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The Effect of Heavy Metal Pollution on Some Haematological Parameters in Domestic Birds: A Study in Camp2 Village, Akamkpa Local Government Area of Cross River State, Nigeria

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Abstract

This study was conducted to evaluate the concentrations of heavy metals in the blood of birds reared for food in Camp2 Community of Akamkpa, Cross River State, Nigeria. Twelve (12) male indigenous jungle fowls (*Gallus domestica*), six each from the control site (Ugep) and from Camp2 were sampled for the studies. Venous blood samples from the wings of the blood were collected and used for hematological and metal analysis. After digestion of the blood, heavy metals [cadmium (Cd), Lead (Pb), Mercury (Hg), Chromium (Cr), Arsenic (As), and Nickel (Ni)] were detected using atomic absorption spectrophotometer (AAS). The results indicated that Cd, Pb, As and Hg) levels in birds from camp2 village was significantly higher ($p < 0.05$; $p = 0.0070$) than those of the Ugep control site. The average value of nickel in the blood of Camp2 birds ($4.72 \pm 1.32 \text{mg/L}$) was significantly higher ($p > 0.05$; $p = 0.003$) than that of the Ugep control site ($4.22 \pm 1.07 \text{mg/L}$). The result shows that nickel, lead and Cadmium in blood samples were significantly ($p < 0.05$; $p = 0.023$) higher in Camp2 compared to the control site. Hence, these values were above WHO standard (0.1mg/l) and FAO/WHO standards of (0.2mg/kg) while others were within joint FAO/WHO standard limit. The studies show that domestic birds can bioaccumulate heavy metals in their blood, which in turn, presents serious health risks to humans who consume these birds within the communities.

Keywords: Quarry, metal toxicity, Atomic Absorption Spectrophotometer, Hematology.

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INTRODUCTION

Mining sites are raising worries in Akamkpa communities, given that they exert considerable influence on the ecosystem, which present adverse effects on the health of the surrounding populations. Individuals residing around such sites are at higher risk because wastes from quarry sites pollute the land, air, and vegetation, and may enter the food and water chain (UN-Habitat, 2008). Akamkpa communities are imperiled, owing to the large quantity of environmental xenobiotics and associated health conditions they are exposed to (Afullo and Odhiambo, 2009). Some of these problems result from the existing incompetence of urban authorities to sufficiently regulate quarry sites, leading to in unrestrained and careless deposition of waste materials in the communities. Increased environmental contamination and pollution by heavy metals is a sequela of this unchecked activity (Uwah et al., 2011), since most of the waste materials are considerable sources of heavy metals (Lisk, 1998). Heavy metals have become a source of universal problem because they are ubiquitous and have multi-faceted outcomes on the biome (Uwah et al., 2011). They cannot be degraded by biotic factors and therefore can accumulate in soil or water, affecting both flora and fauna (Dudka and Miller, 1999). Typical heavy metals include arsenic, mercury, lead, copper, and cadmium. They are aggregate poisons, posited to be extremely toxic, and progressively induce environmental hazards. In addition, they are major promoters of cellular oxidative stress, which has been implicated in the pathogenesis of countless human pathologies, including cancers (USEPA, 2002). Neurodegeneration, impaired cognitive function, cerebral palsy, inflammatory responses, certain types of cancer, and even death of fetus ensues following exposure to heavy metal (Chakarabarti et al., 2001). Many metals alter DNA structure by directly binding to DNA strands to produce DNA adducts, causing chromosomal breaks (Kimanic, 2007). These contaminating heavy metals and other waste materials inevitably enter the food chain of free-range animals, such as chicken, pigs, goats, dogs and cats. These animals can bioaccumulate these toxic matters, which is ultimately transferred to the neighboring households, causing maladies in both animals and humans. One-fourth of the causes of diseases plaguing the human race have been

attributed to protracted exposure to environmental pollutants, according to the World Health Organization (USEPA, 2007). The precarious outcomes of the products of quarry activity on wellbeing cannot be overstressed. Interestingly, the efforts of humans to industrialize have escalated the pollution of aquatic ecosystems. Pollutants from human anthropogenic activities include oil spillage, detergents, industrial dyes, runoffs from agricultural pesticides and fertilizers, sewage and heavy metals. They enter into the food chains of humans and animals, thereby eliciting various harmful effects (USEPA, 2007).

