Assessing the contributions of metals in environmental media to exposure biomarkers in a region of ferroalloy industry

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Abstract

Residential proximity to ferroalloy production has been associated with increased manganese exposure, which can adversely affect health, particularly among children. Little is known, however, about which environmental samples contribute most to internal levels of manganese and other ferroalloy metals. We aimed to characterize sources of exposure to metals and evaluate the ability of internal biomarkers to reflect exposures from environmental media. In 717 Italian adolescents residing near ferromanganese industry, we examined associations between manganese, lead, chromium, and copper in environmental samples (airborne particles, surface soil, indoor/outdoor house dust) and biological samples (blood, hair, nails, saliva, urine). In multivariable regression analyses adjusted for child age and sex, a 10% increase in soil Mn was associated with increases of 3.0% (95% CI: 1.1%, 4.9%) in nail Mn and 1.6% (95% CI: –0.2%, 3.4%) in saliva Mn. Weighted-quantile-sum (WQS) regression estimated that higher soil and outdoor dust Mn accounted for most of the effect on nail Mn (WQS weights: 0.61 and 0.22, respectively, out of a

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total of 1.0). Higher air and soil Mn accounted for most of the effect on saliva Mn (WQS weights: 0.65 and 0.29, respectively). These findings can help inform biomarker selection in future epidemiologic studies and guide intervention strategies in exposed populations.

Keywords

Chromium; Copper; Ferroalloy; Lead; Manganese; Weighted quantile sum regression

1. Introduction

Metals in environmental media resulting from anthropogenic activities have been shown to have adverse health impacts on nearby communities, in particular on children (1–5). Anthropogenic sources of metals include gasoline combustion, mining, fungicide application, e-waste recycling, and emissions from iron and steel industry, such as ferroalloy plants. Ferroalloys are mixtures of iron (Fe), manganese (Mn), chromium (Cr), silica (Si), as well as other metals such as lead (Pb), zinc (Zn), copper (Cu), and cadmium (Cd) (6, 7). Ferroalloys represent one of the most significant anthropogenic sources of Mn to the environment (8). Several studies have identified associations between proximity to ferroalloy plants and increased levels of Mn in environmental media and exposure biomarkers (9–15). Similar relationships may be possible for other metals used in the ferroalloy process.

The body of evidence regarding environmental contamination in proximity to ferroalloy operations is concerning given the potential human health effects of exposure to Mn and other toxic metals. Although dietary Mn is critical for human brain development, studies have reported associations between excess Mn exposure and decrements in neurological function, especially in children (16, 17). Reported health effects include reduced scores on tests of verbal intelligence, learning and memory, and increased hyperactive behaviors (18–21). Some studies have also observed inverse U-shaped relationships between internal Mn and neurological function, consistent with Mn as both a necessary nutrient and a toxicant, in which children with the lowest and highest levels of Mn exhibited the poorest scores on neurobehavioral tests (3, 22, 23). The neurotoxic effects of other metals such as Pb have been well characterized (24, 25), while ferroalloy components such as Cr and Cu are less well studied with regards to their potential effects on the developing brain. Cr and Cu, like Mn, have essential biological functions and are metabolically regulated to meet physiologic need, but excess levels have also been linked with neurobehavioral and other health effects (26–29). Furthermore, health effects from metal mixtures, such as the composition of ferroalloy emissions, may be more severe than from each metal alone (30, 31).

Although there is strong evidence that exposure to metals is higher among individuals living near ferroalloy facilities and that adverse health effects may be linked with exposure to these metals, less is known about sources and pathways of exposure from environmental samples to internal dose (32). There is a need to better understand the relative contributions of different environmental media to metals biomarkers in order to support preventive strategies, even after industrial facilities close (33, 34).

Our study examines associations between levels of Mn, Pb, Cr, and Cu in environmental media (air, soil, dust) around children's homes and schools and their internal levels, estimated using exposure biomarkers (blood, hair, nails, saliva, urine). Using data from a cohort of children residing in Brescia Province, Italy, an area with a longstanding history of industrial ferromanganese activity, we are able to assess these relationships in a higher exposure setting. Previously, we examined household dust as a source of Mn exposure among a subset of our cohort using data available at the time (11). This study expands upon that analysis to include other environmental media and three additional co-occurring metals used in ferroalloy industry among participants of our full cohort. Our objectives were to characterize sources of exposure to Mn, Pb, Cr, and Cu among children, and to evaluate the ability of each biomarker to reflect exposures from environmental media. We employed a recently proposed statistical approach, weighted quantile sum (WQS) regression, to estimate the cumulative impact of all environmental media on each metal biomarker, as well as to examine relative contributions of each environmental medium (35). Understanding exposure sources can help inform risk assessment and exposure reduction strategies, and guide exposure assessment in future epidemiologic studies.

2. Methods

2.1 Description of study population

Subjects of this analysis were participants of the PHIME (Public Health Impact of Metals Exposure) study to examine associations between Mn exposure from anthropogenic emissions and neurodevelopmental outcomes among children. Subjects were enrolled from the northern Italian province of Brescia, from one of three subregions characterized by varying intensity of ferromanganese alloy industry: Bagnolo Mella (BM), with an actively operating plant that has been in operation since 1973; Valcamonica (VC), with three plants in operation historically from 1910 to 2001; and Garda Lake (GL), with no historic or current plant activity. Figure 1 displays a map of this region including locations of ferroalloy plants; an additional map is provided in Lucchini et al. (2007) (36). Participant enrollment took place in the public schools using a community based participatory approach described previously (37). For inclusion into the study, a child must 1) have been born into a family that had been living in the study area since at least the 1970s, 2) have lived in the study area since birth, and 3) be 10–14 years of age. Exclusion criteria included: 1) a pathological condition that may impact performance on neurodevelopmental testing, such as neurologic, hepatic, metabolic, endocrine, or psychiatric disease, 2) use of a medication with known neurological side effects, 3) clinically diagnosed motor impairments to the hands or fingers, 4) clinically diagnosed cognitive or behavioral impairment, 5) visual deficits not receiving adequate corrective measures, and 6) having ever received total parenteral nutrition. Of the 720 children enrolled in the study, 717 had metals data on at least one environmental sample and at least one biomarker, and comprise the final sample. Eligible children received a detailed explanation of study procedures before consenting to participate. The Institutional Review Boards at the Ethical Committee of the Department of Health of Brescia, Italy; the University of California, Santa Cruz; and the Icahn School of Medicine at Mount Sinai reviewed and approved all study materials.

