HOKKAIDO UNIVERSITY

Title	Metals and arsenic distribution in stray dogs' tissues around a lead-zinc mine in Kabwe, Zambia
Author(s)	Soe, Nyein Chan; Yohannes, Yared Beyene; Kataba, Andrew et al.
Citation	Environmental science and pollution research, 31(8), 12083-12093 https://doi.org/10.1007/s11356-024-31948-4
Issue Date	2024-01-16
Doc URL	https://hdl.handle.net/2115/93924
Rights	This version of the article has been accepted for publication, after peer review (when applicable) and is subject to Springer Nature's AM terms of use, but is not the Version of Record and does not reflect post-acceptance improvements, or any corrections. The Version of Record is available online at: http://dx.doi.org/10.1007/s11356-024-31948-4
Type	journal article
File Information	Heavy metals distribution in stray dogs_ESPR_revised_231218.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

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 MM Nakayama[1,3]

[1] Laboratory of Toxicology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan

[2] Department of Pharmacology and Parasitology, University of Veterinary Science, Yezin, 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, Japan

[7] One Health Research Center, Hokkaido University, Sapporo, Japan

[8] Water Research Group, School of Environmental Sciences and Development, North-West University, Potchefstroom, South Africa

Corresponding author: Shouta MM Nakayama

E-mail address: *shouta-nakayama@vetmed.hokudai.ac.jp* *or* *shoutanakayama0219@gmail.com*

Tel.: +81 11 706 5105

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), 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 (ρ= 0.534) and Zn (ρ= 0.600), whereas in the liver, Cu correlated with Zn (ρ= 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.

Key words: 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 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).

63 Moreover, Pb levels in the children's blood who reside close to the mine exceeded the Pb reference
64 value set by the CDC (Yabe et al. 2015, 2020). Dogs share the same habitat as humans, and exposure
65 to environmental pollutants reported in human habitats is unavoidable for them (Reif 2011; Severino
66 et al. 2012; Esposito et al. 2019). For instance, a high mean concentration of blood lead level (525.3
67 ± 230.8 µg/l), which exceeded the toxic level (400 µg/l) was observed in dogs from Kasanda, Kabwe,
68 a township that located about 1 km from the mine (Toyomaki et al. 2020). However, reports on tissue
69 metal distribution in stray dogs, especially around the mining area to better understand the
70 phenomenon of metals in the body are lacking.

71 Therefore, this study was carried out to assess the accumulation of metals and arsenic in
72 several tissues of stray dogs from Kabwe and to compare metal accumulation between dogs from
73 townships close to the mine (within 3 km from the mine) and those 7 km away from the mine.
74 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 U.S. 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 (Figure 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 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% – 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 (≤ 2:>2) from locations close to the mine and distant from the mine were 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 Tables 1 and 2. Among the tissues analyzed, the lungs, kidney, 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

149 near the mine, the pattern was bone> liver> kidney> lungs> stomach> brain> spleen> heart> small
150 intestine.

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

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

164 The relationship of metals and arsenic in the kidney and liver of dogs from the mine's vicinity
165 is depicted in Table 3. A significant positive correlation ($\rho = 0.600$, $p < 0.05$) was observed between
166 Cd and Zn in the kidney Cd and Pb showed positive correlation ($\rho = 0.534$, $p < 0.05$) in the kidney.
167 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 were found to be exceeded 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 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 hours (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 which produced 235 tons 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 (Satoru 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 (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 (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).

241 In this study, the concentration of As in the small intestine of dogs that were distant from the
242 mine was significantly higher in males than in females, but the concentration of As in the heart of
243 dogs that were close to mine exhibited an opposite tendency. In case of Pb, male dogs residing far
244 from the mine had more Pb in their brains and livers than female dogs did. A separate substantial
245 trend (female dogs > male dogs) was observed in the content of Cu in the stomachs of dogs living far
246 from the mine. A previous study also revealed that the effect of sex was reversed between townships
247 near the mine (Toyomaki et al. 2020). Such variations might be influenced by the type of food or
248 feed supply. The subjects in this study were stray dogs that roamed in different places for food. Both
249 female and male dogs may wander from one location to another. As a result, the exposure to metals
250 and arsenic in this study may not have been significantly affected by differences in sex.

251 In this study, significant positive correlation was observed between Zn and Cd in the kidney.
252 Yabe et al. (2011) reported that a similar interaction was observed in the kidney of cattle from
253 Kabwe, with a positive correlation for Cd and Zn in the kidney. It seems that these interactions are
254 regulated by metallothionein, metal-binding proteins that are induced by many metals, including Cd
255 (Nordberg and Nordberg 2022). It has been proven that Zn can also bind to this protein (Tokar et al.
256 2012). A significant positive correlation between Pb and Cd was also observed in the kidney, which
257 is no surprise as both metals can induce metallothionein in the kidney. A significant positive
258 correlation was observed among trace elements in the liver. This agrees with a previous report on
259 cattle from Kabwe (Yabe et al. 2011). In fact, Cu and Zn share chemical properties and interact with
260 each other during tissue metal accumulation (Irato and Albergoni 2005).

261 Limitations of the study included limited information regarding the home range of stray dogs.
262 While the observation that dogs sampled 7 km distance from the mine site exhibited lower levels of
263 Pb in their tissues is noted, it falls short of establishing the actual extent of the dogs' home range.
264 Moreover, the study lacks information on the movement of animals from the reference area. Without
265 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 concentration observed in stray dogs in Kabwe were high in comparison to those found in dogs inhabiting minimal pollution. This study revealed an approximately 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.

References

Agneta Å, Lars B, A BI, et al (2014) Non-Renal Effects and the Risk Assessment of Environmental Cadmium Exposure. Environ Health Perspect 122:431–438. https://doi.org/10.1289/ehp.1307110

Ahmed SF, Kumar PS, Rozbu MR, et al (2022) Heavy metal toxicity, sources, and remediation techniques for contaminated water and soil. Environ Technol Innov 25:102114. https://doi.org/10.1016/J.ETI.2021.102114

Bolognin M, Kirschvink N, Leemans J, et al (2009) Characterisation of the acute and reversible airway inflammation induced by cadmium chloride inhalation in healthy dogs and evaluation of the effects of salbutamol and prednisolone. The Veterinary Journal 179:443–450. https://doi.org/10.1016/J.TVJL.2007.10.004

Briffa J, Sinagra E, Blundell R (2020) Heavy metal pollution in the environment and their toxicological effects on humans. Heliyon 6:e04691. https://doi.org/10.1016/J.HELIYON.2020.E04691

Cafazzo S, Valsecchi P, Bonanni R, Natoli E (2010) Dominance in relation to age, sex, and competitive contexts in a group of free-ranging domestic dogs. Behavioral Ecology 21:443–455. https://doi.org/10.1093/beheco/arq001

