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The effect of heavy metal air pollution arising from local metallurgical activities on albino rat

Adamu Yunusa Ugya[1], Tijjani Sabiu Imam[2], Agamuthu Priatamby[3]

[1]Department of Environmental Management, Kaduna State University, Kaduna, Nigeria [2]Department of Biological Sciences, Bayero University Kano, Kano, Nigeria, [3]Institute of Biology, University of Malaya, Kaula Rumpur, Malaysia.

ABSTRACT

Introduction: The ecological equilibrium of the earth is endangered by the chemical process industries as most industries continue to spew chemical toxicants into the environment. Since local metallurgical activity is inevitable in Nigeria.

Aim: This research is aimed at assessing the effect of heavy metals air pollution arising from local metallurgical activities on Albino rat.

Methods: Particular matter samples were collected for 24h at four different sites twice a month using a high volume air sampler. Point A is Bakin Dogo Market, Point B is Old Panteka market, Point C is new Panteka market and Point D is U/Ma'azu. All the collected samples were analyzed for heavy metals using standard laboratory procedures. Six hundred newly born male albino rats were collected from National Institution for Trypanosomiasis Research (NITR) and were exposed to the sampling points twenty four hours daily via out the experimental period. Mortality, haematological and histological parameters were investigated. The rat tissues were also digested for the determination of Bioconcentration factor (BCF).

Result: The result obtained shows high Concentration of heavy metals (Pb, Cd, Cu and Ni) above the recommended limit by European commission (EC), World health organization (WHO) and NEPM (National Environmental Protection Measures). Higher BCF was obtained for all the heavy metals (Pb, Ni, Cu and Cd) which was attributed to the higher changes in Mortality, haematological and histological parameters if compare to the control.

Conclusion: The studies shows how heavy metal air pollution could cause Mortality and also bring about changes in haematological and histological parameters, which resulted due to high BCF. It is thereby recommended that local metallurgical activities should be closely monitored by environmental agencies such as Kaduna state environmental protection agencies (KEPA) and Federal environmental protection agencies (FEPA). The government should also make regulations that will mandate the paying of environmental degradation tax owing to the daily multiplication of vehicles and other automobile materials.

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Introduction

Environmental pollution is a major hazard facing the world today and there is an increasing awareness of the fact that a clean environment is necessary for smooth living and the better health of human beings [1]. The ecological equilibrium of the earth is endangered by the chemical process industries as most industries continue to spew chemical toxicants into the environment [2]. The release of toxic and harmful substance into air by natural forces, man and other animal has become a great threat to urban areas of developing countries and is increasing in positive

Contact Yunusa Adamu Ugya ugya88@yahoo.com Department of Biology, Ahmadu Bello University Zaria, Kaduna State, Nigeria.

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correlation to urbanization and industrialization [3,4,5,6]. The quality of air quality is compromised by day to day anthropogenic activities resulting from human activities such as indiscriminate use of chemical based substance and this is of a great concern not only in mega cities but developing cities because these chemicals are passed on from one organism to another through all the links can ultimately be accumulated in the fat of human body and can increase in lungs or cardiopulmonary injuries [7,8]. Anthropogenic emission of heavy metals have been linked to rapid urbanization and industrialization since both develop and developing countries are now in mad race to exploit every bit of natural resources to convert them into goods for their comfort and to export them to needy under developing world [9]. Leili, *et al*., [10] reported that heavy metals emissions can be due to combustion of fossil fuel, mineral processing industries, power stations using fossil fuels, refineries, brickworks and motor vehicles, forest fires, industrial metallurgical processes and oceans [11,12,13]. Researchers such as Ostro and Chestnut [14], Pope *et al* [15], Lighty *et al* [16] and Mage *et al* [17] have shown a strong association between particulate exposure and adverse health effects. Pollution of air by heavy metals is responsible for a number of pollution diseases and health conditions such as respiratory infections, heart diseases and lungs cancer [18,19]. Metal air pollution is an emerging risks factor for stroke in developing countries, since pollutants concentration are highest [20]. The cases of cancer is also under the rise in developing countries, since the rate of exposure to traffic related sir pollutants is unavoidable owing to bad roads and higher vehicles [21]. WHO in 2014 estimated that about 7 million people worldwide die premature death as a result of air pollution with developing countries having the highest numbers? Researchers such as Gauderman [22], Chen *et al* [23], Pope *et al*., [24], have also reported death cases arising from exposure to air pollution. Owing to the high rate of unemployment in Nigeria, most inhabitants of Kaduna state solemnly depend on local Metallurgical activities as occupation, They locally works with metals and alloys in the development, production and manufacturing of metal items and structures. They work with a wide range of products including copper sheet, precious metals, iron, steel, zinc, copper and aluminum alloys and are ignorant of the negative impact of these activities on the environment and their health in general. This research thereby aimed at assessing the effect of local metallurgical activity on the quality of air.

