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Metal and metalloid contamination in roadside soil and wild rats around a Pb-Zn mine

in Kabwe, Zambia

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Abstract

Metal (Cr, Co, Cu, Zn, Cd, Pb, Ni) and metalloid (As) accumulation was studied in

roadside soil and wild rat (Rattus sp.) samples from near a Pb-Zn mine (Kabwe,

Zambia) and the capital city of Zambia (Lusaka). The concentrations of the seven

metals and As in the soil samples and Pb in the rat tissue samples were quantified using

atomic absorption spectroscopy. The concentrations of Pb, Zn, Cu, Cd, and As in Kabwe

soil were much higher than benchmark values. Geographic Information System analysis

indicated the source of metal pollution was mining and smelting activity. Interestingly,

the area south of the mine was more highly contaminated even though the prevailing

wind flow was westward. Wild rats from Kabwe had much higher tissue concentrations

of Pb than those from Lusaka. Their body weight and renal Pb levels were negatively

correlated, which suggests that mining activity might affect terrestrial animals in

Kabwe.

Capsule:

The area around Kabwe, Zambia is highly polluted with metals and As. Wild rats from

this area had high tissue concentrations of Pb and decreased body weight.

Keywords: Metal; Metalloid; Soil pollution; GIS; wild rat

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1. Introduction

The African continent has experienced rapid economic development during the last decade. Unfortunately, this has also led to an increase in environmental pollution (Akiwumi and Butler, 2008). Among the chemical pollutants, metal contaminants are now a major health hazard in many African countries (Nweke and Sanders, 2009). Humans and wildlife are exposed to metals in drinking water, air and soil through contamination from anthropogenic activities such as mining and metal smelting. In many instances, mining operations have no containment measures (Blacksmith Institute, 2007). Generally, unless countermeasures are taken, metal pollution is long lasting because the metals do not biodegrade (Li *et al.*, 2004). Exposure to toxic elements such as lead (Pb), cadmium (Cd), and arsenic (As) can cause serious health problems in humans and animals (Chowdhury *et al.*, 2000; Järup, 2003; Lockitch, 1993). Therefore, it is important to assess the level of environmental metal contamination.

The Republic of Zambia holds vast mineral resources, including copper (Cu), cobalt (Co), zinc (Zn), and Pb, and has seen significant growth in the mining industry. In 1997, Zambia contributed 3 % and 20 % of the global annual production of Cu and Co, respectively (Stockwell *et al.*, 2001). Furthermore, the majority of these metals

were smelted within the country (Stockwell *et al.*, 2001). Kabwe mine was operational for over 90 years until June 1994, and was Zambia's main producer of Pb and Zn (Copperbelt Environment Project, 2006). Kabwe mine produced 1.8 million tonnes (t) of Zn, 0.8 million t of Pb, and relatively small amounts of the by-products Cd (235 t), Ag (79 t), Cu (64 t), and fused vanadium (V) oxide (7820 t) (Kamona, 1993; Kamona and Friedrich, 2007). Consequently, metal pollution has become a serious environmental problem in Zambia, with health implications for humans and animals (Blacksmith Institute, 2007; Water Management Consultants, ZCCM Investments Holdings PLC, and Copperbelt Environment Project, 2006; Nwankwo and Elinder, 1979).

An earlier comprehensive study on Pb pollution in Kabwe found soil over a substantial area was contaminated with high levels of toxic metals. Townships such as Kasanda, Makandanyama, Chowa, Mutwe Wansofu, Makululu and Luangwa in close proximity to the mine were particularly affected (Water Management Consultants, ZCCM Investments Holdings PLC, and Copperbelt Environment Project, 2006). The authors noted that there could be 650 cases in the surrounding communities with blood Pb levels >65 µg dL⁻¹, which could induce sub-acute toxicological effects (Water Management Consultants, ZCCM Investments Holdings PLC, and Copperbelt

Environment Project, 2006). Given these facts, the city of Kabwe ranks among the ten most polluted places in the world (Blacksmith Institute, 2007). Despite such alarming reports of metal pollution in Kabwe, investigations of the distribution of metals in the area are scarce (Tembo *et al.*, 2006; Nwankwo and Elinder, 1979). Furthermore, the relationship between metal contamination in soil and that in terrestrial wildlife in Kabwe has not been assessed.