2.2 Environmental and biological samples

Detailed descriptions of sample collection, preparation, and analysis have been provided elsewhere (11, 38). Environmental samples included air, surface soil, and indoor and outdoor household dust. For air sampling, children wore 24-hour Personal Environmental Monitors connected to Leland Legacy pumps (SKC, Inc., Eighty-Four, PA, USA) and personal air samples were analyzed for Mn, Pb, Cr, and Cu by TXRF (39). The children carried the pump in their backpacks, with the sampler attached to the front strap near the breathing zone. While at school, the children had the monitor placed near them; while sleeping, the monitor was in the child's bedroom (39). Soil samples were analyzed for concentrations of Mn, Pb, and Cu *in situ* using portable X-ray fluorescence (Thermo Scientific Niton, model XL3t) (38). Measurements were made on available bare, undisturbed surface soil near each home (e.g., front or back yard area). Indoor and outdoor household dust samples were collected by sweeping a measured horizontal area with a plastic brush into a plastic bag, or using a cyclone vacuum that deposited the sample into a collection jar. Outdoor dust was collected from available and accessible horizontal surfaces outside the home, such as windowsills or door frames. Dust samples were analyzed for concentrations and loading of Mn, Pb, Cr, and Cu using inductively coupled plasma optical emission spectroscopy (ICP-OES; Perkin-Elmer model Optima 4300 DV series).

Biological samples (blood, hair, fingernails, saliva, and urine) were collected from children concurrent with collection of environmental samples. The collection and analysis of biomarkers has been previously described (11, 38–40). Briefly, whole blood samples were collected with butterfly catheters into trace metal free vacutainers; hair samples were collected from the occipital lobe region proximate to the scalp using stainless steel scissors; fingernail clippings were collected with stainless steel nail clippers; and passive saliva samples were drawn into trace metal free microfuge tubes through a plastic straw. Hair and nail samples were cleaned with Triton and weak nitric acid, a cleaning method that has been demonstrated to effectively remove exogenous contamination (40). Metals concentrations (Mn, Pb, Cr, Cu) in all biomarkers were measured using magnetic sector inductively coupled plasma mass spectrometry (Thermo Element XR ICP-MS), as described elsewhere (40, 41). Most measurements were above the limits of detection (LODs); those below were assigned a value of one half the LOD. LODs for metals in environmental and biological samples are presented in Supplemental Table S1.

2.3 Covariate data

Standardized questionnaires were administered by trained study staff either at in-person visits or over the telephone to obtain information on sociodemographic factors. Data were collected on area of residence (Bagnolo Mella, Valcamonica, Garda Lake); birth order (first, second, third or higher); and years of mother's residence in study area. Socioeconomic status (low, medium, high) was calculated using methodology developed in Italy that combines occupation and parental education (37, 42).

2.4 Statistical analysis

Summary statistics and distributional plots were generated for all variables. Distributions of metals concentrations in all environmental samples and most biomarkers were right skewed;

therefore, we report medians and interquartile ranges. We natural log-transformed (ln-transformed) all metals concentrations to satisfy the model assumption of normality of residuals. Pairwise relationships between environmental media and biomarkers were examined using Spearman's correlations.

We estimated associations between metals concentrations in environmental samples and exposure biomarkers. For each metal, we first fit linear regression models of (ln-transformed) concentrations in all environmental media predicting (ln-transformed) concentrations in each biomarker (i.e., separate models for each biomarker). Because most correlations between environmental samples for each metal were moderate (Spearman r_s = 0–0.65), we were able to include all environmental media together in a single model for each biomarker. For example, blood Mn concentrations were predicted with the variables air Mn, soil Mn, indoor dust Mn, and outdoor dust Mn. This multivariable modeling approach is advantageous over simple pairwise correlations because it estimates associations for each environmental sample type, adjusting for other sample types. This approach, however, serves only as a first step to examining associations between environmental samples and biomarkers, because standard regression techniques are limited for examining independent and joint contributions of predictors with complex correlation structures (43–45).

Next, we employed weighted quantile sum (WQS) regression, an approach that estimates the cumulative contribution of a set of correlated predictors on an outcome and identifies which components in the set of predictors have the strongest associations with the outcome. This method has been described in detail previously (35, 44–47). Briefly, WQS regression simultaneously estimates empirical weights for each component in the set of predictors based on the associations between the individual component and the outcome, deriving a weighted index of predictors. The index can then be used as a variable in a standard linear regression model to estimate the association between the combined predictors and the outcome. Here, we used WQS regression to identify, for each metal, 1) the relative contributions of each environmental medium (air, soil, indoor dust, outdoor dust) to each biomarker, and 2) the association of a combined environmental exposure index ("cumulative environmental exposure") with each biomarker. The advantage of the WQS regression approach is that it estimates empirical weights for the contribution of each environmental medium to the internal biomarker, accounting for the complex correlation structure between concentrations of the same metal across different environmental media. Our *a priori* hypothesis was that the WQS index of cumulative environmental exposure would be positively associated with levels in biological samples. Effect estimates generated from WQS models represent the percentage change in biomarker level for a 25% (quartile) increase in the environmental exposure index. We ran separate WQS regression models for each biomarker (i.e., five models per metal), as we did using standard regression. All models were adjusted for child's sex and age, as these variables may influence non-dietary ingestion of soil and/or dust and may affect metabolism of some metals.

We conducted sensitivity analyses to evaluate the robustness of our findings. 1) To examine dust surface loadings versus dust concentrations, we fit all models using indoor and outdoor dust surface loadings, in place of concentrations. 2) To evaluate whether exposure sources may be different in higher exposure settings compared to lower exposure settings, we fit

WQS models including only children who lived in areas with current or past ferroalloy industry (subregions BM and VC). Similarly, we fit WQS models including only children who lived in areas with low current exposure to ferroalloy (subregions VC and GL). We also stratified by distance to nearest ferroalloy plant (median distance of 3.2 kilometers vs. > 3.2 kilometers) and fit WQS models for Mn. All statistical analyses were conducted using SAS 9.4 (SAS Institute, Cary, North Carolina, USA) and R version 3.3.2 (The R Foundation for Statistical Computing, www.r-project.org). The R package 'gWQS' was used to fit WQS models (48).

3.   Results

3.1   Descriptive Statistics

Characteristics of study participants are summarized in Table 1. The average age ± SD of participants at time of enrollment and sampling was 12.8 ± 0.9 years. Males and females comprised 52% and 48%, respectively, of the sample. Over half (52.9%) of the participants were from medium socioeconomic status (SES) families, while 23.9% were from low and 23.2% were from high SES families. Participants' mothers had resided in the study area for an average of 39 years, and approximately one-third of the children resided in each of the three subregions of Valcamonica (36.1%), Garda Lake (34.3%) and Bagnolo Mella (29.6%). Median distance to nearest ferroalloy plant was 1 to 2 km in Bagnolo Mella and Valcamonica, compared to 33 km in Garda Lake.

