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RESEARCH ARTICLE

Metals and arsenic distribution in stray dogs' tissues around a lead–zinc mine in Kabwe, Zambia

Nyein Chan Soe[1,2] · Yared Beyene Yohannes[1] · Andrew Kataba[1,3] · Madalitso Tembo[3] · John Yabe[3,4] · Golden Zyambo[3] · Kenneth Chawinga[5] · Kaampwe Muzandu[3] · Yoshinori Ikenaka[1,6,7,8] · Mayumi Ishizuka[1] · Shouta M. M. Nakayama[1,3]

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Abstract
Metal contamination poses a threat to human, animal, and environmental health. The purpose of the current study was to assess the accumulation of toxic and trace metal concentrations in tissues of stray dogs in Kabwe, a town known for lead pollution due to a long history of lead and zinc mining. Brain, spleen, heart, stomach, stomach content, small intestine, kidney, liver, and bone samples were collected from 29 stray dogs (14 from locations within 3 km of the mine and 15 from sites 7 km away from the mine) after necropsy. Inductively coupled plasma mass spectrometry was used to analyze toxic metals, arsenic (As), cadmium (Cd), and lead (Pb), and trace elements, copper (Cu) and zinc (Zn). The lungs (0.117 ± 0.114 mg/kg dry weight), kidney (7.515 ± 8.830 mg/kg dry weight), and bone (41.68 ± 66.83 mg/kg dry weight) were found to have the highest concentrations of As, Cd, and Pb, respectively. In contrast, higher Cu and Zn concentrations were measured in the liver than in other tissues. In all tissues analyzed, tissues sampled from dogs near the mine had significantly higher mean concentrations of Cd and Pb than dogs far away. Neither sex nor age-related differences were observed in the distribution of metals in most tissues. There were significant associations among toxic (Pb and Cd) and trace metals (Cu and Zn). In the kidney, Cd positively correlated with Pb ($\rho = 0.534$) and Zn ($\rho = 0.600$), whereas in the liver, Cu correlated with Zn ($\rho = 0.565$). The current study's findings suggest that environmental pollution is still a problem in Kabwe, and environmental remediation is needed to address the pollution.

Keywords Stray dog · Accumulation · Metals · Tissue · Mine · Kabwe

Introduction

In nature, heavy metals are present in the Earth's crust. Their emergence in the environment is due to anthropogenic activities by humans and natural causes such as soil erosion and geological weathering (He et al. 2005; Ahmed et al. 2022). The main anthropogenic sources of heavy metal pollution are foundries, mines, and smelting operations (Masindi and Muedi 2018; Briffa et al. 2020). Animals living in polluted areas may experience exposure to heavy metals by different routes, including ingestion of contaminated food or water, inhalation of contaminated dust, and inadvertent ingestion

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✉ Shouta M. M. Nakayama
shouta-nakayama@vetmed.hokudai.ac.jp

1 Laboratory of Toxicology, Faculty of Veterinary Medicine, Hokkaido University, Kita 18 Nishi 9, Kita-ku, Sapporo 060-0818, Japan

2 Department of Pharmacology and Parasitology, University of Veterinary Science, Yezin 15013, Myanmar

3 School of Veterinary Medicine, The University of Zambia, P.O. Box 32379, Lusaka 10101, Zambia

4 School of Veterinary Medicine, University of Namibia, P.O. Box 13301, Windhoek 10005, Namibia

5 Ministry of Fisheries and Livestock, Central Province Veterinary Office, Kabwe, Zambia

6 Translational Research Unit, Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan

7 One Health Research Center, Hokkaido University, Sapporo 060-0818, Japan

8 Water Research Group, School of Environmental Sciences and Development, North-West University, Private Bag X6001, Potchefstroom 2531, South Africa

of contaminated soil (Li et al. 2017; Zhang et al. 2019). The metals may be detoxified or deposited to be excreted or stored in organs by the organisms once they enter the body (Thompson 2018a). Toxic metals including As, Cd, Hg, and Pb are harmful even in small amounts and are not required in the body. Some heavy metals that are classified as essential elements, such as Cu and Zn, are important to organisms for their biological functions. However, excess or deficient concentrations cause health effects (Briffa et al. 2020).

Biomonitoring of heavy metals in the environment allows us to pinpoint the source of pollutants (Zhou et al. 2008; Parmar et al. 2016). Tissues or body fluids are typically used to detect the bioavailability of heavy metals in animals (Tataruch and Kierdorf 2003). They are more favorable than environmental samples such as soil, water, and air because animals in the polluted environment have exposure to contaminants spatially, temporally, and across media (Talmage and Walton 1991).

From 1902 to 1994, Pb–Zn mining activities were operated in Kabwe, a mining town in the central province of Zambia. They left extensive Pb pollution in the environment due to an inadequate regulation to manage the pollutant emissions from the mine (Nakata et al. 2022). Studies have reported that high accumulations of heavy metals were found in soil, wild rats, chickens, cattle, goats, and lizards (Nakayama et al. 2011; Yabe et al. 2011, 2013; Nakata et al. 2016; Doya et al. 2020). Moreover, Pb levels in the children's blood who reside close to the mine exceeded the Pb reference value set by the CDC (Yabe et al. 2015, 2020). Dogs share the same habitat as humans, and exposure to environmental pollutants reported in human habitats is unavoidable for them (Reif 2011; Severino et al. 2012; Esposito et al. 2019). For instance, a high mean concentration of blood lead level (525.3 ± 230.8 µg/l), which exceeded the toxic level (400 µg/l), was observed in dogs from Kasanda, Kabwe, a township that located about 1 km from the mine (Toyomaki et al. 2020). However, reports on tissue metal distribution in stray dogs, especially around the mining area, to better understand the phenomenon of metals in the body are lacking.

Therefore, this study was carried out to assess the accumulation of metals and arsenic in several tissues of stray dogs from Kabwe and to compare metal accumulation between dogs from townships close to the mine (within 3 km from the mine) and those 7 km away from the mine. Furthermore, factors like age and sex that may affect a dog's exposure to metals are reported.