Hematological studies are used to assess the stress or disease state induced by contaminants and environmental changes. Hematological evaluations in domestic birds have garnered more attention, given the heightened cognizance of the adverse effects associated with the pollution of natural resources. Stress is a broad, non-specific, and biologic response to any factor that may disrupt homeostasis, which entails several physiological alterations, such as changes in immune cells and other components of the blood (Svoboda, 2001; Witeska, 2003)

Thus, this study intends to appraise the effect of heavy metal contamination on domestic birds using hematological indices in camp2 village Akamkpa Local Government Area (LGA) of Cross River State, Nigeria. In addition, this study aimed to fill the dearth of information regarding each exposure pathway and assess the probable adverse effects of these metals on the surrounding population. Results of this study will assist the establishment of regulatory and environmental-friendly policies for the affected communities close to the quarry sites in Cross River State, Nigeria.

MATERIALS AND METHODS

Study area

The study area is situated in the Cross River South Senatorial District (Longitude 8°12'E and Latitude 50241 N). The landscape is undulating, with rainforest vegetation, which is progressively deteriorating due to the quarry activities in these areas. Situated in Akamkpa LGA, the Camp2 site of Mfamosing village is thirty-six (36) kilometers from Calabar, which is the capital city of Cross River State, Nigeria. This community has high industrial activities, being located close

to the quarry site of Mfamosing. Ugep, the control site, is situated in the Cross River central senatorial district (longitude 8°51'E and latitude 5°04'18" N) and is one hundred and twenty-two kilometers from Calabar. The Yakurr people are the major inhabitants of Ugep, which is thought to be largest village in Africa by land mass (Sembulingam, 2002).

Relief, drainage and topography

Both study areas had good drainage and are surrounded by streams, which are used mainly for agricultural and drinking purposes. The land is mountainous (~ 250 m above sea level).

Field work Sampling points were chosen to assess the characteristic features. Twelve domestic birds, six (6) each, were sampled from

the two test areas Camp2 and Ugep. Ugep is the control site where there is no quarry activity. It is located far away from Camp2 in the central senatorial district in Cross River State. This was to allow us to investigate the potential source of heavy metal contaminants and determine the effect of the waste discharge from the quarry site on the study communities (Melville and Welsh, 2001). The selection was done by sampling randomly within the two study sites as bird's population are especially vulnerable and as such, a good bio-monitor of the effects of anthropogenic activities on the environment. Numerous biotic and physical processes, such as feeding habits, modulate the concentration and distribution of metals in the birds that are exposed to them, which have consequences in human health, as humans are the primary consumers of these domestic birds.