Metals concentrations in environmental media and biomarkers are summarized in Table 2. Within environmental samples, Mn levels were positively correlated, with a strong correlation between outdoor dust and indoor dust ($r_s = 0.65$, $p < 0.001$), and moderate correlations between air and dust (outdoor dust: $r_s = 0.34$, $p < 0.001$; indoor dust: $r_s = 0.27$, $p < 0.001$) (Table 3). Other correlations of Mn between environmental samples were weak. Several positive correlations were estimated between Mn in environmental samples and biological samples: nail Mn was moderately correlated with all environmental media (air: $r_s = 0.17$, $p < 0.001$; soil: $r_s = 0.22$, $p < 0.001$; indoor dust: $r_s = 0.23$, $p < 0.001$; outdoor dust $r_s = 0.27$, $p < 0.001$). Saliva Mn was moderately correlated with air Mn ($r_s = 0.23$, $p < 0.001$). Weaker correlations were found between environmental media and hair as well as urine. Blood Mn was not correlated with any environmental medium and weakly *negatively* correlated with other biomarkers (Table 3). Correlations for Pb, Cr, and Cu were generally weaker and less consistent than those for Mn (Supplemental Table 2). Pb levels in outdoor dust were positively correlated with levels in indoor dust ($r_s = 0.24$, $p < 0.05$), and blood Pb was moderately correlated with Pb in other biological samples (e.g., hair: $r_s = 0.22$, $p < 0.05$; urine: $r_s = 0.26$, $p < 0.05$) (Supplemental Table 2). There were also several moderate negative correlations, e.g., soil and saliva Pb: $r_s = -0.20$; blood and saliva Cr: $r_s = -0.23$; indoor dust and blood Cu: $r_s = -0.23$ (Supplemental Table 2).

3.2   Standard regression approach

In adjusted linear regression models estimating associations between Mn in environmental samples and biomarkers, nail Mn was associated with all environmental samples, with strongest associations estimated for soil (Table 4). After adjusting for air Mn, indoor and

outdoor dust Mn, child sex and age, a 10% increase in soil Mn was associated with a 3.0% increase in nail Mn (β=0.30, 95% CI: 0.11, 0.49; percent change calculated as $[(1.01)^\beta - 1] * 100$). Saliva Mn was also positively associated with air and soil Mn: a 10% increase in air or soil Mn was associated with a 2.2% or 1.6% increase, respectively, in saliva Mn (air: β=0.22, 95% CI: 0.05, 0.39; soil: β=0.16, 95% CI: −0.02, 0.34). Of the five Mn biomarkers (blood, hair, nails, saliva, urine), variability in saliva Mn was best explained by model predictors (adjusted $R^2 = 0.13$), suggesting that an estimated 13% of the variability in saliva Mn concentrations could be attributed to Mn levels in air, soil, indoor dust, and outdoor dust, as well as child age and sex. Other associations of Mn in environmental and biological samples were weak and close to null.

For Pb, Cr, and Cu, most associations were weaker than for Mn. Outdoor dust Cr was positively associated with blood Cr: a 10% increase in outdoor dust Cr was associated with a 1.0% increase in blood Cr (β=0.10, 95% CI: 0.01, 0.18) (Table 4). Pb levels in environmental media were *negatively* associated with saliva Pb (e.g., soil: β= −0.24, 95% CI: −0.47, −0.01; indoor dust: β= −0.23, 95% CI: −0.48, 0.02). Cu in indoor dust was also negatively associated with blood Cu, but the magnitude of the association was small (β= −0.06, 95% CI: −0.10, −0.03).

3.3 Weighted Quantile Sum Regression

Results from WQS regression analyses of environmental media predicting internal biomarkers are presented in Table 5. For Mn, increases in the weighted environmental exposure index were associated with increases in nail, saliva, and hair Mn (nail: β=0.45, 95% CI: 0.26, 0.64; saliva: β=0.30, 95% CI: 0.11, 0.48; hair: β=0.18, 95% CI: 0.05, 0.31). Soil Mn accounted for 61% of the weight (mean weight=0.61) in models of nail Mn, followed by outdoor dust Mn (22%). In models of saliva Mn, air received the majority of the weight (65%), followed by soil (29%). Similarly, in models of hair Mn, air Mn accounted for 42% of the weight, followed by soil Mn (36%). In contrast, no association was found between the weighted environmental exposure index and blood Mn. Figure 2A summarizes the weighted contributions of each environmental medium on each biomarker, where the height of the bar for each biomarker is scaled by (i.e., multiplied by) the effect estimate for the association between the weighted environmental exposure index and the given biomarker.

For Pb, increases in the weighted environmental exposure index were marginally associated with increases in blood Pb (blood: β=0.12, 95% CI: −0.01, 0.25) (Table 5). The greatest contributor in the model for blood Pb was soil Pb (mean weight=0.40), followed by indoor dust Pb (0.27) (Figure 2B). Notably, we observed a strong, *negative* association between saliva Pb and the weighted environmental exposure index (β= −0.48, 95% CI: −0.80, −0.16), with soil and indoor dust as dominant contributors, consistent with results from the standard regression approach. For Cr, increases in the weighted environmental exposure index were associated with increases in all biomarkers, with the strongest association estimated for nails (β= 0.14, 95% CI: −0.01, 0.30) (Table 5). Dust was the greatest contributor for all Cr biomarkers except saliva, where air was the greatest contributor (Figure 2C). For Cu, a

negative association was estimated between the weighted environmental exposure index and saliva, although this was imprecise (β= −0.22, 95% CI: −0.56, 0.13) (Table 5, Figure 2D).

We conducted several sensitivity analyses with WQS models. When indoor and outdoor dust concentrations (μg/g) were replaced with surface loadings (μg/m), associations remainedsimilar for Mn biomarkers, became slightly weaker for Cr biomarkers, and changed considerably for Pb biomarkers (Supplemental Table 3). Specifically, the negative association estimated between the WQS index and saliva Pb was no longer apparent (using surface loadings: β= 0.02, 95% CI: −0.33, 0.37 vs. concentrations: β= −0.48, 95% CI: −0.80, −0.16), and positive associations with blood, nails, and urine Pb became stronger. Minor differences in results were also found when restricting analyses to lower exposure settings without current ferroalloy activity (i.e., excluding Bagnolo Mella children): Mn findings were similar; estimates for Cr were less precise; and the association with saliva Pb became positive, while positive associations with blood and urine Pb became stronger (Supplemental Table 4). Similarly, in analyses of higher exposure settings in areas with current or historic ferroalloy activity only (i.e., excluding Garda Lake children), the negative association estimated between the WQS index and saliva Pb changed direction (β= 0.08, 95% CI: −0.24, 0.40 vs. all participants: β= −0.48, 95% CI: −0.80, −0.16) (Supplemental Table 5). When models were stratified by distance to nearest ferroalloy plant, findings were similar, but the hair Mn association was weaker, and the association for nail Mn was stronger among children who live farther from a plant, compared to those who live closer (Supplemental Table 6).

4. Discussion

In this adolescent population residing near historic or currently active ferromanganese alloy industries, we characterized exposure sources for four metals used in ferroalloy production by examining associations between metals concentrations in environmental samples and exposure biomarkers. This study begins to fill an important knowledge gap about the connection between environmental and internal measures of exposure. We used an innovative approach for estimating cumulative environmental impact on each metal biomarker and determining relative contributions from each environmental medium. These findings may help inform which biological samples best reflect environmental levels of Mn, Pb, Cr, and Cu in the setting of community exposures to ferroalloy emissions, which can help guide the prioritization of interventions for environmental remediation.