Donnelly PA, Monty DE (1977) Toxicological effects of cadmium chloride on the canine testis following various routes of administration. Toxicol Lett 1:53–58. https://doi.org/10.1016/0378-4274(77)90021-2

Doya R, Nakayama SMM, Nakata H, et al (2020) Land Use in Habitats Affects Metal Concentrations in Wild Lizards around a Former Lead Mining Site. Environ Sci Technol 54:14474–14481. https://doi.org/10.1021/acs.est.0c00150

Esposito M, De Roma A, Maglio P, et al (2019) Heavy metals in organs of stray dogs and cats from the city of Naples and its surroundings (Southern Italy). Environmental Science and Pollution Research 26:3473–3478. https://doi.org/10.1007/s11356-018-3838-5

Garland T (2018) Zinc. In: Veterinary Toxicology: Basic and Clinical Principles: Third Edition. Academic Press, pp 489–492

He ZL, Yang XE, Stoffella PJ (2005) Trace elements in agroecosystems and impacts on the environment. Journal of Trace Elements in Medicine and Biology 19:125–140. https://doi.org/10.1016/J.JTEMB.2005.02.010

Irato P, Albergoni V (2005) Interaction between copper and zinc in metal accumulation in rats with particular reference to the synthesis of induced-metallothionein. Chem Biol Interact 155:155–164. https://doi.org/10.1016/J.CBI.2005.06.005

Kamona AF, Friedrich GH (2007) Geology, mineralogy and stable isotope geochemistry of the Kabwe carbonate-hosted Pb–Zn deposit, Central Zambia. Ore Geol Rev 30:217–243. https://doi.org/10.1016/J.OREGEOREV.2006.02.003

Langlois DK, Kaneene JB, Yuzbasiyan-Gurkan V, et al (2017) Investigation of blood lead concentrations in dogs living in Flint, Michigan. J Am Vet Med Assoc 251:912–921. https://doi.org/10.2460/javma.251.8.912

Li T, Wan Y, Ben Y, et al (2017) Relative importance of different exposure routes of heavy metals for humans living near a municipal solid waste incinerator. Environmental Pollution 226:385–393. https://doi.org/10.1016/J.ENVPOL.2017.04.002

López-Alonso M, Miranda M, García-Partida P, et al (2007a) Use of dogs as indicators of metal exposure in rural and urban habitats in NW Spain. Science of The Total Environment 372:668–675. https://doi.org/10.1016/J.SCITOTENV.2006.10.003

López-Alonso M, Miranda M, García-Partida P, et al (2007b) Toxic and trace metal concentrations in liver and kidney of dogs. Biol Trace Elem Res 116:185–202. https://doi.org/10.1007/BF02685930

Lovásová E, Racz O, Cimboláková I, et al (2013) Effects of Chronic Low-Dose Cadmium Exposure on Selected Biochemical and Antioxidant Parameters in Rats. J Toxicol Environ Health A 76:1033–1038. https://doi.org/10.1080/15287394.2013.828249

Masindi V, Muedi KL (2018) Environmental Contamination by Heavy Metals. In: Heavy Metals. InTech

Nakata H, Nakayama SMM, Yabe J, et al (2022) Interdisciplinary approach to addressing lead pollution caused by mining activity in Kabwe, The Republic of Zambia. Environmental Monitoring and Contaminants Research 2:94–111. https://doi.org/10.5985/emcr.20220004

Nakata H, Nakayama SMM, Yabe J, et al (2016) Reliability of stable Pb isotopes to identify Pb sources and verifying biological fractionation of Pb isotopes in goats and chickens. Environmental Pollution 208:395–403. https://doi.org/10.1016/J.ENVPOL.2015.10.006

Nakayama SMM, Ikenaka Y, Hamada K, et al (2011) Metal and metalloid contamination in roadside soil and wild rats around a Pb–Zn mine in Kabwe, Zambia. Environmental Pollution 159:175–181. https://doi.org/10.1016/j.envpol.2010.09.007

Nordberg M, Nordberg GF (2022) Metallothionein and Cadmium Toxicology—Historical Review and Commentary. Biomolecules 12

Parmar TK, Rawtani D, Agrawal YK (2016) Bioindicators: the natural indicator of environmental pollution. Front Life Sci 9:110–118. https://doi.org/10.1080/21553769.2016.1162753

Paßlack N, Mainzer B, Lahrssen-Wiederholt M, et al (2015) Concentrations of strontium, barium, cadmium, copper, zinc, manganese, chromium, antimony, selenium, and lead in the liver and kidneys of dogs according to age, gender, and the occurrence of chronic kidney disease. J Vet Sci 16:57–66. https://doi.org/10.4142/jvs.2015.16.1.57

Puls R (1994) Mineral Levels in Animal Health: Diagnostic Data. Sherpa International

Rabinowitz PM, Scotch ML, Conti LA (2010) Animals as Sentinels: Using Comparative Medicine To Move Beyond the Laboratory

Reif JS (2011) Animal Sentinels for Environmental and Public Health

359 Satarug S, Baker JR, Urbenjapol S, et al (2003) A global perspective on cadmium pollution and
360 toxicity in non-occupationally exposed population. Toxicol Lett 137:65–83.
361 https://doi.org/10.1016/S0378-4274(02)00381-8

362 Severino L, Serpe FP, Russo R, et al (2012) Levels of heavy metals in liver and kidney of dogs
363 from urban environment. Open Vet J 2:2226–4485

364 Talmage SS, Walton BT (1991) Small Mammals as Monitors of Environmental Contaminants
365 1 ,2

366 Tataruch F, Kierdorf H (2003) Chapter 20 Mammals as biomonitors. In: Trace Metals and other
367 Contaminants in the Environment. Elsevier, pp 737–772

368 Thompson LJ (2018a) Lead. In: Veterinary Toxicology: Basic and Clinical Principles: Third
369 Edition. Academic Press, pp 439–443

370 Thompson LJ (2018b) Copper. In: Veterinary Toxicology: Basic and Clinical Principles: Third
371 Edition. Academic Press, pp 425–427

372 Tokar EJ, Boyd WA, Freedman JH, Waalkes MP (2012) Toxic Effects of Metals. In: Casarett
373 and Doull's Toxicology: The Basic Science of Poisons, 8e. McGraw-Hill Education, New
374 York, NY

375 Toyomaki H, Yabe J, Nakayama SMM, et al (2020) Factors associated with lead (Pb) exposure
376 on dogs around a Pb mining area, Kabwe, Zambia. Chemosphere 247:125884.
377 https://doi.org/10.1016/J.CHEMOSPHERE.2020.125884

378 Yabe J, Nakayama SM, Nakata H, et al (2020) Current trends of blood lead levels, distribution
379 patterns and exposure variations among household members in Kabwe, Zambia.
380 Chemosphere 243:125412. https://doi.org/10.1016/J.CHEMOSPHERE.2019.125412

