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Research Article

Keywords: Environmental pollution, Prefrontal brain performance, Heavy metals, Natural protected area, Abandoned mining area

Posted Date: June 22nd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9919878/v1

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From Mines to Biosphere Reserves: Persistent Metal Burdens and Prefrontal Cognitive Function in Children Three Decades After Mine Closure

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Abstract

Environmental pollution from abandoned mines remains an underrecognized health hazard. We quantified hair concentrations of toxic metals (lead [Pb] and mercury [Hg]) and essential trace elements (zinc [Zn] and copper [Cu]) in children from Huautla, Mexico, a rural community located near tailings from a silver mine that closed more than 30 years ago. Although the site was later designated a federally protected natural reserve, no environmental remediation was undertaken. We recruited approximately one-third of all children aged 5–11 years in Huautla, quantified their heavy metal concentrations, and assessed associations with executive brain functions (anterior prefrontal, orbitomedial, and dorsolateral prefrontal subsystems). We found that all children had at least one toxic metal, and over 80% of them had both. Boys exhibited higher Pb and Hg concentrations and lower Zn than girls, and boys with higher Hg concentration had lower scores in their higher-order executive brain functions. In girls, Zn potentially buffered this association, and Hg did not correlate with their cognitive skills performance. These findings demonstrate that children living within a protected natural area remain exposed to heavy metals that chronically accumulate in their bodies and that, for boys, decrease their cognitive skills. Our results highlight the long-term toxic legacy of mining, even decades after mine closure, and show the neurodevelopmental consequences of exposure to toxic metals.

Keywords: Environmental pollution, Prefrontal brain performance, Heavy metals, Natural protected area, Abandoned mining area.

1 Introduction

Mining plays a critical role in the global economy, contributing to more than 10% of the world's Gross Domestic Product, and is expected to grow substantially due to demand for raw materials essential to cleaner energy transitions. However, mining releases heavy metals (HMs) into water, soil, and air, leading to human exposure. Although the negative health consequences of HMs are well-known, less is known about the health effects of chronic exposure in communities living near mines that ceased operations decades ago and were left unremediated (Durán et al. 2013; Kaninga et al. 2020). This type of toxic legacy represents an understudied public health concern, particularly in low-resource rural settings. In mining-affected environments, including those abandoned for decades, the release of HMs into the environment occurs as complex mixtures rather than as single elements. The geological composition of ore deposits determines which metals co-occur, and weathering of tailings disperses these mixtures into soil, water, and air. Thus, human exposure in such settings is typically to multiple metals simultaneously, a feature that distinguishes real-world contamination from single-metal laboratory or modeling studies.

This exposure to complex metal mixtures might be particularly concerning during early childhood, when the central nervous system is still developing (Kothapalli, 2021; Hermida et al. 2019; Tooley et al. 2021), since HMs are neurotoxic even at low concentrations (Balali-Mood et al. 2021; Jomova et al. 2025).

The prefrontal cortex (PFC), which supports higher-order cognitive and executive functions (EFs), undergoes rapid and prolonged postnatal development, especially before age 12 (Kolk & Rakic, 2022; Stokes et al. 2013). EFs include planning, inhibition, impulse control, working memory, and cognitive flexibility (Anderson, 2002; Diamond, 2013); these skills are essential for academic performance, emotional regulation, and social adaptation (Blair & Cybele Raver, 2015; Zelazo & Carlson, 2012). Because the PFC is both structurally immature and functionally complex during early childhood, it depends critically on its integrity and is therefore especially susceptible to environmental neurotoxicants.

The Sierra de Huautla Biosphere Reserve in south-central Mexico (CONANP, 2025) has a documented history of silver mining. Mining operations ceased in the early 1990s without remediation, leaving an estimated 780,000 tons of open-air tailings. These residues contain elevated concentrations of several heavy metals (HMs), including mercury (Hg; 0.8 mg/kg), lead (Pb; 2297.9 mg/kg), arsenic (As; 139.2 mg/kg), and vanadium (V; 3.9 mg/kg). Concentrations of Pb, As, and V exceed the Mexican regulatory limits for residential soils by 5.7-, 6.3-, and 3.9-fold, respectively, whereas Hg concentrations remain below the corresponding regulatory threshold (NOM-147-SEMARNAT/SSA1-2004). Despite the cessation of mining activities nearly three decades ago, the tailings remain a persistent source of environmental contamination through the dispersion of pollutants in dust, soil, and water. To evaluate the potential neurodevelopmental consequences of this legacy contamination, we examined whether children residing in Huautla (or San Francisco de Huautla), a rural community located approximately 1 km from the abandoned mine tailings, exhibited evidence of chronic exposure to HMs. We quantified hair concentrations of Pb and Hg, and subsequently assessed whether variation in these HMs concentration, was

associated with executive function performance. According to the 2020 national census (INEGI, 2020), the community had 179 children under 12 years of age. Between 2022 and 2023, a total of 53 children (28 girls and 25 boys) aged 5–11 years were recruited, representing approximately one-third of the local child population.

Specifically, we focused on three prefrontal subsystems, each associated with particular cognitive processes (Kolk & Rakic, 2022; Carlén, 2017): the anterior prefrontal cortex (aPFC), implicated in metacognition and abstract reasoning and hierarchical goal organization (Ramnani & Owen, 2004); the orbitomedial cortex (OMC), region covering medial prefrontal and orbitofrontal cortices, involved in emotional regulation, social judgment and decision-making (Euston et al. 2012; Rolls, 2023); and the dorsolateral prefrontal cortex (DLPFC), essential for working memory, cognitive flexibility, planning and decision making (Levy & Goldman-Rakic, 2000; Pochon et al. 2001).