In these data, among the four metals evaluated, we found Mn to have the strongest and most consistent associations between environmental media and exposure biomarkers. This is congruent with Mn being a principal component of ferromanganese alloy (6) and the primary exposure of concern in this region of long-standing ferromanganese alloy activity. Findings from standard multivariable regression and WQS regression were similar, with both approaches estimating positive associations between Mn in environmental samples and nails, saliva, and hair. Our results confirm findings of a previous analysis in a subset of participants, in which household dust Mn was associated with ferroalloy plant activity and with Mn levels in children's hair and nails (11). The present analysis provides additional insight about relative contributions of additional environmental samples to internal levels.

Here, soil was estimated to be the biggest contributor to nail Mn, while air was the largest contributor to both saliva and hair Mn, suggesting that the optimal exposure biomarker for Mn may depend on exposure pathway. Nail Mn also integrates exposure over a longer time period than hair (49), and may therefore better reflect longer-term exposures. For this population, airborne emissions from ferroalloy industry are the primary source, but airborne particles also deposit into the environment and can persist in soils and dusts long after initial deposition. Furthermore, re-suspension of dust particles can cause exposure for many years after the cessation of active emissions. We observed stronger associations between nail Mn and environmental samples, in particular soil and outdoor dust, among participants without current exposure to ferromanganese alloy plant activity (i.e., residents of Valcamonica and Garda Lake). Exposure in these participants is more likely from historic emissions than from active airborne emissions, which supports the notion that nails reflect historically emitted Mn that persists in soils.

Pb, Cr, and Cu in environmental media were less consistently associated with exposure biomarkers than Mn. Cr associations were generally weak, but were more apparent in areas with current or historic ferroalloy industry, which suggests that ferroalloy emissions may be a source of internal Cr levels. Pb levels in environmental media, in particular soil and dust, were marginally associated with blood Pb levels. This is a noteworthy finding given the relatively low levels of blood Pb (e.g., median = 1.3 μg/dL) in this cohort compared to other populations (33, 50, 51). We note that few studies have examined this relationship among adolescents, in whom exposure sources and pathways are less well studied compared to young children. Overall, the associations for Pb, Cr, and Cu suggest that levels of these metals in environmental media, which may have resulted from ferroalloy activity, contribute weakly to internal levels, and that other sources of exposure, including diet (52, 53), are likely important.

Some unexpected negative associations were also estimated in these data. In particular, in the main analysis, saliva Pb was strongly negatively associated with levels in environmental samples. Saliva is not generally accepted as a reliable biomarker of Pb exposure (54), and a limited number of prior studies have evaluated its utility (55–58). Consistent with our findings, Gil et al. (56) reported negative associations between saliva and blood Pb, despite positive associations between blood Pb and hair and urine Pb. It has been suggested that the direction of the saliva-blood Pb association may depend on the blood Pb levels, with positive associations estimated at higher blood Pb levels but negative associations estimated at lower levels (56–58). Pb in saliva is thought to result from the direct excretion of Pb in plasma (58), but the toxicokinetics of saliva Pb have not been well described. It is worth noting that in all sensitivity analyses, the negative saliva Pb association was attenuated and, in some models, changed to a *positive* association. Further, for the more widely accepted Pb biomarkers (e.g., blood, urine), *stronger* positive associations with environmental media were estimated in sensitivity analyses than in main analyses. These shifts in the magnitude and direction of the saliva Pb associations may also indicate a lack of robustness in the relationship.

In contrast to Pb, for which well-validated exposure biomarkers such as blood are widely accepted (54, 59, 60), there is a lack of consensus on an optimal biomarker for Mn (61, 62).

Blood, hair, nails and teeth have all been used as biomarkers of Mn exposure, but advantages and disadvantages have been cited for each (61, 63, 64). In a review of the literature on Mn exposure measures and health outcomes, Coetzee et al. (63) have called for novel biomarkers of Mn exposure, in particular for children. Saliva is promising as a noninvasive sample, but relatively little is known about its utility as a biomarker of Mn exposure. Weak to modest correlations between saliva Mn and other Mn measures have been reported in both environmental and occupational exposure studies (56, 65–67). In a Canadian cohort of school-age children consuming well water, water Mn was weakly correlated with Mn in saliva supernatant (r=0.14), although strong correlations (r=0.60) were reported between water and hair Mn in the same subjects (68). In occupational settings, however, stronger positive correlations were reported for saliva Mn with airborne Mn (r=0.65), as well as with length of employment, plasma Mn, and red blood cell Mn (56, 65–67), suggesting saliva Mn reasonably reflects Mn levels in the air and/or work environment. Heterogeneity in findings between studies regarding the utility of saliva as a biomarker may be explained in part by different analytical methods for processing and measuring saliva Mn, as well as by differences in the type of saliva collected, because saliva is secreted by the parotid, submandibular, and sublingual glands (69). In our study, the use of gently centrifuged whole saliva integrates any potential variability in composition from the three glands (56). More studies examining the potential use of saliva as a biomarker of Mn exposure, however, are warranted. For Cr and Cu, optimal biomarkers have not been established, but previous studies have relied primarily on blood, plasma, or urine (70–72).

The use of dust loading as an alternative to dust concentration to represent metals content in the dust microenvironment has been considered previously (73, 74). Both approaches are informative but represent different metrics: dust concentration represents the mass of the metal per gram of dust, while dust loading represents the mass of metal per unit of surface area and is influenced by the amount of dust and the metal concentration of the dust (74). In our study, Mn results were similar using concentrations and loading; other studies have also reported correlations between Mn concentrations, loading rates and nearby sources (13, 73). Pb associations with the more widely accepted Pb biomarkers (i.e., blood, urine, nails) were stronger using dust loading than dust concentrations, suggesting that loading may be more informative than concentrations for Pb, which is consistent with prior studies (e.g., (75)). In contrast, associations for Cr using dust loading rather than dust concentrations were weaker.

Our finding that metals, in particular Mn, in environmental samples are related to internal levels is consistent with some prior studies. Among children living near a mining-impacted Superfund site, positive associations were reported between metals in house dust and infant's internal biomarkers, specifically blood Pb and hair Mn, e.g., a doubling of dust Pb concentration was associated with a 13–29% increase in blood Pb (34). Blood Pb was also associated with soil Pb levels, although associations were weaker than for dust (34). Similarly, blood Pb was positively associated with both dust and soil Pb levels in an Australian cohort residing in a major urban area (76). Blood Mn, the only Mn biomarker assessed in that study, was not associated with dust, soil, water, or dietary Mn levels; this is similar to our finding of null associations between Mn in blood and environmental media. In a cohort of children living near the largest U.S. ferromanganese refinery, no associations were reported between personal air Mn and blood or hair Mn (10). In a subsequent analysis

of the same cohort, structural equation models of the inhalation exposure pathway demonstrated that air and soil Mn contributed significantly to dust Mn, but that only dust Mn was a predictor of hair Mn (32). None of the aforementioned studies measured Mn in saliva, and most prior studies except Fulk et al. (32) did not examine all environmental media concurrently, due in part to high correlations. It is important to note, however, that variability in biomarker levels and in findings between studies may also relate to host factors, such as genetic variability that can alter absorption and retention of metals (77, 78).

