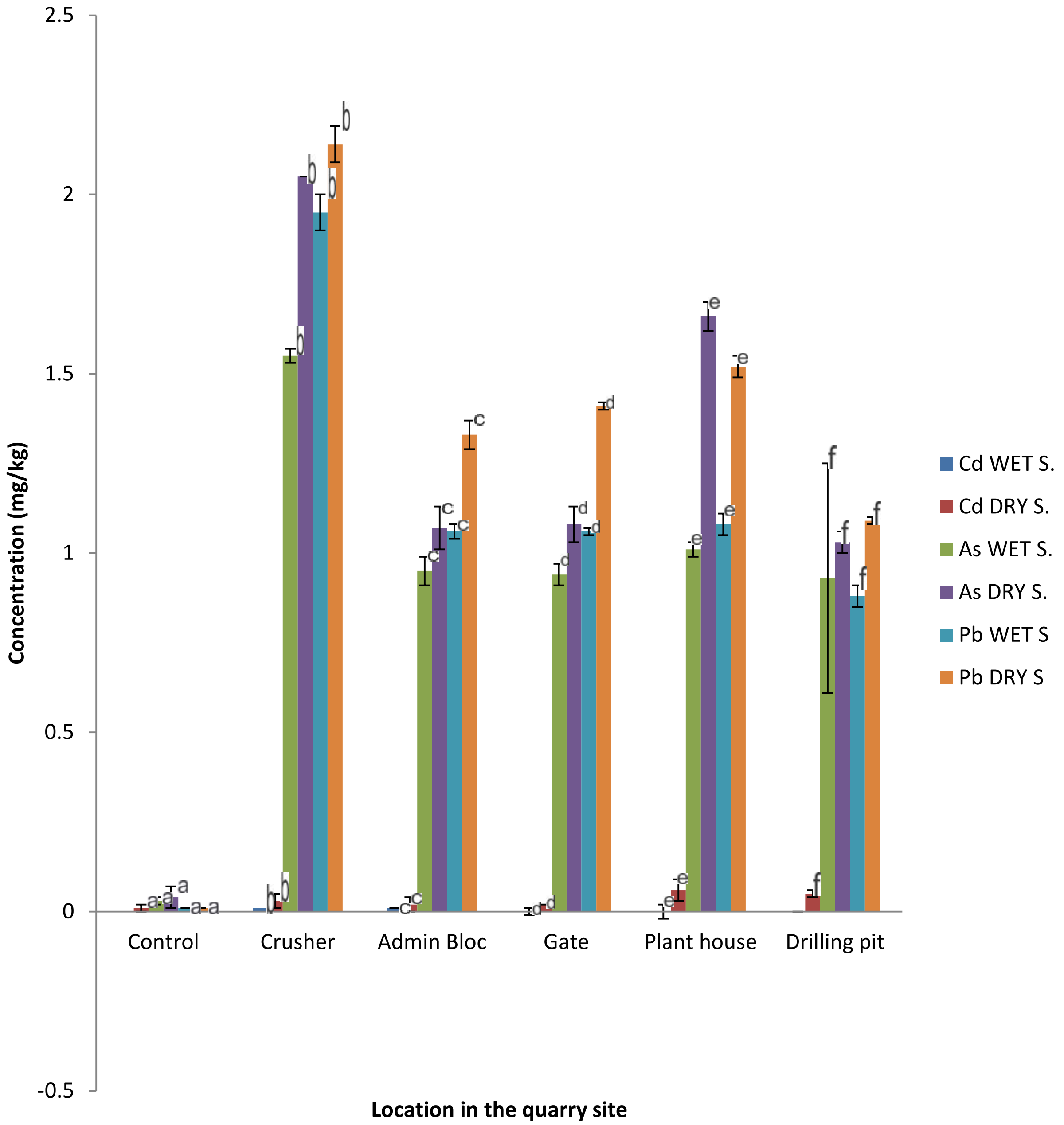
Arsenic (As): During the wet season, the concentration of arsenic from the test samples ranged from 0.93±0.32 (from the drilling pit) to 1.55±0.02 (from the crusher). The least recorded value (0.03±0.01) was from the control sample. In the dry season the concentration ranged from 1.03±0.03 (from the drilling pit) to 2.05±0.04 (from the crusher). The least recorded value (0.04±0.03) was from the control sample.