In addition to measuring toxic HMs, we also included the essential trace elements zinc (Zn) and copper (Cu) in our analysis. Epidemiological and experimental studies suggest that exposure to toxic metals such as Pb and Hg may disrupt the homeostasis of essential metals, thereby amplifying neurotoxic effects and increasing the risk of cognitive dysfunction (Fu et al. 2024; Liu et al. 2025). Although the mechanistic interactions between essential and toxic metals remain poorly understood, they may involve competition for transporters, oxidative stress, and interference with neuronal signaling (Błażewicz & Grabrucker, 2022; Kothapalli, 2021). These complex interactions can lead to additive or antagonistic effects, modifying the toxicological profile of each metal, a dimension that single-metal or toxic-only studies cannot capture.

Therefore, the current study examines the association between coexposure to essential and non-essential metals and executive function performance in children living near a historically contaminated mining site. By focusing on a non-occupational, real-world exposure context in a pediatric population, this research addresses an underexplored area in environmental neurotoxicology and provides insights into the long-term neurodevelopmental risks associated with unmanaged legacy contamination.

2 Results

2.1 Metal exposure and co-exposure different by sex

Hair HMs concentrations, revealed marked sex differences in all HMs determined (Fig. 1); Zn concentrations were significantly lower in boys compared to girls ($\beta = -1.14$, 95% CI [−1.93, −0.34], $p < 0.001$; Fig. 1a), while the other HMs concentration were significantly higher in boys; Cu ($\beta = 0.88$, 95% CI [0.40, 1.36], $p < 0.001$; Fig. 1b), Pb ($\beta = 0.46$, 95% CI [0.04, 0.88], $p = 0.030$; Fig. 1c), and Hg ($\beta = 1.13$, 95% CI [0.24, 2.17], $p = 0.018$; Fig. 1d).

Among girls, Zn was detected in 92.9% of samples, with 32.1% exceeding the upper reference value (240 mg/kg). Cu was detected in 85.7%, although none exceeded the upper reference value (100 mg/kg). Among toxic HMs, Pb was detected in 96.4% of girls, with 75% exceeding the reference value (2 mg/kg), whereas Hg was detected in 85.7% of girls and no exceedances were observed. (1 mg/kg; see Fig. 2a, 2b). Among boys, Zn was detected in 68% of participants, and 8% had concentrations exceeding the upper reference value. Cu

was detected in 92% of boys, although none exceeded the upper reference value. For toxic HMs, Pb was detected in all boys' participants, with 92% presenting concentrations above the reference value, whereas Hg was detected in 92% and 24% exceeded the reference threshold (see Fig. 2a, 2b). Overall, exceedances of reference values for Pb and Hg were more frequent among boys than girls.

Patterns of co-exposure to the toxic HMs Pb and Hg differed by sex. Detectable concentrations of both metals were observed in 82.1% of girls and 92% of boys (Fig. 2c). However, while no girls simultaneously exceeded the reference values for Pb and Hg, 24% of boys exceeded both thresholds concurrently (Fig. 2d).

Patterns of co-occurrence for the essential metals also differed by sex. Detectable concentrations of Zn (160 mg/kg) and Cu (10 mg/kg) were observed in 78.6% of girls and 60% of boys (Fig. 2c). However, when considering concentrations above the lower reference values for both essential elements, only 10.7% of girls and 4% of boys met this criterion (Fig. 2d).

Finally, patterns of co-occurrence between essential and toxic HMs also varied by sex; Pb and Zn co-occurred in 35.7% of girls and 20% of boys, whereas Pb and Cu co-occurred more frequently in boys (64%) than in girls (28.6%). Likewise, Hg and Zn co-occurred in 35.7% of girls and 20% of boys, while no participants met the established criteria for concurrent occurrence of Hg and Cu (Fig. 2d).

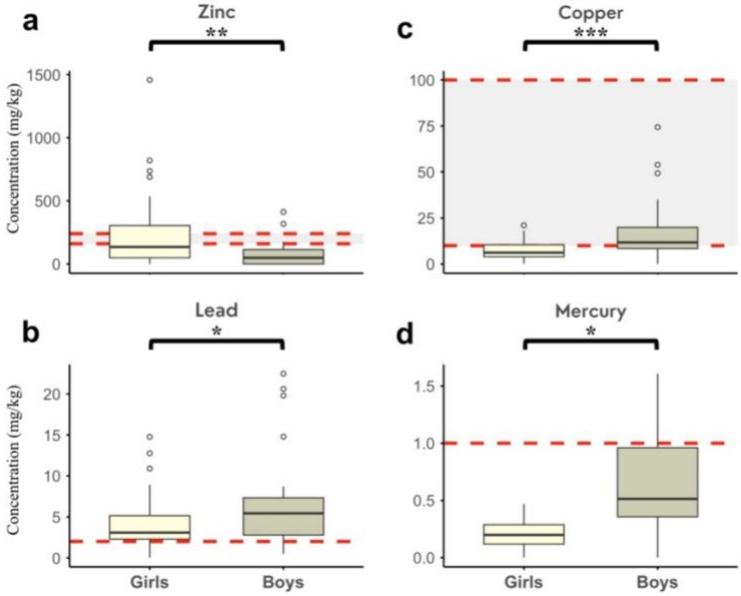


Fig. 1 Metal concentrations and co-occurrence patterns in girls (n = 28) and boys (n = 25). Display boxplots of **a** Zn, **b** Cu, **c** Pb, and **d** Hg concentrations, stratified by sex (girls: light yellow; boys: olive gray). Red dashed lines indicate reference thresholds. For essential metals, both lower and upper reference limits are shown and marked with the gray areas, while for toxic HMs, only a reference limit is provided, and significant associations in bold *p < .05, **p < .01, ***p < .001.