Materials and methods

Sampling sites

Kabwe (latitude 14° 26′ 48.84″ S, longitude 28° 26′ 47.18″ E) is the provincial capital of Central Province of Zambia where a Pb–Zn mine that closed in 1994 occupies southwest of the town center. Even though the mine is closed, illegal artisanal activities at the old dump tailing which is adjacent to residential area have continued to contaminate the surrounding areas with dust via wind flow (Nakayama et al. 2011; Yabe et al. 2015). In a previous study, it was reported that the median values of Pb in the soil near the mine were higher than the reference values for mammals from US EPA Eco-SSL (Nakayama et al. 2011; Doya et al. 2020). Moreover, concentrations of Pb reported in agricultural crops and drinking water near the mine exceeded the reference values for Pb in vegetables (Nakata et al. 2022). It was also observed that distance and wind direction from the mine affected dogs' exposure to Pb and other metals. A decreasing trend in the levels of Pb in their blood was reported particularly after 5 km from the mine (Toyomaki et al. 2020). Therefore, the present study was a case–control study using stray dogs near the mine (Kansada and Chowa; townships within 3 km from the mine) as exposure and dogs away from the mine (Bwacha and Nakoli; townships 7 km from the mine) as control (Fig. 1).

Sampling

The Zambian Ministry of Fisheries and Livestock granted clearance for the sampling, which was carried out strictly in accordance with its regulations. Approval for ethical clearance was obtained from the University of Zambia Biomedical Research Ethics Committee (UNZABREC) under the reference number 012–04-16. The sampling was done during the rabies control effort conducted by the Kabwe District Veterinary Office that targeted at reducing stray dog population in August 2022. Stray dogs were humanly euthanized followed by necropsy by local veterinarians. The brain (cerebrum), spleen, heart, lungs, stomach, stomach contents, small intestine, kidney (cortex), liver, and bone were collected in polypropylene tubes and stored at − 20 °C. The samples were transported to Japan in an icebox for further analysis at the Laboratory of Toxicology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo. Sex was recorded during sample collection, and their age was estimated by tooth eruption and tooth wear (Cafazzo et al. 2010).

Determination of metals

Brain, spleen, heart, lungs, stomach, stomach contents, small intestine, liver, kidney, and bone samples were digested for metals and arsenic detection by wet digestion method presented in a previous study (Yohannes et al. 2017) with some modifications. Briefly, approximately 300 mg of each tissue sample was dried in an oven for 48 h at 50 °C. After drying, acid-wet digestion of the dry

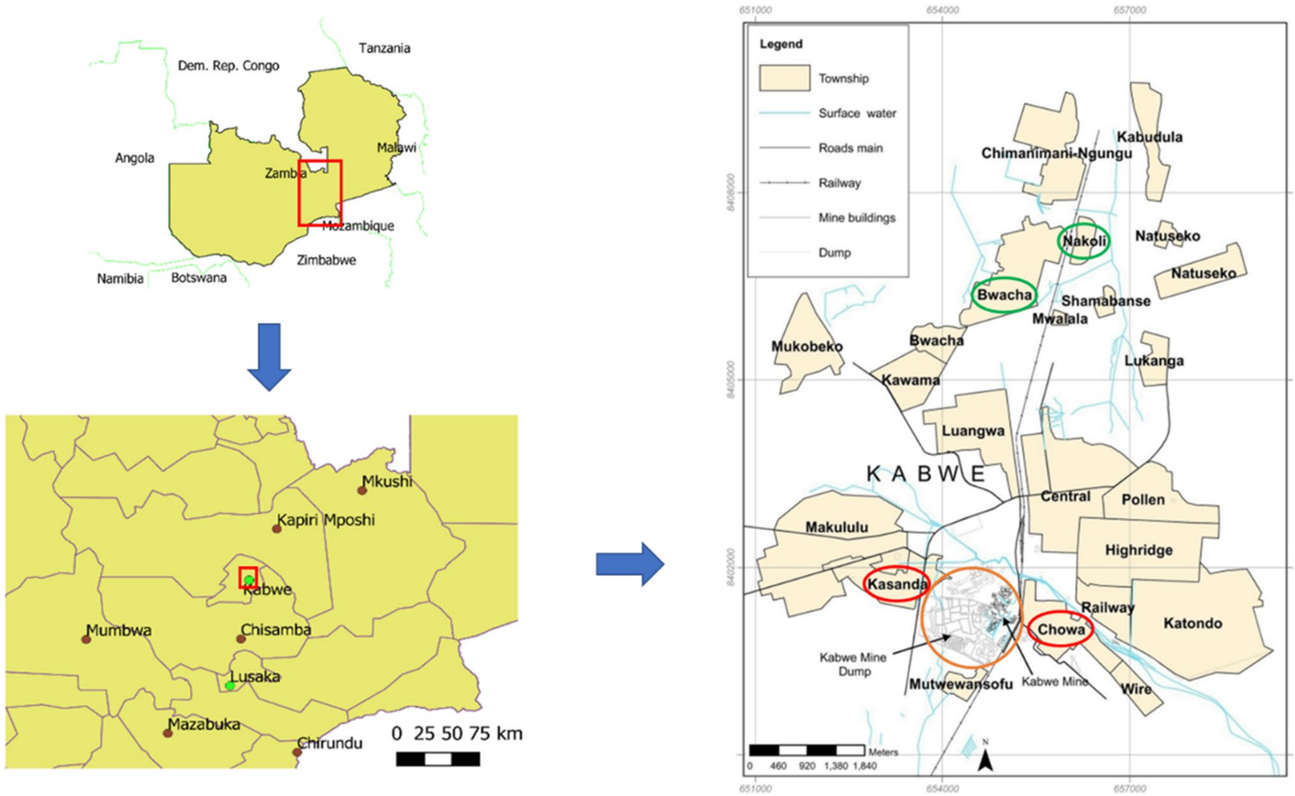

Fig. 1 Location of the study area in Kabwe, Zambia (source: Joseph Makumba/ZCCM-IH); townships within 3 km from the mine (in red color circles), townships 7 km distance from the mine (in green color circles), and Pb–Zn mine (in orange color circles)

samples was done using 5 mL of 30% nitric acid and 1 mL of 30% hydrogen peroxide. A Speed Wave MWS-2 digestion system was used for tissue digestion for metal analysis. After cooling, digested solutions were transferred and filled it up to 10 mL with Milli-Q in 15-mL plastic tube. A reagent blank sample was prepared following the same procedure.

