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Assessing the mediating role of iron status on associations between an industry-relevant metal mixture and verbal learning and memory in Italian adolescents

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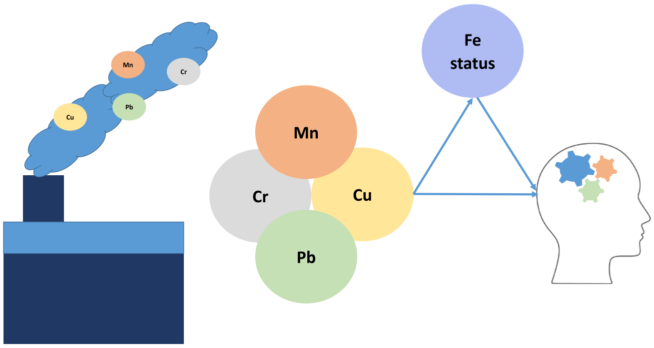
HIGHLIGHTS

- Metals have been linked with neurodevelopment, but less is known about the role of iron (Fe) status.
- We examined Fe status as a mediator of associations between a metal mixture and learning and memory.
- The mixture was associated with neurodevelopment; there was no evidence of mediation by Fe status.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Metals, including lead (Pb), manganese (Mn), chromium (Cr) and copper (Cu), have been associated with neurodevelopment; iron (Fe) plays a role in the metabolism and neurotoxicity of metals, suggesting Fe may mediate metal-neurodevelopment associations. However, no study to date has examined Fe as a mediator of the association between metal mixtures and neurodevelopment.

Objective: We assessed Fe status as a mediator of a mixture of Pb, Mn, Cr and Cu in relation to verbal learning and memory in a cohort of Italian adolescents.

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Mediation
Iron status

Methods: We used cross-sectional data from 383 adolescents (10–14 years) in the Public Health Impact of Metals Exposure Study. Metals were quantified in blood (Pb) or hair (Mn, Cr, Cu) using ICP-MS, and three markers of Fe status (blood hemoglobin, serum ferritin and transferrin) were quantified using luminescence assays or immunoassays. Verbal learning and memory were assessed using the California Verbal Learning Test for Children (CVLT-C). We used Bayesian Kernel Machine Regression Causal Mediation Analysis to estimate four mediation effects: the natural direct effect (NDE), natural indirect effect (NIE), controlled direct effect (CDE) and total effect (TE). Beta (β) coefficients and 95 % credible intervals (CIs) were estimated for all effects.

Results: The metal mixture was jointly associated with a greater number of words recalled on the CVLT-C, but these associations were not mediated by Fe status. For example, when ferritin was considered as the mediator, the NIE for long delay free recall was null (β = 0.00; 95 % CI = −0.22, 0.23). Conversely, the NDE (β = 0.23; 95 % CI = 0.01, 0.44) indicated a beneficial association of the mixture with recall that operated independently of Fe status.

Conclusion: An industry-relevant metal mixture was associated with learning and memory, but there was no evidence of mediation by Fe status. Further studies in populations with Fe deficiency and greater variation in metal exposure are warranted.

1. Introduction

Environmental exposure to metals, including lead (Pb), manganese (Mn), copper (Cu), chromium (Cr), and iron (Fe), is common among children, and can occur through air emissions, drinking water, and contact with contaminated soils (Agency for Toxic Substances and Disease Registry, 2020, 2012a, 2012b, 2004). Children living in close proximity to industry, such as the steel-producing ferroalloy industry, may be more highly exposed to metals, and prior research in the U.S., Canada, Brazil, and elsewhere has reported higher biomarker concentrations of these metals among children living near ferroalloy plants (Boudissa et al., 2006; Butler et al., 2019; Haynes et al., 2012; Menezes-Filho et al., 2016, 2009; Riojas-Rodríguez et al., 2010). These findings are of public health concern because metals have been consistently associated with adverse neurodevelopment (Amorós et al., 2019; Caparros-Gonzalez et al., 2019; de Carvalho et al., 2018; García-Chimalpopoca et al., 2019; Oulhote et al., 2014; Torres-Agustín et al., 2013; Wright et al., 2006; Yorifuji et al., 2011), and the ferroalloy industry is expanding globally ("Ferroalloy Market Share 2018-2025 Industry Growth Outlook Report,", n.d.).

Children are exposed to multiple environmental metals (Agency for Toxic Substances and Disease Registry, 2020, 2012b, 2012a, 2004), and many metals share similar neurotoxic pathways (e.g., dopaminergic toxicity, oxidative stress, mitochondrial disruption) (Ahamed and Siddiqui, 2007; Akinyemi et al., 2019; Gaetke et al., 2014; Kalita et al., 2018; Neal and Guilarte, 2013; O'Neal et al., 2014; Wise et al., 2022). These toxicological data suggest that concurrent exposure to multiple metals can induce joint or interactive neurodevelopmental effects. Findings in animals are generally consistent with studies in pediatric cohorts, which have reported cumulative effects of multiple metals on cognition (Chandra et al., 1981; Merced-Nieves et al., 2021; Shukla and Chandra, 1987; Stein et al., 2022). In the Public Health Impact of Metals Exposure (PHIME) study, we previously found that a mixture of Mn, Pb, Cu and Cr was jointly associated with indices of neurodevelopment, though the direction of effect varied by cognitive domain. For example, the metal mixture was adversely associated with verbal intelligence quotient (IQ) and visuospatial abilities (Bauer et al., 2020a; Rechtman et al., 2020), but beneficially associated with verbal learning and memory measured on the California Verbal Learning Test for Children (CVLT-C) (Schildroth et al., 2023).

Recent epidemiological studies have investigated the role of Fe status, clinically measured through biomarkers like ferritin, hemoglobin, and transferrin (Gibson, 2005), as a modifier of the neurotoxicity of environmental metals in children (Schildroth et al., 2023, 2022b). Fe is a metal and essential nutrient required for cellular oxygen transport, neurotransmitter synthesis, and metabolic activity, and is therefore important for brain development and maturation (McCann et al., 2020). Perturbations to Fe status in gestation, early childhood, and adolescence have been consistently associated with worse scores on assessments of attention-related behaviors, memory, visuospatial abilities, IQ, and academic achievement (Halterman et al., 2001; Jáuregui-Lobera, 2014; Ji et al., 2017; Lukowski et al., 2010; Parkin et al., 2020; Roy et al., 2011; Tseng et al., 2018). A recent study of 922 adolescents observed reduced cognitive abilities among participants with lower brain Fe concentrations (measured using R2* relaxometry from magnetic resonance imaging) (Larsen et al., 2020). On the other hand, Fe overload has similarly been associated with decreased IQ scores in children, reflecting the neurotoxic impacts (i.e., oxidative stress) of Fe in excess (Salvador et al., 2011; Sammallahti et al., 2022). In addition, the toxicokinetics and toxicodynamics of Fe are similar to those of other metals. Fe and environmental metals like Pb and Mn share neurotoxic mechanisms (oxidative stress, mitochondrial disruption, altered neurotransmission and disruption of myelination) (Barkur and Bairy, 2015; Borchard et al., 2018; Fernsebner et al., 2014; Galaris et al., 2019; McCann et al., 2020; Neal and Guilarte, 2013; Soares et al., 2020; Walter et al., 2002), and also compete for transporters (e.g., the divalent metal transporter 1) in the duodenum, brain, and liver (Brain et al., 2006; Kordas, 2010; Kordas and Stoltzfus, 2004; Peraza et al., 1998). Environmental metals tend to deposit more into Fe-deficient, compared to Fe-replete, brain tissues in animal models, and this tendency has been further shown to impact neurotransmitter concentrations (Erikson et al., 2002; Kordas, 2010). These data support a body of evidence indicating that altered Fe status, in particular deficiency, impacts the neurotoxicity of environmental metals in children.

Environmental metal exposure may also affect Fe dynamics in the body. The hematologic toxicity of certain metals has been established: Pb, for example, disrupts *heme* synthesis, transferrin expression, erythrocyte membranes, and erythropoietin production, particularly at concentrations >10 μg/dL (Agency for Toxic Substances and Disease Registry, 2020; McCann et al., 2020; Peters et al., 2021; Rossander-Hultén et al., 1991). Though the hematologic toxicity of metals has been shown primarily with respect to hemoglobin, there is some evidence that environmental metals adversely impact other metrics of Fe status (e.g., ferritin concentrations) even at low concentrations (e.g., blood Pb <5 μg/dL), which is supported by mechanistic data illustrating the ability of metals to interfere with Fe loading onto proteins (e.g., ferritin) (Liu et al., 2020); however, many prior studies in children were limited by methodological constraints (e.g., cross-sectional design) (Choi and Kim, 2005; Henríquez-Hernández et al., 2017; Jain et al., 2005; Kutllovci-Zogaj et al., 2014; Schildroth et al., 2022a; Wang et al., 2012; Weinhouse et al., 2017). This suggests that, in addition to acting as a modifier of metals-induced toxicity, Fe status may also mediate associations between environmental metals and neurodevelopment (Fig. 1, see also: (Schildroth et al., 2022b)).

A mediator is a variable that lies on the causal pathway between an exposure and outcome of interest. Identifying mediating variables can help to 1) elucidate underlying biological mechanisms of the exposure(s) of interest and 2) identify relevant pathways for potential interventions

(Hafeman and Schwartz, 2009). Therefore, characterizing Fe status as a mediator of metal-neurodevelopment associations is an important public health objective, particularly because Fe deficiency is the most common nutritional deficiency in the world (Stevens et al., 2013). Prior work assessing Fe status as a mediator of metals neurotoxicity is limited (Jeong et al., 2015), and no study to date has assessed Fe status as a mediator of environmental metals or of a complex metal mixture.

Our previous study in the PHIME cohort examined Fe status as a modifier of the associations between metal mixture exposure and verbal learning and memory (Schildroth et al., 2023); the goal of the current analysis was to expand on this work and evaluate Fe status as a potential mediator of the industry-relevant metal mixture. We used a novel statistical approach to evaluate associations of a mixture of Pb, Mn, Cu and Cr with verbal learning and memory among adolescents, and to assess Fe status as a mediator of these associations. We hypothesized that the metal mixture was associated with altered Fe status (e.g., reduced ferritin concentrations), and that Fe status was a mediator of the associations between the metal mixture and neurodevelopment.

2. Methods

2.1. Study population

We used cross-sectional data from the Public Health Impact of Metals Exposure (PHIME) study, a cohort designed to assess the impact of metal exposures from ferroalloy industry on neurodevelopment among early adolescents (aged 10–14 years). The study population, recruitment procedures, and study protocols have been described in detail elsewhere (Lucchini et al., 2012a). In brief, we recruited 721 adolescents from three sites in Brescia, Italy with varying historical ferroalloy activity. The first site, Bagnolo Mella, had ferroalloy activity since 1974; the second site, Garda Lake, had no historical ferroalloy industry activity; and the third site, Valcamonica, had continuous ferroalloy activity that ended in 2001. Enrollment into PHIME occurred in two distinct waves that reflected two periods of funding: the first wave (2007–2010) enrolled 311 participants and the second wave (2010–2014) enrolled 410 participants. All study protocols were consistent between the study phases. During the second phase, we recruited participants from the Bagnolo Mella site, collected additional biomarkers (saliva, urine, nails), and administered selected items from the Home Observation Measurement of the Environment (HOME) Short Form questionnaire (National Longitudinal Surveys, 1979).

Participants were eligible for enrollment into PHIME if they 1) were 10–14 years at the time of enrollment, 2) lived in the study region since birth, and 3) were born into families that lived in the study region since the 1970s. Exclusion criteria were: 1) neurologic, hepatic, metabolic, endocrine, or psychiatric disease, or clinically relevant motor deficits that may have impacted testing, 2) use of medication with neurologic side effects, 3) clinically diagnosed cognitive or behavioral impairment, 4) visual deficits without corrective measures, or 5) having ever received parenteral nutrition. Potential participants were given detailed information on all study protocols and gave informed consent prior to enrollment. PHIME study protocols were approved by Institutional Review Boards at the Icahn School of Medicine at Mount Sinai, University of California Santa Cruz, and the Ethical Committee of Brescia.

2.2. Covariate measurements

Information on covariates was collected by trained study staff either in person or via the phone using standardized questionnaires. We collected data on sociodemographic variables, including age (continuous, in years), biological sex (male or female), area of residence, birth order (first, second, third, or higher), parental occupation, and parental education level. Using World Health Organization and Italian National Institute for Statistics classifications (World Health Organization, 1988), education and occupation levels of both parents were classified as low, medium, or high. A socioeconomic status (SES) index (low, medium, or high) for each participant's family was constructed based on education and occupation levels for the parent with the highest educational and vocational attainment using previously developed methodology for Italian populations (Cesana et al., 1995; Lucchini et al., 2012b). A HOME score (range: 0–9) was calculated for each participant using ten selected items from the HOME Short Form (National Longitudinal Surveys, 1979).

2.3. Biomarker collection and measurement

Biomarkers were collected from participants at enrollment. We selected blood as the primary biomarker for Pb because it is the gold standard biomarker of exposure in the epidemiological literature and reflects total body Pb (Barbosa et al., 2005). There is a lack of consensus on the optimal biomarker for Mn, Cu, and Cr (Bertinato and Zouzoulas, 2009; Coetzee et al., 2016; Jursa et al., 2018; Lukaski, 1999). We selected hair as the primary biomarker for these metals because there was little missing hair data, hair has been used to characterize metals exposure previously in the literature (Bauer et al., 2020a), hair metal concentrations have been consistently associated (both beneficially and adversely) with cognitive outcomes in children (Bauer et al., 2020a; Coetzee et al., 2016), and hair metal concentrations have been correlated with environmental exposures (Bauer et al., 2020a; Coetzee et al., 2016). Methodology for quantification of metals in each of these biomarkers has been described previously in depth (Eastman et al., 2013; Lucas et al., 2015; Lucchini et al., 2012a; Smith et al., 2007). Briefly, 4 mL whole blood samples were collected using 19-gauge butterfly

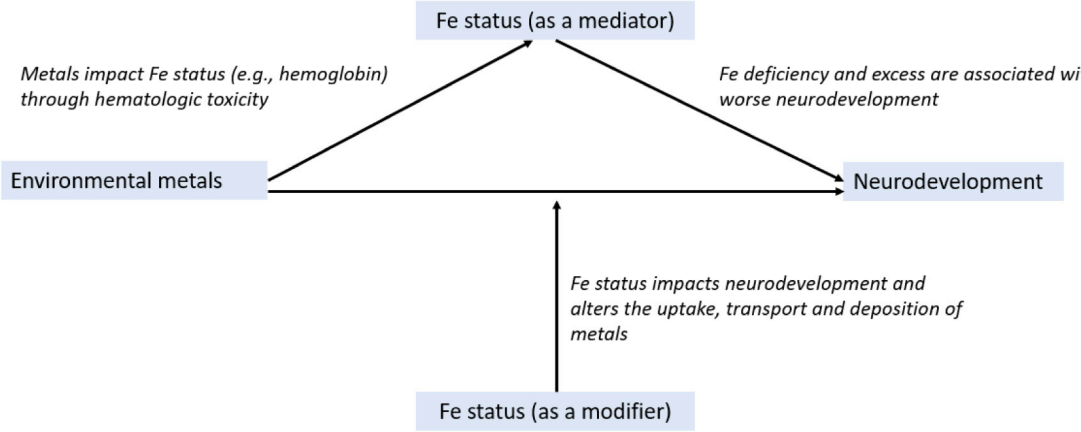


Fig. 1. A schematic illustrating the hypothesized role of Fe status as a modifier and mediator of metal-induced neurotoxicity.

catheters and stored in lithium-heparin Sarstedt Monovette Vacutainers. Hair samples were collected from the occipital region of the scalp with stainless steel clippers and cleaned extensively using validated methods; exogenous metal contamination was removed using Triton detergent, nitric acid, and sonification, and dried hair samples were then digested using distilled nitric acid (Eastman et al., 2013; Lucas et al., 2015). All biomarker metal concentrations were quantified using magnetic sector inductively coupled plasma mass spectrometry (Eastman et al., 2013; Lucchini et al., 2012a; Smith et al., 2007). Limits of detection (LODs) were defined based on repeated measures of procedural blanks (Butler et al., 2019); LODs ranged from 0.08 to 0.12 μg/g for hair Mn, Cr, and Cu, and the LOD for blood Pb was 0.01 μg/dL. There were few biomarker values below the LOD (hair Mn: n = 1, hair Cr: n = 1); those that were below the LOD were imputed as the LOD/2.