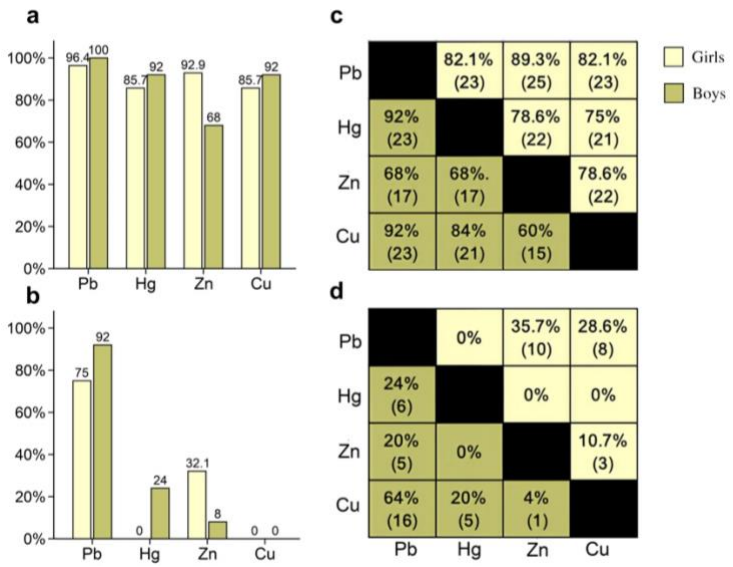


Fig. 2 Detection frequencies, reference value exceedances, and co-occurrence patterns of essential and toxic metals in girls and boys. Panels **a** and **b** summarize sex-specific frequencies for individual HM. Panel **a** shows the percentage of participants with detectable concentrations of Pb, Hg, Zn, and Cu. Panel **b** shows the percentage of participants whose concentrations exceeded the corresponding reference values (or upper reference values for essential metals). Bars are grouped by sex (girls and boys), and values above bars indicate percentages. Panels **c** and **d** present pairwise co-occurrence matrices. Panel **c** displays the percentage and number of individuals with simultaneous detection of each pair of metals. Panel **d** shows the percentage and number of individuals presenting simultaneous exceedances of reference values for each pair of metals, or concurrent exceedance/adequate levels in the case of essential metals. Values in the upper and lower triangles correspond to girls and boys, respectively.

2.2 Association of HMs concentration on Executive Function Performance

Baseline model (BM) selection varied across executive function (EF) outcomes and between sexes (Table S2). The outcome-specific BMs were subsequently used as reference models for evaluating candidate models that incorporated heavy metal (HM) concentrations (Table S3). Comparison of HM-containing models revealed EF- and sex-specific associations (Table 1). For each EF outcome, the top-listed model in Table 1 was selected as the final best-fitting model.

The best-fitting models revealed distinct patterns across sexes. Among girls, the inclusion of HMs variables did not improve model fit for STS or DLPFC scores (Table 1). In contrast, significant associations were identified for other EF domains. Specifically, performance on the aPFC task was significantly associated with the interaction between Hg and Zn. The interaction indicated that no association between Hg concentration and aPFC performance ($\beta = 0.0179$, 95% CI [-0.079, 0.115], $p = 0.719$; Fig. 3a). However, at higher Zn concentrations, aPFC performance improved significantly ($\beta = 0.326$, 95% CI [0.092, 0.561], $p = 0.0063$), suggesting that higher Zn concentration modifies the relationship between Hg exposure and executive functioning (Fig. 3a)

Table 1. Information-theoretic model selection for the associations between heavy metal concentrations and executive function subscores in girls (n = 28) and boys (n = 25).

Task Score	Girls (n = 28)					Boys (n = 25)				
	Predictor	**AIC**	**ΔAIC**	**AICw**	**logLik**	**Predictor**	**AIC**	**ΔAIC**	**AICw**	**logLik**
STS	1	240.79	0	0.228	-118.4	age + pb * zn	218.11	0	0.252	-103.05
	1 + pb	242.12	1.33	0.118	-118.06	age	218.89	0.78	0.171	-106.44
aPFC subscore	1 + hg * zn	226.73	0	0.495	-108.37	1 + hg * zn + pb + cu	225.03	0	0.173	-105.51
	1 + hg * zn + pb + cu	229.42	2.68	0.13	-107.71	1	226.66	1.63	0.077	-111.33
OMC subscore	age + hg * zn + pb + cu	235.6	0	0.382	-109.8	age	227.42	0.64	0.085	-110.71
	age	238.5	2.9	0.09	-116.25	age + pb + hg	226.79	0	0.117	-108.39
DLPFC subscore	1	239.25	0	0.178	-117.62	age + pb * zn	216.96	0	0.163	-102.48
	1 + cu	239.76	0.51	0.138	-116.88	age	217.11	0.15	0.151	-105.55
Working memory	age + hg * zn	188.77	0	0.553	-88.39	age + hg	170.43	0	0.258	-81.22
	age	192.06	3.28	0.107	-93.03	age + pb + hg	170.88	0.45	0.206	-80.44
Executive Function	HC + hg	245.07	0	0.196	-118.54	1	222.5	0	0.245	-109.25
	HC	246.34	1.27	0.104	-120.17	1 + zn	224.06	1.56	0.112	-109.03

Note: For each EF outcome, candidate models with ΔAIC < 2 are presented, with the top-listed model selected as the best fit based on the principle of parsimony. HC: Head Circumference; AICw: Akaike weight; logLik: Log-likelihood; ΔAIC: AIC difference relative to the model with the lowest absolute AIC value. Asterisks (*) denote interaction terms.

