Impact of chronic heavy metal exposure on anemia and respiratory health in children living in Tumbes, Peru

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A DISSERTATION

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ABSTRACT OF THE DISSERTATION

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Heavy metal (HM) exposure is a pressing yet under-addressed global public health issue that contributes substantially to morbidity and mortality, particularly in low- and middle-income countries. Gold mining is an important source of exposure in these settings, as it can release HMs into rivers and affect downstream communities. The Puyango-Tumbes River, which originates in a gold mining region of southern Ecuador and flows into northern Peru, serves as the main source of freshwater for the Tumbes region. Given reports of elevated concentrations of HMs in water and sediment samples from the river, there is growing concern in the community about potential impacts on health, particularly among children.

In this dissertation, I aimed to characterize HM exposure in children from Tumbes, Peru (Aim 1) and evaluate associations of HM exposure with respiratory health (Aim 2) and anemia (Aim 3) through a series of cross-sectional analyses conducted in collaboration with the Center of Global Health Tumbes (CGH)-Universidad Peruana Cayetano Heredia and the local health authority (Dirección Regional de Servicios de Salud Tumbes, DIRESAT).

Between January and March 2023, CGH personnel recruited 409 children aged 4 to 17 years living in Tumbes. I assessed children's internalized exposure to arsenic (As), lead (Pb), cadmium (Cd), and manganese (Mn) using hair samples, analyzed via inductively coupled plasma mass spectrometry (ICP-MS) at the Oregon Health & Science University Elemental Analysis Core Lab. I measured respiratory health outcomes using spirometry—specifically, percent predicted forced vital capacity (ppFVC)—and caregiver-reported respiratory symptoms from the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire. I identified anemia in children using hemoglobin concentrations in whole blood. Environmental exposure was assessed using publicly available water quality data from DIRESAT, which included HM concentrations (also measured by ICP-MS) and water quality parameters.

To address Aim 1, I conducted an exposure assessment to characterize HM levels in water and hair samples, identify potential exposure pathways, and evaluate the association between river proximity and hair HM concentrations. I used geospatial approaches to link water quality measures from geocoded water

samples (n=40) with geocoded home addresses and each study participant's corresponding individual-level biomarker and behavioral data (n=404). A substantial proportion of water samples exceeded national safety thresholds for As (30%), Pb (18%), and Mn (25%), particularly in surface water and within the Puyango-Tumbes watershed region. I found that children living in villages closer to the river (0.6-3.4 km) had higher hair concentrations of As (exponentiated β 3.9, 95% confidence interval [CI] 1.16, 6.73) and Pb (exponentiated β 1.77; 95% CI 0.91, 3.43) compared to those living farther away (20.5-27.5 km). However, only the association with As was statistically significant, as its 95% CI excluded the null. Correlation analysis showed modest positive correlations between water and hair concentrations for As ($\rho = 0.291$) and Mn ($\rho = 0.236$). Overall, findings from Aim 1 analyses identified surface water—particularly from the Puyango-Tumbes River—as a likely source of HM exposure, especially for As, and to a lesser extent, Pb.

For Aim 2, I evaluated the association between As exposure—alone and in combination with other HM (Pb, Cd, and Mn), all log-transformed—and respiratory health (n=399), using multivariable linear models and quantile-based G-computation mixture analysis. I observed inverse associations between hair As and lung function (ppFVC) (β 0.32; 95% CI -0.94, 1.58), as well as between HM mixtures and ppFVC (Ψ -0.44; 95% CI -2.33, 1.45), although these findings did not reach statistical significance. However, I found that higher As levels were significantly associated with increased odds of nasal symptoms suggestive of allergic rhinitis (adjusted odds ratio [aOR] 1.59; 95% CI 1.32, 1.91). I also observed an overall prevalence of restrictive spirometry patterns (13–23%) that exceeded expectations, highlighting the need for further investigation.

Finally, to fulfill Aim 3, I assessed the association between Pb exposure—alone and as part of a HM mixture (Pb, As, Cd, and Mn)—and anemia (n = 404), using the same analytical approach as in Aim 2. Given that previous studies reported lower serum ferritin levels—an iron storage protein—among children living close to the Puyango-Tumbes River compared to those living more than 50 km away with no HM exposure, I explored whether the association between Pb and anemia could be mediated by impaired iron storage. I conducted a causal mediation analysis using natural effects to assess ferritin's mediating role in the Pb-anemia association. Higher Pb was significantly associated with increased odds of anemia (aOR 1.31; 95% CI 1.05, 1.65), and the HM mixture including Pb was more strongly associated with anemia than Pb alone (aOR 1.55; 95% CI 1.02, 2.08). The mediation analysis indicated that ferritin did not mediate this association, suggesting that other biological mechanisms—such as inflammation or disrupted iron transport—may be involved.

This work is among the first in Peru to evaluate the combined effects of HMs on respiratory and hematologic outcomes in children using biomarker-based exposure assessment and mixture modeling. It highlights the need for improved water treatment, food safety monitoring, and targeted health interventions, particularly in river-adjacent communities. My findings also support the need for enhanced respiratory and anemia screening and long-term monitoring of children's health in the region. Future research should incorporate mercury exposure, evaluate additional biomarkers, and use longitudinal designs to clarify causal pathways and inform effective public health interventions.

DEDICATION

To the children of Tumbes

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ABBREVIATIONS

AIC	Akaike information criterion
Al	Aluminum
ALAD	Aminolaevulinic acid dehydratase
As	Arsenic
ASGM	Artisanal and small-scale gold mining
ATS	American Thoracic Society
ATP	Adenosine triphosphate
B	Boron
Ba	Barium
Be	Beryllium
BKMR	Bayesian kernel machine regression
Ca	Calcium
CDC	Centers for Disease Control and Prevention
Cd	Cadmium
CGH	Center for Global Health
CI	Confidence interval
Cl	Chlorine
COPD	Chronic obstructive pulmonary disease
Cr	Chromium
Cu	Copper
DAG	Directed acyclic graph
DALY	Disability-adjusted life year
DE	Direct effects
DIRESAT	Dirección Regional de Salud de Tumbes (Regional Health Authority of Tumbes)
DMT-1	Divalent metal transporter
ERS	European Respiratory Society
Fe	Iron
FEV_1	Forced expiratory volume in the first second
fL	Femtoliter
FVC	Forced vital capacity
GIS	Geographical information systems
GLI	Global Lung Initiative
GPS	Global Positioning System
Hb	Hemoglobin
Hg	Mercury
HM	Heavy metals
HNO3	Nitric acid
IQR	Interquartile range
ICC	Intraclass correlation coefficient
ICP-MS	Inductively coupled plasma mass spectrometry
IE	Indirect effect
ISAAC	International Study of Asthma and Allergies in Childhood
K2 EDTA	Dipotassium ethylenediaminetetraacetic acid
LASSO	Least absolute shrinkage and selection operator
LLN	Lower limit of normality
LMIC	Low- and middle-income countries
LRT	Likelihood ratio test
MCV	Mean corpuscular volume

MCHC	Mean corpuscular hemoglobin concentration
MeHg	Methylmercury
Mn	Manganese
Mo	Molybdenum
Na	Sodium
NDE	Natural direct effect
NHANES	National Health and Nutrition Examination Survey
NHBCS	New Hampshire Birth Cohort Study
Ni	Nickel
NID	National Identity Document
NIE	Natural indirect effect
NIST	National Institute of Standards and Technology
NTU	Nephelometric turbidity units
OHSU	Oregon Health & Science University
OR	Odds ratio
PCA	Principal component analysis
PC	Principal component
Pb	Lead
pg	Picograms
ppFVC	Percent predicted forced vital capacity
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
Sb	Antimony
SD	Standard deviation
Se	Selenium
SES	Socioeconomic status
SPS	Sample Preparation System
TE	Total effect
U	Uranium
US	United States
USEPA	United States Environmental Protection Agency
V	Vanadium
WHO	World Health Organization
WQSR	Weighted quantile sum regression
YLD	Years lived with disability
Zn	Zinc

CHAPTER 1. INTRODUCTION AND RESEARCH AIMS

Exposure to heavy metals (HM) has led to substantial health consequences worldwide, including over one million cases of disease, 56,000 deaths, and more than nine million disability-adjusted life years in 2015 alone.[1] A major source of HM is gold mining, which has been increasing globally, especially in low and middle-income countries.[2] Once deposited into water bodies, HM can bioaccumulate in organisms,[3] making it possible for people living near rivers that are downstream from mining sites to be chronically exposed to HM through water and food. The Puyango-Tumbes River, originating in Ecuador and serving as the primary source of fresh water for the Tumbes region in Peru, exemplifies this issue. Although residents in Tumbes are not directly involved in gold mining activities, they may be chronically exposed to HM due to contamination likely resulting from upstream mining.[4]

Exposure to HM induces oxidative stress and impaired immune response, potentially affecting any tissue in the human body.[5] Compared to adults, children are more vulnerable to these effects because they are in a critical stage of development, during which their bodies are especially susceptible to environmental stressors.[6,7] As a result, children living in Tumbes may be harmed by chronic exposure to HM through direct and indirect contact with the polluted waters of the Puyango-Tumbes River. Despite this potential risk, the magnitude, spatial distribution, and combined nature of HM exposure in this population remain poorly characterized.

The lungs are a highly susceptible organ for some HM, such as Arsenic (As),[8,9] and take longer to develop compared to other organs, which can potentially lead to higher total exposure doses of HM over longer periods of development.[10] Given that the lungs are crucial for oxygenating the blood that nourishes all other organs of the human body, their correct function is vital for systemic health.[10] Although evidence linking HM exposure to respiratory disease risk is growing, important knowledge gaps persist. Few studies have focused on HM exposure in marginalized populations, the cumulative effects of multiple HM on lung function, or the specific impacts on children.[11]

In addition to altering lung function, several HM—including mercury (Hg), As, lead (Pb), and cadmium (Cd)—have been linked with an increased risk of anemia, potentially through disrupted hemoglobin (Hb) synthesis and altered iron metabolism.[5,12] Identifying the specific mechanisms by which these HM induce anemia in our study setting is crucial for developing targeted interventions. Our team's previous research in the Tumbes region indicates that individuals living closer to the Tumbes-Puyango River have lower serum ferritin concentrations—an iron-storage protein—compared to those living farther away (Fernandez et al., in preparation). This suggests that HM exposure may affect iron metabolism and contribute to anemia. However, the extent to which ferritin depletion explains the HM-anemia association in our population remains to be fully determined.

By leveraging collaborative efforts with the Center of Global Health (CGH)-Universidad Peruana Cayetano Heredia and the local health authority (DIRESAT), this dissertation aims to assess HM exposure in water and investigate how chronic exposure to As, Pb, Cd, and manganese (Mn) affects anemia and respiratory health in children living in Tumbes, Peru. To achieve this, CGH personnel recruited 409 children aged 4-17 years from the Tumbes region and collected whole hair samples, from which the 3 proximal cm were analyzed to estimate approximately 3 months of prior exposure to HM. Blood samples were collected to determine anemia status, while survey and spirometry data were used to evaluate respiratory symptoms and lung function. Questionnaires were also administered to capture demographic and lifestyle factors that may influence HM exposure patterns. Finally, publicly available data on water quality, including HM concentrations, were geospatially linked to participants' residential locations to support exposure assessment of HM in water.

This dissertation begins with a review of the literature (**Chapter 2**), which contextualizes HM exposure historically and globally, discusses the value of exposure assessments, and outlines the toxicological mechanisms by which HM affects health. Special attention is given to the effects of HM exposure on lung function and anemia, as well as the specific vulnerabilities—both physiological and socioeconomic—of affected populations in Tumbes.

Chapter 3 addresses the first specific aim: **to perform a comprehensive exposure assessment of HM in Tumbes.** This included geospatially linking water quality metrics with individual-, household-, and village-level data. I used principal component analysis to identify patterns of HM exposure from water measurements and caregiver-reported questionnaire data. I used spatial analysis to detect geographic patterns of exposure to HM and evaluate how proximity to the Puyango-Tumbes River is associated with internalized HM levels.

The second specific aim, presented in **Chapter 4**, was **to assess the impact of chronic As exposure on lung function and respiratory symptoms in children**. I modeled hair As both separately and in combination with other HM to estimate their associations with lung volumes and respiratory symptoms. I hypothesized that higher levels of hair As and hair HM mixtures would be linked to lower lung volumes and a greater prevalence of respiratory symptoms.

Chapter 5 focuses on the third specific aim: **to assess the association between hair Pb and anemia in children**. This specific aim was divided into two sub-aims: Aim 3a **evaluated the association between hair Pb and anemia**, while Aim 3b **examined the role of ferritin as a mediator of the Pb-anemia association.** I started by modeling hair Pb both individually and in combination with other hair HM to explore their impact on anemia, hypothesizing that higher hair Pb levels and higher HM across a mixture would be associated with increased odds of having anemia. Then, I applied a causal mediation analysis to estimate the indirect effect of Pb exposure on anemia through ferritin.

Finally, **Chapter 6** summarizes the main findings of this dissertation, discusses its overall strengths, limitations, and public health implications, and concludes by highlighting directions for future research and alternative methodological approaches to further explore these questions.

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CHAPTER 2. REVIEW OF THE LITERATURE

1. The burden of heavy metals

The global burden of heavy metals (HM) exposure is substantial—resulting in over 1 million cases of disease, 56,000 deaths, and more than 9 million disability-adjusted life years (DALYs) worldwide in 2015.[1] HM are defined as chemical elements that exhibit metallic properties and have a relatively high density compared to water (≥5 g/cm³).[2,3] Examples include arsenic (As), lead (Pb), cadmium (Cd), and manganese (Mn), which can be found in various environmental sources such as contaminated water, soil, and air.[4] Chronic exposure to these metals has been associated with a myriad of health conditions, including neurodevelopmental disorders, cardiovascular diseases, kidney damage, and various forms of cancer.[5–12] Beyond these broader health impacts, HM exposure has also been associated with anemia[13–18] and respiratory dysfunction,[19–21] which are the central outcomes investigated in this dissertation. This burden disproportionately affects low- and middle-income countries (LMICs), where industrialization and mining are widespread, often impacting vulnerable populations who may have limited access to healthcare and preventive measures.[22–24]

2. Historical and current context of HM exposure

HM exposure is closely linked to industrialization and the global expansion of mining activities.[25] Although metals have been extracted and utilized since ancient times, the Industrial Revolution of the mid-18th to early 19th centuries substantially increased metal production and environmental contamination.[26] Gold mining, in particular, has played a major role in economic development but has also contributed substantially to environmental degradation,[27] including the release of HM such as As, Pb, Cd, and Mn as by-products.[5,28–32] This is especially concerning because once released into the environment, these metals can contaminate soil, water, and air—creating long-lasting exposure risks for surrounding populations.[26]

In recent decades, gold mining has expanded substantially in LMICs,[33] driven in part by the sharp increase in gold prices—from $430 per ounce in 2004 to $2,420 per ounce in 2024.[34] This mining expansion has led to widespread environmental issues, including deforestation, biodiversity loss, and increased pollution from HM.[33] In regions such as the Amazon basin, artisanal and small-scale gold mining (ASGM) has become a major economic activity with severe environmental and health consequences.[35] Water pollution from these activities poses serious risks, as it can affect not only nearby communities but also those downstream who depend on these water sources for their livelihoods and well-being. The Puyango-Tumbes River, the focus of this dissertation, exemplifies these risks.

3. Tumbes and the Puyango-Tumbes River

3.1. The Puyango-Tumbes River

Located in the southwestern Andes, the Puyango-Tumbes river originates at the confluence of the Calera, Amarillo, and Pindo rivers in the Portovelo region, near El Oro Province, Ecuador (**Figure 1**).[36] From there, it flows into Peru, crossing the Tumbes region before discharging into the Pacific Ocean.[36] Stretching approximately 160 km, with an annual outflow of 3,400 x 10^6 cubic meters and a drainage area of 4,850 km^2, it serves as a crucial—and sometimes sole—water source for many residents in Tumbes.[37]

However, the river's importance as a water source is increasingly threatened by upstream gold mining activities. The Portovelo region in Ecuador is known for its extensive mining activity. By 2015, over 10,000 people were involved in mining, with about 87-100 small-scale mining sites that collectively produced approximately 1.9 million tons of tailings annually, often discharged directly into local rivers.[36,38] Environmental monitoring studies have shown that levels of As, Pb, Cd, and Mn in the river's water and sediment exceed United States Environmental Protection Agency (USEPA) recommendations at various locations along the river's downstream stretch.[36,37] These concentrations peak near gold processing sites in Ecuador and gradually decrease downstream; however, levels remain elevated—well above baseline—even over 140 kilometers into the Tumbes region.[36] This exposure extends beyond drinking water, as high levels of HM are also found in river fish, rice, and crops irrigated with river water, and seafood from areas near the river's mouth.[39,40]

This sustained contamination reflects not only the scale of mining activity but also the region's limited capacity to prevent and remediate environmental damage. The persistence of HM contamination is largely driven by historical gaps in environmental regulation and enforcement,[41] which have contributed to the ongoing public health threat. These risks have raised growing concern among the Tumbes community and local authorities, particularly regarding the potential health impacts on children, who may be especially vulnerable to chronic HM exposure.

3.2. The Tumbes population

Tumbes, the second smallest region in Peru, has a population of approximately 260,000.[429/5/2025 4:54:00 PM] The region faces challenges, including limited access to basic sanitation and low education levels. In 2017, 32% of the population lacked an in-house water supply, 41% lacked access to a sewer system, 24% had only completed elementary education, and 16% lacked access to healthcare—conditions that disproportionately affect rural areas.[43] About 69% of households have access to public water managed by 35 entities, most of which provide minimally treated water.[44] Some entities use groundwater, while others rely on the Puyango-Tumbes River, including the main provider, "Unidad Ejecutora de Servicios Sanitarios Tumbes."[44] Inspections in 2021 revealed that As, Pb, and other toxicants exceed safety standards, especially in surface water sources like the El Milagro supply, which serves over 100,000 residents, mainly on the left (west) margin of the watershed.[44]

4. Toxicology of heavy metals

4.1 Exposure routes and bioavailability

Although HM are naturally occurring elements, most human exposure arises from human activities such as industrial emissions, agricultural runoff, improper waste disposal, and mining operations.[3,5,25] The bioavailability of HM, or their capacity to be absorbed by living organisms, is shaped by a variety of physical, chemical, and biological factors (**Table 1**).[3] HM can accumulate in organisms over time (bioaccumulation).[45] This bioaccumulation is especially dangerous when HM are released into aquatic environments, where they can remain for extended periods and infiltrate the food web.[45] As a result, both nearby residents and downstream communities face substantial risks from using contaminated water for drinking, agriculture, and fishing.

4.2 Toxicological mechanisms

While elements like As, Pb, and Cd are known for their toxicity at low concentrations and their ability to accumulate in living organisms and the environment,[3] others, such as Mn, are essential for metabolic processes but become toxic at high doses.[46] HM exert their toxic effects through several mechanisms across a wide range of organisms. At a molecular level, they have an affinity for sulfhydryl (thiol) groups, allowing them to bind and inactivate proteins, including enzymes crucial for metabolism, detoxification, and damage repair.[3,47–49] This interaction also leads to the depletion of glutathione, a critical cellular antioxidant that contains thiol groups.[47,50] In addition, HM generate free radicals, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), which contribute to oxidative stress.[3,45,47,51] This oxidative stress causes damage to lipids and affects proteins, leading to their malfunction and inactivation.[47] Metal ions can also interact with DNA and nuclear proteins, causing DNA damage and conformational changes that may lead to cell-cycle modulation, carcinogenesis, or apoptosis (**Figure 2**).[47]

Beyond these molecular effects, HM impact various cellular structures, including membranes, mitochondria, lysosomes, endoplasmic reticulum, and nuclei.[3] They disrupt cellular signaling and regulatory networks, affecting metabolism, immune responses, and hormonal balance, contributing to organ dysfunction.[3,47,52] Furthermore, HM can alter gene expression through epigenetic modifications, potentially leading to long-term health effects.[53]

Some HM exhibit more specific toxic effects. For instance, As can mimic and replace phosphate in various biochemical processes, disrupting pathways that rely on phosphate.[3] Additionally, it interferes with cellular respiration by inhibiting pyruvate dehydrogenase, disrupting the Krebs cycle and oxidative phosphorylation, leading to decreased adenosine triphosphate (ATP) production and cellular damage.[47] Pb mimics and interferes with the actions of calcium (Ca) while also inhibiting key enzymes involved in heme biosynthesis, such as aminolaevulinic acid dehydratase (ALAD) and ferrochelatase, which can result in anemia and reduced oxygen transport capacity (see **section 8**).[3,13,47]

4.3 Nutritional status

Poor nutritional status can both increase heavy metal (HM) exposure and worsen its health effects. Nutrient deficiencies make individuals more vulnerable to HM toxicity;[54] for example, Fe deficiency enhances Pb absorption,[54] and Ca deficiency increases Cd uptake.[55] Additionally, malnutrition impairs the body's ability to handle oxidative stress from HM, raising the risk of adverse effects.[56] This dual burden is especially common in LMICs, where both HM exposure and malnutrition are widespread.[57,58]

4.4 Combined exposure and interactions

Aside from sharing multiple harmful mechanisms,[47] HM often co-occur, making exposure to a single HM uncommon, especially from environmental sources like water.[22] Additionally, HM can interact, meaning that people exposed to water polluted with multiple HM may experience more severe health outcomes compared to those with no exposure or single HM exposure. Moreover, our general lack of knowledge regarding how these co-exposures interact—whether their effects are additive, multiplicative, or even counteracting—complicates this issue further. This understanding is particularly relevant for the Tumbes population, where the public water system provides a mix of HM.[59] Therefore, using models that incorporate multiple exposures is fundamental for understanding these combined effects and addressing the complexities of real-world exposure scenarios.