An inductively coupled plasma–mass spectrometer (ICP-MS 7700 series; Agilent Technologies Inc., Tokyo, Japan) was used to measure the concentration of As, Cd, Pb, Cu, and Zn in the digested solutions. The certified reference material of DOLT-4 (fish liver; National Research Council of Canada) was used as the analytical quality control. Replicate analyses of the reference material revealed good recoveries ranging from 94 to 105%. The instrumental detection limit (IDL) (μg/g) of As, Cd, Pb, Cu, and Zn were 0.001, 0.0002, 0.001, 0.01, and 0.02, respectively.

Statistical analysis

All statistical analyses were conducted using JMP Pro 16.0.0 (SAS Institute, USA), with a significance level of $p < 0.05$. The Mann–Whitney *U* test, a non-parametric test, was used to compare the variations in As, Cd, Pb, Cu, and Zn with respect to age, sex, and location. The relationship between metals and arsenic in the kidney and liver of dogs living close to the mine was examined using Spearman's rank correlation.

Results

Characteristics of sampled dogs

A total of 29 stray dogs (14 near the mine and 15 far away the mine) were sampled. Sex ratio (female/male) was 8:6 for sites near the mine and 6:9 for far from the mine. Age was estimated by dental pattern and color wearing (Cafazzo et al. 2010) and divided into two groups (≤ 2 years old vs > 2 years old). The age ratio ($\leq 2 : > 2$) from locations close to the mine and distant from the mine was 10:4 and 9:6, respectively.

Concentration of metals and arsenic in tissues of dogs

The concentration of metals and arsenic in tissues of dogs from Kabwe is shown in Table 1 and 2. Among the tissues analyzed, the lungs, kidney, and bone showed the highest mean concentration of As (0.117 mg/kg dry weight), Cd (7.515 mg/kg dry weight), and Pb (41.68 mg/kg dry weight), respectively, whereas the liver had a higher concentration of Cu and Zn than other tissues analyzed.

The concentrations of toxic metals in dogs per site are described in Table 1. There were significant differences in the concentrations between the dogs near and away from the mine. The significantly higher concentrations of As in dogs near the mine were observed in the brain ($p < 0.01$), stomach ($p < 0.01$), small intestine ($p < 0.001$), and kidney ($p < 0.01$) compared to dogs about 7 km away from the mine. Dogs close to the mine showed significantly higher Cd concentrations than dogs far away from the mine in all tissues ($p < 0.01$). Except in the heart tissues, the concentrations of Pb were significantly higher in all tissues of dogs near the mine than in those of dogs far from the mine.

The accumulation pattern of As in tissues of dogs near the mine was observed as follows: lungs > stomach > small intestine > kidney > heart > brain > spleen > liver > bone. For Cd in dogs around mining areas, the highest concentration was found in the kidney followed by liver, small intestine, spleen and/or lungs, stomach, brain, heart, and bone. In the case of Pb concentration in dogs living near the mine, the pattern was bone > liver > kidney > lungs > stomach > brain > spleen > heart > small intestine.

Essential metal concentrations in dogs from the two locations are summarized in Table 2. Cu concentrations in dogs near the mine differed significantly ($p < 0.05$) in brain, spleen, heart, lungs, stomach contents, small intestine, kidney, and liver compared with those far from the mine. While the mean concentrations of Zn in all tissues were significantly higher in dogs near the mine than those far from the mine, except in the bone.

Comparing the influence of sex on metal accumulation, female and male dogs far from the mine showed significantly different concentration for As, Pb, and Cu. The male dogs had significantly higher concentration of As in small intestine and Pb in brain and liver, while female dogs had higher concentration of Cu in the stomach (Fig. 2 a, c, d). Except for the concentration of As in the heart ($p < 0.05$), the concentration of metals in tissues of female and male dogs from near the mine was not different ($p > 0.05$) (Fig. 2 a – e). Regarding age, no significant difference was observed between groups from both locations of dogs ($p < 0.05$), except for Cu concentration in the stomach of dogs far from the mine (Fig. 3 a–e).

The relationship of metals and arsenic in the kidney and liver of dogs from the mine's vicinity is depicted in Table 3. A significant positive correlation ($\rho = 0.600$, $p < 0.05$) was observed between Cd and Zn in the kidney; Cd and Pb showed positive correlation ($\rho = 0.534$, $p < 0.05$) in the kidney. Between Cu and Zn, a significant positive correlation ($\rho = 0.565$, $p < 0.05$) was observed in the liver.

Discussion

Studies about heavy metal environmental exposure in tissues of dogs around mining area are scarce. In our understanding, this is the first study to examine metals and arsenic in tissues of dogs around a mining area in the world. The recommended levels of Cd in healthy dogs was < 0.7 mg/kg dry weight in kidney and < 0.35 mg/kg dry weight in liver. In contrast, at the elevated levels, the concentration of Cd ranged from 14 to 68 mg/kg dry weight in the kidney and from 3.5 to 28 mg/kg dry weight in the liver (Puls 1994). In the present study, the mean concentration of Cd in kidney (7.515 mg/kg dry weight) and liver (0.598 mg/kg dry weight) of Kabwe dogs was found to exceed the recommended levels of Cd in healthy dogs. Moreover, mean concentration of Cd concentrations in kidneys (0.755 mg/kg dry weight) and liver (0.245 mg/kg dry weight) exceeded concentrations reported in stray dogs from Naples, Italy (Esposito et al. 2019). Accumulation levels of Pb in kidney (5.550 mg/kg dry weight) and liver (10.54 mg/kg dry weight) were also higher than those of Pb in Naples' stray dogs (0.324 mg/kg dry weight in kidney and 0.520 mg/kg in liver) (Esposito et al. 2019). The study's findings therefore demonstrated that environmental pollution has an impact on the accumulation of heavy metals in animals from Kabwe, Zambia.