OMC performance exhibited a similar pattern, being significantly associated with the interaction between Hg and Zn. As observed for aPFC, no association between Hg concentration and OMC subscore varied according to Zn (β = 0.0179, 95% CI [-0.079, 0.115], p = 0.719), whereas higher Zn concentrations were associated with better OMC performance (β = 0.288, 95% CI [0.090, 0.486], p = 0.0044; Fig. 3b), suggesting that Zn modifies the relationship between Hg exposure and executive functioning. In addition to this interaction, Pb was negatively associated with OMC subscore (β = −0.114, 95% CI [−0.22, −0.01], p = 0.029; Fig. 3c), while Cu showed a positive association (β = 0.138, 95% CI [0.04, 0.23], p = 0.005; Fig. 3d).

A comparable Hg and Zn interaction was also retained in the best-fitting model for WM-DLPFC domain. Similar to the patterns observed for aPFC and OMC, the association between Hg and WM-DLPFC performance have not relation with Zn (β = −0.022, 95% CI [−0.157, 0.112], p = 0.742), but higher Zn concentrations were associated with improved WM-DLPFC scores (β = 0.489, 95% CI [0.184, 0.792], p = 0.0016; Fig. 3e). In contrast, no HMs effects were retained in the final model for EF-DLPFC following model selection.

Among boys, the only HM retained in the best-supported models was Hg, which showed a negative association with WM-DLPFC performance after accounting for age (β = −0.209, 95% CI [−0.368, −0.052], p = 0.0092; Table 1 Figure 4). No HM effects were supported for the remaining executive function outcomes.

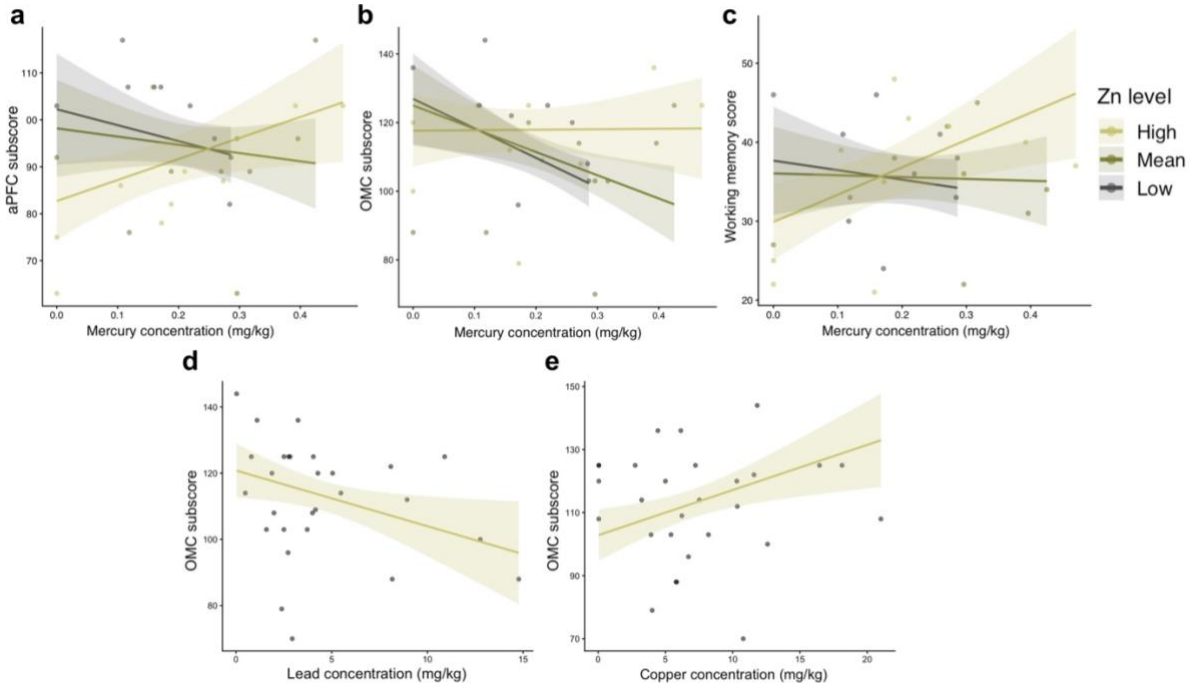


Fig. 3 Associations between essential, toxic HMs and executive function outcomes in girls (n = 28). The figure highlights the moderating role of Zn in the negative effect between Hg concentration and EFs performance, with higher Zn levels associated with more favorable predicted outcomes across multiple domains. Plots display estimated effects derived from Negative Binomial Generalized Linear Models (GLM.nb). Panels **a**-**c** illustrates the moderating effects of Zn levels on the association between Hg concentration (mg/kg) and, **a** anterior Prefrontal Cortex (aPFC) subscore, **b** Orbitomedial Prefrontal Cortex (OMC) subscore, and **c** Working Memory (WM-DLPFC) domain. For these interaction plots, lines represent model-predicted estimates at low (dark grey), mean (olive green), and high (yellow/ochre) Zn concentrations. Panels **d**-**e** show the main effects on the OMC subscore: **d** a significant negative association with Pb concentration; and **e** significant positive association with Cu concentration ($\beta = 0.138$, 95% CI [0.04, 0.23], $p = 0.005$). Shaded areas in all panels indicate 95% confidence intervals.