5. Children's vulnerability to HM exposure

Children are particularly susceptible to the toxic effects of HM because they are going through a developmental period that makes their organs more vulnerable to any insult.[19,21,60,61] Toxic substances can cause more damage in children than adults because they consume more water and food proportionally to their body weight, have higher respiratory rates, and have larger alveolar surfaces.[19] Additionally, some HM, like Pb, are absorbed faster in children's than adults' gastrointestinal tracts.[62] Further, some childhood behaviors, such as spending more time outdoors, increased contact with the ground, and putting objects into their mouths, place children at higher risk of exposure to HM.[19,63] This combination of heightened exposure and biological susceptibility places children at a higher risk of experiencing irreversible or fatal health outcomes in adulthood, such as cancer, bronchiectasis, lung fibrosis,[64] or even early death.[60]

6. Exposure assessment

Exposure assessment quantifies the intensity, frequency, and duration of human exposures to environmental contaminants, including HM.[65] Its goal is to provide accurate, precise, relevant, and cost-effective estimates of exposure across study populations.[66] Identifying exposure pathways to accurately estimate dose-response relationships is a critical step in this process. To this end, behavioral and dietary surveys provide insights into habits and consumption patterns, helping to improve exposure estimates based on how individuals in the population behave and alter their exposure (i.e., dose) through ingestion (e.g., food or water), inhalation (e.g., airborne particles), or dermal contact (e.g., soil).[66] Understanding these potential exposure pathways informs the choice of measurement methods, which vary in precision and cost. Imputed or modeled exposures can reduce costs and limit the burden on study populations, but can introduce measurement errors. Directly measuring environmental exposures—whether through area sampling at fixed locations or personal sampling with portable devices—is more precise but can be costly and may still introduce measurement error (e.g., each study participant's exposure may differ from the monitoring site measurement value). Personal monitoring can place a heavy burden on participants who must carry and care for monitoring equipment and may only be feasible for short periods. Biomarkers of exposure, which measure the amount of a chemical present in the body,[67] involve sampling body fluids or tissues (e.g., blood and hair) and offer the most precise assessment of the internalized dose of an environmental exposure within a specific time window. However, these methods are typically the most expensive.[68]

Beyond these measurement methods, tools such as Geographical Information Systems (GIS) and remote sensing help identify spatial patterns of exposure, including contamination hotspots.[69] These tools also model exposure concentrations between sample sites.[66] In addition to spatial assessments, temporal assessments evaluate whether exposure levels are stable, increasing, or decreasing over time.[69] Risk assessment integrates exposure and toxicity data to assess the likelihood of adverse health effects.[70] Together, these approaches provide a comprehensive evaluation of how, where, and to what extent individuals in a study population are exposed to environmental contaminants and associated health risks. This information is essential for informing public health interventions, shaping regulatory policies, and guiding risk management strategies aimed at reducing exposures—such as to HM—and mitigating its health impacts.

7. HM and lung function

Unlike other organs, the lungs continue their development into adolescence or even early adulthood.[21] During this extended developmental period, the lungs are more susceptible to damage from HM exposure compared to other fully developed organs.[21] This vulnerability is concerning because the lungs are crucial for oxygenating the blood, which is then circulated to nourish all other organs and tissues in the human body.[71] When the lungs are compromised, their ability to transfer oxygen into the bloodstream can be reduced.[71] This reduction in oxygen availability can adversely affect the function of other organs and overall systemic health.[71] Therefore, maintaining optimal lung health is essential not only for respiratory well-being but also for the health of the entire body.

The lungs are a primary target organ for certain HM, particularly As.[72,73] Besides the toxicological effects described above (**section 4**), As disrupts the immune response, epithelial barrier function, and mucociliary function, leading to respiratory issues.[73] These alterations, along with oxidative stress and impaired DNA repair, contribute to the development of respiratory infections and colonization, bronchoconstriction, reduced lung function, and lung cancer.[64,73]

7.1 Previous evidence linking HM exposure with lung function

Studies show that HM exposure can impair lung function, reducing lung volumes and increasing respiratory symptoms.[19–21,52,60,61,74–85] However, children from LMICs living in settings like Tumbes are underrepresented in current evidence, limiting generalizability. Methodological and knowledge gaps—such as the lack of assessment of multiple exposures and our limited understanding of how co-exposures affect health—make it difficult to fully comprehend the impact of HM on lung health, particularly in Latin American children.

For instance, in Mexico, childhood As exposure has been associated with a higher prevalence of restrictive lung patterns.[72] Similarly, in-utero As exposure has been linked to increased mortality from bronchiectasis and lung cancer in adulthood in a Chilean population.[60] Studies from the US[74,81] and Bangladesh[21,61] also reported associations between early-life As exposure and increased respiratory symptoms, as well as reduced lung function in children. However, methodological limitations—like the lack of comprehensive evaluation of co-exposures,[21,60,61,72,74,81] and limited availability of individual-level exposure and health outcome data[60]—reduce the strength and reliability of this evidence. In addition, substantial variability in how As exposure and lung function are measured across studies complicates direct comparison and limits the ability to synthesize findings. Furthermore, differences in geographic, environmental, and demographic characteristics of the study populations reduce the generalizability of existing evidence to populations such as those in Tumbes. These limitations highlight the need to investigate these associations in populations that more closely resemble the children of Tumbes, using standardized methods for exposure and outcome assessment. Another area of uncertainty involves possible sex-based differences in susceptibility to As exposure. While some studies suggest stronger associations among girls,[81] others have found greater effects in boys,[61] highlighting the need for further research to clarify these patterns.

Research on other HM, like Pb, Mn, Cd, and others, has produced mixed results. For example, findings from the PROGRESS cohort in Mexico City,[78] using the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire[86]—a validated tool for assessing asthma and allergic rhinitis symptoms[87,88] —identified a positive association between maternal blood Pb levels and wheezing in school-aged children. Similarly, results from the National Health and Nutrition Examination Survey (NHANES) revealed an inverse association between Pb and Mn and lung function in US children,[19] while a study of Chinese children found an inverse Pb-lung function association.[82] In addition, a study in US children found inverse associations between several metals—including copper (Cu), Mn, vanadium (V), and nickel (Ni)—and lung function.[77] However, many of these studies have failed to explore the HM-lung function association while accounting for the effects of combined exposures. Given that exposure to a single HM in real-world scenarios is unlikely,[89] it is crucial to investigate combined HM effects.

7.2 Exposure to multiple HM and lung function

One of the studies mentioned above reported an inverse association between blood Pb levels and lung function among Chinese children, along with a positive interaction between blood Pb and Cd, suggesting that co-exposure to these HM may result in greater lung toxicity than either metal alone.[82] Recent work has begun to assess the joint effects of HM mixtures using advanced statistical methods. A 2023 cross-sectional US study using quantile-based G-computation found an overall inverse association between a urinary mixture of twelve metals—including As, Pb, and Cd—and lung function.[90] Additionally, two cohort studies—one from Mexico[91] and another from the US[92]—used weighted quantile sum regression (WQSR) to assess perinatal exposure to HM mixtures. In the Mexican study, exposures were measured in deciduous teeth, while the US study relied on maternal urinary biomarkers; both studies found that HM mixtures were associated with decreased lung function in children. However, studies applying mixture-based approaches remain limited, and the populations examined in existing studies likely differ from that of Tumbes, limiting the generalizability of these findings to this setting.

8. HM and anemia

The World Health Organization (WHO) identifies whole blood hemoglobin (Hb) levels as the most reliable indicator of anemia. The Hb threshold for defining anemia varies by age group and gender, being <10.9 g/dL for children <5 years old, <11.4 g/dL for children 5-11 years, <11.9 g/dL for children 12-14 years old and girls ≥15 years old, and <12.9 g/dL for boys ≥15 years old (**Table 2**).[93] Anemia leads to impaired oxygen delivery throughout the body, which can cause irreversible morbidity and mortality in both children and adults.[94,95] In 2021, approximately 25% of the global population had anemia,[95] with a higher prevalence among females (31.2%) than males (17.5%). Children under five years old were the most affected age group, with a prevalence of 41.4%.[95] This condition accounted for an estimated 52 million years lived with disability (YLDs), representing 5.7% of all YLDs that year, with more cases observed in LMICs and lower socioeconomic groups.[95] In Peru, the prevalence of anemia among children 6-25 months old in 2023 was 43.1%,[96] representing a severe public health problem, according to the WHO's classification of anemia (**Table 3**).[97]

Anemia can be classified by erythrocyte size as microcytic (often due to iron deficiency) or macrocytic (often due to vitamin B12 or folic acid deficiencies).[98] It can also be categorized by erythrocyte color as hypochromic, normochromic, or hyperchromic, which helps identify specific causes of anemia, such as hypochromic anemia linked to iron deficiency.[99]

A leading cause of anemia worldwide is iron deficiency,[95] which can result from low iron intake, poor iron absorption in the gastrointestinal tract, and periods of high iron requirement, such as during growth and pregnancy.[97] Other important causes of anemia include hemoglobinopathies, hemolytic anemia, and neglected tropical diseases like malaria, intestinal nematode infections, and schistosomiasis.[95] In addition, emerging evidence suggests that environmental exposure to HM could also be a cause of anemia.[13–18]

HM have been linked to an increased risk of anemia through mechanisms that disrupt Hb synthesis, increase hemolysis, and alter iron metabolism, including reduced iron absorption.[13,47] Common pathways by which various HM induce anemia include the inactivation of enzymes, such as glutathione peroxidase and reductase, ROS generation, oxidative stress, and the weakening of the antioxidant defense system.[47] Specific effects of HM in the hematopoietic system include direct damage to bone marrow, where blood cells originate and differentiate, inhibition of enzymes involved in cell division and maturation, impaired erythrocyte transport, and immune-mediated cell destruction.[13]

Pb, one of the most studied HM in this context,[13,100,101] induces anemia by disrupting heme synthesis.[13] It inhibits key enzymes, such as ALAD and ferrochelatase, which are essential for heme production, thereby impairing erythrocyte formation.[13] Additionally, Pb causes oxidative damage due to the accumulation of aminolaevulinic acid, leading to free radical formation.[102] Pb further compromises the integrity of erythrocyte membranes, making them more fragile,[3] and promotes hemolysis by inhibiting phosphoribosyl transferases.[13]

Arsenic induces oxidative stress and binds to Hb, lowering its concentration and contributing to anemia.[18,103] Although less studied in humans, evidence from animal studies suggests Cd may be associated with anemia through impaired iron absorption in the gut following gastrointestinal exposure,[104] disrupted erythropoiesis,[105] hemolysis from erythrocyte deformities, and renal anemia due to decreased erythropoietin production.[106]

8.1 Ferritin as a mediator

Ferritin, an iron-storage protein, is essential for iron homeostasis and serves as a marker for iron deficiency anemia.[107] Our research in the Tumbes region indicates that children living closer to the Puyango-Tumbes River, a potential source of HM exposures, have lower serum ferritin levels compared to those living farther away.[108] This suggests that HM exposure could disrupt iron metabolism and contribute to anemia. However, the extent to which disrupted iron metabolism explains the prevalence of anemia in the Tumbes region remains unclear.

8.2 Exposure to multiple HM and anemia

As described in **section 4.4**, individuals are often simultaneously exposed to multiple HM, which may lead to more severe health outcomes, including reduced Hb levels and increased risk of anemia.[22] Recent studies have begun to explore these effects using mixture-based analytical approaches. A 2023 study in Uganda using WQS regression found a non-significant inverse association between a blood HM mixture and Hb.[109] A 2024 Chinese study using quantile-based G-computation reported a significant inverse association between a urinary HM mixture and Hb.[110] In contrast, a 2022 study in US adolescents using Bayesian kernel machine regression (BKMR) found a positive association between a blood HM mixture and Hb.[111] Findings from animal studies further support the potential for joint toxicity. For instance, erythrocyte damage was greater in mice exposed to both Cd and Pb through food compared to those exposed to individual metals or unexposed controls.[112] Likewise, combined exposure to multiple HM (Hg, As, Pb, Cd, Mn, Cr, Fe, Ni) has been associated with bone marrow genotoxicity in rat models.[113] These studies underscore the importance of considering co-exposure to HMs when evaluating anemia risk.

9. Equity issues related to HM exposure

LMICs and marginalized communities are disproportionately affected by HM exposure.[22–24,114] Limited resources, weak regulatory frameworks, and inadequate infrastructure in these regions hinder their ability to regulate and minimize toxicant exposures, leading to elevated environmental pollutants.[115] For instance, about 632 million children in LMICs have blood Pb levels above the US Centers for Disease Control and Prevention's (CDC) action level of 5 µg/dL,[116] over 4.5 million people in Latin America are exposed to As levels in water more than 200 times the WHO limit of 10 µg/L,[117] and in more than 50 countries Mn levels in drinking water exceed 400 µg/L.[57] These pollutants, often stemming from poorly regulated agriculture, mining, and industry, cause long-lasting and wide-reaching health impacts.[3]

Additionally, these communities face challenges such as water insecurity, malnutrition, food insecurity, and inadequate healthcare access, which, combined with socioeconomic factors like poverty and low educational attainment, further worsen the effects of HM exposure.[118–120] In many cases, affected communities receive no benefit from the industrial activities responsible for their exposure. For instance, in the Tumbes region of Peru, communities are chronically exposed to HM likely originating from upstream gold-mining operations in Ecuador.[36] Although they are not directly involved in or profiting from mining, they bear the environmental and health burdens. This situation illustrates the unjust and unequal distribution of risks and rewards—where one population benefits economically from gold extraction while another suffers the consequences. Research in marginalized regions like Tumbes is therefore essential to understanding the specific impacts of HM exposure and developing targeted interventions that improve health outcomes and advance environmental justice.

10. Summary of knowledge gaps

Despite the substantial burden of HM exposure and its well-documented health impacts, several knowledge gaps remain regarding its effects on the health status of the Tumbes pediatric population:

- **Health equity:** There is a lack of representation of marginalized communities—particularly in LMICs like Peru—in studies evaluating HM exposure and its health consequences.
- **Combined effects**: Limited research has explored how concurrent exposure to multiple HM impacts anemia and lung function, particularly in pediatric populations.
- **Exposure assessment**: Current measures may not accurately reflect the sources of HM exposure as well as the intensity, frequency, and duration of HM exposure in Tumbes.
- **Lung function:** There is a lack of conclusive evidence on the association between HM and lung function in children.
- **Underlying mechanisms:** The role of ferritin as a potential mediator in the association between HM exposure and anemia remains poorly understood in this setting.

By addressing these gaps, this dissertation seeks to advance the understanding of environmental health risks in the Tumbes population, which may help inform targeted public health interventions.

11. Tables and figures

Table 1. Factors that influence the bioavailability of heavy metals (HM)[3]

Category	Factors	Description
Physical	Temperature, phase association, adsorption, sequestration	Factors like temperature, surface binding, and environmental containment affect HM mobility and availability
Chemical	Speciation, complexation, kinetics, lipid solubility, partition coefficient	Chemical forms of HM, how they bind with other substances, the speed of these interactions, and their solubility in fats versus water
Biological	Species traits, trophic interactions, physiological adaptations	How organisms absorb and process HM, movements trough food chains and biological responses to HM exposure

Table 2. Hemoglobin (Hb) thresholds for anemia by age group and gender according to the World Health Organization[93]

Age group/gender	Hb threshold
Children <5 years	<10.9 g/dL
Children 5–11 years	<11.4 g/dL
Children 12–14 years	<11.9 g/dL
Girls ≥ 15 years	<11.9 g/dL
Boys ≥ 15 years	<12.9 g/dL

Table 3. Classification of anemia as a problem of public health (PH) significance

Prevalence of anemia (%)	Category of PH significance
≤ 4.9	No PH problem
5 – 19.9	Mild PH problem
20 – 39.9	Moderate PH problem
≥ 40	Severe PH problem

Adapted from Worldwide prevalence of anaemia 1993–2005: WHO global database on anaemia[97]

Figure 1 Map of the Peruvian-Ecuadorian border on the Pacific coast showing the Puyango-Tumbes River (blue line), which flows from the Oro region in Ecuador through the Tumbes region in Peru to the Pacific Ocean. The Zaruma-Portovelo area is a hotspot of gold mining operations. Inset: Location of Peru (green) and Ecuador (purple) in South America.

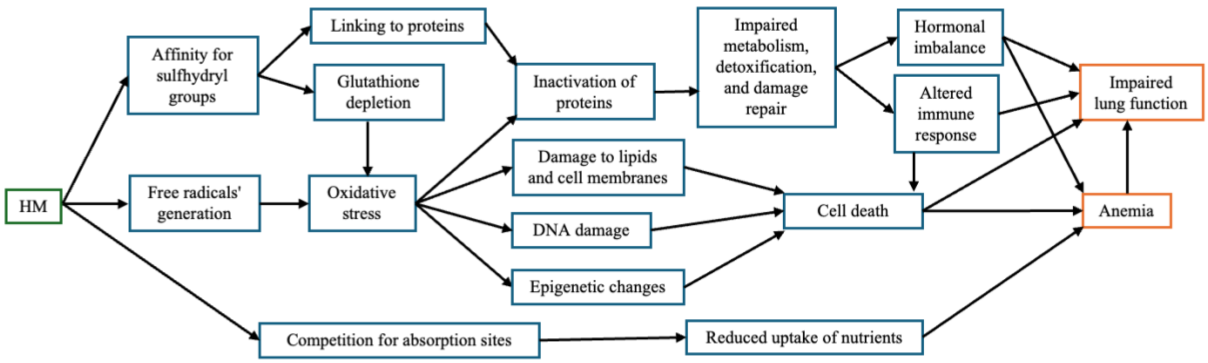

Figure 2. Mechanisms of heavy metal (HM) toxicity. HMs can bind to sulfhydryl groups, inactivating proteins; induce oxidative stress that damages DNA, cells, and tissues; and compete with essential nutrients for absorption sites in the gut. These mechanisms can lead to anemia, impaired respiratory function, and other adverse health effects, particularly in children.

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CHAPTER 3. RESEARCH PAPER #1

Exposure assessment of heavy metals in children living downstream from a gold mining region: the case of Tumbes, Peru

1. ABSTRACT

Background: Children living in the Tumbes region of northern Peru may face elevated exposure to heavy metals (HM) due to contamination of the Puyango-Tumbes River—a transboundary waterway originating in Ecuador and impacted by upstream mining activities. This study aimed to assess environmental and internal HM exposure among children living near the river, identify geographic exposure patterns, and evaluate the association between river proximity and hair HM concentrations.

Methods: We conducted a cross-sectional exposure assessment combining water sampling (n=40) and hair HM analysis (n=404 children aged 4-17 years) across communities in Tumbes. Potential exposure patterns were identified through descriptive analysis and principal component analysis. Spatial maps and regression models were used to examine associations between proximity to the river and hair HM levels.

Results: A substantial proportion of water samples exceeded safety thresholds for As (30%), Pb (17.5%), and Mn (25%), with higher concentrations observed in surface water, water not intended for human consumption, and samples taken closer to the river. Among children, 17.8% had elevated hair As, 26% had elevated Pb, 61.1% had elevated Cd, and 79.7% had elevated Mn levels. Children living closer to the river had substantially higher hair As and Pb concentrations. In adjusted mixed-effects models, the geometric mean of hair As among children near the river was 3.9 times that of children living farther away (95% CI 1.16, 6.73). A similar, though not statistically significant, association was observed for hair Pb (exponentiated coefficient 1.77; 95% CI 0.91, 3.43). Correlation analysis showed modest positive correlations between water and hair concentrations for As and Mn.

Conclusion: Children residing near the Puyango-Tumbes River are exposed to elevated levels of HM, particularly through contaminated surface water. Spatial and statistical analyses support the river as a primary exposure pathway, especially for As, and to a lesser extent, Pb.

2. INTRODUCTION

The burden of heavy metals. Heavy metals (HM) such as arsenic (As), lead (Pb), cadmium (Cd), and manganese (Mn) are widely recognized environmental contaminants linked to severe health effects, including neurodevelopmental disorders, cardiovascular disease, kidney damage, and various forms of cancer.[1–8] In 2015, HM exposure contributed to over 1 million disease cases, more than 50,000 deaths, and over 9 million disability-adjusted life years (DALYs).[9]

Most affected subpopulations. The burden of HM exposure is not evenly distributed. It disproportionately affects low- and middle-income countries (LMICs), where industrial activities such as mining and inadequate environmental regulation increase exposure risks, particularly among populations with limited access to healthcare and prevention.[10–12] Children are especially vulnerable due to their ongoing physiological development, which makes their organs more susceptible to toxic insults.[13–16]

Exposure pathways. Although HM are naturally occurring elements, most human exposure to HM results from anthropogenic sources, including industrial emissions, agricultural runoff, improper waste disposal, and mining operations.[1,17,18] Their capacity to bioaccumulate in organisms makes them particularly hazardous when released into water systems, where they can persist and enter the food chain.[19] This threatens not only nearby populations but also downstream communities that rely on contaminated water for drinking, irrigation, and fishing.

Regional context & knowledge gaps. Tumbes, a region in northern Peru with approximately 260,000 residents,[20] faces substantial socioeconomic challenges, including poor access to basic services.[21] It lies downstream of the Puyango-Tumbes River, a transboundary waterway originating in Ecuador that serves as the main—and sometimes sole—water source for many communities in the region.[22] However, the river is heavily contaminated with HM, including As, Pb, and Cd, often exceeding safety thresholds set by the US Environmental Protection Agency (USEPA).[22,23] Spatial assessments have shown that HM concentrations in river water and sediments peak near gold processing sites in Ecuador and decline downstream, but remain elevated—without returning to baseline levels—even more than 140 km into the Tumbes region.[23] This exposure extends beyond drinking water, as high levels of HM are also found in river fish, rice and crops irrigated with river water, and seafood from areas near the river's mouth,[24] posing chronic exposure risks to populations relying on these sources. Prior studies in the region have reported higher levels of As, Pb, and Mn in the hair of children living near the river compared to children with similar socioeconomic characteristics living in more distant locations, suggesting the Puyango-Tumbes River may be a major source of exposure (Fernandez et. al., in preparation). Yet, the magnitude, spatial distribution, and combined nature of these exposure**s** remain poorly characterized.

Aims and implications. This study addresses these knowledge gaps by conducting a comprehensive exposure assessment of HM in the Tumbes region and evaluating how proximity to the Puyango-Tumbes River is associated with internalized HM levels. It quantifies concentrations of HM in both environmental (water) and human (hair) samples, identifies geographic areas with higher exposure through spatial analysis, and explores exposure patterns. By geospatially linking water quality metrics with individual-, household-, and village-level data, this assessment aims to improve our understanding of real-world, multi-pathway exposures in a region affected by environmental degradation and limited access to basic services and infrastructure. The findings will contribute evidence for identifying at-risk populations and developing public health strategies to reduce HM exposure and its health consequences among children in Tumbes.