Material and Method

Air sampling and study area

The samples were collected from for site twice a month for the period of 12 month (January 2016 to December 2016). Particular matter samples were collected for 24h at four different sites twice a month using a high volume air sampler (Envirotech APM 410-411) according to the method employed by Bhaita, [1]. Point A is Bakin Dogo Market which is characterizing by combustion of substances belonging to stationary and mobile categories, it is also characterize with high local metallurgical activities. Point B is Old Panteka market which is characterize with local metallurgical activities including material handling, ore sintering and pelletising and high metallurgical work in which metal scraps are convert into different objects by the use of heating, hammering and other processes e.t.c. Point C is new Panteka market which is characterize by removing of all the parts of a vehicle by roasting, hammering and heating processes. Point D is U/ma'azu which is characterized with separation of large vehicles into metal part for transportation to new Panteka market for conversion into other metal objects. All the collected samples were packed in polyethylene bag and transported immediately to the laboratory and analyzed for heavy metals in the particulate matter using standard laboratory procedures.

Extraction of heavy metals from air

The method of Pruseth *et al.*, [25] was used to digest the sampled particulate matter before subjected to atomic absorption spectrometer. The exposed fiberglass filter, was cut and put in a Teflon crucible, then a mixture of 5 ml of Hydroflouric acid (HF), 10ml of conc. Nitric acid (HNO_3) and 1 ml of Perchloric acid ($HClO_4$) was taken in the same crucible and was kept on a hot plate at a temperature range of 85˚C - 90˚C with the lid on for 4 hours to ensure complete reaction. After 4 hours the lid was removed and the solution was evaporated to dryness. In next step, 10 ml HF, 5 ml HNO_3 and 1 ml $HClO_4$ was added to above and then heated to dryness. After cooling, in the next stage, 5 ml concentrated Nitric acid was added and heated to dryness. In next step, 20 ml of 1 N Hydrochloric acid (HCl) was added to about 100˚C to bring the digested sample into solution and then transferred to 50 ml flask. This sample was cooled and raised up to 100 ml by adding milli-Q water. The obtained solution was filtered through a Whatman 42 filter paper and stored in a clean and sterile plastic bottle at 4˚C until further analysis. The digested samples were analyzed for target heavy metals by Atomic absorption spectrometer.

One-way analysis of variance (One-way ANOVA) was used to test for significant difference between the concentration of heavy metals in air across the sampling point and year at $P < 0.05$.

Animal collection

Six hundred newly born male albino rats were collected from National Institution for Trypanosomiasis Research (NITR). The Animals were divided into 5 groups (Group 1: one hundred and twenty albino rat exposed to point A, Group 2: one hundred and twenty albino rat exposed to point B, Group 3: one hundred and twenty albino rat exposed to point C, Group 4: one hundred and twenty albino rat exposed to point D, Group 5: one hundred and twenty albino rat use as control).

Exposure and treatment

Four Hundred and eighty albino rat (one hundred and twenty each) were exposed to the sampling points twenty four hours daily via out the experimental period and provided with food and water always and closely observed in their cages for any mortality/any adverse signs of severe toxic effects like hypo-activity, anorexia, salivation, diarrhea, syncope, muscle cramping, convulsions,alopecia, barbering, head tilt, Paralysis, facial dermatitis, eye problems, degloving injury if any, one hundred and twenty albino rat were keep in the zoology laboratory of Kaduna State university as the control. The general behavior of animals and symptoms developed by the animals during the study were continuously monitored [26,27].