381 Yabe J, Nakayama SMM, Ikenaka Y, et al (2011) Uptake of lead, cadmium, and other metals in
382 the liver and kidneys of cattle near a lead-zinc mine in Kabwe, Zambia. Environ Toxicol
383 Chem 30:1892–1897. https://doi.org/10.1002/etc.580

384 Yabe J, Nakayama SMM, Ikenaka Y, et al (2013) Metal distribution in tissues of free-range
385 chickens near a lead-zinc mine in Kabwe, Zambia. Environ Toxicol Chem 32:189–192.
386 https://doi.org/10.1002/etc.2029

387 Yabe J, Nakayama SMM, Ikenaka Y, et al (2015) Lead poisoning in children from townships in
388 the vicinity of a lead–zinc mine in Kabwe, Zambia. Chemosphere 119:941–947.
389 https://doi.org/10.1016/J.CHEMOSPHERE.2014.09.028

390 Yohannes YB, Ikenaka Y, Nakayama SMM, et al (2017) Trace Element Contamination in
391 Tissues of Four Bird Species from the Rift Valley Region, Ethiopia. Bull Environ Contam
392 Toxicol 98:172–177. https://doi.org/10.1007/s00128-016-2011-4

393 Zhang H, Mao Z, Huang K, et al (2019) Multiple exposure pathways and health risk assessment
394 of heavy metal(loid)s for children living in fourth-tier cities in Hubei Province. Environ Int
395 129:517–524. https://doi.org/10.1016/J.ENVINT.2019.04.031

Zhou Q, Zhang J, Fu J, et al (2008) Biomonitoring: An appealing tool for assessment of metal pollution in the aquatic ecosystem. Anal Chim Acta 606:135–150. https://doi.org/10.1016/J.ACA.2007.11.018

Ethical 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.

Data availability: Available from the corresponding author on reasonable request.

Acknowledgements

The Authors thank to the veterinary office of Kabwe for their technical support: in the collection of dogs' tissues.

Funding

This work was supported by the Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan awarded to Mayumi Ishizuka (Nos. 22KK0163, 21H04919), and Shouta MM Nakayama (Nos. 20K20633 and 23H03545). This work was also supported by the foundation of JSPS Bilateral Open Partnership Joint Research Projects (JPJSBP120209902; SMMN). This work was also supported by JSPS CORE to CORE program (MI,

SMMN), The Japan Prize Foundation (SMMN); and Hokkaido University Specific Research Projects (MI). This research was also supported by JST/JICA, SATREPS (Science and Technology Research Partnership for Sustainable Development; No. JPMJSA1501), aXis (Accelerating Social Implementation for SDGs Achievement; No. JPMJAS2001), and AJ-CORE project funded by JST.

Author contribution

Nyein Chan Soe: conceptualization, data analysis, investigation, methodology, and writing both drafting the original paper.

Yared Beyene Yohannes: conceptualization, analysis, investigation, and writing—review and editing.

Andrew Kataba: sample collection, material preparation

Madalitso Tembo: sample collection

John Yabe: Participated in sample collection, material preparation.

Kenneth Chawinga: Participated in sample collection, material preparation.

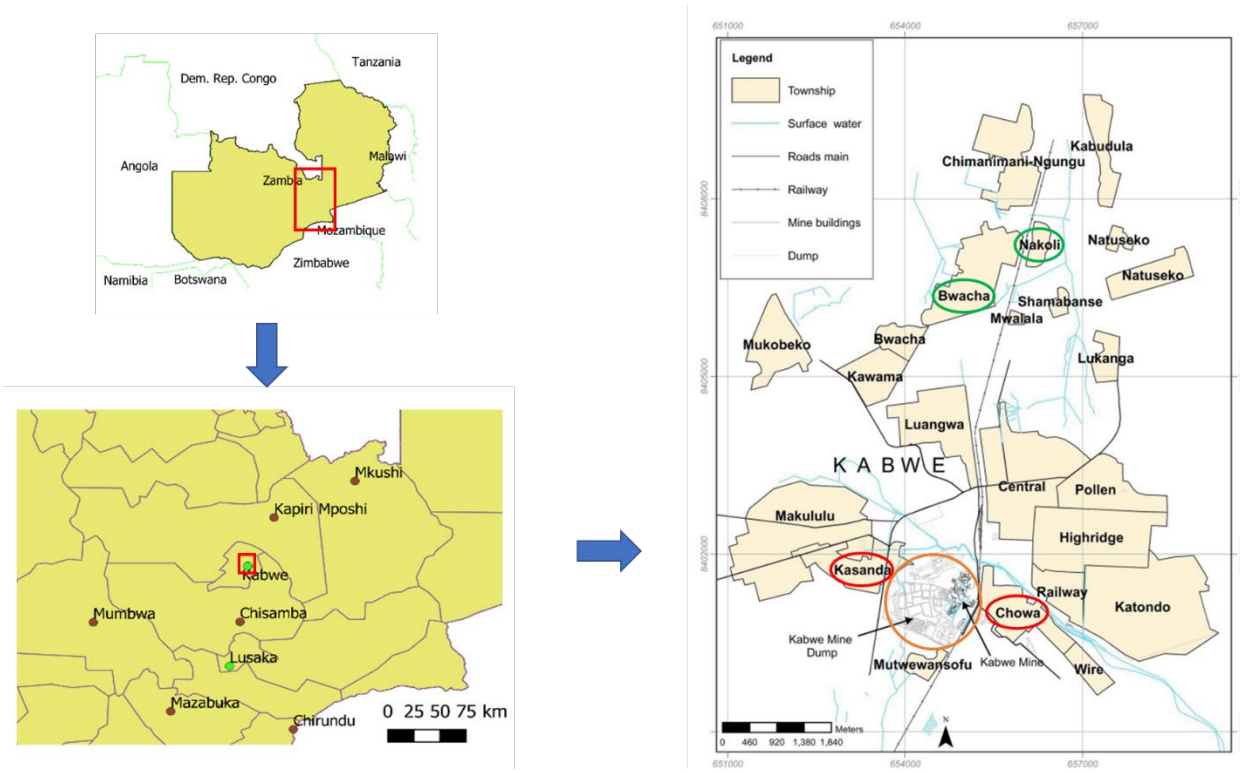
Golden Zyambo: Participated in sample collection, material preparation.

Kaampwe Muzandu: Supervision

Yoshinori Ikenaka: Supervision

Mayumi Ishizuka: funding acquisition, supervision, and reviewing the manuscript and Supervision.

Shouta MM Nakayama: conceptualization, analysis, funding acquisition, investigation, supervision, writing and reviewing the manuscript and corresponding author.