In the present study, we investigated roadside soils because they accumulate metals and other environmental pollutants (Ikenaka *et al.*, 2005; Li *et al.*, 2004). Geographic Information System (GIS) analysis has previously been used as a powerful method for displaying pollution patterns (Li *et al.*, 2004). We used GIS to determine the source of pollution and predict distributions of the metals in soil samples. Furthermore, to investigate the effect of Pb pollution on terrestrial wildlife, we captured wild rats and determined Pb levels in their tissues. Wild rats are ideal as bio-indicators of Pb pollution for several reasons (Ceruti *et al.*, 2002; Pereira *et al.*, 2006): (1) they are widely distributed all over the world; (2) their habitats are close to those of humans and they accumulate metals by ingestion of contaminated food or soils and grooming; (3) they are a terrestrial species and a component of the food chain; and (4) there is a lot of experimental data available from laboratory rats for comparison.

Our objective was to identify the relationship between Pb accumulation in wild rats and the soil contamination in Kabwe. This could be used as a typical model for studies focused on assessing environmental exposures to metals by mining activity.

2. Materials and Methods

2.1. Study area and sampling

Kabwe is situated at approximately 14°27'S and 28°26'E. Roadside soils and wild rats were collected from this area in May 2009. The sampling locations were accurately located (Fig. 1) using a global positioning system (GPS). Soil samples were collected in Kabwe (n=101), and reference soil samples in the Mwangule area (n=3), which is located 80 km north of the mine, and the capital city of Zambia, Lusaka (n=7) (not shown in Fig. 1). Samples were previously collected from similar locations by Nwankwo and Elinder (1979). Approximately 50 g of soil from a depth of 0–5 cm was collected for each sample and kept in a plastic bottle. At least three composite soil samples were collected from each sampling point. Each sample was passed through a 2 mm sieve before extraction. The dry weight of each sample was measured after 12 h in an oven at 105 °C. To determine the soil organic matter (SOM) content, the ignition loss of each sample was measured after 5 h in an oven at 600 °C.

Wild rats (*Rattus sp.*) were captured using live traps baited with food in Kabwe (n=32, Fig. 1) and at the University of Zambia, Lusaka (n=20, as control). The

university is very wide and expected as non-contaminated area because of little traffic within university and far from industrial, agricultural and mining areas. Rats were collected from a number of places, including roadsides, dairy pasture, residential gardens, and the university. The university is very large and should be a non-contaminated area because there is little traffic on campus and it is located far from any industrial, agricultural or mining areas. Mitochondrial DNA sequencing suggested the rat species were Rattus rattus or Rattus tanezumi (Robins et al., 2007), and these species are closely related. Consequently, we treated our rat samples as coming from the same group. Body weight and sex were determined for each rat. Liver, kidney, and eye tissues were collected for metal analysis and age estimation. The liver and kidney samples were stored separately at -20 °C for metal analysis or water content measurement. For measurement of water content, the tissue samples were dried in an oven at 105 °C for 24 h.

Ages of the wild rats were determined from the weight of the eye lens (Okamoto, 1980). Both eyes were removed and kept in 10 % neutral buffered formaldehyde solution after sacrifice. These eyes were fixed in formalin at room temperature for 4 weeks. After fixation, the tissue around the lens was removed, and the lenses were washed with distilled water. The lenses were then dried at 60 °C for 3 days in an oven,

and weighed to estimate the age using the formula from a previous report (Okamoto, 1980). Regression lines of the mean lens weight (y) to the age (x, log scale) were obtained as follows: y = 17.68 x + 8.39 (male), and y = 17.18 x + 8.36 (female).