Compared to other studies of children residing in areas of increased metals exposure, biomarker concentrations measured here were comparable for most metals. Blood Mn levels among 7- to 9-year old Ohio children living near a ferromanganese alloy refinery were similar (geometric mean: 9.7 µg/L vs. median in our study: 10.9 µg/L), although hair Mn levels were higher (geometric mean: 0.4 µg/g vs. median in our study: 0.08 µg/g) and blood Pb levels were lower (geometric mean: 0.8 µg/dL vs. median in our study: 1.3 µg/dL) (3). Among 1- to 2-year old children living near a former lead and zinc mine in Oklahoma, blood Pb levels were similar (median 1.4 µg/dL); blood Mn levels were slightly higher (median 12 µg/L), as expected given the younger ages of the children; and hair Pb and Mn levels were higher (Pb: median 4.4 µg/g vs. median in our study 0.2 µg/g; Mn: median 0.5 µg/g vs. median in our study: 0.1 µg/g) (34). Fewer studies have reported copper and chromium levels in children. Among children living in Mumbai, India, mean blood Cu level was 797 µg/L (79), similar to levels measured in our study (median: 838 µg/L). Cr levels in hair were higher among adolescents of an industrial region of Spain (mean: 0.5 µg/g vs. median in our study: 0.05 µg/g) (80). It is worth noting that differences in hair metal levels between studies may also be related to differences in sample cleaning methods (40). Hair samples in our study were effectively cleaned for exogenous contamination and thus represent primarily endogenous metals (40), although minor residual external contamination may remain.

There are several limitations to this study. Although metal concentrations were measured in drinking water samples from the homes of a small subset of participants in 2008 (n=92) and 2012 (n=15), Mn concentrations were low (mean [SD] = 0.005 [0.002] mg/L, range: <0.001 – 0.02 mg/L) and lacked variability (96% of samples < 0.01 mg/L). Therefore, we did not consider tap water as a source of exposure in this analysis. We also lack adequate data on dietary habits to account for dietary exposures to these metals, which can be important for some metals (52). For Cr, data on soil concentrations were not available and could therefore not be evaluated as a contributor to biomarker levels. Cr concentrations in air, indoor dust, outdoor dust, and biomarkers were not speciated, although there is evidence that toxicokinetics (and toxicodynamics) may differ by valence state (81). Species may similarly play a role in Mn and Pb absorption, as some forms (e.g., sulfides) are not bioaccessible. This may have contributed to measurement error in explanatory and outcome variables. Although any error is likely to be nondifferential, it is difficult to predict the direction of this bias. Last, the cross-sectional study design, in which environmental and biological samples were collected at a single point in time, does not allow us to account for or examine temporal variability. It should be noted that interpretation of the internal biomarkers should factor in biological properties. For example, nail concentrations reflect metals deposited in the cuticle 6–9 months prior to collection of the nail (49). Hair reflects secretory properties of metals and, while cumulative, because hair grows at ~1 cm per month, variability in hair

length will add variability to measures unless exposure/secretion is constant (82, 83). Blood Mn is under physiologic regulation, unlike metals such as Pb, and while blood Mn levels may reflect biologically active Mn, it is maintained in a relatively tight concentration range in the blood with larger pools of Mn being found in the liver and bones (84). The use of blood Mn as an exposure biomarker is therefore controversial. Urine reflects excretory Mn, although bile, rather than urine, is the major source of excretion (84). All of these properties will introduce variability in the associations between exposure biomarkers and environmental samples.

Despite these limitations, our study has several unique strengths. First, our robust data set allowed us to examine multiple environmental sources of metals exposure on five different biomarkers. We were able to confirm previous findings in a subset of our sample that Mn exposure in this community is a concern. We were also able to characterize exposure to other metals, addressing residents' questions about concomitant exposures to metals. We implemented an innovative statistical approach to examine the impact of exposure to metals from multiple environmental sources, while simultaneously using a traditional approach to compare findings. To our knowledge this is the first time that the WQS regression approach has been used to examine the contributions of different environmental sources to exposure biomarkers.

In conclusion, these results suggest that environmental factors can influence levels of metals in internal biomarkers. Our analysis confirms that Mn exposure in this population is a primary exposure of concern and that levels of Mn in air and soil are the largest contributors to levels in biomarkers. In this setting of community exposure to ferromanganese alloy emissions, our data suggest that nails and saliva best represent Mn levels in the environment. Future reductions and eventual cessation of ferroalloy industry activity are planned, thereby reducing exposure from active emissions. However, it will remain important to implement sustainable interventions for reducing house dust and particle re-suspension from historic emissions. Findings from this study can help prioritize intervention strategies in exposed populations and guide the selection of metals biomarkers in future epidemiologic studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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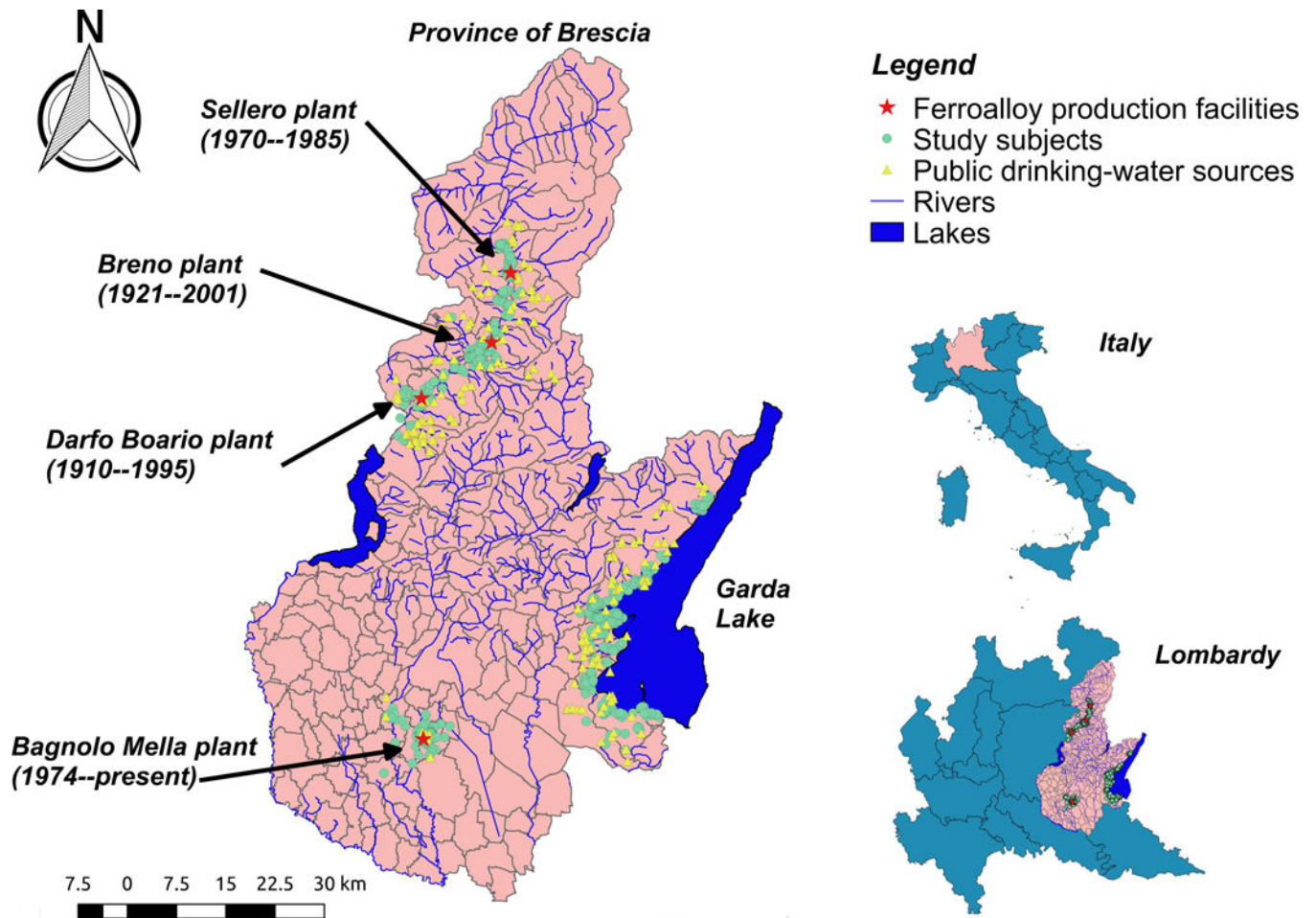
Figure 1.
Map of the province of Brescia, Italy. Ferroalloy plants in Valcamonica (Sellero, Breno, and Darfo Boario plants) and in Bagnolo Mella are shown with their respective operating periods. Residential drinking water is provided from public drinking water supply systems that are fed by the drinking water sources depicted on the map.

