Metal mining and birth defects: a case-control study in Lubumbashi, Democratic Republic of the Congo

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Summary

Background Widespread environmental contamination caused by mining of copper and cobalt has led to concerns about the possible association between birth defects and exposure to several toxic metals in southern Katanga, Democratic Republic of the Congo (DRC). We therefore aimed to assess the possible contribution of parental and antenatal exposure to trace metals to the occurrence of visible birth defects among neonates.

Methods We did a case-control study between March 1, 2013, and Feb 28, 2015, in Lubumbashi, DRC. We included newborns with visible birth defects (cases) and healthy neonates born in the same maternity ward (controls). Mothers were interviewed about potentially relevant exposures, including their partners' jobs. Various trace metals were measured by inductively coupled plasma mass spectrometry in maternal urine, maternal blood, umbilical cord blood, placental tissue, and surface dust at home. Multivariable logistic regression analyses were done to calculate adjusted odds ratios and their 95% CIs (CI).

Findings Our study included 138 neonates with visible birth defects (about 0·1% of the 133 662 births in Lubumbashi during the study period) and 108 control neonates. Potential confounders were similarly distributed between cases and controls. Vitamin consumption during pregnancy was associated with a lower risk of birth defects (adjusted odds ratio 0·2, 95% CI 0·1–0·5). Mothers having paid jobs outside the home (2·8, 1·2–6·9) and fathers having mining-related jobs (5·5, 1·2–25·0) were associated with a higher risk of birth defects. We found no associations for trace metal concentrations in biological samples, except for a doubling of manganese (Mn; 1·7, 1·1–2·7) and zinc (Zn; 1·6, 0·9–2·8) in cord blood. In a separate model including placentas, a doubling of Mn at the fetal side of the placenta was associated with an increased risk of birth defects (3·3, 1·2–8·0), as was a doubling of cord blood Zn (5·3, 1·6–16·6).

Interpretation To our knowledge, this is the first study of the effects of mining-related pollution on newborns in sub-Saharan Africa. Paternal occupational mining exposure was the factor most strongly associated with birth defects. Because neither Mn nor Zn are mined in Lubumbashi, the mechanism of the association between their increased prenatal concentrations and birth defects is unclear.

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For the French translation of the abstract see Online for appendix 1

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Introduction

The southern part of the (former) province of Katanga in the Democratic Republic of the Congo (DRC) is part of the African Copperbelt and has been an area of intensive mining activities for centuries. In the colonial period, the mining of copper (Cu), cobalt (Co), and uranium (U), represented one of Belgian Congo's main sources of revenue.[1] After Congo's independence in 1960, mining activities and metal processing continued by the state-owned company Gécamines. These activities have increased again, since the beginning of the 21st century, largely because of the need for Cu, Co, and other metals for the industrial development of fast-growing Asian economies and lithium ion batteries.[2] Artisanal mining has become widespread in Katanga in the past 20 years, with tens of thousands of young people (including children) working as so-called diggers (or creuseurs) or in ancillary jobs in poorly regulated and often dangerous working conditions.[2,3] Metal furnaces have also been built, often in close vicinity of residential areas, with little regard for environmental issues.

These activities have resulted in widespread contamination of the environment by various trace metals. Our group has previously shown that people living near mining or metallurgical activities in south Katanga are highly exposed to Co, arsenic (As), and U.[4–7] In a case study[5] among mineworkers and residents of an urban neighbourhood that had been transformed into an artisanal cobalt mine, we documented very high concentrations of Co, U and manganese (Mn) in urine and blood, especially among children, in whom there was also evidence of exposure-related oxidative DNA damage.

Research in context

Evidence before this study

The African Copperbelt, a mining region in Zambia and the Democratic Republic of the Congo (DRC), is one of the ten most polluted areas worldwide, mainly because of metal mining for electric cars and mobile phone batteries. The population of Lubumbashi is subject to serious health threats owing to a combination of poverty, poor governance, and major environmental pollution. High concentrations of several toxic metals have been reported for the general population of Katanga, but the adverse health effects of this pollution have hardly been investigated.

Worldwide, major birth defects occur in one in 33 births, and the aetiology is unknown in 80% of cases. The incidence of birth defects is rising worldwide, with the increase of risk factors, such as maternal diabetes and obesity, and new threats such as the Zika epidemic and air pollution. Even if they are likely only a small component of a complex multifactorial system, environmental pollutants, including toxic metals, might have a contributing role. We searched PubMed for studies related to effects of toxic metals on birth defects between database inception and Aug 1, 2019, using the terms "metals," and "birth defects" or "congenital malformations". We used the reference lists of related articles to identify other studies.

Maternal exposure to lead (Pb) during pregnancy has been associated with congenital abnormalities among offspring, including cardiac malformations. Exposure to other trace elements that are associated with modern industrial processes and are absorbed from cigarette smoke, such as cadmium and chromium, were also reported to increase the likelihood of birth defects in animals and possibly in humans. Copper (Cu) is an essential element required in trace concentrations for normal physiology. Small case-control studies reported greater Cu and Pb concentrations among mothers who had an elective abortion because of a neural tube defect, and greater placental manganese (Mn) concentrations in babies with a neural tube defect. A suggestive association was found between increased placental mercury concentrations (mostly because of maternal seafood consumption) and higher risk of neural tube defect in offspring from a Chinese population. Generally, very little is known about the effects of prenatal metal exposure on neonatal outcomes.

Added value of this study

To our knowledge, this is the first epidemiological study addressing a long-existing data gap—the association between congenital malformations and prenatal metal exposure through paternal mining jobs in sub-Saharan Africa. Metal concentrations measured in different matrices of our study population were greater than reported in previous studies of pregnant women. We show an association between birth defects and fathers with a mining-related job, mothers with a paid job, and concentrations of Mn and Zn in cord blood and placental tissue.

Implications of all the evidence available

While further evidence is accumulating, it is imperative to develop sustainable mining and metal processing practices, to minimise prenatal exposure to heavy metals, especially in low-income and middle-income countries.

Fetal damage is one of the potential effects of exposure to toxic metals and might present in the form of intrauterine growth retardation or congenital malformations, resulting from various mechanisms (eg, teratogenesis or chromosomal damage). Evidence of reproductive or developmental toxicity of metals exists in animal models—often at high doses.[8] Metals like lead (Pb), cadmium (Cd), chromium (Cr), Cu, and mercury (Hg) have received most attention as possible causes of human congenital malformations.[9–15] One study[10] also found an association between Mn and neural tube defects, but the evidence in humans remains sparse. Owing to concerns expressed by clinicians, media, and civil society, we aimed to assess the possible contribution of parental and antenatal trace metal exposure to the occurrence of visible birth defects among neonates in Lubumbashi, DRC.