2.2. Extraction and measurement of metals

Metals were extracted from the soil samples using a method modified from our previous study (Nakayama *et al.*, 2010). Briefly, 1 g of each soil sample was placed in a 200 mL flask. Nitric acid (15 mL, atomic absorption spectrometry grade, 60 %, Kanto Chemical Corp., Tokyo, Japan) was then added. The mixture was heated at 180 °C for 5 h on a hotplate. After the mixture cooled, 1 g of ammonium chloride (Wako Pure Chemical Industries Ltd., Osaka, Japan) was added. The samples were reheated at 180 °C for 1 h and evaporated to approximately 5 mL. After the samples cooled, they were filtered into plastic bottles through ash-less filter paper 5B (Advantec, Tokyo, Japan). Lanthanum chloride (1 mL, atomic absorption spectrometry grade, 100 g/L, Wako) was then added. The sample volume was standardized to 100 mL using 2 % HNO₃. A reagent blank was produced following the same process.

Metals were extracted from the liver and kidney of the wild rats by acid digestion

using a method modified from Seymore *et al.* (1996). Briefly, 1 g of fresh tissue was placed in a 200 mL flask. Nitric acid (20 mL) and perchloric acid (5 mL) were added. The samples were gradually heated to 225 °C on a hotplate, and left for 12 h to evaporate to approximately 5 mL. When the samples became clear liquids, 0.2 mL of lanthanum chloride (100 g /L) was added. The volume was then made up to 20 mL with 2 % HNO₃. A reagent blank was produced using the same procedure.

We measured the concentrations of eight elements (Cr, Co, Cu, Zn, Cd, Pb, Ni and As) in the soil samples using an atomic absorption spectrophotometer (AAS, Z-2010, Hitachi High-Technologies Corporation, Tokyo, Japan) with an acetylene flame or argon non-flame, after preparation of the calibration standard. Because quite high concentrations of Pb were observed in the soil samples and Pb is one of the most toxic metals, we focused on the effect of Pb on wild rats. The overall recovery rates (mean S.D.) of Cr, Co, Cu, Zn, Cd, Pb, Ni and As were 91±3.0, 92±3.4, 89±5.6, 91±2.3, 111±8.3, 90±3.5, 92±4.2 and 62±3.7 %, respectively. It should be noted that the recovery rate of As was lower than the other seven elements. The detection limits (μg kg⁻¹) of Cr, Co, Cu, Zn, Cd, Pb, Ni and As were 0.5, 0.5, 1.0, 0.1, 0.2, 1.0, 0.5 and 2.0, respectively. Each metal concentration was converted from mg kg⁻¹ wet-weight (wt) to mg kg⁻¹ dry-wt using the water content of each tissue. For soil samples, we compared

our results to the benchmark values from the US Environmental Protection Agency (EPA) (2003, 2004) and world ranges in non-polluted soils (Kabata-Pendias and Pendias, 1992).

2.3. Statistical analysis

Statistical analyses were performed using JMP 7.0.1 (SAS Institute, Cary, NC, USA). Data were normalized by a base 10 logarithm transformation. We analyzed for differences among the groups using F-test and a Mann-Whitney U test. Correlation coefficients were analyzed between each metal and SOM. The Spearman's Rank correlation was used to analyze the relationship between metal levels in the soil and distance from the mine. Correlation coefficients between tissue Pb levels and age and weight of wild rats were also analyzed (Ma 1989). Mapping of each metal concentration was performed using ArcGIS 9.3 (ESRI, New York, USA). Kriging was adopted for the interpolation of geographical data.

3. Results

3.1. Metal concentrations in soil samples

Concentrations of eight elements were measured in soil samples from Kabwe and reference sites (Table 1, Figs. 2–4). Concentrations of Pb, Zn, Cu, Cd, and As in Kabwe soils were quite high. These values exceeded both the benchmark values from the US EPA (2003, 2004) and previously published results for global ranges in non-polluted soils (Kabata-Pendias and Pendias, 1992). In contrast, concentrations of Cr, Co, and Ni were below the US EPA benchmark values(2003, 2004). Soils from the reference sites (Mwangule and Lusaka) contained lower amounts of all the elements compared with the samples from Kabwe (Table 1).