Fe status was measured from blood samples using three clinically relevant biomarkers: ferritin (ng/mL), hemoglobin (g/dL), and transferrin (mg/dL). Ferritin is considered a sensitive marker of Fe-deficiency and reflects long-term Fe tissue storage; transferrin transports Fe to tissues and reflects Fe availability; and hemoglobin reflects functional Fe status, where low concentrations are indicative of Fe-deficient anemia (Gibson, 2005). Hemoglobin was measured in whole blood samples during a complete blood count (CBC) with the Beckman Coulter LH Series (Beckman Coulter, Inc. Diagnostics Division, CA, USA). Blood samples were collected into tubes containing EDTA coagulant. Ferritin and transferrin were measured in serum using immunoassays with the Architect *i*2000*SR* – Abbott Laboratories (Abbott Park, IL, USA) and ADVIA (Siemens Healthcare GmbH, Germany), respectively.

2.4. Cognitive assessment

The California Verbal Learning Test for Children (CVLT-C) was administered to PHIME participants by trained neuropsychologists to assess verbal learning and memory (Delis et al., 1994). The CVLT-C was only administered to participants during the second phase of PHIME (n = 403). The CVLT-C consists of 5 recall trials (trials 1-5) of a list of 15 verbally presented words (List A). The 15 words include 5 semantically related words from three separate categories (e.g., fruits). Participants then perform a recall trial from an interference list of 15 words (List B). This is followed by additional recall trials of List A, including free (i.e., not cued) and cued recall trials immediately following the interference list (short delay recall) and after 20 minutes (long delay recall). Lastly, participants complete a recognition trial, where they are asked to select target words (i.e., those on List A) from a written list of 44 words.

CVLT-C outcomes available for analysis included scores for the correct number of target words recalled on trial 1, trial 5, the inference list, the short (free and cued) delay trials, the long (free and cued) delay trials, and the recognition trial. Higher scores (i.e., a higher number of words correctly recalled) for these trials were indicative of better learning and memory. We calculated the number of intrusions, defined as the total number of non-target words recalled across all trials, and the number of perseveration errors, defined as the total number of target words repeated across all trials. Higher scores for both intrusions and perseverations were indicative of worse cognitive function. Lastly, we calculated a metric of forgetting by subtracting the number of correct words on the short delay free recall trial from the number of correct words on the long delay free recall trial (Kreutzer et al., 2011; Strauss et al., 2006). Negative scores for forgetting were indicative of worse memory performance, while positive scores reflected better performance (i.e., better retention, or remembering). We a priori selected five CVLT-C outcomes for analysis in the current study, including trial 5 recall, long delay free recall, long delay cued recall, perseveration errors, and forgetting, because these outcomes represent varying aspects of learning and memory function (Table S1).

2.5. Statistical analysis

All analyses were performed in R version 3.6.1.

2.5.1. Multiple imputation

The analytic sample for this study was restricted to adolescents with complete outcome data (n = 403). There was little missing data (<6 % for covariates and biomarkers, Table S2). Missing biomarker and covariate data were imputed using Markov chain Monte Carlo methods (Zhou et al., 2001) with the *mice* package in R (van Buuren and Groothuis-Oudshoorn, 2011), where missing data were assumed to be missing at random. Twenty datasets were imputed using all covariate data, and one imputed dataset was randomly selected for all subsequent analyses.

2.5.2. Confounder selection

Confounders were identified a priori using knowledge of the literature, biologic plausibility, and directed acyclic graphs (DAGs) (Bauer et al., 2020b; de Carvalho et al., 2018; Kordas, 2010; Torres-Agustín et al., 2013). All models were adjusted for age (continuous), biological sex (binary), HOME score (continuous) and socioeconomic status (ordinal, classified as low, medium, or high).

2.5.3. Summary statistics

Summary statistics, including medians, 25th percentiles, 75th percentiles, means and standard deviations (SDs), were calculated for all variables. Summary statistics for the randomly selected imputed dataset were similar to the complete data (Table S3). Distributions of all continuous variables were examined; based on visual inspection of histograms and boxplots, we identified several extreme values for metal concentrations. Participants with concentrations of any metal that were ±3 SDs from the mean (n = 20) were excluded from further analyses (final sample size = 383). All metals (Pb, Mn, Cu, Cr), ferritin, and one CVLT-C outcome (perseveration errors) were right-skewed, and we natural log-transformed these variables to reduce the influence of outlier values and to satisfy assumptions of normality of residuals for statistical modeling. Metals, Fe status metrics, and CVLT-C outcomes were z-standardized prior to regression modeling. We estimated Spearman correlation coefficients between the metals and between the CVLT-C outcomes.

2.5.4. Bayesian Kernel Machine Regression

We used Bayesian Kernel Machine Regression Causal Mediation Analysis (BKMR-CMA) as our primary model to investigate the mediating role of Fe status on associations between the metal mixture (Pb, Mn, Cr, Cu) and verbal learning and memory. BKMR is a highly flexible model that uses a kernel function to model the exposure response surface of the mixture, where individuals in the study population with similar exposure profiles are assumed to have similar neurodevelopment scores (Bobb et al., 2018, 2015). The flexible kernel function allows for non-linearity, metal-metal interactions, higher order interactions (e.g., interactions of an individual metal with the rest of the mixture), and estimation of cumulative effects (Bobb et al., 2018, 2015).

BKMR-CMA is an extension of BKMR used to quantify the mediation of the mixture (Pb, Mn, Cr, Cu) by a third variable, where the mediating variable is also allowed to interact with the mixture by including the mediator (i.e., ferritin, transferrin, hemoglobin) in the kernel function (Devick et al., 2022). Three BKMR models were fit to estimate the mediation effects: [1] the outcome model, which quantified the association of the mixture (Pb, Mn, Cr, Cu) with the CVLT-C outcomes when Fe status was included in the kernel function; [2] the mediator model, which quantified the association of the metal mixture (Pb, Mn, Cr, Cu) with Fe status; and [3] the total effects model, which quantified the association of the metal mixture (Pb, Mn, Cr, Cu) with the CVLT-C outcomes when Fe status was excluded from the model. These models took the following form:

$$CVLT\ score = h(Mn_i, Pb_i, Cr_i, Cu_i, Fe\ status\ metric_i) + \beta_1{*}Sex_i + \beta_2{*}Age_i + \beta_3{*}SES_i + \beta_4{*}HOME\ score_i + e_i \tag{1}$$

$$Fe\ status\ metric = h(Mn_i, Pb_i, Cr_i, Cu_i) + \beta_1{*}Sex_i + \beta_2{*}Age_i + \beta_3{*}SES_i + \beta_4{*}HOME\ score_i + e_i \tag{2}$$

$$CVLT\ score = h(Mn_i, Pb_i, Cr_i, Cu_i\ ) + \beta_1{*}Sex_i + \beta_2{*}Age_i + \beta_3{*}SES_i + \beta_4{*}HOME\ score_i + e_i \tag{3}$$

where *h* represents the kernel function, e_i is the error term, and confounders were assumed to have linear associations with the outcomes.

Each of these models was fit utilizing the default non-informative prior specifications and 50,000 iterations with a 50 % burn-in. The component-wise variable selection option was used to estimate posterior inclusion probabilities (PIPs), which describe the relative importance of each component in the kernel function with the outcome while accounting for multiple testing.

Using Eqs. (1)–(3), we estimated four mediation effects using previously developed methodology (Devick et al., 2022): the controlled direct effect (CDE), natural direct effect (NDE), natural indirect effect (NIE), and total effect (TE). The CDE described the association of the metal mixture (Pb, Mn, Cr, Cu) with each CVLT-C outcome for an increase in the mixture from its 25th to 75th percentile, holding the mediator (e.g., ferritin) at its 25th, 50th, and 75th percentiles. The NDE quantified the direct association (i.e., not mediated through Fe status) of the mixture (Pb, Mn, Cr, Cu) with each CVLT-C outcome for an increase in the mixture from its 25th to 75th percentiles, holding the mediator constant at the level it would take if the mixture was held at its 25th percentile. The NIE described the indirect association (i.e., mediated through Fe status) of the mixture (Pb, Mn, Cr, Cu) with each CVLT-C outcome when the mixture was held at its 75th percentile, and Fe status was set to the concentration it would have taken when the mixture was set at its 25th percentile compared to its 75th percentile. The TE reflected the sum of both the direct (NDE) and indirect (NIE) pathways. These mediation effects are visualized in Fig. 2A. Beta coefficients and 95 % credible intervals (CIs) were estimated for all mediation effects.

We also estimated summary measures to describe associations of the mixture (Pb, Mn, Cr, Cu, Fe status metric) with each CVLT-C outcome (using Eq. (1)) and the association of the metal mixture (Pb, Mn, Cr, Cu) with Fe status metrics (using Eq. (2)). These summary measures included 1) exposure-response profiles for each exposure variable included in the kernel function, holding all other exposure variables at their medians; 2) exposure-response profiles of each exposure variable included in the kernel function at the 25th, 50th, and 75th percentile of a second exposure variable, while holding other exposure variables at their 50th percentiles; 3) the association for a percentile increase in all exposure variables simultaneously, compared to the 50th percentile of all exposure variables; and 4) the association for each exposure variable included in the kernel function for an increase from its 25th to 75th percentiles, holding all other exposure variables at their 25th, 50th, or 75th percentiles.

2.5.5. *Sex-stratified analyses*

Epidemiological evidence suggests that associations of metals with neurodevelopmental outcomes can be sex-specific, and that females are more susceptible to Fe deficiency in adolescence (Bauer et al., 2017; Kounnavong et al., 2020; Llop et al., 2013; Rechtman et al., 2020; Shaw, 1996; Zhu et al., 2021). In exploratory analyses, we fit BKMR-CMA models for each CVLT-C outcome in sex-stratified datasets to examine potential sex-specific effects.

2.5.6. *BKMR-CMA sensitivity analyses*

We investigated the sensitivity of our findings to the specification of the priors by fitting the BKMR-CMA models 1) using the gamma prior distribution instead of the default inverse uniform distribution, and 2) changing the degree of smoothness of the *h* function from the default (100) to 50 and 1000 (Bauer et al., 2020a; Valeri et al., 2017). We also performed a complete case analysis, where we fit BKMR-CMA models for one *a priori* selected CVLT outcome (trial 5 recall) in a restricted sample that included only adolescents with complete data for all biomarkers,

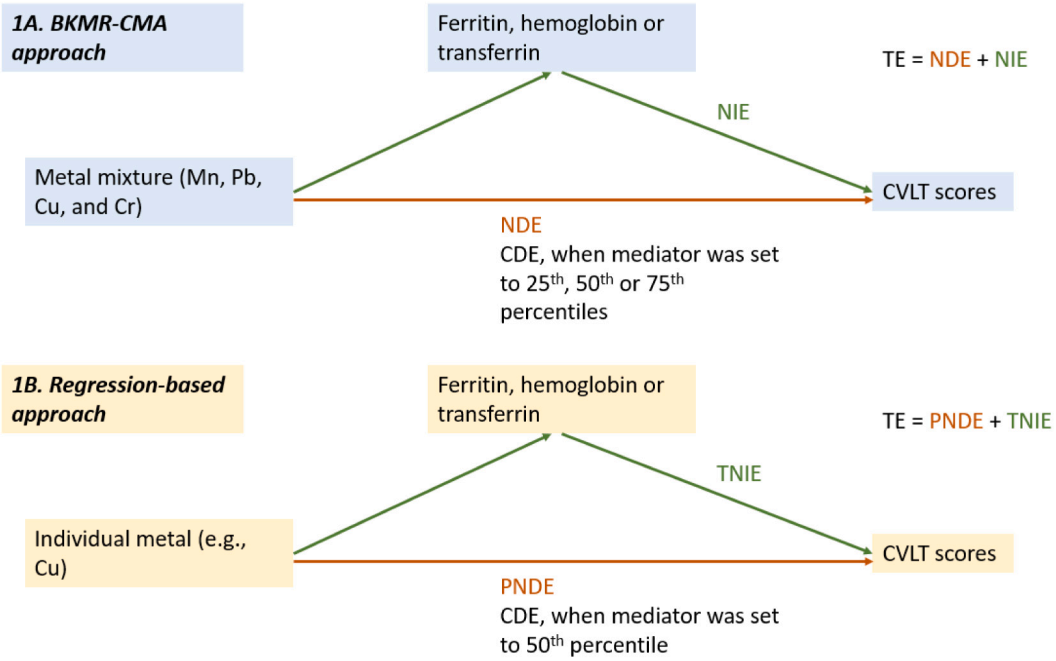


Fig. 2. Visualization of the mediation effects estimated from the (A) BKMR-CMA and (B) regression-based models.
BKMR-CMA, Bayesian Kernel Machine Regression Causal Mediation Analysis; TE, total effect; NDE, natural direct effect; NIE, natural indirect effect; CDE, controlled direct effect; Mn, manganese; Pb, lead; Cu, copper; Cr, chromium; CVLT, California Verbal Learning Test; PNDE, pure natural direct effect; TNIE, total natural indirect effect.