Methods

Study design and participants

We did a case-control study in Lubumbashi, DRC. Given the absence of previous information on the subject of our study, we were unable to do a power calculation. In such an explorative setting, and given the logistical and practical conditions, we intended to include as many cases in a period of 2 years as possible, with a 1:1 proportion for cases and controls.

A reporting system was set up to include all neonates born with birth defects in all maternity wards in Lubumbashi in a 2-year period (from March 1, 2013, to Feb 28, 2015). With the approval and help from the city's health authorities, the midwives from all state-run and private health stuctures were instructed to call a central phone number for each neonate (livebirths, stillbirths, or late fetal deaths defined as >22 weeks gestational age) born with a visible birth defect, except isolated polydactyly and club foot. We did not include isolated post-axial polydactyly because this anomaly is frequent in Africans, and isolated club foot because we considered this very unlikely to be caused by metal exposure.[16] For each included case, the midwife received US$10. Within 24 h of receiving a phone call, one of five study doctors (TKK, TLK, LKN, SMM, and PMO) went to the maternity ward to examine and photograph the neonate in a systematic way, administer a questionnaire to the mother, and take samples. During this visit, the study doctor would also try to recruit a normal neonate born in the same maternity ward just before or within 3 days of the birth of the case, from a mother of preferably similar age (±3 years) as that of the case. For some cases, no control

could be recruited because of logistical reasons or because no woman was available or willing to participate. Anthropometrics (birthweight, length, and head circumference) were expected to differ between newborns with birth defects and healthy controls (either because of the malformation itself or because of associated prematurity).[17] Therefore, we did not include these parameters as outcomes or covariates.

The study protocol was developed after consultations with various stakeholders (including representatives of local health authorities and civil society) and approved by the ethical committee of the University of Lubumbashi. Oral and written, informed consent was obtained from mothers before administering the questionnaire.

Procedures

Mothers were interviewed face-to-face on the day of the delivery or the day after, using a questionnaire in French or Swahili, about their medical and obstetrical history, socioeconomic variables, personal habits during pregnancy, and residential and occupational exposures. Respondents received $10.

We calculated a socioeconomic score on the basis of various household characteristics (appendix 2 pp 5–6). To define occupational exposures, the jobs reported in the questionnaire were later coded into relevant categories (appendix 2 pp 5–6).

Within 24 h of delivery, mothers received a 40 mL polystyrene vial with screw cap (Plastiques-Gosselin, Hazebrouck, France) at the maternity ward where they delivered and were instructed to urinate directly into the vessel, avoiding external contamination. Urine was aliquoted later into 1·5 mL cryovials. Venous blood was also obtained from mothers from a brachial vein using Beckton Dickinson (Franklin Lakes, NJ, USA) Vacutainer tubes (4 mL in a spray-coated K_2EDTA tube, 4 mL in a lithium heparin tube, and 4 mL in a dry tube). Whenever possible, umbilical venous cord blood was also sampled within 4 h of birth or, if no cord blood could be obtained (not available or coagulated), blood was obtained by peripheral venipuncture. If the placenta was still available upon arrival of the study doctor, samples of a few grams were taken from a central spot at the fetal side of the placenta and from a central spot at the maternal side and placed in the same type of vessel as for urine samples. All samples were kept frozen (–20°C) at the laboratory of the Unit of Toxicology and Environment of the University of Lubumbashi (Lubumbashi, DRC) from the day of sampling until their transportation in isothermal boxes, by commercial flights in successive batches (last flight in November, 2015), to Belgium, where they were again kept frozen (–80°C) until analysis.

In about half of the participants, home visits were done (by DKwK) within a few weeks after their inclusion. During these visits, surface dust was collected, as in our previous surveys,[7] with a dustpan (by sweeping a surface of approximately 1 m²) from the yard in front of the house (outdoor dust), and also from inside the house (usually the living room) if the participants consented. Samples of drinking water were obtained as indicated by the participants (communal tap water or wells).

Congenital malformations were grouped by an experienced clinical geneticist (KD) on the basis of clinical descriptions and detailed photographs into a group of clearly recognisable genetic syndromes (eg, trisomy 13 or 21), malformations of unknown cause (possible genetic or multifactorial cause), and in-utero acquired malformations (eg, caused by amniotic bands). Regardless of these categories, cases were also classified according to the organ systems affected (multiple

	Cases (N=138)	Number of valid answers	Controls (N=108)	Number of valid answers	p value
Mother					
Age, years	28·5 (7·0)	131	26·5 (6·8)	106	0·028
Previous pregnancies	..	130	..	105	0·081
0	28 (22%)	..	32 (30%)	..	..
1–4	59 (45%)	..	51 (49%)	..	..
≥5	43 (33%)	..	22 (21%)	..	..
Education	..	117	..	101	0·15
None	10 (9%)	..	3 (3%)	..	..
Up to secondary	39 (33%)	..	30 (30%)	..	..
Above secondary	68 (58%)	..	68 (57%)	..	..
Paid job*	..	126	..	108	0·056
No paid job	72 (57%)	..	78 (72%)	..	..
White collar job	49 (39%)	..	27 (25%)	..	..
Blue collar job	5 (4%)	..	3 (3%)	..	..
Socioeconomic status†	..	120	..	104	0·16
Low	39 (33%)	..	22 (21%)	..	..
Medium	75 (63%)	..	75 (72%)	..	..
High	6 (5%)	..	7 (7%)	..	..
Father					
Age, years	35·1 (8·2)	101	33·7 (7·8)	93	0·21
Education	..	98	..	86	0·32
None	2 (2%)	..	3 (3%)	..	..
Up to secondary	27 (28%)	..	16 (19%)	..	..
Above secondary	67 (70%)	..	67 (78%)	..	..
Paid job	..	123	..	105	0·094
No paid job	13 (11%)	..	6 (6%)	..	..
White collar job	48 (39%)	..	55 (52%)	..	..
Blue collar job	62 (50%)	..	44 (42%)	..	..
Newborn					
Gestational age, weeks	38·2 (3·4)	108	38·3 (3·7)	91	0·84
Sex	..	127	..	103	0·19
Male	54 (43%)	..	51 (50%)	..	..
Female	70 (55%)	..	52 (50%)	..	..
Ambiguous	3 (2%)	..	0 (0%)	..	..