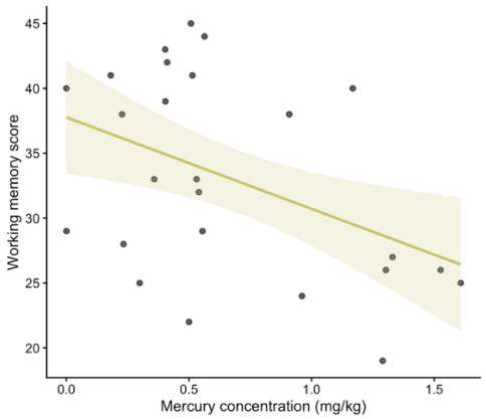


Fig. 4 Association between Hg concentration and WM-DLPFC performance in boys (n = 25). We observed significant negative association between Hg concentration (mg/kg) and WM-DLPFC domain performance ($\beta = –0.209$, 95% CI [–0.368, –0.052], $p = 0.0092$). Lines represent model-

predicted estimates derived from Generalized Linear Models with Negative Binomial distribution (GLM.nb), with shade areas indicating 95% confidence intervals.

3 Materials and Methods

3.1 Study site and sampling

This study focused on children from "Huautla", a rural community located within the Sierra de Huautla Biosphere Reserve in southern Morelos, Mexico (18°26'29.6" N, -99°01'20.4" W). The village lies in a mountainous region with limited accessibility. Critically, Huautla is located near exposed tailings, which present a potential source of chronic HMs exposure. According to the 2020 national census (INEGI, 2020), the community had 179 children under the age of 12. Between 2022 and 2023, data was collected, resulting in a total of 53 children (28 girls and 25 boys) between 5 to 11 years old, approximately one-third of the local child population. Eligibility criteria included continuous residence in the area since birth, no history of relocation, and current enrollment in the local elementary school. Participation was voluntary; informed consent was obtained from parents or legal guardians, and assent was provided by the children. The study protocol was reviewed and approved by the Ethics Committee of the Faculty of Psychology, National Autonomous University of Mexico (FPSI/422/CEI/432/2025).

3.2 Data collection and measures

Sociodemographic and eligibility information were obtained through a caregiver-administered questionnaire. This instrument confirmed inclusion criteria such as continuous residence in the community since birth and current enrollment in the local elementary school, including school grade for appropriate task selection and testing. It also collected data on the household environment of each child. Subsequently, anthropometric assessments were conducted by trained personnel following standardized procedures. In this case, children head circumference (HC) was also measured, were taken in accordance with World Health Organization35,36 (WHO, 2006; Nicolaou et al. 2020) child growth standards.

3.3 Hair sampling and heavy metal concentration analysis

Hair samples were used as a biomarker to assess long-term exposure to HMs (Rafiee et al. 2020; Nakaona et al. 2020), given their wide acceptance for this purpose. Approximately 100 strands of hair were collected from the occipital region of each child, cut as close as possible to the scalp with stainless-steel scissors. Samples were aligned to preserve root orientation, labeled accordingly, and stored in sealed plastic bags at room temperature until analysis. All instruments were disinfected with 70% isopropyl alcohol before and after each use to minimize contamination.

Samples were later transported to the Research Center in Food and Development in Guaymas, Sonora, for analysis. Upon arrival, each sample underwent a standardized decontamination protocol to eliminate surface pollutants. HMs concentrations including toxic (Pb and Hg) and essential metals (Zn and Cu), were quantified using stripping voltammetry. For quality assurance, each analytical batch included procedural blanks, sample duplicates, and certified reference materials limits: Pb > 0.029 mg/kg, Hg > 0.00001 mg/kg, Zn > 0.172 mg/kg and Cu > 0.047 mg/kg.

Although there are no internationally harmonized cutoffs for hair metal concentrations in children, we compared our findings to established reference ranges from previous studies in general and health populations without known exposure (Hair Mineral Analysis, 2012; Mikulewicz et al. 2013). These reference values were: Pb < 2.00 mg/kg, Hg < 1.00 mg/kg, Zn between 160-240 mg/kg, and Cu between 10-100 mg/kg. These thresholds were used to classify individuals by exceedance status, as well as to identify cases of co-exceedance, defined as concurrent concentrations of two or more metals above reference values.

We further examined patterns of co-occurrence among HMs using classification matrices, estimating the proportion of participants with detectable concentrations of one or more HMs. For essential metals, participants were also classified based on whether their levels fell below reference thresholds (Cu = 10 mg/kg and Zn = 160 mg/kg), indicating potentially optimal concentration. For toxic HMs, children were categorized as exceeding the reference limits (Pb = 2 mg/kg and Hg = 1 mg/kg) to capture toxicologically relevant exposures. This approach allowed us to characterize exposure not only in terms of detection but also relative to biologically meaningful thresholds, distinguishing between mean, deficient low, and high levels. In addition, we evaluated patterns of co-occurrence across these categories, including: a) co-detection of toxic HMs (Pb+Hg); b) co-occurrence of essential metals (Zn+Cu) within acceptable ranges; and c) cross-category combinations between essential and toxic HMs to explore potential additive or antagonistic interactions relevant to neurotoxicity.

