Pathways of human exposure to cobalt in Katanga, a mining area of the D.R. Congo.

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Abstract

Human exposure biomonitoring in the African Copperbelt (Katanga, southern D.R. Congo) revealed elevated cobalt (Co) exposure in the general population. This study was designed to identify the Co exposure routes for the non-occupationally exposed population in that area. The concentration of Co was measured in environmental and urine samples collected in urban and rural communities close to metal mining and/or refining plants, villages near a lake receiving effluents from metal refining plants, and control rural areas without industrial pollution. Drinking water, uncooked food items (maize flour, washed vegetables, fish and meat), indoor and outdoor dust samples were collected at each location. A food questionnaire was used to estimate dietary Co intake for adults and children. Geometric mean urine-Co (U-Co) concentrations were 4.5-fold (adults) and 6.6-fold (children) higher in the polluted than in the control area, with U-Co values being intermediate in the lakeside area. Average Co concentrations in environmental samples differed 6-40-fold between these areas. U-Co was positively correlated with most environmental Co concentrations, the highest correlations being found with Co in drinking water, vegetables and fruit. Estimated average total Co intake for adults was 63 (±42) µg/day in the control area, 94 (±55) µg/day in the lakeside villages and 570 (±100) µg Co/day in the polluted areas. U-Co was significantly related to modelled Co intake (R^2=0.48, adults and R^2=0.47, children; log-log relationship). Consumption of legumes, i.e. sweet potato leaves (polluted) and cereals+fish (lakeside) were largest contributors to Co intake in adults, whereas dust ingestion appeared to contribute substantially in children in the polluted area. In conclusion, dietary Co is the main source of Co exposure in the polluted area and Co is efficiently transferred from soil and water in the human food chain.

1 Introduction

Almost 55% of the world's cobalt (Co) supply in 2012 was mined in the Katanga Copperbelt, a mining area in the southern part of the province of Katanga, DR Congo (USGS 2013). The Katanga province hosts a world-class sediment-hosted stratiform copper (SHSC) deposit, in which copper (main ore) is intimately and naturally mixed with accessory cobalt. Two important specificities of this deposit are that: (1) it exposes secondary oxidized Cu and Co ore in thick surface deposits (De Putter et al. 2010; Decrée et al. 2010); and (2) exploitation there began early in the 20thC., leaving huge masses of tailings and large tracks of unprotected polluted soils. Hence, the topsoil is contaminated with Co and copper (Cu) at concentrations sufficient to alter the composition of natural plant communities (Noel et al. 2012). Moreover, past and current industrial and artisanal smelting and refining activities have increased the environmental concentrations of Co. A number of studies have identified high concentrations of Co in plants, fish, water and soil in the region (Faucon et al. 2009; Manda et al. 2010; Narendrula et al. 2012). A recent study has further demonstrated high Co concentrations in volatile efflorescence in the Likasi area (Mees et al. 2013).

Recently, we performed a human biomonitoring study of metal exposure in this region and identified the highest ever reported urinary concentrations of Co (U-Co) in adults and children from the general population (Banza et al. 2009). In subjects from the Copperbelt area living within 3 to 10 km from mines or smelting plants, (geometric) mean U-Co was 5.7 µg/g creatinine and in subjects living close to (within 3 km) mines or smelting plants mean U-Co was 15.7 µg/g creatinine with almost 90% of the children (<14 years) exceeding the ACGIH's occupational Biological Exposure Index of 15 µg Co/L (ACGIH 2013). These values are several fold higher than the mean values found in the capital of D.R. Congo (0.39 µg/g creatinine) (Tuakuila et al. 2012), the US National Health and Nutrional Examination Survey (0.36 µg /g creatinine) (Richter 2009), and a recent survey in Belgium (0.15 µg/g creatinine) (Hoet et al. 2013).

In non-polluted areas, Co exposure originates mainly from food sources, including vitamin B-12. Dietary Co intake is estimated to range 5–40 µg/day in the general population (Kim et al. 2006). A total-diet survey in Canada in 1986-1988 estimated an average dietary intake of cobalt over all ages of 11 µg/day (Dabeka and McKenzie 1995) with bakery goods and cereals (29.8%) and vegetables (21.9%) contributing most to the dietary intake. Five total diet studies in the UK (1977-1994) estimated Co dietary exposures ranging 12-31 µg/day (Ysart et al. 1999). The dietary Co intake of the French population was estimated at 7-13 µg Co (mean and P95), with cereals constituting a large fraction of the daily source, in the first Total Diet Study (Leblanc et al. 2005), but dietary intake was about 30 to 40% higher in the second Total Diet Study (Arnich et al. 2012). The average daily Co intake estimated with duplicate meals in France (Noel et al.

2003) was only 4 µg Co. A recent duplicate diet study in Catalonia, Spain estimated a dietary intake of 19.8 µg Co/day (Domingo et al. 2012). The Co exposure routes in polluted environments remain unexplored. Soil concentrations in the mining area of Katanga can exceed 1000 µg Co/g (Narendrula et al. 2012), hence inadvertent soil ingestion, typically estimated at 100 mg/day (EPA 2008), could yield 100 µg Co/day. Cobalt is known to be transferred from soil to plants in the food chain (Alloway 2013), however, no data have been found on Co uptake in food crops in the affected area. Drinking water and the consumption of fish may also contribute. Manda et al. (2010) collected water and fish samples from the polluted Lufira river and Tshangalele lake, downstream of an intensive mining and refining site. Concentrations in river and lake water ranged 1->500 µg Co/L and Co in the commonly consumed tilapia fish (*Oreochromis macrochir*) exceeded 30 µg/g dry weight in contaminated rivers, i.e. about 30-fold above a background value found upstream of the pollution source.

The gastrointestinal (GI) absorption of Co is between 5 and 20% of ingested Co (although it may be much higher under depleted iron stores) and about 80-90% of absorbed Co is eliminated in a few days (Barceloux 1999). Based on an average Co intake of 7 µg/day for the general population in a reference area, a 5% GI absorption and 90% elimination, a concentration of 0.3 µg/g creatinine can be predicted for U-Co, assuming a daily excretion of 1 g creatinine. This predicted concentration agrees well with measured reference values (0.36 µg Co/g creatinine; US National Health and Nutrional Examination Survey). The about 100-fold larger U-Co values found in the population of the mining area of Katanga hence point to a large dietary Co intake and/or higher GI absorption.

Metal exposure routes in mining or smelting areas vary largely among metals. Thus, dietary intake is the major route for cadmium, whereas soil and dust ingestion contribute most for lead and drinking water is the major source of arsenic (Alloway 2013). No such information is available for Co, which has been mainly studied in the context of radiological protection against radioactive isotopes (Leggett 2008) or the ingestion of dietary supplements (Kerger et al. 2013)). The objectives of this study were to identify Co exposure routes in the general population living in a Co polluted area.

2 Materials and methods

2.1 Study areas and sample collection

Figure 1 shows the location of the sampling sites and Table 1 presents further details (including dates) of these samplings. The sites were initially chosen to verify the hypothesis that people consuming fish from Lake Tshangalele, which receives industrial effluents from the Likasi mines and metal smelters, might be highly exposed to trace metals, including cobalt. Consequently, we

selected three villages and grouped these as the lakeside area (Shinangwa, Kansalabwe, Kibangu) close to Lake Tshangalele, where people consume fish caught from the lake. For comparison, we selected two urban neighbourhoods situated near the metal smelters of Likasi (Shituru and Panda), where our previous biomonitoring study (Banza et al, 2009) had already revealed very high exposures to metals. In addition, we also obtained samples from a rural village very close to a copper-cobalt mine (Shamitumba) and from an urban neighbourhood (Kabecha) close to a metal smelter in Lubumbashi. All these locations were grouped as highly polluted areas to contrast with the lakeside area. Finally, as reference area within the Copperbelt, we selected a rural location (Sando) without industrial or mining activities. From outside the Copperbelt area, we obtained samples from a village (Lovoj) near Kamina in northern Katanga, as well as from villages near Mbuji Mayi, the capital of Kasai-Oriental Province, where there is no metal mining, but diamond mining.