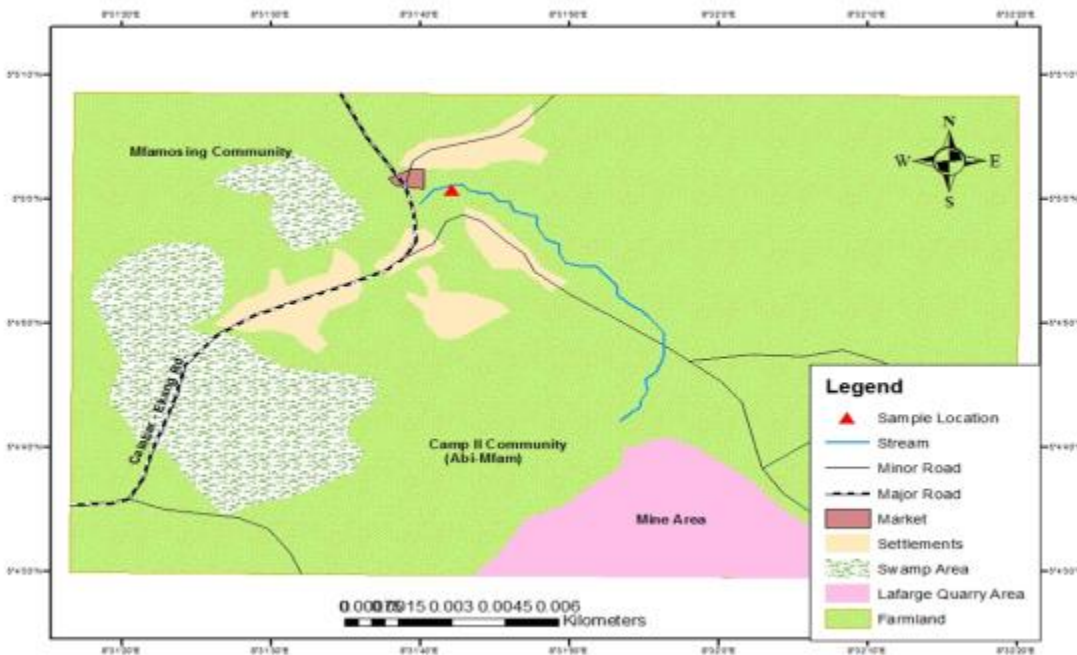


FIG. 1: Map of Camp2 showing the geographical characteristics of the quarry site (Source: Geographic Information System (GIS) Laboratory, Department of Geography and Environmental Science, University of Calabar).

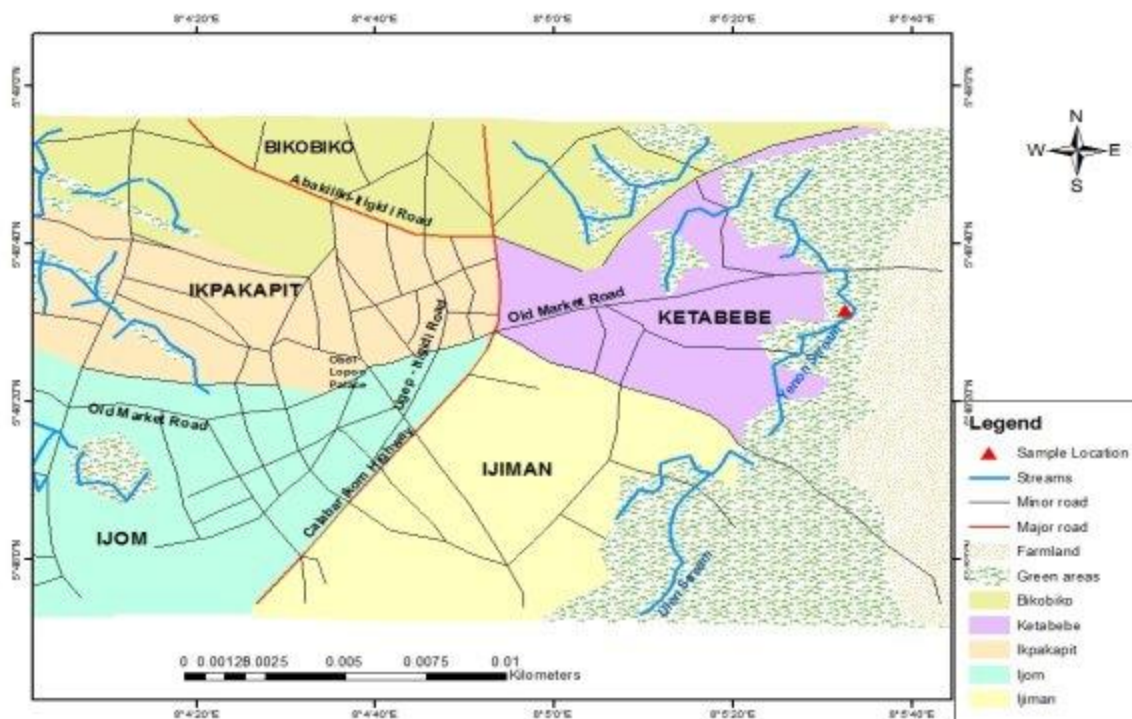


Fig. 2: Map of the Control Site (Ugep) (Source: Geographic Information System (GIS) Laboratory, Department of Geography and Environmental Science, University of Calabar).