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

444

445

446

447

448

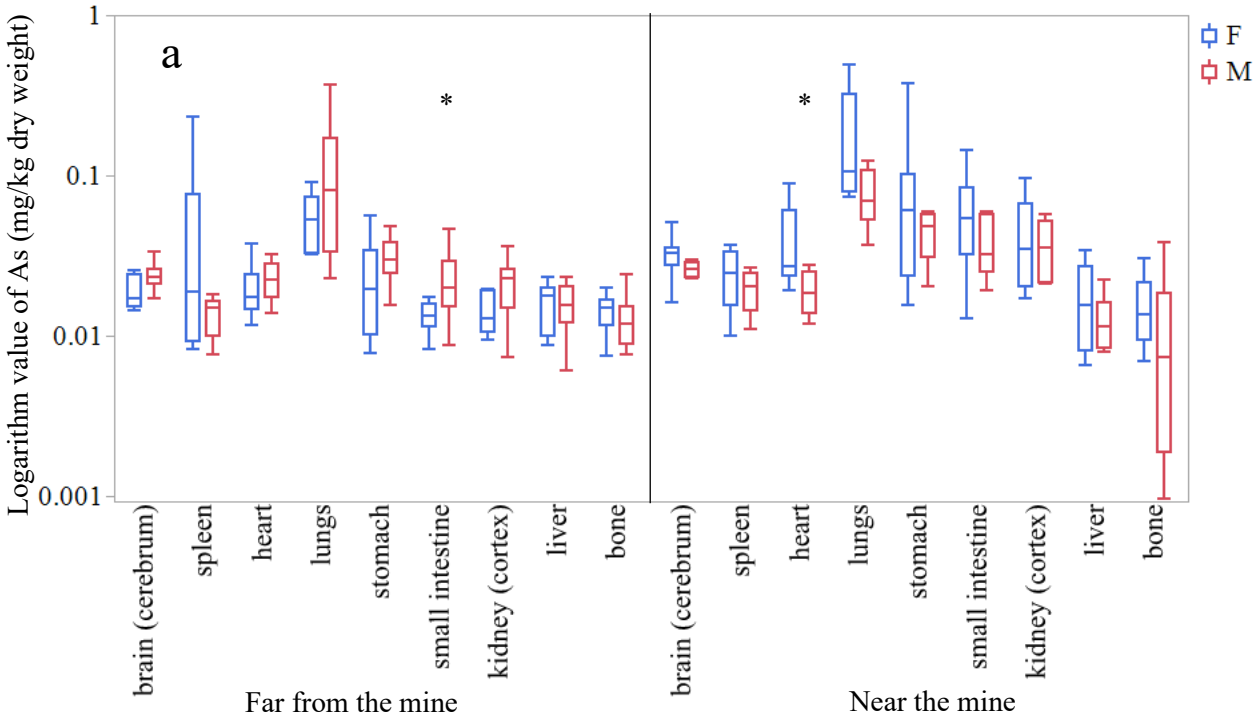
449

450

451

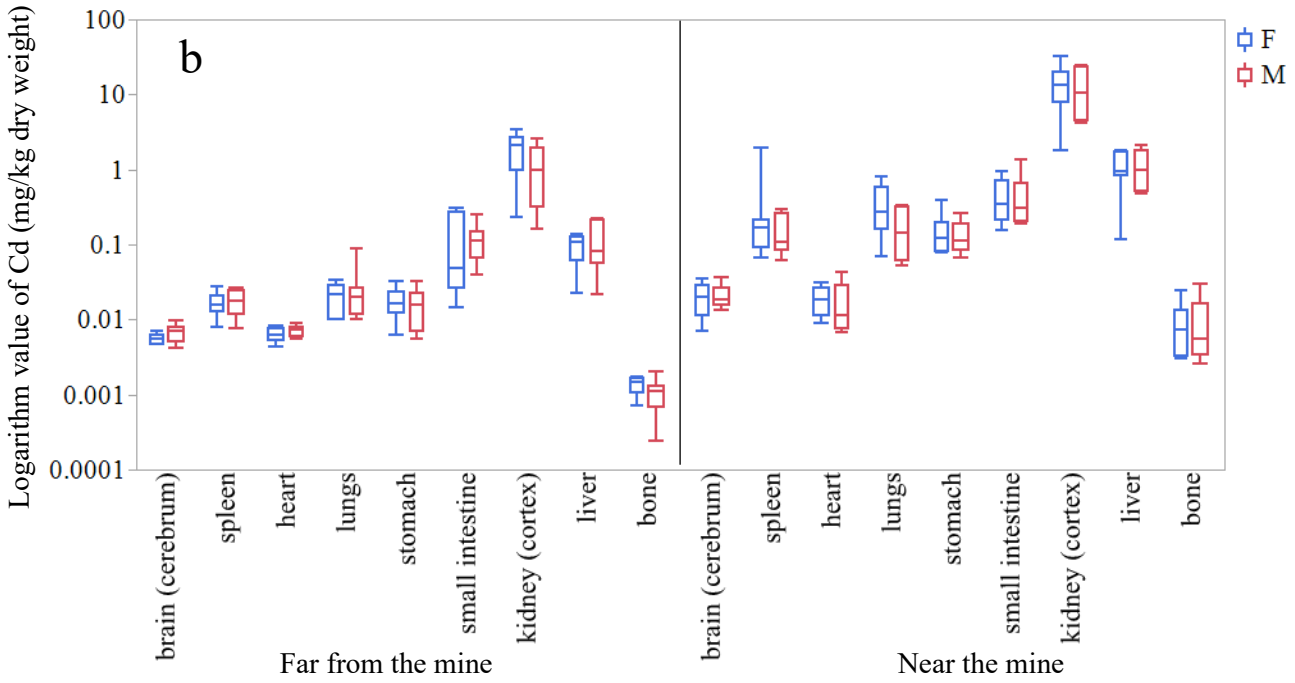
452

453



454

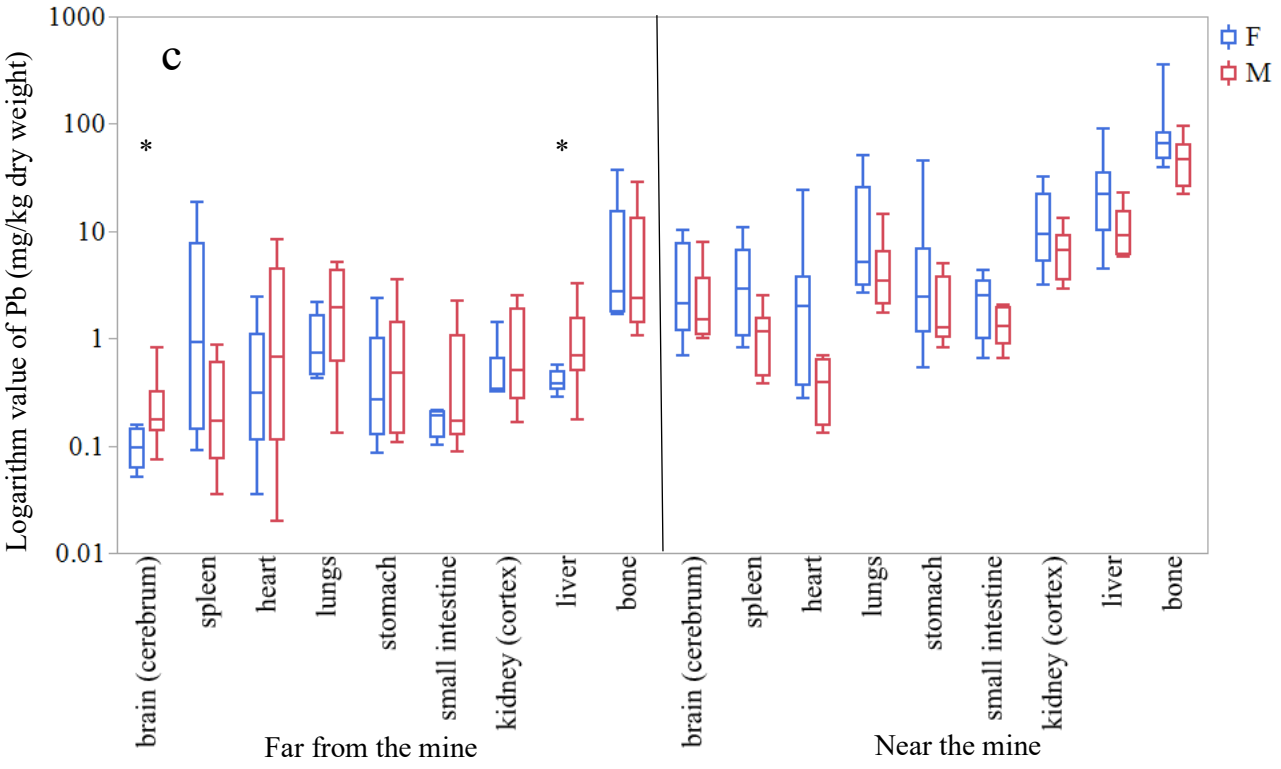
455



456

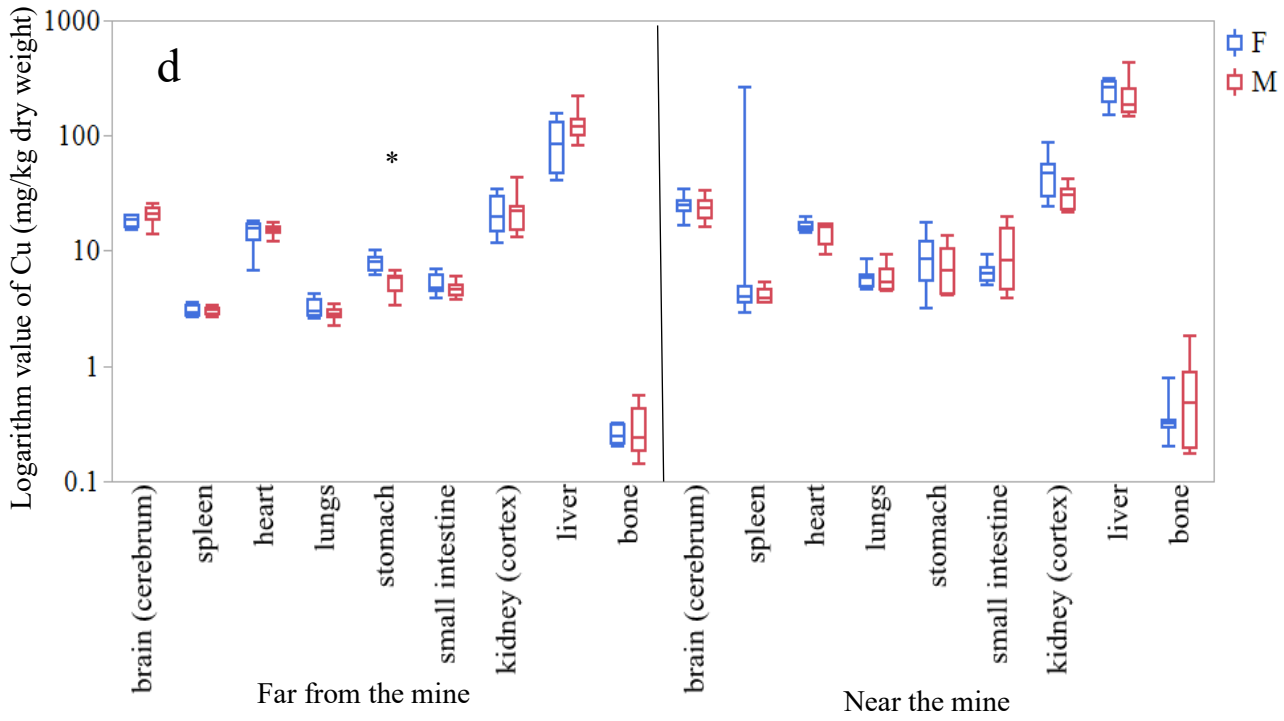
457

458


c
Logarithm value of Pb (mg/kg dry weight)
1000
100
10
1
0.1
0.01
*
*
F
M
brain (cerebrum)
spleen
heart
lungs
stomach
small intestine
kidney (cortex)
liver
bone
Far from the mine
Near the mine

459

460


d
Logarithm value of Cu (mg/kg dry weight)
1000
100
10
1
0.1
*
F
M
brain (cerebrum)
spleen
heart
lungs
stomach
small intestine
kidney (cortex)
liver
bone
Far from the mine
Near the mine