Dogs close to the mine had higher As concentrations in their brain, stomach, small intestine, and kidney than dogs farther away. The highest mean As concentration was found in the stomach content of dogs living close to the mine (1.325 mg/kg dry weight), followed by the lungs (0.144 mg/kg dry weight) suggesting that stray dogs had a high risk of exposure through ingesting contaminated food and inhaling in polluted air (Rabinowitz et al. 2010). Our findings give further corroborative evidence to the presence of environmental arsenic contamination in Kabwe from past mining activities of lead-chloride arsenate mineral ore called mimetite, $Pb_5(AsO_4)_3Cl$ found in Kabwe (Kamona and Friedrich 2007). Moreover, our previous study, reported a positive correlation As with Pb in soil from Kabwe (Nakayama et al. 2011). Mean As concentrations in kidney (0.029 mg/kg dry weight) and liver (0.016 mg/kg dry weight), on the other hand, were within the lowest range of As ever reported in dog tissues (< 0.7 mg/kg dry weight in both kidney and liver) (Puls 1994) and below the dogs from NW Spain (0.0636 mg/kg dry weight in kidney and 0.0504 mg/kg dry weight in liver) (López-Alonso et al. 2007a).

Dog Cd poisoning is rare and typically results from chronic exposure. Yet, even low amounts of exposure to Cd can harm the cardiovascular system, kidney, liver, skeletal system, and liver. Bolognin et al. (2009) reported a

Table 1 Toxic metal (As, Cd, and Pb) concentration in dogs (mg/kg dry weight)

	Kabwe			Near the mine			Far from the mine		
	n	Mean ± SD	Median (range)	*n*	Mean ± SD	Median (range)	*n*	Mean ± SD	Median (range)
As									
Brain	29	0.026 ± 0.008	0.024 (0.015 – 0.052)	14	0.029 ± 0.008**	0.028 (0.016 – 0.052)	15	0.022 ± 0.005	0.022 (0.015 – 0.033)
Spleen	29	0.026 ± 0.041	0.017 (0.008 – 0.234)	14	0.022 ± 0.008	0.022 (0.010 – 0.037)	15	0.029 ± 0.057*	0.015 (0.008 – 0.234)
Heart	29	0.026 ± 0.016	0.022 (0.012 – 0.089)	14	0.031 ± 0.022	0.026 (0.012 – 0.089)	15	0.022 ± 0.007	0.019 (0.012 – 0.038)
Lungs	29	0.117 ± 0.114	0.079 (0.023 – 0.488)	14	0.144 ± 0.131	0.092 (0.037 – 0.488)	15	0.093 ± 0.093	0.068 (0.023 – 0.368)
Stomach	29	0.05 ± 0.067	0.031 (0.008 – 0.377)	14	0.074 ± 0.091**	0.054 (0.016 – 0.377)	15	0.028 ± 0.013	0.028 (0.008 – 0.057)
Stomach contents	22	0.856 ± 1.596	0.224 (0.025 – 6.857)	10	1.325 ± 2.222	0.298 (0.053 – 6.857)	12	0.465 ± 0.675	0.191 (0.025 – 2.264)
Small intestine	29	0.035 ± 0.03	0.027 (0.008 – 0.145)	14	0.052 ± 0.034***	0.051 (0.013 – 0.145)	15	0.019 ± 0.009	0.016 (0.008 – 0.047)
Kidney	29	0.029 ± 0.021	0.023 (0.007 – 0.097)	14	0.042 ± 0.023**	0.035 (0.017 – 0.097)	15	0.018 ± 0.008	0.018 (0.007 – 0.036)
Liver	29	0.016 ± 0.007	0.014 (0.006 – 0.034)	14	0.015 ± 0.009	0.014 (0.007 – 0.034)	15	0.016 ± 0.005	0.017 (0.006 – 0.023)
Bone	29	0.014 ± 0.008	0.013 (0.001 – 0.038)	14	0.014 ± 0.011	0.012 (0.001 – 0.038)	15	0.014 ± 0.005	0.014 (0.008 – 0.024)
Cd									
Brain (cerebrum)	29	0.013 ± 0.01	0.009 (0.004 – 0.036)	14	0.021 ± 0.009***	0.019 (0.007 – 0.036)	15	0.006 ± 0.002	0.006 (0.004 – 0.010)
Spleen	29	0.141 ± 0.35	0.027 (0.008 – 1.919)	14	0.275 ± 0.479***	0.136 (0.061 – 1.919)	15	0.017 ± 0.006	0.017 (0.008 – 0.027)
Heart	29	0.012 ± 0.01	0.008 (0.004 – 0.043)	14	0.018 ± 0.010***	0.016 (0.007 – 0.043)	15	0.007 ± 0.001	0.007 (0.004 – 0.009)
Lungs	29	0.145 ± 0.19	0.053 (0.010 – 0.807)	14	0.275 ± 0.221***	0.222 (0.053 – 0.807)	15	0.024 ± 0.019	0.020 (0.010 – 0.088)
Stomach	29	0.079 ± 0.09	0.032 (0.006 – 0.384)	14	0.148 ± 0.024***	0.114 (0.067 – 0.384)	15	0.017 ± 0.009	0.016 (0.006 – 0.032)
Stomach contents	22	0.241 ± 0.54	0.032 (0.004 – 2.249)	10	0.427 ± 0.742**	0.094 (0.032 – 2.249)	12	0.085 ± 0.235	0.015 (0.004 – 0.829)
Small intestine	29	0.275 ± 0.29	0.208 (0.015 – 1.354)	14	0.447 ± 0.348***	0.314 (0.158 – 1.354)	15	0.115 ± 0.024	0.068 (0.015 – 0.310)
Kidney (cortex)	29	7.515 ± 8.83	2.583 (0.160 – 32.38)	14	13.996 ± 8.918***	2.383 (1.827 – 32.38)	15	1.468 ± 1.025	1.434 (0.160 – 3.376)
Liver	29	0.598 ± 0.65	0.221 (0.022 – 2.111)	14	1.119 ± 0.590***	0.942 (0.115 – 2.111)	15	0.113 ± 0.069	0.095 (0.022 – 0.225)
Bone	29	0.005 ± 0.01	0.002 (0.0002 – 0.029)	14	0.009 ± 0.008***	0.006 (0.003 – 0.029)	15	0.001 ± 0.0005	0.001 (0.0002 – 0.002)
Pb									
Brain (cerebrum)	29	1.68 ± 2.69	0.690 (0.05 – 10.13)	14	3.27 ± 3.22***	1.55 (0.69 – 10.13)	15	0.20 ± 0.19	0.15 (0.05 – 0.83)
Spleen	29	2.32 ± 4.01	0.875 (0.04 – 18.73)	14	2.84 ± 3.08**	1.26 (0.38 – 10.91)	15	1.83 ± 4.78	0.20 (0.04 – 18.73)
Heart	29	2.13 ± 4.69	0.56 (0.02 – 24.16)	14	2.75 ± 6.27	0.59 (0.13 – 24.16)	15	1.55 ± 2.61	0.44 (0.02 – 8.34)
Lungs	29	6.05 ± 10.59	2.65 (0.13 – 50.84)	14	10.59 ± 14.00**	3.76 (1.73 – 50.84)	15	1.81 ± 1.61	1.46 (0.13 – 5.16)
Stomach	29	3.07 ± 8.23	0.93 (0.09 – 44.89)	14	5.51 ± 11.51**	2.00 (0.54 – 44.89)	15	0.79 ± 1.02	0.36 (0.09 – 3.55)
Stomach contents	22	47.7 ± 85.02	18.59 (0.28 – 375.8)	10	82.67 ± 115.18*	28.98 (7.07 – 375.8)	12	18.56 ± 30.12	3.16 (0.28 – 98.51)
Small intestine	29	1.14 ± 1.19	0.65 (0.09 – 4.33)	14	1.93 ± 1.15***	1.64 (0.65 – 4.33)	15	0.42 ± 0.67	0.19 (0.09 – 2.22)