3.2. Correlation coefficient (R^2) between each metal and SOM

To determine the pollution sources and their relationship to organic matter, we analyzed the correlation coefficient (R²) between each metal and SOM (Table 2). We found strong positive correlations among Pb, Zn, Cd, and As. We also observed positive

correlations between Cr-Ni, and Ni-Co. There was no correlation between metal concentration and SOM.

3.3. Spearman's rank correlations (ρ) between each metal concentration and distance from the mine

We determined the relationship between the concentration of each metal and distance from the mine (Table 3). For Pb, Zn, Cu, Cd, and As, metal concentration and distance were negatively correlated in all directions from the mine. In contrast, positive correlations were observed for Cr, Ni, and Co to the west.

3.4. GIS analysis to determine source and distribution of each metal

We analyzed the distribution of each metal using GIS analysis (Fig. 5). Higher concentrations of Pb, Zn, Cu, Cd, and As were observed around the mine, and these concentrations decreased with distance from the mine. For these five elements, soils from south of the mine contained higher concentrations than those from other directions. Cr, Ni, and Co had different distribution patterns to the other five elements.

3.5. Body weight, age and Pb concentration in liver and kidney of wild rats

Among the metals and metalloid we tested for, quite high concentrations of Pb were observed in the soil samples. In addition, Pb is one of the most toxic metals. Consequently, we focused on the effect of Pb on the wild rats. We determined the concentration of Pb in liver and kidney of wild rats (Table 4). There was no significant difference between the rats from Kabwe and Lusaka with regard to their sex. The body weight and age of Kabwe rats were significantly lower than those from Lusaka. Tissues from Kabwe rats had significantly higher concentrations of Pb than those from Lusaka (p<0.0001). Pb concentrations were compared between liver and kidney within Kabwe (p<0.0001) and Lusaka (p<0.0001), and results showed kidney accumulated significantly higher levels of Pb than the liver in both areas.

3.6. Correlation coefficients (R) among body weight, age and tissue Pb values

To determine the effect of Pb on wild rats, correlation coefficients (R) among body weight, age and tissue Pb values were analyzed (Fig. 6). Body weight and age were positively correlated. We also found a positive correlation between the log Pb

values of liver and kidney. Interestingly, significantly negative correlation was observed between body weight and log renal Pb. However, we found no correlation between age and log renal Pb.

4. Discussion

4.1. Source and distribution of metals in soil

Our results clearly demonstrate that the source of metal and metalloid pollution in Kabwe is the abandoned Pb-Zn mine (Tables 1–3, Fig. 2–5). Similar trends for Pb and Zn were reported by Tembo *et al.* (2006). However, mining might not be the source of Cr, Ni, and Co, because of the different distribution patterns, lower metal concentrations, and positive correlations of metal concentration with distance from the mine (Tables 1 and 3, Fig. 4 and 5) were observed for these compounds.

Interestingly, the area south of the mine had higher concentrations of metals compared with other directions (Fig. 5). Historically, the Kabwe main canal extended southeast from the mine and was used for discharging water from underground mining activities (Water Management Consultants, ZCCM Investments Holdings PLC, and Copperbelt Environment Project, 2006). Sediment from this canal was dredged and deposited along the banks. Consequently, we hypothesize that humans and animals living in the area south of the mine are more at risk from metal pollution than those living in other areas. In contrast to our results, Tembo *et al.* (2006) noted that soils from the area west of the mine contained higher amounts of metals, mainly due to wind flow

patterns in Kabwe. Therefore, there is also a possibility of increased risk in the area west of the mine.