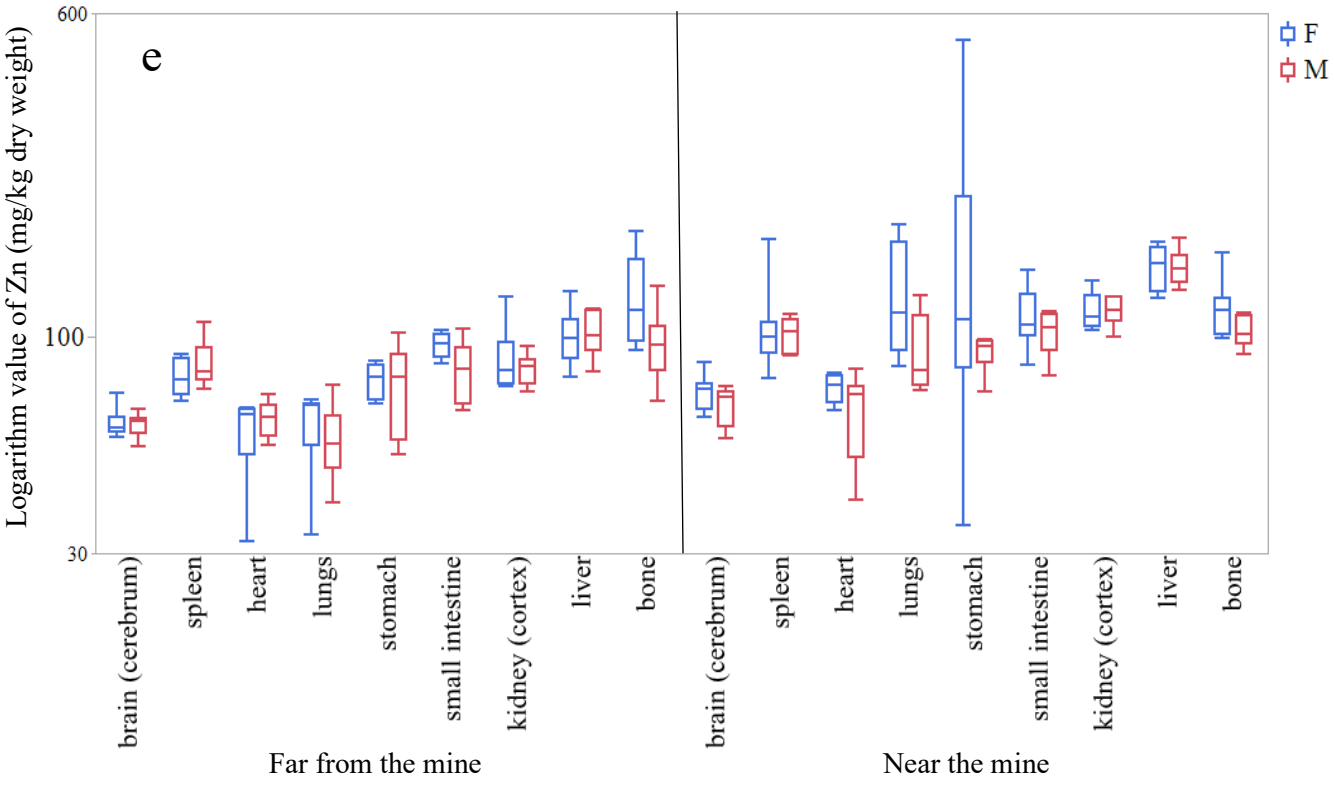
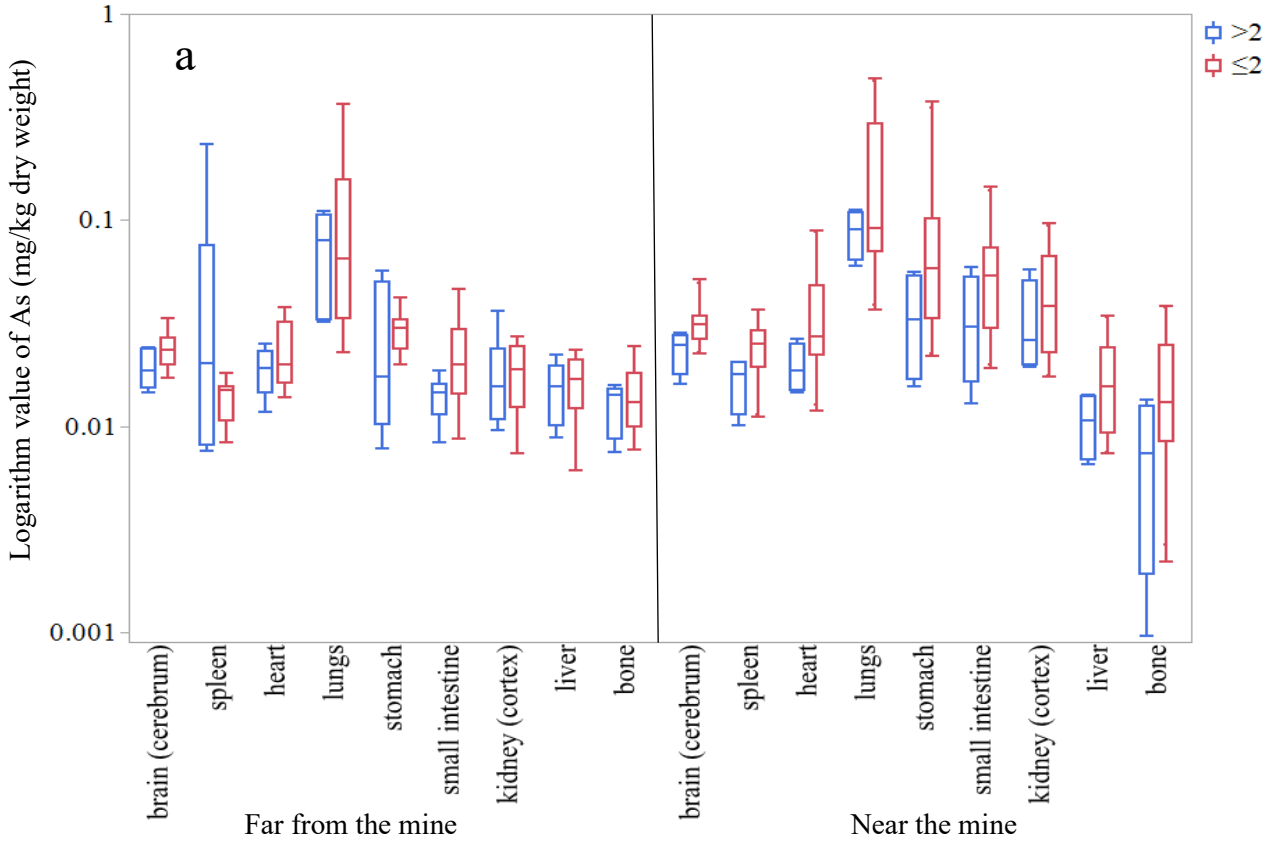
