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Land Use in Habitat Affects Metal Concentrations in Wild Lizards Around a Former Lead Mining Site

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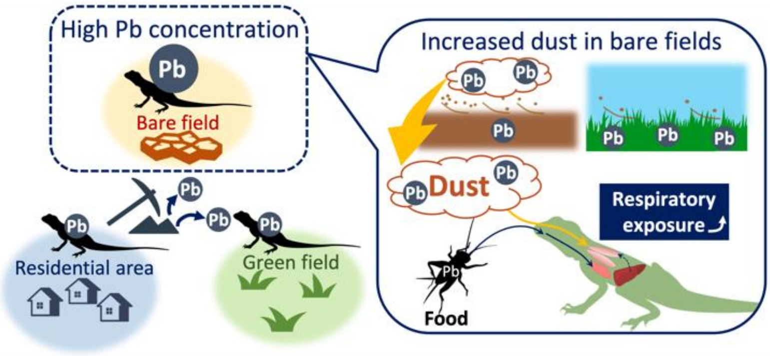
ABSTRACT

We investigated the potential effects of different land use and other environment factors on animals living in a contaminated environment. The study site in Kabwe, Zambia, is currently undergoing urban expansion, while lead contamination from former mining activities is still prevalent. We focused on a habitat-generalist lizards (*Trachylepis wahlbergii*). The livers, lungs, blood and stomach contents of 224 lizards were analyzed for their lead, zinc, cadmium, copper, nickel and arsenic concentrations. Habitat types were categorized based on vegetation data obtained from satellite images. Multiple regression analysis revealed that land use categories of habitats and three other factors significantly affected lead concentrations in the lizards. Further investigation suggested that the lead concentrations in lizards living in bare fields were higher than expected based on distance from the contaminant source, while those in lizards living in green fields were lower than expected. In addition, lead concentration of lungs were higher than that of liver in 19% of the lizards, implying direct exposure to lead via dust inhalation besides digestive exposure. Since vegetation reduces the production of dust from surface soil, it is plausible that dust from the mine is one of the contamination sources, and that vegetation can reduce exposure to this.

ABSTRACT ART



INTRODUCTION

Several studies have described environmental hazards preceding city development. Examples are environmental contamination in developing countries that is caused by poorly managed electronic waste recycling facilities, dumping grounds, and mining sites.[1–5] Since diversification of land use is one of the characteristics of urbanization,[6] the status of long-standing contamination and how the effects thereof differ between different land use patterns should be understood before cities are developed in regions where environmental pollution has already occurred. Existing studies have examined the effects of different land uses on environmental contamination status only by describing the existence of pollution sources, such as intensive crop fields, various industries, power plants, and heavy traffic.[6–9] In contrast, we have considered land use as one of the environmental factors controlling exposure to and accumulation of contaminants in living organisms. Such insight is necessary for creating proper methodologies for city development that can minimize the potential negative environmental and health effects. Although environmental remediation is the most fundamental solution for heavily contaminated regions, it costs an enormous amount of money and is not always feasible for addressing problems in widely spread populations.[10,11] Until remediation is complete, humans and animals continue to be exposed to toxic substances. Moreover, even if the environment is not suitable for people to live in, since contamination sources often correspond with local economic drivers, social communities and economic activities continue to flourish and fuel urbanization regardless. Therefore, appropriate city planning should be conducted before mass construction begins, so that people can receive the benefits of urbanization while their exposure to environmental pollutants is mitigated.

Kabwe, in the Republic of Zambia, is a remarkable example of a city undergoing urban expansion in an environment that has long been contaminated.[12] In Kabwe, the primary contamination source is a lead (Pb) and zinc (Zn) mine. After the mine closed in 1993, slags were deposited in the open environment on the premises (S14º27′44″, E28º25′51″).[13] Even though official operation had stopped, high concentrations of Pb, Zn, copper (Cu), cadmium (Cd), and arsenic (As) were detected in the soil around the mine in 2009.[14] Leaded gasoline has been eliminated from fuel distribution in Zambia since 2008, and the majority of electricity powering of Kabwe is provided by hydroelectric plants. There is no major heavy industry in Kabwe. Considering these points, the mining site is regarded as the predominant contaminant source in Kabwe. Samples from humans and animals (chicken, cattle, goats, rats and dogs) in Kabwe have revealed accumulation of high concentrations of metals in the blood and liver, kidneys, and muscles.[5,12,14–18]

The Pb contamination in Kabwe has been intensively analyzed, due to the high toxicity of Pb and the associated health risk to animals. Pb is a nonessential element and can cause various toxic effects, including neurodevelopmental and cardiovascular disorders, renal failure, and hypochromic anemia.[19] Recent guidelines require exposure to Pb to be minimized as much as possible.[20,21] To achieve this goal, it is necessary to understand the exposure pathways and factors that affect the amount of exposure.