Equipment and instruments

Atomic Absorption Spectrophotometer (model SOLAAR 969AA) was procured from Schemduu Company, Tokyo, Japan; Centrifuge (802 electric table centrifuge) from B-Bran Scientific Instrument Company, England; Semi-auto Biochemistry Analyzer (Model Aj-1222) was a product of Easy-way Medical Equipment Ltd, West Midlands, England; Automated Hematology analyzer (Model Bc-2600Kx-21N) was procured from Mid-Ray Ltd, Cambridgeshire England; while water bath was from Searl Instruments, Satchwell, Sunvic Ltd, Cambridge, England.

Reagents/chemicals used

All the reagents and chemicals used were of analytical grade and include coded International Atomic Energy Agency (IAEA-336) from Sigma, USA Nitric acid (Riedel Haen, Seelze, Germany), perchloric acid (Sigma-Aldrich, Baden-Wurtemberg, Germany), Distilled deionized water obtained from Central Analytical Laboratory Institute of Oceanography, University of Calabar. All biochemical assays were carried

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out using Agape Kit (Shanghai, China) and Aj-semi-auto Biochemistry Analyzer (Kalstein, Lichtenstein, Germany), while the hematological parameters were determined using Auto Haematology Analyzer Biobase Biozone Co. Ltd, Jinan, China)

Animal protocols

A total of twelve (12) local domestic birds/fowl, (males) (6 from Camp2 and 6 from Ugep respectively) that were sexually mature (between 10-12 months of age) with normal feather, obtained from local farmers in the control site and Camp2 village, both of Cross River State, were used for the study.

Blood sample collection

The blood samples were collected from the wing veins of the feather because they are considered to be exposed to heavy metals (Burger, 1993). Disposable syringes (2ml) were used and a portion of the blood was directly transferred into a labeled bottle containing EDTA (Ethylenediaminetetraacetic acid anti-coagulant) while the remaining portion blood was allowed to

clot. The non-coagulated blood was used to determine the hematological profile. Afterward the same quantity of the blood was subjected to centrifugation at 300 rpm for 15 mins, to get the blood serum samples that were used to measure toxicological indices using 5ml syringe and needle.

Sample preparation

Standard methods for blood digestion were followed (Stanley *et al.*, 2005). The samples were thoroughly mixed, 5ml of blood serum was transferred into a conical flask, 25ml concentration. Nitric acid and 5ml of perchloric acid was added and brought to slow boiling on an evaporating plate to lowest volume. Digestion was completed as shown by light colour and a clear solution. The solution was not allowed to get dried during digestion. The digest was filtered into 50ml volumetric flask diluted with distilled water for elemental analysis using Atomic Absorption Spectrophotometer (SOLAAR, 969 AA).

Estimation of Hematological Indices

The full blood counts including Hb, RBC, WBC and platelet count were estimated using the Sysmex Automated Haematology Analyzer (Biobase Biozone Co. Ltd, Jinan, China). The method was previously described by (Mbosso *et al.*, 2014). The instrument uses whole blood and pre-diluted blood samples. In this analyzer, the blood is fed into the transducer chamber (which contains electrodes between which flows direct current). The blood cells that pass through the aperture cause direct current resistance to change between the electrodes. As the direct current passes, the blood cell size is detected as electric pulses. The blood cell count is then detected by counting the pulses and a histogram of blood cell sizes is plotted by determining the pulse sizes.

Determination of white blood cell (WBC) and haemoglobin (HB) concentration.

The blood was aspirated from the sample probe into the sample rotor valve. About 6 μ l of blood measured by the sample rotor valve was transferred into the WBC transducer chamber along with 1.99-ml diluent. Simultaneously, 1.0ml of WBC lyse was added to prepare 1:500 dilution sample. The solution was made to react

with this for about 10 seconds. The red blood cell (RBC) was hemolyzed and platelets (PLT) shrink with white blood cell (WBC) membrane. Also, haemoglobin was transferred into red coloured methemoglobin. Of the diluted haemolysed sample in the WBC transducer chamber, approximately 1ml was transferred to haemoglobin (Hb) flow cell. A known quantity 500 μ l of sample in the WBC transducer is aspirated through the aperture. The pulses of the blood cells when passing through the aperture are counted by the DC method (Mobso *et al.*, 2014). In the Hb flow cell, 555nm beam irradiated from the light emitting diode was applied with the sample in the Hb flow cell. The concentration of this sample was measured as absorbance. This absorbance was compared with that of the diluent alone that was measured before the addition of the sample, thereby calculating Hb value