Continuous variables are presented as mean (SD) and their p values were calculated by *t* test. Categorical variables are presented as n (%) and their p values were calculated by χ^2 or Fisher exact tests. *Retained in the multivariable analyses shown in table 4 and 5. †Socioeconomic status is based on education of the mother, meat consumption, and family assets (appendix 2 pp 5–6).

Table 1: **Sociodemographic characteristics of parents and newborns**

See Online for appendix 2

	Cases (N=138)	Number of valid answers	Controls (N=108)	Number of valid answers	p value
Previous abortions (for multiparae)*	..	102	..	69	0·41
None	69 (68%)	..	46 (67%)	..	..
One	22 (22%)	..	19 (28%)	..	..
More than one	11 (11%)	..	4 (6%)	..	..
Previous stillbirths (for multiparae)*	..	103	..	73	0·36
None	86 (83%)	..	65 (89%)	..	..
One	9 (9%)	..	6 (8%)	..	..
More than one	8 (8%)	..	2 (3%)	..	..
Dead infants (for multiparae)*	..	106	..	77	0·37
None	99 (93%)	..	73 (95%)	..	..
One	7 (7%)	..	3 (4%)	..	..
More than one	0 (0%)	..	1 (1%)	..	..
Congenital malformations in family*	8 (7%)	111	0 (0%)	92	N/A
Diabetes	0 (0%)	130	1 (1%)	107	0·27
Hypertension	1 (1%)	128	3 (3%)	108	0·24
Pre-eclampsia third trimester	15 (13%)	114	12 (12%)	99	0·82
Antenatal care followed	111 (87%)	128	97 (92%)	106	0·25
Antenatal care start	..	88	..	69	0·64
First trimester	13 (15%)	..	13 (19%)	..	..
Second trimester	61 (69%)	..	48 (70%)	..	..
Third trimester	14 (16%)	..	8 (12%)	..	..
At least one ultrasound in pregnancy	46 (37%)	124	50 (49%)	103	0·082
Urogenital infections	81 (67%)	121	58 (56%)	103	0·10
Malaria	89 (68%)	131	70 (65%)	107	0·68
Vitamins before pregnancy*	12 (10%)	118	7 (13%)	95	0·48
Vitamins during pregnancy†	68 (56%)	122	73 (74%)	99	0·0056
Geophagy	111 (83%)	132	86 (80%)	108	0·561
Geophagy categories	..	132	..	108	0·611
Never	21 (16%)	..	22 (20%)	..	..
Occasionally	42 (32%)	..	30 (28%)	..	..
Often	69 (52%)	..	56 (52%)	..	..
Any medicine	107 (86%)	124	96 (93%)	103	0·017
Traditional medicines	12 (10%)	126	5 (5%)	102	0·19
Vaccines (at least one)†	62 (49%)	126	66 (64%)	103	0·024
X-rays	0	120	0	97	N/A
Smoking mother	2 (2%)	130	3 (3%)	105	0·49
Passive smoking	26 (27%)	96	13 (17%)	75	0·13
Alcohol	..	95	..	87	0·23
Never	75 (79%)	..	62 (71%)	..	..
Occasionally	18 (19%)	..	19 (22%)	..	..
Often	2 (2%)	..	6 (7%)	..	..
Antiseptic soap	61 (54%)	113	62 (67%)	92	0·051
Whitening cream	60 (62%)	97	44 (58%)	76	0·59
Fish consumption	..	118	..	101	0·068
Never	13 (11%)	..	3 (3%)	..	..
Occasionally	23 (19%)	..	20 (20%)	..	..
Often	81 (69%)	..	77 (76%)	..	..
Meat consumption	..	121	..	100	0·097
Never	41 (34%)	..	16 (16%)	..	..
Occasionally	68 (56%)	..	73 (73%)	..	..
Often	12 (10%)	..	11 (11%)	..	..

(Table 2 continues on next page)

defects, neural tube defects, orofacial defects, and other defects).

The concentrations of 13 elements (aluminium [Al], vanadium [V], Cr, Mn, Co, nickel [Ni], Cu, zinc [Zn], As, molybdenum [Mo], Cd, Pb, and U) were measured by inductively coupled argon plasma mass spectrometry, with quality control or assurance procedures (appendix 2 p 6), in the laboratory of the Division of Soil and Water Management of KU Leuven (Leuven, Belgium). Six metals (Al, V, Cr, Ni, Mo, and U) were not considered in the analyses because more than 40% of biological samples and more than 50% of blood samples had concentrations below the limit of quantification. For the other elements, a value of half the batch-specific limit of quantification was assigned for concentrations below the limit of quantification .

The concentration of creatinine in urine was measured in the central laboratory of Leuven University Hospital (Leuven, Belgium), and used to correct metal concentrations for urinary dilution. As recommended, urine samples with creatinine concentrations of less than 0·3 g/L or more than 3·0 g/L were excluded from analysis.[18] Samples were discarded after review by DVB and BN if they had very high metal concentrations, suggesting external contamination at some stage in sample processing.

Statistical analysis

Data management and statistical analyses were done with SPSS (version 24.0). Many variables had missing values, because some questionnaires were not completed fully or adequately, and because a full set of biological samples was not always available for each participant (eg, sample not taken, insufficient volume, or broken or lost tube), but we did not attempt to impute values.

We compared cases and controls by χ^2 or Fisher's exact test for categorical variables, and by Student's *t* test for continuous variables. Because metal concentrations were not normally distributed (Kolmogorov-Smirnov test), we log-transformed all metal values for statistical analyses. To compare subcategories of congenital malformations, we did a one-way analysis of variance, followed by Dunnett's post-hoc test.

We then analysed data by multivariable analysis. We built stepwise forward logistic regression models to calculate adjusted odds ratios (aORs) and their 95% CIs, adjusting for potential confounders—ie, sociodemographic and environmental variables differing between cases and controls with a p value of less than 0·2 in the bivariate analysis. In the first model, we included metal concentrations in maternal blood and urine and in cord blood stepwise. We included placentas only in a second model, because placentas were available in fewer subjects, thus reducing power. All possible explanatory variables, except those mentioned in the final model, were eliminated from the logistic regression during the stepwise process. An additional model included

interactions between the retained metals. We made no adjustments for multiple comparisons.

