

Potentially toxic metals in dust, blood, and hairs from exposed security dogs in an oil and gas industry

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Dog,
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Summary

Environmental pollutants pose a health risk to animals and humans. We evaluated levels of some potentially toxic metals in environmental dust, blood, and hair samples of apparently healthy security dogs from a crude oil well drilling site (A) and liquefied natural gas production site (B) industrial environments in Nigeria. These samples were routinely digested and analyzed for lead, cadmium, nickel, chromium, and zinc using atomic absorption spectrophotometry assay. Mann-Whitney U test was used to compare concentrations of the metals in different samples. Dust samples contained a high amount of the metals considered. There was no significant difference between levels of heavy metals in blood and hair samples from dogs guarding both sites, except for blood ($p=0.034$) and hair ($p=0.015$) chromium which were higher in those securing site A compared with site B. Higher nickel ($p=0.001$) and zinc ($p=0.001$) with lower chromium ($p=0.004$) levels occurred in the hair samples than in the blood. Lead was not detected in blood and hair samples suggesting safety. There was no correlation between the same metal in blood and hair. Hair chromium and nickel levels were above the reference suggesting toxic exposure. There is a need for regular monitoring and decontamination of air pollutants within similar facilities for environmental safety.

Introduction

Heavy metals are defined as metals and metalloids having densities $>5 \text{ g cm}^{-3}$ (Hawkes 1997). Heavy metals and metalloids are often used directly or indirectly at home, in agriculture, medicine, technology, and industries (Hawkes 1997). They are found naturally in the environment, certain foods, medicines, and water (Hawkes 1997; Zhuang et al. 2009; Renner 2010; Vodyanitskii 2016; Nworu et al. 2019). Their wide distribution in the environment has potential effects on the environment, plant, animal, and human health (Slotnick et al. 2000; McBride et

al. 2003; Zhuang et al. 2009, Orisakwe et al. 2012). Some heavy metals considered to be potentially toxic are required by the body in trace quantities for biochemical and physiological processes (Singh et al. 2018; Pourret and Hursthouse, 2019). However, at higher concentrations and depending on the dose, route of exposure, chemical speciation, age, gender, and nutritional status of the exposed animal or human being, they can have deleterious effects (Jaishankar et al. 2014; Pourret and Hursthouse, 2019). Animals and humans get exposed through ingestion of contaminated food, drinking of

contaminated water, inhalation of contaminated air, exposure to contaminated soils and industrial wastes, and absorption through the skin (Airey 1983; Alexander and Davidson 2006; Babalola *et al.* 2007; Renner 2010; Sharma *et al.* 2016).

High blood levels of heavy metals imply high exposure levels, while lower levels of trace elements and essential minerals represent insufficient intake which may be a sign of nutritional deficiency (Flora *et al.* 2008). A whole blood level of heavy metals is representative of extracellular and intracellular metal concentrations, while plasma and serum concentrations depict extracellular concentrations (Flora *et al.* 2008). Heavy metal toxicity is primarily due to oxidative damage to the biological macromolecules following the binding of metals to the DNA and nuclear proteins (Flora *et al.* 2008). Consequently, this distorts the normal functions of the brain, lungs, kidneys, liver, and hematopoietic organs (Broun *et al.* 1990; Järup 2003; Dorne *et al.* 2011). Some of the degenerative conditions associated with heavy metal toxicity are Parkinson's and Alzheimer's diseases among others (Flora *et al.* 2012). Chronic exposure to some of these potentially toxic metals could lead to varying types of cancers, while some metallic elements induce multiple organ damage even at lower levels of exposure (Aquino *et al.* 2012; Chervona *et al.* 2012). Some common heavy metals with potentially high occupational and environmental adverse effects are mercury (Hg), cadmium (Cd), lead (Pb), copper (Cu), vanadium (V), chromium (Cr), cobalt (Co), nickel (Ni), selenium (Se), arsenic (As), manganese (Mn), silver (Ag), zinc (Zn), and uranium (U) (Jaishankar *et al.* 2014; Vodyanitskii 2016). In Nigeria, crude oil and gas exploration, exploitation, and production, as well as gas flaring are common sources of environmental pollution (Nworu *et al.* 2016). Crude oil and gas pollution occurs in form of spillage from accidental discharges, corrosion of pipelines, oil well blowout, oil pipeline vandalism, and gas flaring. Dogs are used to support securing many of the crude oil and gas production facilities in Nigeria and are exposed to the environmental pollutants associated with these anthropogenic activities just as human beings working or living within those locations. Also, the pathophysiology of the adverse effects of these toxic metals in animals and human beings are similar (Airey 1983; Jaishankar *et al.* 2014; Santin *et al.* 2005). Animals are valuable sentinel for environmental contamination; however, they are often ignored. Consequently, this study was designed to assess the levels of some potentially toxic heavy metal elements in environmental dust, blood, and hair of dogs used for securing a crude oil well drilling and liquefied natural gas production facility, generally regarded as one of the environments at higher risk of elemental pollutants.