Haematologicalical analysis and histopathology

The blood of the albino rats were sampled as described by Omnia [28] for the assessment of the various blood parameters such as Haemoglobin concentration, Packed Cell Volume (PCV), The Red Blood Cell (RBC) counted, total white Blood Cell Counts (WBC). The RBC indices including Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) were calculated. Liver, kidney, lungs and heart of the rats were removed and fixed in formal-saline [29]. The tissues were washed in running tap water for at least 2 hours to remove traces of formalin. This was followed by dehydration using successive percentages of alcohol (30, 50, 70, 90 and 100%). They were then infiltrated in chloroform and blocked in paraffin wax 58-60˚C melting point. Samples were embedded in fresh molten wax using L-shaped embedding moulds. Sections of 8µm thickness were cut and stained in haematoxylin and eosin (H&E). Permanent slides were prepared with these sections and microphotographs taken with a magnification of X400. This were examined and compared with those for control. Data were subjected to one-way analysis of variance (ANOVA) using SPSS software to test for the significant differences between means and where significant differences are found, the Duncan's Multiple Range Test (DMRT) was used to separate the significantly different means. The tissues of the animals were digested using acid mixtures (HNO_3+$H2SO_4$+$HClO_4$) in Teflon (PTFE) vessels for 60–120 min on hot plate (125–140°C) until a clear solution was obtained [30]. Heavy metal (Pb, Cu, Ni and Cd) analysis was done by flame Atomic Absorption Spectrophotometer. Degree of metal accumulation in the tissues was assessed by bioaccumulation factor (BAF) for the rats as described by [31]. Where, BAF = [metal concentration in rats organ (µg/1)/metal concentration in air (µg/1)] [32].

Result

Heavy metals pollution

The result of the studies shows mean Pb concentration of between 15 µg/m^3 and 47 µg/m^3 throughout the year across the entire points, with point A having the highest concentration of 47 µg/m^3 (Figure 1). The mean concentration of Cu was also noticed to be between 9.5 µg/m^3 to 26.1 µg/m^3 throughout the year across the points, point A in the month of December recorded the highest of 26.1 (Figure 2). Comparison of mean Cu concentration obtain in this studies to Standard for ambient air quality by WHO [33] which clearly indicate high Cu pollution in all the month and with high possibility of deleterious effects to inhabitants of the area. The mean concentration of Ni and Cd were noticed to be between 5-47 µg/m^3 and 28-58µg/m^3 respectively, with the month of July having highest of 47 µg/m^3 and 58µg/m^3 respectively and at point A (Figure 3) and (Figure 4). Comparison of Ni and Cd concentration obtains in these studies to Standard for ambient air quality by EC (European commission) [34] clearly indicate high Ni and Cd pollution in all the month and with high possibility of deleterious effects to inhabitants of the area.

Mortality and general symptoms

Mice mortality was observed in all the cages including control. This mortality rate increases with increase with the time frame for the study. The study shows a total of 70 mortality cases in animals exposed in point A, with point D with the second highest mortality rate of 50 cases (Figure 5). Although,

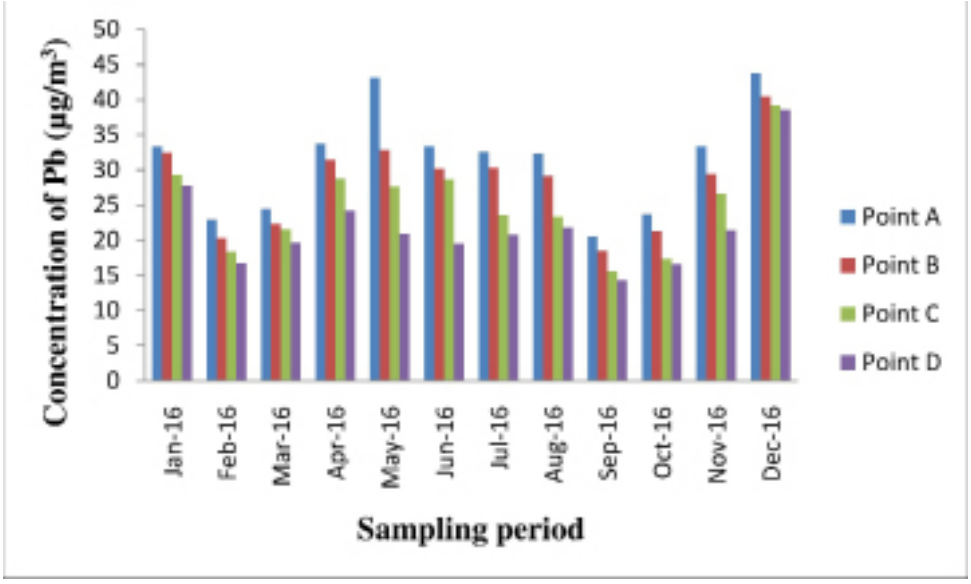

Figure 1. Mean concentration Pb in sampled air.