In all these locations we obtained spot urine samples from adults and children following the same general procedures as in our previous survey (Banza et al. 2009). No systematic sampling strategy was followed, but we generally started our sampling at the home of a village elder or representative, who had given us permission to carry out the survey, and then approached families at various distances from this first home. In each household, we recruited participants of either sex among children (<14 y) and adults (≥14 y). For each participant we obtained – via a questionnaire administered face-to-face in vernacular language by local collaborators (usually students in public health) – demographic data, a brief residential and occupational history, and a brief medical history, and we also recorded blood pressure (not reported). Sterile polystyrene containers (40 mL) with screw caps (Plastiques-Gosselin, Hazebrouck, France) were used for the collection of urine. Participants were instructed to avoid contamination of the urine by the hands. After collection, the samples were stored in a cool box, and then in a freezer until transportation to Belgium, using commercial flights.

Environmental samples, grouped in 17 classes (Table 1), were collected usually on the day of urine sampling. Samples of drinking water were obtained as indicated by the participants (communal tap water or wells for the urban locations; wells or containers for the villages). Surface dust was collected with a dustpan (by sweeping over a surface of approximately 1 m^2) from the yard in front the home (outdoor dust) and from inside the home (usually the sitting room). Maize flour was collected from the households store and kept in polyethylene bags. Sweet potato leaves were collected from the kitchen garden, with the tubers and associated soil being also collected whenever possible. Other vegetables and fruit were also collected from local kitchen gardens or from the local market, depending on circumstances. These samples were stored in polyethylene or paper bags, and rinsed with tap water and then demineralized water

147 before being oven dried. Fish was purchased directly from fishermen or from local markets;
148 control fish came from Lubumbashi market and from Sando and other locations in the Lufira
149 basin upstream from the industrial contamination areas; scales and internal organs were
150 removed (using plastic knives), but since the entire fish (including gills and head) is then
151 normally consumed, the specimens were not further dissected into specific anatomical regions.
152 Samples of meat (chicken, goat and pork) and consumed offal were purchased at markets where
153 the origin was requested.
154 The protocol of the surveys was approved by the committee for medical ethics of the University
155 of Lubumbashi (UNILU).

156 *2.2 Preparation of environmental samples*

157 Most samples of vegetable or animal origin were oven dried (24 h at 60°C) and ground with
158 pestle and mortar; soil and dust samples were also oven dried and sieved (2 mm). These sample
159 preparations were done at the Institut Supérieur Pédagogique in Lubumbashi for most samples,
160 but some samples underwent these same procedures in Belgium.

161 Further sample processing took place in the Centre for Environmental Sciences, Hasselt
162 University for samples collected before 2011 (indicated in italics in Table 1) and in the Division
163 Soil and Water Management, KU Leuven for samples collected in 2011.

164 In Hasselt, ground oven-dried vegetable material (200 mg) was digested in HNO_3 [3 successive
165 runs of dissolution in HNO_3 (1 mL suprapur 70%) followed by evaporation to dryness for 4 h at
166 110°C] and then dissolution in HCl (1 mL suprapur 37%) followed by evaporation to dryness for
167 3 h at 110°C]; ground oven-dried animal tissues [500 mg in HNO_3 (6 mL suprapur 70%) + H_2O_2
168 (1 mL suprapur 30%)] were digested in a microwave oven (250W x 1 min; 250W x 5 min; 400W
169 x 5 min; 650W x 5 min); soil or dust samples (500 mg) were solubilized by adding HNO_3 (1 mL
170 suprapur 70%) and HCl (3 mL suprapur 37%) followed by microwave heating (250Wx5min;
171 400Wx5min; 650Wx10min; 250Wx5min) and then filtered. The samples were kept in a final
172 solution of 2% HCl and analysed by ICP-MS (see below) in the Louvain centre for Toxicology and
173 Applied Pharmacology (Université catholique de Louvain, Belgium).

174 In Leuven, oven dried plant, fish or meat material was crushed, digested in duplicate (50 mg in 2
175 mL boiling 65% HNO_3) and analyzed for total metal content by inductively coupled
176 plasma/optical emission spectroscopy (ICP-OES, Perkin-Elmer Optima 3300 DV, Norwalk, CT,
177 USA). At concentrations < 5 µg Co/L in the digest, measurements were made by ICP-MS (Thermo
178 X-series I) in He mode using Ga internal standard. Certified reference material NIST SRM-1573a
179 (tomato leaves, National Institute of Standards and Technology, Gaithersburg, Germany, certified
180 value=0.57 mg Co/kg; measured value=0.54 ± 0.01 mg Co/kg) was included in duplicate with

each analysis. Aqua regia–soluble metal concentrations in 0.1 g soil samples were determined by boiling aqua regia extraction and analyzed by ICP-OES. Certified reference material BCR-142R (uncontaminated light sandy soil, Institute for Reference Material Measurement, Joint Research Center, European Commission, certified value=12.1 mg Co/kg, measured value=12.1 ± 0.4 mg Co/kg) was included in duplicate with each analysis. Water samples were filtered (0.45µm), and measured by ICP-OES. The NIST certified water was included (certified value= 27 µg Co/L, measured value= 26 ± 1 µg Co/L). Detection limits for all environmental samples are calculated as twice the standard deviation of 6 preparation blank sample measurements (table 2). Soil pH was measured in deionised water at a soil:liquid volume ratio of 1:5.

2.3 Analysis of urinary Co

Urine samples and the environmental samples that had been solubilized in Hasselt were analyzed in the Louvain centre for Toxicology and Applied Pharmacology (Université catholique de Louvain, Belgium) as previously published (Banza et al. 2009, Hoet et al. 2013) without knowledge of their exact provenance in relation to exposure (blind analysis). In all samples, Co was quantified (together with other elements) by means of inductively coupled argon plasma mass spectrometry (ICP-MS) with an Agilent 7500 ce instrument. Briefly, urine specimens (500 µl) were diluted quantitatively (1+9) with a HNO_3 1%, HCl 0.5% solution containing Sc, Ge, Rh and Ir as internal standards and Co was measured in the He mode. Using this validated and ISO15189 certified method, the laboratory has obtained successful results in external quality assessment schemes organized by the Institute for Occupational, Environmental and Social Medicine of the University of Erlangen, Germany (G-EQUAS program), and by the Institut National de Santé Publique, Québec (PCI and QMEQAS programs). Creatinine was determined using a Beckman Synchron LX 20 analyser (Beckman Coulter GmbH, Krefeld, Germany). In all samples, other elements besides cobalt were also measured, but these data will not be presented here.

2.4 Food questionnaires

A (self-made) ad hoc food questionnaire was administered, by a member of the investigating team in the local language, to an adult (generally the mother) of the household. The information in the questionnaire included the number of adults and children living at the household and the food consumption and preference of the last 7 days at the household. Maize flour intake was quantified as the number of buckets (estimated weight 2.5 kg) prepared per week. Vegetable and fruit intake was quantified as portions (estimated 25 g dry weight/portion) prepared per week and the same was done for meat and fish (estimated 10 g dry weight/portion). The average food intake per adult was then calculated as the total quantity of food consumed per

household divided by the sum of adults and half the number of children. The median caloric intake per adult in the household, assuming 3 kcal/g cereals, 4 kcal/g vegetables and 5 kcal/g fish or meat (dry weight based) was 1650 kcal/day in the entire study area, range 641-4266 kcal/day. Suspected misreporting of consumption was, hence, corrected: if the caloric intake per adult in a household differed by more than 20% from the average caloric intake per adult over all questionnaires, then the recorded intake was normalized to 2000 kcal/day. Drinking water consumption was set at 1.5 L/day for adults and 0.75 L/day for children. Dust ingestion was estimated 100 mg/day for adults and 200 mg/day for children, these assumptions are discussed below.