Lead (Pb): During the wet season, the concentration of lead in the test samples ranged from 0.88±0.03 (from the drilling pit) to 1.95±0.05 (from the crusher). The least recorded value for the season was 0.01±0.00 (from the control), whereas, in the dry season, the concentration ranged from 1.09±0.01 (from the drilling pit) to 2.14±0.05 (from the crusher). The least recorded value (0.01±0.00) was from the control sample. Concentrations of all the analysed heavy metals in the serum of the albino rats housed at different locations of the quarry site increased significantly ($p<0.05$) compared to the control.



Results are mean ± standard deviation of triplicate results obtained. Bars of the same color having different letters of alphabet are statistically different ($p < 0.05$) using Least Significant Difference (LSD). **Legend:** Wet S. = Wet season, Dry S. = Dry season.

Figure 3: Concentrations (mg/kg) of heavy metals (Co, Zn and Cr) in the blood samples of albino rats exposed to quarry dust at various locations in Ugwuele quarry site, Uturu.



Results are mean ± standard deviation of triplicate results obtained. Bars of the same color having different letters of alphabet are statistically different ($p < 0.05$) using Least Significant Difference (LSD). **Legend:** Wet S. = Wet season, Dry S. = Dry season.

Figure 4: Concentrations (mg/kg) of heavy metals (Cd, As and Pb) in the blood samples of albino rats exposed to quarry dust at various locations in Ugwuele quarry site, Uturu.

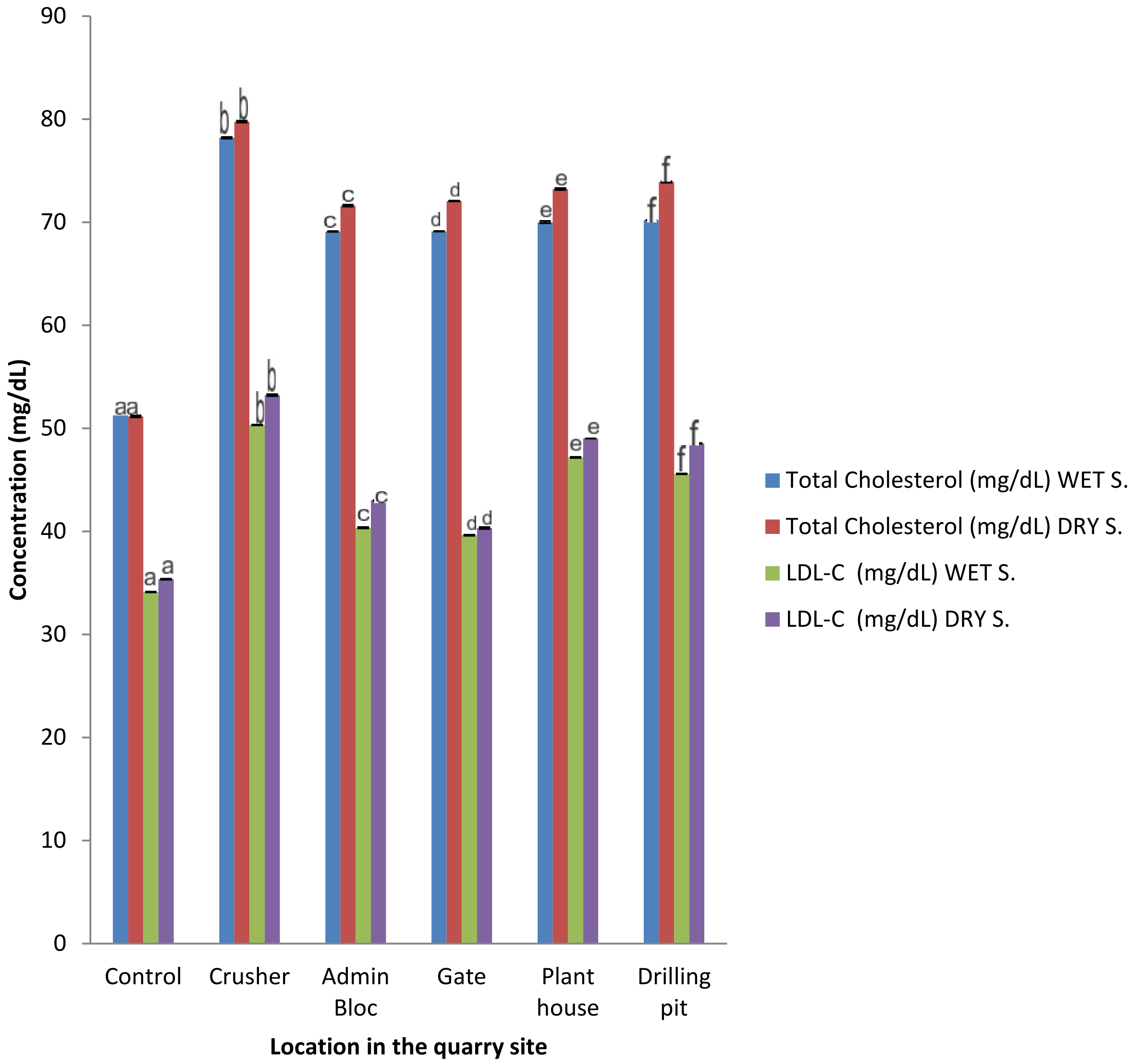
Concentrations (mg/dL) of lipid profile parameters in the serum samples of the albino rats are presented in figures 5 and 6 below.