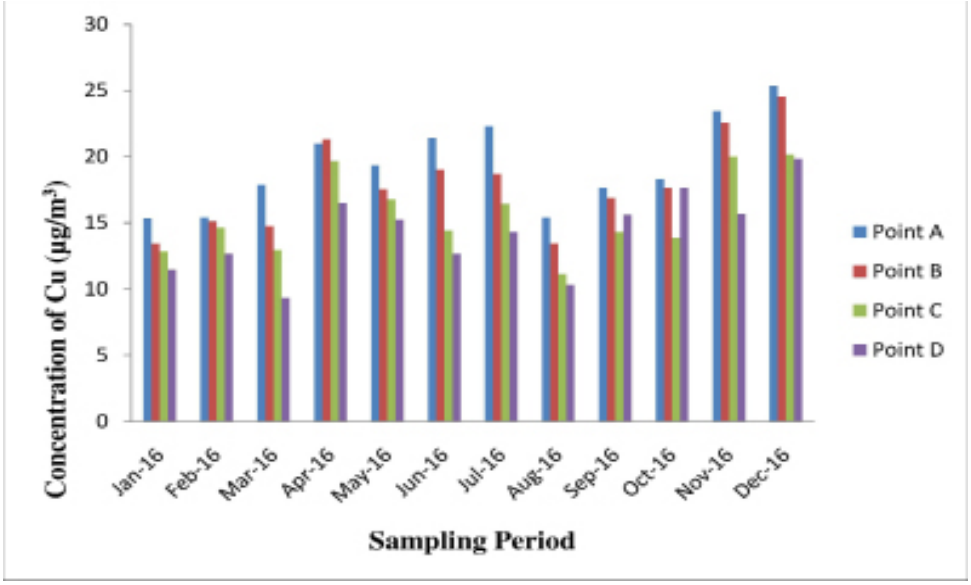

Figure 2. Mean concentration Cu in sampled air.

cases of mortality was reported in the control (9 cases) it could be deducted that exposure to the air pollutants to the various point contributed to the rates of mortality recorded since similar result was recorded by Bhatnagar [35]. Adverse effect resulting from rat exposure to air heavy metals show high percentage of symptoms such as hypo-activity, anorexia, salivation, diarrhea, muscles cramping, convulsion, alopecia, barbering, head tilt, paralysis, facial dermatitis, eye problem and degloving injury in the exposed animal compare to the control (Table 2).

Haematological analysis and histopathology

High decrease in packed cell volume (mm^3), total red blood cell (RBC) and haemoglobin (Hb) was recorded for the exposed mice compare to the control (Table 1) but an increase in total white blood cells (WBC), was recorded for all the exposed mice. Mice exposed shows to point A show lower packed cell volume, RBC and Hb compare to other extracts signifying high toxicity by caused by high heavy metal pollution in the point. The tissues (liver, kidney, adrenal glands, heart, brain, testes and spleen) exposed to the sampling points are slightly different from those of the control. Some of the observed changes include marked loss of secondary lamellae, attenuated primary lamellae, necrosis, marked fatty changes and loss of epithelial cells (Plate 1). The