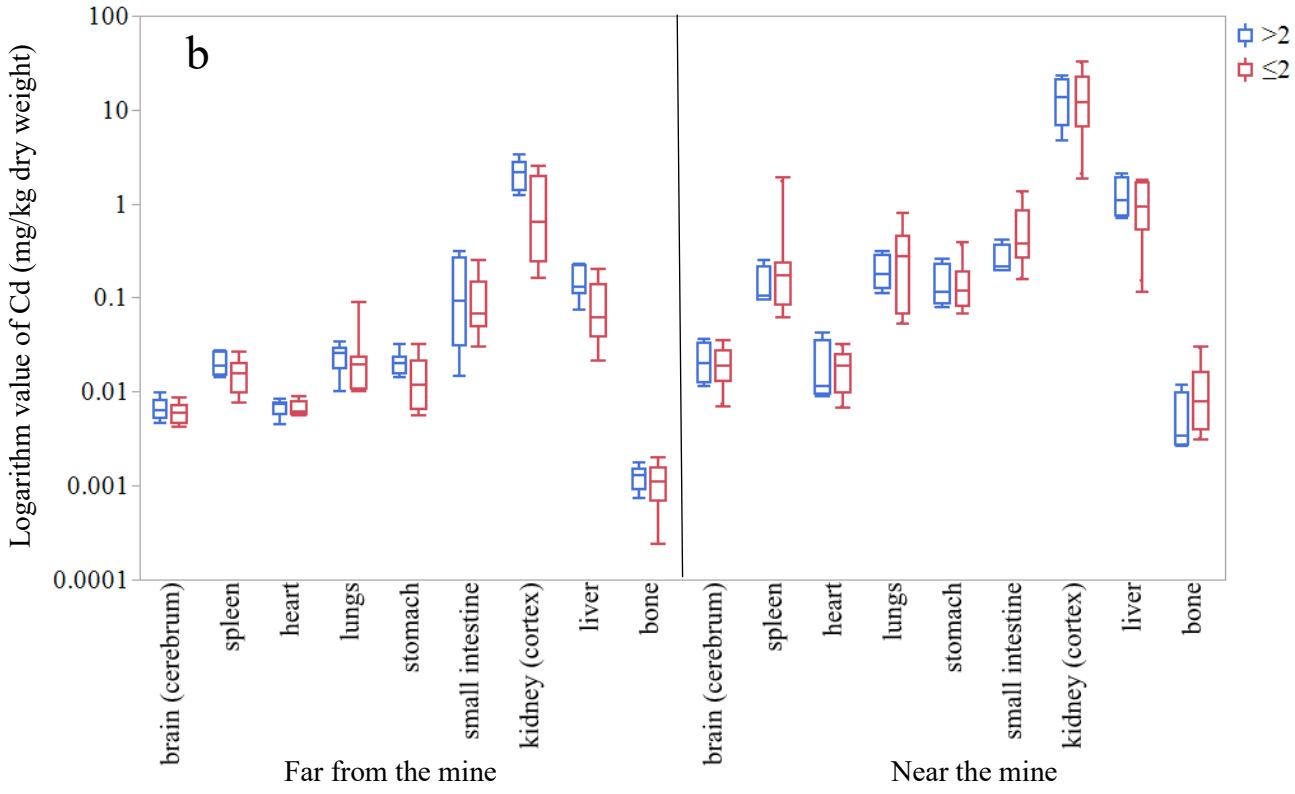
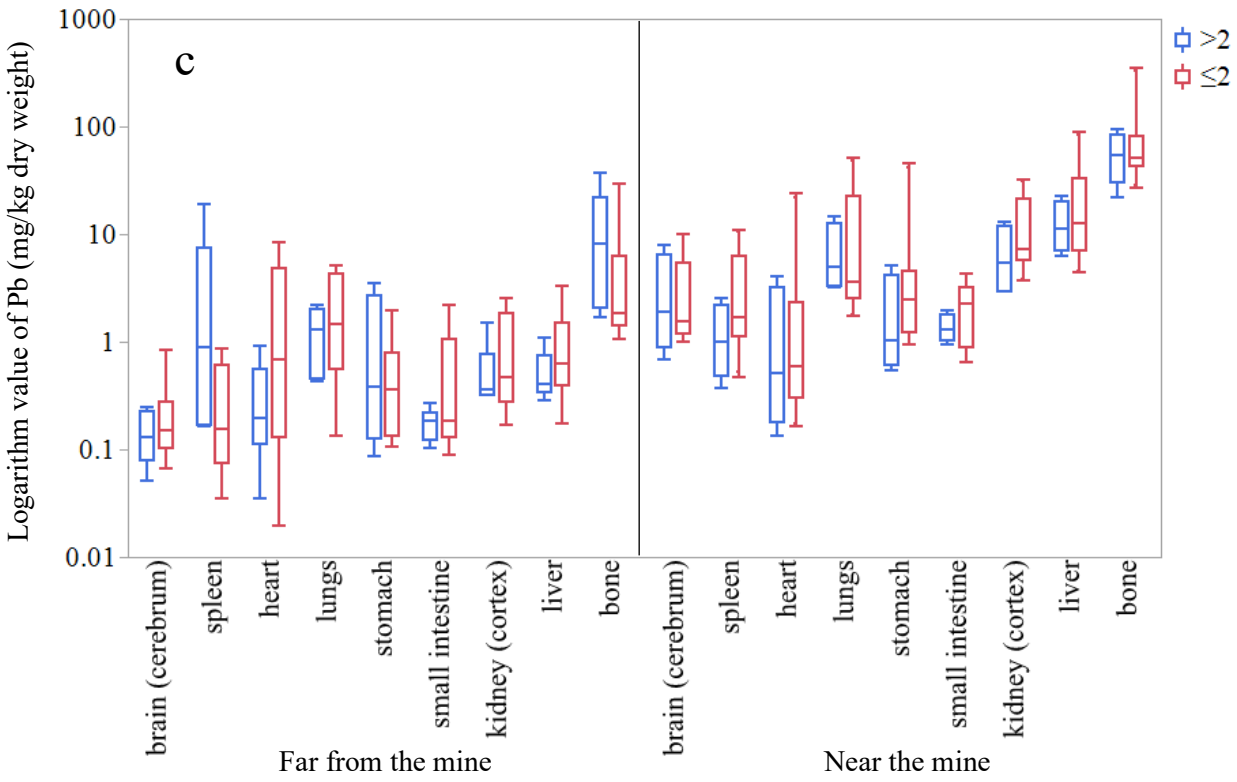