4.2. Cd, As and Cu pollution in soil

Previously, Tembo et al. (2006) noted that Kabwe soils had high concentrations of Pb and Zn. In this study, we found that in addition to Pb and Zn, the residue levels of Cd, As, and Cu were also quite high (Table 1). We also found positive correlations among Pb, Zn, Cd and As (Table 2). Cd and As are frequently found with Pb (Tembo et al., 2006; Ratnaike, 2003). Moreover, Kamona and Friedrich (2007) reported that ore in Kabwe contained sphalerite (ZnS) with Cd, galena (PbS), briarite [Cu₂(Fe,Zn)GeS₄], and mimetite [Pb₅(AsO₄)₃Cl]. Consequently, we believe that the source of these metals in the Kabwe area is Pb-Zn mining and smelting activity. Renal dysfunction and bone loss have been reported in humans with chronic Cd toxicity (Järup, 2003). Due to this severe toxicity, the Agency for Toxic Substances and Disease Registry (ATSDR) has ranked Cd among the top seven of the 275 most hazardous substances in the environment (ATSDR, 2008). As is known to cause severe skin cancer and hyperkeratosis (Chowdhury et al., 2000). The reported benchmark values for Cd, As and Cu in soil are 1.6, 18, and 70 mg kg⁻¹ (US EPA, 2003, 2004), respectively. Therefore,

the pollution with these metals and metalloid in Kabwe should not be ignored.

4.3. Pb pollution in soil and wild rat samples

We observed that Kabwe soil contained quite high concentrations of Pb (Table 1, Fig. 2), which is similar to previously reported results (Water Management Consultants, ZCCM Investments Holdings PLC, and Copperbelt Environment Project, 2006). In the previous study, it was noted that soils from townships close to the mine were highly contaminated with Pb. Median concentrations of Pb (mg kg⁻¹) in soils from the townships of Kasanda (3,008), Makandanyama (1,613), Chowa (1,233), Mutwe Wansofu (1,148), Makululu (870), and Luangwa (507) exceeded the US EPA benchmark value (120 mg kg⁻¹) (2003, 2004).

In addition to the differences observed in Pb levels in soil, both liver and kidney from rats in Kabwe had significantly higher Pb concentrations than those from Lusaka (Table 4). This result indicates that soil pollution causes metal accumulation in wild rats. In this study, a significantly higher concentration of Pb was found in kidney than in liver (Table 4), and Pb concentrations of liver and kidney were positively correlated (Fig. 6). Furthermore, a significant negative correlation between body weight and renal Pb was found (Fig. 6). These results suggest that wild rats from Kabwe have been chronically

exposed to Pb, and that Pb might affect growth of these rats. A reduction in body weight is a known typical toxic effect of Pb in rats, and similar results have been reported in small wild mammals (Ma, 1989). However, because the body weight of wild animals is affected by many environmental factors, it remains unclear whether Pb stunted the growth of these rats. An additional explanation for the negative correlation is that there is a possibility of transfer of Pb to other tissues, such as femur.

Ma (1989) reported that a kidney Pb level of >25 mg kg⁻¹ dry-wt was diagnostic of Pb intoxication in small wild mammals. In histopathological studies, changes in the kidney, such as Pb intranuclear inclusion bodies and karyocytomegaly in proximal tubular cells, are detected at renal Pb concentrations >2.5 mg kg⁻¹ dry-wt (Ceruti *et al.*, 2002). In our study, several of the Kabwe rats had Pb levels higher than the histopathological threshold (2.5 mg kg⁻¹ dry-wt), while none exceeded the diagnostic level (25 mg kg⁻¹ dry-wt). This may be due to fact that most rats were collected in Northern Kabwe, while our soil contamination results indicated metal concentrations were highest to the south. To determine whether these metals affect rat health, we need to analyze the biological responses of these rats using biomarkers such as metallothionein, heme oxygenase, δ-aminolevulinic acid dehydratase (Buekers, 2009).

It has been reported that children are more susceptible to Pb toxicity because

intestinal absorption is five times greater in children than in adults (Lockitch, 1993). Pb toxicity can cause hematological, gastrointestinal, and neurological diseases, and nephropathy, abortion, stillbirth, and neonatal death (Lockitch, 1993). Consequently our result indicates that children who play in the soil around Kabwe, and mine-workers who scavenge for scrap metal, could potentially suffer Pb poisoning and adverse health effects. Furthermore, geophagy (consumption of soil or earth) and pica are also risk factors for metal poisoning, especially for pregnant woman in Kabwe (Water Management Consultants, ZCCM Investments Holdings PLC, and Copperbelt Environment Project, 2006).