Reptiles are the least-studied group in ecotoxicology[22], however, lizards are increasingly regarded as important in ecotoxicological field research. There are increasing reports on field research which feature lizards including some geckoes as bioindicator of pollutants.[23–25] These studies take advantage of the species' insect-based feeding habits in estimating the quantities of contaminants entering into the vertebrate food web from invertebrate level. In addition, some species of lizards are abundant throughout a region, while the areas of individual habitats area tend to be small. There are also increasing numbers of laboratory studies using lizards as model species or investigating contaminant kinetics in lizards.[26,27] In this study, the lizard *Trachylepis wahlbergii* (Wahlberg's striped skink; Scincidae) was selected as the target species in an investigation of the relationship between land use and Pb exposure. *T. wahlbergii* is a diurnal lizard with a snout–vent length (SVL) of around 10 cm. This species is common and widespread throughout southern and eastern Africa and is a habitat generalist. The lizards are terrestrial, arboreal or rock-living. They have also become habituated themselves to humans and settle in houses.[28] Therefore, this species can be used to monitor a wide range of geographic areas. In addition, its home range is thought to be less than 500 m^2,[29] which is relatively small compared to its body size,[30] so it can be used to compare the status of locations that are close to each other. This is important, because few other species have such small home ranges. The lizards eat a variety of insects, such as beetles, flies, and grasshoppers. They forage actively but also bask in strategic position so that they can dart forward to catch passing prey.[28] Although they may sometimes accidentally eat soil or small stones, their main source of contaminants via oral exposure is assumed to be the insects they eat. This feature is optimal for identifying exposure sources. Together, these characteristics make *T. wahlbergii* an ideal species for investigating differences in metal accumulation among individual animals living in different environments.

The objective of this study is to explore the environmental factors affecting the contamination status of living organisms by comparing metal (Pb, Zn, Cd, Cu, nickel [Ni]) and As concentrations in lizards inhabiting various locations and a range of environments.

MATERIALS and METHODS

1. Sampling

The primary ore mineral assemblage and metal production history in Kabwe are described in SI (Supporting Information) 1.

The sampling of lizards and soil was conducted in the vicinity of the Kabwe Pb-Zn mine from May to September 2017, which corresponded to the dry season in the region (SI2, Figure S1). The distance from the mine to the sampling sites ranged from 0.26 to 21.2 km (SI2, Figure S2). The sampling sites were accurately located using global positioning system (GPS) coordinates. The sampling took place under a permit from the Zambian Ministry of Fisheries and Livestock, as well as the Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan (Approval Number: Vet-17010). A total of 224 lizards were captured by hand or using adhesive traps, and their body weight and SVL were measured. Juveniles were assigned to the ‘unknown sex’ category, since it was difficult to sex them. The lizards were carried in ventilated plastic cases to a laboratory in the Central Province Veterinary Office of the Ministry of Fisheries and Livestock in Kabwe, and dissected after being euthanized with isoflurane (Isotroy, Troikaa Pharmaceuticals, Gujarat, India). First, blood was collected from the heart with a 27-gauge needle and syringe, which had been flushed with heparin (Mochida Pharmaceutical, Tokyo, Japan). Subsequently, the livers, lungs, and stomach contents were placed in sampling tubes and stored at −20 °C until metal analysis. A small portion of the heart was preserved in RNAlater (Sigma-Aldrich, St. Louis, US) for species identification. Kidneys were not collected because they were too small to differentiate. Surface soils (n = 29) were collected from each sampling site and stored at −20 °C until analysis. Biological samples and soils were transported to the Laboratory of Toxicology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan, under permits from both the Zambian and the Japanese governments, for the following analyses.

2. Species identification

Whole genomic DNA was extracted from the hearts using the Wizard Genomic DNA Purification Kit (Promega, Fitchburg, US). The 12S rRNA region was amplified via polymerase chain reaction (PCR) using Tks Gflex DNA Polymerase (Takara Bio, Kyoto, Japan). The primers and PCR conditions are shown in SI3, Table S1. After purification, PCR products were sequenced using the same primers. All nucleotide sequences were confirmed by the Fasmac sequencing service (Kanagawa, Japan). Sequences were aligned and the phylogenetic tree was constructed using MEGA7 software (Molecular Evolutionary Genetics Analysis version 6.0).[31,32] Information on the 12S rRNA genes used in the phylogenic analysis is shown in SI3, Table S2.

3. Metal and As analysis

Metal and As analysis was conducted based on an existing method[5] with minor modifications. After being dried at 50 °C for 48 h, the 29 soil samples were sieved to eliminate particles larger than diameter of 2 mm. The average dry weight used for analysis was 51.0 mg. With the lizard tissue samples, parts of organs or whole organs were analyzed. The average wet weight of samples was 82.7 mg for liver and 51.5 mg for lung. Whole volumes of blood and stomach contents were used in the analysis. The mean wet weight of blood was 68.0 mg, while that of stomach contents was 182.3 mg. Samples were dried in an oven at 50 °C for 48 h. For stomach content samples whose dry weight exceeded 100 mg, the dried samples were homogenized and a dry weight of approximately 50 mg was used. Dried samples were placed in prewashed digesting vessels with 5 ml of 30% HNO_3 (nitric acid for atomic absorption spectrometry, 60%, Kanto Chemical, Tokyo, Japan) in distilled deionized water (DDW) and 1 ml of 30% H_2O_2 (hydrogen peroxide for atomic absorption spectrometry, 30%, Kanto Chemical, Tokyo, Japan) and digested in a microwave digestion system (Speed Wave MWS-2, Berghof, Eningen, Germany). Parameters for digestion are shown in SI4, Table S3.