Determination of red blood cell (RBC) concentration and platelet count

The blood was aspirated from the sample probe into the sample rotor valve. Four microliters (4 μ l) of blood measured by the sample rotor valve were diluted 1:500 with 1.996ml of diluent and brought to the mixing chamber as diluted sample. Out of the 1:500 dilution sample, 40 μ l was measured by the sample rotor valve, diluted into 1:25000 with 1.960-ml diluent and then transferred to the RBC/PLT transducer chamber. Two hundred and fifty microliters (250 μ l) of the sample in the RBC/PLT transducer chamber was aspirated through the aperture. At this time, RBC and PLT were counted in the DC detection method. At the same time, HCT is calculated by RBC pulse height detection method.

Data analysis

Data obtained were expressed as mean \pm standard deviation and was analysed using the student's t-test where applicable. Mean values of $p < 0.05$ were regarded as significant in comparison to appropriate controls. Statistical analysis was also carried out using predictive analytic software for window (SPSS package 19.0). Values at $p < 0.05$ were regarded as significant in comparison with appropriate controls.

Ethical consideration

All animals experiment were approved by the Nigeria animal and use committee (NACUC) of the Faculty of Basic Medical Science, University of Calabar, and the methods were conducted in accordance with the approved guidelines

RESULTS

Comparison of blood sample parameters in domestic birds (*Gallus domestica*) in Camp2 and control site (Ugep).

The result of the red blood cell (RBC) count was 4.86×10^6 cells/ μ l for the domestic birds (*Gallus domestica*) in the control site. The results in Table 1 show a significant increase in hematological parameters in the control site (Ugep) compared to that of camp2.

Similarly, the white blood cell (WBC) counts ($\times 10^3$ cells/ μ l) of control site birds (39.82) were significantly higher ($p < 0.05$) than those of Camp2 birds. The packed cell volume (PCV) and haemoglobin (Hb) values of control site birds (43.40% and 14.44mg/dL respectively) were significantly higher ($p < 0.05$) than the corresponding values of Camp2 birds (29.40% and 9.78mg/dL respectively). Although the platelet count ($\times 10^3$ cells/ μ l) of the domestic birds were higher at Camp2 (261.60 ± 6.87 mg/dl)

compared to value recorded for the control site (249.20 ± 4.61 mg/dl), the difference was not statistically significant ($p > 0.05$). Also, the mean concentration of neutrophils in the control site had a mean value of 78.60 ± 1.36 compared to the study sites at Camp2 with a mean value of 58.60 ± 12.36 . The mean concentration of lymphocytes was significantly higher at Camp2 study sites with a mean value of 41.40 ± 12.36 compared to the control site with a mean value of 21.40 ± 1.36 .

Comparison of heavy metals in blood samples in domestic birds in camp2 and control site

The following elements were analyzed Cd, Pb, As, Hg, Cr and Ni. The results indicated that, Cr, Pb, As and Hg levels in camp2 village were significantly higher ($p < 0.05$) than those of the control site in Table 2. The Cd, Pb, As and Hg levels in (mg/L) were 3.19, 0.23, 0.22 and 0.02 respectively while the corresponding values in the control site were 1.15, 0.04, 0.01 and 0.01 respectively. The Cr levels were similar in both sides. The mean value of Ni in Camp2 (4.72 ± 1.32 mg/L) was significantly higher ($p > 0.05$) than that of the control site (4.22 ± 1.07 mg/L).