(A) Manganese

(B) Lead
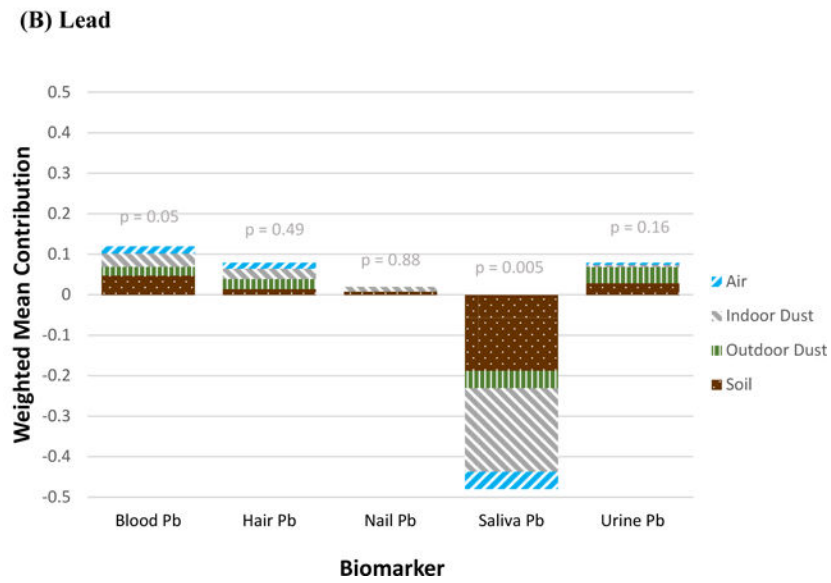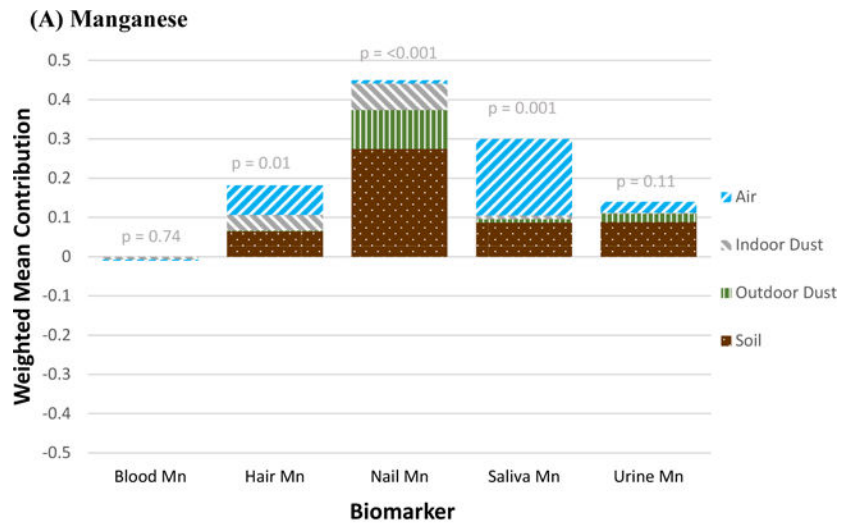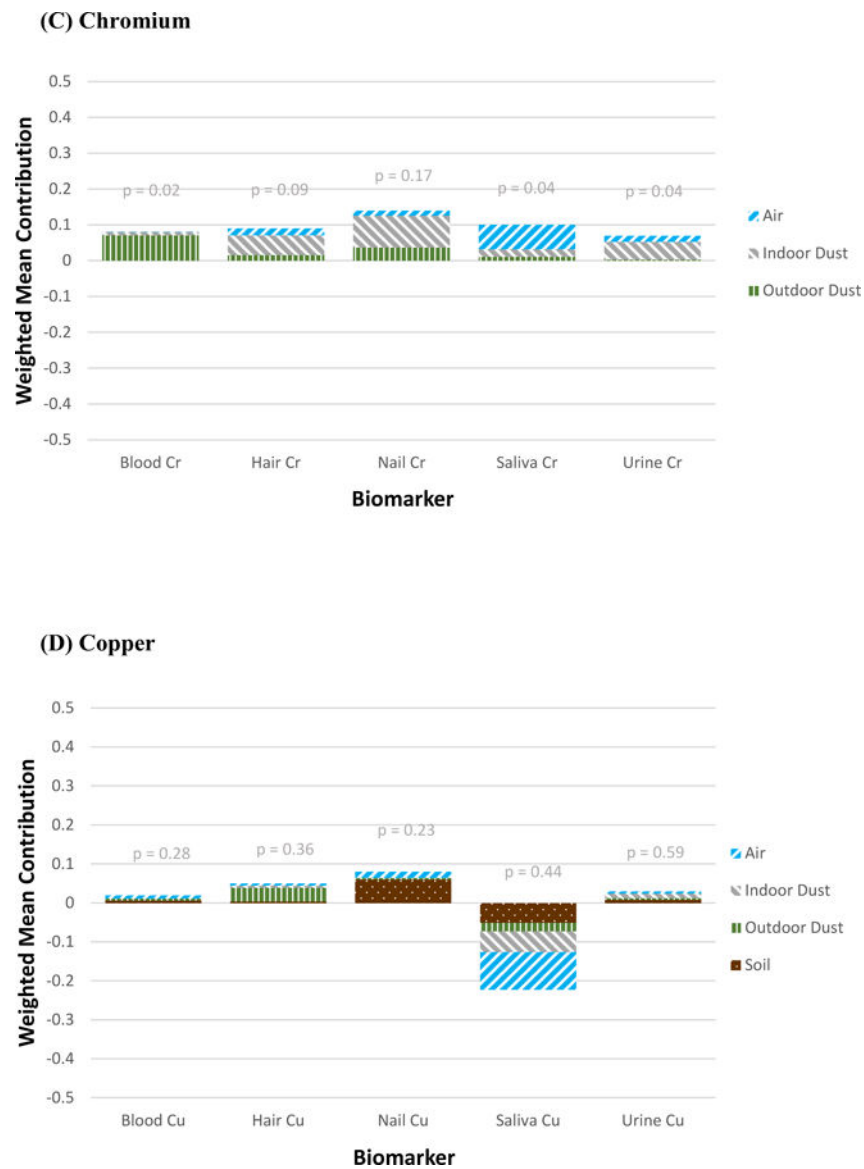
(C) Chromium