Table 1 (continued)

	Kabwe			Near the mine			Far from the mine		
	n	Mean ± SD	Median (range)	*n*	Mean ± SD	Median (range)	*n*	Mean ± SD	Median (range)
Kidney (cortex)	29	5.55 ± 7.74	2.54 (0.17 – 31.87)	14	10.66 ± 8.61***	7.36 (2.93 – 31.87)	15	0.78 ± 0.77	0.39 (0.17 – 2.54)
Liver	29	10.54 ± 18.38	3.3 (0.17 – 89.69)	14	20.95 ± 22.38***	12.23 (4.40 – 89.69)	15	0.82 ± 0.82	0.57 (0.17 – 3.29)
Bone	29	41.68 ± 66.83	27.24 (1.07 – 354.99)	14	77.35 ± 82.71***	53.36 (22.29 – 354.99)	15	8.39 ± 10.97	2.41 (1.07 – 36.84)

SD standard derivation, *n* number of dogs

*Significant difference between dogs near the mine and dogs distant from the mine at < 0.05 (Mann–Whitney *U* test)

**Significant difference between dogs near the mine and dogs distant from the mine at < 0.01 (Mann–Whitney *U* test)

***Significant difference between dogs near the mine and dogs distant from the mine at < 0.001 (Mann–Whitney *U* test)

bronchial inflammation and an elevated neutrophil count in an experimental exposure of dogs to nebulized 0.2% Cd solution within a week. Moreover, intramuscular administration of cadmium chloride (8 mg/kg body weight) caused hemorrhagic, necrotic foci in the testes while intravenous injection of same dose cadmium chloride resulted in mortality within 24 h (Donnelly and Monty 1977). In the present study, Cd concentrations in all tissues of dogs from near the mine showed approximately 10 times higher in concentrations compared with those far from the mine. The mean concentration of Cd in the kidney of dogs in Kabwe (13.996 mg/kg dry weight) was higher than the lowest value (< 0.7000 mg/kg dry weight) (Puls 1994). A similar pattern was also observed in the liver of dogs from near the mine (1.119 mg/kg dry weight vs < 0.350 mg/kg dry weight). The mean Cd concentrations were higher in the kidney of dogs from both locations when compared with other tissues. Our findings provided that the presence of Cd contamination produced 235 t as one of the by-products in the past mining in Kabwe (Kamona and Friedrich 2007). Once Cd is absorbed, it rapidly leaves the bloodstream and accumulates in various tissues, especially in the liver and kidney, due to their richness in metallothionein (Satarug et al. 2003). Given these situations and pervasive nature of Cd in the environment, dogs living close to the mine area may have potential Cd-induced impairments, including increased oxidative stress and detrimental effects on bone health (Lovásová et al. 2013; Agneta et al. 2014). Therefore, the detection of Cd in dog tissues could be a concern to the overall health of dogs from households within the vicinity of the former Pb–Zn mine.

Pb poisoning in dogs primarily manifests as gastrointestinal and neurological problems (Wismer 2013). Moreover, it affects the cardiovascular system, the renal system, and the hematological system (Fine et al. 1988; Huerter 2000; Zook 1972). In Kabwe, high concentrations of Pb were observed in all tissues from dogs near the mine compared to those far from the mine. Similar patterns have been reported in other animals from Kabwe (Nakayama et al. 2011; Yabe et al. 2011, 2013; Nakata et al. 2016; Doya et al. 2020). The mean concentrations of Pb in the kidney and liver of dogs near the mine were above the lowest level (< 14 mg/kg dry weight for the liver; < 10 mg/kg dry weight for the kidney) as reported by Puls (1994). Meanwhile, the mean Pb concentration was found to be low in the liver (0.82 mg/kg dry weight) and kidney (0.78 mg/kg dry weight) of dogs far from the mine, which is consistent with previous reports for dogs from unpolluted areas (López-Alonso et al. 2007a; Paßlack et al. 2015; Esposito et al. 2019). Once Pb is absorbed, it is widely distributed in the body, including the brain after crossing the blood–brain barrier (Thompson 2018a) and then can cause subtle changes in the immune system, organ function, and cognitive abilities even at low levels of exposure (Langlois et al. 2017). Among tissues analyzed in dogs from Kabwe, the highest concentration of Pb was observed in the bone. The mean Pb bone concentration in dogs from near the mine (77.35 mg/kg dry weight) was higher than the range (2 – 5 mg/kg dry weight as normal and 20–40 mg/kg dry weight as high) reported by Puls (1994). Once Pb enters the body, it binds to a variety of proteins as well as metallothionein in the soft tissues but mostly accumulates in the active bone matrix, serving as a reservoir (Thompson 2018a).