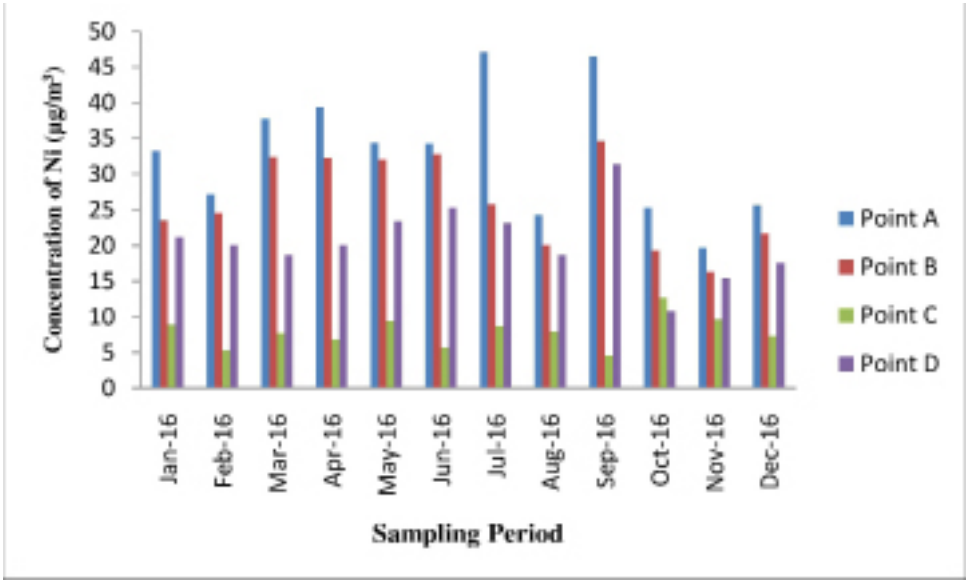

Figure 3. Mean concentration Ni in sampled air.

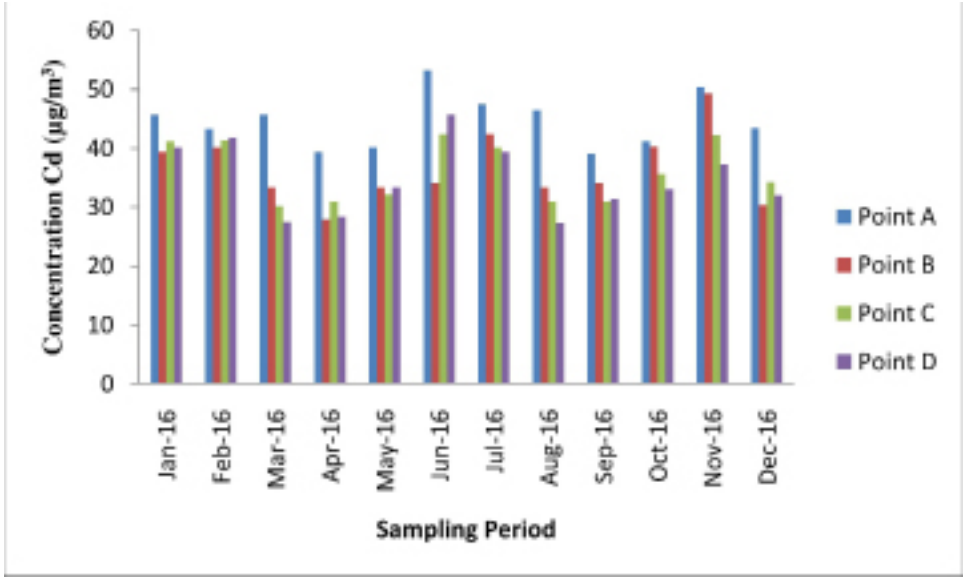

Figure 4. Mean concentration Cd in sampled air.

results obtain also show high BCF of Pb, Cu, Ni and Cd in the liver compared to other organs (Figure 6).

Discussion

Heavy metal pollution

The high Pb concentration recorded via out the month of December could be attributed to the fact that in the month of December more metallurgical activities were performed also this high Pb content could be supported by the high combustion of gasoline containing lead additives which is a primary source of lead in urban atmosphere [1,7]. This is also the reason why Pb concentration is higher in point A compare to point B, C and D in all the months, since point a is not just an area of metallurgical activities only but also an of intense combustion of stationary and mobile categories [36]. The high Pb concentration in point A is also a product of vehicular emission since the pollutants from reciprocating engine include Pb and its compound [37]. Comparison of Pb concentration obtain in this studies to Standard for ambient air quality by NEPC (National Environment Protection Council) [38] and WHO [33] clearly indicate Pb pollution in the month of December and high possibility of deleterious effects to inhabitants of the area. Pb pollution was also experiences in other months at some points.