Variance inflation factors for metal concentrations within matrices and between matrices did not indicate multicollinearity problems, except in the case of metals in surface dust, where concentrations were very similar in indoor and outdoor dust samples. Therefore, indoor dust was not accounted for in the logistic regression models. Furthermore, standard errors did not indicate multicollinearity problems while building the logistic regression models.

To check the robustness of our findings, we repeated all comparisons done by *t* test on logarithmically transformed metal values by a non-parametric Mann-Whitney test. We also did a paired analysis for the bivariate comparisons. In a sensitivity analysis we repeated the analyses without a batch of cord blood samples that had been analysed later than the majority of samples. In an additional analysis, confounders possibly associated with birth defects in literature (also those with p>0·2 in bivariate analysis)—ie, infections, diabetes, alcohol consumption, and malaria—were added to our logistic regression models.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

We enrolled 138 cases with visible external malformations (about 0·1% of the 133 662 births in Lubumbashi during the study period) and 108 normal controls over a period of 2 years. As indicated in the methods, complete data were not available for all participants, due to occasional refusals or inability to respond to some questions and a number of incompletely or poorly filled out questionnaires.

The mothers of cases were on average 2 years older (mean age 28·5 [SD 7·0]) than the mothers of controls (mean age 26·5 [SD 6·8]; table 1). Besides the proportion of primiparae and grand multiparae, there were no significant differences in personal or obstetrical history or environmental exposures between cases and controls (table 2). However, mothers of cases and controls did differ significantly regarding vaccinations during pregnancy (49% of cases received at least one vaccine *vs* 64% of controls, p=0·024) and consumption of vitamins during pregnancy (56% among cases *vs* 74% among controls, p=0·0056; table 2). The proportion of mothers with a paid job outside the home was larger among cases (43%) than among controls (28%), but not significantly (p=0·056). None of the women declared that they were working or had worked in mines or smelters (table 2).

Fathers were, on average, about 7 years older than the mothers (although many women could not tell how old their partners were exactly) with no difference in mean age between cases and controls. However, the fathers did differ by occupation, with 22 (18%) having a mining-related job among cases compared with eight men (8%) among controls (p=0·022; table 2). We provide details on the jobs of the 30 men with mining exposure, with a description of their infants, in appendix 3.

See Online for appendix 3

Mean (reported) gestational ages did not differ between cases (38·2 weeks, SD 3·4, n=108) and controls (38·3 weeks, SD 3·7, n=91; table 1). All congenital malformations were grouped into a group of 12 neonates with clinically recognisable genetic syndromes (eg, trisomy 13 or 21), 118 neonates with malformations of unknown cause (including 16 with a possible genetic cause and 70 with a multifactorial cause), five neonates with in-utero acquired malformations (eg, caused by amniotic bands), and three cases without photographs and insufficient clinical descriptions to be classified. Categorised by the organ systems affected, 45 cases had multiple defects, 21 had neural tube defects (mainly spina bifida, anencephaly, or encephalocele), 21 had orofacial defects (mainly cleft lip or palate), and 48 had other defects (appendix 2 p 2; appendix 3).

As recommended, urine samples with creatinine concentrations less than 0·3g/L (n=14) or more than 3g/L (n=8) were excluded from analysis.[18] One maternal blood sample and four cord blood samples were discarded after review by DVB and BN, because they had very high metal concentrations, suggesting external contamination. In general, cases and controls did not differ with regard to metal concentrations in maternal blood, maternal urine (creatinine-corrected), cord blood, and placenta (appendix 3). However, Mn and Zn in cord blood and Mn at the fetal side of the placenta were significantly more concentrated in cases than in

	Cases (N=138)	Number of valid answers	Controls (N=108)	Number of valid answers	p value
(Continued from previous page)					
Cooking with charcoal	116 (100%)	116	100 (100%)	100	N/A
Cooking outside	70 (93%)	75	62 (100%)	62	0·038
Cooking inside	71 (93%)	76	45 (98%)	46	0·28
Live close to mining‡	22 (19%)	117	11 (12%)	95	0·15
Asphalt road nearby	14 (13%)	112	17 (17%)	99	0·34
Non-asphalt road nearby	90 (81%)	111	81 (83%)	98	0·77
Mother's mining exposure§	..	126	..	108	N/A
No obvious exposure	126 (100%)	..	108 (100%)	..	..
Definite exposure	0 (0%)	..	0 (0%)	..	..
Father's mining exposure father†§	..	123	..	105	0·022
No obvious exposure	101 (82%)	..	97 (92%)	..	..
Definite exposure	22 (18%)	..	8 (8%)	..	..

Data are n (%). p values were calculated by χ^2 or Fisher exact tests. N/A=not applicable. *Data not relating to the current pregnancy. †Retained in multivariable analysis. ‡Close to mining was defined as mining visible from home. §Definite exposure in the mining exposure category included diggers, ore smelters, metallurgical workers, or people selling or transporting minerals.

Table 2: **Personal data obtained from the questionnaire**

	Manganese				Zinc			
	<LOQ	Cases, geometric mean (IQR)	Controls, geometric mean (IQR)	p value	<LOQ	Cases, geometric mean (IQR)	Controls, geometric mean (IQR)	p value
Mother's blood, µg/L	4%	16·4 (12·1–24·0); n=126	15·9 (10·6–21·3); n=100	0·68	0%	5056 (4145–6184); n=126	4967 (4101–5882); n=100	0·70
Cord blood, µg/L	12%	48·6 (33·3–84·4); n=80	33·5 (26·2–46·7); n=60	0·0036	0%	4692 (3129–6096); n=80	3288 (2238–4225); n=60	0·0020
Mother's urine, µg/g creatinine	18%	0·9 (0·5–1·7); n=91	0·9 (0·4–1·8); n=83	0·97	0%	478 (326–861); n=91	448 (306–746); n=83	0·61
Placenta fetal side, mg/kg wet matter	0%	0·2 (0·12–0·24); n=39	0·1 (0·08–0·15); n=48	0·0027	0%	11 (8·6–12·2); n=39	9·9 (7·4–12·1); n=48	0·17
Placenta maternal side, mg/kg wet matter	0%	0·15 (0·10–0·20); n=19	0·14 (0·11–0·18); n=38	0·46	0%	11·8 (8·8–13·5); n=19	10 (8·1–11·9); n=38	0·15

All other measured metals with their bivariate significance are shown in appendix 3. LOQ=limit of quantification.