3.4 Executive Functions Assessments

EFs were assessed using the Neuropsychological Battery of Executive Functions and Frontal Lobes – Third Edition (BANFE-3; Flores-Lázaro et al. 2021), a standardized and normed instrument designed specifically for the Mexican population, with normative adjustments based on age and educational level. The battery includes culturally adapted versions of internationally recognized executive function tasks, such as the Wisconsin Card Sorting, Stroop, verbal fluency, and the Tower of Hanoi tests, that have been validated to ensure psychometric equivalence and cultural relevance.

The number of tasks administered varied according to the age group of participants, as outlined in the BANFE-3 protocol. In the present study, between 7 and 13 tasks were applied per child, depending on their developmental stage and in accordance with standardized procedures. The assessment lasted approximately 60 to 90 minutes, depending on the pace of participant.

Scoring followed the official guidelines of the BANFE-3, higher scores reflect better executive performance. We obtained six scores: 1) standardized total score (STS); three subscores corresponding to anatomo-functional division of the PFC: 2) anterior prefrontal cortex (aPFC); 3) orbitomedial cortex (OMC); and 4) dorsolateral prefrontal cortex (DLPFC); in line with the theoretical framework of this instrument, the DLPFC was further divided into two specific functional domains, 5) Working Memory (WM-DLPFC) and 6) Executive Functions (EF-DLPFC). This division enhances the precision of cognitive profiling. Furthermore, normalized scores from the corresponding subtests were summed for each domain using the same tasks across all participants, ensuring consistency and comparability. Finally, these composite scores were subsequently used in statistical analyses to examine individual differences in executive functioning and their relationship with HMs co-exposure.

3.5 Statistical analyses and visualization

To examine the potential neurotoxic and modulatory roles of HMs in EFs outcomes, we fitted negative binomial generalized linear models (GLMs) using the glm.nb function from the MASS package in R (v4.5.0; Venables & Ripley, 2013). Candidate GLMs were compared using a two-stage information-theoretic model-selection approach based on the Akaike Information Criterion corrected for small sample size (AICc; Burnham & Anderson, 2004). Prior to model selection, we examined whether HMs concentrations differed between boys and girls. This analysis was conducted to assess potential sex differences in exposure levels, given that such differences could contribute to variation in executive function performance and provide additional support for subsequent sex-stratified analyses.

In the first stage, we identified an outcome-specific baseline model for each of the six BANFE-III scores. Three candidate models were evaluated: a null model (intercept only), a model including age, and a model including head circumference (HC). Both age and HC are indicators of child growth and development that may influence executive function performance (Rolls, 2023; WHO, 2006; Nicolaou et al., 2020). The model with the lowest AICc was selected as the baseline model. Consequently, the baseline model could differ among BANFE-III outcomes.

In the second stage, we assessed the contribution of HMs concentrations to executive function performance. Starting from the selected baseline model for each outcome, we constructed a set of 20 a priori candidate models representing alternative biological hypotheses. These models included single-metal effects, additive effects of multiple metals, and interactions among essential and non-essential metals. Candidate models were evaluated separately for males and females because sex may influence both executive function performance and HMs concentrations (see Table S1). For each outcome and sex, the candidate model with the lowest AICc was considered the best-supported model. When competing models differed by less than two AICc units, the more parsimonious model was preferred to avoid retaining uninformative parameters (Arnold, 2010). Biological interpretation focused on outcomes for which the best-supported model included at least one HM predictor, either as a main effect or as part of an interaction term, thereby indicating support for an association between HMs exposure and executive function performance.

Statistical significance was defined as $p < 0.05$. Results are reported as parameter estimates, standard errors, and 95% confidence intervals. To evaluate potential multicollinearity among predictors, variance inflation factors (VIFs) were calculated for models containing multiple HMs variables. All VIF values were below 2, indicating low collinearity among explanatory variables (Zuur et al., 2010).

To facilitate the interpretation of significant interaction effects identified in the negative binomial GLMs, we generated predictive plots using the ggeffects package (Lüdecke, 2021). These plots illustrate the estimated relationship between a focal predictor (e.g., Pb concentration) and executive function outcomes across three representative values of the moderating variable (e.g., Zn concentration), corresponding to low, mean, and high levels. Predicted values and their 95% confidence intervals were calculated using the ggpredict function and visualized with ggplot2 (Wickham & Sievert, 2009). This approach allowed us to examine how the association between one metal and executive function performance varied across different concentrations of the interacting metal.

4 Discussion

The legacy of mining contamination in Huautla, a community located within a protected natural reserve, has left a persistent imprint on human health. Our study shows that, despite the cessation of mining activities more than three decades ago, a complex mixture of essential and toxic HMs remains in the environment and continues to exert measurable neurotoxic effects during childhood. These effects were not homogeneous across the population: we observed marked sex-specific differences in both exposure patterns and in their associations with EFs performance, particularly across distinct regions of the PFC. Together, these findings underscore the need to evaluate neurotoxic risk not only in terms of individual contaminants but also as interactive mixtures, while considering key contextual factors such as sex.