Total Cholesterol: In the wet season, the concentration of total cholesterol in the test samples varied from 69.08±0.01 (from the admin. block) to 78.18±0.03 (from the crusher). The least recorded value (51.25±0.06) for the wet season was from the control sample. In the dry season, the concentration varied from 71.61±0.09 (from the admin. block) to 79.74±0.09 (from the crusher). The least recorded value (51.16±0.09) was from the control. Values of total cholesterol in the serum of the albino rats housed at different locations of the quarry site increased significantly ($p<0.05$) compared to the control.

LDL-C: During the wet season, the values of low density Lipoprotein cholesterol (LDL-C) in the test sample ranged from 39.62±0.04 (from the gate) to 50.31±0.03 (from the crusher). The least value of 34.12±0.03 was from the control. During the dry season, the values ranged from 40.31± 0.09 (from the gate) to 53.22±0.09 (from the crusher). The least recorded value of 35.35±0.05 was from the control sample. Values of LDL-C in the serum of the albino rats housed at different locations of the quarry site increased significantly ($p<0.05$) compared to the control.

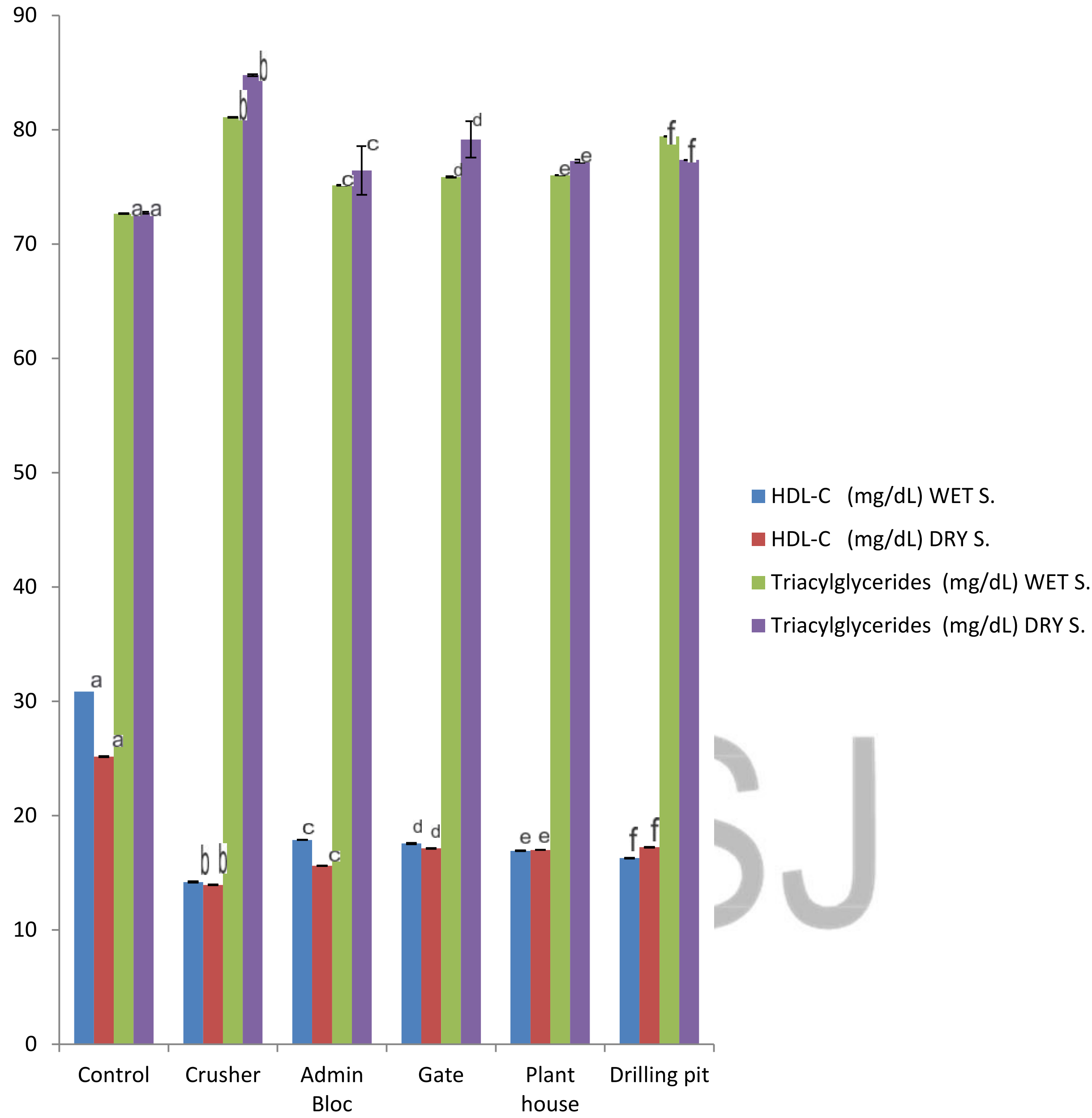
HDL-C: In the wet season, the values of high density lipoprotein cholesterol (HDL-C) in the test samples varied from 14.19±0.01 (from the crusher) to 17.88±0.07 (from the admin. block). The highest recorded value (30.84±0.06) was from the control sample. During the dry season, the values ranged from 13.94±0.04 (from the crusher) to 17.24±0.04 from the drilling pit. The highest value (25.16±0.04) was from the control samples. Values of HDL-C in the serum of the albino rats housed at different locations of the quarry site decreased significantly ($p<0.05$) compared to the control.