3. METHODS

3.1. Study setting and design

We conducted a cross-sectional exposure assessment in the Tumbes province of Peru, targeting HM exposure through drinking water in children under 18 years of age. Fourteen rural villages across four regions were purposively selected based on their distance from the Puyango-Tumbes River to capture varying levels of HM exposure: some located within the river's watershed at a distance of 0.6 to 3.4 km (classified as "close") and others located 20.5 to 27.5 km (classified as "far"). The geographic extent of the study area ranged approximately from 80.56°W to 80.10°W longitude and from 3.80°S to 3.45°S latitude (**Figure 1**).

3.2. Data collection

Children's dataset

Between January and March 2023, the Center for Global Health (CGH) enrolled 409 children as part of this study. Data collection was conducted in participants' homes, and informed consent was obtained before enrollment. This investigation formed part of a broader study examining the association between HM exposure and lung function. Accordingly, eligible participants were children aged ≥4 years or older who were capable of performing forced spirometry[25,26] and willing to provide a hair or blood sample.

CGH personnel collected hair samples, recorded GPS coordinates at the household and village level, and administered a structured questionnaire capturing demographic and behavioral data. To enhance participation, up to three follow-up visits were made if households were unavailable during initial contact. Coordination with local authorities was essential to foster community trust, facilitate site access, and support participant recruitment across the selected villages.

Water Dataset
The regional health authority in Tumbes (Dirección Regional de Salud Tumbes– DIRESAT) routinely collects water samples to monitor quality and safety. Data from 2013-2023 (including GPS coordinates) are publicly available on DIRESAT's website.[27] For this study, we selected samples collected between October and December 2022[28]—a period 1-5 months prior to participant recruitment—that included all HM measured in hair samples (As, Pb, Cd, and Mn) as well as additional elements and water quality parameters. We restricted our analysis to samples collected within the geographical boundaries of the defined study region.

Variables

Children's dataset
Hair HM (As, Pb, Cd, and Mn): Internalized exposure to HM was assessed through hair concentrations of As (µg/kg), Pb (mg/kg), Cd (µg/kg), and Mn (mg/kg). Trained staff from the CGH collected three hair samples (~100 strands each) from the occipital region using stainless steel scissors, cutting as close to the scalp as possible. Two samples were sent to the Elemental Analysis Core Lab at Oregon Health & Science University (OHSU) for analysis, and one was stored at CGH. The proximal 3 cm of hair (~3 months of exposure) was processed.[29] Following standard protocols, samples were washed, then 200 µl of concentrated HNO_3 was added, and samples were heated to 90°C for 45 minutes. After cooling, 1% HNO_3 was added to reach a total volume of 2000 µl, followed by digestion at room temperature for 12 hours. HM levels were measured using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) with Agilent 8900 triple quad equipped with a Sample Preparation System (SPS) autosampler. Data were quantified using weighed, serial dilutions of a multi-element standard for Mn and Pb, and single-element standards for Cd and As, with a 12-point calibration curve. Measurements were performed in triplicate and averaged. Precision was assessed via the coefficient of variation, while internal standards (scandium, germanium, bismuth) corrected for detector fluctuations and monitored plasma stability. Controls and standards, including National Institute of Standards and Technology (NIST) SRM 1643f, NIST SRM 1683f (8x dilution), and Bovine Liver NIST SRM 1577c, ensured accuracy. Recovery for NIST water and bovine liver SRMs ranged from 83-112%, with spikes and repeats within 5% of expected values, indicating robust analytical performance.

Hair HM concentrations were dichotomized as low or high using the following thresholds: As ≥1,000 µg/kg,[30] Pb ≥5 mg/kg,[31] Cd ≥0.1 µg/g,[32] Mn ≥1.1 mg/g.[32]

Other covariates: We collected data on several covariates that may influence HM exposure levels. Demographic information included age (years), gender (male or female), and residence time (years). Socioeconomic status (SES) was assessed through parental occupation (housewife, service sector, agriculture/fishing, construction/mining) and the use of durable housing materials (yes or no). Smoke exposure was characterized by household exposure to secondhand tobacco smoke, use of wood stoves, field burning, and trash burning, each coded as yes or no. Dietary factors included whether children consumed fish from the river (yes or no), and the frequency of consumption of 20 common food items—tuna, chicken, fish, bone marrow, eggs, lentils, rice, spinach, broccoli, tomato, potato, cassava, garlic, onion, banana, beetroot, lemon, orange, carrot, and plantain—coded as frequent (≥3 times per week) or infrequent (<3 times per week). Breastfeeding duration (months) was also recorded. Water consumption patterns were documented, including the use of purified water (yes or no), and the type of water consumed (bottled, tap, well, river, cistern, filtered, or boiled). We also recorded each household's water source (public network, well, river, or other), and the type of water (surface or groundwater) distributed by the public water network. Information on agricultural practices included whether households grew food for self-consumption (yes or no), the kind of product grown, and whether they used river water for irrigation. This variable was categorized as: no cultivation, cultivation using river water, or cultivation without river water. The type of water distributed to households was determined based on DIRESAT's reports on water quality,[27] while CGH personnel recorded age and gender from national identity documents and collected all other data through a questionnaire administered to the participants' parents or guardians.

Water dataset

Water HMs (As, Pb, Cd, Mn, Al, Sb, Ba, Be, B, Cu, Cr, Fe, Mo, Ni, Se, Na, U, and Zn): Environmental exposure was assessed through HM levels in water samples (mg/L), including As, Pb, Cd, Mn, aluminum (Al), antimony (Sb), barium (Ba), beryllium (Be), boron (B), copper (Cu), chromium (Cr), iron (Fe), molybdenum (Mo), nickel (Ni), selenium (Se), sodium (Na), uranium (U), and zinc (Zn). Samples were collected by DIRESAT personnel from multiple points (intake points, water treatment plant entrances, reservoirs, and the public network). Analyses were performed by the Advanced Global Quality Labs Peru using ICP-MS following USEPA protocols.[33] Lower detection limits (LDL) in mg/L were as follows: As=0.00004, Pb=0.00006, Cd=0.00001, Mn=0.00006, Al=0.002, Sb=0.00002, Ba=0.0003, Be=0.00001, B=0.002, Cu=0.0003, Cr=0.001, Fe=0.03, Mo=0.00003, Ni=0.0009, Se=0.00004, Na=not applicable, U=0.00001, and Zn=0.002. Values below these limits were imputed as half the corresponding LDL.

***Water quality indicators**:* Additional water quality parameters included pH, residual chlorine (Cl, mg/L), turbidity (Nephelometric Turbidity Units [NTU]), temperature (°C), conductivity (µmho/cm), altitude (meters above sea level [masl]), total dissolved solids (mg/L), and whether water is intended for human consumption or not (yes or no) all measured by DIRESAT and publicly reported.

Each HM and selected water quality parameters (dissolved solids, turbidity, and electrical conductivity) were classified as normal or high based on whether they exceeded thresholds established in national regulations.[34,35] Residual Cl was categorized as normal or low depending on whether it fell below the recommended level, and pH was classified as normal or abnormal based on whether it fell within the recommended range specified in the same regulations. This classification followed DIRESAT's standard approach, which is based on two national regulations: DS 031-2010-SA[34] and DS 004-2017-MINAM.[35] The former (SA) sets maximum permissible limits for water intended for human consumption and was applied to samples from reservoirs and the public water network. The latter (MINAM) defines environmental quality standards for water that can be made potable—either through disinfection (ECA1) or conventional treatment (ECA2). Surface and groundwater samples from intake points were compared to ECA1, while surface water samples collected at treatment plant entrances were compared to ECA2. Not all parameters were covered by these regulations: SA does not provide a threshold for Be, ECA2 does not include thresholds for Mo and Ni, and neither ECA1 nor ECA2 includes reference values for Na and residual Cl. Values for these parameters were coded as missing when no reference value was available. See **Table S1** for the specific thresholds used.

Linking children's and water datasets

We converted the coordinates of villages and water sampling locations into spatial data and mapped them using open-access vector geospatial layers. Water samples were identified and mapped in relation to village locations. Each child was then linked to the nearest water sample intended for human consumption based on their recorded village of residence (**Figure 2**). All characteristics of the assigned water sample—including HM concentrations and water quality indicators—were attributed to each child, resulting in a merged dataset that integrated individual- and environmental-level exposure information.

3.3. Statistical analysis

Descriptive analysis, missing data, and transformations

We began with separate descriptive analyses of the children's and water datasets. Continuous variables were summarized as means and standard deviations (SD) or medians and interquartile ranges (IQR), as appropriate; categorical variables were summarized as frequencies.

In the children's dataset, participants with missing values on hair HM were excluded. In the water dataset, samples with missing data were also excluded from the analysis, and variables with more than 20% missingness were excluded from multivariate analyses but included in descriptive summaries. To address right-skewed distributions and enhance comparability with other studies, HM levels were log-transformed (natural logarithm) in both datasets. Distributions of transformed water and hair HMs are shown in **Figures S1** and **S2**.

Spatial visualization

We created a series of spatial maps to visualize HM exposure patterns in both hair and water samples. First, we generated four maps of water sampling locations—one for each metal (As, Pb, Cd, and Mn)—indicating whether concentrations exceeded recommended thresholds. Next, we produced four maps displaying household locations coded by participants' hair HM levels (high or low), with each map corresponding to one metal. To assess spatial overlap between environmental and internalized exposure, we created four composite maps—again, one per metal—showing both water and household locations. These overlays allowed for visual examination of the proximity between children with elevated hair HM levels and nearby water sources with elevated HM concentrations.

Univariate comparisons

In the water dataset, we examined the distribution of HM concentrations and other water metrics by water quality (for human consumption, yes/no), water origin (surface/groundwater), and distance from the Puyango-Tumbes River (close/far). Likewise, we compared the distribution of variables in the children's dataset by household distance to the river (close/far). Additionally, we evaluated the distribution of water HM by agricultural water use category (no self-production, self-production without river water, self-production with river water) in the merged dataset.

Correlation between water and hair HM concentrations

Using the merged dataset, we calculated Spearman coefficients between each HM in hair and the corresponding concentration in the nearest water sample for human consumption.

Association models: Distance to river and hair HM

To test whether hair HM concentrations differed by proximity to the Puyango-Tumbes River, we fit crude and adjusted linear regression models using the children's dataset. The exposure variable was proximity to the river (far or close), and the outcomes were log-transformed concentrations of hair As, Pb, Cd, and Mn. Potential confounders were identified based on the literature and a directed acyclic graph (DAG) (**Figure 3**) and included parental occupation and housing materials as proxies for SES. Crude and adjusted beta coefficients and their 95% CI were estimated and exponentiated to reflect the change in the geometric mean of a given hair HM associated with living close to the river. To account for clustering within villages, models included village-level random intercepts. Model fit was assessed using likelihood ratio tests (LRTs) and the Akaike Information Criterion (AIC), while clustering was evaluated using the intraclass correlation coefficient (ICC). Mixed-effects models were retained when the LRT P-value was <0.05 or the ICC exceeded 10%.

Principal component analysis (PCA)

We performed PCA on the merged dataset to reduce dimensionality and identify patterns of HM exposure that may reflect shared sources and help elucidate pathways through which waterborne HM reach the human body. Variables included in the PCA were water concentrations of HM, water quality indicators (pH, temperature, dissolved solids, and residual Cl), dietary factors (frequent consumption of 20 food items, fish consumption from the river, and breastfeeding duration), drinking water source, household's water supply, and agricultural practices. Variable categorization is described above. Categorical variables were converted to dummy variables, and all variables were standardized. We used a scree plot and parallel analysis to determine the number of components to retain and applied oblique rotation to account for potential correlations among components. Component loadings were examined to interpret grouped variables. Pairwise correlations among variables were visualized using a correlation matrix. Our sample size (n=404) exceeded the minimum threshold of 50 recommended for recovering principal components.[36]

Model comparison using PCA-derived components

To explore whether PCA-derived scores improved the prediction of hair HM concentrations, we added them to the adjusted association models described above and compared model fit using AIC. Lower AIC values indicated better model performance.

Ethics statement

This study was approved by the Institutional Ethics Committee for Humans at the Universidad Peruana Cayetano Heredia and the Institutional Review Board at OHSU.

4. RESULTS

Descriptive analysis

Water dataset: Forty samples met the inclusion criteria. Of these, 34 (85%) were intended for human consumption, most of which were collected from reservoirs (n = 29), with smaller numbers from households (n = 3) and other water treatment plant sites (n = 2). The remaining 6 samples (15%), not intended for human consumption, were all taken from intake points at water treatment plants. Twenty-six samples (65%) were groundwater, and 14 (35%) were surface water. Median (IQR) concentrations of HM were as follows: As 0.003 (0.001, 0.02) mg/L, Pb 0.00003 (0.00003, 0.0032) mg/L, Cd 0.000005 (0.000005, 0.0002) mg/L, and Mn 0.08 (0.013, 0.315) mg/L. Compared to reference limits, 12 samples (30%) exceeded the limit for As, 7 (17.5%) for Pb, 2 (5%) for Cd, and 10 (25%) for Mn. Descriptive summaries of this and other water sample characteristics are presented in **Table 1**. Data on residual Cl and Na were not collected for the 6 samples not intended for human consumption, as these parameters are not included in the corresponding Peruvian standard. Similarly, Be is not included in the standard for water intended for human consumption, resulting in data for only 6 samples (85% missing). In addition, altitude data were missing for 18 samples (45%). These two variables were excluded from further analysis.

Children's dataset: A total of 409 children met the inclusion criteria and consented to participate in the study. Of these, 5 were excluded due to missing hair HM data, resulting in an analytical sample of 404 children. The participants were 52.7% female, with a mean age of 10.2 years (SD 3.9). On average, children had lived in their current residence for 9.6 years (SD 4.2). Median concentrations (IQR) of hair HM were 291.5 (94.8, 729.0) μg/kg for As, 1.9 (0.6, 5.3) mg/kg for Pb, 152.5 (51.8, 376.5) μg/kg for Cd, and 4.5 (1.5, 12.3) mg/kg for Mn. When compared to reference thresholds, elevated levels were observed in 72 children (17.8%) for As, 105 (26%) for Pb, 247 (61.1%) for Cd, and 322 (79.7%) for Mn.

Spatial visualization

Figures 4-7 show the overlaid geographic distribution of water and hair samples, highlighting those that exceed reference limits. A greater proportion of elevated As, Pb, Cd, and Mn concentrations in water was observed within the Puyango-Tumbes watershed compared to more distant areas. For hair samples, a higher proportion of children living near the river had As and Pb concentrations above recommended limits compared to those living farther away. In contrast, no clear geographic pattern was observed for hair Cd and Mn. Several participants with elevated levels of hair As, Pb, and Cd resided near water sources with high concentrations of the same metals, whereas those with hair levels within reference ranges were generally located farther from these high-concentration water sources.

Univariate comparisons

Water for human consumption. **Table 2** presents the distribution of water quality parameters by whether the sample was intended for human consumption. Overall, water not intended for human consumption had markedly higher concentrations of all measured chemical elements—except boron—compared to water for human consumption. However, levels of HM in water intended for human consumption were still high in some samples, with 20.6% exceeding the threshold for As, 5.9% for Pb, 2.9% for Cd, and 14.7% for Mn. Water not intended for human consumption had substantially higher turbidity levels, but lower concentrations of dissolved solids and lower conductivity. All non-consumption water samples were collected from intake points at surface water treatment plants. In contrast, the majority (74%) of water samples intended for human consumption were taken from groundwater.

Surface vs. groundwater. **Table 3** summarizes the characteristics of water samples by origin—groundwater or surface water. Surface water samples exhibited consistently higher concentrations of most chemical elements, except for B and Na, often exceeding recommended levels. Surface water also had lower levels of dissolved solids, conductivity, and temperature, but higher turbidity. Residual Cl levels were higher in surface water compared to groundwater. Fifty-seven percent of surface water samples were intended for human consumption.

Distance from the Puyango-Tumbes River. Water samples collected from areas closer to the Puyango-Tumbes River had substantially higher median concentrations of several HM, including As, Pb, Mn, and Fe (**Table 4**). Consistent with spatial visualizations, a greater proportion of these samples exceeded recommended safety thresholds for As, Pb, Cd, and Mn. Turbidity was also elevated in samples near the river, indicating poorer water quality.

We also examined children's characteristics by proximity to the Puyango-Tumbes River (**Table 5**). Children living closer to the river had higher mean concentrations of As (1160 µg/kg vs. 273 µg/kg) and Pb (7.71 mg/kg vs. 3.02 mg/kg) in hair compared to those living farther away. Cd and Mn levels were more evenly distributed between groups. A larger proportion of children living farther from the river came from families engaged in fishing or agriculture (87% vs. 53%) and reported food cultivation (84% vs. 49%). The most commonly cultivated crops farther from the river were banana (79%), lemon (42.7%), and plantain (25.2%), whereas those closer to the river more frequently reported growing lemon (72.6%), banana (48.7%), and cassava (33.6%). Growing rice for self-consumption was reported 11 times among participants living farther from the river and twice among those living closer. Second-hand tobacco smoke exposure was more common among children living closer to the river (15% vs. 2%), whereas field burning for cultivation was more frequently reported among those living farther away (70% vs. 38%). Households closer to the river more often used wells as a water source (25% vs. 13%). Dietary patterns were generally similar across groups, though children living farther from the river reported a more varied diet, with higher consumption of spinach, broccoli, beetroot, tomato, and cassava. In contrast, fish consumption from the river was more common among those living closer. Finally, a higher proportion of families living closer to the river reported drinking tap water (63.7% vs. 43.3%), while a lower proportion reported using safer drinking water sources such as bottled water (16.3% vs. 29.9%).

Agricultural practices. **Table 6** compares the median (IQR) concentrations of chemical elements in the nearest water samples linked to 404 children in Tumbes, Peru, according to household agricultural practices and the type of irrigation water used. Households that used river water for irrigation had the highest median As concentrations (0.027 mg/L), compared to those that cultivated with non-river water

(0.019 mg/L) or did not cultivate crops (0.003 mg/L), suggesting a potential link between river water use and As exposure. For other elements—such as Al, Se, and Mo—concentrations were also highest in households engaged in cultivation, regardless of the irrigation source, compared to those not cultivating. In contrast, concentrations of Mn were higher among households not involved in cultivation. Many elements, including Pb, Cd, Cr, Ni, and Zn, showed little to no variation across groups.

Correlation between water and hair HM concentrations
Scatterplot graphs (**Figure 8**) suggested that hair concentrations of hair ln-As, ln-Pb, and ln-Mn increased with rising levels of the same metals in water. Spearman correlation coefficients were 0.291 for As, 0.089 for Pb, -0.011 for Cd, and 0.236 for Mn. A full matrix of correlation between water and hair HM concentrations is provided in **Figure 9**.

Association models: distance to river and hair HM
A visual inspection (**Figure 10)** revealed higher hair ln-As and ln-Pb levels among children living close to the river compared to those living in more distant locations. In mixed-effects models, the geometric mean of hair ln-As concentration for children living close to the Puyango-Tumbes River was 3.58 times (95% CI 2.11, 6.07) the geometric mean for those living farther away. This association retained its direction, magnitude, and statistical significance after adjusting for confounders (exponentiated coefficient 3.97, 95% CI 2.31, 6.83). We also observed higher hair ln-Pb levels among children living close to the river, with an adjusted exponentiated coefficient of 1.77 (95% CI: 0.91, 3.43); however, this association was not statistically significant. Conversely, hair levels of ln-Cd and ln-Mn were lower among children living closer to the river, but these associations were not significant (**Table 7**).

Principal component analysis (PCA)
Based on scree plots and parallel analysis (**Figure 11**), we determined that the ideal number of components to retain from the oblique PCA was nine, which together explained 63% of the total variance. **Figure 12** shows the variables loading onto each component. The first component reflected general water mineral content and water quality, while the second captured toxic metal and particulate contamination in water. The third component primarily represented staple diet, the fourth described water access and water safety practices, and the fifth reflected leafy and root vegetable intake. **Table 8** provides a full list of the variables loading on each component, interpretations of the identified patterns, and the proportion of variance explained. **Figure S3** presents a correlation matrix showing pairwise correlations among the studied variables, while **Figure S4** illustrates the direction and relative contribution of variable loadings for the first two components.

Model comparison using PCA-derived components
The addition of PCA scores to the models that evaluated the association between distance from the river and hair HMs did not improve model fit for hair As, Pb, or Cd, as evidenced by higher AIC values in models that included PCA scores compared to those that did not (As: 1445.9 vs. 1443.4; Pb: 1509.9 vs. 1506.8; Cd: 1523.4 vs. 1518.9). For Mn, the model including PCA scores showed a negligible improvement in fit (AIC: 1476.4 vs. 1476.9), suggesting that the added complexity offered minimal predictive value.

5. DISCUSSION

5.1. Key findings
A considerable proportion of water samples exceeded recommended safety thresholds for As, Pb, and Mn, with higher concentrations generally observed in surface water and in samples not intended for human consumption. Hair As, Pb, Cd, and Mn concentrations in children's hair were also elevated. Spatial analyses revealed that water As, Pb, Cd, and Mn concentrations were higher in areas closer to the

Puyango-Tumbes River, while hair As and Pb levels were elevated among children living near the river—suggesting the river as a likely source of environmental exposure. Adjusted mixed-effects models confirmed a statistically significant association between proximity to the river and hair ln-As levels, and a non-significant but positive association for hair ln-Pb. Correlation analysis demonstrated a modest positive correlation between water and hair concentrations for As and Mn, further supporting water as a potential exposure pathway. PCA identified distinct exposure patterns, separating components related to water quality and dietary behaviors. Taken together, these findings suggest that the Puyango-Tumbes River may be an important source of HM exposure, particularly As, in this population.