This study's results showed that the Cu and Zn levels in dogs were within normal ranges (Puls 1994). The livers of dogs from both sites were found to have higher amounts of Cu and Zn than other organs. After being absorbed, Cu and Zn are stored in the liver (Garland 2018; Thompson 2018b). Moreover, these metals have shown significant individual variation among individuals, and this variation may be influenced by diet and age (López-Alonso et al. 2007b).

In this study, the concentration of As in the small intestine of dogs that were distant from the mine was significantly higher in males than in females, but the concentration of As in the heart of dogs that were close to mine exhibited an opposite tendency. In case of Pb, male dogs residing far from the mine had more Pb in their brains and livers than female dogs did. A separate substantial trend (female dogs > male dogs) was

Table 2 Trace metal (Cu and Zn) concentration in dogs (mg/kg dry weight)

	Kabwe			Near the mine			Far from the mine		
	n	Mean ± SD	Median (range)	*n*	Mean ± SD	Median (range)	*n*	Mean ± SD	Median (range)
Cu									
Brain (cerebrum)	29	21.9 ± 4.8	21.64 (13.95 – 34.31)	14	24.28 ± 5.14*	24.67 (16.27 – 34.31)	15	19.71 ± 3.23	19.17 (13.95 – 25.98)
Spleen	29	12.5 ± 48.3	3.32 (2.67 – 263.41)	14	22.55 ± 69.33***	3.99 (2.95 – 263.41)	15	3.01 ± 0.26	2.89 (2.67 – 3.55)
Heart	29	15.3 ± 2.6	15.88 (6.66 – 19.59)	14	15.78 ± 2.53	16.31 (9.27 – 19.59)	15	14.92 ± 2.69	15.44 (6.66 – 18.07)
Lungs	29	4.4 ± 1.8	4.19 (2.21 – 9.37)	14	5.91 ± 1.44***	5.76 (4.48 – 9.37)	15	2.98 ± 0.48	2.86 (2.21 – 4.19)
Stomach	29	7.3 ± 3.3	6.34 (3.16 – 17.81)	14	8.38 ± 4.16	7.89 (3.16 – 17.81)	15	6.35 ± 1.75	6.09 (3.38 – 10.08)
Stomach contents	22	8.1 ± 8.4	4.56 (1.97 – 27.32)	10	10.94 ± 9.52*	6.29 (2.64 – 27.32)	12	5.79 ± 6.81	2.54 (1.97 – 25.52)
Small intestine	29	6.4 ± 3.3	5.10 (3.76 – 19.54)	14	7.94 ± 4.22**	6.54 (3.89 – 19.54)	15	4.89 ± 0.88	4.75 (3.76 – 6.96)
Kidney (cortex)	29	30.6 ± 16.3	26.32 (11.88 – 86.11)	14	40.09 ± 17.49***	35.28 (21.55 – 86.11)	15	21.81 ± 8.53	21.01 (11.88 – 43.58)
Liver	29	171.4 ± 89.5	149.83 (40.66 – 433.68)	14	235.30 ± 80.37***	203.93 (148.52 – 433.68)	15	111.82 ± 46.11	119.07 (40.66 – 221.29)
Bone	29	0.4 ± 0.3	0.30 (0.14 – 1.80)	14	0.47 ± 0.42	0.34 (0.17 – 1.80)	15	0.28 ± 0.12	0.25 (0.14 – 0.56)
Zn									
Brain (cerebrum)	29	66.3 ± 7.9	63.3 (54.4 – 86.9)	14	71.5 ± 7.5***	72.2 (56.7 – 86.9)	15	61.5 ± 4.5	60.9 (54.4 – 73.1)
Spleen	29	93.6 ± 19.4	89.9 (69.7 – 171.4)	14	104.3 ± 21.4***	100.7 (79.5 – 171.4)	15	83.7 ± 10.3	82.1 (69.67 – 108.25)
Heart	29	66.2 ± 11.5	67.2 (32.0 – 83.5)	14	71.2 ± 11.5**	73.5 (40.3 – 83.5)	15	61.5 ± 9.5	64.2 (32.0 – 72.7)
Lungs	29	84.0 ± 37.3	73.9 (33.2 – 186.5)	14	111.2 ± 36.1***	36.1 (73.9 – 186.5)	15	58.7 ± 12.4	60.6 (33.2 – 76.2)
Stomach	29	104.7 ± 87.2	87.1 (35.0 – 517.8)	14	133.8 ± 112.0**	97.9 (35.0 – 517.8)	15	77.6 ± 14.3	79.9 (51.8 – 101.8)
Stomach contents	22	1324 ± 5598	47.2 (9.1 – 26,331.1)	10	2870.4 ± 8260.8*	71.5 (17.2 – 26,331.1)	12	35.4 ± 25.2	24.3 (9.1 – 79.5)
Small intestine	29	97.4 ± 17.9	97.9 (66.4 – 144.8)	14	108.0 ± 16.7**	106.4 (80.4 – 144.8)	15	87.5 ± 12.9	89.9 (66.4 – 104.4)
Kidney (cortex)	29	99.9 ± 18.9	99.8 (73.5 – 136.6)	14	115.4 ± 10.3***	115.0 (99.8 – 136.6)	15	85.5 ± 12.3	83.6 (73.5 – 124.7)
Liver	29	123.6 ± 27.2	123.9 (79.6 – 172.7)	14	146.9 ± 16.4***	145.3 (123.9 – 172.7)	15	101.9 ± 13.6	99.9 (79.6 – 128.4)
Bone	29	109.2 ± 22.9	104.0 (70.0 – 179.4)	14	111.2 ± 17.1	108.0 (90.5 – 159.6)	15	107.4 ± 27.8	99.7 (70.0 – 179.4)

SD standard derivation, *n* number of dogs

*Significant difference between dogs near the mine and dogs distant from the mine at < 0.05 (Mann–Whitney *U* test)

**Significant difference between dogs near the mine and dogs distant from the mine at < 0.01 (Mann–Whitney *U* test)

***Significant difference between dogs near the mine and dogs distant from the mine at < 0.001 (Mann–Whitney *U* test)

observed in the content of Cu in the stomachs of dogs living far from the mine. A previous study also revealed that the effect of sex was reversed between townships near the mine (Toyomaki et al. 2020). Such variations might be influenced by the type of food or feed supply. The subjects in this study were stray dogs that roamed in different places for food. Both female and male dogs may wander from one location to another. As a result, the exposure to metals and arsenic in this study may not have been significantly affected by differences in sex.