Triacylglycerides: During the wet season, the values of triacylglycerides in the test samples ranged from 75.14±0.04 (from the admin. block) to 81.08±0.03 (from the crusher). The least recorded value (72.66±0.04) was from the control sample. In the dry season, the values ranged from 76.43±2.12 (from the admin. block) to 84.77±0.09 (from the crusher). The least recorded value (72.74±0.09) was from the control. Values of triacylglyceride in the serum of the albino rats housed at different locations of the quarry site increased significantly ($p<0.05$) compared to the control.



Results are mean ± standard deviation of triplicate results obtained (n=3). Bars of the same colour having different letters of alphabet are statistically different ($p < 0.05$) using Least Significant Difference (LSD). **Legend:** Wet S. = Wet season, Dry S. = Dry season.

Figure 5: Concentrations of lipid profile test parameters (Total cholesterol and LDL-C) in the albino rats exposed to quarry dust at various locations in Ugwuele quarry site, Uturu.



Results are mean ± standard deviation of triplicate results obtained (n=3). Bars of the same color, having different letters of alphabet are statistically different ($p < 0.05$) using Least Significant Difference (LSD). **Legend:** Wet S. = Wet season, Dry S. = Dry season.

Figure 6: Concentrations of lipid profile test parameters (HDL-C and triacylglycerol) in the albino rats exposed to quarry dust at various locations in Ugwuele quarry site, Uturu

DISCUSSION

The results from the present study show that there was decrease in the body mass of the test albino rats during the study period and throughout the studied areas of the quarry. The decrease in body mass could be as a result of inhaled particulate matter and higher concentrations of toxic heavy metals in the serum of the studied rats. However, the body mass was lower in the dry season compared ($p<0.05$) to the wet season. This may be due to the comparatively higher concentrations of heavy metals detected in the serum of the rats during the dry season. However, the control rats showed increase in body mass, in both wet and dry seasons. Farida *et al*. [16] reported similar results.