2.5 Exposure estimation

The Co intake values for adults and children were modelled at the household level. Consumption of 16 different classes of environmental samples (all data except soil), sorted in 6 exposure groups (table 3), were combined with corresponding Co concentrations (table 4). As noted above, not all environmental samples or food consumption data were available at the household level and average (aggregated) data were used instead in these cases. There are three levels of aggregation: household, location and area. The aggregation of data was made stepwise for each environmental sample with preference for the highest resolution possible, i.e. household level. Aggregation level varied with the type of environmental sample. For example, Co intake via maize flour and in dust was generally estimated with household specific data. Intake from drinking water, vegetables and fruits were based on location mean values, whereas Co in meat was aggregated at an area level. The Co-fish data was aggregated at an area level for control and polluted area, but not for the lake area. All intake values yielded data per household but data is represented per location. The variance of the intake per location (n=10) was calculated from the combined variances in consumption and in concentrations within the location assuming independent variances.

2.6 Statistical analysis

The U-Co and environmental Co concentrations were logarithmically transformed for statistical analysis after testing original data for normality with the Kolmogorov-Smirnov test. Analysis of variance (ANOVA) was used followed by Duncan's test for post-hoc comparisons of means (SAS 9.3). The correlation analysis (Pearson regression) between U-Co and Co exposure or between U-Co and environmental concentrations was based on household or location arithmetic mean values. Levels of statistical significance were set at 0.05 or below unless indicated.

3 Results

3.1 Cobalt concentrations in urine

Participation rates exceeded 95% of those approached. A total number of 372 subjects [236 adults (defined here as ≥ 14 y) and 109 children (defined as < 14 y)] provided a urine sample, but only 252 urine samples were retained for the present analysis, because subjects vulnerable for occupational exposure (miners) and subjects whose urine samples had a creatinine concentration below 0.3 g/L were excluded (Cocker et al. 2011). The participants were aged between 2 and 80 years (mean 29 years). The male/female ratios were 40/60 in adults and 43/57 in children. Neither age nor gender distributions differed among areas ($p>0.05$). The geometric mean U-Co concentrations ranked control<lakeside<polluted for adults and children (table 5) and were significantly different for the adults between control and lakeside/polluted area and for children between control/lakeside and polluted area. Children had significantly higher U-Co than adults in the polluted area but not in the control and lakeside area. The U-Co (log transformed values) significantly decreased with age in all areas and age explained between 7% and 20% of the variance depending on the area. The value of 15 µg/g creatinine was exceeded in 52% of children from the lakeside area and in 72% of children from the polluted areaswhere extreme values (up to 370 µg Co/g creatinine) were reached, well above those of adults collected at the same time in the same location. Gender did generally not influence U-Co, except in adults from the lakeside and polluted areas where females had slightly larger U-Co than males (details not shown). Effects of smoking were not analysed as previous assessment did not always find a significant association and smoking prevalence and intensity were low anyway (Banza et al., 2009).

3.2 Cobalt concentrations in environmental samples

Except for the concentration of Co in locally caught fish, there were no systematic differences between environmental samples obtained from the lakeside villages and those from the control areas. This can be explained by the fact that the lakeside area is situated too far away from mines or industrial operations to be affected by atmospheric deposition of metals. We have, therefore, grouped the results from the control and lakeside areas into a single category, except for fish (Table 4). The Co concentrations in the environmental samples differed substantially and significantly between the polluted areas and the other two areas. The average soil Co concentration in the polluted areas (150 µg/g) was 7-fold larger than that in the control area (20 µg/g), with concentrations up to 860 µg Co/g. Concentrations of Co were very similar in outdoor and indoor dust, and much higher in the polluted areas (average of 330 µg/g and 490 µg/g) than

in the control areas (11 µg/g and 11 µg/g), Concentrations of Co in drinking water were also higher in the polluted sites (12 µg/L) than in other areas (<1 µg/L).

Concentrations of Co in the food items were up to a factor 40 higher in the polluted area than in corresponding samples of control+lakeside area (Table 4). This was most marked for leafy vegetables that are grown locally. The paired sampling of soil and sweet potato leaves showed a significant soil pH dependent association with soil Co (R^2=0.41 of multiple regression model). At pH=6.0, Co concentrations in these leaves increased from 1 to 18 µg Co/g with increasing soil Co from background (10 µg Co/g) to polluted values (200 µg Co/g). At soil pH values that are 2 units lower, these values roughly doubled. The fish caught in the lakeside area contained, on average, 18-fold higher Co concentrations than in the control area (6 µg/g dry weight vs 0.3 µg/g dry weight). No fish was collected in the polluted areas because no fish is being caught there. The lake Tshangalele is currently still fed by contaminated water. A spot water sample taken in May, 2011 in the river Panda feeding this lake, between Likasi and lake Tshangalele, showed 32,000 µg total Co/L whereas recent data of lakewater showed water Co concentration >300 µg Co/L (Manda et al. 2010). Hence, the lakeside area has a particularly high contamination of food derived from the water whereas soil grown food is not contaminated.

Pearson correlation analysis between log(U-Co) and log(Co) in environmental samples was made per household (Table 6). Maize flour and dust data were always collected at that resolution level, whereas most other environmental data were only available per location. The correlation coefficients were almost always positive and were strongest for environmental matrices that are grown or present locally, i.e. leafy vegetables, fruit vegetables and drinking water.

3.3 Estimation of the intake of cobalt

Food questionnaires showed no substantial dietary preferences among the areas (Table 3). The diet is mainly vegetarian, with caloric intake being mainly derived from maize flour, and only little contribution from cassava flour or sweet potatoes, at least during the early dry season when we performed the surveys. The estimated Co intake at household level was calculated and illustrated (Figure 2 and Figure 3) as pie diagrams. In adults, the average (± standard deviation of location means) total Co intake was estimated at 63 (±42) µg/day in the control area, 94 (±55) µg/day in the lakeside villages and 570 (±100) µg Co/day in the polluted areas. For adults, the main contribution of Co intake in the control area comes from cereals (57 %), followed by vegetables (37 %). Consumption of vegetables (dry weight) is almost 20-fold below that of maize flour but Co concentrations are up to 20-fold higher in the former than in the latter. Fish consumption, albeit very low (Table 3), significantly contributes to daily Co intake in the

lakeside area (22 %). The Co intake originating from vegetables increases disproportionally in the polluted areas, reaching > 50%. Cobalt in dust also contributes substantially in the polluted areas, especially at the Likasi-Panda location where concentrations of Co in dust exceeded 1000 µg /g. In children, intake values and their contributing factors were similar to those derived for adults except that, by virtue of the default values chosen for the calculations, dust ingestion contributes relatively more in the polluted area than for adults, because food intake of children was considered half of that of adults, whereas dust ingestion was assumed double for children than for adults.

In adults, the U-Co concentrations averaged per location correlate well with corresponding intake values up to about 200 µg Co/day intake, beyond which the U-Co appears to saturate (Figure 4). In children, this association is less obvious due to the very large U-Co values of the two most contaminated locations. Based on the mean data obtained in the 10 locations, the following significant ($p<0.05$) log-linear regression model can be derived for adults and children:

$$\log(\text{U-Co}) = 0.06 + 0.45 \log(\text{Intake-Co}),\ R^2 = 0.47 \text{ (adults)} \quad (1)$$

$$\log(\text{U-Co}) = -0.02 + 0.62 \log(\text{Intake-Co}),\ R^2 = 0.46 \text{ (children)} \quad (2)$$

with U-Co and daily Co intake values in units as shown in Figure 4. Linear regression on the adult data of control and lakeside area only (n=6) yielded $R^2 = 0.58$ with a slope of 0.20 day g^{-1} creatinine beyond which U-Co probably saturates.