Table 1
Characteristics of PHIME study participants included in present analysis (n = 383).[a]

Characteristic	N (percent) or mean ± SD
Sex	
Female	182 (47.5 %)
Male	201 (52.5 %)
Age (years)	12.3 ± 1.0
Socioeconomic status index	
Low	89 (23.2 %)
Medium	201 (52.5 %)
High	93 (24.3 %)
HOME score	6.0 ± 1.7
Site	
Bagnolo Mella	204 (53.3 %)
Garda Lake	79 (20.6 %)
Valcamonica	100 (26.1 %)
CVLT-C outcomes (raw scores)	
Long delay free recall	11.5 ± 2.1
Long delay cued recall	11.8 ± 2.1
Trial 5	12.3 ± 1.9
Perseveration errors	7.2 ± 6.1
Forgetting	0.3 ± 1.6
Metal biomarkers (median, 25th, 75th percentile)	
Hair Mn (μg/g)	0.07 (0.04, 0.12)
Hair Cu (μg/g)	9.4 (6.6, 14.8)
Hair Cr (μg/g)	0.04 (0.03, 0.06)
Blood Pb (μg/dL)	1.3 (1.0, 1.7)
Iron biomarkers (median, 25th, 75th percentile)	
Ferritin (ng/mL)	32.0 (21.0, 44.0)
Transferrin (mg/dL)	283.0 (260.0, 308.0)
Hemoglobin (d/dL)	13.8 (13.2, 14.4)

[a] PHIME, Public Health Impact of Metals Exposure Study; HOME, Home Observation Measurement of the Environment; CVLT-C, California Verbal Learning Test for Children; Mn, manganese; Cu, copper; Cr, chromium; Pb, lead.

CVLT outcomes, and confounders (n = 329).

2.5.7. Generalized additive models (GAMs) and multivariable linear regression

To investigate the robustness of our BKMR-CMA findings, we additionally used a regression-based causal mediation approach to examine mediation of the association of the individual metals by Fe status. There was limited evidence that the Fe status markers (ferritin, hemoglobin, transferrin) were associated with CVLT-C scores on any subtest, or of mediation of the overall mixture by Fe status, in BKMR-CMA models. We therefore present findings from regression-based approaches only for the association of Cu with trial 5 because Cu was found to drive the beneficial association of the metal mixture with the recall trial outcomes in our previous work (Schildroth et al., 2023).

Because there was evidence of non-linear associations of the metals with Fe status metrics in BKMR-CMA models, we first used Generalized Additive Models (GAMs) to visually inspect the shape of the associations between each metal with the CVLT-C outcomes, each metal with the Fe status metrics, and each Fe status metric with the CVLT-C outcomes. These models were fit using penalized splines (knots = 4) to allow for non-linear associations, and likelihood ratio tests (LRTs) were used to compare fits between the models with and without the penalized splines. GAM plots and LRTs confirmed non-linear associations of the nutrient transition metals (Mn, Cu, Cr) with markers of Fe status observed in the BKMR-CMA models, which is consistent with prior literature (Schildroth et al., 2022a). These essential metals were therefore modeled using GAMs with penalized splines (knots = 4) to account for non-linearity in the mediator regression models that characterized the association between the individual metals and markers of Fe status (Eq. (4)). Pb was modeled continuously in these models. Although non-linear associations of the nutrient metals with neurodevelopmental outcomes have been reported previously in the literature (Bauer et al., 2020a; Claus Henn et al., 2010), there was no evidence of non-linear associations of any of the metals or Fe status markers with the CVLT scores. Therefore, all biomarker concentrations were modeled continuously in the outcome linear regression models in Eq. (5).

Regression models were fit using the regression-based approach in the *CMAverse* package in R (Shi et al., 2021; Valeri and VanderWeele, 2013). Mediation effects for the regression-based approach were estimated by fitting a mediator model and an outcome model, specified in Eqs. (4) and (5), respectively. These models were fully adjusted for all *a priori* selected confounders, and adjusted for all other metals (Pb, Mn, and Cr).

$$Fe\ status\ metric = \beta_0 + \beta_1 {*} s(Cu) + \beta_2 {*} s(Cr) + \beta_3 {*} s(Mn) + \beta_4 {*} Pb + \beta_5 {*} Sex + \beta_6 {*} Age + \beta_7 {*} SES + \beta_8 {*} HOME\ score \tag{4}$$

[a] These models were fit using GAMs; s() denotes a penalized spline (knots = 4).

$$Trial\ 5\ recall = \beta_0 + \beta_1 {*} Cu + \beta_2 {*} Cr + \beta_3 {*} Mn + \beta_4 {*} Pb + \beta_5 {*} Sex + \beta_6 {*} Age + \beta_7 {*} SES + \beta_8 {*} HOME\ score + \beta_9 {*} Fe\ status\ metric \tag{5}$$

[a] These models were fit using linear regression.

As we have previously reported (Schildroth et al., 2023), there was evidence of an interaction between Cu and ferritin for trial 5 recall. Allowing for exposure-mediator interaction is pertinent for correctly specifying the outcome model (VanderWeele, 2016). Therefore, we included an interaction term between Cu and ferritin in the outcome linear regression model when ferritin was considered the mediator.

In the regression-based approach, the total effect (TE) is decomposed into the pure natural direct effect (PNDE) and the total natural indirect effect (TNIE). The PNDE reflects the direct association of the exposure with the outcome when the mediator is set to its natural value at low concentration levels of the exposure, and the TNIE reflects the indirect association of the exposure with the outcome due to both mediation and mediator-exposure interaction (Shi et al., 2021; Valeri and VanderWeele, 2013; Vanderweele, 2014). We report the PNDE and TNIE for the association of Cu with trial 5 when ferritin, transferrin, and hemoglobin were considered the mediators. We also report the CDE for these associations, setting the mediators at their 50th percentiles. These mediation effects are visualized in Fig. 2B.

3. Results

About half the participants in the analytic sample were male (53 %), lived in the Bagnolo Mella study site (53 %) and came from families characterized as medium SES (53 %, Table 1). The mean age was 12.3 years (SD: 1.0) and the mean HOME score was 6.0 (SD: 1.7). Median (25th–75th percentile) hair concentrations for Mn, Cu, and Cr were 0.07 μg/g (0.04–0.12 μg/g), 9.4 μg/g (6.6–14.8 μg/g), and 0.04 μg/g (0.03–0.06 μg/g), respectively. The median blood Pb concentration was 1.3 μg/dL (1.0–1.7 μg/g). Females had higher median concentrations of hair Cu (10.3 μg/g; 25th–75th percentiles: 7.6–16.5 μg/g) compared to males (8.6 μg/g; 25th–75th percentiles: 6.3–13.7 μg/g), but males had higher median concentrations of blood Pb (1.4 μg/dL; 25th–75th percentiles: 1.1–2.0 μg/dL) compared to females (1.1 μg/dL; 25th–75th percentiles: 0.9–1.4 μg/dL). Median concentrations of Mn and Cr were similar in males and females (Table S4). Spearman correlation coefficients between the metals were weak and ranged from 0.01 (blood Pb – hair Cu) to 0.35 (hair Mn – hair Cr).

Median concentrations for serum ferritin (32.0 ng/mL; 25th–75th percentile: 21.0–44.0 ng/mL), serum transferrin (283.0 mg/dL; 25th–75th percentile: 260.0–308.0 mg/dL), and blood hemoglobin (13.8 g/dL; 25th–75th percentile: 13.2–14.4 g/dL) were within normal clinical ranges for adolescents (Gibson, 2005). Males had higher median concentrations of ferritin (33.0 ng/mL vs. 30.0 ng/mL), transferrin

(284.0 mg/dL vs. 282.5 mg/dL) and hemoglobin (14.0 g/dL vs. 13.6 g/dL) (Table S4) compared to females, which is typical during the adolescent period (Gibson, 2005).

3.1. Associations of the metal mixture with Fe status

The metal mixture was inconsistently associated with concentrations of Fe status markers. Compared to the 50th percentile of the overall mixture, setting the metal mixture (Pb, Mn, Cr, Cu) to its 90th percentile was associated with lower serum ferritin concentrations (β = −0.19, 95 % CI = −0.46, 0.07). Conversely, the 90th percentile of the mixture was associated with a 0.33 SD increase (β = 0.33, 95 % CI = 0.03, 0.63) in hemoglobin concentrations, compared to when the metal mixture was set to its 50th percentile. The metal mixture was not materially associated with transferrin concentrations (Fig. 3).

The association of the mixture with ferritin was driven by Pb (PIP = 0.83), where a change in Pb from its 25th to 75th percentiles was associated with a 0.33 SD decrease (95 % CI = −0.58, −0.07) in serum ferritin concentrations when the rest of the mixture was held at its 50th percentile (Fig. 4). The association of the mixture with hemoglobin concentrations was also driven by Pb; however, a change in Pb from its 25th to 75th percentiles was associated with higher concentrations of hemoglobin (β = 0.43, 95 % CI = 0.18, 0.68) when the mixture was held at its 50th percentile (Fig. S1). None of the metals in the mixture were materially associated with transferrin concentrations (Fig. S2).

3.2. Associations of the metal mixture with CVLT-C outcomes

Consistent with our previous findings in PHIME (Schildroth et al., 2023), the metal mixture was jointly associated with better performance on the recall trials, especially long delay free recall, when each of the Fe status metrics was included in the mixture (Figs. S3–S11, panel C). Compared to the 50th percentile, the 90th percentile of the mixture was associated with higher scores for long delay free recall scores when ferritin (β = 0.20, 95 % CI = −0.15, 0.55), transferrin (β = 0.22, 95 % CI = −0.12, 0.55), and hemoglobin (β = 0.22, 95 % CI = −0.08, 0.53) were included in the model, respectively (Fig. S3). Similar associations were observed for trial 5 recall and long delay cued recall (Figs. S6–S11, panel C), though the beta coefficients were smaller in magnitude. The positive association of the mixture with the recall trials was driven primarily by copper (Figs. S3–S11).

The metal mixture was also associated with higher scores for perseveration errors, reflecting worse cognitive performance, when each of the Fe status metrics was included as a component of the mixture. The 90th percentile of the mixture, compared to the 50th percentile, was associated with a 0.25 SD increase (95 % CI = −0.07, 0.56), 0.23 SD increase (95 % CI = −0.15, 0.60), and 0.22 SD increase (95 % CI = −0.10, 0.54) in ln-transformed perseveration errors when ferritin, transferrin, and hemoglobin were included in the model, respectively. These associations were primarily driven by the association between Pb and higher number of perseveration errors (Figs. S12–S14, panel C).

3.3. Mediation of the metal mixture by Fe status

Fig. 5 shows the mediation effects for trial 5, long delay free, and long delay cued recall in BKMR-CMA models. Notably, there was no evidence of mediation by Fe status: the NIE, which reflects the indirect association of the mixture with neurodevelopment mediated through Fe status, was null across all three Fe status metrics for trial 5 (Fig. 5, panel A), long delay free (Fig. 5, panel B), and long delay cued (Fig. 5, panel C) recall. Conversely, the NDE, which reflects the direct association of the mixture (for a change from its 25th to 75th percentiles) not mediated by Fe status, was positive for all three recall trials when ferritin, hemoglobin, or transferrin were considered the mediators (Fig. 5, panels A–C), suggesting better cognitive performance with increasing metal concentrations, independent of Fe status. The strongest associations were observed for LDFR: the NDE was 0.23 (95 % CI = 0.01, 0.44), 0.23 (95 % CI = 0.02, 0.44), and 0.23 (95 % CI = 0.01, 0.45) when ferritin, hemoglobin, and transferrin, respectively, were considered the mediators (Fig. 5, panel B). These findings suggest that associations of the metal mixture with the recall trials in these data operate exclusively through the direct pathway and are not mediated by Fe status.

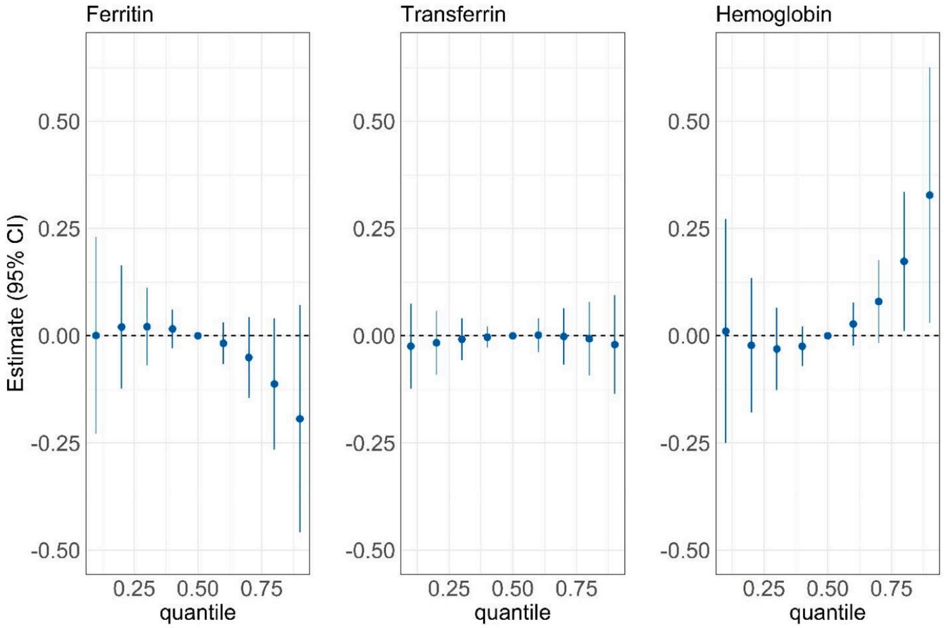


Fig. 3. Joint associations of the metal mixture (Pb, Mn, Cr, Cu) with ferritin, transferrin, and hemoglobin at increasing percentiles (10th, 20th, 30th, 40th, 60th, 70th, 80th, and 90th) of all metals compared to the medians from BKMR models. Metal and ferritin concentrations were log-transformed and Z-standardized. Transferrin and hemoglobin concentrations were also Z-standardized. Models were adjusted for age, sex, SES, and HOME score (n = 383).

Pb, lead; Mn, manganese; Cr, chromium; Cu, copper; BKMR, Bayesian Kernel Machine Regression; SES, socioeconomic status; HOME, Home Observation Measurement of the Environment.