(D) Copper

Figure 2.
Results of weighted quantile sum regression for (A) manganese, (B) lead, (C) chromium, and (D) copper. The y-axis, Weighted Mean Contribution, indicates the relative contributions of environmental sources to each biomarker. The height of the bar for each biomarker is scaled by the magnitude of that biomarker's beta coefficient in the weighted regression model. The colors within the bar indicate the proportion contributed by each environmental source.

Table 1.

Sociodemographic characteristics of study participants (N=717)[a]

Characteristic	Mean ± SD or n (%)
Age (years)	12.8 ± 0.9
Mother's years of residence in study area	39.0 ± 9.5
Sex	
Male	373 (52.0%)
Female	344 (48.0%)
Socioeconomic status	
Low	166 (23.9%)
Medium	368 (52.9%)
High	161 (23.2%)
Area of residence	
Bagnolo Mella	212 (29.6%)
Valcamonica	259 (36.1%)
Garda Lake	246 (34.3%)
Distance to nearest ferroalloy plant (km), median	3.2
Distance to nearest ferroalloy plant (km), median, by area of residence:	
Bagnolo Mella	1.1
Valcamonica	2.4
Garda Lake	33.4
Birth order	
1	355 (50.2%)
2	272 (38.5%)
3	69 (9.8%)
4	11 (1.5%)

[a] - Missing data on mother's years of residence in study area (n=14), socioeconomic status (n=22), and birth order (n=10).

Table 2.

Metals concentrations in environmental media and children's exposure biomarkers

Metal	Environmental medium or Biomarker	n	% >LOD[a]	Median	Interquartile Range
Manganese	Air ($\mu g/m^3$)	526	100	26.02	12.91– 50.38
	Soil ($\mu g/g$)	697	100	699.78	473.21– 987.93
	Indoor dust ($\mu g/g$)	334	100	379.50	250.45– 722.30
	Outdoor dust ($\mu g/g$)	324	100	1432.28	661.12–4970.09
	Blood ($\mu g/L$)	687	100	10.90	8.83–13.27
	Hair ($\mu g/g$)	638	99	0.08	0.05– 0.15
	Nails ($\mu g/g$)	521	99	0.19	0.10– 0.38
	Saliva ($\mu g/L$)	389	100	4.91	1.99–13.05
	Urine ($\mu g/L$)	642	98	0.12	0.09– 0.28
Lead	Air ($\mu g/m^3$)	439	100	12.11	5.62– 21.86
	Soil ($\mu g/g$)	253	99	35.80	24.76– 47.35
	Indoor dust ($\mu g/g$)	334	97	71.16	47.80–107.27
	Outdoor dust ($\mu g/g$)	324	100	157.91	100.25– 229.65
	Blood ($\mu g/L$)	687	100	13.33	10.00– 19.00
	Hair ($\mu g/g$)	638	99	0.17	0.07– 0.38
	Nails ($\mu g/g$)	521	97	0.10	0.04– 0.27
	Saliva ($\mu g/L$)	389	99	0.55	0.19– 1.70
	Urine ($\mu g/L$)	531	100	0.54	0.34– 0.79
Chromium	Air ($\mu g/m^3$)	526	100	7.00	4.12–11.29
	Soil ($\mu g/g$)[b]	-	-	-	-
	Indoor dust ($\mu g/g$)	334	100	42.13	32.95– 53.46
	Outdoor dust ($\mu g/g$)	324	100	55.16	40.95– 80.44
	Blood ($\mu g/L$)	388	100	0.63	0.43– 0.85
	Hair ($\mu g/g$)	638	99	0.05	0.03– 0.08
	Nails ($\mu g/g$)	521	96	0.15	0.08– 0.30
	Saliva ($\mu g/L$)	389	99	0.40	0.20– 0.91
	Urine ($\mu g/L$)	531	100	0.19	0.12– 0.26
Copper	Air ($\mu g/m^3$)	526	100	21.63	12.65– 34.95
	Soil ($\mu g/g$)	251	100	45.09	28.27– 70.87
	Indoor dust ($\mu g/g$)	334	100	43.70	33.65– 56.02
	Outdoor dust ($\mu g/g$)	324	100	276.88	187.34– 388.47
	Blood ($\mu g/L$)	276	100	837.92	759.85– 940.29
	Hair ($\mu g/g$)	638	100	9.57	7.08–15.38
	Nails ($\mu g/g$)	521	99	2.66	2.10– 3.26
	Saliva ($\mu g/L$)	389	100	21.58	9.55–49.88

Metal	Environmental medium or Biomarker	n	% >LOD[a]	Median	Interquartile Range
	Urine (μg/L)	531	100	7.85	5.8–10.73

[a] – Limit of detection

[b] – Soil chromium data not available.

Table 3.

Spearman correlation coefficients for manganese in environmental media and biomarkers

	Air Mn	Soil Mn	Indoor dust Mn	Outdoor dust Mn	Blood Mn	Hair Mn	Nail Mn	Saliva Mn
Soil Mn	0.13*							
Indoor dust Mn	0.27**	0.16*						
Outdoor dust Mn	0.34**	0.17*	0.65**					
Blood Mn	−0.05	−0.02	−0.07	−0.10				
Hair Mn	0.12*	−0.01	0.12*	0.11	−0.06			
Nail Mn	0.17**	0.22**	0.23**	0.27**	−0.06	0.18**		
Saliva Mn	0.23**	0.08	−0.03	0.00	−0.11*	0.22**	−0.04	
Urine Mn	0.01	0.14**	0.04	0.08	−0.06	−0.05	0.06	0.09

*
p-value < 0.05

**
p-value <0.001

Table 4.