5. Conclusions

High concentrations of Pb, Zn, Cu, Cd and As were found in soil samples taken near Kabwe, Zambia. The source of this metal pollution was historical mining activity at the now abandoned Kabwe Pb-Zn mine. The area south of the mine had particularly high contamination levels when compared with other areas around the mine. Wild rats from Kabwe had significantly higher tissue concentrations of Pb than those from Lusaka. Interestingly, body weight and renal Pb level were negatively correlated. These

results suggest that pollution due to historical mining might affect terrestrial animals in Kabwe. Further investigation of metal pollution in animals and humans in Kabwe is required.

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Figure Captions

Fig. 1. Maps of Africa and Zambia (upper panel). Sampling locations of roadside soils (yellow symbols) and wild rats (red symbols) in Kabwe. The satellite image was modified from Google Earth.

Fig. 2. Vertical and box plot of Cu, Zn and Pb concentrations (mg kg⁻¹ dry-wt) in Kabwe soil.

Fig. 3. Vertical and box plot of Cd and As concentrations (mg kg⁻¹ dry-wt) in Kabwe soil.

Fig. 4. Vertical and box plot of Cr, Ni and Co concentrations (mg kg⁻¹ dry-wt) in Kabwe soil.

Fig. 5. Geographical maps of the concentrations of eight elements in Kabwe soil.

Fig. 6. Correlation coefficients (R) among body weight (g), age (month), and tissue Pb concentrations (mg kg⁻¹ dry-wt) for wild rats.

Table 1: Metal and metalloid concentrations (range and median, mg kg⁻¹ dry-wt) and soil organic matter (SOM, %) in soils from Kabwe, and reference sites at Lusaka and Mwangule

Element or SOM ^(a)	Kabwe (n=101)	Lusaka (n=7)	REF (b) (n=3)	Recommend (c)	World range in non- polluted soils ^(d)
Cr	3-110 (42)	27-52 (39)	1-5	240	5-120
Co	2-93 (13)	3-31 (9)	1-2	-	0.1-20
Cu	2-5727 (37)	19-1873 (31)	3-4	70	6-60
Zn	5-91595 (607)	17-777 (48)	4-115	50-120	17-125
Cd	0.01-139(1)	0.03-0.33 (0.08)	0.01-0.2	1.6	0.07-1.1
Pb	9-51188 (282)	8-134 (26)	7-39	120	10-70
Ni	2-74 (12)	7-31 (21)	2-3	30-200	1-200
As	0.04-141 (7)	1-5 (4)	0.1-0.7	18	1-15
SOM	0.4-20(3)	1.2-8.5	0.1-2.1	-	-

⁽a) soil organic matter, (b) Mwangule region as reference, (c) Soil cleanup criteria and ECO-Soil Screening Levels (US EPA, 2003, 2004) (d) Kabata-Pendias and Pendias (1992)

Table 2: Correlation coefficients (R^2) between each metal concentration and soil organic matter (SOM) in Kabwe soil

	Cr	Co	Cu	Zn	Cd	Pb	Ni	As	SOM (a)
Cr	1.00								
Co	0.23	1.00							
Cu	0.00	0.05	1.00						
Zn	0.00	0.12	0.27	1.00					
Cd	0.00	0.05	0.23	0.78	1.00				
Pb	0.00	0.08	0.27	0.93	0.91	1.00			
Ni	0.33	0.52	0.12	0.09	0.06	0.08	1.00		
As	0.02	0.16	0.33	0.78	0.74	0.77	0.19	1.00	
SOM	0.00	0.02	0.02	0.09	0.06	0.07	0.06	0.10	1.00

⁽a) soil oraganic matter, bold and italic indicate p < 0.0001 and p < 0.05, respectively.