In this study, significant positive correlation was observed between Zn and Cd in the kidney. Yabe et al. (2011) reported that a similar interaction was observed in the kidney of cattle from Kabwe, with a positive correlation for Cd and Zn in the kidney. It seems that these interactions are regulated by metallothionein, metal-binding proteins that are induced by many metals, including Cd (Nordberg and Nordberg 2022). It has been proven that Zn can also bind to this protein (Tokar et al. 2012). A significant positive correlation between Pb and Cd was also observed in the

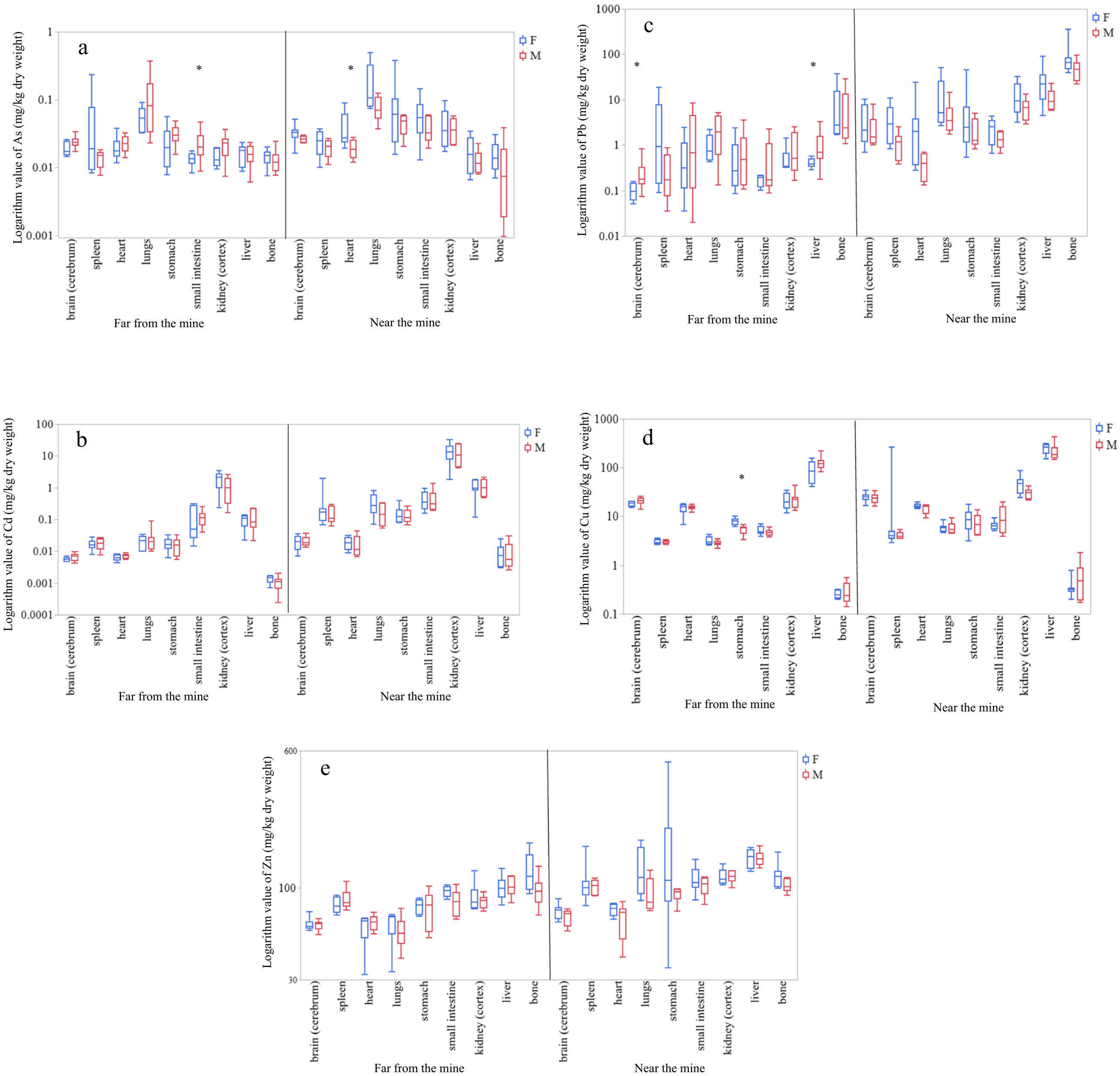

Fig. 2 Box plots of toxic element and trace element concentration in tissues of dogs by sex: **a** As, **b** Cd, **c** Pb, **d** Cu, and **e** Zn (* denoted $p < 0.05$)

kidney, which is no surprise as both metals can induce metallothionein in the kidney. A significant positive correlation was observed among trace elements in the liver. This agrees with a previous report on cattle from Kabwe (Yabe et al. 2011). In fact, Cu and Zn share chemical properties and interact with each other during tissue metal accumulation (Irato and Albergoni 2005).

Limitations of the study included limited information regarding the home range of stray dogs. While the observation that dogs sampled 7 km distance from the mine site exhibited lower levels of Pb in their tissues is noted, it falls short of establishing the actual extent of the dogs' home range. Moreover, the study lacks information on the movement of animals from the reference area. Without comprehensive data on the behavior and movement patterns of dogs from both areas, the study's capacity to make definitive conclusions regarding the boundaries of their habitats and exposure to contaminants remains limited.