4 Discussion

Cobalt is an essential trace element present in vitamin B12 (cyanocobalamin) and cobalamin, and plays an important role in human nutrition. The toxicology and biokinetics of cobalt have been recently reviewed (Simonsen et al. 2012). The toxicity of Co has been mainly studied in occupational settings, e.g. Co as a factor in hard metal lung disease (Nemery et al. 2001) and in occupational asthma (Nemery 1990). Excessive Co exposure has also been linked with erythrocytosis (Jefferson et al. 2002), heart damage (Alexander 1972) and thyroid disruption (Prescott et al. 1992). Cardiomyopathy related to Co has been identified in heavy beer drinkers in Canada where Co had been added in beer as foam stabiliser (Barceloux 1999). The possible consequences of such Co related effects in environmentally exposed populations remain to be demonstrated.

The U-Co data in this study (n=252) reinforces our previous biomonitoring study (n=351) that showed U-Co to be extremely high in this mining area, especially in children (Banza et al. 2009). The values recorded here for children are even larger than in our previous assessment. The U-Co

obtained here in the control area (~3 µg Co/g creatinine) exceeds that of the U.S. reference value (0.4 µg/g creatinine, see introduction) and that found in Kinshasa, capital of D.R. Congo (0.4 µg/g creatinine (Tuakuila et al. 2012), and even that of our previous assessment (~1.3 µg/g creatinine) where the control area was located at a larger distance (about 400 km) of the mining area. Clearly, the control area in the current study is not representative as a more generally applicable background for Co-exposure. Our exposure assessment estimates that daily Co intake is about 60-70 µg Co for adults in the control area, well above most recent estimates in Europe (~7-20 µg Co/day; see introduction). Both the environmental data and the food habits may explain this difference: maize-flour samples of the control area contained, on average, 50 µg Co/kg thus conferring 30 µg Co/kg for a daily consumption of about 0.6 kg. In contrast, the lower Co concentration in cereals on the EU market (<20 µg Co/kg) and lower consumption (typically less than 0.3 kg/day) only confer <6 µg Co/day (Leblanc et al. 2005).

As far as we are aware, this is the first study linking environmental Co concentration data with Co biomonitoring in humans. No special attention was given in this study to the seasonal effects of human consumption, however, the different sampling dates account partly for the uncertainty associated with seasonal effects. Also no special attention was given to the cooking processes of the food. For example, cooking may reduce metal content although some foods can absorb metals if the cooking water is contaminated. Especially for the processing of cassava which involves some wetting and drying steps this might influence the Co concentration.

The data analysis aggregated at location level found a significant association between modelled exposure and body burden (Figure 4 and Equations 1-2). Since Co has a short biological half life in humans, it is justified to make the analysis based on environmental samples collected at the same time as the biomonitoring. The exposure assessment suggests that intake of maize flour and legumes (mainly leafy vegetables) are main vectors for adults, and that fish consumption largely contributes for the population residing around the contaminated lake. For children, similar trends appear but dust ingestion becomes relatively more important. The correlation analysis confirms the substantial contribution of vegetables in the Co-exposure but not that of maize flour or fish. Maize flour is not always grown locally and is collected from a wider area than vegetables, i.e. maize flour Co does not correlate well with exposure routes that are intimately linked with the location.

In our previous study (Banza et al. 2009) we hypothesized that dust exposure (via hand-to-mouth contact) contributed to the high urinary metal concentrations found among children. This pathway of exposure seems to be confirmed in the present study. Drinking water Co correlates well with U-Co, however that association is unlikely to be causal: the highest Co in water in the

polluted area was 17 µg Co/L yielding maximally 50 µg Co daily intake, even accounting for an excessive daily consumption intake of 3 L. Hence, the correlation between U-Co and drinking water Co is likely due to covariance of drinking water Co with other, more important, local Co sources such as vegetables, dust or cereals.

Ingestion of Co via dust is likely the most uncertain factor in the exposure assessment. Soil and dust ingestion rates can be estimated using fecal excretion data of conservative tracers. Key studies for children in U.S.A. have suggested that arithmetic mean and 95th percentiles are about 60 and 240 mg soil and dust ingestion/day whereas these values may amount to 1000 and 1200 mg/day for children with pica behaviour (Bierkens et al. 2011) and references therein). For risk assessments, recommended soil and dust ingestion rates for children range between 20 and 200 mg/day, the most recent US-EPA value recommends 100 mg/day, whereas we used 200 mg/day. As far as we are aware, no soil or dust ingestion rates have been estimated for the local population of Katanga, albeit Co-rich volatile dust components were identified around the ore treatment plants. Ingestion of dust, especially in the dry season, is likely to be much higher in most rural and even urban areas in Africa, including Katanga, where roads and home yards are generally not covered by hard materials. Most dwellings in the areas included in our surveys did not have hard floors. Hence, dust exposure must be considerable, especially in small children who constantly play in dusty places. Moreover, pica behaviour is likely prevalent in children. Geophagy, i.e. the deliberate eating of soil, has been estimated for in schoolchildren in Lusaka (Zambia, about 400 km south of Katanga) and was found in 74% of the interviewed children, mean age of 10 years with mean daily soil ingestion of 25 g (Nchito et al. 2004). Such uncertainty has a high impact on the estimated Co exposure in the contaminated areas: increasing dust ingestion from 200 mg/day to only 1000 mg/day, would increase the average Co-intake for children from 334 µg Co to 605 µg Co in the polluted areas. Geophagy would have an even a larger effect but this would likely be counteracted by lower Co bioavailability than Co derived from food. Eventually, at the start of the rainy season, peaks in heavy metals can be expected in the Lufira River and downstream basin, as described in other mining areas (Keith et al. 2001). This is because Co-rich dust particles deposited along the river banks are then dissolved and drained into the river (Mees et al. 2013), hence contributing to the metal-load in surface waters and thus possibly in fish.

The existing toxicokinetic information for Co (see introduction) has been added as a dashed line in Figure 4. The data for adults match this model well that is independent of these data: all location mean values were predicted within a factor 3 (one exception), and the ratio of observed/predicted U-Co values is only, on average, factor 1.9. The logarithmic association in the Figure 4 suggests that U-Co saturates at higher Co intake. Reduced GI absorption of Co may

occur at larger trace metal intake but may also be related to a lower GI absorption factor of soil or dust-borne Co that contribute more to the theoretical Co intake. It is well established that GI absorption of metals, such as lead (Pb), bound to soil is well below that of metal salts (Freeman et al. 1992) but no studies have yet been made for Co. Restricting the data of Figure 4 to the linear part, i.e. within the control and lakeside areas only, yielded a strong association with a slope of 0.20 day g^{-1} creatinine. This value is more than 4-fold above the slope of the toxicokinetic model (0.045 day g^{-1} creatinine) which may suggest a somewhat larger Co bioavailability due to trace metal deficiency. The GI absorption ranges 5% to 20% and exceeds 40% under Fe deficiency (Barceloux 1999). For children, the toxicokinetic model largely underestimates U-Co at almost all locations, the mean factor underestimate is 5.0 in the present study. Again, this suggests that soil/dust ingestion was underestimated in our assessment and/or that the Co absorption from food is larger than that in the existing toxicokinetic model.

5 Conclusion

This study identified a significant association between Co exposure, modelled on the basis of comprehensive environmental and dietary data, and the Co concentration in urine. The study shows that dietary Co exposure is the main exposure route of Co for adults, mainly via cereals, leafy vegetables and fish where applicable. In this respect, the situation is analogous to that of cadmium (Cd) for which dietary Cd is the main vector controlling body burden and risk to humans (Smolders and Mertens 2013). The limited soil-plant data for sweet potato leaves identified a significant soil pH dependent association that is also commonly found for Cd (Smolders and Mertens 2013). For children, dust and soil ingestion is likely a major additional source of body burden Co in the polluted area, as suggested here by the quantitative analysis. Tracer studies on fecal samples are needed to corroborate this hypothesis and to refine exposure assessments. The health significance of high Co intake for the affected populations is unknown at this stage. Epidemiological studies are currently being conducted in various populations in Katanga to evaluate the possible health impacts of environmental exposures to Co (and accompanying elements). A refined exposure assessment can help local authorities to create dietary recommendations for the local population to minimise these health risks correlate with Co intake.