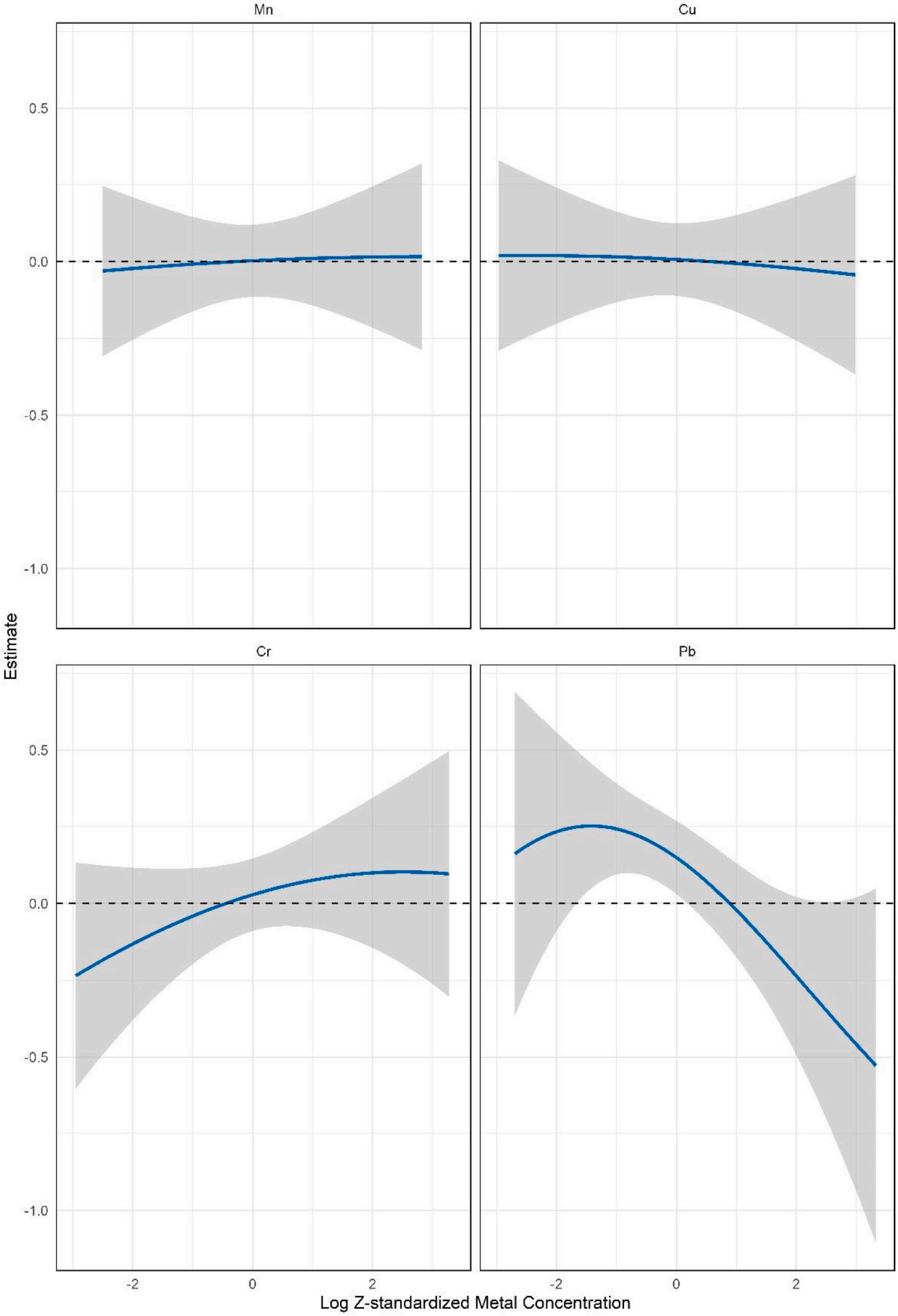
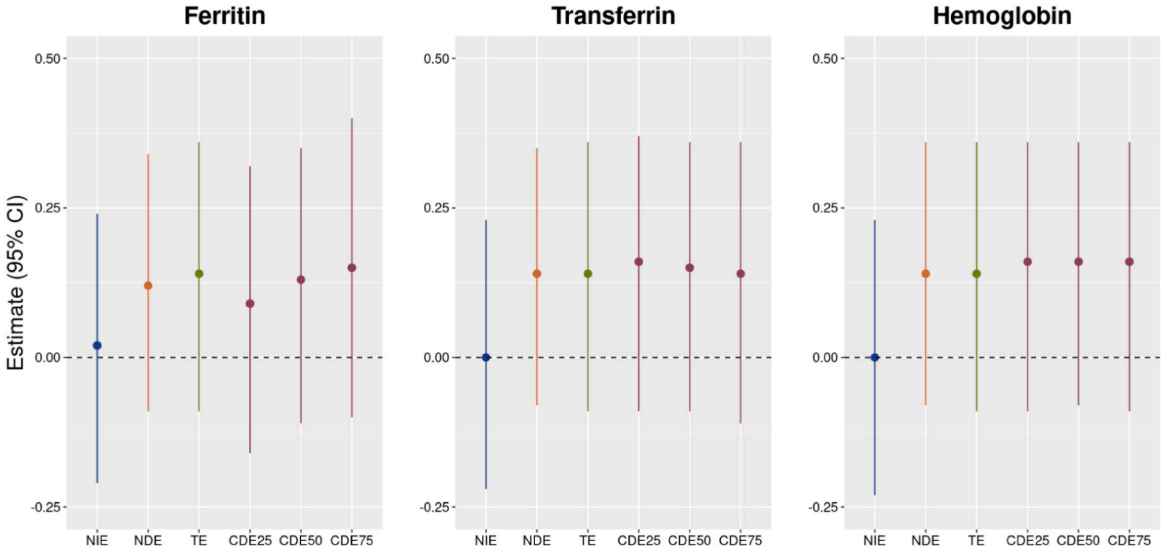
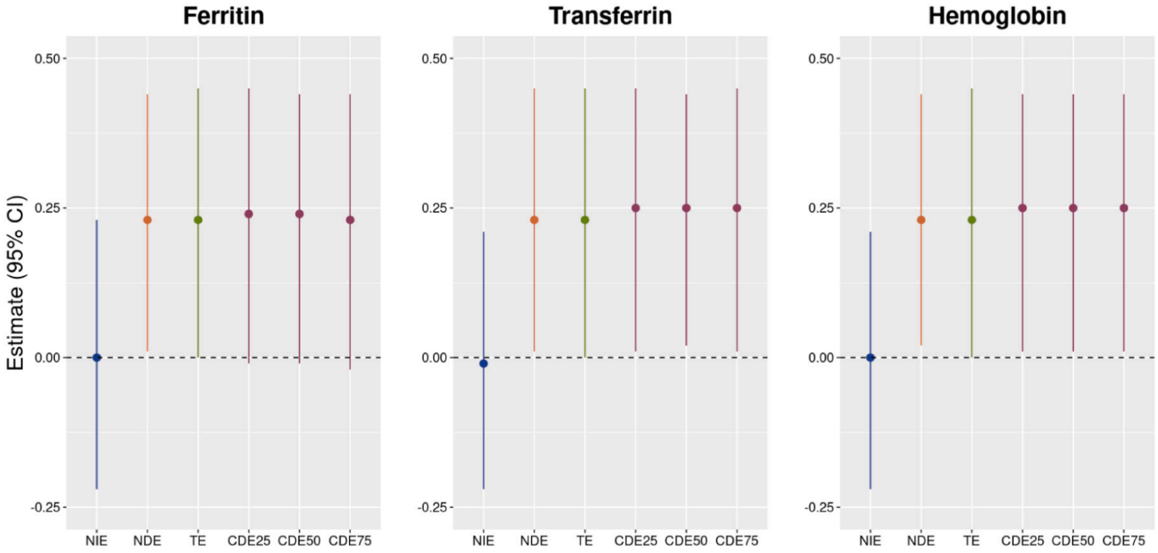


Fig. 4. Exposure response profiles from BKMR models for each metal with ferritin for participants in the PHIME cohort (n = 383). Gray shading represents 95 % credible intervals. Metal and ferritin concentrations are log-transformed and Z-standardized. Associations are adjusted for age, sex, HOME score and SES.
Pb, lead; Mn, manganese; Cr, chromium; Cu, copper; BKMR, Bayesian Kernel Machine Regression; SES, socioeconomic status; HOME, Home Observation Measurement of the Environment.

(A) Trial 5 Recall



(B) Long Delay Free Recall



(C) Long Delay Cued Recall

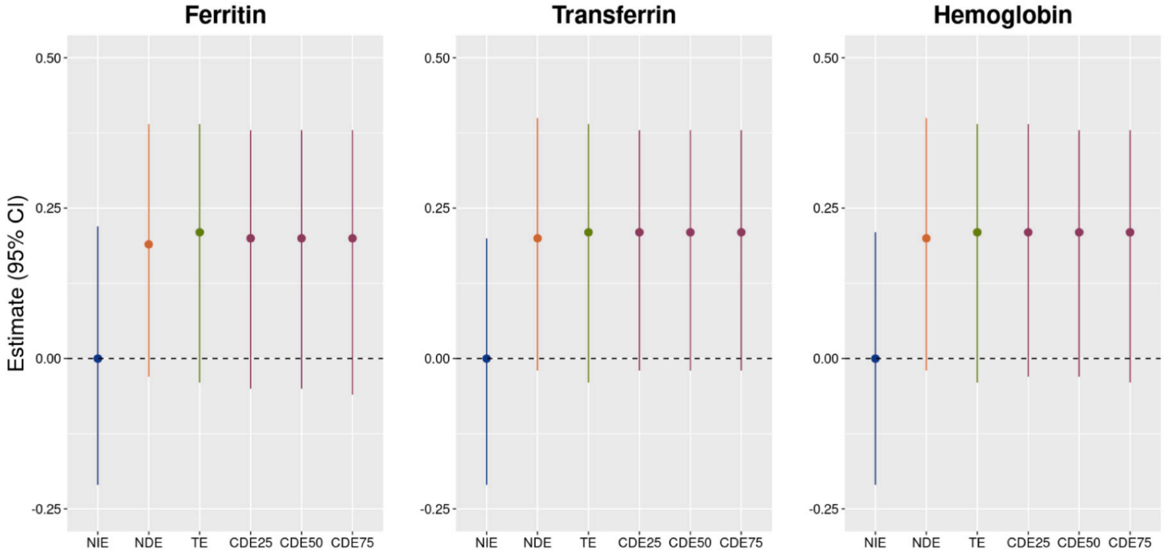


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Fig. 5. Mediation effects of ferritin, transferrin, and hemoglobin on associations between the metal mixture and (A) trial 5 recall, (B) long delay free recall, and (C) long delay cued recall in the PHIME cohort (n = 383). Mediation effects are estimated for a change in the metal mixture from its 25th percentile to its 75th percentile. The CDEs are estimated holding the mediator (ferritin, transferrin, or hemoglobin) at its 25th, 50th, or 75th percentiles. Metals and ferritin are log-transformed and Z-standardized. Hemoglobin and transferrin concentrations are Z-standardized. Associations are adjusted for age, sex, HOME score and SES.
BKMR, Bayesian Kernel Machine Regression; SES, socioeconomic status; HOME, Home Observation Measurement of the Environment; NIE, natural indirect effect; NDE, natural direct effect; TE, total effect; CDE, controlled direct effect.

There was also no evidence of mediation by any Fe status marker for the association between Cu and trial 5 in the regression-based approach (Table S5). For example, when ferritin was considered the mediator, the TNIE was null (β = 0.00, 95 % CI = −0.01, 0.01), while the PNDE suggested a beneficial association of Cu with trial 5 (β = 0.21, 95 % CI = 0.11, 0.30) that operated exclusively on the direct pathway. Similar associations were also found when hemoglobin or transferrin was considered the mediator (Table S5).

The NIE was also null for perseveration errors, suggesting no evidence of mediation by any Fe status metric (Fig. 6). However, the NDE (for a change in the mixture from its 25th to 75th percentiles) was positive for perseveration errors, indicating adverse associations with cognitive performance. The NDE was 0.13 (95 % CI = −0.08, 0.37), 0.14 (95 % CI = −0.08, 0.38), and 0.13 (95 % CI = −0.08, 0.36) when ferritin, transferrin, or hemoglobin were considered the mediator. As with the recall trials, these findings suggest that the adverse association of the mixture with perseveration errors operated exclusively via the direct pathway.

The mixture was not materially associated with forgetting, and there was similarly no evidence of mediation by any of the Fe status markers (Fig. S15).

3.4. *Sex-stratified analyses: associations of the metal mixture with Fe status*

In sex-stratified BKMR-CMA models, the negative association of the mixture (Pb, Mn, Cr, Cu) at its 90th percentile, compared to the 50th percentile, with ferritin was stronger in males (β = −0.21; 95 % CI = −0.69, 0.28) compared to females (β = −0.08; 95 % CI = −0.44, 0.29, Fig. 7, panels A and B). The positive association of the mixture at its 90th percentile, compared to the 50th percentile, with hemoglobin was also stronger in males (β = 0.34; 95 % CI = −0.03, 0.70) compared to females (β = 0.16; 95 % CI = −0.26, 0.59, Fig. 7, panels C and D). Higher concentrations of the mixture (Pb, Mn, Cr, Cu) were not materially associated with transferrin in males (at the 90th percentile, compared to the 50th percentile: β = −0.09; 95 % CI = −0.46, 0.28) or females (at the 90th percentile, compared to the 50th percentile: β = −0.12; 95 % CI = −-0.52, 0.28, Fig. 7, panels E and F). As with our main findings, the joint associations of the mixture with ferritin and hemoglobin among males were driven primarily by Pb (Fig. S16).

3.5. *Sex-stratified analyses: associations of the metal mixture with CVLT-C outcomes*

As we have previously reported (Schildroth et al., 2023), there was evidence of sex-specific associations of the mixture with our indices of neurodevelopment, though these associations tended to be imprecise. Notably, joint increases in the overall mixture were associated with better recall scores only among females. For example, the 90th percentile of the mixture (compared to the 50th percentile) was associated with a 0.25 SD increase (95 % CI = −0.39, 0.90) in trial 5 recall scores among females, but a 0.14 decrease (95 % CI = −0.63, 0.36) in males when ferritin was included in the model. Similar associations were observed for the other recall trials, and these sex-specific associations were driven primarily by stronger associations of Cu with the recall trials among females (data not shown). As in the main findings, there was no evidence of mediation by any Fe status metric in either sex (Figs. S17–S21).

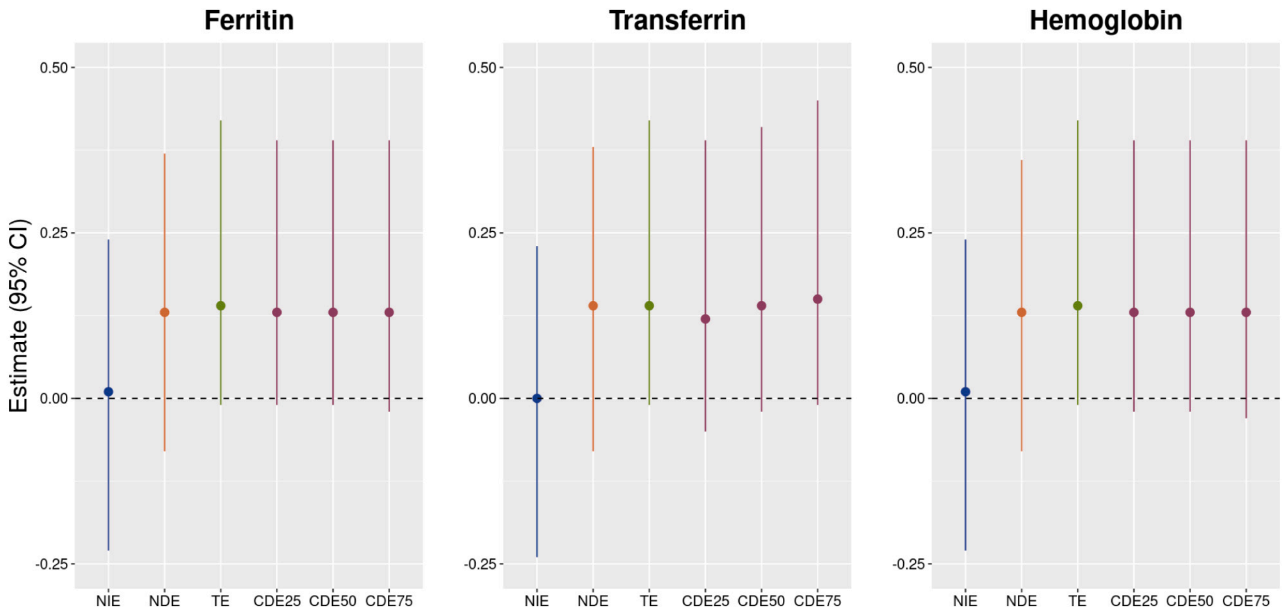

Fig. 6. Mediation effects of ferritin, transferrin, and hemoglobin on associations between the metal mixture and perseveration errors in the PHIME cohort (n = 383). Mediation effects are estimated for a change in the metal mixture from its 25th percentile to its 75th percentile. The CDEs are estimated holding the mediator (ferritin, transferrin, or hemoglobin) at its 25th, 50th, or 75th percentiles. Metals and ferritin are log-transformed and Z-standardized. Hemoglobin and transferrin concentrations are Z-standardized. Associations are adjusted for age, sex, HOME score and SES.
Pb, lead; Mn, manganese; Cr, chromium; Cu, copper; BKMR, Bayesian Kernel Machine Regression; SES, socioeconomic status; HOME, Home Observation Measurement of the Environment; NIE, natural indirect effect; NDE, natural direct effect; TE, total effect; CDE, controlled direct effect.