Analysis of Pb, Zn, Cd, Cu, Ni, and As content was performed using inductively coupled plasma–mass spectrometry (ICP-MS 7700 series, Agilent Technologies, Tokyo, Japan). Detailed operating conditions are shown in SI4, Table S4. The procedure for verifying analytical quality is described in SI5. All concentrations are expressed in micrograms per gram on a dry-weight basis (μg/g DW) in the following results.

4. Categorization of land use

Land use was categorized based on the analysis of satellite images taken in the rainy season (January to March, SI2, Figure S1) in QGIS 2.14.14 Essen (QGIS Development Team, 2016). Datasets of spectral reflectance were obtained from the Sentinel-2 (European Space Agency) database via the EO browser (https://apps.sentinel-hub.com/eo-browser/?lat=41.9000&lng=12.5000&zoom=10). Satellite images with cloud coverage of less than 10% were selected. Datasets containing band 3 (green), 4 (visible red), and 8A (near infrared) were imported to QGIS and merged to create new raster graphics, which indicate the status of vegetation. By comparing these with true-color images which are also created in QGIS from datasets containing band 2 (blue), 3 and 4, the coloration of the merged band images was linked to the actual land use pattern (Figure 1). Changes in surface land cover during the rainy season were compared to categorize land use into the following four groups: bare field (no vegetation cover throughout the rainy season) , green field (vegetation cover present throughout the rainy season), open field (vegetation cover present for part of the rainy season), and residential area (several buildings present).

5. Calculation of the normalized difference vegetation index (NDVI)

The normalized difference vegetation index (NDVI) indicates the amount of vegetation by measuring the activity of photosynthetic pigments. Calculation of the NDVI is based on aerial imagery of spectral reflectance in the visible red (665 nm) and the near infrared (865 nm) wavelengths, as shown in SI6.

6. Defining the angle toward the prevailing wind

Since the prevailing wind in Kabwe is from the east-southeast (ESE), the angle of the sampling sites from the mine with respect to the ESE wind direction was calculated to investigate the effect of wind, as shown in SI7.

7. Measurement of soil pH

Soil pH was measured according to an existing method,[33] as shown in SI8.

8. Statistical analysis

All statistical analyses were conducted using JMP Pro 14 software (SAS Institute, NC, US). Spearman's rank correlation test was used to determine the relationships between distance from the mine and the metal/As concentrations in the soil (SI10, Figure S8), and Pb concentrations among biological samples (SI11, Table S9). Multiple regression analysis was used to investigate the effects of various environmental factors on the Pb concentrations in lizards organ (Table 1). To examine the confounding effect of distance from the mine to the sampling site, we used the residuals between measured liver Pb concentration and the concentrations modelled based on the distance from the mine (Figure 3). Estimated Pb concentration was calculated with least-squares method between measured liver Pb concentration and distance from the mine (SI12, Figure S10). The Steel-Dwass test was used to evaluate the differences among the above-mentioned residuals (Figure 3), metal concentrations (SI11, Figure S9; SI12, Figure S11) and NDVI annual averages (SI12, Figure S12).

RESULTS and DISCUSSION

1. Species identification

A total of 224 lizards captured in Kabwe were identified as *Trachylepis wahlbergii* (Wahlberg's striped skink; Scincidae) based on coloration, distribution,[34] and phylogenetic analysis of their 12S rRNA (SI3, Figure S3). Sex distribution and SVLs are shown in SI9, Table S6. The median SVL of captured lizards was 8 cm and there was no significant difference in SVL between males and females.

2. Metal and As concentrations in the soil

The concentrations of the elements (Pb, Zn, Cd, Cu, Ni, and As) in the soil samples (median and range, μg/g DW) are shown in SI10, Table S7, alongside values for Zambian soils obtained from other studies, as well as the Ecological Soil Screening Levels (Eco-SSL) for birds and mammals suggested by the US Environmental Protection Agency (EPA).[14,35–40] The medians of the Pb, Zn, and Cd concentrations were higher than the US EPA Eco-SSL values for both birds and mammals. Although the median concentration of Cu was lower than the Eco-SSL for mammals, the maximum value exceeded the Eco-SSL. These patterns of accumulation in the soil were in agreement with previous research,[14,41] which means that soil contamination is still a cause for concern in Kabwe. In contrast, even the maximum concentrations of Ni and As were lower than the Eco-SSL values. As shown in SI10, Figure S8, concentrations of Pb, Zn, Cd, Cu, and As in the soil were negatively correlated with distance from the mine ($p < 0.0001$). Therefore, the mine can be identified as the primary metal contamination source in Kabwe. For Ni, however, the concentrations in the soil were low, and this was the only element that did not show significant correlation with the distance from the mine. Thus, of the six elements investigated, only Ni can be excluded as a potential metal contaminant originating from the Kabwe mine. There were no significant differences in the concentrations of any of the elements among the land use categories.