Table 1. Hematological parameters of domestic birds (*Gallus domestica*) of Camp2 and control site

	RBC ($\times 10^6$ cells/ μ l)	PCV (%)	Hb (g/dl)	PLT ($\times 10^3$ cells/ μ l)	WBC ($\times 10^3$ cells/ μ l)	N (%)	L (%)	E (%)	M (%)	B (%)
Camp2	3.80	29.40	9.78	261.60	29.16	58.60	41.40	0.00	0.00	0.00
	± 0.44	± 4.26	± 1.42	± 6.87	± 0.31	± 12.36	± 12.36	± 0.00	± 0.00	± 0.00
Control site (Ugep)	4.86	43.40	14.44	249.20	39.82	78.60	21.40	0.00	0.00	0.00
	$\pm 0.09^*$	$\pm 0.51^*$	$\pm 0.17^*$	± 4.61	$\pm 1.00^*$	± 1.36	± 1.36	± 0.00	± 0.00	± 0.00

Values are expressed as mean \pm SEM, n = 5; * = significantly different from Camp2 at $p < 0.05$
RBC – erythrocytes; PCV – Packed cell volume; Hb – Haemoglobin; PLT – Platelets; WBC – leukocytes
N – Neutrophils; L – Lymphocytes; E – Eosinophil; M – Monocytes; B - Basophils

Table 2: Concentration of heavy metals in blood samples in domestic birds of the study areas
Heavy metal concentration (mg/L)

	Cd	Pb	As (mg/L)	Hg	Cr	Ni
Camp2	3.19±0.61	0.23±0.07	0.02±0.00	0.02±0.00	0.05±0.01	4.72±1.32
Control site (Ugep)	1.15±0.66*	±0.04±0.01*	0.01±0.00*	0.01±0.00*	0.05±0.01	4.22±1.07

Values are expressed as mean ±SEM, n = 6; * = significantly different from Camp2 at p<0.05

DISCUSSION

The results of the hematological parameters of the domestic birds are presented in Table (1). The red blood cell count ($\times 10^6$ cells/ μ l) of the animal in Camp2 site and that of the control site were compared appropriately. The results in the control site were significantly higher ($P < 0.05$) than those of Camp2 respectively. These finding is an indication of some activities going on within the study area Camp2 and emission of dust in to the environment. The pack cell volume (PCV) also follows the same increase recorded for RBC for the animals in Camp2 comparing with that of the control sites. Pack cell volume, haemoglobin and red blood cell counts are important parameters that indicate the oxygen carrying capacity of the cells, and have the organism's ability to oxidatively clear normally by the liver, it's also a series of good biomarker for the kidneys filtration and clearance ability and is a good analyst than urea (Harman, 1981). Heavy metals are potent inhalator of data-aminolaevulinic acid oxidase, which is significant in haemoglobin synthesis (Beyersmann, 2002). A decrease in haemoglobin concentration will eventually result to a consequent reduction in red blood cell. Haemoglobin concentrations in blood samples from Camp2 study site were above the WHO range of $4.2 \times 10^2/l$ to $5.4 \times 10^2/l$ accepted for normal subjects (Stanley et al., 2005). This suggestion is in lines with other research work carryout in an environment exposed to heavy metals. The platelet count ($\times 10^3$ cells/ μ l) for animals in Camp2 site were compared to that of the animal at the control site. The result further shows that there was a significant increase in the two study sites indicating thrombocytosis which is usually result to an existing condition such as cancer or lymphoma. Also, this increase in the platelet

count may be followed by hemorrhage, surgery or bone fractures. In this present study the platelet counts for both study sites were above the range value for normal subject captured. The total white blood cells (WBC) count ($\times 10^3$ cells/ μ l) show a significant increase in the control site compared to the Camp2 sites. This decrease in Camp2 may be due to the fact that the body responds to contaminated food from the surrounding environment that may trigger the production of more antibodies. White blood cells constitute to the body defense mechanism. They constitute granulocytes, neutrophils, eosinophils and basophils and the agranulocytes constitute lymphocytes and monocytes. Their function is to fight against disease infection in order to protect the immune system. The percentage lymphocytes count of the animals in the study sites shows a significant increase in Camp2 compared to that of control site, though the changes in the two study sites were not significant. The present result is in line with that of (Latuente et al., 2003). The percentage eosinophil's, monocytes and basophils were not significant though they all play a vital role in immunity. They also secrete different substances e.g., factors that affect lymphocytes prostaglandins and dot promoting factors. A mean blood cadmium level of $3.4 \mu\text{g}/\text{dl}$ was measured in a remote unpopulated area in South Africa in accordance with the present results recorded in cadmium level in camp2 ($3.19 \mu\text{g}/\text{l}$). Compared to the control site Ugep (Molatlhegi, 2005). The heavy metal concentration in blood of animal (domestic birds) presented in Table 2 was significantly higher than the control. Interestingly all the heavy metals analyzed were however, within the permissible WHO limit except for cadmium nickel that were above WHO permissibility limit for metals. Human and animal models have indicated the carcinogenicity of nickel compounds. The effect of Ni may indirectly