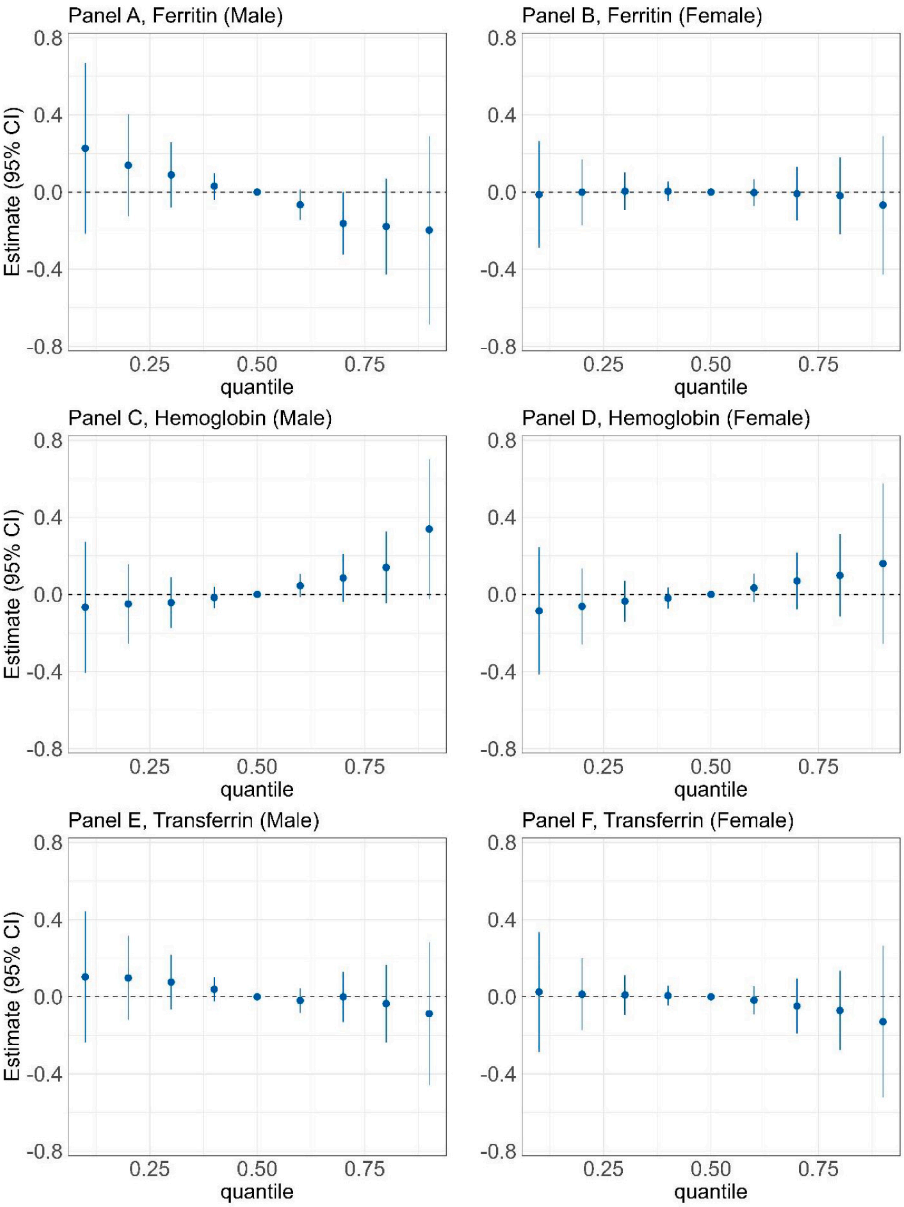

Fig. 7. Joint associations of the metal mixture (Pb, Mn, Cr, Cu) with ferritin, transferrin, and hemoglobin at increasing percentiles (10th, 20th, 30th, 40th, 60th, 70th, 80th, and 90th) of all metals compared to the medians from BKMR models among males (n = 201) and females (n = 182). Metal and ferritin concentrations were log-transformed and Z-standardized. Transferrin and hemoglobin concentrations were also Z-standardized. Models were adjusted for age, SES, and HOME score.
Pb, lead; Mn, manganese; Cr, chromium; Cu, copper; BKMR, Bayesian Kernel Machine Regression; SES, socioeconomic status; HOME, Home Observation Measurement of the Environment.

3.6. BKMR-CMA sensitivity analyses

We performed a series of sensitivity analyses to examine the robustness of our main BKMR-CMA findings: 1) a complete case analysis, where we restricted the analytic sample to adolescents with no missing data (n = 329); 2) an analysis changing the default uniform prior distribution to a gamma distribution; and 3) an analysis changing the default smoothness of the *h* function (100) to 50 and 1000.

The mediation effects for trial 5 recall, including the NDE, NIE, TE, and CDEs, from sensitivity analyses that used the gamma prior distribution and changed the smoothness of the *h* function to 50 or 1000 were consistent with our main findings (Figs. S22–S24). Findings for the mediation effects for the complete case analysis were also similar to our main findings, though associations tended to be stronger (Fig. S25). For example, the NDEs for trial 5 in the complete case analysis for ferritin, transferrin, and hemoglobin were 0.19 (95 % CI = −0.04, 0.41; main model: β = 0.12; 95 % CI = −0.09, 0.34), 0.18 (95 % CI = −0.05, 0.40; main model: β = 0.14; 95 % CI = −0.08, 0.35), and 0.18 (95 % CI = −0.04, 0.41; main model: β = 0.14; 95 % CI = −0.08, 0.36), respectively. There was no evidence of mediation by any Fe status metric in any of the sensitivity analyses.

Associations of the metals with ferritin, transferrin, and hemoglobin also tended to be similar in sensitivity analyses (Figs. S26–S37), with one notable exception: the association of Pb with ferritin that we observed in the main model (Fig. 3) was null in the analysis that used the gamma distribution (Fig. S26) and in the complete case analysis (Fig. S35).

4. Discussion

In this study of Italian adolescents residing near ferroalloy production sites, we found that a mixture of Pb, Mn, Cu, and Cr was jointly associated with aspects of verbal learning and memory. As we have previously reported, the associations of the overall mixture with the recall trials and perseveration errors were driven primarily by Cu (beneficial) and Pb (adverse), respectively (Schildroth et al., 2023). The metal mixture was also associated with markers of Fe status, (i.e., ferritin and hemoglobin), but there was no evidence that Fe status mediated the association of the metal mixture with neurodevelopment.

When considering the association of the overall mixture with verbal learning and memory, we found that a joint increase in all components of the mixture (Pb, Mn, Cu, Cr, marker of Fe status) was positively associated with the recall trials when any of the three markers of Fe status (ferritin, hemoglobin, or transferrin) was included in the mixture. These associations were primarily driven by Cu, which we have reported on previously (Schildroth et al., 2023), and may reflect the role of Cu as an essential nutrient needed for catecholamine synthesis, cellular respiration, formation/maintenance of myelin, and long-term potentiation (Gaetke et al., 2014; Opazo et al., 2014). It should also be noted that, although we observed beneficial associations of Cu with cognition, previous studies have reported beneficial, adverse and nonlinear associations of Cu with neurodevelopment outcomes (Amorós et al., 2019; Bauer et al., 2020a; Liu et al., 2018; Zhou et al., 2015), likely reflecting differences in dose, among other factors. Conversely, the overall mixture was concurrently associated with increased perseveration errors, reflecting worse cognitive performance. This association was driven primarily by Pb, which is consistent with the known toxicological mechanisms of Pb (e.g., disruption of neurotransmitter release and neuronal plasticity) and previous research in pediatric cohorts (Neal and Guilarte, 2013; Sanders et al., 2009).

We further found that the mixture was jointly associated with lower concentrations of serum ferritin, and that this association was primarily due to Pb. Increased blood Pb concentrations have previously been associated with decreased concentrations of markers of Fe status in children (Choi and Kim, 2005; Hegazy et al., 2010; Jeong et al., 2015). The hematologic toxicity of Pb is well established: Pb shares mechanisms of uptake and transport with Fe, disrupts enzymes involved in *heme* synthesis, interferes with the expression of transferrin, disrupts cellular membranes, and has been associated with decreased concentrations of erythropoietin (EPO), the primary hormone that stimulates the production of erythrocytes (Kordas, 2010; Levander, 1979). Although most studies have found Pb-induced hematologic toxicity at concentrations >10 μg/dL, several studies with blood Pb concentrations ≤10 μg/dL have reported decreased platelet counts and inhibition of δ-ALAD, an enzyme active in the production of *heme* (Agency for Toxic Substances and Disease Registry, 2020). These studies suggest that Pb hematologic toxicity may occur at Pb concentrations <10 μg/dL; however, most prior studies were cross-sectional in design and conducted in adults or in populations with small sample sizes. Other studies in children have alternatively reported higher blood Pb concentrations among children who were Fe-deficient (Ataur Rahman et al., 2012; Bradman et al., 2001; Khan et al., 2011). Because the toxicokinetics and toxicodynamics of Pb are closely related to those of Fe (Agency for Toxic Substances and Disease Registry, 2020), further research in larger pediatric cohorts with longitudinal designs, particularly in populations with low environmental Pb exposure, is warranted.

Conversely, increasing percentiles of the mixture were jointly associated with higher concentrations of hemoglobin in the current analysis, and this association was again driven by Pb. However, given the cross-sectional nature of this study and known hematologic toxicity of Pb, this finding should not be interpreted as a beneficial association of Pb on hematologic function. One alternative explanation of this finding is that EPO production was higher in response to low-level Pb exposure in our Fe-replete population. EPO is secreted by the kidneys, and concentrations increase under certain conditions (e.g., low oxygen, blood loss, damaged or malfunctioning erythrocytes) (Agency for Toxic Substances and Disease Registry, 2020; Suresh et al., 2020). This suggests that EPO may increase following toxicant exposure as a compensatory mechanism against the hematologic toxicity of Pb (e.g., disruption of *heme* synthesis). Although we did not have EPO concentrations to further investigate this hypothesis in the PHIME cohort, a compensatory mechanism was observed in one study of 5-year-old Fe-replete children, where environmental Pb exposure was associated with increased EPO concentrations (Factor-Litvak et al., 1998). Thus, the hypothesized compensatory mechanism could explain the positive association of blood Pb with Hb in our analysis. Alternatively, this finding could be explained by residual confounding by other nutrients (e.g., vitamin A, vitamin B6, vitamin B12, riboflavin, or folic acid) or dietary factors that have been associated with both Fe status and metals exposure (Al-Attar, 2011; Ferri et al., 2015, 2012; Fishman et al., n.d.; Levander, 1979). For example, we have previously shown that Pb was detectable in soils and Fe-rich vegetables (e.g., spinach) grown in gardens near ferroalloy industry, suggesting diet may be an important confounder in this population (Ferri et al., 2015, 2012). It is also possible that this is a spurious statistical correlation given the cross-sectional nature of our data and the known kinetics of Pb in the body (e.g., >90 % of whole blood Pb is bound to hemoglobin) (Collin et al., 2022), highlighting the need for additional studies with longitudinal designs.

Although the overall mixture was associated with indicators of neurodevelopment and markers of Fe status, there was no evidence that Fe status mediated the associations between the metal mixture and verbal learning and memory. These results were consistent across three clinical markers of Fe status. We are aware of only one prior study that considered metals and Fe status in a mediation analysis of neurodevelopmental outcomes. Using cross-sectional data from a population of 5-year old Korean children, Jeong et al. found that blood Pb was a partial mediator of the positive association between ferritin and verbal IQ, whereby exposure to Pb attenuated the beneficial effect of ferritin on neurodevelopment (Jeong et al., 2015). We likely did not observe any mediation by Fe status in the current analysis because Fe status was, on average, sufficient and not strongly related to the neurodevelopmental outcomes in this study population. Fe-deficiency has been associated with worse neurodevelopment in other pediatric cohorts because Fe is required for processes of neuronal development and function (e.g., metabolism, myelination, neurotransmitter synthesis) (Halterman et al., 2001; Jáuregui-Lobera, 2014; Ji et al., 2017; Lukowski et al., 2010; Parkin et al., 2020; Roy et al., 2011; Tseng et al., 2018; Wang et al., 2019, 2017). Jeong et al. classified nearly half of their participants as having either low (<15 ng/mL) or low-normal ferritin levels (15.0 < 30.0 ng/mL) (Jeong et al., 2015), whereas the PHIME population had minimal indication of Fe deficiency (median serum ferritin: 32.0 ng/mL). This likely explains, at least in part, the null mediation findings in our study. However, because Fe-deficiency, and specifically anemia, has been consistently associated with neurodevelopment in children (Halterman et al., 2001; Jáuregui-Lobera, 2014; Ji et al., 2017; Lukowski et al., 2010; Parkin et al., 2020; Roy et al., 2011; Tseng et al., 2018; Wang et al., 2019, 2017), mediation by Fe status is still possible, and other prospective studies with larger variations in Pb and Fe levels are warranted.

In sex-stratified models, the beneficial associations of the mixture with the recall trials were stronger among females. Sex-specific associations of Cu with neurodevelopment have been previously reported, such that Cu was more strongly associated with adverse cognitive scores in males (Amorós et al., 2019; Zhou et al., 2015). Animal data are inconsistent: studies have reported higher susceptibility to Cu toxicity among both males and females (Chen et al., 2006; Lamtai et al., 2020). These findings may relate to differences in hormonal modulation of Cu-induced neurotoxicity (Lamtai et al., 2020). In the current study, sexual dimorphic findings may also be due to differential Cu concentrations between the sexes (median: females, 10.3 μg/g; males, 8.6 μg/g). However, further research is needed to better understand sex-specific impacts of Cu on neurodevelopment.