5.2. Interpretation of the results and consistency with other studies

Water characteristics

Heavy metals. The concentrations of HM in surface water samples from our study are consistent with those reported in a previous study that collected water samples between 2012 and 2014 at various locations along the Puyango-Tumbes River, including the Tumbes region.[23] In particular, As concentrations during the wet season—the same season in which our samples were collected—were similar, ranging from approximately 0.005 to 0.1 mg/L (5–100 µg/L). In our study, the median As concentration was 0.028 mg/L (IQR: 0.02, 0.13 mg/L). In contrast, the previously reported levels of Pb and Cd were higher than those observed in our study, with approximate ranges of 0.1–0.3 mg/L (100–300 µg/L) for Pb and 0.001–0.01 mg/L (1–10 µg/L) for Cd. In comparison, our median concentrations were 0.0126 mg/L for Pb (IQR: 0.003, 0.26) and 0.0006 mg/L for Cd (IQR: 0.0002, 0.0026). The prior study, however, analyzed water samples taken directly from natural water bodies, whereas our samples were primarily collected from reservoirs containing treated water for human consumption, which likely explains the differences.

A study that collected water samples from irrigation canals on the west (left) margin of the Tumbes watershed between March and July 2018 reported median concentrations of As (0.012 mg/L; IQR: 0.011–0.021), Pb (0.022 mg/L; IQR: 0.014–0.060), Cd (0.019 mg/L; IQR: 0.014–0.019), Mn (0.031 mg/L; IQR: 0.020–0.034), Cr (0.014 mg/L; IQR: 0.014–0.014), and Zn (0.027 mg/L; IQR: 0.026–0.031).[37] In comparison, water samples collected near the Puyango-Tumbes River in our study showed similar median As concentrations (0.010 mg/L; IQR: 0.00002–0.266), but lower median levels of Pb (0.0003 mg/L; IQR: 0.00003–0.806), Cd (0.000005 mg/L; IQR: 0.000005–0.007), Cr (0.0005 mg/L; IQR: 0.0005–0.013), and Zn (0.008 mg/L; IQR: 0.001–1.16), and a higher median concentration of Mn (0.088 mg/L; IQR: 0.00003–1.71). These differences may reflect distinct water use and treatment: the previous study sampled irrigation canals likely receiving little or no treatment, while most of our samples were taken from reservoirs containing water intended for human consumption, often subjected to some level of treatment or filtration.

Water for human consumption. Water not intended for human consumption had consistently higher HM concentrations and poorer quality indicators, such as turbidity, compared to water for human use. This aligns with our expectations, as water for human consumption typically undergoes more treatment and, in our study, was more often sourced from groundwater, which is less exposed to surface contaminants.

Surface vs. groundwater. Our finding that surface water samples had higher concentrations of most other chemical elements than groundwater contrasts with studies from other regions of Peru. For example, a 2014 study in central and southern Peru reported that 86% of groundwater samples exceeded the 0.01 mg/L As threshold, compared to 50% of surface water samples.[38] In contrast, 86% of surface water samples in our study exceeded this threshold, while none of the groundwater samples did. Similarly, a 2019–2020 study in the Rimac River basin found higher levels of HM and other chemicals (Al, As, Ba, B, Cd, Cu, Fe, Mn, Pb, and Zn) in groundwater, whereas we observed consistently higher concentrations in surface water.[39] A likely explanation for these discrepancies is the presence of upstream gold mining operations in Ecuador, which discharge contaminated residues into the Puyango-Tumbes River, elevating

HM levels in surface water. Meanwhile, groundwater in our study area may be less affected due to natural geological barriers or limited contaminant infiltration. Future research should examine hydrogeological characteristics, soil permeability, and the depth of aquifers in the region to better understand why groundwater in Tumbes appears less contaminated than surface water and to determine whether this protective pattern holds across seasons and broader geographic areas.

Hair HM

Hair As concentrations in our study (median: 866 µg/kg; SD: 1,850) are broadly consistent with those reported in a 2003–2004 Bolivian study (n=78) conducted among individuals 11 to 56 years living near mining sites (range: 37–2,110 µg/kg).[40] This suggests that the children in Tumbes are experiencing exposure levels comparable to those in mining-affected populations. Regarding Pb, a 2010 study in Uruguay (n=180) among children 5–37 months living in an urban setting reported a higher median hair Pb level (13.7 mg/kg) compared to our sample (1.9 mg/kg).[41] Conversely, a 2012 study in Pakistani children aged 6-60 months (n=216) found lower Pb levels (mean: 0.16 mg/kg vs. 6.16 mg/kg in our study).[42] These discrepancies may reflect variances in age (4-17 years in our study), settings (urban vs. rural), exposure sources (air and soil vs. water pollution), and geographic locations. Finally, our mean hair Mn level (13.9 mg/kg; SD: 38.1) exceeded levels reported in a 2015 study (n=93) on the Ecuadorian side of the Puyango-Tumbes watershed (1–7.5 mg/kg),[43] suggesting greater exposure in Tumbes.

HM by proximity to the river

Spatial visualizations and descriptive analyses provided evidence of a geographic gradient in HM exposure. Elevated concentrations of As, Pb, Cd, and Mn in water were more frequently observed within the Puyango-Tumbes watershed, suggesting a regional pattern of contamination that could be influenced by upstream mining activities. Importantly, children living closer to the river were more likely to have higher hair As and Pb concentrations. This was reflected in a statistically significant positive association between proximity to the river and hair ln-As and a non-significant positive association with ln-Pb in mixed-effects models. These findings align with previous research conducted in the Tumbes region (n=264) among children 6 to 59 months old (Fernandez et. al., in preparation), which compared hair HM levels in a district adjacent to the Tumbes-Puyango River, with those in a district located over 50 km away. That study found significantly higher median levels of hair As (400 µg/kg vs. 60 µg/kg, $P<0.001$) and Pb (2.8 mg/kg vs. 1.3 mg/kg, $P<0.001$) in the river-adjacent district. These patterns underscore the need to prioritize river-adjacent communities for exposure mitigation and environmental monitoring.

Correlation between water and hair HM concentrations

Correlation analyses showed a modest positive correlation between water and hair concentrations of As (Spearman's $\rho = 0.291$) and Mn ($\rho = 0.236$), and a weak correlation for Pb ($\rho = 0.089$), suggesting water may be a relevant exposure pathway for these metals in children. However, these results highlight that not all hair-metal levels directly reflect water concentrations, likely due to differences in metal-specific toxicokinetics, and alternative exposure routes (e.g., diet, air, soil), and potential home water treatment practices, such as filtering water. Still, the findings support water as a likely contributor to internal exposure, especially in communities using untreated or minimally treated water.

HM and agricultural practices

Households using river water for cultivation had the highest median As concentrations in nearby water samples, suggesting that agriculture involving river water for irrigation may be a key pathway of As exposure—through both contaminated produce ingestion and household use of irrigation water. Most participants reported growing bananas, and previous studies from the region have identified this crop as a potential source of both As and Pb.[44] This highlights the importance of future research to evaluate bananas as a possible source of HM exposure in the region.

Rice is another area of concern because rice plants can absorb and transfer As into the grain,[45] creating a dietary risk. Although only a small number of participants reported growing rice for personal self-consumption, it is the main crop in Tumbes, representing 60.5% of the region's agricultural production and 67.2% of cultivated land from 2008 to 2017.[46] Rice consumption is also nearly universal, with 99% of participants reporting frequent intake. This makes exposure to As through rice a plausible and possibly widespread route in this population. Future studies are needed to assess the extent of this exposure.

PCA

PCA identified distinct water-related exposure patterns, with PC1 reflecting general water mineral content and quality, and PC2 capturing a cluster of toxic metals (e.g., As, Pb, Cd, Cr, Ni) and turbidity—suggesting contamination likely tied to anthropogenic sources such as upstream mining. Other components reflected dietary habits, water use behaviors, and agricultural practices. These results provide additional insight into how various exposures co-occur and may interact within this population. We incorporated the PCA-derived scores into regression models to evaluate whether they could better explain variation in hair HM levels. While the PCA captured a more comprehensive exposure profile—combining water quality parameters (e.g., HM, pH, turbidity), household water sources, dietary patterns, and food cultivation practices—it did not improve model fit. This suggests that, in this context, proximity to the river remained a more effective predictor of internalized HM exposure than the composite PCA-based approach. Future steps could include the application of more advanced methods, such as the least absolute shrinkage and selection operator (LASSO) or elastic net regression, to better model complex exposure patterns and identify key predictors of internalized exposure.

5.3. Limitations and strengths.

Limitations

Temporality. Hair samples were collected exclusively during the rainy season in Tumbes, a period that may elevate HM exposure due to increased rainfall, runoff, and changes in water sources. Although hair reflects roughly three months of exposure and smooths short-term fluctuations, it may not capture seasonal variations. Thus, measured HM levels may represent a worst-case scenario, with limited generalizability to drier seasons. To improve temporal alignment, water samples were restricted to the 1–5 months preceding hair collection.

Measurement error. Hair was used to assess internal HM exposure due to its practical advantages: it is non-invasive, easy to store and transport, and reflects longer-term exposure.[47] Hair binds As effectively and is particularly informative in populations with high fish or seafood intake, where elevated levels of non-toxic organic As are common.[48–51] Hair also excretes a small portion of body Pb, making it a viable alternative to blood or urine for Pb assessment.[49] However, its utility for measuring internalized Cd and Mn is limited.[29,49] External contamination from environmental sources or cosmetic products can affect hair HM levels, [29,47,48] especially in settings like Tumbes, where access to washing facilities is limited,[21] potentially leading to overestimation of the exposure. To address this, we restricted our analysis to the 3 proximal centimeters of hair and employed a standardized hair washing protocol. Nevertheless, some residual contamination may remain, and the extent of overestimation is uncertain.

Unmeasured variables. We did not directly measure socioeconomic status (SES), which can influence HM exposure through differences in water access, diet, or housing. As a proxy, we used parents' occupation and housing materials. Reports from the local government suggest income is relatively homogeneous in the region,[21] which may limit variation in SES. However, if unmeasured SES differences exist, they could affect exposure estimates. We also did not assess participants' own smoking status. While unlikely among younger children, older participants may have smoked. Still, national data from 2023 show that only 1.3% of Peruvians aged 15 and older reported daily smoking,[52] suggesting a limited influence on overall HM levels.

Strengths
A key strength of this study is its comprehensive assessment of HM exposure using multiple data sources and methods. We applied standardized protocols for hair HM measurement and collected extensive information on water quality, behaviors, demographics, diet, and hygiene practices. By combining geographic tools (e.g., spatial mapping) with statistical models, we captured different dimensions of exposure and strengthened the overall evaluation.

Generalizability
This study's findings are most relevant to children residing in the Tumbes region during the rainy season. The results may also apply to similar rural, non-mining communities in northern Peru that share environmental and socioeconomic conditions. However, the extent to which these findings apply to adults, urban populations, or groups with different dietary habits, healthcare access, or exposure pathways (e.g., less reliance on surface water) is limited.

Implications
Our findings underscore the need for environmental monitoring and regulation in regions affected by upstream mining activity, particularly along the Puyango-Tumbes River. The spatial gradient observed in water and hair HM levels points to surface water—and specifically the Puyango Tumbes River—as an important pathway for exposure, especially for As and Pb. Public health strategies should prioritize reducing exposure in river-adjacent communities through targeted interventions for at-risk populations, such as improved water treatment and agricultural guidance. Longitudinal studies are also needed to monitor health impacts over time and to evaluate the effectiveness of mitigation efforts. These findings further emphasize the importance of regional cooperation between Peru and Ecuador to address transboundary environmental pollution and protect child health.

Conclusion
This exposure assessment provides evidence that children living near the Puyango-Tumbes River in northern Peru are exposed to elevated levels of HM, likely due to contamination of surface water sources. A substantial proportion of water samples exceeded national safety thresholds, and hair As and Pb concentrations in children were higher in communities located near the river. Spatial and statistical analyses support the river as a primary exposure pathway—particularly for As, and to a lesser extent, Pb—likely driven by upstream anthropogenic activities such as mining. The findings also highlight agricultural practices, such as the use of river water for irrigation, as a potential source of HM exposure, especially for As. These findings underscore the need for targeted public health interventions, improved water treatment, and environmental regulations to mitigate exposure risks. However, effectively protecting this vulnerable population will require coordinated, cross-border efforts to monitor and manage HM contamination throughout the Puyango-Tumbes watershed.

6. TABLES AND FIGURES

Table 1. Characteristics of water samples in Tumbes, Peru (October-December 2022)				
Variable	**n**	**Mean (SD)**	**Median (IQR)**	**Outside reference limits n (%)**
Arsenic (mg/L)	40	0.030 (0.063)	0.003 (0.001, 0.020)	12 (30.0)
Lead (mg/L)	40	0.062 (0.169)	0.00003 (0.00003, 0.0032)	7 (17.5)
Cadmium (mg/L)	40	0.001 (0.002)	0.00001 (0.00001, 0.0002)	2 (5.0)
Manganese (mg/L)	40	0.287 (0.454)	0.080 (0.013, 0.315)	10 (25.0)
Aluminum (mg/L)	40	0.579 (1.404)	0.001 (0.001, 0.108)	5 (12.5)
Antimony (mg/L)	40	0.005 (0.008)	0.00001 (0.00001, 0.012)	2 (5.0)
Barium (mg/L)	40	0.070 (0.058)	0.052 (0.035, 0.084)	0 (0.0)
Beryllium (mg/L)	6	0.000005 (0.000005)	0.000005 (0.000005, 0.000005)	0 (0.0)
Boron (mg/L)	40	0.187 (0.182)	0.128 (0.084, 0.227)	0 (0.0)
Copper (mg/L)	40	0.052 (0.136)	0.001 (0.0001, 0.015)	0 (0.0)
Chromium (mg/L)	40	0.002 (0.003)	0.001 (0.001, 0.001)	0 (0.0)
Iron (mg/L)	40	1.224 (2.984)	0.094 (0.027, 0.331)	11 (27.5)
Molybdenum (mg/L)	40	0.001 (0.001)	0.001 (0.001, 0.002)	0 (0.0)*
Nickel (mg/L)	40	0.002 (0.002)	0.0004 (0.000, 0.003)	0 (0.0)*
Selenium (mg/L)	40	0.002 (0.003)	0.002 (0.001, 0.004)	0 (0.0)
Sodium (mg/L)	34	123.4 (116.0)	85.0 (44.3, 167.5)	8 (23.5)
Uranium (mg/L)	40	0.0004 (0.0012)	0.000005 (0.000005, 0.0004)	0 (0.0)
Zinc (mg/L)	40	0.084 (0.230)	0.001 (0.001, 0.018)	0 (0.0)
Dissolved solids	40	501.0 (414.1)	401.0 (178.5, 668.3)	4 (10.0)
Temperature (°C)	40	28.4 (2.5)	28.0 (27.0, 29.0)	NA
pH	40	7.2 (0.4)	7.1 (6.9, 7.6)	1 (2.5)
Turbidity (NTU)	40	81.0 (245.1)	1.3 (0.6, 6.7)	12 (30.0)
Conductivity (µmho/cm)	40	934.3 (735.4)	690.5 (339.5, 1337.0)	9 (22.5)
Residual Cl (mg/L)	34	0.77 (0.78)	0.57 (0.09, 1.25)	16 (47.1)
Altitude (masl)	22	44 (26)	38 (25, 60)	NA

SD: standard deviation; IQR: Interquartile range; NTU: Nephelometric turbidity units, Cl: Chloride, masl: meters above sea level

* The denominator for this calculation was 35, corresponding to samples with available reference values based on Peruvian standards for water for human consumption (LMP DS 031-2010-SA) and for waters that can be made potable through disinfection (D.S. N° 004-2017-MINAM)

Table 2. Characteristics of 40 water samples in Tumbes, Peru (October-December 2022) by water for human consumption expressed as medians (IQR) or counts (%)		
	For human consumption (n=34)	**Not for human consumption** (n=6)
High arsenic	7 (20.6)	5 (83.3)
High lead	2 (5.9)	5 (83.3)
High cadmium	1 (2.9)	1 (16.7)
High manganese	5 (14.7)	5 (83.3)
Arsenic (mg/L)	0.003 (0.001, 0.006)	0.127 (0.104, 0.168)
Lead (mg/L)	0.00003 (0.00003, 0.00003)	0.248 (0.185, 0.364)
Cadmium (mg/L)	0.000005 (0.000005, 0.000005)	0.0024 (0.0018, 0.0031)
Manganese (mg/L)	0.046 (0.010, 0.144)	0.562 (0.525, 0.971)
Aluminum (mg/L)	0.001 (0.001, 0.033)	2.97 (2.04, 3.27)
Antimuonium (mg/L)	0.00001 (0.00001, 0.0004)	0.0163 (0.0123, 0.0191)
Barium (mg/L)	0.048 (0.032, 0.065)	0.087 (0.075, 0.110)
Boron (mg/L)	0.148 (0.088, 0.244)	0.076 (0.069, 0.114)
Copper (mg/L)	0.0006 (0.0002, 0.0139)	0.197 (0.151, 0.244)
Chromium (mg/L)	0.0005 (0.0005, 0.0005)	0.007 (0.005, 0.009)
Iron (mg/L)	0.064 (0.015, 0.153)	7.29 (5.03, 8.23)
Molybdenum (mg/L)	0.0012 (0.0008, 0.0019)	0.0021 (0.0017, 0.0023)
Nickel (mg/L)	0.0005 (0.0005, 0.0012)	0.0032 (0.0024, 0.0053)
Selenium (mg/L)	0.0006 (0.00002, 0.0029)	0.0042 (0.0033, 0.0043)
Sodium (mg/L)	85 (44.3, 168)	NA
Uranium (mg/L)	0.000005 (0.000005, 0.00051)	0.000005 (0.000005, 0.000005)
Zinc (mg/L)	0.001 (0.001, 0.009)	0.307 (0.061, 0.390)
Dissolved solids (mg/L)	460 (241, 743)	162 (115, 223)
Temperature (°C)	28.0 (27.0, 29.8)	27.5 (27.0, 28.8)
pH	7.14 (6.92, 7.62)	7.25 (6.97, 7.52)
Turbidity (NTU)	0.8 (0.6, 3.0)	269 (175, 358)
Conductivity (µmho/cm)	900 (406, 1490)	288 (192, 447)
Residual Chlorine (mg/L)	0.57 (0.09, 1.25)	NA
Water origin		
Groundwater	26 (76.6)	0 (0)
Surface	8 (23.5)	6 (100)
Sampling point		
Household	3 (8.8)	0 (0)
Outlet point	1 (2.9)	0 (0)
Plant	1 (2.9)	0 (0)
Reservoir	29 (85.3)	0 (0)
Intake point	0 (0)	6 (100)
IQR: Interquartile range; NTU: Nephelometric turbidity units; NA: Not applicable		

Table 3. Characteristics of 40 water samples in Tumbes, Peru (October-December 2022) by water origin expressed as medians (IQR) or counts (%)		
	Groundwater (n=26)	**Surface water** (n=14)
High arsenic	0 (0)	12 (85.7)
High lead	0 (0)	7 (50.0)
High cadmium	0 (0)	2 (14.3)
High manganese	4 (15.4)	6 (42.9)
Arsenic (mg/L)	0.001 (0.001, 0.003)	0.028 (0.020, 0.129)
Lead (mg/L)	0.00003 (0.00003, 0.00003)	0.0126 (0.0032, 0.258)
Cadmium (mg/L)	0.000005 (0.000005, 0.000005)	0.0006 (0.0002, 0.0026)
Manganese (mg/L)	0.028 (0.005, 0.144)	0.219 (0.077, 0.577)
Aluminum (mg/L)	0.001 (0.001, 0.001)	0.324 (0.108, 2.99)
Antimuonium (mg/L)	0.00001 (0.00001, 0.00001)	0.0163 (0.0115, 0.0192)
Barium (mg/L)	0.0474 (0.0315, 0.0778)	0.0522 (0.0464, 0.0872)
Boron (mg/L)	0.165 (0.108, 0.286)	0.076 (0.050, 0.098)
Copper (mg/L)	0.0002 (0.0002, 0.0007)	0.0151 (0.0120, 0.204)
Chromium (mg/L)	0.0005 (0.0005, 0.0005)	0.0005 (0.0005, 0.007)
Iron (mg/L)	0.054 (0.015, 0.145)	0.556 (0.139, 6.48)
Molybdenum (mg/L)	0.0009 (0.0005, 0.0018)	0.0018 (0.0014, 0.0021)
Nickel (mg/L)	0.0005 (0.0005, 0.0005)	0.0020 (0.0005, 0.0033)
Selenium (mg/L)	0.00002 (0.00002, 0.0015)	0.0042 (0.0032, 0.0050)
Sodium (mg/L)	122 (77.3, 242)	17.0 (9.05, 21.8)
Missing	0 (0%)	6 (42.9%)
Uranium (mg/L)	0.000005 (0.000005, 0.0006)	0.000005 (0.000005, 0.000005)
Zinc (mg/L)	0.001 (0.001, 0.001)	0.026 (0.008, 0.340)
Dissolved solids (mg/L)	576 (381, 844)	155 (125, 179)
Temperature (°C)	28.0 (27.3, 30.8)	27.5 (26.3, 28.0)
pH	7.14 (6.92, 7.70)	7.20 (6.97, 7.47)
Turbidity (NTU)	0.7 (0.5, 1.2)	9.7 (6.6, 279)
Conductivity (µmho/cm)	1120 (676, 1690)	310 (227, 359)
Residual Chlorine (mg/L)	0.37 (0.02, 1.10)	1.16 (0.63, 1.41)
Missing	0 (0%)	6 (42.9%)
For human consumption	26 (100%)	8 (57.1%)
Sampling point		
Household	2 (7.7%)	1 (7.1%)
Outlet point	1 (3.8%)	0 (0%)
Plant	23 (88.5%)	6 (42.9%)
Reservoir	0 (0%)	6 (42.9%)
Intake point	0 (0%)	1 (7.1%)
IQR: Interquartile range; NTU: Nephelometric turbidity units		