Table 3: Spearman's rank correlations (ρ) between each metal concentration and distance from the mine

Element	East (n=33)	West (n=14)	North (n=45)	South (n=9)	Total (n=101)
Cr	-0.56	0.60	-0.40	-0.78	-0.42
Co	-0.72	0.51	-0.69	-0.75	-0.60
Cu	-0.89	-0.66	-0.72	-0.68	-0.75
Zn	-0.91	-0.59	-0.72	-0.36	-0.72
Cd	-0.92	-0.74	-0.72	-0.78	-0.77
Pb	-0.91	-0.50	-0.75	-0.80	-0.79
Ni	-0.89	0.35	-0.64	-0.76	-0.68
As	-0.81	-0.32	-0.64	-0.78	-0.66

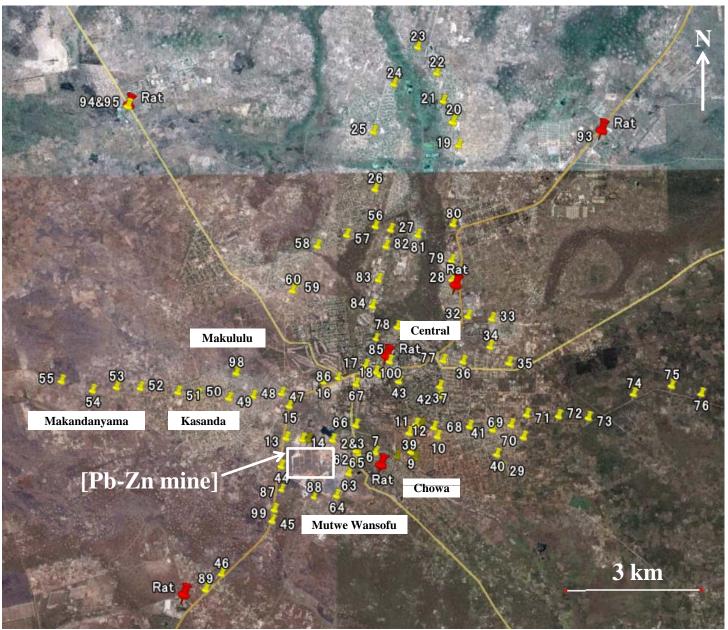
Bold indicates p < 0.05.

Table 4: Body weight (g), age (months) and Pb concentrations (mg kg⁻¹ dry-wt) in liver and kidney of wild rats

			Pb concentration (mg kg ⁻¹ dry-wt)		
	Body weight (g)	Age (month)	Liver	Kidney	
Kabwe	21-170 (86) ^B	0.9-20 (4) ^B	0.009-7.3 (0.5) Ab	0.3-22.1 (2.2) Aa	
Lusaka	28-213 (136) ^A	0.9-15 (8) ^A	0.003-0.5 (0.1) Bb	$0.08-1.9(0.2)^{\mathrm{Ba}}$	

Large and small character indicate comparison between Kabwe and Lusaka, and liver and kidney, respectively.