We also found sexual dimorphic associations between the metal mixture and markers of Fe status: the association of the overall mixture, driven by Pb, with lower ferritin concentrations and higher hemoglobin concentrations was stronger in males compared to females. These findings are contrary to a previous epidemiological study in adolescents that found stronger adverse associations of metals (Mn, Pb, cadmium, selenium) or their mixture in females (Schildroth et al., 2022a). However, stronger associations of metals with Fe status among females in this prior analysis were driven primarily by Mn and cadmium, and biomarker concentrations of these metals tended to be higher in females compared to males (Schildroth et al., 2022a). In contrast, the sex-specific associations in the current analysis were driven by Pb, where median Pb concentrations were modestly higher in males (1.4 μg/dL) than in females (1.1 μg/dL). This might partly explain why we observed stronger associations of the metal mixture with markers of Fe status in males compared to females in our study. As with our main analysis in the full cohort, there was no evidence that Fe status mediated the association of the metal mixture with neurodevelopment in sex-stratified analyses.

This analysis had several strengths. Notably, we were among the first to use the novel BKMR-CMA approach to examine mediation of any chemical mixture, and this study was the first to assess mediation of any metal or a metal mixture by Fe status in relation to neurodevelopmental outcomes. We were also able to examine mediation by multiple clinically relevant biomarkers for Fe status that each quantifies different aspects of Fe status with various sensitivities for reflecting Fe deficiency (Gibson, 2005). We further quantified metals (Pb, Mn, Cr, and Cu) using biomarkers that have been consistently utilized in previous epidemiological studies of neurodevelopment (Bauer et al., 2020a; Caparros-Gonzalez et al., 2019; de Carvalho et al., 2018; Oulhote et al., 2014; Torres-Agustín et al., 2013; Wright et al., 2006; Yorifuji et al., 2011). Our study also focused on the adolescent period, an understudied yet critical period for neuronal maturation, physical growth, and possible Fe deficiency (Anttila et al., 1997; Arain et al., 2013; Das et al., 2017; Mesías et al., 2013; Shaw et al., 2020). Lastly, we utilized the CVLT-C to assess neurodevelopment, which is an objective and commonly used test of verbal learning and memory in children and adolescents (Lezak et al., 2012).

The primary limitation of this analysis was its cross-sectional design, where the metals, Fe status, and neurodevelopment were assessed concurrently. Reverse causation is therefore possible, and longitudinal studies are needed to confirm our findings. Residual confounding is also possible as we were missing data on key covariates that may be associated with metals, Fe status, and neurodevelopment. For example, we were not able to control for biomarkers of inflammation (e.g., C-reactive protein) that may impact levels of Fe status markers (Gibson, 2005), other co-exposures (e.g., nickel), or past exposures (e.g., in the prenatal and early childhood periods) that have similarly been associated with neurodevelopment in adolescence (Bauer et al., 2021; Lamtai et al., 2018; Rechtman et al., 2022). We also had a limited sample size ($n = 383$), which likely impacted the precision of our estimates.

5. Conclusion

In conclusion, we found that an environmentally relevant metal mixture was associated with Fe status and aspects of verbal learning and memory, though mediation of the mixture by Fe status was not observed. Fe status should nonetheless be considered as a possible mediator of metal mixtures in future studies of neurodevelopment, especially in populations with Fe deficiency or higher environmental metal exposure given the known hematologic toxicity of metals like Pb, and the mechanistic overlap of Fe with other metals.

Abbreviations

Pb	lead
Mn	manganese
Cu	copper
Cr	chromium
Fe	iron
PHIME	Public Health Impact of Metals Exposure
IQ	intelligence quotient
CVLT-C	California Verbal Learning Test for Children
SES	socioeconomic status
HOME	Home Observation for Measurement of the Environment
LOD	limit of detection
CBC	complete blood count
DAG	directed acyclic graph
BKMR-CMA	Bayesian Kernel Machine Regression Causal Mediation Analysis
PIP	posterior inclusion probability
CDE	controlled direct effect
NDE	natural direct effect
NIE	natural indirect effect
TE	total effect
CI	credible interval
GAM	generalized additive model
LRT	likelihood ratio test
PNDE	pure natural direct effect
TNIE	total natural indirect effect
SD	standard deviation

CRediT authorship contribution statement

Samantha Schildroth: conceptualization, formal analysis, software, writing- original draft, writing- review + editing; Birgit Claus Henn: conceptualization, methodology, writing- review + editing, supervision, funding acquisition; Linda Valeri: methodology, writing- review + editing; Baoyi Shi: methodology, software, writing- review + editing; Alexa Friedman: writing- review + editing, software, validation; Roberta White: writing- review + editing, supervision; Katarzyna Kordas: writing- review + editing; Donatella Placidi: writing- review + editing; methodology, project administration, data curation; Robert Wright: writing- review + editing, methodology, project administration, funding acquisition; Donald Smith: writing- review + editing, methodology, project administration, funding acquisition; Roberto Lucchini: writing- review + editing, methodology, project administration, funding acquisition; Megan Horton: writing- review + editing, methodology, project administration, funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

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References

Agency for Toxic Substances and Disease Registry, 2004. Toxicological Profile for Copper.
Agency for Toxic Substances and Disease Registry, 2012a. Toxicological Profile for Manganese.

Agency for Toxic Substances and Disease Registry, 2012b. Toxicological Profile for Chromium.

Agency for Toxic Substances and Disease Registry, 2020. Toxicological Profile for Lead.

Ahamed, M., Siddiqui, M.K.J., 2007. Low level lead exposure and oxidative stress: current opinions. Clin. Chim. Acta. https://doi.org/10.1016/j.cca.2007.04.024.

Akinyemi, A.J., Miah, M.R., Ijomone, O.M., Tsatsakis, A., Soares, F.A.A., Tinkov, A.A., Skalny, A.V., Venkataramani, V., Aschner, M., 2019. Lead (Pb) exposure induces dopaminergic neurotoxicity in Caenorhabditis elegans: involvement of the dopamine transporter. Toxicol. Rep. 6, 833–840. https://doi.org/10.1016/J.TOXREP.2019.08.001.

Al-Attar, A.M., 2011. Vitamin E attenuates liver injury induced by exposure to lead, mercury, cadmium and copper in albino mice. Saudi J. Biol. Sci. 18, 395. https://doi.org/10.1016/J.SJBS.2011.07.004.

Amorós, R., Murcia, M., González, L., Soler-Blasco, R., Rebagliato, M., Iñiguez, C., Carrasco, P., Vioque, J., Broberg, K., Levi, M., Lopez-Espinosa, M.J., Ballester, F., Llop, S., 2019. Maternal copper status and neuropsychological development in infants and preschool children. Int. J. Hyg. Environ. Health 222, 503–512. https://doi.org/10.1016/j.ijheh.2019.01.007.

Anttila, R., Cook, J.D., Siimes, M.A., 1997. Body iron stores decrease in boys during pubertal development: the transferrin receptor-ferritin ratio as an indicator of iron status. Pediatr. Res. 41, 224–228. https://doi.org/10.1203/00006450-199702000-00012.

Arain, M., Haque, M., Johal, L., Mathur, P., Nel, W., Rais, A., Sandhu, R., Sharma, S., 2013. Maturation of the adolescent brain. Neuropsychiatr. Dis. Treat. 9, 449. https://doi.org/10.2147/NDT.S39776.

Ataur Rahman, M., Rahman, B., Saeed Ahmad, M., Blann, A., Ahmed, N., 2012. Blood and hair lead in children with different extents of iron deficiency in Karachi. Environ. Res. 118, 94–100. https://doi.org/10.1016/J.ENVRES.2012.07.004.

Barbosa, F., Tanus-Santos, J.E., Gerlach, R.F., Parsons, P.J., 2005. A critical review of biomarkers used for monitoring human exposure to lead: advantages, limitations, and future needs. Environ. Health Perspect. https://doi.org/10.1289/ehp.7917.

Barkur, R.R., Bairy, L.K., 2015. Assessment of oxidative stress in hippocampus, cerebellum and frontal cortex in rat pups exposed to lead (Pb) during specific periods of initial brain development. Biol. Trace Elem. Res. 164, 212–218. https://doi.org/10.1007/S12011-014-0221-3, 2015 164:2.

Bauer, J.A., Claus Henn, B., Austin, C., Zoni, S., Fedrighi, C., Cagna, G., Placidi, D., White, R.F., Yang, Q., Coull, B.A., Smith, D., Lucchini, R.G., Wright, R.O., Arora, M., 2017. Manganese in teeth and neurobehavior: sex-specific windows of susceptibility. Environ. Int. 108, 299–308. https://doi.org/10.1016/j.envint.2017.08.013.

Bauer, J.A., Devick, K.L., Bobb, J.F., Coull, B.A., Bellinger, D., Benedetti, C., Cagna, G., Fedrighi, C., Guazzetti, S., Oppini, M., Placidi, D., Webster, T.F., White, R.F., Yang, Q., Zoni, S., Wright, R.O., Smith, D.R., Lucchini, R.G., Claus Henn, B., 2020a. Associations of a metal mixture measured in multiple biomarkers with IQ: evidence from Italian adolescents living near ferroalloy industry. Environ. Health Perspect. 128, 97002. https://doi.org/10.1289/EHP6803.

Bauer, J.A., Fruh, V., Howe, C.G., White, R.F., Claus Henn, B., 2020b. Associations of metals and neurodevelopment: a review of recent evidence on susceptibility factors. Curr. Epidemiol. Rep. 7, 237–262. https://doi.org/10.1007/s40471-020-00249-y.

Bauer, J.A., White, R.F., Coull, B.A., Austin, C., Oppini, M., Zoni, S., Fedrighi, C., Cagna, G., Placidi, D., Guazzetti, S., Yang, Q., Bellinger, D.C., Webster, T.F., Wright, R.O., Smith, D., Horton, M., Lucchini, R.G., Arora, M., Claus Henn, B., 2021. Critical windows of susceptibility in the association between manganese and neurocognition in Italian adolescents living near ferro-manganese industry. Neurotoxicology 87, 51–61. https://doi.org/10.1016/J.NEURO.2021.08.014.

Bertinato, J., Zouzoulas, A., 2009. Considerations in the development of biomarkers of copper status. J. AOAC Int. https://doi.org/10.1093/JAOAC/92.5.1541. Oxford Academic.

Bobb, J.F., Valeri, L., Claus Henn, B., Christiani, D.C., Wright, R.O., Mazumdar, M., Godleski, J.J., Coull, B.A., 2015. Bayesian Kernel Machine Regression for Estimating the Health Effects of Multi-pollutant Mixtures. J. F. BOBB AND OTHERS, 16, pp. 493–508. https://doi.org/10.1093/biostatistics/kxu058.

Bobb, J.F., Claus Henn, B., Valeri, L., Coull, B.A., 2018. Statistical software for analyzing the health effects of multiple concurrent exposures via Bayesian kernel machine regression. Environ. Health 17, 67. https://doi.org/10.1186/s12940-018-0413-y.

Borchard, S., Bork, F., Rieder, T., Eberhagen, C., Popper, B., Lichtmannegger, J., Schmitt, S., Adamski, J., Klingenspor, M., Weiss, K.H., Zischka, H., 2018. The exceptional sensitivity of brain mitochondria to copper. Toxicol. in Vitro 51, 11–22. https://doi.org/10.1016/J.TIV.2018.04.012.

Boudissa, S.M., Lambert, J., Müller, C., Kennedy, G., Gareau, L., Zayed, J., 2006. Manganese concentrations in the soil and air in the vicinity of a closed manganese alloy production plant. Sci. Total Environ. 361, 67–72. https://doi.org/10.1016/j.scitotenv.2005.05.001.

Bradman, A., Eskenazi, B., Sutton, P., Athanasoulis, M., Goldman, L.R., 2001. Iron deficiency associated with higher blood lead in children living in contaminated environments. Environ. Health Perspect. 109, 1079–1084. https://doi.org/10.1289/ehp.011091079.

Brain, J.D., Heilig, E., Donaghey, T.C., Knutson, M.D., Wessling-Resnick, M., Molina, R. M., 2006. Effects of iron status on transpulmonary transport and tissue distribution of Mn and Fe. Am. J. Respir. Cell Mol. Biol. 34, 330–337. https://doi.org/10.1165/rcmb.2005-0101OC.

Butler, L., Gennings, C., Peli, M., Borgese, L., Placidi, D., Zimmerman, N., Hsu, H.H.L., Coull, B.A., Wright, R.O., Smith, D.R., Lucchini, R.G., Claus Henn, B., 2019. Assessing the contributions of metals in environmental media to exposure biomarkers in a region of ferroalloy industry. J. Expo. Sci. Environ. Epidemiol. 29, 674–687. https://doi.org/10.1038/s41370-018-0081-6.

van Buuren, S., Groothuis-Oudshoorn, K., 2011. mice: multivariate imputation by chained equations in R. J. Stat. Softw. 45, 1–67. https://doi.org/10.18637/JSS.V045.I03.

Caparros-Gonzalez, R.A., Giménez-Asensio, M.J., González-Alzaga, B., Aguilar-Carduño, C., Lorca-Marín, J.A., Alguacil, J., Gómez-Becerra, I., Gómez-Ariza, J.L., García-Barrera, T., Hernandez, A.F., López-Flores, I., Rohlman, D.S., Romero-Molina, D., Ruiz-Pérez, I., Lacasaña, M., 2019. Childhood chromium exposure and neuropsychological development in children living in two polluted areas in southern Spain. Environ. Pollut. 252, 1550–1560. https://doi.org/10.1016/j.envpol.2019.06.084.

de Carvalho, C.F., Oulhote, Y., Martorelli, M., de Carvalho, C.O., Menezes-Filho, J.A., Argollo, N., Abreu, N., 2018. Environmental manganese exposure and associations with memory, executive functions, and hyperactivity in Brazilian children. Neurotoxicology 69, 253–259. https://doi.org/10.1016/j.neuro.2018.02.002.

Cesana, G.C., Ferrario, M., De Vito, G., Sega, R., Grieco, A., 1995. Evaluation of the socioeconomic status in epidemiological surveys: hypotheses of research in the Brianza Area MONICA Project. Work Environ. Health 86, 16–26.

Chandra, S.V., Ali, M.M., Saxena, D.K., Murthy, R.C., 1981. Behavioral and neurochemical changes in rats simultaneously exposed to manganese and lead. Arch. Toxicol. 49, 49–56. https://doi.org/10.1007/BF00352071.

Chen, Z., Meng, H., Xing, G., Chen, C., Zhao, Y., Jia, G., Wang, T., Yuan, H., Ye, C., Zhao, F., Chai, Z., Zhu, C., Fang, X., Ma, B., Wan, L., 2006. Acute toxicological effects of copper nanoparticles in vivo. Toxicol. Lett. 163, 109–120. https://doi.org/10.1016/J.TOXLET.2005.10.003.

Choi, J., Kim, S., 2005. Relationships of lead, copper, zinc, and cadmium levels versus hematopoiesis and iron parameters in healthy adolescents. Ann. Clin. Lab. Sci. 35, 428–434.

Claus Henn, B., Ettinger, A.S., Schwartz, J., Téllez-Rojo, M.M., Lamadrid-Figueroa, H., Hernández-Avila, M., Schnaas, L., Amarasiriwardena, C., Bellinger, D.C., Hu, H., Wright, R.O., 2010. Early postnatal blood manganese levels and children's neurodevelopment. Epidemiology 21, 433–439. https://doi.org/10.1097/EDE.0b013e3181df8e52.