Table 3: **Manganese and zinc in biological matrices in mother-child pairs with a congenital malformation (cases) versus controls**

	Adjusted odds ratio (95% CI)	p value
Mining exposure father*	5·5 (1·2–25·0)	0·025
Mother with paid job	2·8 (1·2–6·9)	0·020
Vitamins during pregnancy	0·2 (0·1–0·5)	0·0010
Doubling of manganese in cord blood	1·7 (1·1–2·7)	0·026
Doubling of zinc in cord blood	1·6 (0·9–2·8)	0·086

N=118. Nagelkerke R^2=0·36. All possible explanatory variables with a bivariate p value <0·2, except those mentioned in this table, were eliminated from the logistic regression during the stepwise process and thus not retained in the final model. *Mining-related jobs include diggers, ore smelters, metallurgical workers, or people selling or transporting minerals.

Table 4: **Adjusted odds ratios of variables retained in a multivariable logistic regression model (not including placentas)**

	Adjusted odds ratio (95% CI)	p value
Vitamins during pregnancy	0·1 (0·02–0·5)	0·0076
Doubling of manganese at foetal side of placenta	3·3 (1·2–8·0)	0·021
Doubling of zinc in cord blood	5·3 (1·6–16·6)	0·0049

N=62. Nagelkerke R^2=0·54. All possible explanatory variables with a bivariate p-value <0·2, except those mentioned in this table, were eliminated from the logistic regression during the stepwise process and thus not retained in the final model.

Table 5: **Adjusted odds ratios of variables retained in a multivariable logistic regression model including placentas**

controls, in both parametric and non-parametric tests (table 3). Similarly, no differences in residential surface dust and drinking water were found, except that Cu and Co were more concentrated in residential dust (indoor and outdoor) obtained from controls than from cases (appendix 3), but these associations were not maintained in the multivariable models.

In the first stepwise logistic regression model, which included all metal concentrations in blood or urine, we found that a doubling of cord blood Mn (aOR 1·7, 95% CI 1·1–2·7) and Zn (1·6, 0·9–2·9) was associated with a greater risk of visible birth defects. In the same model vitamin consumption during pregnancy was associated with a lower risk of birth defects (0·2, 0·1–0·5), whereas mothers with a paid job outside the home (2·8, 1·2–6·9) and fathers with a mining-related job (5·5, 1·2–25·0) were associated with a higher risk of birth defects (table 4). In the second logistic regression model, which also included data from placentas, a doubling of Mn at the fetal side of the placenta (3·3, 1·2–8·0) and, doubling of Zn in cord blood (5·3, 1·6–16·6) were associated with an increased risk of birth defects, while taking vitamins during pregnancy (0·1, 0·02–0·5) was associated with a decreased risk (table 5). When interactions were added to the first model (without placentas), the interaction Mn×Zn in cord blood was significantly associated with birth defects (1·2, 1·1–1·3), as well as the previously retained variables from the questionnaire with similar aORs (appendix 2 p 1). When interactions were added to the second model (including placentas), Mn×Zn in cord blood was again associated with a greater risk of birth defects (4·0, 1·5–10·8), as was the mining exposure of the father (9·1, 1·2–66·7). Taking vitamins during pregnancy (0·05, 0·01–0·3) was again associated with a reduced risk of birth defects (appendix 2 p 1).

We obtained similar results with a pairwise analysis of cases and controls and in a sensitivity analysis in which we left out 14 cord blood samples (that had been analysed separately) from the logistic regression models (appendix 2 p 3). The addition of confounders possibly associated with birth defects in the published literature (also those with p>0·2 in bivariate analysis)—ie, infections, diabetes, alcohol consumption and malaria—did not have significant effect on our final logistic regression model.

Similar associations were also observed between Mn and Zn concentrations and the general categories of congenital anomalies. Mn in cord blood and at the fetal side of the placenta were higher in cases for both the genetic cause (p=0·0065 and p<0·0001) and the unknown cause category (p=0·030 and p=0·068), but Zn in cord blood was significantly higher only for the unknown cause category (p=0·0029; appendix 2 p 4).

Subgroup analysis did not reveal one specific organ or developmental anomaly to be responsible for the significant differences found in the overall case-control analysis. Mn in cord blood was slightly more concentrated in cleft lip or palate cases, but not significantly so (p=0·078; n=11 *vs* 60 controls). Zn in cord blood (p=0·0085; n=45 *vs* 60 controls) and Mn on the fetal side of the placenta

(p=0·018; n=18 *vs* 48 controls) were significantly more concentrated for multiple malformations (appendix 2 p 4). Paternal occupational mining exposure was only significantly associated with birth defects in the unknown cause category (p=0·014), but not with any specific organ category. Taking vitamins during pregnancy was protective for neural tube defects (p=0·036) and multiple malformations (p=0·0080).

Discussion

This case-control study was designed to address widely expressed concerns in the Lubumbashi community about the impact of mining-related pollution on the occurrence of birth defects. We found that paternal mining jobs, maternal paid jobs, and prenatal Mn and Zn concentrations were associated with congenital malformations. However, we did not find higher metal concentrations in blood or urine of mothers (at the moment of delivery) of neonates with birth defects, than in control mothers. Only the concentrations of Mn and Zn in cord blood and at the fetal side of the placenta differed between cases and controls.

Before discussing these results, we wish to acknowledge the technical limitations, but also the merits of our study. Our study took place in a medically underserved setting. Therefore, a first shortcoming is that we were only able to include neonates born with visible external malformations, and not those with cardiac, renal, or other anomalies, let alone subtle neurodevelopmental defects. Our cases include a wide variety of external malformations, but, as in most African settings, we were unable to refine the diagnoses of congenital malformations by genetic investigations. The routine registration of birth defects at the time of delivery is known to often be very poor.[19] The conditions in which we had to administer the questionnaires to mothers (at a traumatic moment—ie, shortly after they had given birth to a child with a severe malformation), take photographs, and obtain biological samples were often precarious, especially during the night. These logistical constraints explain why our study was not perfect, with missing controls (only 108 controls for 138 cases), missing replies, and missing samples (cord blood was only available in 80 cases and 60 controls and a sample of the fetal side of the placenta in 39 cases and 48 controls). Of note, our study was done with little funding. Nevertheless, we are confident that these imperfections did not compromise the validity of our diagnoses, available questionnaire data, metal analyses, and statistical analyses.