Materials and Methods

Sampling and sample preparation

This study was carried out on dogs used for security purposes in crude oil well drilling (site A) and liquefied natural gas production (site B) in Nigeria. Blood (5 ml) and hair (2 g) samples opportunistically collected from 13 dogs (in July 2016) from blood used for health parameters evaluation, i.e., for routine clinical and laboratory screening were used for some heavy metal analysis. Technically, no animal was specifically sampled for this study. Ethical approval from the University of Ibadan Animal Care Use and Research Ethical Committee (UI-ACUREC/App/2015/027) was obtained for this study after reviewing the panel and the listed guidelines and principles of animal handling and care were strictly adhered to during and after sampling. The dogs sampled were all males, with a mean age of 9.7 ± 1.4 years (range 8.0 - 13.0), and a mean body weight of 33.2 ± 5.3 kg (range 18.0 - 38.0) which have spent at least 7 years in that environment. Also, environmental dust within the vicinity of the kennels (sites A and B) was collected for heavy metal analysis. Dust was collected manually by using clean dry cotton wool and plain paper to gather dust on windows and dry hard surfaces in the kennels. Dust samples were collected from each of the 16 and 10 kennel partitions from site A and site B, respectively. Subsequently, the dust samples from each site were pooled together into two sets before the levels of metals were quantified. All the samples collected were labeled appropriately and stored for further processing. Blood samples collected in heparinized bottles were stored at -20°C until heavy metals were quantified. The hair samples were repeatedly washed in the laboratory with metal-free distilled water, properly rinsed and dried in a special drying oven, and kept in a humid-free plastic container at room temperature before digestion and heavy metals analysis.

The samples were digested following a standard procedure (Metals and Others, Method 971.21, Chapter 9) as described by the Association of Official Analytical Chemists (AOAC, 2000) in a laboratory shared by Veterinary Physiology, Biochemistry, Pharmacology, and Toxicology units of the Federal University of Agriculture, Makurdi, Nigeria. We measured 1 ml of blood, 1 g of hair, and 1 g of the dust samples and digested them in three separate reactions with 2 ml of HNO_3 , HClO_4 , and H_2SO_4 mixture in a ratio of 3:1:1 (v/v/v), and microwaved in a closed container until fumes became clear. Subsequently, the volume was made up to 50 ml with deionized water and stored in clean plastic test tubes for metal analysis.

Analyses of heavy metal concentrations in the samples

The levels of Pb, Cd, Cr, Ni, and Zn in the dust, hair, and blood samples were determined employing atomic absorption spectrophotometer (Buck Scientific-210VGP AAS, Norwalk, CT, United States) in the Department of Soil Science, University of Ibadan, Nigeria. Standard salt preparations for each metal were used to calibrate the AAS machine. Limits of detection for the analyzed samples, expressed as a wet weight (w/w) were - Cd 0.00024 mg/kg, Cr 0.0015 mg/kg, Pb 0.013 mg/kg, Ni 0.004 mg/kg, and Zn 0.014 mg/kg for both hair and dust. Those of blood were - Cd 0.00013 mg/L, Cr 0.0021 mg/L, Pb 0.002mg/L, Ni 0.0041mg/L, and Zn 0.053mg/L.