3. Metal and As concentration in lizards

The metal and As concentrations in the liver, lungs, blood, and stomach contents of lizards in the current study are shown in SI11, Table S8, along with concentrations reported in previous studies for other animals collected in Kabwe (µg/g DW).[12,14,16] Box-and-whisker plots are shown in SI11, Figure S9. Liver concentrations of Pb, Cd, and Cu showed the widest variability among the tissue samples. The liver samples that displayed the highest concentrations of Pb, Zn, Cd, and Cu were all taken from lizards living inside the mine. The wide range of Pb concentrations could be a consequence of the broad coverage and differences in the habitats of the analyzed lizards. Although further investigation of the biological and toxicological aspects of lizards is required, the current results highlight the potential for lizards to be indicators of pollution status in a range of environments.

Pb concentrations in the livers, lungs, blood, and stomach contents showed significant positive correlations with each other, as shown in SI11, Table S9 ($p < 0.0001$). The strongest correlations were between livers and blood and between lungs and blood (both $\rho = 0.91$, $p < 0.0001$). Therefore, it is reasonable to assume that the accumulation of Pb in livers and lungs reflects blood Pb levels. Due to the limited number of blood samples ($n = 102$), liver Pb levels were used as an indicator of systemic contamination in the following analyses.

4. Effects of environmental parameters on metal and As exposure

As shown in SI12, Figure S11, lizards living in Hamududu, which is 21.2 km away from the mine, had accumulated significantly lower concentrations of Pb than lizards living near the mine ($p < 0.01$). Therefore, Hamududu could be considered not polluted by the mine. In contrast, liver Pb concentrations of lizards captured inside the mine site were significantly higher than those of lizards captured at other sites ($p < 0.01$). Since extremely low or high contamination levels may conceal other underlying factors, the results from lizards from Hamududu and inside the mine were excluded from the following analyses.

Figure 2 demonstrates the distribution of sampling sites together with mean liver Pb concentrations and sample sizes. The height of each bar represents the mean concentration and its color indicates the land use category. It is clear that the mean Pb concentrations in lizards were higher in the areas closer to the mine. In addition, sites categorized as bare fields also tended to show high Pb concentrations.

In order to evaluate the effects of possible environmental factors (land use category, distance from the mine, wind direction, average annual NDVI, and soil Pb concentration and pH), multiple regression analysis was performed (Table 1). Land use category affected liver Pb concentration, especially bare fields and green fields, which were positively (t = 5.00) and negatively (t = -4.02) associated with Pb concentration, respectively. The absolute values of t for these two categories were largest among all the potential environmental factors. Therefore, it is assumed that lizards living in bare field accumulate more Pb than lizards living in other habitats, while those living in green fields accumulate less Pb. In addition, distance from the mine negatively affected liver Pb concentration, and soil Pb concentration was positively correlated with liver Pb concentrations (t =-3.20 and 2.63 respectively; $p < 0.01$). These results show that Pb accumulation is enhanced in surface soils adjacent to the mine, which results in a higher Pb concentration in the lizards inhabiting these areas.

The pH values of most of the soil samples were close to neutral (median 7.56), as shown in SI8, Figure S7, although one sample exhibited an extremely high pH (11.5). This might have been caused by fire, since high temperatures and ash promote alkalization of soil.[42,43] In fact, controlled burns are commonly used in Kabwe as a way of reducing the growth of wild plants. Since alkaline conditions suppress metal mobility,[44] it is reasonable that the high soil pH value would negatively affect liver Pb concentration as shown in Table1.

The frequency distribution of the angle of the sites between the mine and ESE, the prevailing wind direction is shown in SI7, Figure S6. The distribution indicated that 64% of all sampling sites were located downwind of the mine (angle from ESE > 90º). Although Figure 2 suggests that lizards on the west side of the mine showed higher concentrations of Pb than those living on the east side, there was no significant effect of wind direction (angle from ESE in Table 1).