Table 4. Characteristics of 40 water samples in Tumbes, Peru (October-December 2022) by distance to the Puyango-Tumbes River expressed as medians (IQR) or counts (%)		
	Far (n=16)	**Close** (n=24)
High arsenic	0 (0%)	12 (50.0%)
High lead	0 (0%)	7 (29.2%)
High cadmium	0 (0%)	2 (8.3%)
High manganese	2 (12.5%)	8 (33.3%)
Arsenic (mg/L)	0.00139 (0.000468, 0.00281)	0.00955 (0.00332, 0.0471)
Lead (mg/L)	0.00003 (0.00003, 0.00003)	0.000280 (0.00003, 0.0551)
Cadmium (mg/L)	0.000005 (0.000005, 0.000005)	0.000005 (0.000005, 0.000963)
Manganese (mg/L)	0.0606 (0.0107, 0.139)	0.0879 (0.0134, 0.546)
Aluminum (mg/L)	0.001 (0.001, 0.001)	0.0375 (0.001, 0.722)
Antimuonium (mg/L)	0.00001 (0.00001, 0.00001)	0.0101 (0.00001, 0.0164)
Barium (mg/L)	0.0474 (0.0391, 0.0837)	0.0526 (0.0315, 0.0826)
Boron (mg/L)	0.135 (0.0973, 0.172)	0.111 (0.069, 0.268)
Copper (mg/L)	0.00015 (0.00015, 0.000525)	0.0146 (0.00259, 0.0477)
Chromium (mg/L)	0.0005 (0.0005, 0.0005)	0.0005 (0.0005, 0.00138)
Iron (mg/L)	0.015 (0.015, 0.138)	0.151 (0.0645, 1.24)
Molybdenum (mg/L)	0.000785 (0.000383, 0.00114)	0.00179 (0.00131, 0.00213)
Nickel (mg/L)	0.00045 (0.00045, 0.00045)	0.00108 (0.00045, 0.0031)
Selenium (mg/L)	0.00002 (0.00002, 0.0018)	0.00274 (0.00002, 0.00426)
Sodium (mg/L)	89.0 (53.5, 126)	61.0 (19.5, 242)
Missing	0 (0%)	6 (25.0%)
Uranium (mg/L)	0.000188 (0.000005, 0.000753)	0.000005 (0.000005, 0.000005)
Zinc (mg/L)	0.001 (0.001, 0.0025)	0.0075 (0.001, 0.0528)
Dissolved solids (mg/L)	481 (327, 600)	215 (150, 710)
Temperature (°C)	28.0 (27.8, 31.5)	28.0 (27.0, 29.0)
pH	6.94 (6.73, 7.3)	7.30 (7.10, 7.73)
Turbidity (NTU)	0.710 (0.415, 0.958)	5.55 (1.01, 46.0)
Conductivity (µmho/cm)	918 (618, 12)	429 (293, 1420)
Residual Chlorine (mg/L)	0.510 (0.103, 0.838)	0.780 (0.0575, 1.28)
Missing	0 (0%)	6 (25.0%)
For human consumption	16 (100%)	18 (75.0%)
Sampling point		
Household	1 (6.3%)	0 (0%)
Outlet point	15 (93.8%)	14 (58.3%)
Plant	0 (0%)	3 (12.5%)
Reservoir	0 (0%)	6 (25.0%)
Intake point	0 (0%)	1 (4.2%)
IQR: Interquartile range; NTU: Nephelometric turbidity units		

Table 5. Characteristics of 404 children 4-17 years old from Tumbes, Peru (2023) by distance to the Puyango-Tumbes River expressed as means and standard deviations (SD) or counts (%)			
	Far (n=134)	**Close** (n=270)	**Overall** (n=404)
Hair arsenic (µg/kg)	273 (471)	1160 (2180)	866 (1850)
Hair lead (mg/kg)	3.02 (4.60)	7.71 (17.5)	6.16 (14.7)
Hair cadmium (µg/kg)	440 (1190)	392 (876)	408 (988)
Hair manganese (mg/kg)	18.7 (52.7)	11.5 (28.0)	13.9 (38.1)
Age (years)	9.85 (4.02)	10.3 (3.81)	10.2 (3.88)
Female gender	67 (50.0)	146 (54.1)	213 (52.7)
Residence time (years)	9.05 (4.34)	9.84 (4.04)	9.58 (4.16)
Parent or guardian's occupation			
Housewife	1 (0.7)	10 (3.7)	11 (2.7)
Service sector	13 (9.7)	101 (37.4)	114 (28.2)
Fishing or agriculture	117 (87.3)	144 (53.3)	261 (64.6)
Construction or mining	3 (2.2)	15 (5.6)	18 (4.5)
Mining (any family member)	3 (2.2)	1 (0.4)	4 (1.0)
Durable house material	70 (52.2)	125 (46.3)	195 (48.3)
Household's water supply			
Public network	57 (42.5)	126 (46.7)	183 (45.3)
Well	18 (13.4)	68 (25.2)	86 (21.3)
River	5 (3.7)	2 (0.7)	7 (1.7)
Other	54 (40.3)	74 (27.4)	128 (31.7)
Drinking purified water	22 (16.4)	61 (22.6)	83 (20.5)
Drinking water type			
Bottled	40 (29.9)	44 (16.3)	84 (20.8)
Tap	58 (43.3)	172 (63.7)	230 (56.9)
Well	19 (14.2)	27 (10.0)	46 (11.4)
River	5 (3.7)	2 (0.7)	7 (1.7)
Cistern	0 (0)	15 (5.6)	15 (3.7)
Filtered	0 (0)	1 (0.4)	1 (0.2)
Boiled	12 (9.0)	9 (3.3)	21 (5.2)
Food cultivation	113 (84.3)	131 (48.5)	244 (60.4)
Second-hand tobacco smoke	2 (1.5)	40 (14.8)	42 (10.4)
Wood stove	66 (49.3)	122 (45.2)	188 (46.5)
Burning trash	41 (30.6)	76 (28.1)	117 (29.0)
Burning fields	94 (70.1)	103 (38.1)	197 (48.8)
Fish consumption from the river	29 (21.6)	83 (30.7)	112 (27.7)
Frequent chicken consumption	110 (82.1)	228 (84.4)	338 (83.7)
Frequent fish consumption	110 (82.1)	211 (78.1)	321 (79.5)
Frequent tuna consumption	101 (75.4)	187 (69.3)	288 (71.3)
Frequent bone marrow consumption	11 (8.2)	24 (8.9)	35 (8.7)
Frequent eggs consumption	121 (90.3)	212 (78.5)	333 (82.4)
Frequent lentils consumption	102 (76.1)	181 (67.0)	283 (70.0)
Frequent rice consumption	134 (100)	265 (98.1)	399 (98.8)
Frequent spinach consumption	46 (34.3)	63 (23.3)	109 (27.0)
Frequent broccoli consumption	63 (47.0)	55 (20.4)	118 (29.2)
Frequent tomato consumption	124 (92.5)	201 (74.4)	325 (80.4)
Frequent potato consumption	127 (94.8)	226 (83.7)	353 (87.4)

Frequent cassava consumption	111 (82.8)	166 (61.5)	277 (68.6)
Frequent garlic consumption	133 (99.3)	263 (97.4)	396 (98.0)
Frequent onion consumption	133 (99.3)	238 (88.1)	371 (91.8)
Frequent banana consumption	120 (89.6)	232 (85.9)	352 (87.1)
Frequent beetroot consumption	69 (51.5)	70 (25.9)	139 (34.4)
Frequent lemon consumption	133 (99.3)	237 (87.8)	370 (91.6)
Frequent orange consumption	86 (64.2)	160 (59.3)	246 (60.9)
Frequent carrot consumption	100 (74.6)	165 (61.1)	265 (65.6)
Frequent plantain consumption	123 (91.8)	238 (88.1)	361 (89.4)
Breastfeeding (months)	15.9 (8.79)	14.4 (8.42)	14.9 (8.57)

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Table 6. Median (IQR) concentrations of chemical elements (mg/L) in the nearest water sample linked to the households of 404 children (ages 4–17) in Tumbes, Peru (2003), stratified by household cultivation status and use of river water for irrigation				
	No cultivation (n=118)	**Cultivation with non-river water** (n = 126)	**Cultivation with river water** (n = 160)	**Overall** (n=404)
Arsenic	0.003 (0.001, 0.003)	0.019 (0.003, 0.027)	0.027 (0.003, 0.027)	0.019 (0.003, 0.027)
Lead	0.00003 (0.00003, 0.00003)	0.00003 (0.00003, 0.0024)	0.00003 (0.00003, 0.00003)	0.00003 (0.00003, 0.00003)
Cadmium	0.000005 (0.000005, 0.000005)	0.000005 (0.000005, 0.00017)	0.000005 (0.000005, 0.000005)	0.000005 (0.000005, 0.000005)
Manganese	0.060 (0.013, 0.060)	0.002 (0.00003, 0.088)	0.00003 (0.00003, 0.015)	0.002 (0.00003, 0.060)
Aluminum	0.001 (0.001, 0.001)	0.031 (0.031, 0.121)	0.031 (0.031, 0.031)	0.031 (0.001, 0.031)
Antimuonium	0.00001 (0.00001, 0.00001)	0.017 (0.00001, 0.017)	0.017 (0.00001, 0.017)	0.017 (0.00001, 0.017)
Barium	0.067 (0.044, 0.067)	0.046 (0.046, 0.052)	0.046 (0.046, 0.046)	0.046 (0.046, 0.067)
Boron	0.142 (0.138, 0.142)	0.051 (0.051, 0.1)	0.051 (0.051, 0.141)	0.096 (0.051, 0.142)
Copper	0.0002 (0.0002, 0.003)	0.003 (0.003, 0.014)	0.003 (0.003, 0.012)	0.003 (0.0002, 0.012)
Chromium	0.0005 (0.0005, 0.0005)	0.0005 (0.0005, 0.0005)	0.0005 (0.0005, 0.0005)	0.0005 (0.0005, 0.0005)
Iron	0.031 (0.031, 0.031)	0.056 (0.015, 0.151)	0.015 (0.015, 0.056)	0.031 (0.012, 0.056)
Molybdenum	0.0012 (0.0008, 0.001)	0.0017 (0.0008, 0.0017)	0.0017 (0.0008, 0.0017)	0.0013 (0.0008, 0.0017)
Nickel	0.0005 (0.0005, 0.0005)	0.0005 (0.0005, 0.0014)	0.0005 (0.0005, 0.0029)	0.0005 (0.0005, 0.0005)
Selenium	0.00002 (0.00002, 0.004)	0.004 (0.002, 0.004)	0.004 (0.002, 0.004)	0.004 (0.00002, 0.004)
Sodium	81.0 (77.3, 81.0)	15.0 (15.0, 77.0)	15.0 (15.0, 53.0)	21.0 (15.0, 81.0)
Uranium	0.0004 (0.000005, 0.0004)	0.000005 (0.000005, 0.000005)	0.000005 (0.000005, 0.000005)	0.000005 (0.000005, 0.000005)
Zinc	0.001 (0.001, 0.001)	0.001 (0.001, 0.008)	0.001 (0.001, 0.001)	0.001 (0.001, 0.001)
IQR: Interquartile range				

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Table 7. Crude and adjusted coefficients and 95% confidence intervals (CI) for the association between distance from the Puyango-Tumbes River and hair heavy metals concentrations among 404 children aged 4-17 years in Tumbes, Peru (2023)				
	Crude models		**Adjusted models**[*]	
Outcome for each model	Coefficient	95% CI	Coefficient	95% CI
Hair ln-arsenic	1.27	0.74, 1.80	1.38	0.84, 1.92
Hair ln-lead	0.57	-0.09, 1.23	0.57	-0.09, 1.23
Hair ln-cadmium	-0.17	-0.71, 0.37	-0.10	-0.65, 0.46
Hair ln-manganese	-0.72	-1.43, -0.01	-0.56	-1.24, 0.11
[*]All models were adjusted for parental occupation and house material as proxies for socioeconomic status				

Table 8. Summary of the first nine components from an oblique principal component analysis of variables representing potential sources and pathways of heavy metal exposure among 404 children aged 4–17 years in Tumbes, Peru (2023)				
PC	**Variables**	**Concept**	**Explained variance (%)**	
			Each PC	**Cumulative**
1	Water antimony, boron, selenium, uranium, manganese, arsenic, molybdenum, aluminum, copper, iron, barium, dissolved solids, residual chlorine, conductivity, temperature, and pH	General water mineral content and quality	18.0	18.0
2	Water zinc, chromium, lead, cadmium, iron, nickel, aluminum, copper, manganese, barium, arsenic, turbidity, temperature, residual chlorine	Heavy metals water contamination	13.6	31.6
3	Frequent lemon, onion, potato, plantain, tomato, garlic, banana, cassava, carrot, eggs, and fish consumption Drinking water source: cistern	Staple diet and cistern water use	8.4	40.0
4	Water barium, nickel, copper, uranium, and temperature Water supply: well and other (neighbor, common tap, house connection) Drinking water: well and boiled	Water safety/water access	6.0	46.0
5	Frequent broccoli, spinach, beetroot, carrot, orange and lentils consumption.	Leafy and root vegetable diet	4.5	50.5
6	Drinking water: River Water supply: River	Reliance on river water	3.7	54.2
7	Water barium Water supply: other (neighbor, common tap, house connection) Drinking water: tap, well, cistern, boiled	Household water sources	3.1	57.3
8	pH Food cultivation (with and without using river water)	Household agriculture	2.9	60.2
9	Water barium, pH Frequent chicken, fish, tuna, eggs, and rice consumption	Animal protein and rice diet	2.6	62.8
PC: Principal component				

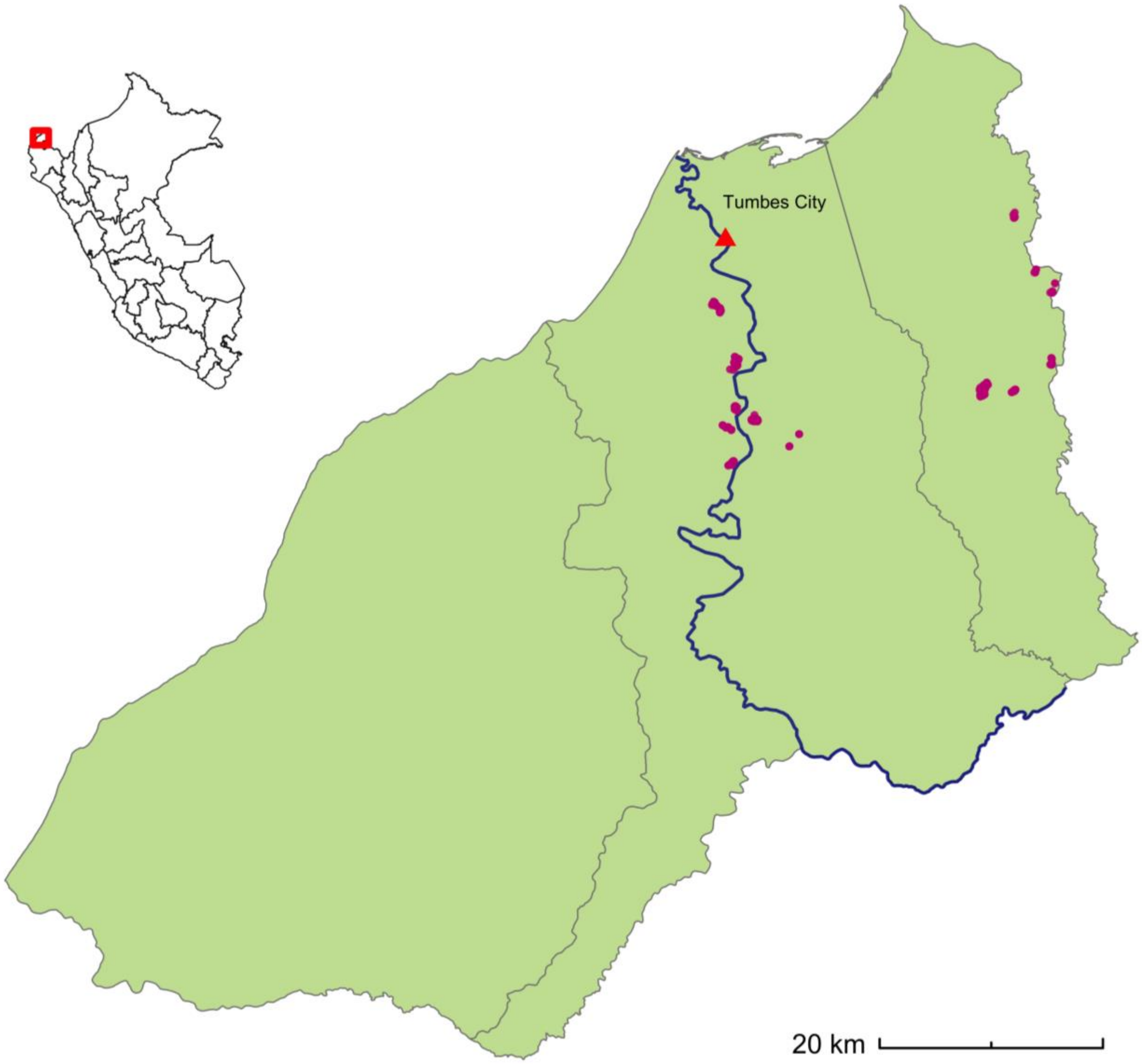

Figure 1. Map of the Tumbes region in northern Peru. The blue line represents the Puyango-Tumbes River, and the magenta dots indicate participants' households. Some households are located within the river watershed, at distances of 0.6–3.4 km from the river, while others are outside the watershed, at distances of 20.5–27.5 km. Inset: location of Peru (green) in South America.

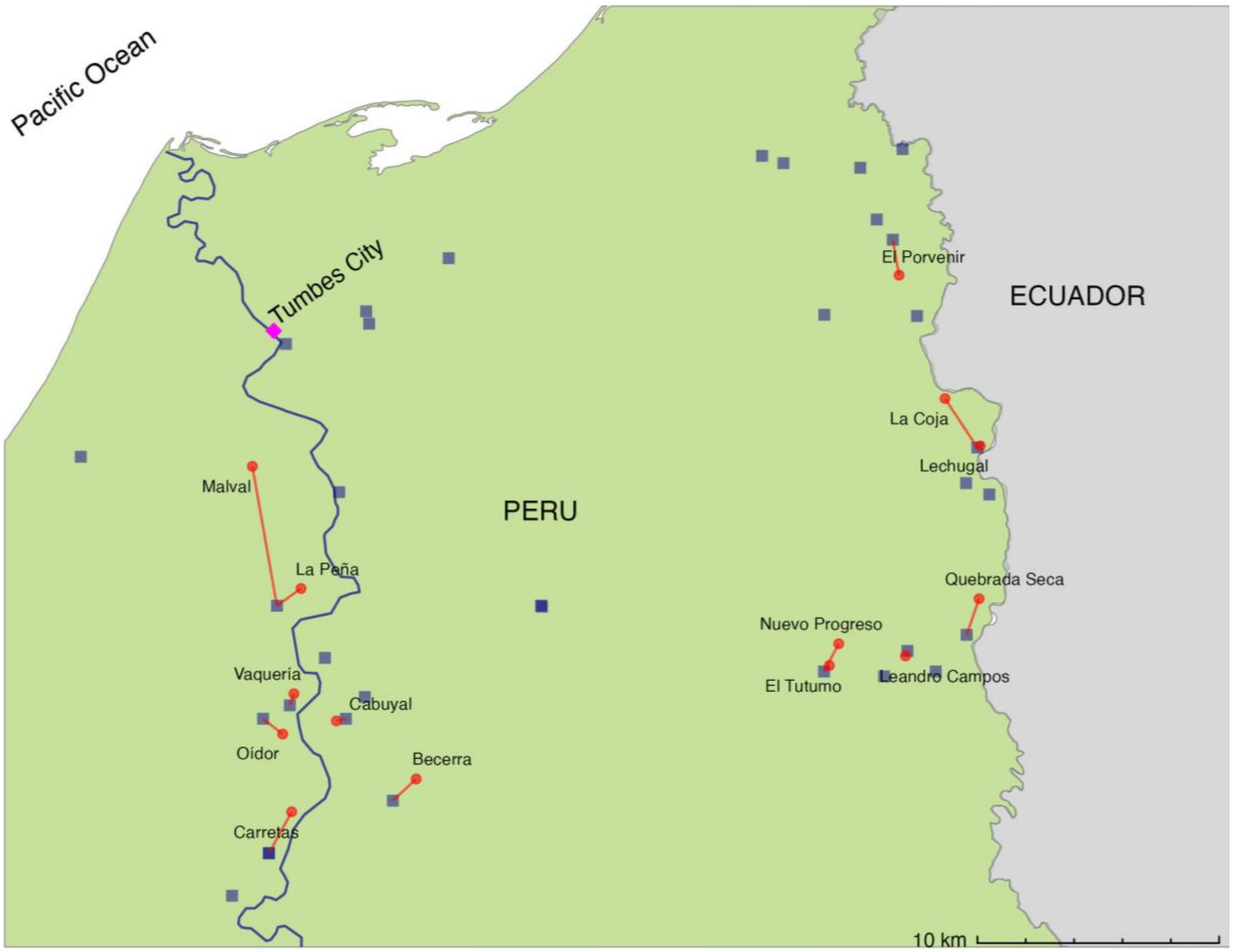

Figure 2. Map of the study region showing the water-to-village linkage. Red dots indicate the included villages, blue squares represent water samples intended for human consumption, and red lines show the link between each village and its nearest water sample. The blue line represents the Puyango-Tumbes River.

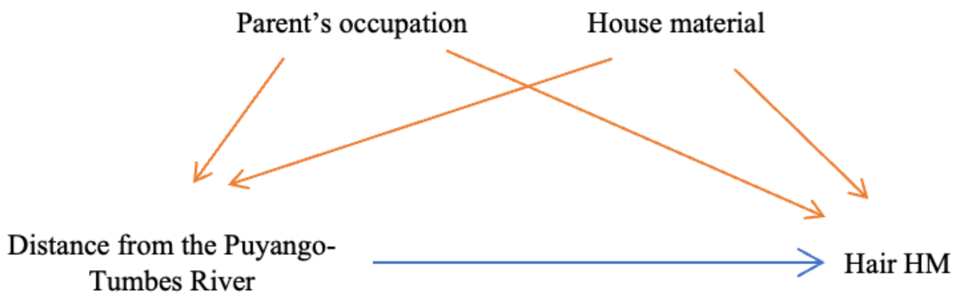

Figure 3. Directed acyclic graph for the association between location and hair heavy metals (HM). The blue arrow represents the main association, and the orange arrows represent confounding, which includes parents' occupation and house material as proxies for socioeconomic status.