6 Tables

Table 1: Locations and areas of the sampling sites with details about urine sampling, food questionnaires and environmental sampling.

Location	MAP Figure 1	Area	Sampling date(s) (DD/MM/YYYY)	Urine	Food questionnaire	Environmental samples
				Number (adults + children)	Number of households	Type of samples*, **
SANDO	F	Control (rural)	*10/04/2009* & 10/05/2011	27 (17 + 10)	6	1, 2, 3, 4, 5, 13, 14, 15, 16, 17
KAMINA, Lovoj	B	Control (rural)	01/03/2011	22 (13 + 9)	ND	5, 6, 9, 14, 15
MBUJI MAYI	C	Control (rural)	26/04/2011	32 (27 + 5)	ND	3, 5, 6, 7, 8, 9
LUBUMBASHI[$]	A	Control (urban)	09/05/2011	ND	ND	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 17
SHINANGWA	G	Lakeside	*17/03/2009*	*17 (11 + 6)*	ND	*4, 5, 6, 7, 8, 9, 14, 15, 17*
KIBANGU	H	Lakeside	*23/08/2010* & 10/05/2011	*17 (11 + 6)*	5	1, 4, 6, *14, 15*, 16, 17
KANSALABWE	I	Lakeside	*05/06/2009* & 07/05/2011	*26 (25 + 1)*	4	1, 2 ,3, *4, 5, 6, 7*, 16
LIKASI, Shituru	D	Polluted (urban)	*25/08/2010* & 07/05/2011	*37 (28 + 9)*	ND	1 ,3 ,4 ,5 ,6 ,7 ,*14 ,15*
LIKASI, Panda	D	Polluted (urban)	*25/08/2010* & 07/05/2011	*28 (21 + 7)*	5	1, 2, *3*, 4, 5, *7, 14, 15,* 16
SHAMITUMBA	E	Polluted (rural)	06/05/2011	22 (13 + 9)	5	1, 2, 3, 4, 6, 7, 9, 13, 14, 15, 16
KABECHA	A	Polluted (urban)	11/05/2011	25 (17 + 8)	5	1, 2, 3, 4, 5, 6, 7, 8, 9, 13, 14, 15, 16

* font (normal or italics) corresponds with font of date of sampling; if number is underlined, the sampling was done at both times.

** (1) maize flour; (2) cassava tubers; (3) cassava leaves; (4) sweet potato leaves; (5) other leafy vegetables; (6) fruit vegetables; (7) beans; (8) fruit; (9) other vegetables; (10) meat (muscle); (11) meat (liver); (12) meat (kidney); (13) drinking water; (14) indoor dust; (15) outdoor dust; (16) soil, (17) fish. [$]Only environmental samples were purchased on a local market in Lubumbashi, no urine samples were collected.

452

453 Table 2: Limits of detection for cobalt in environmental and urine samples.

	ICP-OES		ICP-MS	
Water	0.3	µg /L		
Plant	0.27	µg /g dry weight	0.005	µg /g dry weight
Soil	0.07	µg /g dry weight		
Urine			0.018	µg/L

454

Table 3: Average estimated consumption for adults (g dry weight/day or mL/day; standard deviations in brackets) based on food questionnaires taken at different locations per area. The last column shows the distribution of the different fractions of specific substances.

	control area n=5	**lakeside area** n=9	**polluted area** n=15	**fraction of total**	
cereals (g/day)	610 (50)	620 (26)	610 (47)	maize flour	95 %
				tubers	5%
vegetables (g/day)	25 (11)	28 (14)	23 (17)	cassava leaves	25 %
				sweet potato leaves	25 %
				other leafy vegetables	20 %
				fruit vegetables	15 %
				beans	5 %
				fruit	5 %
				other vegetables	5 %
meat (g/day)	0.6 (11)	0.1 (0.2)	0.5 (0.5)	meat (muscle)	80 %
				meat (liver)	10 %
				meat (kidney)	10 %
water (mL/day)	1500 (500)	1500 (500)	1500 (500)	drinking water	100 %
dust[$] (g/day)	0.1 (0.05)	0.1 (0.05)	0.1 (0.05)	indoor dust	33 %
				outdoor dust	67 %
fish (g/day)	4.1 (1.0)	3.1 (0.8)	2.5 (1.6)	fish	100 %

[$]0.2 g/ for children; assumption, see discussion.

462

463 Table 4: Average cobalt concentrations (µg Co/g oven dry weight) in environmental samples in
464 the different areas with standard deviations in brackets. Samples from the lakeside area are
465 grouped together with the control area with the exception of the fish samples. Difference in
466 mean concentrations (log transformed) were tested between the areas

	control + lakeside area	**polluted area**	**p value$**
maize flour	0.05 (0.05) n=12	0.10 (0.11) n=10	*
tubers	0.21 (0.16) n=4	2.6 (2.6) n=5	**
cassava leaves	1.5 (2.3) n=8	12 (8.1) n=16	***
sweet potato leaves	1.1 (0.98) n=9	6.5 (4.2) n=28	***
other leafy vegetables	1.2 (1.6) n=54	46 (73) n=52	***
fruit vegetables	0.58 (0.55) n=19	12 (10) n=14	***
beans	0.84 (1.35) n=15	22 (32) n=11	***
fruit	0.58 (0.52) n=3	-	-
other vegetables	0.53 (0.54) n=12	5.8 (5.4) n=10	***
meat (muscle)	0.38 (0.23) n=4	-	-
meat (liver)	0.59 n=1	-	-
meat (kidney)	0.11 n=1	-	-
drinking water	<0.001 n=4 µg/L	0.012 (0.007) n=5 µg/L	*
indoor dust	11 (12) n=26	490 (660) n=26	***
outdoor dust	11 (14) n=26	330 (390) n=26	***
soil	20 (11) n=5	150 (200) n=17	**

	control	**lakeside**	**polluted**	
fish	0.34 (0.61) n=44	6.2 (12) n=54	-	***

467 $: t-test on log-transformed values assuming equal variance*:p<0.05; **: p<0.01; ***: p<0.001;

468

469

470 Table 5: Cobalt concentrations in urine for adults and children, corrected per g creatinine in the
471 urine samples (µg Co/g creatinine): geomean and P25-P75 in brackets. Values followed by a
472 different character are significantly different within columns (first character) or row (second
473 character) at $p<0.05$ using Duncan or t-test.

	adults (≥14 years)		**children (<14 years)**	
	U-Co	n	U-Co	n
control	$2.7^{a,A}$ (1.3-5.3)	57	$4.2^{a,A}$ (2.6-7.2)	24
lakeside	$9.4^{b,A}$(4.0-19.8)	47	$14.0^{a,A}$ (7.5-21)	13
polluted	$11.7^{b,A}$ (7.1-22.0)	79	$27.9^{b,B}$ (13.4-62.7)	32

474

475

476 Table 6: Pearson correlation between household the mean U-Co and mean environmental Co
477 concentrations, both logtransformed. *: p<0.1; **p<0.05; ***: p<0.01.

	adult (n=30)	**children (n=24)**
maize flour	0.04	-0.02
cassava leaves	**0.31***	**0.50****
sweet potato leaves	0.19	**0.38***
leafy vegetable	**0.37****	**0.58*****
fruit vegetable	**0.32***	**0.54*****
beans	-0.05	0.02
other vegetables	-0.26	-0.31
tuber	0.26	**0.41****
fish	0.15	0.03
indoor dust	0.25	**0.43****
outdoor dust	0.24	**0.44****
drinking water	**0.36****	**0.52*****
soil	0.13	0.25