Figure 2 also suggests that bare fields tend to be located near the mine. To further assess the effect of land use while controlling the effect of distance from the mine, the residuals between measured liver Pb concentrations and the concentrations predicted by a model based on distance from the mine were compared among land use categories (Figure 3). These residuals were positive for bare fields and negative for green fields. This suggests that the lizards living in bare fields accumulated more Pb than expected based on the location of their habitat, while those living in green fields accumulated less. Since the median values of the residuals of open fields and residential areas were almost zero and there were no significant differences between these two categories, the extent of accumulation in these areas was assumed to be intermediate between the extent observed for bare and green fields. This result suggests that vegetation status is an important factor affecting Pb accumulation in living organisms, supporting the result shown in Table 1. Previous reports have concluded that both distance and direction from the source of pollution are important factors affecting Pb accumulation in terms of dust scattering.[11,12] The present findings support these results, since vegetation can suppress both the production and the remobilization of dust.[45] In regions that have long dry seasons, like Kabwe, the role of dust both in spreading pollutants from the pollution source to surrounding areas and in allowing pollutants to travel through the air even when distant from the source cannot be dismissed.

The median NDVI value was 0.315 and the range was from 0.050 to 0.486 (SI6, Figure S4). To confirm the validity of the link between vegetation status and the land use categories defined in this study, the NDVI value was compared among different land use categories (SI12, Figure S12). The NDVI value increased in the following order; bare fields ≈ residential areas < open fields < green fields ($p < 0.05$). There was no significant difference between bare fields and residential areas. Since the average annual NDVI was consistent with the land use categories, we attempted to use it as a parameter to describe land use. However, despite the significant differences in the effects of different land use categories on liver Pb concentrations (Table 1 and Figure 3) **エラー！参照元が見つかりません。**, the effect of NDVI was not significant (Table 1). This striking difference between the effects of the land use categories used in this study and that of NDVI can be explained by the fact that the calculation of NDVI does not reflect the existence of houses. Thus, it is possible that not only the amount of vegetation but also human activities, such as the construction of buildings or frequent traffic on unpaved roads, affect the contamination status of the animals inhabiting those areas. In fact, the variation in the residuals between the measured and predicted liver Pb concentrations shown in Figure 3 was largest in the residential areas. This implies that there are other potential factors affecting the accumulation of Pb in this type of habitat. Therefore, in order to further investigate the effect of land use on contamination status, additional parameters that reflect these differences should be developed and used.

5. Pathway of exposure of lizards to Pb

Among the four biological sample types, concentrations of most elements (except for Cu) were highest in the stomach contents (SI11, Table S8 and Figure S9). Stomach content concentrations can be considered to reflect the quantity of ingested contaminants. In order to compare the present results with *in vivo* studies in which the dosage is expressed as µg/g body weight (BW), our Pb concentrations in the stomach content samples were converted to µg/g BW. The results are shown in SI13, Figure S13. Generally, the stomach contents contained < 10 µg/g BW Pb. The highest Pb concentration (1478 µg/g DW) was equivalent to 32.9 µg/g BW. Since the Pb concentration of the stomach contents was positively correlated with that of the livers

(SI11, Table S9), ingestion may be an important Pb exposure pathway. Salice et al. performed toxicity tests of inorganic Pb administered via oral exposure in *Sceloporus occidentalis* (Western fence lizard).[46] They reported that the approximate lethal dose was > 2000 µg/g BW, with sub-acute effects, such as weight loss, seen in the 62.5 µg/g BW/day group, and sub-chronic effects, such as decreased hematocrit, in the 10 µg/g BW/day group. Holem et al. also conducted acute toxicity tests on *S. occidentalis*.[47] After a single administration of 1000 µg/g BW Pb via gavage, 30% of the lizards died within 24 hours and 50% of the surviving lizards exhibited a significant increase in skin pigmentation (they became darker). Dark coloration was also seen in the acute exposure test conducted by Salice et al.,[46] although its biological meaning remains unknown. In the current study, only two individuals had > 10 µg/g BW Pb in their stomach contents (Figure S13). In fact, a small fragment with a stone-like structure was found in the stomach of the lizard that had the highest Pb concentration in its stomach contents (32.9 µg/g BW). That fragment was removed prior to the analysis, but the Pb concentration was nevertheless high. The Pb concentrations in the tissues of this lizard were not especially high compared to other lizards captured at the same site. It should be noted that while the Pb concentration of stomach contents can be considered to represent oral exposure levels, they capture only a limited timeframe. Although these Pb concentrations overall tended to be much lower than the reported toxicity levels, they should not be disregarded, particularly because there has been limited research on the consequences of chronic exposure to such levels of Pb for lizards.