An intrinsic limitation of our study is its case-control design and the absence of measurements before delivery, which precludes drawing causal inferences. Solving the issue of causality would require assessment of the incidence of congenital malformations in a longitudinal design. Case-control designs are appropriate for finding risk factors for relatively rare outcomes, such as congenital malformations, but they do not allow conclusions about their frequency of occurrence, unless all incident cases can be ascertained in the community. Because no previous data were available on mining exposure and congenital malformations, we did not want to limit our investigation to one category of malformations. Our target was to include all neonates born with visible malformations in Lubumbashi over a period of 2 years, but not all cases of congenital malformations were reported to our reporting system (and some reported cases could not be ascertained and included in the study for various reasons). In a separate study, we found that the birth registries of 63 of the 109 maternity wards in Lubumbashi—covering nearly half of births during our recruitment period (62 288 of 133 662 births)—included 282 neonates with birth defects, thus indicating that our case-control study captured less than half of the cases that had occurred in the Lubumbashi area. The same study revealed that the incidence of congenital malformations was higher in wards situated in health districts containing a mine or a smelter, when compared with health districts without mining.[20] Household air pollution (associated with neonatal mortality[21] but not with birth defects) cannot be completely ruled out as a potential confounder. The use of charcoal for cooking was reported by 100% of cases and controls, so this cannot be a confounder in itself. However, cooking indoors (probably more representative for household air pollution) was also a separate variable obtained from the questionnaire, but did not affect our statistical analysis. The HIV-status of mothers is a sensitive issue in Lubumbashi, which is why we did not include it in our questionnaire. Having to reveal HIV status could have led to false answers or to refusals to participate in the study and could, therefore, have introduced bias. However, HIV and antiretroviral therapy are not typically associated with congenital malformations nor with the exposure, and dolutegravir had not yet been introduced in Lubumbashi at the time of our study.[22,23]

For several metals (Al, V, Cr, Ni, Mo, and U), more than half of the measurements were under the limit of quantification in most matrices, which is why we could not include them in the analysis. This is particularly unfortunate for U, because there is much concern about this element in Katanga. Another limitation of our study is that the control group was also highly exposed to trace metals. This high background exposure is not surprising in view of our previous findings[4,7] and the fact that, by design, controls were recruited from the same maternity ward (and, therefore, the same community) as cases. We chose this procedure for obtaining controls, because a case-control design requires that cases and controls are recruited from the same source population, and we reasoned that recruiting controls from another town or from more affluent areas with less mining would be inappropriate. However, we recognise that selecting controls from the same area possibly masked the contribution of environmental contamination to the occurrence of birth defects. We are also aware that

the timing of our study might have obscured an association between prenatal exposure to pollutant metals and birth defects, because samples for measuring trace metals were obtained at delivery, and not during the crucial windows of exposure for causing congenital malformations, namely in early pregnancy or even before. The timing of sampling, as well as scientific ignorance of the toxicokinetics of most metals during pregnancy, is a well known and frequent difficulty in epidemiological studies into the causes of birth defects.[24] Even if potential confounders were accounted for sufficiently in our models, the very specific and highly polluted environment of Lubumbashi means that we have to be cautious about extrapolating our results to other mining areas and other regions in the world.

Nevertheless, our study has several strengths, which lend validity to the results. The distribution of measured potential confounders was similar in cases and controls, and adjustment for potential confounders in multivariable analyses did not affect the differences found between cases and controls. A paired comparison between cases and their controls confirmed the findings of the unpaired analysis. Reassuringly, because it is well known in medical literature,[25] taking vitamins during pregnancy proved to be a strong protective factor against congenital malformations in our study. Mothers could usually not specify which vitamins they had received from their health-care providers, but it is a local (and global) policy to prescribe folic acid for neural tube defect prevention. This finding lends credence to the questionnaire findings.

The cause of 80% of birth defects is unknown.[26] The early stages of life are vulnerable to exogenous chemical exposures because of rapid development and incomplete metabolic activities.[26,31,32] High toxic metal exposure has been reported previously in people living in the Katanga Copperbelt, including in Lubumbashi,[4] which is confirmed in our study where both case and control women had metal concentrations that are among the highest ever reported for pregnant women.[27,28] There are no standard reference values for metals like Mn, Zn, and Cu in cord blood, but we observed higher concentrations than reported in the published literature.[29] The metals discussed in this study might cross the placenta towards a developing fetus.[30] Possible biological mechanisms underlying the teratogenic effects of these metals include oxidative damage to the DNA and modification of epigenetic patterns.[31,32] Metals like Pb, Cd, Cr, Cu, and Hg have received most attention as possible causes of congenital malformations.[9,11–14]

Our cases and controls did not differ with regard to the biological concentrations of locally mined metals (Co and Cu) and the toxic metals we analysed (Cu, Co, As, Cd, and Pb). It is possible that the timing of sampling (at delivery) and the spatial proximity of cases and controls (recruited from the same maternity wards) explain the absence of any association between these metals and the risk of birth defects in our study. However, the simplest explanation is that there is no relationship between in-utero exposure to these metals and birth defects. The positive associations found for Mn and Zn were unexpected and difficult to explain. Mn and Zn concentrations differed between cases and controls solely in cord blood and at the fetal side of the placenta; their differences were consistent but not pronounced (less than the interquartile ranges within each group). Neither Mn nor Zn are mined in Lubumbashi, but both elements can accompany the copper and cobalt ores that are extracted and processed locally.[33] The high fetal concentrations of Mn and Zn could be proxies for the exposure to other metals in the area, either because of a high maternal exposure to these elements or, perhaps more likely, because of body distribution shifts of these elements. Even if we doubt that the mathematical interaction found in our study between Mn and Zn has biological relevance, growing evidence suggests that co-exposure to multiple metals can result in increased toxicity compared with single-metal exposure, particularly early in life.[34]

Mn is a trace element that is necessary for physiological processes, such as neuronal function, protein and energy metabolism, bone growth, and enzyme activation. During fetal and neonatal development, there is an increased need for Mn owing to its role in brain function and skeletal development.[35] High environmental Mn exposure has been associated with various negative neurodevelopmental outcomes among newborns and school-age children.[36–39] Increased Mn concentrations were also associated with an increased risk of neural tube defects in a case-control study from China.[10] In Kolwezi—a town 300 km from Lubumbashi where a neighbourhood was transformed into an artisanal cobalt mine—our group showed very high Co, U, and Mn values in urine in residents of that neighbourhood compared with a nearby control area.[5]

Zn is also an essential trace element in the body that is responsible for numerous structural and biochemical functions. Zn deficiency is closely associated with stunting, respiratory infections, diarrhoea, and dermatitis in children.[40] Several studies have also reported an association between maternal Zn deficiency and increased risk of birth defects, such as non-syndromic cleft lip or palate.[41] However, Zn toxicity is rare and has not, to our knowledge, been associated with congenital malformations.[40] Our results suggest that an association might exist between high Zn exposure and birth defects. The biological mechanisms of possible teratogenic effects of Mn and Zn are unknown.[31,32]

We have no explanation for the finding that newborns with birth defects appeared to have more mothers with a paid job outside the home. None of these women worked or had worked in mining or smelting. Our data do not indicate that socioeconomic or lifestyle factors could be responsible. Pregnant women in the Copperbelt of

South Katanga practice geophagy—the practice of soil eating—for pregnancy-related malaise. An exploratory study of 48 mother-child pairs in another region of DRC found that geophagy leads to prenatal lead exposure.[42] Geophagy is widespread in the Lubumbashi area, but we did not find a significant association between geophagy and congenital malformations in our study.