Data analysis

The reading obtained from the AAS was multiplied by the dilution factor (50) to obtain the actual amount of heavy metal in each of the samples. Statistical analysis was performed with SPSS v20 Program. The normality of data was assessed by applying the Kolmogorov-Smirnov test. As the data were not normally distributed, non-parametric statistics were applied. Mann-Whitney U test statistic by ranks on blood and hair results of the sampling sites (A and B) was considered. Because the data were not normally distributed, the geometric mean of the quantified metals was computed to take care of outliers in order to avoid overestimating the means or skewness of the mean values towards higher values. Furthermore, a correlation test within the

tissue samples to check if any relationship existed between blood and hair metals concentrations was applied. A statistical test of significance was set at $p < 0.05$.

Results

Results (Table I) showed a higher level of Pb, Cr, and Ni in the dust samples from the kennel located within site B compared to site A where crude oil well drilling activities take place. On the other hand, dust samples from the kennel in site A contained a higher level of Cd and Zn as compared with dust samples from site B. Furthermore, there was no significant difference between the mean amount of heavy metals in blood and hair samples from dogs securing sites A and B, except for blood ($p = 0.034$) and hair ($p = 0.015$) Cr levels which were significantly higher in dogs guarding site A compared with those in site B. The level of Cr in the hair and blood of the dogs guarding site A were 1.6- and 1.7-folds, respectively higher than the mean level in those operating within site B. Regarding the body tissues, significantly higher levels of Ni ($p = 0.001$) and Zn ($p = 0.001$) were observed in the hair than in the blood samples. No amount of Pb was detected in the hair and blood samples from the investigated dogs. Correlation analysis between blood and hair metal levels showed no connection between the same metal in blood and hair. However, some interesting correlations observed were that between blood Cr and hair Cd ($r = 0.64$; $p = 0.045$); and that between Cr and Zn in the blood ($r = 0.57$; $p = 0.041$).

Table I. Heavy metals mean values \pm SD (ppm) in kennel dust, hair and blood of dogs from crude oil well drilling (site A) and liquified natural gas production (site B). Different letters in the same column means statistical difference ^{a,b}($p = .015$), ^{a,c}($p = .034$) for site; ^{a,d}($p = .001$), ^{a,e}($p = .004$), ^{a,f}($p = .001$)). Statistical comparison was not applied to the dust samples since they were pooled together before elements quantification; LOD denotes limit of detection.

Site	Mean + SD				
	Pb	Cd	Ni	Cr	Zn
Dust (ppm)					
A	109.0	1.7	89.3	128.6	4620.0
B	300.0	0.1	304.3	248.1	3095.0
Hair (ppm)					
A (n = 7)	< LOD	<LOD ^a	18.68 \pm 1.51 ^a	4.53 \pm 1.45 ^a	113.31 \pm 1.81 ^a
B (n = 3)	< LOD	0.02 \pm 0.03 ^a	26.04 \pm 1.58 ^a	2.79 \pm 1.36 ^b	103.69 \pm 2.57 ^a
Blood (ppm)					
A (n = 8)	< LOD	0.01 \pm 0.02 ^a	11.83 \pm 1.36 ^a	12.08 \pm 1.31 ^a	51.08 \pm 1.28 ^a
B (n = 5)	< LOD	0.02 \pm 0.04 ^a	11.12 \pm 1.12 ^a	7.01 \pm 1.49 ^c	48.62 \pm 1.20 ^a
Body tissue (ppm)					
Hair (n = 10)	< LOD	0.01 \pm 0.03 ^a	20.64 \pm 1.54 ^a	3.92 \pm 1.51 ^a	110.33 \pm 1.93 ^a
Blood (n=13)	< LOD	0.01 \pm 0.03 ^a	11.55 \pm 1.26 ^d	9.80 \pm 1.51 ^e	50.12 \pm 1.22 ^f