inhibit DNA repair system (Silbergeld, 2003). Report has shown that accumulation of Ni in the breast tissue could result to malignant growth process (Hartwig and Sdiwerdtle, 2002). People who refine or utilize Ni are at risk of nasal cancers, following occupational exposure to Ni compounds, particularly nickel carbonyl (NiCO₄). NiCO₄ is produced following the reaction of Ni with carbon monoxide and is an unstable compound. It is the most noxious form of Ni and a potent carcinogen. Exposure to ~30 ppm NiCO₄ for thirty minutes could be lethal to humans. Although, empirical and epidemiological investigations have also implicated nickel sulphide (Ni₃S₂) in the carcinogenesis of Ni and has been reported to induce local tumors at points of exposure, such as lungs (following inhalation) and injection sites (Kasprzak et al., 2003). *In vitro* studies, involving mammalian cell, demonstrated that both Ni₃S₂ and nickel sulphate induced mammalian cell transformation (WHO, 2004). Exposure to large dose of Ni results in dermatitis and respiratory disorders. While its powder or dust is carcinogenic, in the cell, Ni inhibits the activities enzymes, such as cytochrome oxidase, maleic dehydrogenase and isocitrate dehydrogenase (Kasprzak et al., 2003).

Lead is a well-known neurotoxin, which can impair brain function in infants. Lead can accumulate in the tissues to aid mobilization from bones during pregnancy and lactation (ATSDR, 2007). Pb has been recounted to promote or enhance carcinogenic events, at a cellular and molecular level, and possibly involving DNA damage, as well inhibition of DNA repairs and deregulation of tumor suppressor genes (Deknudt, 1977). Important sources of Pb include food, drinking water and air (mainly lead dust originating from petrol). General feeling of illness, lethargy and pain in limbs are hallmarks of chronic Pb toxicity. Anemia is typically the first symptom of chronic exposure to low levels of Pb, in humans and animals. Pb induces oliguria and has been linked to the development of gouty conditions in exposed subjects. High Pb blood levels have been positively correlated with hypertension (Barttop and Smith, 1971). Lead poisoning, also known as plumbism, may instigate the occurrence of distinctive nuclear inclusions, which under light microscopes; appear as condensed, homogenous, eosinophilic bodies. Cells containing these nuclear inclusions typically appear swollen. Investigational studies posit that these inclusions

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are the initial sign of Pb toxicity, observable even before any of the functional alterations in the system become noticeable (Abadin et al., 2007). Furthermore, heavy metals, ever-present in the environment, could react with endogenous target molecules such as receptors, enzymes, DNA, proteins and lipids to alter their biochemical functions and thereby producing changes that would result oxidative damage.

CONCLUSION

The studies show that domestic birds have the potentials to accumulate heavy metals in their tissues. Thus, the concentrations of heavy metals present worrisome health hazards to humans who consumed birds within the communities. And also, could bring about an irrevocable damage to humans that feed directly from the food chain via assimilation, bioaccumulation and biomethylation processes. Thus, this study further suggest that more research should conducted on the distribution of these noxious metals in different parts of the tissues of the birds (blood, feather and other internal organs) in the affected sites both in the blood feather and other internal organs. Therefore, these studies recommend a biochemical chelating agent and the consumptions of vegetables and fruits, which are major sources of vitamin C, to facilitate in the excretion and chelation of heavy metals from human that may otherwise result in heavy metals-induce toxicity within their system.

Conflict of interest

The authors have no conflict of interest to declare.

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