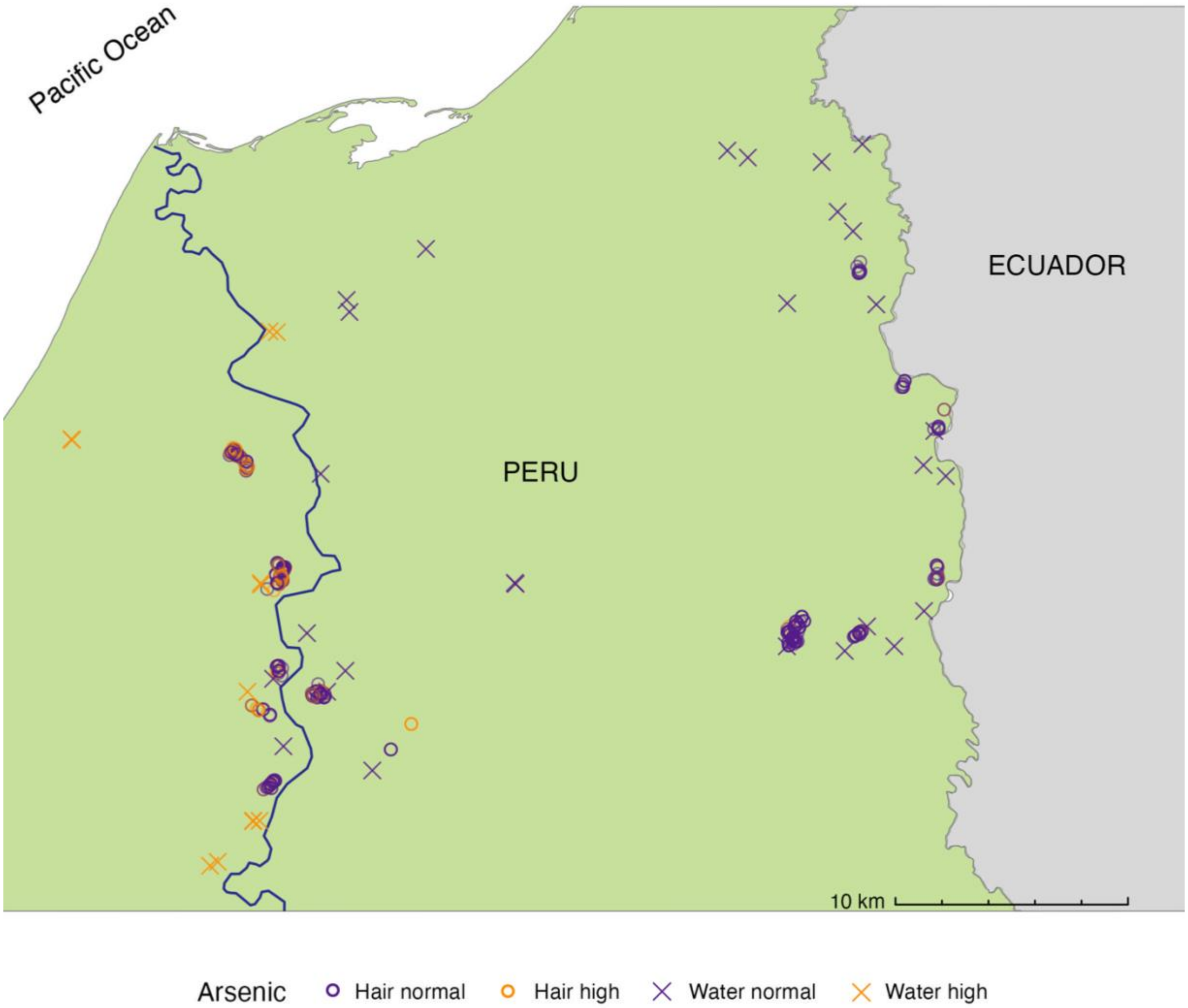

Figure 4. Map of the study region showing arsenic (As) concentrations in 40 water samples and in hair samples from 404 children aged 4–17 years. Orange crosses and orange circles indicate As levels above the reference limit in water and hair samples, respectively, while purple crosses and circles indicate levels within the recommended range—crosses for water and circles for hair. The blue line represents the Puyango-Tumbes River.

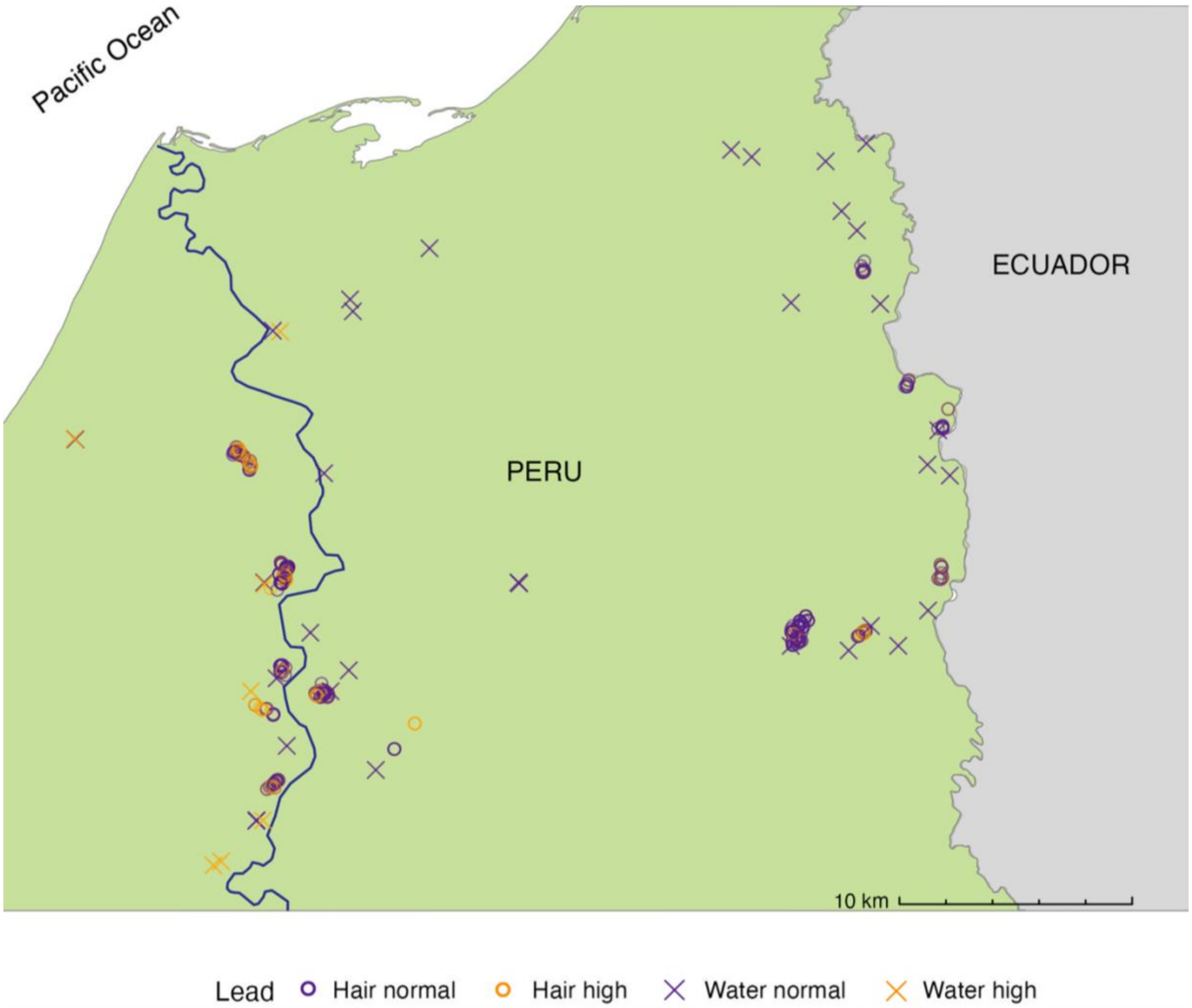

Figure 5. Map of the study region showing lead (Pb) concentrations in 40 water samples and in hair samples from 404 children aged 4–17 years. Orange crosses and orange circles indicate Pb levels above the reference limit in water and hair samples, respectively, while purple crosses and circles indicate levels within the recommended range—crosses for water and circles for hair. The blue line represents the Puyango-Tumbes River.

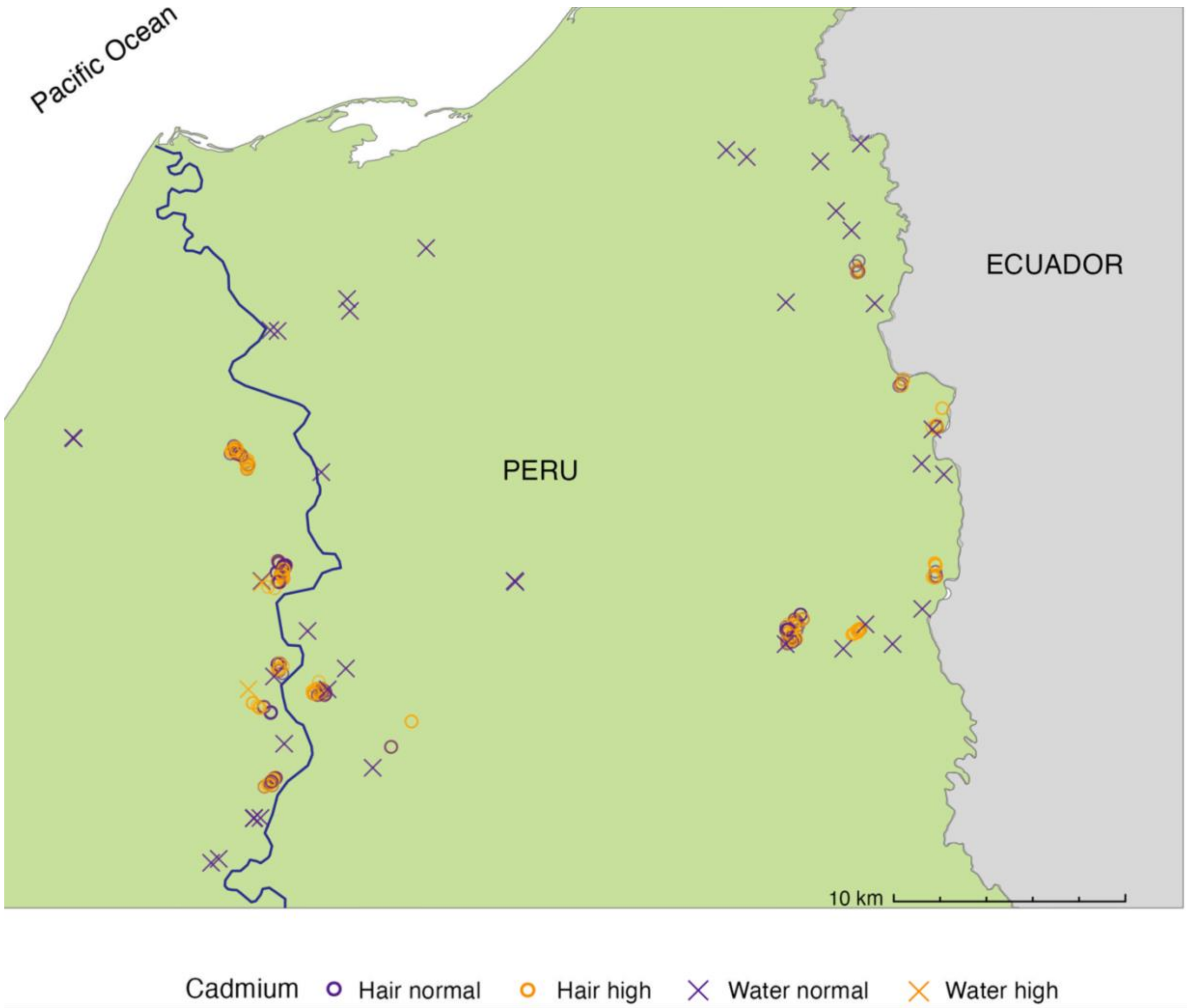

Figure 6. Map of the study region showing cadmium (Cd) concentrations in 40 water samples and in hair samples from 404 children aged 4–17 years. Orange crosses and orange circles indicate Cd levels above the reference limit in water and hair samples, respectively, while purple crosses and circles indicate levels within the recommended range—crosses for water and circles for hair. The blue line represents the Puyango-Tumbes River.

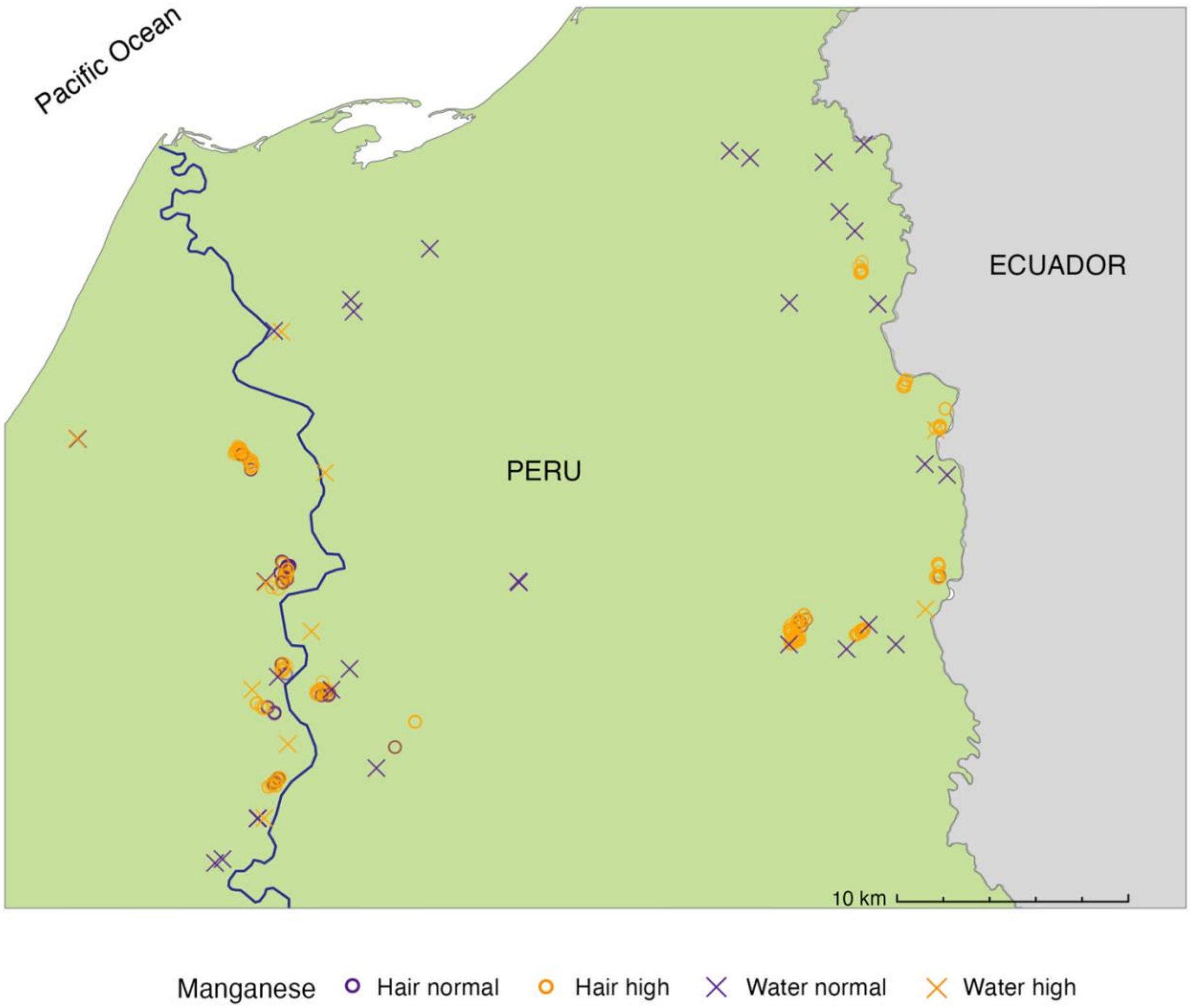

Figure 7. Map of the study region showing manganese (Mn) concentrations in 40 water samples and in hair samples from 404 children aged 4–17 years. Orange crosses and orange circles indicate Mn levels above the reference limit in water and hair samples, respectively, while purple crosses and circles indicate levels within the recommended range—crosses for water and circles for hair. The blue line represents the Puyango-Tumbes River.

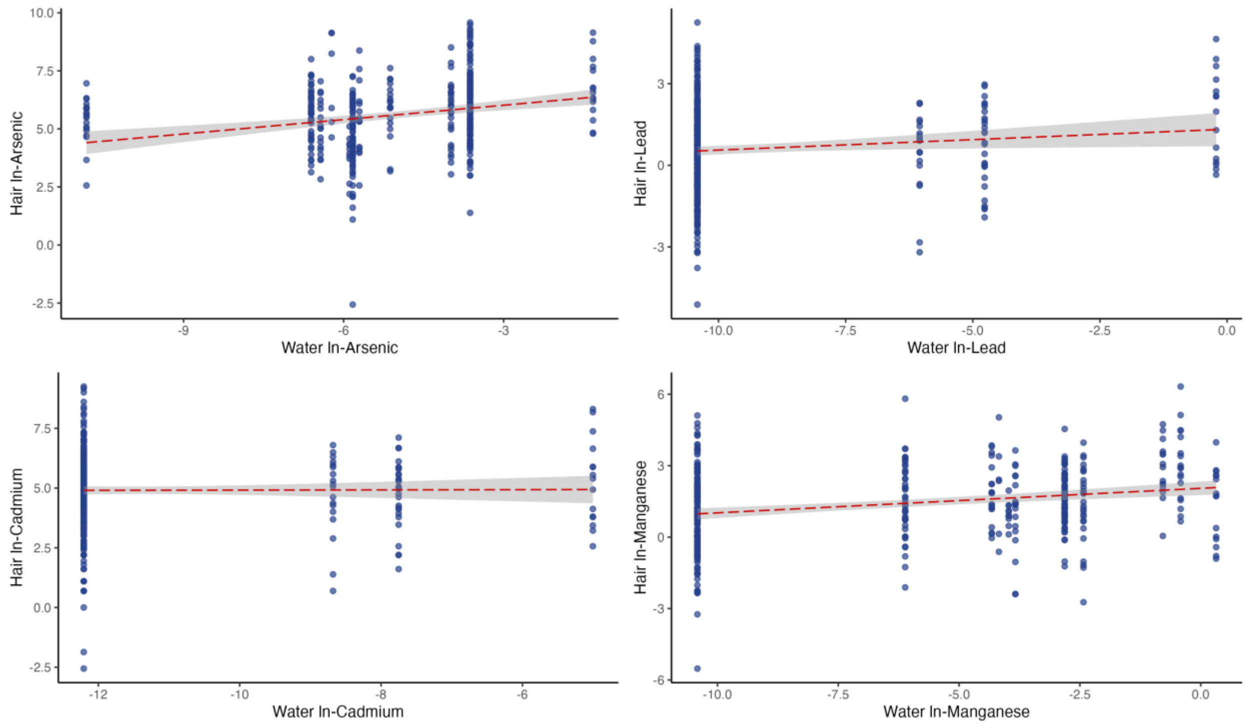

Figure 8. Scatter plots comparing log-transformed concentrations of arsenic, lead, cadmium, and manganese in water and hair (n = 404). Each point represents an individual participant. Red dashed lines show linear regression fits with 95% confidence intervals (gray shading).

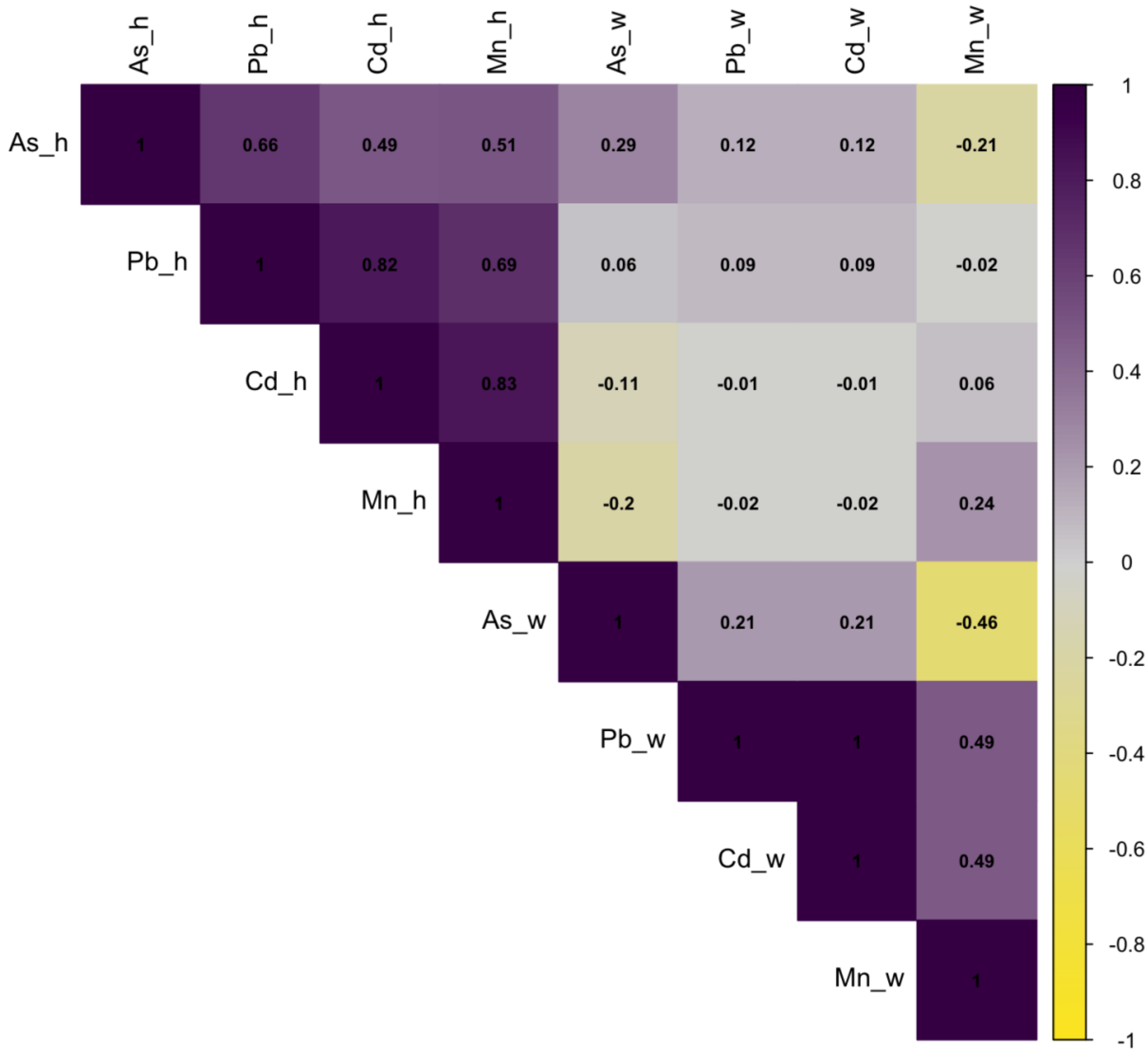

Figure 9. Spearman correlation matrix of arsenic (As), lead (Pb), cadmium (Cd), and manganese (Mn) concentrations in water and hair samples. Variables ending in "_w" refer to metal concentrations in water, and those ending in "_h" refer to concentrations in hair. Color gradients range from yellow (strong negative correlations) to purple (strong positive correlations), with gray representing weak or no correlation.