In the case of oral exposure, Pb is absorbed from the digestive tract, enters systematic circulation, and is subsequently delivered to every organ, including the lungs. Generally, Pb accumulation in the lungs via blood circulation is subtle. Winiarska-Mieczan and Kwiecień have reported the distribution of Pb among the organs of rats after sub-acute oral exposure.[48,49] In their studies, the concentration of Pb in the lungs was only 4% of that in the livers. However, in the present results, the median ratio of lung Pb concentration to liver Pb concentration was 0.6 (Figure 4). Remarkably, 19% of the lizards had accumulated higher concentrations of Pb in their lungs than in their livers (*i.e.* ratios of lung Pb concentration to liver Pb concentration > 1). Similar patterns have been reported for goats and chickens in Kabwe (SI11, Table S8).[12] If exposure via ingestion were the only pathway of Pb exposure in Kabwe, the concentration of Pb in the lungs would be much lower than that in the liver. Therefore, it is suspected that not only distribution via blood circulation but also direct exposure to Pb via dust inhalation is responsible for the high accumulation of Pb in the lungs of lizards in Kabwe. In fact, a prolonged (one year) study on the exposure of rats to Pb-contaminated soil has shown that Pb can accumulate in tissues without exposure via food sources.[50] In that study, the Pb accumulation ratio of lungs to liver was also found to be high (0.432). Studies in the field of occupational exposure show that the absorption rate of Pb from the lungs is 40–50% after the Pb reaches the alveolar region,[51] while that from the digestive tract is only 10%.[52] Considering the high absorption rate from the lungs, Pb exposure via dust inhalation should be considered an important pathway, in addition to digestive exposure.

In rapidly developing countries, land-use patterns are altered and diversified through urbanization. Topsoil is often exposed to wind at construction sites or along temporary roads. Our results suggest that these bare lands produce dust that is contaminated with metals from pollution sources, thus increasing Pb exposure via inhalation. Apart from dust generation, several studies have reported that changes in land use can affect soil properties, altering the leaching rate and bioavailability of pollutants.[53,54] In these studies, both vegetation cover and the type of vegetation, which was not considered in this study, affected the transfer of trace elements from the environment to organisms. Overall, the influence of land use and vegetation patterns on exposure to contaminant warrants further research. Moreover, to take effective measures to mitigate the impact of contaminated land on organisms in the course of city development, exposure pathways should be well understood and taken into account from the planning phase onwards. Our results contribute to both of these needs by demonstrating the significance of land use categorization in a contaminant study and indicating the implications of respiratory exposure to metal contaminants.

FIGURES and TABLES

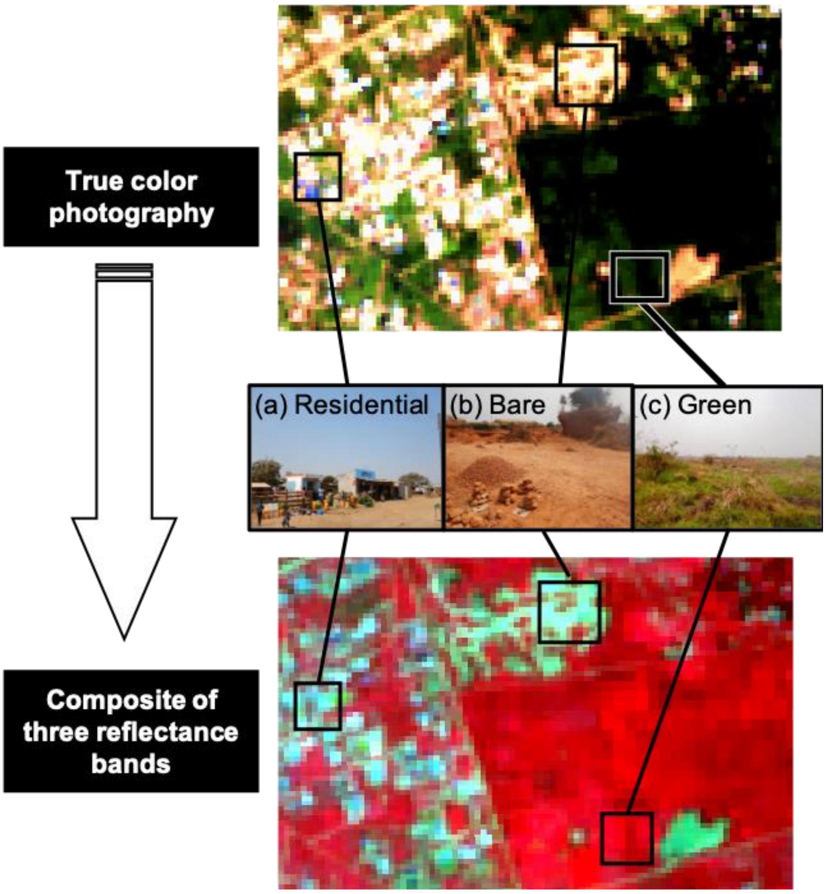


Figure 1. Process of land use categorization.

Composite images were created from three reflectance band images that had been separately colored and categorized. Photographs are representative of (a) residential areas, (b) bare fields, and (c) green fields. Containing modified Copernicus Sentinel data [2017].