First, we observed that boys carried a higher toxic burden than girls, particularly with respect to Pb and Hg, with significantly higher rates of reference exceedances. This finding aligns with previous research reporting a greater biological and/or behavioral susceptibility of males to accumulate HMs (Gade et al., 2021; Llop et al., 2013), possibly due to differences in metabolic and hormonal activity, as well as distinct patterns of environmental interaction (Rafiee et al., 2020; Arnold & Liu, 2020). The high prevalence of toxic HMs co-exposure among boys is especially concerning, as both metals have been consistently linked to adverse effects on the PFC and broader neurodevelopmental outcomes (Joo et al., 2018; Karri et al., 2016; Oluyemi et al., 2024; Ouyang et al., 2023; Santa Maria et al., 2019). These results suggest that boys may face a heightened cumulative risk from legacy contamination, underscoring the importance of considering sex-specific vulnerability in environmental health assessments.

In contrast, among girls we identified modulatory effects of essential metals, particularly Zn, on the relationship between exposure to toxic elements. Although sex-specific differences in the physiological functions of these elements remain unclear, dysregulation of Zn and Cu may compromise neuronal excitability, redox balance, and the function of glutamatergic, GABAergic, and dopaminergic systems (Crăciun et al., 2016; Kawahara et al., 2024; Sowa-Kućma et al., 2011), as well as immune processes within the brain (Zheng et al., 2023; Shen et al., 2023). Our results reflected this pattern in performance across specific EF domains. The most consistent finding was the positive interaction of Zn levels on Hg concentration and their effects on aPFC, OMC, and WM-DLPFC performance, where high Zn levels appeared to attenuate, or even reverse, the neurotoxic effects of Hg. Interestingly, this interaction was not observed in boys, suggesting that the modulatory role of essential metals may be shaped by sex-dependent factors, and girls significatly more Zn levels. One possibility is compensatory biological capacity of Zn, which may exert greater influence on functional domains dependent on PFC networks in girls. In this context, biological sex emerges as a critical moderator, potentially mediated by hormonal differences that influence cortical maturation, the expression of metal transporters, and the integrity of the blood–brain barrier (Gade et al., 2021; Oluyemi et al., 2024; Shinohara & Moriguchi, 2021) besides having an interaction with the function of the PFC by means of immune response and neurodevelopmental processes participating in glutamate receptor regulation (Pochwat et al., 2015; Truby et al., 2024).

As for negative associations, Pb showed an adverse effect on OMC performance in girls, consistent with prior literature linking Pb exposure to cognitive and behavioral impairments (Joo et al., 2018; Karri et al., 2016; Oluyemi et al., 2024; Ouyang et al., 2023; Santa Maria et al., 2019). Mechanistically, Pb disrupts calcium homeostasis, interferes with NMDA receptor signaling, and alters glutamatergic and dopaminergic pathways (Buha et al., 2021; Neal et al., 2010; Xiao et al., 2006), while simultaneously promoting oxidative stress, neuroinflammation, and apoptosis (Gąssowska-Dobrowolska et al., 2023; Zheng et al., 2023). These processes are known to particularly compromise PFC-related networks (Arnold & Liu, 2020; Cecil et al., 2008; Neal et al., 2010; Xiao et al., 2006). In contrast, Cu showed a positive association with OMC performance, which may reflect its essential role in maintaining dopaminergic signaling and enzymatic neurotransmission homeostasis (Li et al., 2023). Together, these findings suggest that in real-world contexts of multiple exposures, essential metals do not act in isolation but can modulate the neurotoxic effects of Pb and Hg in either a protective or detrimental direction, generating risk profiles that vary across brain regions and between sexes (Karri et al., 2016; Kawahara et al., 2024).

Among boys, associations were less consistent and largely dominated by the effect of age on score variability. The only robust finding was the negative relationship between Hg and WM-DLPFC, confirming the particular vulnerability of working memory and the DLPFC to neurotoxic metal exposure (Levy & Goldman-Rakic, 2000; Pochon et al., 2001). Although mean Hg levels were differed by sex, being greater in boys, its neurotoxic potential is well established, including the disruption of glutamate homeostasis, Na^+/K^+-ATPase activity, and redox balance (Farina et al., 2011; Huang et al., 2008). The absence of more complex interactions in boys could reflect sex-specific differences in the absorption and regulation of metals, or suggest that the direct neurotoxic effects of Hg and Pb outweigh any potential modulation by essential metals in this group.

Taken together, these results emphasize the importance of analyzing exposure to HMs in terms of mixtures rather than isolated contaminants, as interactions between essential and toxic metals may produce outcomes that cannot be predicted from their individual toxicological profiles. The identification of differential patterns by sex aligns with emerging evidence pointing to distinct neurodevelopmental vulnerabilities in boys and girls (Hermida et al., 2019; Tooley et al., 2021). As the central hub for EFs integration, the PFC is especially sensitive to environmental insults due to its prolonged postnatal maturation (Anderson, 2002; Diamond, 2013; Blair & Cybele Raver, 2015; Zelazo & Carlson, 2012). Accordingly, the combined exposure to essential and non-essential metals should not be interpreted as a simple additive effect. Rather, our findings suggest that the impact of HMs on executive development may manifest as the result of dynamic interactions between neurotoxic and modulatory elements. Such interactions can compromise fundamental neurodevelopmental processes, including synaptic pruning, myelination, and the regulation of neurotransmitter systems (e.g., glutamatergic, GABAergic, and dopaminergic) potentially leading to functional immaturity that becomes evident under conditions of high cognitive demand (Balali-Mood et al., 2021; Karri et al., 2016). This may in turn interfere with the normative developmental trajectory of EF, affecting gradually emerging abilities such as planning, self-regulation, inhibitory control, cognitive flexibility, and decision-making.