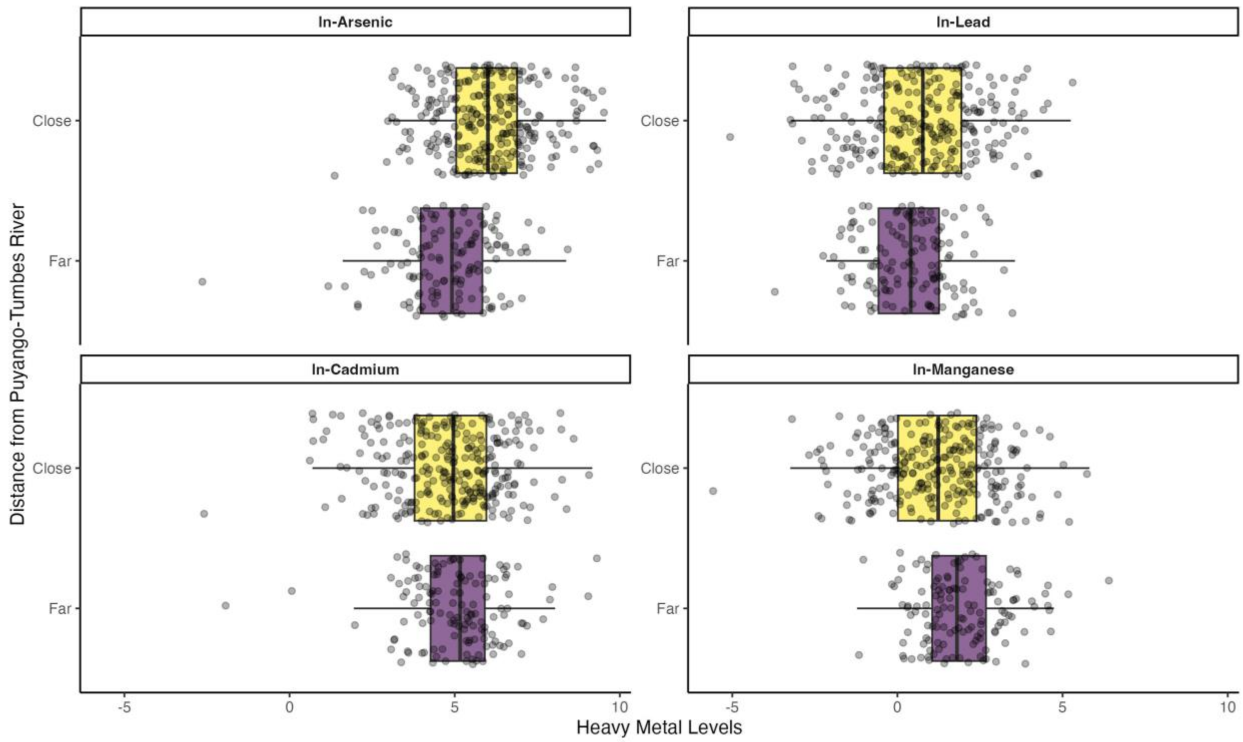

Figure 10. Box plot of log-transformed arsenic, lead, cadmium, and manganese by distance from the Puyango-Tumbes River in 404 children aged 4-17 years in Tumbes, Peru (2023). Yellow boxes represent children living closer to the river, while purple boxes represent those living farther from the river. Each box shows the interquartile range (IQR) with the left and right edges indicating the 25th and 75th percentiles, respectively. The line inside the box represents the median. Whiskers extend to the smallest and largest values within 1.5 times the IQR from the lower and upper quartiles. Gray dots represent individual observations.

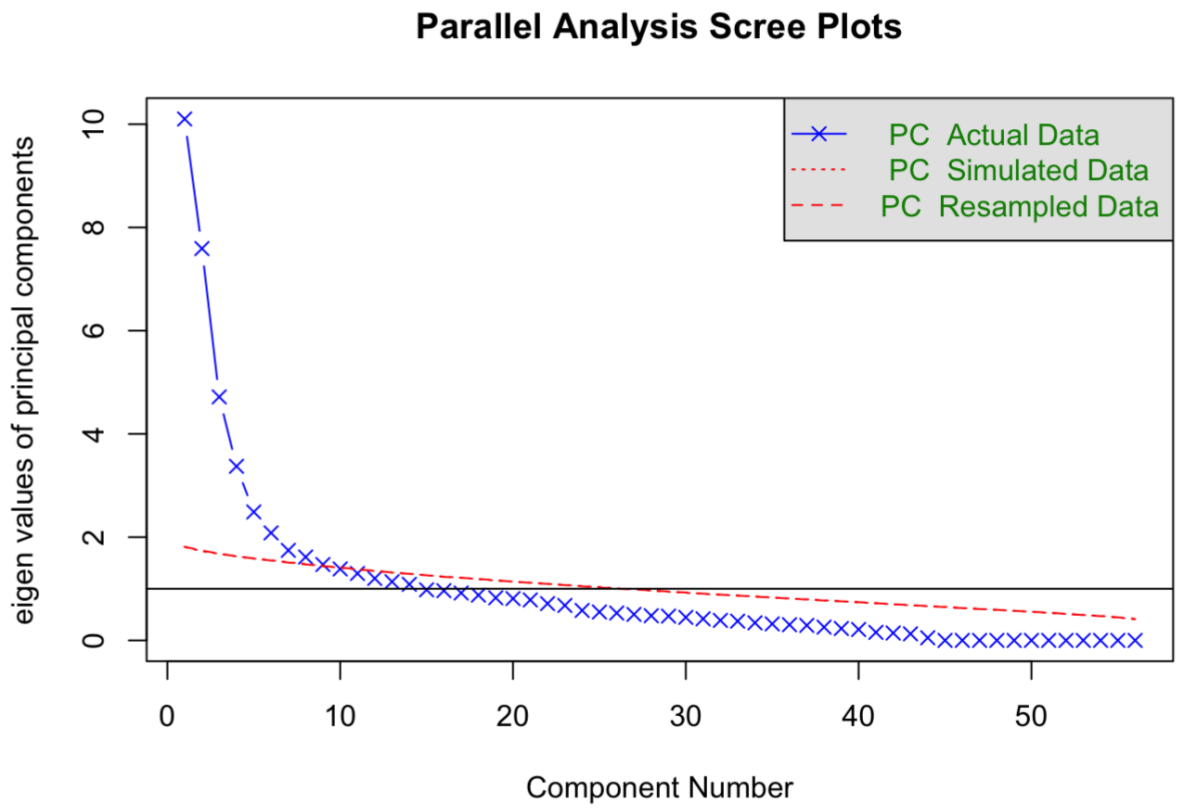

Figure 11. Scree plot from parallel analysis of principal components. Blue crosses represent eigenvalues from the actual dataset, red dotted line shows simulated random data eigenvalues, and red dashed line shows resampled data eigenvalues (overlapped in this figure). Components with actual eigenvalues (blue crosses) above the comparison thresholds (red lines) are retained.

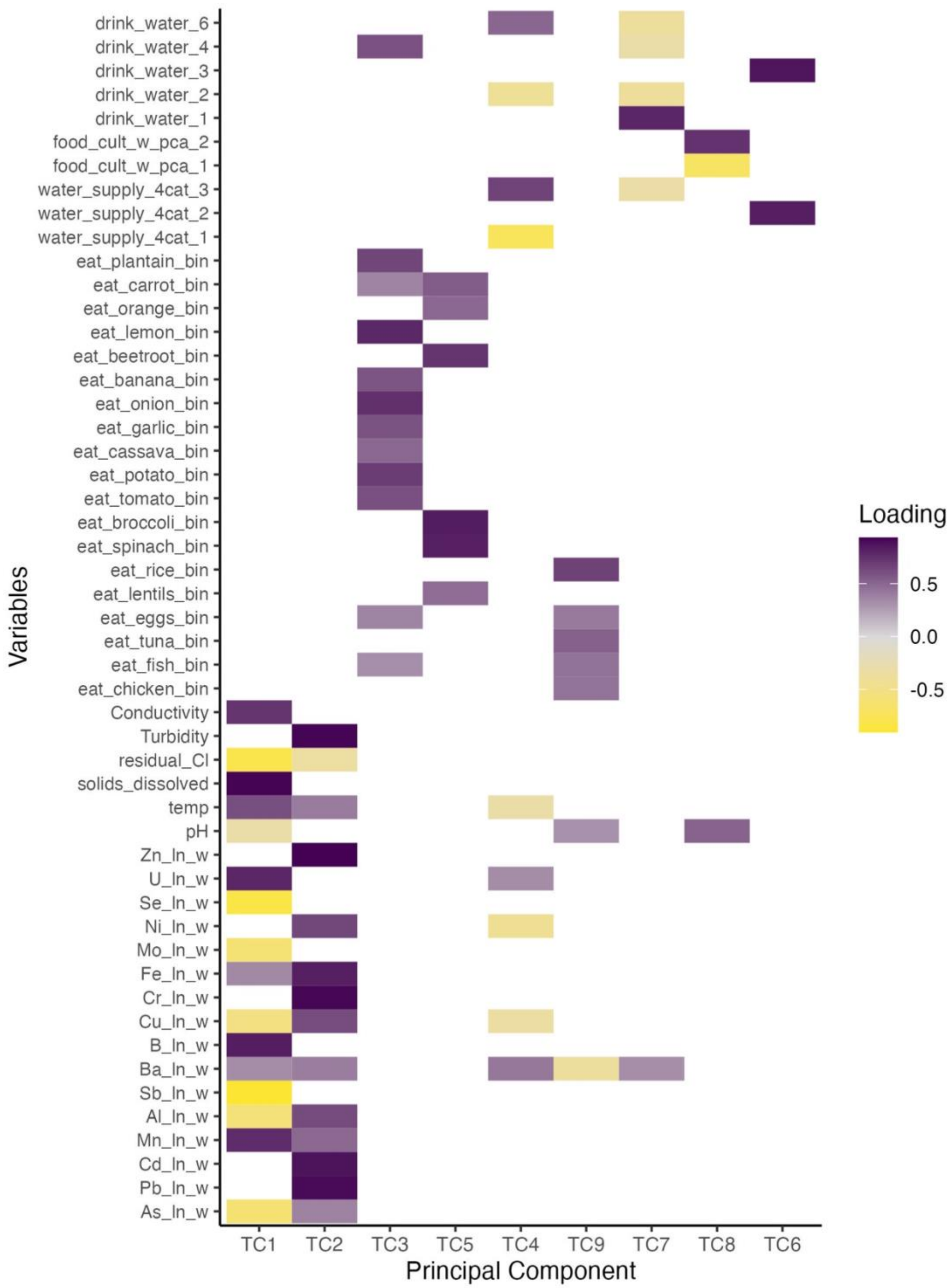

Figure 12. Component loadings heatmap showing variable contributions to the retained principal components. Loading coefficients are color-coded from negative (yellow) to positive (purple) values. Only loadings with absolute values ≥0.3 are displayed.

7. SUPPLEMENTAL TABLES AND FIGURES

Table S1. Thresholds used to classify water quality variables based on Peruvian guidelines

Parameter	**SA**	**ECA1**	**ECA2**
Arsenic (mg/L)	≥0.01	≥0.01	≥0.01
Lead (mg/L)	≥0.01	≥0.01	≥0.05
Cadmium (mg/L)	≥0.003	≥0.003	≥0.005
Manganese (mg/L)	≥0.4	≥0.4	≥0.4
Aluminum (mg/L)	≥0.2	≥0.9	≥5.0
Antimony (mg/L)	≥0.02	≥0.02	≥0.02
Barium (mg/L)	≥0.7	≥0.7	≥1.0
Beryllium (mg/L)	—	≥0.012	≥0.04
Boron (mg/L)	≥1.5	≥2.4	≥2.4
Copper (mg/L)	≥2.0	≥2.0	≥2.0
Chromium (mg/L)	≥0.05	≥0.05	≥0.05
Iron (mg/L)	≥0.3	≥0.3	≥1.0
Molybdenum (mg/L)	≥0.07	≥0.07	—
Nickel (mg/L)	≥0.02	≥0.07	—
Selenium (mg/L)	≥0.1	≥0.4	≥0.4
Sodium (mg/L)	≥200	—	—
Uranium (mg/L)	≥0.015	≥0.02	≥0.02
Zinc (mg/L)	≥3.0	≥3.0	≥5.0
pH	6.5–8.5	6.5–8.5	5.5–9.0
Dissolved solids (mg/L)	≥1,000	≥1,000	≥1,000
Turbidity (NTU)	≥5	≥5	≥100
Electrical conductivity (μmho/cm)	≥1,500	≥1,500	≥1,600
Residual chlorine (mg/L)	<0.5	—	—

SA: Thresholds for water intended for human consumption, based on DS 031-2010-SA; ECA1: Environmental quality standards for water that can be made potable through disinfection, based on DS 004-2017-MINAM; ECA2 Environmental quality standards for water that can be made potable through conventional treatment, based on DS 004-2017-MINAM; NTU: Nephelometric turbidity units
"—" indicates that no threshold was provided in the corresponding guideline

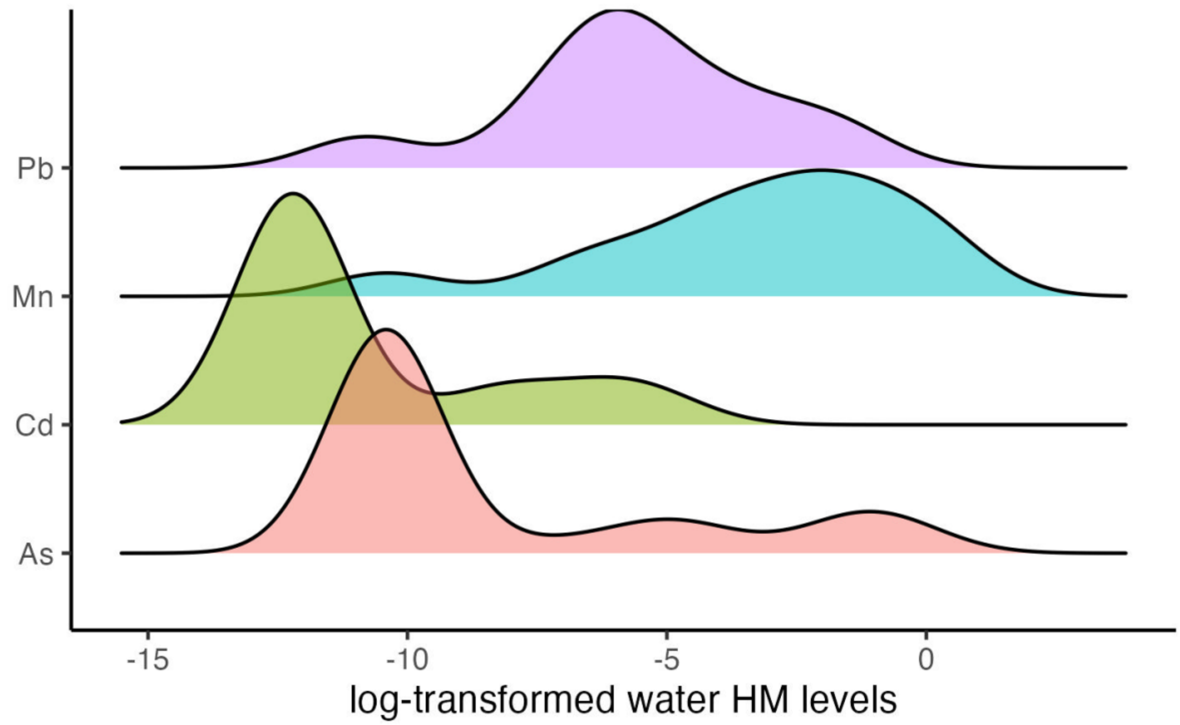

Figure S1. Density plots of the distribution of log-transformed water heavy metals in 40 water samples in Tumbes, Peru (October- December 2022)

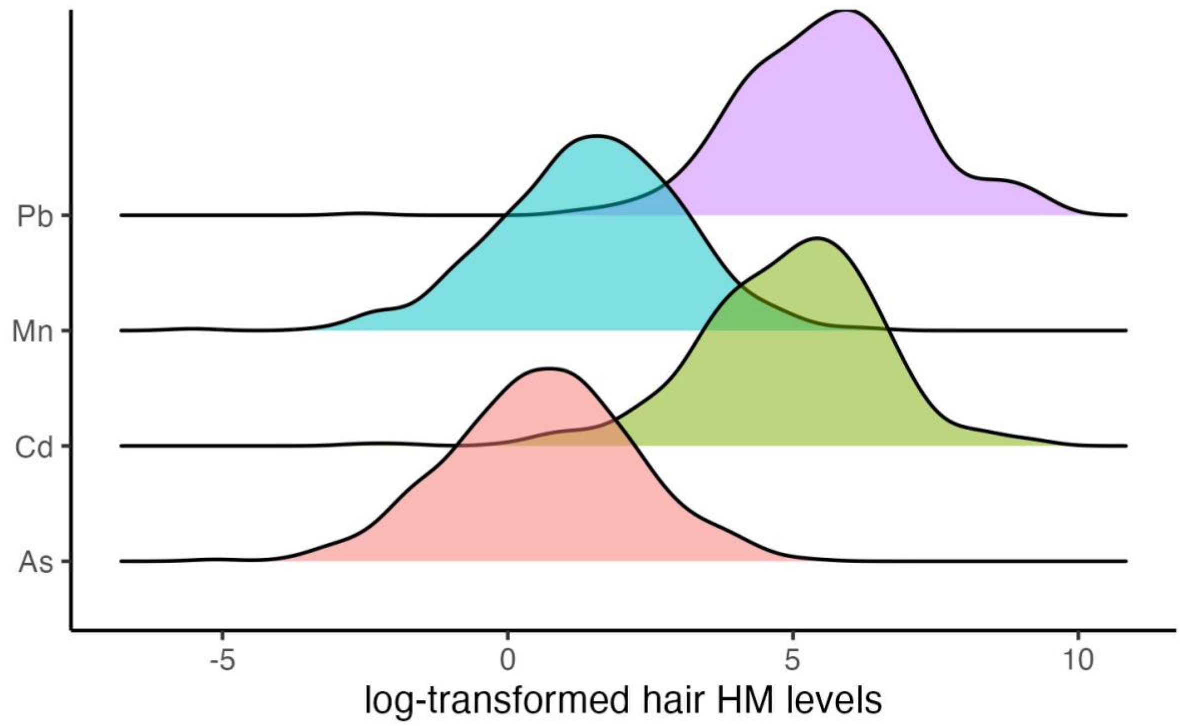

Figure S2. Density plots of the distribution of log-transformed hair heavy metals in 404 children aged 4-17 years in Tumbes, Peru (2023)

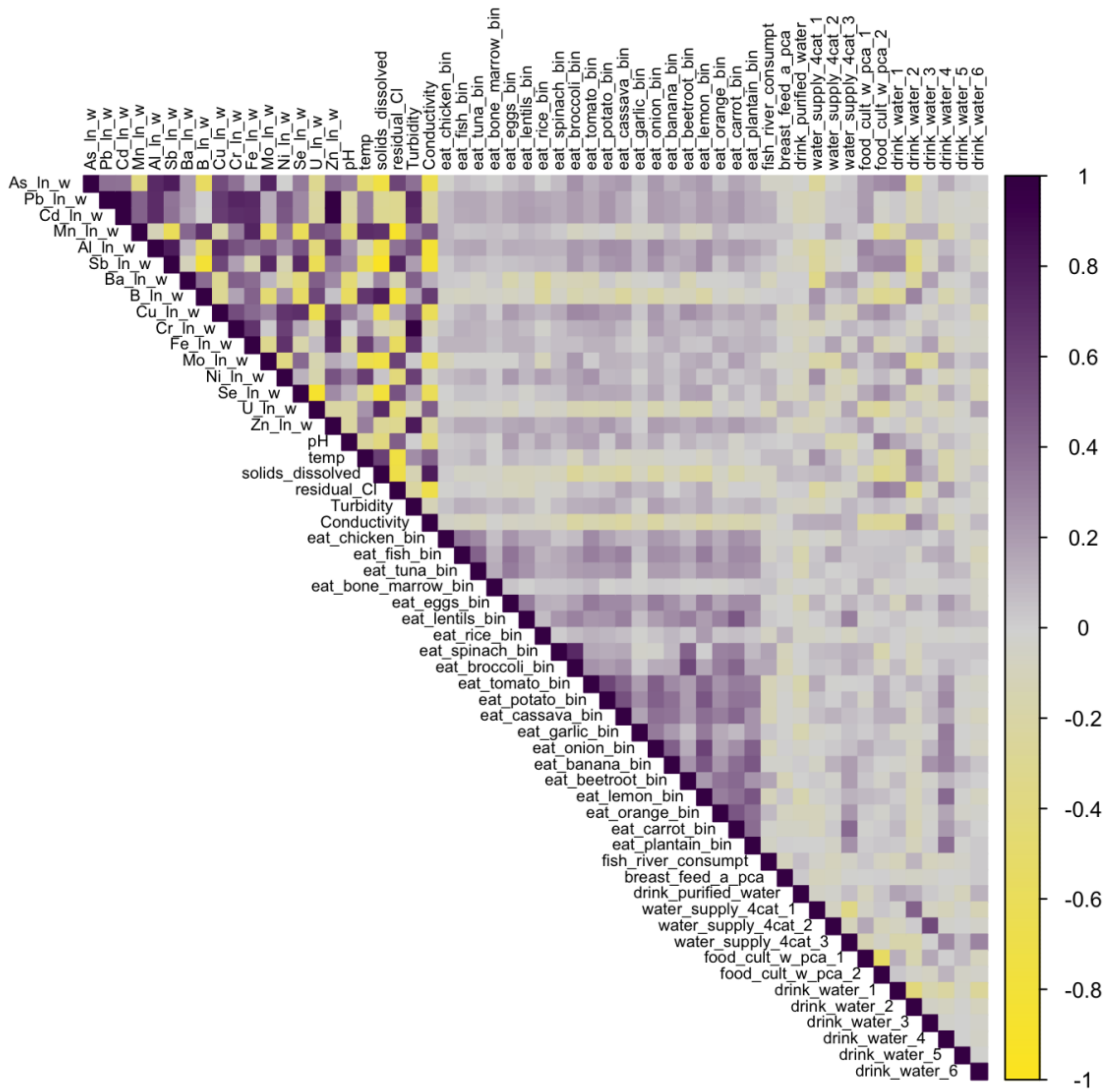

Figure S3. Correlation matrix heatmap showing pairwise correlations between all variables considered during principal component analysis. Color gradients range from yellow (strong negative correlations) to purple (strong positive correlations), with gray representing weak or no correlation.