Conclusion

The Cd and Pb concentrations observed in stray dogs in Kabwe were high in comparison to those found in dogs inhabiting minimal pollution. This study revealed an approximately

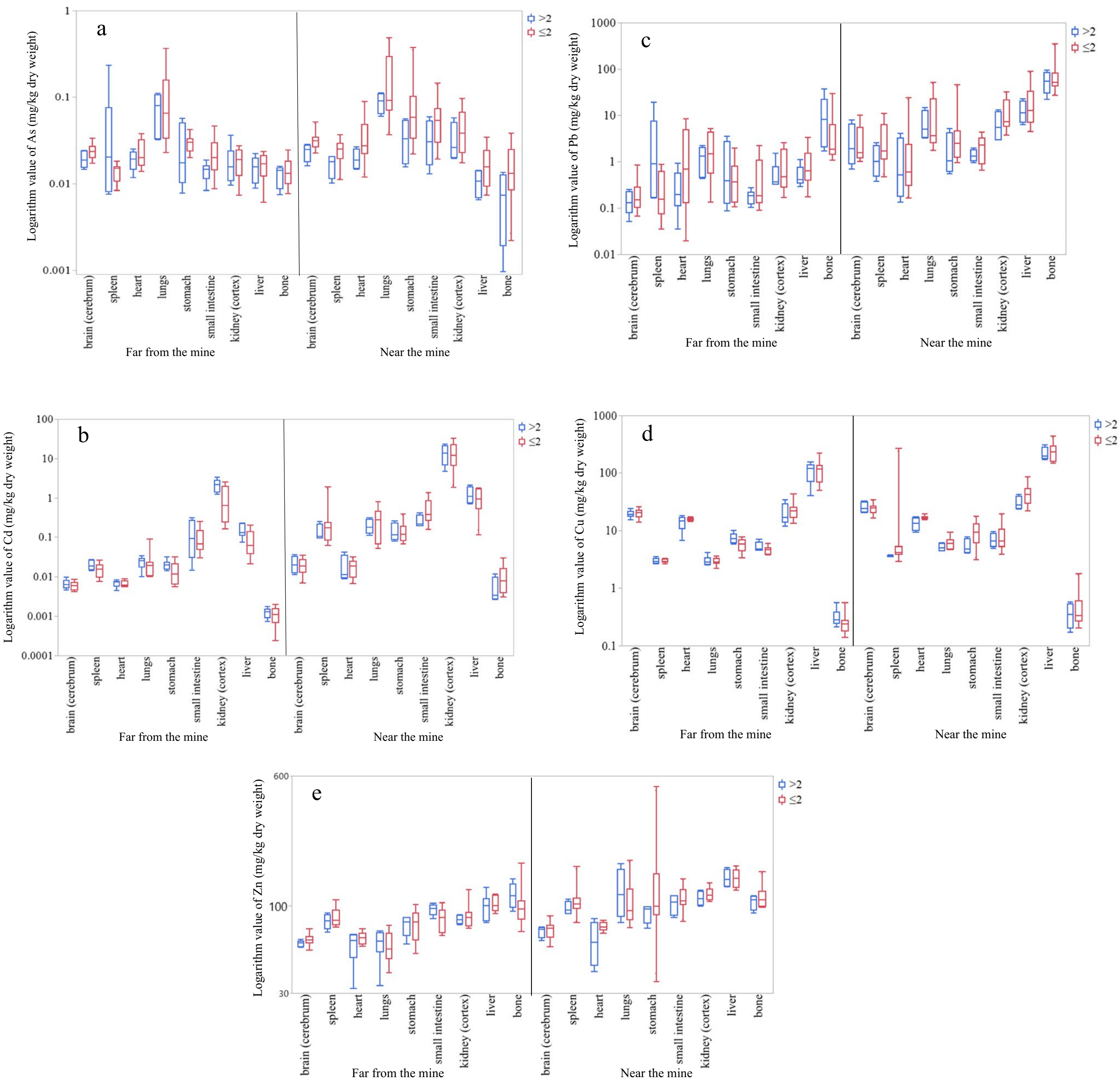

Fig. 3 Box plots of toxic element and trace element concentration in tissues of dogs by age: **a** As, **b** Cd, **c** Pb, **d** Cu, and **e** Zn (* denoted $p < 0.05$)

10 times increase in accumulation of toxic metals, specifically Cd and Pb, in several tissues of dogs living close to the mine compared to dogs living farther away. Especially, the highest concentration of As, Cd, and Pb was found in lungs, kidney, and bone, respectively. Metals may accumulate in the dog's body because of continuous exposure, suggesting that the pattern of metal accumulation may be similar in humans residing around the mine. Therefore, metal toxicity must not be taken lightly and to better understand the metal species, present in the tissues of various other mammals for remediation reasons, more research should be done. Considering this, environmental remediation such as applying soil amendments, phytoremediation, bioremediation, and covering and capping the mine tailing is urgently required for human, animal, and environmental health concerns in Kabwe where environmental pollution is still a problem.

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Author contribution Nyein Chan Soe: conceptualization, data analysis, investigation, methodology, and writing both drafting the original paper. Yared Beyene Yohannes: conceptualization, analysis, investigation, and

Table 3 Spearman's rank correlation (ρ) between metals in kidneys and livers of dogs from near the mine

	As	Cd	Pb	Cu	Zn
Kidney					
As	1.000				
Cd	0.244	1.000			
Pb	0.481	0.534*	1.000		
Cu	0.376	-0.037	0.481	1.000	
Zn	0.446	0.600*	0.411	0.257	1.000
Liver					
As	1.000				
Cd	-0.086	1.000			
Pb	0.200	0.486	1.000		
Cu	-0.011	-0.323	-0.055	1.000	
Zn	-0.310	-0.301	-0.231	0.565*	1.000

*$p < 0.05$

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Data availability Available from the corresponding author on reasonable request.

Declarations

Ethics approval Ethical approval for this study was granted by the Zambian Ministry of Fisheries and Livestock. Approval for ethical clearance was obtained from the University of Zambia Biomedical Research Ethics Committee (UNZABREC) under the reference number 012–04-16. The sampling was carried out strictly in accordance with its regulations.

Consent to participate All authors consent to participate.

Consent to publication All authors consent to publication.

Competing interests The authors declare no competing interests.

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