Paternal occupational mining exposure was the factor most strongly associated with birth defects in our study. Subgroup analysis showed significance specifically for the unknown cause category, which is more likely linked with exogenous causes. Thus, our study seems to support the widely held belief in the Lubumbashi area that men working in mining are at higher risk of having a child with a malformation. This paternal effect might explain why we found little to no association with metals in maternal blood or urine. Men working in mines might bring home metal-containing dust and thus contaminate their domestic environment, but this should have been reflected in higher metal concentrations among their wives, which was not the case, at least not at the time of delivery. One might speculate that the paternal effect on their offspring could have occurred preconceptionally through mutagenesis or epigenetic changes at the spermatozoid level.[31,32,43] Additional research is warranted to assess whether metal exposure before conception affects gene-specific DNA methylation profiles or other epigenetic processes and to characterise their effect on fetal development.[15]

Environmental metal exposure is a global health concern, especially in highly polluted areas, such as the African Copperbelt. Metal concentrations measured in mothers in our study were greater than previously reported in pregnant women.[27,28,44] Our study found associations between visible birth defects and paternal occupational mining exposure, maternal paid jobs, and prenatal Mn and Zn concentrations. These findings add to the growing concern about the toxicity of prenatal exposure to mining-related pollution. However, if environmental pollutants, such as metals, have a role in the aetiology of birth defects, they will be one component in a complex multifactorial system. Whether Mn and Zn, as by-products of the mining process, proxies of the entire metal mixture, or another unknown factor, have a role in possible genetic or epigenetic alterations, is unclear. Following the precautionary principle, our results call for sustainable mining practices to minimise exposure of fathers of childbearing age to toxic metals. Future studies should not only address the association between metal exposure of pregnant women throughout pregnancy longitudinally, but also focus on the father.

Contributors

BN and CBLN conceived and designed the study. TK-K, SM-M, TLK, LKN, and PMO went to the maternity wards to examine and photograph the neonates, administer a questionnaire to the mothers, and take samples under the supervision of CB. DKwM did the home visits and sampling. TK-K, PMO and DKwM were involved in the pre-analytical treatment of samples. DVB, TK-K, and BN assigned job categories by consensus and without knowledge of case or control status. KD diagnosed congenital malformations using clinical descriptions and detailed photographs, with help from TK-K. ES supervised the measurements of metal concentrations in blood, urine, placenta, dust, and water samples. DVB and TK-K cleaned and analysed the data. DVB did the statistical analysis under supervision of KVH and DA. DVB wrote successive drafts of the paper, with input from TK-K and under the supervision of KVH and BN. All authors approved the final version of the manuscript.

Declaration of interests

We declare no competing interests.

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References

1 Vellut JL. Mining in the Belgian Congo. In: Birmingham D, Martin PM, eds. Longman History of Central Africa. London: Longman, 1983: 126–63.
2 Global Witness. Digging in corruption. Fraud, abuse and exploitation in Katanga's copper and cobalt mines. Global Witness, 2006. https://cdn.globalwitness.org/archive/files/import/kat-doc-engl-lowres.pdf (accessed March 10, 2020).
3 Sovacool BK. The precarious political economy of cobalt: balancing prosperity, poverty, and brutality in artisanal and industrial mining in the Democratic Republic of the Congo. *Extr Ind Soc* 2019; **6:** 915–39.
4 Banza CL, Nawrot TS, Haufroid V, et al. High human exposure to cobalt and other metals in Katanga, a mining area of the Democratic Republic of Congo. *Environ Res* 2009; **109:** 745–52.
5 Banza Lubaba Nkulu C, Casas L, Haufroid V, et al. Sustainability of artisanal mining of cobalt in DR Congo. *Nat Sustain* 2018; **1:** 495–504.
6 Cheyns K, Banza Lubaba Nkulu C, Ngombe LK, et al. Pathways of human exposure to cobalt in Katanga, a mining area of the D.R. Congo. *Sci Total Environ* 2014; **490:** 313–21.
7 Smolders E, Roels L, Kuhangana TC, et al. Unprecedentedly high dust ingestion estimates for the general population in a mining district of DR Congo. *Environ Sci Technol* 2019; **53:** 7851–58.
8 Apostoli P, Telisman S, Sager PS. Reproductive and developmental toxicity of metals. In: Nordberg GF, Fowler BA, Nordberg M, Friberg LT, eds. Handbook on the toxicology of metals, 4th edn. Amsterdam: Academic Press-Elsevier, 2015: 214–19.
9 Jin L, Zhang L, Li Z, Liu JM, Ye R, Ren A. Placental concentrations of mercury, lead, cadmium, and arsenic and the risk of neural tube defects in a Chinese population. *Reprod Toxicol* 2013; **35:** 25–31.
10 Liu J, Jin L, Zhang L, et al. Placental concentrations of manganese and the risk of fetal neural tube defects. *J Trace Elem Med Biol* 2013; **27:** 322–25.
11 Goldberg SJ, Lebowitz MD, Graver EJ, Hicks S. An association of human congenital cardiac malformations and drinking water contaminants. *J Am Coll Cardiol* 1990; **16:** 155–64.
12 Thompson J, Bannigan J. Cadmium: toxic effects on the reproductive system and the embryo. *Reprod Toxicol* 2008; **25:** 304–15.
13 Liu Z, Yu Y, Li X, et al. Maternal lead exposure and risk of congenital heart defects occurrence in offspring. *Reprod Toxicol* 2015; **51:** 1–6.
14 Cengiz B, Söylemez F, Oztürk E, Cavdar AO. Serum zinc, selenium, copper, and lead levels in women with second-trimester induced abortion resulting from neural tube defects: a preliminary study. *Biol Trace Elem Res* 2004; **97:** 225–35.
15 Ou Y, Bloom MS, Nie Z, et al. Associations between toxic and essential trace elements in maternal blood and fetal congenital heart defects. *Environ Int* 2017; **106:** 127–34.
16 Watt AJ, Chung KC. Duplication. *Hand Clin* 2009; **25:** 215–27.
17 Kishimba RS, Mpembeni R, Mghamba J. Factors associated with major structural birth defects among newborns delivered at Muhimbili National Hospital and Municipal Hospitals in Dar Es Salaam, Tanzania 2011–2012. *Pan Afr Med J* 2015; **20:** 153.