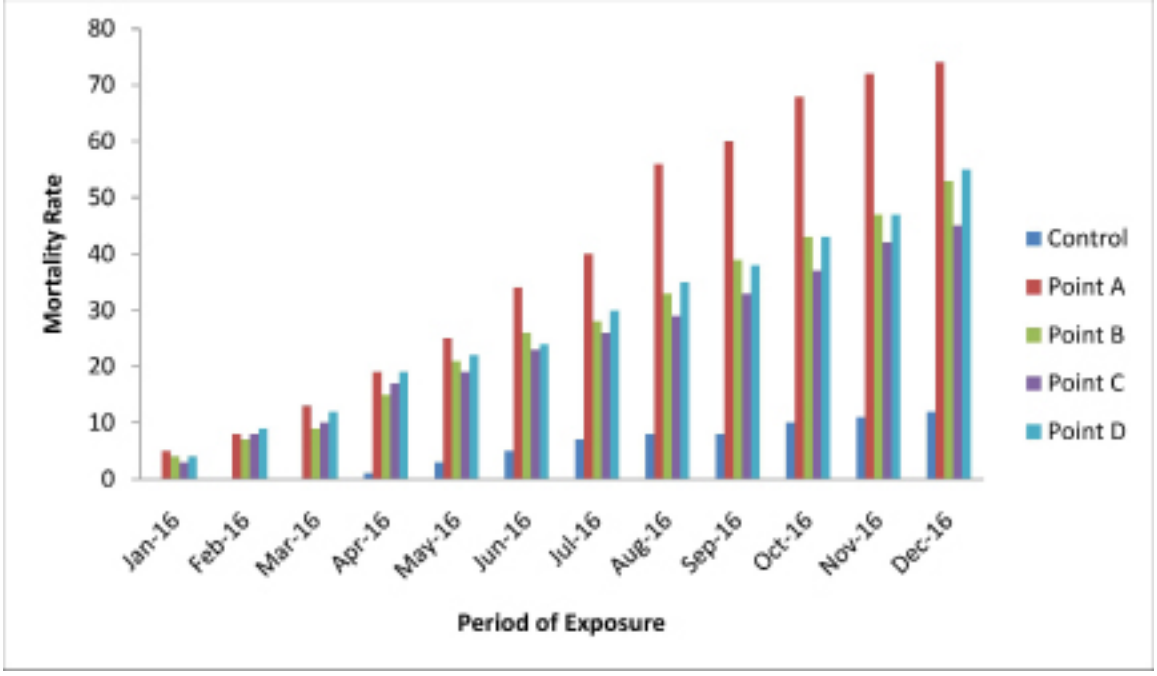

Figure 5. Mortality Rate of Expose Rats.

Table 1. Haematological parameters of Exposed Rat.

Conc. (mg/l)	Hb	WBC	PCV	RBC	MCV	MCH	MCHC
Control	7.33 ± 0.78^{a}	22.6 ± 0.35^{f}	21.57 ± 0.88^{a}	245.5 ± 0.058^{a}	0.86 ± 0.008^{c}	0.23 ± 0.025^{d}	32.5 ± 0.08^{d}
Point A	5.5 ± 0.25^{b}	23.39 ± 0.19^{e}	16.56 ± 0.88^{b}	241.6 ± 0.63^{b}	0.673 ± 0.27^{d}	0.16 ± 0.0125^{e}	32.2 ± 0.03^{d}
Point B	5.3 ± 0.215^{c}	24.42 ± 0.16^{d}	15 ± 0.446^{c}	177.3 ± 0.79^{c}	0.82 ± 0.022^{c}	0.263 ± 0.02^{d}	34.9 ± 0.034^{c}
Point C	4.85 ± 0.55^{d}	25.57 ± 0.09^{c}	11.32 ± 0.77^{d}	125.6 ± 0.55^{d}	1.14 ± 0.025^{b}	0.36 ± 0.0125^{c}	35.8 ± 0.036^{c}
Point D	4.53 ± 0.14^{d}	26.64 ± 0.02^{b}	10.20 ± 0.77^{e}	112.9 ± 1.76^{e}	1.17 ± 0.016^{a}	0.41 ± 0.021^{b}	38.4 ± 0.039

Means with the same superscript along the columns are not significantly different (P>0.05).

Table 2. Adverse Effect Resulting from Rat Exposure to Air Heavy Metals.

S/N	Adverse Sign	Exposed Animals (%)	Control Animals (%)
1	Hypo-activity	30	1
2	Anorexia	12	0
3	Salivation	73	49
4	Diarrhea	59	12
5	Muscles cramping	75	15
6	Convulsion	54	16
7	Alopecia	82	53
8	Barbering	61	33
9	Abscesses	0	0
10	Head Tilt	9	0
11	Paralysis	15	0
12	Facial Dermatitis	38	0
13	Eye problems	43	0
14	Degloving injury	13	0