Results from linear regression models for associations between environmental media and exposure biomarkers[a]

Biomarker	n	Air (µg/m³) Beta (95%CI)	Soil (µg/g)[a] Beta (95%CI)	Indoor dust (µg/g) Beta (95%CI)	Outdoor dust (µg/g) Beta (95% CI)	Adjusted R²
Manganese						
Blood (µg/L)	248	−0.01 (−0.04,0.03)	0.01 (−0.03, 0.06)	−0.01 (−0.06, 0.05)	−0.003 (−0.06, 0.05)	0.001
Hair (µg/g)	241	0.03 (−0.07, 0.13)	0.04 (−0.07, 0.16)	0.04 (−0.09, 0.17)	−0.04 (−0.18, 0.09)	0.03
Nails (µg/g)	216	0.02 (−0.15, 0.19)	0.30 (0.11, 0.49)**	0.10 (−0.13, 0.33)	0.02 (−0.21, 0.25)	0.06
Saliva (µg/L)	249	0.22 (0.05, 0.39)**	0.16 (−0.02, 0.34)*	−0.11 (−0.33,0.10)	−0.04 (−0.26, 0.19)	0.13
Urine (µg/L)	210	0.02 (−0.13, 0.17)	0.03 (−0.14, 0.20)	−0.04 (−0.23, 0.15)	−0.05 (−0.25, 0.15)	−0.02
Lead						
Blood (µg/L)	142	0.02 (−0.07, 0.11)	0.07 (−0.02, 0.15)	−0.01 (−0.10, 0.08)	0.05 (−0.03, 0.14)	0.08
Hair (µg/g)	139	−0.03 (−0.25,0.18)	−0.02 (−0.22, 0.19)	0.07 (−0.15, 0.29)	0.06 (−0.15, 0.27)	−0.04
Nails (µg/g)	118	−0.16 (−0.45,0.12)	−0.03 (−0.30,0.23)	−0.11 (−0.39, 0.17)	−0.002 (−0.27, 0.26)	−0.03
Saliva (µg/L)	140	−0.01 (−0.31,0.29)	−0.24 (−0.47, −0.01)**	−0.23 (−0.48, 0.02)*	−0.06 (−0.30, 0.18)	0.04
Urine (µg/L)	117	−0.02 (−0.14,0.11)	0.06 (−0.04, 0.15)	−0.06 (−0.16, 0.04)	0.07 (−0.02, 0.17)	−0.004
Chromium						
Blood (ug/L)	262	−0.06 (−0.14,0.02)		−0.04 (−0.13, 0.04)	0.10 (0.01, 0.18)**	0.02
Hair (ug/g)	255	−0.03 (−0.11,0.05)		0.07 (−0.02, 0.16)	0.01 (−0.08, 0.10)	0.00
Nails (ug/g)	212	−0.01 (−0.13,0.12)		0.08 (−0.04, 0.19)	0.02 (−0.10, 0.15)	−0.01
Saliva (ug/L)	262	0.10 (−0.04, 0.23)		0.03 (−0.11, 0.17)	−0.05 (−0.19, 0.09)	0.01
Urine− (ug/L)	224	0.02 (−0.06, 0.10)		0.04 (−0.05, 0.12)	−0.02 (−0.10, 0.07)	0.01
Copper						
Blood (ug/L)	102	0.02 (−0.01, 0.06)	−0.004 (−0.03, 0.03)	−0.06 (−0.10,−0.03)**	0.002 (−0.02, 0.03)	0.14
Hair (ug/g)	138	0.01 (−0.08, 0.10)	−0.05 (−0.15,0.06)	−0.04 (−0.15, 0.07)	0.07 (−0.03, 0.16)	0.03
Nails (ug/g)	120	−0.02 (−0.10,0.06)	0.06 (−0.03, 0.16)	−0.03 (−0.13, 0.07)	−0.001 (−0.08, 0.08)	−0.02
Saliva (ug/L)	139	−0.11 (−0.31,0.09)	−0.01 (−0.22,0.20)	−0.06 (−0.29, 0.16)	−0.10 (−0.29, 0.09)	0.01
Urine (ug/L)	115	−0.03 (−0.13,0.08)	−0.02 (−0.12,0.09)	−0.01 (−0.11, 0.10)	0.000 (−0.10, 0.10)	−0.04

[a] – All models adjusted for child's sex and age. Each row represents a single model.

*
p-value <0.10

**
p-value < 0.05

Table 5.

Average weights[a] for environmental media and estimated associations between weighted environmental exposure index and exposure biomarkers, from WQS regression[b]

Biomarker	n	Average Weights				Weighted Environmental Exposure Index
		Air	Soil	Indoor dust	Outdoor dust	Beta (95% CI)
Manganese						
Blood	248	0.32	0.00	0.68	0.00	−0.01 (−0.05, 0.03)
Hair	241	0.42	0.36	0.22	0.01	**0.18 (0.05, 0.31)** [**]
Nails	216	0.02	0.61	0.15	0.22	**0.45 (0.26, 0.64)** [**]
Saliva	249	0.65	0.29	0.03	0.03	**0.30 (0.11, 0.48)** [**]
Urine	210	0.20	0.63	0.02	0.15	0.14 (−0.03, 0.31)
Lead						
Blood	142	0.15	0.40	0.27	0.18	**0.12 (−0.01, 0.25)** [*]
Hair	139	0.19	0.18	0.32	0.31	0.08 (−0.23, 0.39)
Nails	118	0.00	0.40	0.58	0.02	0.02 (−0.30, 0.35)
Saliva	140	0.09	0.39	0.43	0.09	**−0.48 (−0.80, −0.16)** [**]
Urine	117	0.06	0.36	0.08	0.50	0.08 (−0.05, 0.22)
Chromium						
Blood	262	0.01	-	0.11	0.89	**0.08 (0.00, 0.16)** [**]
Hair	255	0.21	-	0.62	0.17	0.09 (−0.02, 0.20)
Nails	212	0.10	-	0.64	0.26	**0.14 (−0.01, 0.30)** [*]
Saliva	262	0.68	-	0.21	0.11	0.10 (−0.06, 0.26)
Urine	224	0.25	-	0.71	0.04	0.07 (−0.03, 0.16)
Copper						
Blood	102	0.45	0.33	0.00	0.22	0.02 (−0.02, 0.06)
Hair	138	0.11	0.08	0.10	0.71	0.05 (−0.07, 0.17)
Nails	120	0.20	0.76	0.01	0.04	0.08 (−0.03, 0.19)
Saliva	139	0.44	0.23	0.24	0.10	−0.22 (−0.56, 0.13)

		Average Weights			Weighted Environmental Exposure Index
Urine	115	0.19	0.28	0.40	0.13	0.03 (−0.14, 0.20)

[a] – Weights were generated across 100 bootstrap samples.

[b] – All models adjusted for child's sex and age. Betas represent percent change in biomarker per 25% increase in weighted environmental exposure index.

*
p < 0.10

**
p < 0.05