Coetzee, D.J., McGovern, P.M., Rao, R., Harnack, L.J., Georgieff, M.K., Stepanov, I., 2016. Measuring the impact of manganese exposure on children's neurodevelopment: advances and research gaps in biomarker-based approaches. Environ. Health. https://doi.org/10.1186/s12940-016-0174-4.

Collin, M.S., Venkatraman, S.K., Vijayakumar, N., Kanimozhi, V., Arbaaz, S.M., Stacey, R.G.S., Anusha, J., Choudhary, R., Lvov, V., Tovar, G.I., Senatov, F., Koppala, S., Swamiappan, S., 2022. Bioaccumulation of lead (Pb) and its effects on human: a review. J. Hazard. Mater. Adv. 7, 100094. https://doi.org/10.1016/J.HAZADV.2022.100094.

Das, J.K., Salam, R.A., Thornburg, K.L., Prentice, A.M., Campisi, S., Lassi, Z.S., Koletzko, B., Bhutta, Z.A., 2017. Nutrition in adolescents: physiology, metabolism, and nutritional needs. Ann. N. Y. Acad. Sci. 1393, 21–33. https://doi.org/10.1111/NYAS.13330.

Delis, D.C., Kramer, J.H., Kaplan, E., Ober, B.A., 1994. California Verbal Learning Test: Children's Version (Pearson Education).

Devick, K.L., Bobb, J.F., Mazumdar, M., Claus Henn, B., Bellinger, D.C., Christiani, D.C., Wright, R.O., Williams, P.L., Coull, B.A., Valeri, L., 2022. Bayesian kernel machine regression-causal mediation analysis. Stat. Med. 41, 860–876.

Eastman, R.R., Jursa, T.P., Benedetti, C., Lucchini, R.G., Smith, D.R., 2013. Hair as a biomarker of environmental manganese exposure. Environ. Sci. Technol. 47, 1629–1637. https://doi.org/10.1021/es3035297.

Erikson, K.M., Shihabi, Z.K., Aschner, J.L., Aschner, M., 2002. Manganese accumulates in iron-deficient rat brain regions in a heterogeneous fashion and is associated with neurochemical alterations. Biol. Trace Elem. Res. 87, 143–156. https://doi.org/10.1385/BTER:87:1-3:143.

Factor-Litvak, P., Slavkovich, V., Liu, X., Popovac, D., Preteni, E., Capuni-Paracka, S., Hadzialievic, S., Lekic, V., Lolacono, N., Kline, J., Graziano, J., 1998. Hyperproduction of erythropoietin in nonanemic lead-exposed children. Environ. Health Perspect. 106, 361–364.

Fernsebner, K., Zorn, J., Kanawati, B., Walker, A., Michalke, B., 2014. Manganese leads to an increase in markers of oxidative stress as well as to a shift in the ratio of Fe(II)/(III) in rat brain tissue. Metallomics 6, 921–931. https://doi.org/10.1039/C4MT00022F.

Ferri, R., Donna, F., Smith, D.R., Guazzetti, S., Zacco, A., Rizzo, L., Bontempi, E., Zimmerman, N.J., Lucchini, R.G., 2012. Heavy metals in soil and salad in the proximity of historical ferroalloy emission. J. Environ. Prot. (Irvine, Calif.) 3, 374. https://doi.org/10.4236/JEP.2012.35047.

Ferri, R., Hashim, D., Smith, D.R., Guazzetti, S., Donna, F., Ferretti, E., Curatolo, M., Moneta, C., Beone, G.M., Lucchini, R.G., 2015. Metal contamination of home gardens soils and cultivated vegetables in the province of Brescia, Italy: implications for human exposure. Sci. Total Environ. 0, 507. https://doi.org/10.1016/J.SCITOTENV.2015.02.072.

Ferroalloy Market Share 2018-2025 Industry Growth Outlook Report [WWW Document], n.d. URL https://www.gminsights.com/industry-analysis/ferroalloy-market (accessed 5.24.21).

Fishman, S.M., Christian, P., West, K.P., n.d. The role of vitamins in the prevention and control of anaemia. Public Health Nutr. 3, 125–150. doi:https://doi.org/10.1017/S1368980000000173.

Gaetke, L.M., Chow-Johnson, H.S., Chow, C.K., 2014. Copper: toxicological relevance and mechanisms. Arch. Toxicol. https://doi.org/10.1007/s00204-014-1355-y.

Galaris, D., Barbouti, A., Pantopoulos, K., 2019. Iron homeostasis and oxidative stress: an intimate relationship. Biochim. Biophys. Acta Mol. Cell Res. 1866, 118535. https://doi.org/10.1016/J.BBAMCR.2019.118535.

García-Chimalpopoca, Z., Hernández-Bonilla, D., Cortez-Lugo, M., Escamilla-Núñez, C., Schilmann, A., Riojas-Rodríguez, H., Rodríguez-Dozal, S., Montes, S., Tristán-López, L.A., Catalán-Vázquez, M., Rios, C., 2019. Verbal memory and learning in schoolchildren exposed to manganese in Mexico. Neurotox. Res. 36, 827–835. https://doi.org/10.1007/s12640-019-00037-7.

Gibson, R.S., 2005. Principles of Nutritional Assessment, Second Edi. ed. In: Principles of Nutritional Assessment. Oxford University Press, New York.

Hafeman, D.M., Schwartz, S., 2009. Opening the Black Box: a motivation for the assessment of mediation. Int. J. Epidemiol. 38, 838–845. https://doi.org/10.1093/IJE/DYN372.

Halterman, J.S., Kaczorowski, J.M., Aligne, C.A., Auinger, P., Szilagyi, P.G., 2001. Iron deficiency and cognitive achievement among school-aged children and adolescents in the United States. Pediatrics 107, 1381–1386. https://doi.org/10.1542/peds.107.6.1381.

Haynes, E.N., Ryan, P., Chen, A., Brown, D., Roda, S., Kuhnell, P., Wittberg, D., Terrell, M., Reponen, T., 2012. Assessment of personal exposure to manganese in children living near a ferromanganese refinery. Sci. Total Environ. 427–428, 19–25. https://doi.org/10.1016/j.scitotenv.2012.03.037.

Hegazy, A.A., Zaher, M.M., Abd El-Hafez, M.A., Morsy, A.A., Saleh, R.A., 2010. Relation between anemia and blood levels of lead, copper, zinc and iron among children. BMC Res. Notes 3, 133. https://doi.org/10.1186/1756-0500-3-133.

Henríquez-Hernández, L.A., Boada, L.D., Carranza, C., Pérez-Arellano, J.L., González-Antuña, A., Camacho, M., Almeida-González, M., Zumbado, M., Luzardo, O.P., 2017. Blood levels of toxic metals and rare earth elements commonly found in e-waste may exert subtle effects on hemoglobin concentration in sub-Saharan immigrants. Environ. Int. 109, 20–28. https://doi.org/10.1016/j.envint.2017.08.023.

Jain, N.B., Laden, F., Guller, U., Shankar, A., Kasani, S., Garshick, E., 2005. Relation between blood lead levels and childhood anemia in India. Am. J. Epidemiol. 161, 968–973. https://doi.org/10.1093/aje/kwi126.

Jáuregui-Lobera, I., 2014. Iron deficiency and cognitive functions. Neuropsychiatr. Dis. Treat. https://doi.org/10.2147/NDT.S72491.

Jeong, K.S., Park, H.H., Ha, E., Hong, Y.C., Ha, M., Park, H.H., Kim, B.N., Lee, S.J., Lee, K.Y., Kim, J.H., Kim, Y., 2015. Evidence that cognitive deficit in children is associated not only with iron deficiency, but also with blood lead concentration: a preliminary study. J. Trace Elem. Med. Biol. https://doi.org/10.1016/j.jtemb.2014.08.014. Elsevier GmbH.

Ji, X., Cui, N., Liu, J., 2017. Neurocognitive function is associated with serum iron status in early adolescents. Biol. Res. Nurs. 19, 269. https://doi.org/10.1177/1099800417690828.

Jursa, T., Stein, C.R., Smith, D.R., 2018. Determinants of hair manganese, lead, cadmium and arsenic levels in environmentally exposed children. Toxics 6, 19. https://doi.org/10.3390/toxics6020019.

Kalita, J., Kumar, V., Misra, U.K., Bora, H.K., 2018. Memory and learning dysfunction following copper toxicity: biochemical and immunohistochemical basis. Mol. Neurobiol. 55, 3800–3811. https://doi.org/10.1007/S12035-017-0619-Y.

Khan, D.A., Ansari, W.M., Khan, F.A., 2011. Synergistic effects of iron deficiency and lead exposure on blood lead levels in children. World J. Pediatr. 7, 150–154. https://doi.org/10.1007/s12519-011-0257-9.

Kordas, K., 2010. Iron, lead, and children's behavior and cognition. Annu. Rev. Nutr. 30, 123–148. https://doi.org/10.1146/annurev.nutr.012809.104758.

Kordas, K., Stoltzfus, R.J., 2004. New evidence of iron and zinc interplay at the enterocyte and neural tissues. J. Nutr. 134, 1295–1298. https://doi.org/10.1093/JN/134.6.1295.

Kounnavong, S., Vonglokham, M., Kounnavong, T., Kwadwo, D.D., Essink, D.R., . Anaemia Among Adolescents: Assessing a Public Health Concern in Lao PDR. https://doi-org.ezproxy.bu.edu/10.1080/16549716.2020.1786997, p. 13. https://doi.org/10.1080/16549716.2020.1786997.

Kreutzer, J.S., DeLuca, J., Caplan, B. (Eds.), 2011. Encyclopedia of Clinical Neuropsychology. Springer, New York.

Kutllovci-Zogaj, D., Krasniqi, S., Elezaj, I., Ramadani, N., Gjergji, T., Zogaj, D., Kutllovci, A., Jaka, A., Ukëhaxhaj, A., Gashi, S., Bince, E., 2014. Correlation between blood lead level and hemoglobin level in mitrovica children. Mediev. Archaeol. 68, 324–328. https://doi.org/10.5455/medarh.2014.68.324-328.

Lamtai, M., Chaibat, J., Ouakki, S., Zghari, O., Mesfioui, A., El Hessni, A., Rifi, E.H., Marmouzi, I., Essamri, A., Ouichou, A., 2018. Effect of chronic administration of nickel on affective and cognitive behavior in male and female rats: possible implication of oxidative stress pathway. Brain Sci. 8 https://doi.org/10.3390/BRAINSCI8080141.

Lamtai, M., Zghari, O., Ouakki, S., Marmouzi, I., Mesfioui, A., El Hessni, A., Ouichou, A., 2020. Chronic copper exposure leads to hippocampus oxidative stress and impaired learning and memory in male and female rats. Toxicol. Res. 36, 359. https://doi.org/10.1007/S43188-020-00043-4.

Larsen, B., Bourque, J., Moore, X.T.M., Adebimpe, A., Calkins, M.E., Elliott, M.A., Gur, R. C., Gur, R.E., Moberg, P.J., Roalf, D.R., Ruparel, K., Turetsky, B.I., Vandekar, S.N., Wolf, X.D.H., Shinohara, R.T., Satterthwaite, X.T.D., 2020. Longitudinal development of brain iron is linked to cognition in youth. J. Neurosci. 40, 1810–1818. https://doi.org/10.1523/JNEUROSCI.2434-19.2020.

Levander, O.A., 1979. Lead toxicity and nutritional deficiencies. Environ. Health Perspect. 29, 115. https://doi.org/10.1289/EHP.7929115.

Lezak, M., Howieson, D., Bigler, E., Tranel, D., 2012. Neuropsychological Assessment, Fifth. ed. Oxford University Press, New York.

Liu, S.H., Bobb, J.F., Claus Henn, B., Gennings, C., Schnaas, L., Tellez-Rojo, M., Bellinger, D., Arora, M., Wright, R.O., Coull, B.A., 2018. Bayesian varying coefficient kernel machine regression to assess neurodevelopmental trajectories associated with exposure to complex mixtures. Stat. Med. 37, 4680–4694. https://doi.org/10.1002/sim.7947.

Liu, Z.H., Shang, J., Yan, L., Wei, T., Xiang, L., Wang, H.L., Cheng, J., Xiao, G., 2020. Oxidative stress caused by lead (Pb) induces iron deficiency in Drosophila melanogaster. Chemosphere 243, 125428. https://doi.org/10.1016/J.CHEMOSPHERE.2019.125428.

Llop, S., Lopez-Espinosa, M.J., Rebagliato, M., Ballester, F., 2013. Gender differences in the neurotoxicity of metals in children. Toxicology 311, 3–12. https://doi.org/10.1016/j.tox.2013.04.015.

Lucas, E.L., Bertrand, P., Guazzetti, S., Donna, F., Peli, M., Jursa, T.P., Lucchini, R., Smith, D.R., 2015. Impact of ferromanganese alloy plants on household dust manganese levels: implications for childhood exposure. Environ. Res. 138, 279–290. https://doi.org/10.1016/j.envres.2015.01.019.

Lucchini, R.G., Guazzetti, S., Zoni, S., Donna, F., Peter, S., Zacco, A., Salmistraro, M., Bontempi, E., Zimmerman, N.J., Smith, D.R., 2012a. Tremor, olfactory and motor changes in Italian adolescents exposed to historical ferro-manganese emission. Neurotoxicology 33, 687–696. https://doi.org/10.1016/j.neuro.2012.01.005.

Lucchini, R.G., Zoni, S., Guazzetti, S., Bontempi, E., Micheletti, S., Broberg, K., Parrinello, G., Smith, D.R., 2012b. Inverse association of intellectual function with very low blood lead but not with manganese exposure in Italian adolescents. Environ. Res. 118, 65–71. https://doi.org/10.1016/j.envres.2012.08.003.

Lukaski, H.C., 1999. Chromium as a supplement. Annu. Rev. Nutr. 19, 279–302. https://doi.org/10.1146/annurev.nutr.19.1.279.

Lukowski, A.F., Koss, M., Burden, M.J., Jonides, J., Nelson, C.A., Kaciroti, N., Jimenez, E., Lozoff, B., 2010. Iron deficiency in infancy and neurocognitive functioning at 19 years: evidence of long-term deficits in executive function and recognition memory. Nutr. Neurosci. 13, 54–70. https://doi.org/10.1179/147683010X12611460763689.

McCann, S., Amadó, M.P., Moore, S.E., 2020. The role of iron in brain development: a systematic review. Nutrients. https://doi.org/10.3390/nu12072001.

Menezes-Filho, J.A., Paes, C.R., Ângela, Â.M., Moreira, J.C., Sarcinelli, P.N., Mergler, D., 2009. High levels of hair manganese in children living in the vicinity of a ferro-manganese alloy production plant. Neurotoxicology 30, 1207–1213. https://doi.org/10.1016/j.neuro.2009.04.005.

Menezes-Filho, J.A., de Souza, K.O.F.F., Rodrigues, J.L.G.G., dos Santos, N.R., Bandeira, M. de J., Koin, N.L., Oliveira, S.S. do do P., Godoy, A.L.P.C.C., Mergler, D., 2016. Manganese and lead in dust fall accumulation in elementary schools near a ferromanganese alloy plant. Environ. Res. 148, 322–329. https://doi.org/10.1016/j.envres.2016.03.041.