Discussion

Heavy metals are environmental pollutants that cause harmful effects in animals, and humans following absorption into the body through soft tissues above the specified permissible levels (Flora et al. 2012; WHO, 2020). The most common metals that the human body could absorb in toxic amounts are Hg, V, As, Pb, and Cd (Jaishankar et al. 2014; Renner 2010; Flora et al. 2012). High levels of exposure to these heavy metals could be from contaminated food, air, water, medicine, food containers with improper coating, and industrial exposures (Zhao et al. 2009; Zhuang et al. 2009; Renner 2010; Flora et al. 2012; Wei et al. 2015; Sharma et al. 2016; Singh et al. 2018). In this study, we evaluated levels of Cd, Cr, Ni, Pb, and Zn in the dust from the kennels as well as blood and hairs of some dogs used for security purposes in the oil and gas industry which are zones with potentially high levels of elemental pollutions.

This study revealed that dust, hair, and whole blood samples obtained from the dog kennels and dogs guarding the crude oil drilling and liquified natural gas-producing areas contained varying amounts of Cd, Ni, Cr, and Zn. However, Pb was not detected in any of the blood and hair samples that were analyzed, except for dust samples. The high enrichment of dust with heavy metals observed in this study could be explained by the anthropogenic activities, gas flaring, and non-biodegradability of these metals in the dust medium (Wei 2015; Nworu et al. 2016). There is no reported safe or beneficial level of Pb in blood and other biological tissues for animals or humans (Renner 2010; WHO 2020; Flora et al. 2012). Therefore, the non-detection of Pb in the blood and hair of the investigated dogs also suggests that they could be safe from Pb poisoning and perhaps those working in that environment at the period within which the samples were obtained.

The hair tissue mineral analysis showed that the amount of Cr (3.9 ± 1.5 ppm), Ni (20.6 ± 1.5 ppm), and Zn (110.3 ± 1.9 ppm) recovered from the blood and hairs of the dogs investigated were well above the reference ranges of 0.02 - 0.08 ppm, 0 - 0.1 ppm, and 10 - 21 ppm, respectively considering animal and human reference values (Puls 1994; Renner 2010; Flora et al. 2012). Natural sources of Cr are the burning of oil and coal as well as crude oil well drilling activities (Jaishankar et al. 2014). The later natural source is one of the major activities in the study area which probably explains the basis for the high level of Cr recorded in the hair samples. Two forms of Cr exist, Cr (III) and Cr (VI) also referred to as hexavalent Cr which is highly soluble, extremely toxic to animals and humans and predominates in the environment (Cervantes et al. 2001). Even though Cr is essential for many biological functions, when the concentrations in the body are above the safe level, it reacts with the

physiological reducing agents (thiols and ascorbate) to form reactive oxygen species (superoxide, hydrogen peroxide, and hydroxyl radicals) resulting in oxidative stress and consequent damage to important biological macromolecules, such as the DNA and proteins (Stohs and Bagchi 1995). Again, Ni and Cr are among the carcinogenic metals that are environmental and occupational pollutants (Aquino et al. 2012, Chervona et al. 2012). Even though Ni is an essential physiological mineral, chronic exposure to it has been associated with an increased risk of breast and pulmonary cancers, and cardiovascular, kidney, liver, and neurological diseases due to the release of free radicals that cause oxidative damage (Aquino et al. 2012, Chervona et al. 2012, Das et al. 2008, Zhao et al. 2009).