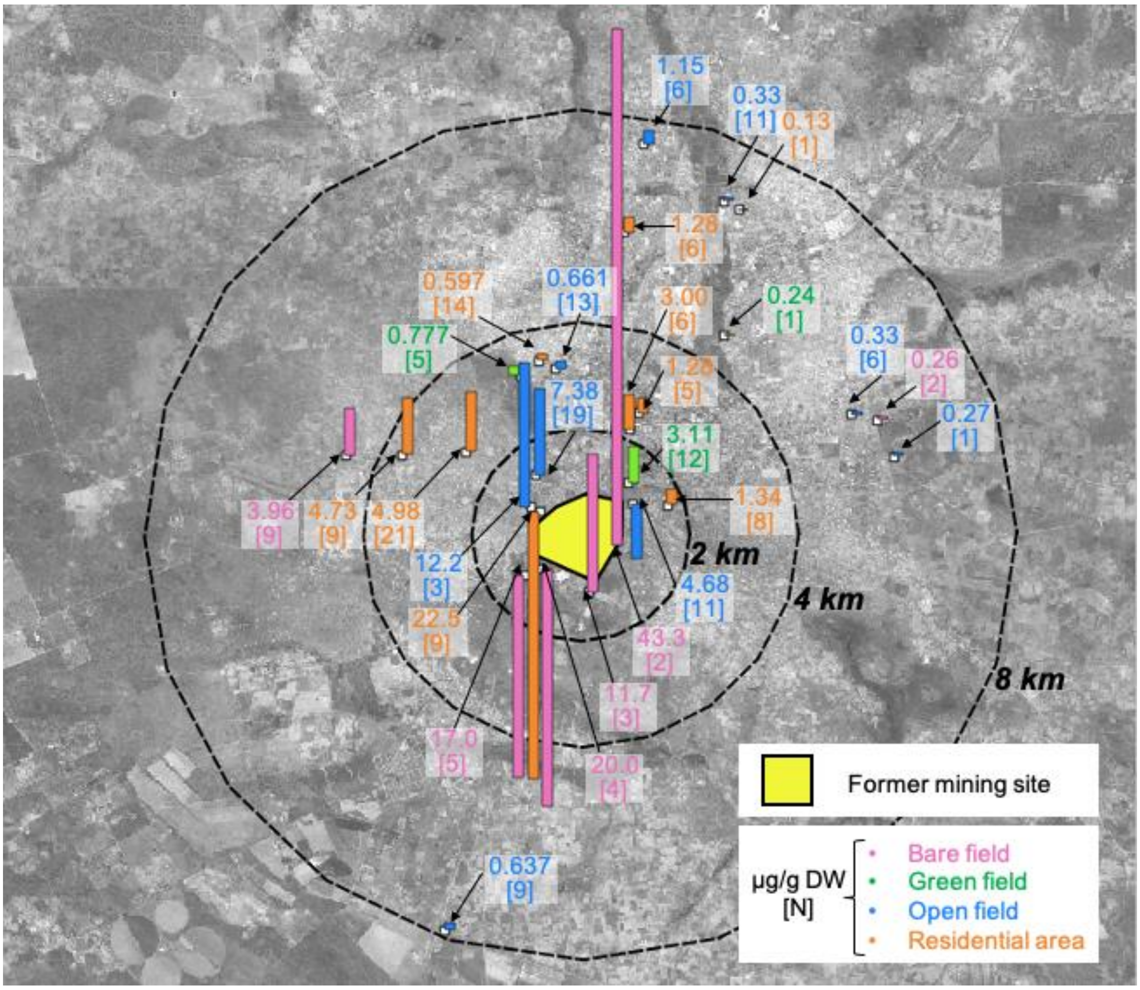


Figure 2. Mean liver Pb concentration (µg/g DW) with sample sizes indicated in brackets. Bars and texts colored according to land use categories (pink; bare field, green; green field, blue; open field, orange; residential area); bar length is proportional to Pb concentration. Containing modified Copernicus Sentinel data [2020] processed by Sentinel Hub.

Table 1. Association between liver Pb concentration and environmental parameters. Standardized partial regression coefficient (*β*) and *t*-values (*β* divided by standard error of each parameter) were obtained from multiple regression analysis (Least squares method).

	β	*t*	*p*
Distance from the mine	-0.382	-3.20	0.0018
Bare field	0.450	5.00	<0.0001
Green field	-0.427	-4.02	0.0001
Open field	-0.028	-0.29	0.7735
Residential area	-0.002	0.09	0.9317
Soil Pb	0.287	2.63	0.0098
Soil pH	-0.165	-2.06	0.0414
Angle from ESE	0.020	0.24	0.8118
Average annual NDVI	0.019	0.21	0.8337