Ultimately, these findings underscore the urgent need to account for both multi-metal co-exposure and sex-specific differences in environmental health research, particularly in rural

communities affected by mining legacies. Although the developing brain exhibits substantial plasticity, our results suggest that this capacity may be insufficient to fully counteract the effects of chronic exposure to toxic metals during critical developmental periods. Rather than supporting the existence of effective adaptive responses, the observed patterns indicate persistent vulnerability of executive function networks to environmental contaminants. In girls, the interaction between Zn and Hg suggests that essential elements may partially modulate the impact of neurotoxic exposures; however, this association should not be interpreted as evidence of compensation, but rather as an indication that the effects of toxic metals depend on the broader elemental context in which exposure occurs.

Despite the strengths of the study design and analytical approach, several limitations must be acknowledged. First, the cross-sectional nature of the study precludes the establishment of causal relationships. Second, metal analysis was conducted solely using hair samples, which, although useful as markers of chronic exposure, do not provide information on tissue distribution or individual toxicokinetic. Finally, the relatively small sample size limits the generalizability of the findings and restricts the ability to explore more complex interactions between multiple metals and executive function domains.

The findings have important implications for both research and public health. From a scientific perspective, this study highlights the need to adopt modeling approaches that account for metal–metal interactions, the modulatory role of biological sex, and the influence of environmental context. Future research should expand toward longitudinal designs to track the developmental trajectory of EF in relation to dynamic environmental exposures. Additionally, incorporating inflammatory biomarkers relevant to nervous system development and function could offer greater clarity regarding the underlying mechanisms. Finally, given the influence of the heavy metals evaluated on excitatory and inhibitory dynamics of neuronal communication, future efforts will include the implementation of complementary techniques, such as electroencephalography (EEG), to obtain a global measure of brain electrical activity.

5 Conclusion

This study provides evidence that chronic exposure to mixtures of essential and toxic metals in a community affected by a mining legacy is associated with sex-specific patterns of EFs performance during childhood. Notable differences were observed in both exposure profiles and the functional impact of metals on PFC-related domains. Among girls, Zn appeared to modulate the association between Hg exposure and performance in domains related to abstract reasoning, cognitive flexibility, emotional regulation, and working memory, suggesting a potential buffering role of this essential element. In contrast, this interaction was not observed among boys, indicating that the neurocognitive effects of metal mixtures may differ according to sex-specific biological and exposure-related factors. Overall, these findings highlight the importance of evaluating environmental exposures as complex mixtures rather than isolated contaminants and support the inclusion of biological sex as a key factor in studies examining the neurodevelopmental consequences of HMs exposure during childhood.

Supplementary information. The supplementary information contains in the online version.

Acknowledgements.

Christian G. Curiel-Guerrero is a doctoral student from the Programa de Doctorado en Ciencias Biomédicas at Universidad Nacional Autónoma de México (UNAM) and has received a fellowship 1191214, from Secretaría de Ciencia, Humanidades, Tecnología e Innovación (SECIHTI, formerly CONAHCYT).

Aída Elizondo García is a doctoral student from the Programa de Doctorado en Ciencias Biomédicas at Universidad Nacional Autónoma de México (UNAM) and has received CONAHCYT fellowship 1121764, from Secretaría de Ciencia, Humanidades, Tecnología e Innovación (SECIHTI, formerly CONAHCYT).

We are grateful to Ornela De Gasperín Quintero for her valuable assistance in reviewing and editing this manuscript. We also thank Lynna Marie Kiere for her contributions to data analysis, particularly in the development of hypotheses and linear models. Finally, we acknowledge Jaqueline García-Hernández for the formal analysis of heavy metal concentrations conducted at the Center for Food and Development Research (CIAD) in Guaymas, Sonora, Mexico.

Declarations

Funding. Funding provided by Secretaría de Ciencia, Humanidades, Tecnología e Innovación (SECIHTI, formerly CONAHCYT), Frontier Science 191975, Mexico.

Conflict of interest. The authors declare no conflicts of interest.

Ethics Approval and Consent to Participate. The study protocol was approved by the Ethics Committee of the Faculty of Psychology, National Autonomous University of Mexico (Approval No. FPSI/422/CEI/432/2025). All procedures were conducted in accordance with the relevant ethical standards. Written and oral informed consent was obtained from all participating children and their parents or legal guardians prior to data collection.

Consent for Publication. Not applicable. This manuscript does not contain any identifiable images or personal information that could compromise participant anonymity.

Data Availability. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Author contributions Christian G. Curiel-Guerrero: Investigation, Methodology, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review and editing. Aída Elizondo García: Investigation, Methodology, Data curation, Visualization, Writing – original draft. Marcela Osorio-Beristain: Funding acquisition and Resources. Moisés Rubio Osornio: Writing – review and editing. Gabriel Roldán Roldán: Writing – review and editing. Isaac G-Santoyo: Conceptualization, Investigation, Funding acquisition,

Project administration, Supervision, Writing – review and editing. All authors read and approved the final manuscript and consented to its publication.

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