18 Cocker J, Mason HJ, Warren ND, Cotton RJ. Creatinine adjustment of biological monitoring results. *Occup Med (Lond)* 2011; **61:** 349–53.

19 Salemi JL, Tanner JP, Sampat DP, et al. Evaluation of the sensitivity and accuracy of birth defects indicators on the 2003 revision of the U.S. birth certificate: has data quality improved? *Paediatr Perinat Epidemiol* 2017; **31:** 67–75.

20 Kayembe-Kitenge T. Incidence of congenital malformations and proximity to mining in Lubumbashi, DR Congo. Annual Conference of the International Society for Environmental Epidemiology; Utrecht, Netherlands; Aug 25–28, 2019 (abstr).

21 Patel AB, Meleth S, Pasha O, et al. Impact of exposure to cooking fuels on stillbirths, perinatal, very early and late neonatal mortality—a multicenter prospective cohort study in rural communities in India, Pakistan, Kenya, Zambia and Guatemala. *Matern Health Neonatol Perinatol* 2015; **1:** 18.

22 Delicio AM, Lajos GJ, Amaral E, Cavichiolli F, Polydoro M, Milanez H. Adverse effects in children exposed to maternal HIV and antiretroviral therapy during pregnancy in Brazil: a cohort study. *Reprod Health* 2018; **15:** 76.

23 Zash R, Holmes L, Diseko M, et al. Neural-tube defects and antiretroviral treatment regimens in Botswana. *N Engl J Med* 2019; **381:** 827–40.

24 Claus Henn B, Bellinger DC, Hopkins MR, et al. Maternal and cord blood manganese concentrations and early childhood neurodevelopment among residents near a mining-impacted superfund site. *Environ Health Perspect* 2017; **125:** 067020.

25 MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. *Lancet* 1991; **338:** 131–37.

26 Feldkamp ML, Carey JC, Byrne JLB, Krikov S, Botto LD. Etiology and clinical presentation of birth defects: population based study. *BMJ* 2017; **357:** j2249.

27 Hoet P, Jacquerye C, Deumer G, Lison D, Haufroid V. Reference values and upper reference limits for 26 trace elements in the urine of adults living in Belgium. *Clin Chem Lab Med* 2013; **51:** 839–49.

28 Woodruff TJ, Zota AR, Schwartz JM. Environmental chemicals in pregnant women in the United States: NHANES 2003-2004. *Environ Health Perspect* 2011; **119:** 878–85.

29 Lewicka I, Kocyłowski R, Grzesiak M, Gaj Z, Oszukowski P, Suliburska J. Selected trace elements concentrations in pregnancy and their possible role—literature review. *Ginekol Pol* 2017; **88:** 509–14.

30 Caserta D, Graziano A, Lo Monte G, Bordi G, Moscarini M. Heavy metals and placental fetal-maternal barrier: a mini-review on the major concerns. *Eur Rev Med Pharmacol Sci* 2013; **17:** 2198–206.

31 Pilsner JR, Hu H, Ettinger A, et al. Influence of prenatal lead exposure on genomic methylation of cord blood DNA. *Environ Health Perspect* 2009; **117:** 1466–71.

32 Ercal N, Gurer-Orhan H, Aykin-Burns N. Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. *Curr Top Med Chem* 2001; **1:** 529–39.

33 Thys T, Decree S, Burlet C, et al. Characterisation of heterogenite (CoOOH) from oxidized copper-cobalt deposits in the Katanga Copperbelt, D.R.Congo. Geologica Belgica Symposium; Ghent, Belgium; Sep 14–15, 2009 (abstr).

34 Valeri L, Mazumdar MM, Bobb JF, et al. The joint effect of prenatal exposure to metal mixtures on neurodevelopmental outcomes at 20–40 months of age: evidence from rural Bangladesh. *Environ Health Perspect* 2017; **125:** 067015.

35 Hurley LS. The roles of trace elements in foetal and neonatal development. *Philos Trans R Soc Lond B Biol Sci* 1981; **294:** 145–52.

36 Wasserman GA, Liu X, Parvez F, et al. Water manganese exposure and children's intellectual function in Araihazar, Bangladesh. *Environ Health Perspect* 2006; **114:** 124–29.

37 Bouchard MF, Sauvé S, Barbeau B, et al. Intellectual impairment in school-age children exposed to manganese from drinking water. *Environ Health Perspect* 2011; **119:** 138–43.

38 Yu XD, Zhang J, Yan CH, Shen XM. Prenatal exposure to manganese at environment relevant level and neonatal neurobehavioral development. *Environ Res* 2014; **133:** 232–38.

39 Takser L, Mergler D, Hellier G, Sahuquillo J, Huel G. Manganese, monoamine metabolite levels at birth, and child psychomotor development. *Neurotoxicology* 2003; **24:** 667–74.

40 Willoughby JL, Bowen CN. Zinc deficiency and toxicity in pediatric practice. *Curr Opin Pediatr* 2014; **26:** 579–84.

41 Jara-Palacios MA, Cornejo AC, Narváez-Caicedo C, et al. Plasma zinc levels in Ecuadorian mothers of infants with nonsyndromic cleft lip with or without cleft palate: a case series. *Birth Defects Res* 2018; **110:** 495–501.

42 Gundacker C, Kutalek R, Glaunach R, Deweis C, Hengstschläger M, Prinz A. Geophagy during pregnancy: is there a health risk for infants? *Environ Res* 2017; **156:** 145–47.

43 Baccarelli A, Bollati V. Epigenetics and environmental chemicals. *Curr Opin Pediatr* 2009; **21:** 243–51.

44 Musa Obadia P, Kayembe-Kitenge T, Haufroid V, Banza Lubaba Nkulu C, Nemery B. Preeclampsia and blood lead (and other metals) in Lubumbashi, DR Congo. *Environ Res* 2018; **167:** 468–71.