ESE, east-southeast; NDVI, normalized difference vegetation index

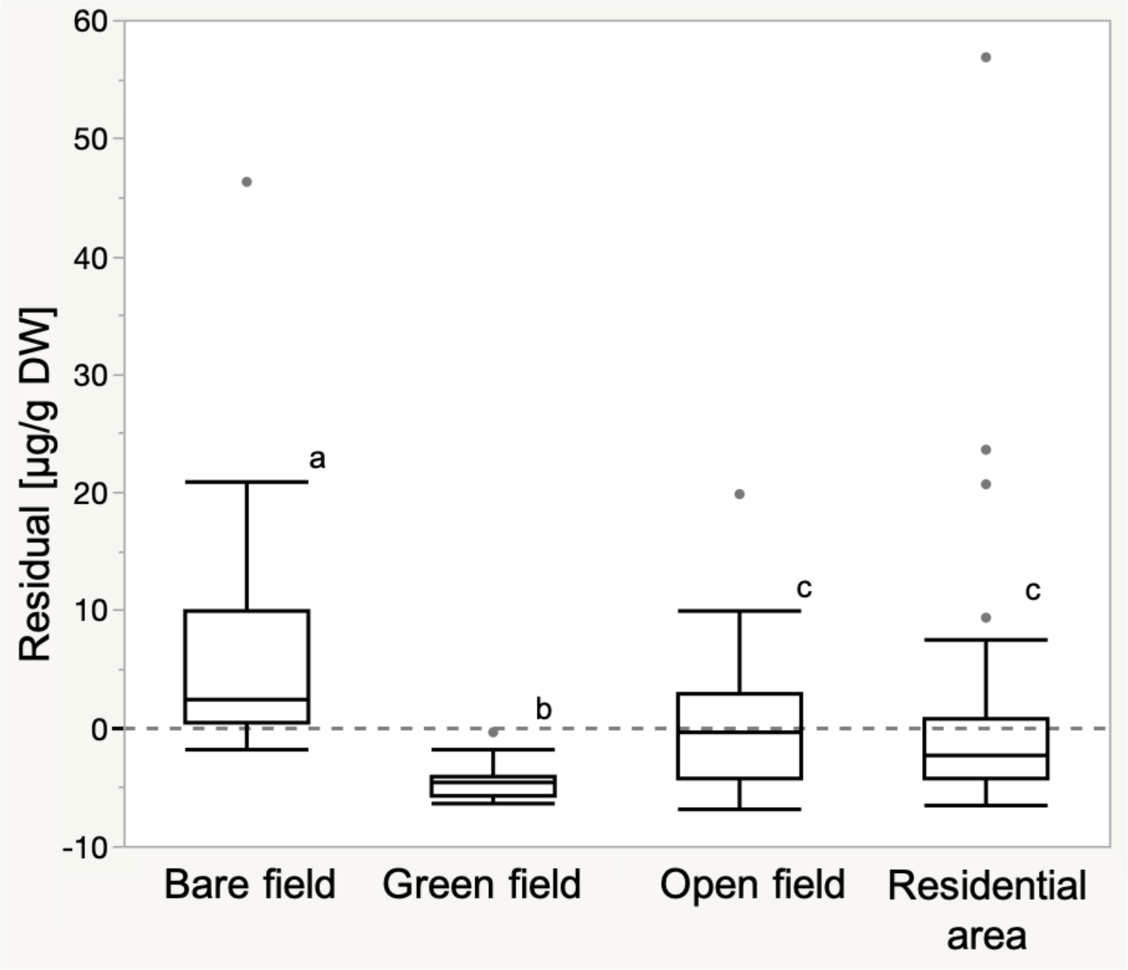


Figure 3. Box-and-whisker plots of the residuals obtained by subtracting the liver Pb concentration predicted based on distance from the mine from the measured values.

The upper and lower boundaries of the box and the line inside each box indicate the upper and lower quartiles and the median, respectively. The whiskers extend to the maximum and minimum values observed, excluding outliers. Significant differences between groups are indicated by different letters (Steel–Dwass test, $p < 0.01$).

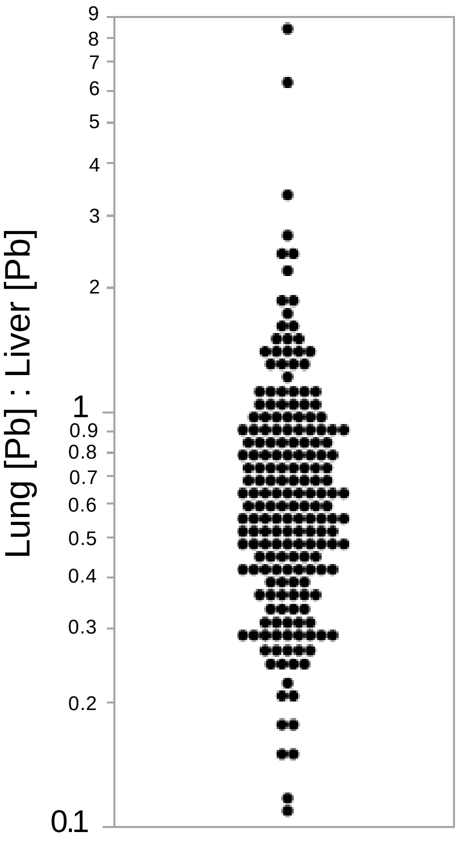


Figure 4. Distribution of the ratios of lung Pb concentration to liver Pb concentration (N = 207)

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SUPPORTING INFORMATION

Detailed description of the sampling period and site locations; analytical methods; analytical results for each trace element, and the determination methods and distributions of environmental parameters.

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