7 Figures

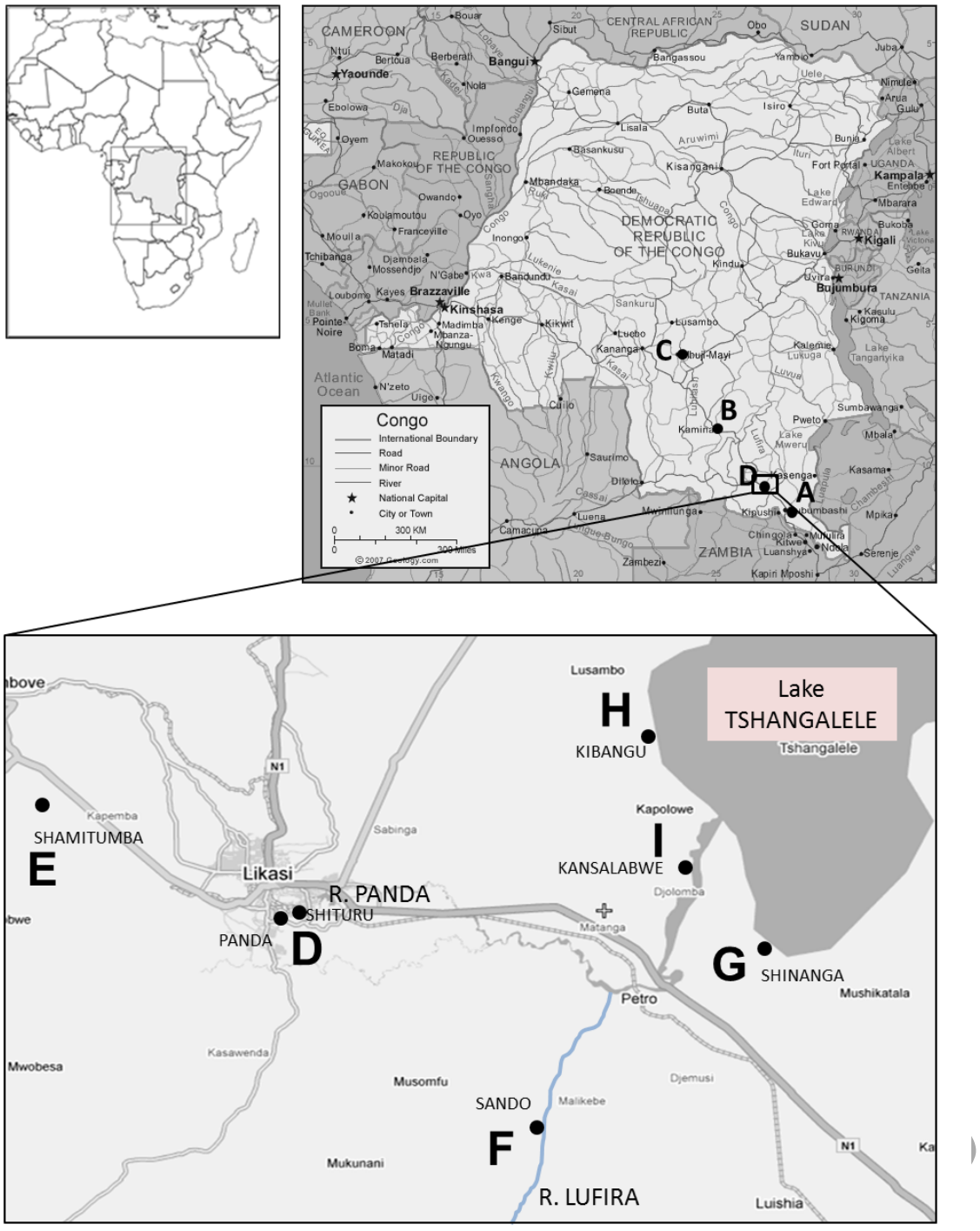
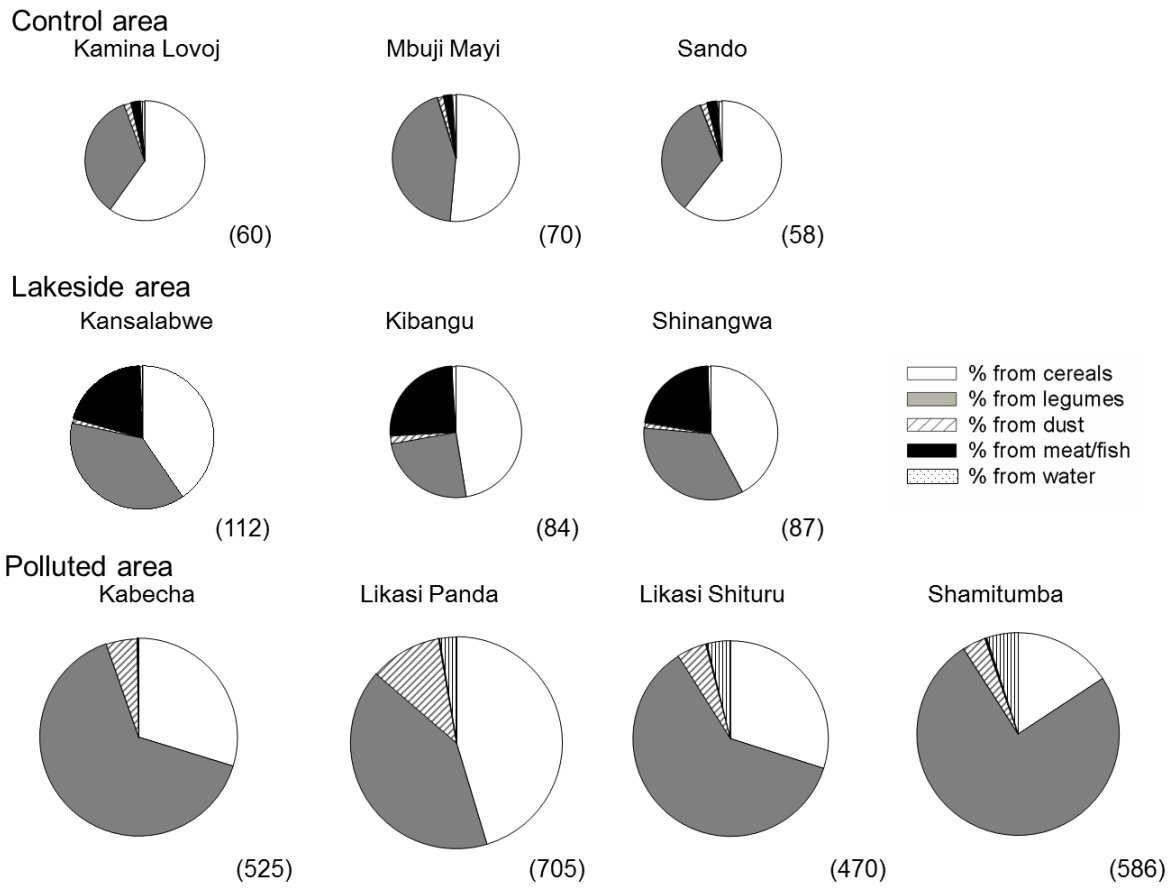
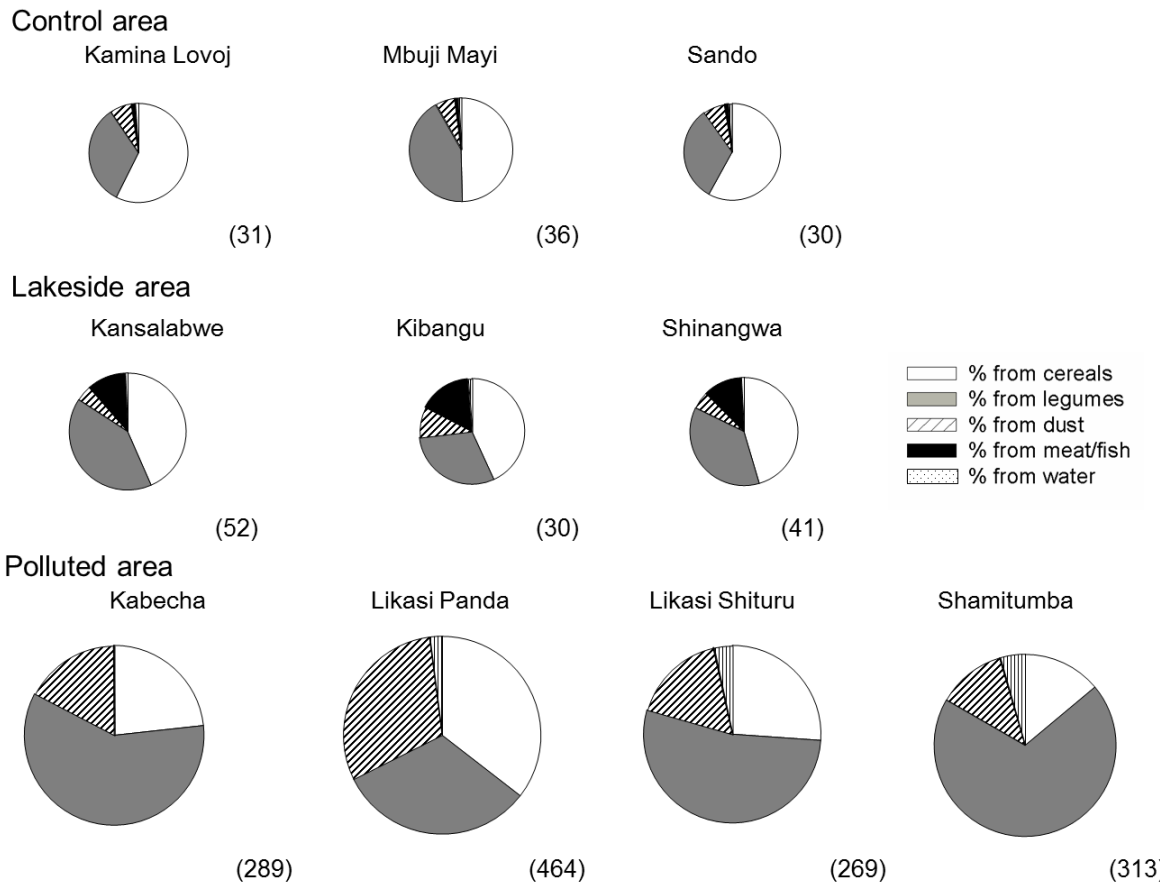