Zinc is an "essential trace element" because very small amounts are required for human health, e.g., immune and thyroid functions as well as vision (Girodon et al. 1999). Hair metal analysis is a toxicological screening that detects and measures essential minerals and metals reflecting tissue stores; however, the body does not store excess Zn hence detection of its high levels in hair samples obtained from the investigated dogs may not necessarily be predictive of its high tissue concentration. Nevertheless, it could suggest that these dogs were previously exposed to high levels of Zn. One of the toxic effects of Zn following inhalation by animals and humans is anosmia which is one of the very important and valuable defensive characteristics of security dogs. Anosmia following intranasal intake of Zn is a result of damage to the olfactory epithelium, inducing overt intracellular edema (Alexander and Davidson 2006; McBride et al. 2003; Slotnick et al. 2000). Exposure of security dogs to toxic levels of Zn would adversely affect their defending and guarding capabilities.

This study also showed no significant ($p > 0.05$) difference in the levels of heavy metals in blood and hair samples of dogs from the two sites investigated, except for Cr which was observed to be significantly higher in the hair ($p = 0.015$) and blood ($p = 0.034$) of dogs guarding site A compared with those in site B. The high levels of Cr in the hair and blood of dogs from site A could be attributed to the higher exposure level due to anthropogenic or crude oil well drilling activities which release these pollutants into the atmosphere and consequently settle as dust and precipitations. Naturally, Cr is found in high concentrations in activities involving the burning of oil, coal, and crude oil well drilling (Jaishankar et al. 2014).

This result also revealed a significant difference in the levels of Ni ($p = 0.001$), Cr ($p = 0.004$), and Zn ($p = 0.001$) (all trace elements) in hair and blood samples from dogs kept in the two units of the company. The levels of Ni and Zn were about 1.8- and 2.2-fold,

respectively higher in the hair samples than what was obtained in the blood samples. Inversely, no significant difference ($p>.05$) was observed for Cd levels in blood and hair samples. Mineral and heavy metal contents of the hair are considered as the spillover from what is in the body. Essential elements and toxic heavy metals are sequestered into the hair from the follicular cells and their blood supply as part of the detoxification mechanism to prevent the expression of their adverse biological effect, thus implying that hair element analysis is a valid means for screening mineral deficiencies and toxic element exposures (Airey 1983).

Correlation analysis between blood and hair metal levels showed no connection between the same metal in blood and hair. This could suggest differences in the kinetics of the metals considered (Zaccaroni *et al.* 2014). Also, it is established that mineral and heavy metal contents of the hair are the spillover from what is in the body (Airey 1983). The existing study recorded a correlation between the same metal (Ni and Cr) in hair and blood (Zaccaroni *et al.* 2014). Two remarkable correlations were that between blood Cr and hair Cd ($r=0.64$, $p=0.045$), and that between Cr and Zn in the blood ($r=0.57$, $p=0.041$). However, the explanation for these connections remains elusive and warrants further investigation. The lack of correlation between blood and hair Cd and Pb is consistent with existing reports (Gyori *et al.* 2005; Patra *et al.* 2007; Zaccaroni *et al.* 2014). Hair is a keratin-rich tissue, with abundant sulfhydryl groups which bind divalent cations such as Pb and Cd, leading to their persistence in hair (Hasan *et al.* 2004). This lack of correlation between blood and hair Pb and Cd may be linked to the saturation

of these binding sites following chronic exposure, leading to a plateau in metals concentrations and consequently the lack of correlation with blood levels (Patra *et al.* 2007). This study was limited by small sample size as only 13 dogs were kept in these facilities.

Conclusions

This study showed that non-invasive analysis of Ni and Zn using hair samples from animals could be used to predict previous exposures, environmental pollution, and the nutritional metabolic activity that has occurred within a given period. Non-detection of Pb and a negligible amount of Cd in hair and blood samples from dogs in the study area suggest environmental safety from these metals in this location within the period considered. The observed higher levels of Cr and Ni in hair samples above the reference values instead suggest the high level of exposure of security dogs and by extension, workers in this industrial area to these environmental pollutants. Consequently, there is a need for routine monitoring and decontamination of potentially toxic environmental metal pollutants for the safety of aquatic lives, security dogs, and workers within the vicinity of related industries.

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