The results from this study reveal that levels of heavy metals recorded from the exposed rats in both seasons were significantly higher ($p<0.05$) compared to those of the control. The concentrations in the dry season were also higher than those in the wet season. These are so because the exposed rats accumulated more heavy metals from the quarry dust through inhalation and / or contaminated food and drinking water. The results of the present study also show that chromium recorded the highest concentration (16.86±0.06mg/L) in the blood samples of the rats as chromium might have been accumulated more than other heavy metals. The fact that chromium is an essential element might have also contributed to its high level in the blood samples of the exposed rats. Cadmium on the other hand recorded the least concentration of 0.01±0.01mg/L. Cobalt is an important element because it is a component of Vitamin B_{12} but too much of it can be toxic. Exposure of industrial workers to toxic levels of cobalt is mainly through inhalation [17]. Excessive levels of cobalt affect not only the respiratory system but also have harmful effects on the kidney, liver, body mass and cardiovascular system [18]. Zinc is also an essential element but excess zinc can be harmful. Hydrochloric acid which is present in the stomach dissolves zinc thereby producing zinc chloride which can be injurious to the lining of the stomach [19]. Chromium is also an important element because of its role in sugar, protein and fat metabolism but excess of it can be detrimental to health [20].Cadmium is a heavy metal poison [21]. The main route of exposure of cadmium is inhalation thereby causing inhalation toxicity. Cadmium may cause kidney and liver injuries [22]. Arsenic can blind thiol or sulfhydryl groups in tissue proteins of the kidney, liver, lung and spleen. It can generate reactive oxygen species which may cause lipid peroxidation and DNA damage [23]. Lead is a toxic metal. It interrupts the biosynthesis of heme thereby causing anemia and other hematological problems [24]. Lead also has negative effects on the proximal tubules of the kidney [17].

Lipids are very important components of plasma membranes and hormones. They are also important source of energy in the body. However, the lipids are not soluble in blood and therefore require low density and high density lipoproteins for their transportation in and out of cells. Results obtained from the present study show that the concentration of total cholesterol (TC), LDL-C and Triacylglycerides (TG) from the exposed rats increased significantly ($p<0.05$) compared to the control. However, the concentrations of HDL-C from the exposed rats were lower compared to those of the control. The increase in TC, LDL-C and TG reported in this study may be due to higher production or low clearance of lipoproteins. Low clearance may occur due to inhibition of the liver lipoprotein lipase activity by heavy metals or alterations of the cell-surface receptors for lipoproteins [25]. Some heavy metals like lead and chromium inhibit cytochrome P450 activity [26, 25]. This can in turn decrease the clearance of cholesterol from the body through the hindrance of the biosynthesis of the bile acids. HDL-C is often referred to as the "good cholesterol" as a result of its role in the catabolism of cholesterol. The low level of HDL-C reported in this study is in the same trend with that of Samarghandian *et al.* [27] who reported that liver dysfunctions caused by exposure to heavy metals such as cadmium decrease the HDL-C content to cause dyslipidemia. The results from this study also indicate that the concentrations of TC, TG and LDL-C were raised significantly ($p<0.05$) in the test samples during the dry season compared to the wet season, while the concentrations of HDL-C were decreased in the dry season compared to the wet season. This may be due to the fact that higher concentrations of heavy metals were determined in the serum of the rats during the dry season compared to the concentration in the wet season. High concentrations of TC, TG, LDL-C and lower concentrations of HDL-C found in the serum of rats used in this study are indications of risk of cardiovascular disease.

CONCLUSION

The data obtained from the present study indicate that the experimental animals used in this study were at a high risk of heavy metal toxicity and cardiovascular disease. The risks are more in the dry season than in the wet season. These results imply that the quarry workers at Ugwuele are at greater risks of heavy metal toxicity and cardiovascular disease. This study also found that workers at the crusher section of the quarry site are at the highest risk of heavy metal toxicity and cardiovascular disease.

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