Figure 1: Maps of the Democratic Republic of Congo (left above), part of the Katanga province (right above) and the region around Likasi (under). The Copperbelt stretches along both sides of the border between Zambia and the D.R. Congo. The locations used in this study in the control (Kamina-B, Mbuji Mayi-C, Sando-F), lakeside (Kansalabwe-I, Kibangu-H, Shinangwa-G) and polluted (Kabecha-A , Likasi Panda-D, Likasi Shituru-D, Shamitumba-E) areas are indicated.



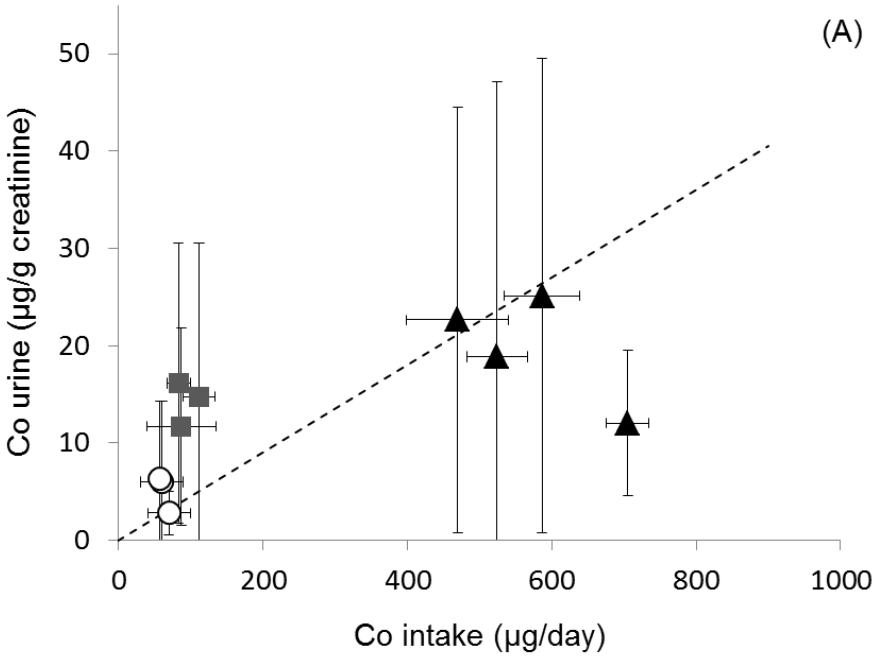
485

486 Figure 2: Cobalt intake for adults per location. Diameters of the pie charts are proportional to
487 total Co intake (µg Co/day, in brackets). The pie charts show the relative contribution of the
488 different fractions for human exposure (cereals, legumes=vegetables, dust, meat/fish, drinking
489 water).



490

491 Figure 3: Cobalt intake for children per location. Diameters of the pies charts are proportional to
492 the absolute exposure value (µg Co/day), given in brackets. The pie charts show the relative
493 contribution of the different fractions for human exposure (cereals, legumes=vegetables, dust,
494 meat/fish, drinking water).



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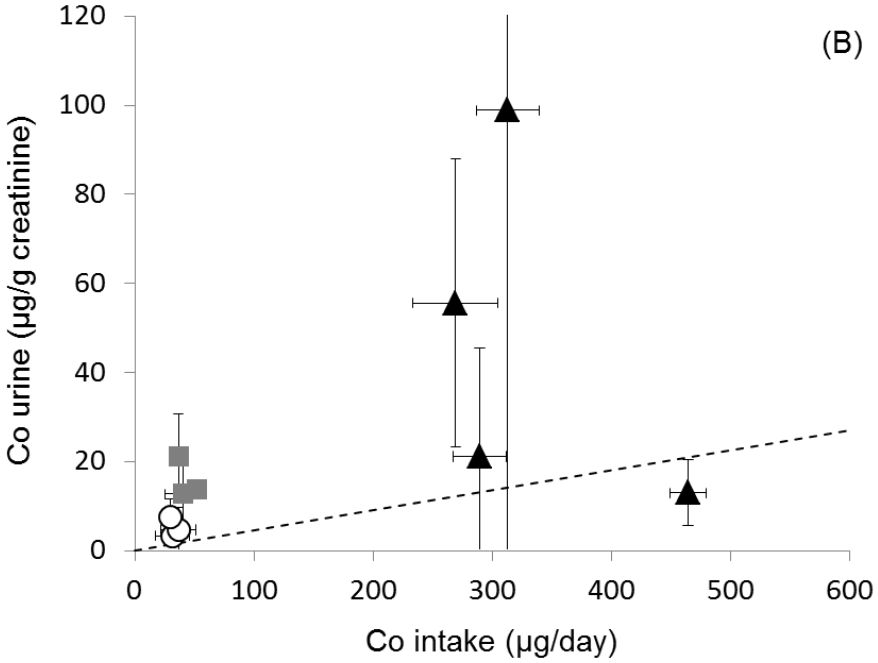

496

497 Figure 4: Urine Co concentrations (µg/g creatinine) in adults (a) and children (b) increase with
498 increasing Co intake (µg/day). Data are averages per location and are shown for the control area
499 (circles), lakeside area (grey squares) and polluted area (black triangles). Error bars show the
500 standard deviations. Note different Y-scales for adults and children. The dashed line is not a
501 regression line but is the predicted trend with the default toxicodynamic parameters, i.e. 5%
502 absorption of ingested dose, 90% elimination and 1 g creatinine excretion per day (Barceloux
503 1999).