Fig. 1



Road to Lusaka

Fig. 2

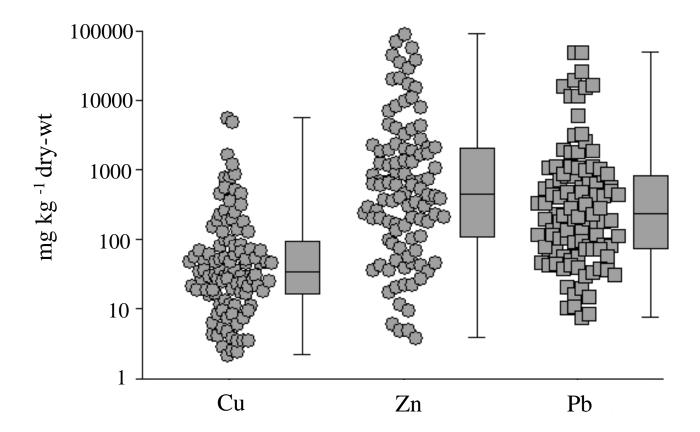


Fig. 3

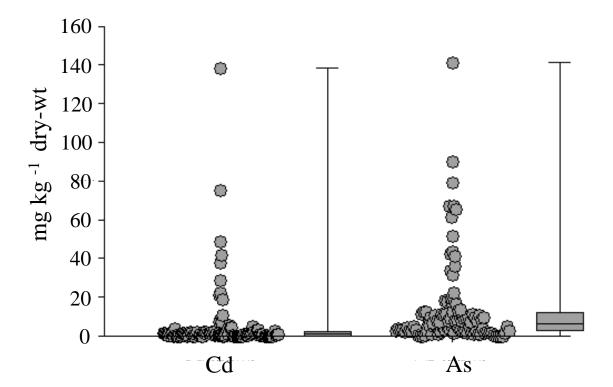


Fig. 4

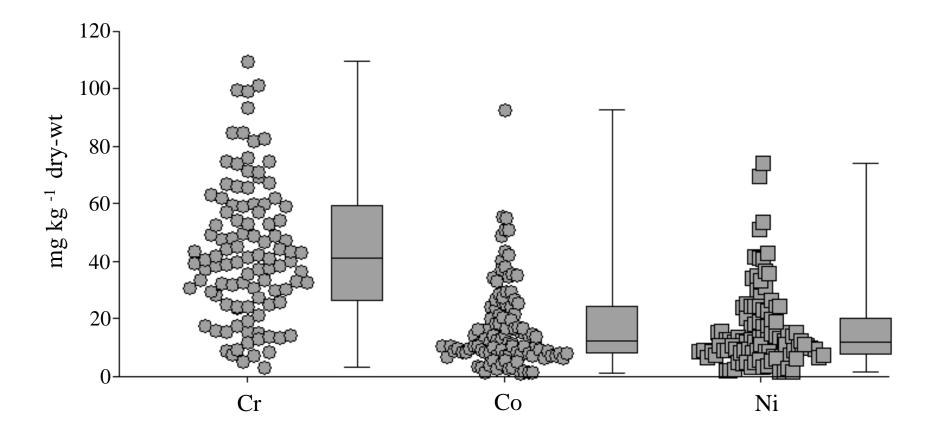


Fig. 5

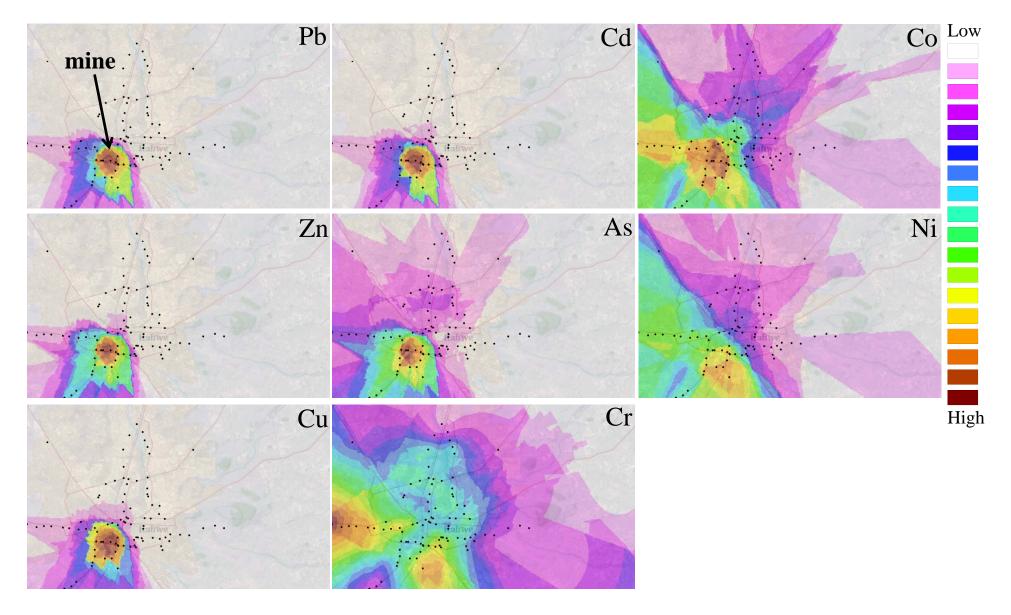


Fig. 6