Figure 2. Box plots of toxic elements and trace elements concentration in tissues of dogs by sex [a] As; [b] Cd; [c] Pb; [d] Cu and [e] Zn; (* denoted $p < 0.05$)

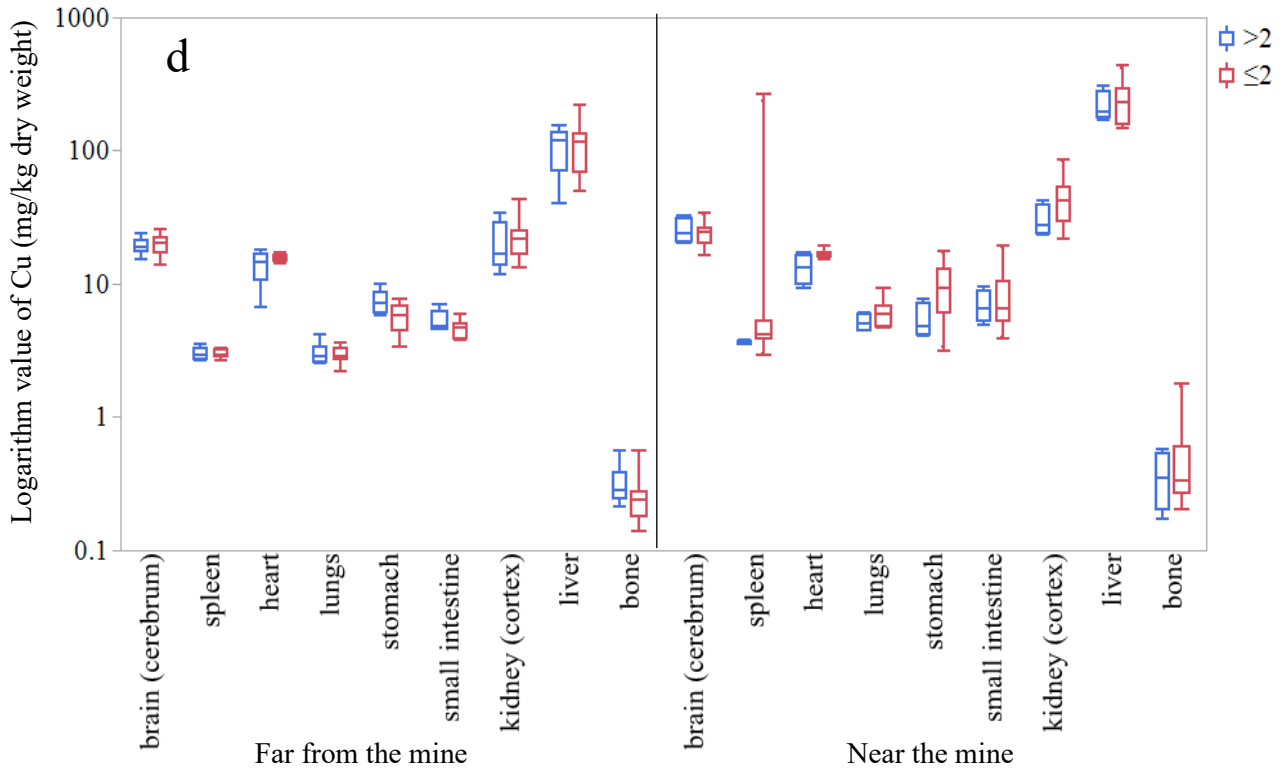

a
Logarithm value of As (mg/kg dry weight)
1
0.1
0.01
0.001
>2
≤2
brain (cerebrum)
spleen
heart
lungs
stomach
small intestine
kidney (cortex)
liver
bone
Far from the mine
Near the mine

465

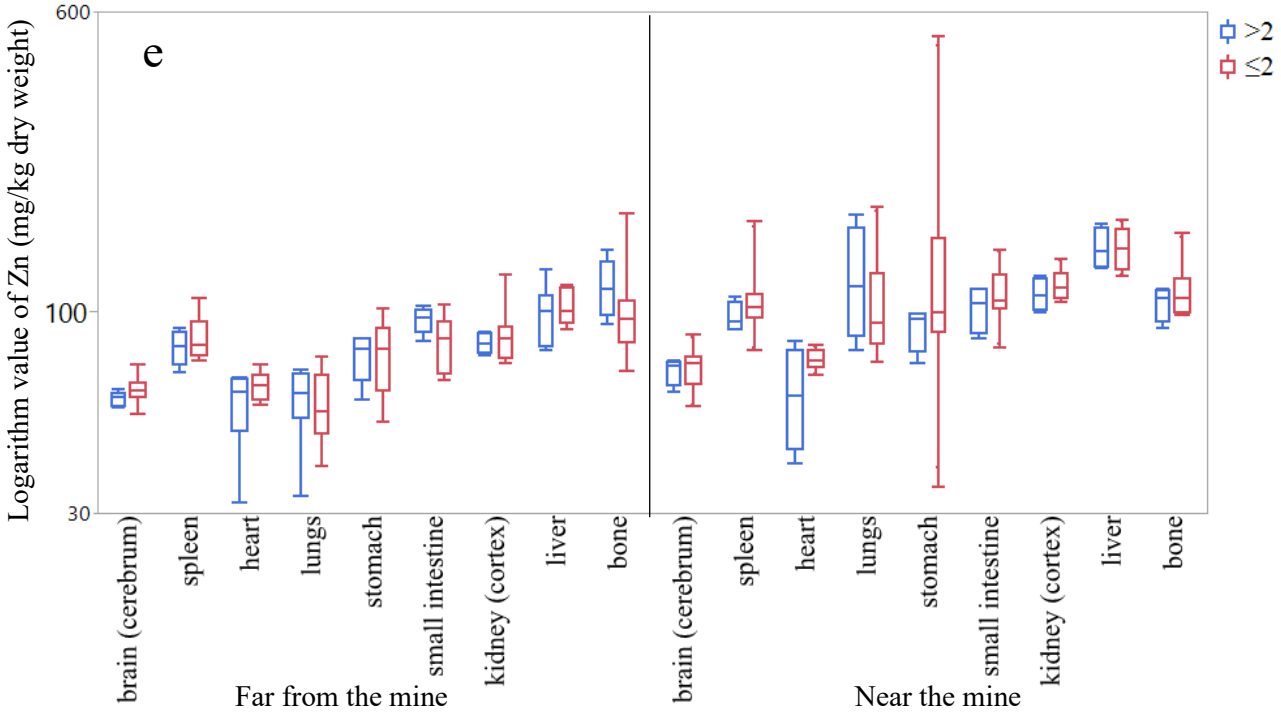

b
Logarithm value of Cd (mg/kg dry weight)
100
10
1
0.1
0.01
0.001
0.0001
>2
≤2
brain (cerebrum)
spleen
heart
lungs
stomach
small intestine
kidney (cortex)
liver
bone
Far from the mine
Near the mine


c
Logarithm value of Pb (mg/kg dry weight)
1000
100
10
1
0.1
0.01
>2
≤2
brain (cerebrum)
spleen
heart
lungs
stomach
small intestine
kidney (cortex)
liver
bone
Far from the mine
Near the mine

466


d
Logarithm value of Cu (mg/kg dry weight)
1000
100
10
1
0.1
>2
≤2
brain (cerebrum)
spleen
heart
lungs
stomach
small intestine
kidney (cortex)
liver
bone
Far from the mine
Near the mine

467



468

469 Figure 3. Box plots of toxic elements and trace elements concentration in tissues of dogs by age [a]

470 As; [b] Cd; [c] Pb; [d] Cu and [e] Zn; (* denoted $p < 0.05$)

471 Table 1. Toxic metals (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)

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)

472 SD: standard derivation; n: number of dogs; *, ** and *** denoted significant difference between dogs near the mine and dogs distant from the

473 mine at <0.05, <0.01 and <0.001, respectively (Mann-Whitney U test)

474 Table 2. Trace metals (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 (9.1–26331.1)	10	2870.4 ± 8260.8*	71.5 (17.2–26331.1)	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)

475 SD: standard derivation; n: number of dogs; *, ** and *** denoted significant difference between dogs near the mine and dogs distant from the

476 mine at <0.05, <0.01 and <0.001, respectively (Mann-Whitney U test)

477 Table 3. Spearman's rank correlation (ρ) between metals in kidneys and livers of dogs from

478 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

479 * denoted $p<0.05$.

480