504

8 References

ACGIH (2013). TLVs and BEIs Based on the Documentation of the Threshold Limit Values for Chemical Substances and Physical Agents & Biological Exposure Indices Cincinnati, Ohio.

Alexander, C. (1972). "Cobalt-beer cardiomyopathy - clinical and pathologic study of 28 cases." American Journal of Medicine **53**(4): 395-&.

Alloway, B. (2013). Heavy Metals in Soils, Springer Netherlands.

Banza, C. l. L. N., T. S. Nawrot, et al. (2009). "High human exposure to cobalt and other metals in Katanga, a mining area of the Democratic Republic of Congo." Environmental Research **109**(6): 745-752.

Banza, C. L. N., T. S. Nawrot, et al. (2009). "High human exposure to cobalt and other metals in Katanga, a mining area of the Democratic Republic of Congo." Environmental Research **109**(6): 745-752.

Barceloux, D. G. (1999). "Cobalt." Journal of Toxicology-Clinical Toxicology **37**(2): 201-216.

Bierkens, J., M. Holderbeke, et al. (2011). Exposure Through Soil and Dust Ingestion. Dealing with Contaminated Sites. F. A. Swartjes, Springer Netherlands**:** 261-286.

Cocker, J., H. J. Mason, et al. (2011). "Creatinine adjustment of biological monitoring results." Occupational Medicine **61**(5): 349-353.

Dabeka, R. and A. D. McKenzie (1995). "Survey of Lead, Cadmium, FLuoride, Nickel, and Cobalt in Food composites and estimation of Dietary Intakes of These Elements by Canadians in 1986-1988." Journal of AOAC International **78**(4): 897-909.

De Putter, T., F. Mees, et al. (2010). "Malachite, an indicator of major Pliocene Cu remobilization in a karstic environment (Katanga, Democratic Republic of Congo)." Ore Geology Reviews **38**(1–2): 90-100.

Decrée, S., É. Deloule, et al. (2010). "Geodynamic and climate controls in the formation of Mio–Pliocene world-class oxidized cobalt and manganese ores in the Katanga province, DR Congo." Mineralium Deposita **45**(7): 621-629.

Domingo, J. L., G. Perelló, et al. (2012). "Dietary intake of metals by the population of Tarragona County (Catalonia, Spain): results from a duplicate diet study." Biological Trace Element Research **146**(3): 420-425.

EPA, U. S. (2008). Child-Specific Exposure Factors Handbook (Final Report) U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-06/096F.

Faucon, M. P., G. Colinet, et al. (2009). "Soil influence on Cu and Co uptake and plant size in the cuprophytes Crepidorhopalon perennis and C-tenuis (Scrophulariaceae) in SC Africa." Plant and Soil **317**(1-2): 201-212.

Freeman, G. B., J. D. Johnson, et al. (1992). Relative bioavailability of lead from mining waste soil in rats. Fundamental and Applied Toxicology. **19:** 388-398.

Hoet, P., C. Jacquerye, et al. (2013). "Reference values and upper reference limits for 26 trace elements in the urine of adults living in Belgium." Clinical chemistry and laboratory medicine : CCLM / FESCC **51**(4): 839-849.

Jefferson, J. A., E. Escudero, et al. (2002). "Excessive erythrocytosis, chronic mountain sickness, and serum cobalt levels." Lancet **359**(9304): 407-408.

Keith, D. C., D. D. Runnells, et al. (2001). "Geochemical models of the impact of acidic groundwater and evaporative sulfate salts on Boulder Creek at Iron Mountain, California." Applied Geochemistry **16**(7–8): 947-961.

Kerger, B. D., B. E. Tvermoes, et al. (2013). "Cobalt speciation assay for human serum, Part II. Method validation in a study of human volunteers ingesting cobalt(II) chloride dietary supplement for 90 days." Toxicological & Environmental Chemistry **95**(4): 709-718.

Kim, J. H., H. J. Gibb, et al. (2006). Cobalt and inorganic cobalt compounds. WHO: Concise International Chemical Assessment

Leblanc, J. C., T. Guerin, et al. (2005). "Dietary exposure estimates of 18 elements from the 1st French Total Diet Study." Food Additives and Contaminants **22**(7): 624-641.

Leggett, R. W. (2008). "The biokinetics of inorganic cobalt in the human body." Science of the Total Environment **389**(2–3): 259-269.

Manda, B. K., G. Colinet, et al. (2010). "Evaluation of Contamination of the Food Chain by Trace Elements (Cu, Co, Zn, Pb, Cd, U, V and As) in the Basin of the Upper Lufira (Katanga/DR Congo)." Tropicultura **28**(4): 246-252.

Mees, F., M. N. N. Masalehdani, et al. (2013). "Concentrations and forms of heavy metals around two ore processing sites in Katanga, Democratic Republic of Congo." Journal of African Earth Sciences **77**(0): 22-30.

Narendrula, R., K. Nkongolo, et al. (2012). "Comparative Soil Metal Analyses in Sudbury (Ontario, Canada) and Lubumbashi (Katanga, DR-Congo)." Bulletin of Environmental Contamination and Toxicology **88**(2): 187-192.

Nchito, M., P. W. Geissler, et al. (2004). "Effects of iron and multimicronutrient supplementation on geophagy: a two-by-two factorial study among Zambian schoolchildren in Lusaka." Transactions of the Royal Society of Tropical Medicine and Hygiene **98**(4): 218-227.

Nemery, B. (1990). "Metal toxicity and the respiratory-tract." European Respiratory Journal **3**(2): 202-219.

Nemery, B., E. K. Verbeken, et al. (2001). "Giant cell interstitial pneumonia (hard metal lung disease, cobalt lung)." Seminars in Respiratory and Critical Care Medicine **22**(4): 435-447.

Noel, L., R. Chekri, et al. (2012). "Li, Cr, Mn, Co, Ni, Cu, Zn, Se and Mo levels in foodstuffs from the Second French TDS." Food Chemistry **132**(3): 1502-1513.

Noel, L., J. C. Leblanc, et al. (2003). "Determination of several elements in duplicate meals from catering establishments using closed vessel microwave digestion with inductively coupled plasma mass spectrometry detection: estimation of daily dietary intake." Food Additives and Contaminants **20**(1): 44-56.

Prescott, E., B. Netterstrom, et al. (1992). "Effect of occupational exposure to cobalt blue dyes on the thyroid volume and function of femal plate painters." Scandinavian Journal of Work Environment & Health **18**(2): 101-104.

Richter, P. A. (2009). "Tobacco smoke exposure and levels of urinary metals in the US youth and adult population: the National Health and Nutrition Examination Survey (NHANES) 1999–2004." International Journal of Environmental Research and Public Health **6**(7): 1930.

Simonsen, L. O., H. Harbak, et al. (2012). "Cobalt metabolism and toxicology—A brief update." Science of the Total Environment **432**(0): 210-215.

Smolders, E. and J. Mertens (2013). Cadmium. Heavy Metals in Soils. B. J. Alloway, Springer. **22:** 283-311.

Tuakuila, J., D. Lison, et al. (2012). "Worrying exposure to trace elements in the population of Kinshasa, Democratic Republic of Congo (DRC)." International Archives of Occupational and Environmental Health **85**(8): 927-939.

USGS. (2013). "Mineral commodity summary, cobalt 2013. Accessible online at http://minerals.usgs.gov/minerals/pubs/commodity/cobalt/mcs-2013-cobal.pdf."

Ysart, G., P. Miller, et al. (1999). "Dietary exposure estimates of 30 elements from the UK Total Diet Study." Food Additives and Contaminants **16**(9): 391-403.