Merced-Nieves, F.M., Arora, M., Wright, R.O., Curtin, P., 2021. Metal mixtures and neurodevelopment: recent findings and emerging principles. Curr. Opin. Toxicol. 26, 28–32. https://doi.org/10.1016/J.COTOX.2021.03.005.

Mesías, M., Seiquer, I., Navarro, M.P., 2013. Iron nutrition in adolescence. Crit. Rev. Food Sci. Nutr. https://doi.org/10.1080/10408398.2011.564333. Taylor & Francis Group.

National Longitudinal Surveys, 1979. Appendix A: HOME-SF Scales (NLSY79 Child).

Neal, A.P., Guilarte, T.R., 2013. Mechanisms of lead and manganese neurotoxicity. Toxicol. Res. (Camb.). https://doi.org/10.1039/c2tx20064c.

O'Neal, S.L., Lee, J.W., Zheng, W., Cannon, J.R., 2014. Subacute manganese exposure in rats is a neurochemical model of early manganese toxicity. Neurotoxicology 44, 303–313. https://doi.org/10.1016/J.NEURO.2014.08.001.

Opazo, C.M., Greenough, M.A., Bush, A.I., 2014. Copper: from neurotransmission to neuroproteostasis. Front. Aging Neurosci. 6, 143. https://doi.org/10.3389/FNAGI.2014.00143/BIBTEX.

Oulhote, Y., Mergler, D., Barbeau, B., Bellinger, D.C., Bouffard, T., Brodeur, M.-È., Saint-Amour, D., Legrand, M., Sauvé, S., Bouchard, M.F., 2014. Neurobehavioral function in school-age children exposed to manganese in drinking water. Environ. Health Perspect. 122, 1343–1350. https://doi.org/10.1289/ehp.1307918.

Parkin, P.C., Koroshegyi, C., Mamak, E., Borkhoff, C.M., Birken, C.S., Maguire, J.L., Thorpe, K.E., Aglipay, M., Anderson, L.N., Keown-Stoneman, C., Kowal, C., Mason, D., 2020. Association between serum ferritin and cognitive function in early childhood. J. Pediatr. 217 https://doi.org/10.1016/j.jpeds.2019.09.051, 189-191. e2.

Peraza, M.A., Ayala-Fierro, F., Barber, D.S., Casarez, E., Rael, L.T., 1998. Effects of micronutrients on metal toxicity. Environ. Health Perspect. https://doi.org/10.1289/ehp.98106s1203.

Peters, J.L., Perry, M.J., McNeely, E., Wright, R.O., Heiger-Bernays, W., Weuve, J., 2021. The association of cadmium and lead exposures with red cell distribution width. PLoS One 16, e0245173. https://doi.org/10.1371/journal.pone.0245173.

Rechtman, E., Curtin, P., Papazaharias, D.M., Renzetti, S., Cagna, G., Peli, M., Levin-Schwartz, Y., Placidi, D., Smith, D.R., Lucchini, R.G., Wright, R.O., Horton, M.K., 2020. Sex-specific associations between co-exposure to multiple metals and visuospatial learning in early adolescence. Transl. Psychiatry 10, 1–10. https://doi.org/10.1038/s41398-020-01041-8, 2020 10:1.

Rechtman, E., Navarro, E., de Water, E., Tang, C.Y., Curtin, P., Papazaharias, D.M., Ambrosi, C., Mascaro, L., Cagna, G., Gasparotti, R., Invernizzi, A., Reichenberg, A., Austin, C., Arora, M., Smith, D.R., Lucchini, R.G., Wright, R.O., Placidi, D., Horton, M.K., 2022. Early-life critical windows of susceptibility to manganese exposure and sex-specific changes in brain connectivity in late adolescence. Biol. Psychiatry Glob. Open Sci. https://doi.org/10.1016/J.BPSGOS.2022.03.016.

Riojas-Rodríguez, H., Solís-Vivanco, R., Schilmann, A., Montes, S., Rodríguez, S., Ríos, C., Rodríguez-Agudelo, Y., 2010. Intellectual function in Mexican children living in a mining area and environmentally exposed to manganese. Environ. Health Perspect. 118, 1465–1470. https://doi.org/10.1289/ehp.0901229.

Rossander-Hultén, L., Brune, M., Sandström, B., Lönnerdal, B., Hallberg, L., 1991. Competitive inhibition of iron absorption by manganese and zinc in humans. Am. J. Clin. Nutr. 54, 152–156. https://doi.org/10.1093/AJCN/54.1.152.

Roy, A., Hu, H., Bellinger, D.C., Mukherjee, B., Modali, R., Nasaruddin, K., Schwartz, J., Wright, R.O., Ettinger, A.S., Palaniapan, K., Balakrishnan, K., 2011. Hemoglobin, lead exposure, and intelligence quotient: effect modifcation by the DRD2 taq IA polymorphism. Environ. Health Perspect. 119, 144–149. https://doi.org/10.1289/ehp.0901878.

Salvador, G.A., Uranga, R.M., Giusto, N.M., 2011. Iron and mechanisms of neurotoxicity. Int. J. Alzheimers Dis. https://doi.org/10.4061/2011/720658.

Sammallahti, S., Tiemeier, H., Reiss, I.K.M., Muckenthaler, M.U., El Marroun, H., Vermeulen, M., 2022. Maternal early-pregnancy ferritin and offspring neurodevelopment: a prospective cohort study from gestation to school age. Paediatr. Perinat. Epidemiol. 36, 425–434. https://doi.org/10.1111/PPE.12854.

Sanders, T., Liu, Y., Buchner, V., Tchounwou, P.B., 2009. Neurotoxic effects and biomarkers of lead exposure: a review. Rev. Environ. Health. https://doi.org/10.1515/REVEH.2009.24.1.15.

Schildroth, S., Friedman, A., Bauer, J., Claus Henn, B., 2022a. Associations of a metal mixture with iron status in U.S. adolescents: evidence from the National Health and Nutrition Examination Survey. New Dir. Child Adolesc. Health. https://doi.org/10.1002/cad.20457.

Schildroth, S., Kordas, K., Bauer, J.A., Wright, R.O., Claus Henn, B., 2022b. Environmental metal exposure, neurodevelopment, and the role of Iron status: a review. Curr. Environ. Health Rep. 2022, 1–30. https://doi.org/10.1007/S40572-022-00378-0.

Schildroth, S., Friedman, A., White, R.F., Kordas, K., Placidi, D., Bauer, J.A., Webster, T. F., Coull, B.A., Cagna, G., Wright, R.O., Smith, D., Lucchini, R.G., Horton, M., Claus Henn, B., 2023. Associations of an industry-relevant metal mixture with verbal learning and memory in Italian adolescents: the modifying role of iron status. Environ. Res. 224 https://doi.org/10.1016/J.ENVRES.2023.115457.

Shaw, G.A., Dupree, J.L., Neigh, G.N., 2020. Adolescent maturation of the prefrontal cortex: role of stress and sex in shaping adult risk for compromise. Genes Brain Behav. 19 https://doi.org/10.1111/GBB.12626.

Shaw, N., 1996. Iron deficiency and anemia in school children and adolescents. J. Formos. Med. Assoc. 95, 692–698.

Shi, B., Choirat, C., Coull, B.A., Vanderweele, T.J., Valeri, L., 2021. CMAverse: a suite of functions for reproducible causal mediation analyses. Epidemiology E20–E22. https://doi.org/10.1097/EDE.0000000000001378.

Shukla, G.S., Chandra, S.V., 1987. Concurrent exposure to lead, manganese, and cadmium and their distribution to various brain regions, liver, kidney, and testis of growing rats. Arch. Environ. Contam. Toxicol. 16, 303–310. https://doi.org/10.1007/BF01054947.

Smith, D., Gwiazda, R., Bowler, R., Roels, H., Park, R., Taicher, C., Lucchini, R., 2007. Biomarkers of Mn exposure in humans. Am. J. Ind. Med. 50, 801–811.

Soares, A.T.G., Silva, A. de C., Tinkov, A.A., Khan, H., Santamaría, A., Skalnaya, M.G., Skalny, A.V., Tsatsakis, A., Bowman, A.B., Aschner, M., Ávila, D.S., 2020. The impact of manganese on neurotransmitter systems. J. Trace Elem. Med. Biol. 61, 126554. https://doi.org/10.1016/J.JTEMB.2020.126554.

Stein, C.R., Wu, H., Bellinger, D.C., Smith, D.R., Wolff, M.S., Savitz, D.A., 2022. Exposure to metal mixtures and neuropsychological functioning in middle childhood. Neurotoxicology 93, 84–91. https://doi.org/10.1016/J.NEURO.2022.09.003.

Stevens, G.A., Finucane, M.M., De-Regil, L.M., Paciorek, C.J., Flaxman, S.R., Branca, F., Peña-Rosas, J.P., Bhutta, Z.A., Ezzati, M., 2013. Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995-2011: a systematic analysis of population-representative data. Lancet Glob. Health 1, e16–e25. https://doi.org/10.1016/S2214-109X(13)70001-9.

Strauss, E., Sherman, E., Spreen, O., 2006. A Compendium of Neuropsychological Tests: Administration, Norms, and Commentary, 3rd ed. Oxford University Press.

Suresh, S., Rajvanshi, P.K., Noguchi, C.T., 2020. The many facets of erythropoietin physiologic and metabolic response. Front. Physiol. 10, 1534. https://doi.org/10.3389/FPHYS.2019.01534/BIBTEX.

Torres-Agustín, R., Rodríguez-Agudelo, Y., Schilmann, A., Solís-Vivanco, R., Montes, S., Riojas-Rodríguez, H., Cortez-Lugo, M., Ríos, C., 2013. Effect of environmental manganese exposure on verbal learning and memory in Mexican children. Environ. Res. 121, 39–44. https://doi.org/10.1016/j.envres.2012.10.007.

Tseng, P.T., Cheng, Y.S., Yen, C.F., Chen, Y.W., Stubbs, B., Whiteley, P., Carvalho, A.F., Li, D.J., Chen, T.Y., Yang, Wei Cheng, Tang, C.H., Chu, C.S., Yang, Wei Chieh, Liang, H.Y., Wu, C.K., Lin, P.Y., 2018. Peripheral iron levels in children with attention-deficit hyperactivity disorder: a systematic review and meta-analysis. Sci. Rep. https://doi.org/10.1038/s41598-017-19096-x.

Valeri, L., VanderWeele, T.J., 2013. Mediation analysis allowing for exposure-mediator interactions and causal interpretation: theoretical assumptions and implementation with SAS and SPSS macros. Psychol. Methods 18, 137. https://doi.org/10.1037/A0031034.

Valeri, L., Mazumdar, M.M., Bobb, J.F., Henn, B.C., Rodrigues, E., Sharif, O.I.A., Kile, M. L., Quamruzzaman, Q., Afroz, S., Golam, M., Amarasiriwardena, C., Bellinger, D.C., Christiani, D.C., Coull, B.A., Wright, R.O., 2017. 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. 125 https://doi.org/10.1289/EHP614.

Vanderweele, T.J., 2014. A unification of mediation and interaction: a four-way decomposition. Epidemiology 25, 749. https://doi.org/10.1097/EDE.0000000000000121.

VanderWeele, T.J., 2016. Mediation Analysis: A Practitioner's Guide. https://doi.org.ezproxy.bu.edu/10.1146/annurev-publhealth-032315-021402, 37, pp. 17–32. https://doi.org/10.1146/ANNUREV-PUBLHEALTH-032315-021402.

Walter, P.B., Knutson, M.D., Paler-Martinez, A., Lee, S., Xu, Y., Viteri, F.E., Ames, B.N., 2002. Iron deficiency and iron excess damage mitochondria and mitochondrial DNA in rats. Proc. Natl. Acad. Sci. 99, 2264–2269. https://doi.org/10.1073/PNAS.261708798.

Wang, H., Shi, H., Chang, L., Zhang, X., Li, J., Yang, Y., Jiang, Y., 2012. Association of blood lead with calcium, iron, zinc and hemoglobin in children aged 0-7 years: a large population-based study. Biol. Trace Elem. Res. 149, 143–147. https://doi.org/10.1007/S12011-012-9413-X.

Wang, Y., Huang, L., Zhang, L., Qu, Y., Mu, D., 2017. Iron status in attention-deficit/hyperactivity disorder: a systematic review and meta-analysis. PLoS One. https://doi.org/10.1371/journal.pone.0169145.

Wang, Y., Wu, Y., Li, T., Wang, X., Zhu, C., 2019. Iron metabolism and brain development in premature infants. Front. Physiol. 10, 463. https://doi.org/10.3389/fphys.2019.00463.

Weinhouse, C., Ortiz, E.J., Berky, A.J., Bullins, P., Hare-Grogg, J., Rogers, L., Morales, A.-M., Hsu-Kim, H., Pan, W.K., 2017. Hair mercury level is associated with anemia and micronutrient status in children living near artisanal and small-scale gold mining in the Peruvian Amazon. Am. J. Trop. Med. Hyg. 97, 1886. https://doi.org/10.4269/AJTMH.17-0269.

Wise, J.P., Young, J.L., Cai, J., Cai, L., 2022. Current understanding of hexavalent chromium [Cr(VI)] neurotoxicity and new perspectives. Environ. Int. 158, 106877. https://doi.org/10.1016/J.ENVINT.2021.106877.

World Health Organization, 1988. MONICA Psychosocial Optional Study: MOPSY Suggested Measurement Instruments (WHO Regional Office for Europe).

Wright, R.O., Amarasiriwardena, C., Woolf, A.D., Jim, R., Bellinger, D.C., 2006. Neuropsychological correlates of hair arsenic, manganese, and cadmium levels in school-age children residing near a hazardous waste site. Neurotoxicology 27, 210–216. https://doi.org/10.1016/j.neuro.2005.10.001.

Yorifuji, T., Debes, F., Weihe, P., Grandjean, P., 2011. Prenatal exposure to lead and cognitive deficit in 7- and 14-year-old children in the presence of concomitant exposure to similar molar concentration of methylmercury. Neurotoxicol. Teratol. 33, 205–211. https://doi.org/10.1016/j.ntt.2010.09.004.

Zhou, G., Ji, X., Cui, N., Cao, S., Liu, C., Liu, J., 2015. Association between serum copper status and working memory in schoolchildren. Nutrients 7, 7185–7196. https://doi.org/10.3390/nu7095331.

Zhou, X.-H., Eckert, G.J., Tierney, W.M., 2001. Multiple imputation in public health research. Stat. Med. 20, 1541–1549. https://doi.org/10.1002/sim.689.

Zhu, Z., Sudfeld, C.R., Cheng, Y., Qi, Q., Li, S., Elhoumed, M., Yang, W., Chang, S., Dibley, M.J., Zeng, L., Fawzi, W.W., 2021. Anemia and associated factors among adolescent girls and boys at 10–14 years in rural western China. BMC Public Health 21, 1–14. https://doi.org/10.1186/S12889-021-10268-Z, 2021 21:1.