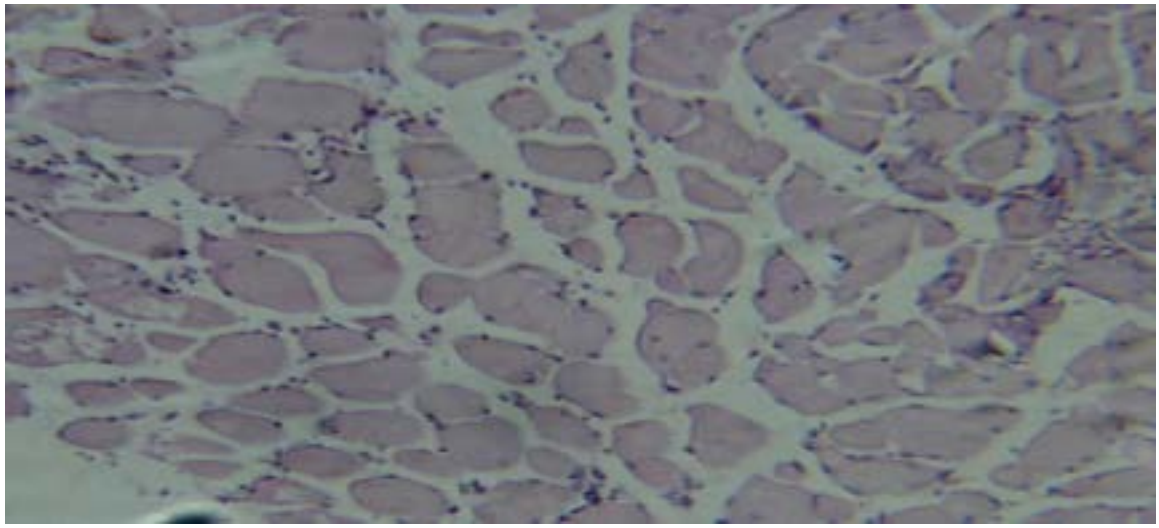

Plate 1. Liver cell of exposed albino rat showing extensive areas of necrosis.

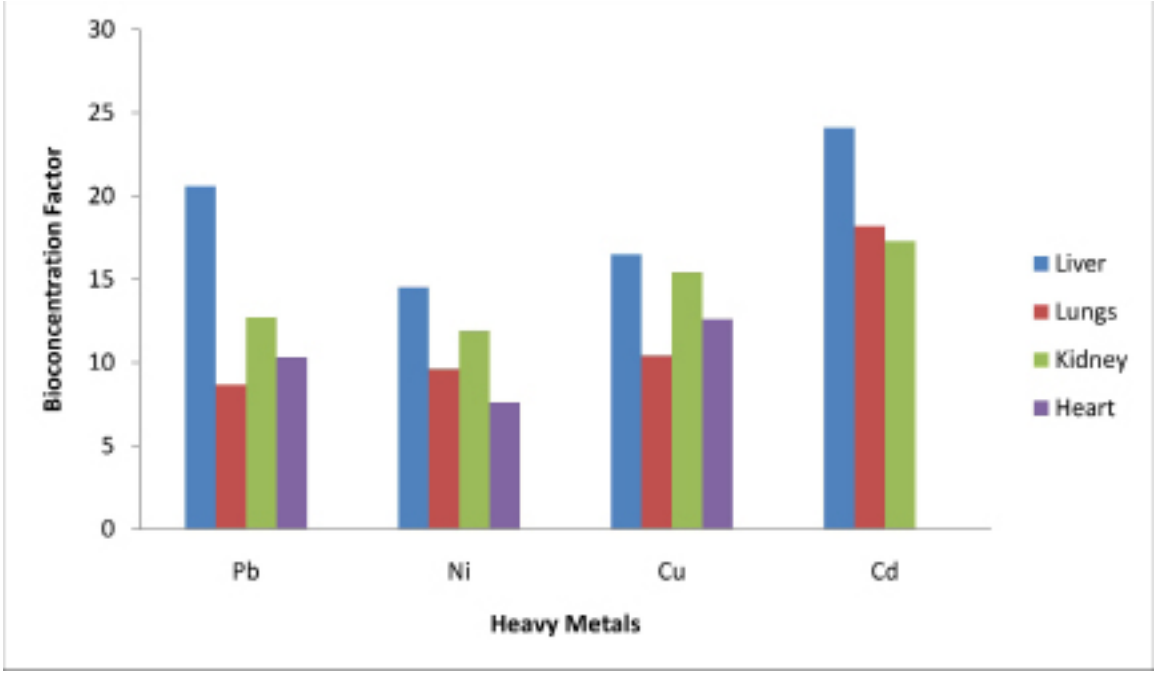

Figure 6. Bioconcentration factor for heavy metals in exposed Rats organs.

The high Cu concentration experienced in the month of December is attributed to high local metallurgical activities that occur in the area. The process of heating, melting and polishing of alloys and other metal substance could be the reason why the concentration of Cu was high in point A and in the month of December [39]. Though, the high Cu concentration in Point A could also be due vehicular and exhaust emissions [40,41]. The high Cd concentration experienced is attributed to high local metallurgical activities that occur in the area, vehicular, exhaust emissions and traffic emissions [39,40,41].

Haematological analysis and histopathology

The decrease in erythrocyte production could be the reason why the mices experienced anaemia, these decrease in erythrocyte production is attributed to the shortage in Hb which could be as a result of exposure to the air pollutants, although the shortage in Hb is critical after exposure to point A [42]. The significant decrease in the packed cell volume (PCV) in this study could be attributed to kidney damage and or impaired osmoregulation causing anaemia and haemodilution [43]. The increase in white blood cell could be associated with an increase in antibody production in response to

the extracts which help in long survival of the mice exposed [44]. Changes in haematological values occur in relation to physiological stress, diseases and toxic environmental conditions [40]. The fluctuation in the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) in the present study, clearly indicates that the concentration of haemoglobin in the red blood cells were much lower in the exposed mice than in the control mice, depicting aneamic condition. Aliyu *et al*., [45] observed similar fluctuations. The Mean Corpuscular Haemoglobin Concentration is a good indicator of red blood cell swelling [46], the Mean Corpuscular Haemoglobin Concentration, which is the ratio of haemoglobin concentration as opposed to the haemotocrit, is not influenced by the blood volume for the number of cells in the blood, but can be interpreted incorrectly only when the new cells, with a different haemoglobin concentration, are released into blood circulation. The fluctuation in neutrophils and lymphocytes could possibly be as result of antibodies released in the circulatory system of the mice as a form of defense. A study shows that lymphocytes consist the majority of white blood cell present in peripheral blood of mice, this study also showed neutrophils and lymphocytes increased as the concentration of the toxicant increased compared with basophils, eosinophils and monocytes [47]. Prentice and Kopp [48] reported lymphopenia on both neutrophils and eosinophils and also granulocytosis in mice after exposure to chrysin. These changes in differential leucocytes count also give evidence for decreased level non-specific immunity in rats after the exposure to toxic substances [49,50]. The higher BCF of Pb, Cu, Ni and Cd in liver compare to other organs could be attributed to the fact that the route of heavy metal entry into the experimental organism is not only via the lungs as the mouth and the skin can also serve as route of entry [1]. The high BCF recorded in this studies could be the reason why vacuolization, necrosis and nucleus condensation were noticed in the liver [51,52,53]. Higher BCF was also obtained in the lungs, kidney and heart, this higher BCF are responsible for the degenerated renal tubules, Bowman's capsule and contraction of glomeruli with reference to the kidney [49], lung and heart irritation [50,51].

Mortality and general symptoms

The effect of the heavy metal exposure could be the reason why high mortality rate was recorded in the exposed animals since less of the control organism die during the study. This high mortality recorded in the study correlate positively with the adverse effect resulting from exposure of the animal to air heavy since symptoms such as hypo-activity, anorexia, salivation, diarrhea, muscles cramping, convulsion, alopecia, barbering, head tilt, paralysis, facial dermatitis, eye problem and degloving injury were recorded in the exposed animal but minimal in the control animals [43].

Conclusion

Air pollution is not a new phenomenon since it existed even before man becomes aware of nature but the sources of this pollution need to be investigated with the view of reducing it, the present study is a step in the direction of air pollution and evaluation. This studies report high metal pollution which was linked to local metallurgical activities. The high heavy metal obtained is higher than recommended standard by EC and WHO so drastic health effect is expected from long time exposure to man and the environment. The studies shows how heavy metal air pollution could cause Mortality and also bring about changes in haematological and histological parameters, which resulted due to high BCF. It is thereby recommended that local metallurgical activities should be closely monitored by environmental agencies such as Kaduna state environmental protection agencies (KEPA) and Federal environmental protection agencies (FEPA). The government should also make regulations that will mandate the paying of environmental degradation tax owing to the daily multiplication of vehicles and other automobile materials.

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