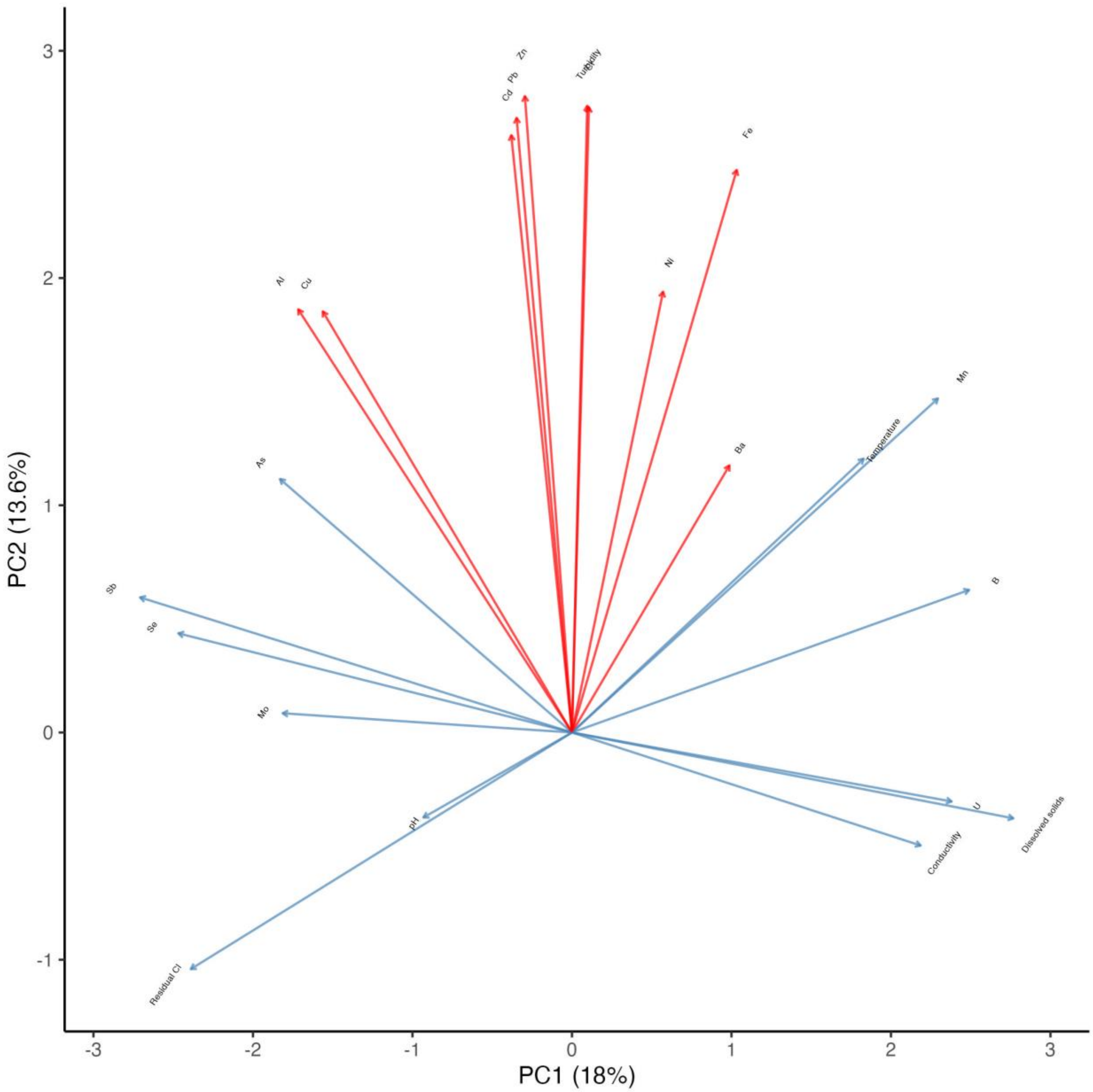

Figure S4. Principal component analysis biplot showing the first two principal components (PC). Arrows (vectors) direction and length indicate the strength and direction of variable contributions to each component. Vectors are color-coded based on whether the variable loads more strongly on PC1 (blue) or PC2 (red). Variables pointing in similar directions are positively correlated, while those pointing in opposite directions are negatively correlated. Only variables with absolute loadings ≥0.3 are displayed to highlight the most influential variables.

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CHAPTER 4. RESEARCH PAPER #2

Association between dietary exposure to arsenic alone and in combination with other heavy metals and respiratory health in children from Tumbes, Peru

1. ABSTRACT

Background: Impaired lung function is a major health concern, particularly in children, as it increases the risk of chronic respiratory diseases and long-term morbidity. Environmental exposure to heavy metals (HM) such as arsenic (As), lead (Pb), cadmium (Cd), and manganese (Mn) has been associated with reduced lung function and respiratory symptoms. However, findings vary by exposure context and population, with limited research in pediatric populations and few studies evaluating the combined effects of multiple HMs. This cross-sectional study investigated the association between dietary arsenic (As) exposure, in isolation and in combination with other HMs (Pb, Cd, and Mn), and respiratory health in children from Tumbes, Peru—a region where the primary freshwater source, the Puyango-Tumbes River, is polluted with HMs, likely due to upstream gold mining.

Methods: This study included 399 children aged 4-17 years from 14 villages in Tumbes in 2023. Hair samples were analyzed for HM levels using inductively coupled plasma mass spectrometry. Lung function was assessed by spirometry and analyzed as percent predicted forced vital capacity (ppFVC) and as lung function patterns. Respiratory symptoms indicative of asthma and allergic rhinitis were evaluated using the International Study of Asthma and Allergies in Childhood questionnaire. We assessed the associations between As and HM exposures and respiratory health using linear models and mixture analysis through quantile-based G-computation, adjusting for sociodemographic and behavioral confounders.

Results: After adjusting for confounders, higher hair ln-As levels (µg/kg) were significantly associated with nasal symptoms suggestive of allergic rhinitis (adjusted odds ratio: 1.59; 95% CI 1.32, 1.91). A non-significant inverse association was found between hair ln-As and ppFVC. Quantile-based G-computation assessing HM mixtures also showed a non-significant inverse association with ppFVC, with As contributing the most to the negative effect.

Conclusion: These findings suggest that while As exposure may be linked to allergic rhinitis, its impact on lung function in this population remains inconclusive.

2. INTRODUCTION

Impaired lung function is a major global health concern, affecting quality of life[1] and increasing the risk of long-term morbidity and mortality.[2,3] Reduced lung function, commonly assessed through spirometry, is associated with an elevated risk of chronic obstructive pulmonary disease (COPD),[4] cardiovascular disease, and all-cause mortality.[2,3] For instance, children with the lowest lung function quartiles at school age face significantly higher odds of developing COPD in adulthood (Odds ratio [OR] 5.76; 95% confidence interval [CI] 1.9, 17.4).[4] Additionally, childhood respiratory conditions, including asthma and allergic rhinitis, are predictors of lung function decline, further increasing the risk of COPD later in life.[1] This burden is particularly concerning for children, as lung development continues into adolescence and early adulthood, leaving the lungs vulnerable to environmental insults during this critical period.[5]

Heavy metals (HM) such as arsenic (As), lead (Pb), cadmium (Cd), and manganese (Mn) are environmental pollutants that potentially impair lung function through mechanisms including oxidative stress, inflammation, immune dysregulation, and damage to epithelial and mucociliary systems.[6–10] These metals bind to sulfhydryl groups, interfering with key metabolic processes, inactivating essential proteins,[7,8,11,12] and depleting antioxidants like glutathione, thereby exacerbating cellular damage.[7–10,13] In real-world settings, HM often co-occur, making single-metal exposures uncommon, especially from environmental sources such as drinking water.[14,15] Co-exposure to multiple HM can amplify lung damage through synergistic mechanisms, further compounding their harmful effects.[15,16]

Research suggests that HM exposure during childhood, particularly As,[6,17] impairs lung function by reducing lung volumes and increasing respiratory symptoms.[16,18–31] Early life As exposure has been associated with increased respiratory symptoms and reduced lung function in children from the US[18,21] and Bangladesh,[19,22] higher mortality from bronchiectasis and lung cancer in adulthood in Chile,[20] and a higher prevalence of restrictive spirometric lung patterns in Mexican children.[23] However, methodological gaps—such as limited evaluation of combined exposures,[18–23] lack of individual-level data,[20] variability in As and lung function assessment, and population differences—limit the generalizability of these findings. Furthermore, conflicting evidence regarding gender differences in susceptibility, with some studies reporting stronger associations among girls[21] and others among boys,[22] further underscores the need for research. Investigations into other HM, such as Pb, Mn, and Cd, show mixed evidence. Results from the PROGRESS cohort in Mexico City,[26] using the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire,[32] reported a positive association between maternal blood Pb and wheezing at school age, whereas a study of Chinese children found an inverse association between blood Pb and lung function, along with a positive interaction between Pb and Cd on lung function.[16] Studies in US children have also reported inverse associations between Pb or Mn and lung function,[27] as well as between several HM—including Copper (Cu), Mn, Vanadium (V), and Nickel (Ni)—and lung function.[28] However, most available evidence has evaluated the effects of metals individually, with only a few recent studies evaluating the joint effects of HM mixtures on impaired lung function.[33–35]

Despite growing evidence of the respiratory risks associated with HM exposure, critical knowledge gaps remain. Limited research has examined HM exposure in marginalized populations, the combined effects of multiple HM on lung function, and the specific impacts on children. Addressing these gaps is essential to identifying high-risk populations and informing environmental remediation efforts. This study aims to assess the association between As exposure—both individually and in combination with other HM—and respiratory health in children from Tumbes, Peru. We hypothesize that higher levels of hair As and HM mixtures will be associated with reduced lung volumes and an increased prevalence of respiratory symptoms. This research will provide valuable insights into the health impacts of HM exposure and guide interventions to mitigate its effects in vulnerable populations.

3. METHODS

3.1. Study setting and design

The Puyango-Tumbes River, which spans 160 km from southwestern Ecuador into northern Peru, is a key water source for many communities in the Tumbes region.[36] Originating in areas of extensive mining in Ecuador, the river is polluted with HM (As, Pb, Cd), often exceeding the safety standards set by the United States Environmental Protection Agency (USEPA) at multiple points along its course.[37] These metals have been detected in water, sediment, fish, crops, and seafood,[38] posing persistent health risks, particularly for children. The Tumbes region is home to approximately 260,000 people,[39] many of whom rely on minimally treated water. In addition, the population faces substantial socioeconomic challenges—including limited access to basic sanitation, education, and healthcare[40]—which may further increase vulnerability to the adverse health effects of chronic exposure to HM through contaminated water.

This cross-sectional study, conducted between January and March 2023, focused on children in the Tumbes province of Peru. We purposefully selected 14 villages from four regions to capture varying levels of potential HM exposure: some villages located within the river's watershed (0.6-3.4 km from the river), while others were farther away (20.5-27.5 km). The sample was restricted to children under 18 years old capable of performing forced spirometry (≥4 years).[41,42] Exclusion criteria included refusal to provide hair samples or undergo spirometry, as these data are critical for the study's analysis.

3.2. Data collection

The Center for Global Health (CGH) personnel recruited 409 children. After obtaining consent, they took hair samples, performed spirometry testing, recorded global positioning system (GPS) coordinates of each participant's household location, and applied a survey to collect information about demographics, background characteristics, and health and behavior status. Enrollment occurred in participants' households. To address potential challenges in reaching participants, the study team adopted a flexible approach, allowing up to three visits if a household was unavailable during the initial visit. The study team collaborated closely with local authorities to navigate community dynamics, facilitate access and engagement, and maximize participation.

Exposures

Hair Arsenic (As) and HM mixture

The primary exposure of interest was hair As (μg/kg), with Pb (mg/kg), Cd (μg/kg), and Mn (mg/kg) also measured for inclusion in the HM mixture analysis. Trained CGH personnel collected three hair samples (~100 strands each) from the occipital region using stainless steel scissors, cutting as close to the scalp as possible. Samples were stored at room temperature; two were sent to the Elemental Analysis Core Lab at Oregon Health & Science University (OHSU) for analysis, while the third was archived at CGH. The proximal 3 cm of hair (~3 months of exposure) was processed.[43,44] After washing, 200 μl of concentrated Nitric Acid (HNO_3) was added, and samples were heated to 90°C for 45 minutes. After cooling, 1% HNO_3 was added to reach a total volume of 2000 μl, followed by digestion at room temperature for 12 hours. HM levels were measured using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) with Agilent 8900 triple quad equipped with a Sample Preparation System (SPS) autosampler. Data were quantified using weighed, serial dilutions of a multi-element standard for Mn and Pb, and single-element standards for Cd and As, with a 12-point calibration curve. Measurements were performed in triplicate and averaged. Precision was assessed via the coefficient of variation, while internal standards (scandium, germanium, bismuth) corrected for detector fluctuations and monitored plasma stability. Controls and standards, including National Institute of Standards and Technology (NIST) SRM 1643f, NIST SRM 1683f (8x dilution), and Bovine Liver NIST SRM 1577c, ensured accuracy. Recovery for NIST water and bovine liver SRMs ranged from 83-112%, with spikes and repeats within 5% of expected values, indicating robust analytical performance.

Outcomes

Main outcome: Lung function (ppFVC). We measured lung function continuously as % predicted forced vital capacity (ppFVC) using spirometry. FVC represents the maximum amount of air that can be forcefully exhaled after a maximum inhalation in liters (L), whereas ppFVC represents the percentage of the predicted value of FVC based on age, sex, and sometimes ethnicity, providing a standard for comparing each participant's lung function with that of a normal population. We selected this lung function measure over others because FVC is a useful metric for identifying restrictive lung function patterns, which are more frequently found among populations exposed to As through food and water.[45] CGH personnel used EasyOne® Air portable spirometers to perform this test. They asked each participant to sit in a steady chair with arm support, maintaining an upright position with a slightly elevated head. After providing a new clean mouthpiece and a nose clip, CGH personnel instructed participants to take a maximum inhalation followed by an explosive exhalation, sustaining the exhalation without inhaling again. This maneuver was repeated up to eight times until three grade A or B maneuvers were achieved, following the American Thoracic Society/European Respiratory Society (ATS/ERS) acceptability and repeatability criteria (**Table 1**).[46,47] We selected the best value for each spirometry metric for analysis.

Secondary outcomes:

Lung function pattern. We categorized lung function into three categories—obstructive, suggestive of restriction (hereafter referred to as restrictive), and normal—using the Global Lung Initiative (GLI) reference equations[48] with a race-neutral approach.[49] To record this variable, we used the following spirometry metrics: FVC in L (defined above), FEV_1 (the volume of air exhaled during the first second of the FVC) in L, and FEV_1/FVC ratio (the proportion of the FVC exhaled in the first second), which were categorized following these criteria: if the FEV_1/FVC ratio was below the lower limit of normality (LLN)—typically the lowest 5% values within the normal range—the test was categorized as obstructive; if the FEV_1/FVC ratio was above the LLN but the FVC was below the LLN, it was categorized as restrictive; and if both the FEV_1/FVC ratio and FVC were above the LLN, it was categorized as normal (**Figure 1**).[50] Additionally, we created the variable "abnormal spirometry pattern," coded as yes if the spirometric pattern was restrictive or obstructive and no if it was normal. To account for the absence of Latin American children in the GLI equations and evaluate the robustness of our findings, we conducted a sensitivity analysis using the Pérez-Padilla equations,[51] designed for Latin American children ≥6 years, and integrated into the EasyOne® Air spirometer.[52]

Respiratory symptoms. Asthma and allergic rhinitis symptoms (yes or no) were evaluated by CGH personnel by administering a survey to the participants' parents or guardians using questions based on the ISAAC core questionnaire for asthma: In the previous 12 months, has your child experienced any of the following symptoms? a) wheezing, b) shortness of breath, c) nighttime cough without a cold, d) wheezing during or after physical activity; as well as for rhinitis: In the past 12 months, has your child experienced nasal secretion, sneezing, or nasal obstruction without a cold?[32,53]

Potential confounders

Using a directed acyclic graph (DAG) (**Figure 2**) and based on the literature, we identified the following confounding variables: age (in years), gender (male or female), secondhand tobacco smoking (yes or no), smoke exposure from wood stoves (yes or no), field burning (yes or no), and trash burning (yes or no), family occupation (housewife, service sector, fishing/agriculture, and construction/mining for all models—except in the model with spirometry pattern as the outcome, where "housewife" and "service sector" were merged due to sparse data in the "housewife" and "restriction" category) and durable house material (yes or no) as proxies for socioeconomic status (SES), fish consumption from the river (yes or no), and fish consumption frequency (daily, 3 times per week, and ≤ 1 time per week). CGH personnel recorded participants' age and gender using their national identity document (NID) and collected the other variables through a questionnaire administered to the parents or guardians.

3.3. Statistical analysis

Descriptive analysis. In the descriptive analysis, we calculated frequencies for categorical variables and means with standard deviations (SD) or medians with interquartile ranges (IQR) for continuous variables, as appropriate. Participants with missing values for exposures, outcomes, or confounders were excluded, with missingness below 10% considered acceptable. For missing data ≥10%, a sensitivity analysis was planned to assess potential selection bias. All HM concentrations exhibited a right-skewed distribution and were log-transformed using the natural logarithm (**Figure S1**). To improve model stability, all continuous variables were centered on their mean. Arsenic was categorized into quartiles to summarize the distribution of variables across As levels in a table. The mean (SD) of ppFVC, along with the prevalence of lung function patterns and respiratory symptoms, was calculated.

Association models. To assess the association between ln-As and ppFVC, we performed multivariable linear regression to estimate crude and adjusted beta coefficients with 95% CI. For binary outcomes, such as abnormal lung function or respiratory symptoms, we used multivariable logistic regression to compute crude ORs and adjusted ORs (aOR) with 95% CI. For lung function patterns with more than two categories, we applied multinomial logistic regression to estimate OR and aOR with 95% CIs. Dose-response was assessed by constructing a quartile-based multivariable regression model, examining regression coefficients across ln-As quartiles to identify monotonic changes in the associations between As and ppFVC. Considering a potential clustering effect at the village level, we evaluated the need for random effects by measuring the intraclass correlation coefficient (ICC) and by comparing models with and without random effects using the Akaike Information Criterion (AIC). Based on these assessments, we included random intercepts for villages in our final models where the clustering effect was significant.

Mixture analysis. To model the impact of multiple HM exposures on ppFVC, we applied quantile-based G-computation including HMs (As, Pb, Cd, Mn) with Pearson correlation coefficients >0.3, transformed into quartiles, and adjusted for confounders.[54] The marginal effect of the mixture was calculated to reflect the change in ppFVC per quartile increase in the mixture. Each HM's contribution to the mixture effect was weighted to estimate both the overall effect of the mixture and the individual HM contributions. The 95% CI was computed using a nonparametric bootstrap approach.

Sensitivity analysis. Sensitivity analysis involved recategorizing spirometry results using the Pérez-Padilla reference equations for Latin American children and restricting the analysis to children with available reference values (>5 years old).

Sample size. The study was designed to achieve 80% power to detect a correlation of −0.15 (small to moderate effect) between As and ppFVC, with a two-sided α=0.05, requiring a minimum of 347 children. To account for an anticipated low non-participation rate (~5%), we aimed to approach 365 children for inclusion. Based on census data, we projected ~1,500 children aged 4–17 years in the selected villages, meeting the sample size requirements. All statistical tests were two-tailed with a significance level of P <0.05.

Ethics statement
This study was approved by the Institutional Ethics Committee for Humans at the Universidad Peruana Cayetano Heredia and the Institutional Review Board at OHSU.

4. RESULTS

Descriptive analysis. Of the 409 children who met the inclusion criteria and agreed to participate, 9 were excluded due to missing spirometry data, and 1 was excluded for missing hair HM data. None of the missing data exceeded 10%. The final analytical sample consisted of 399 children, 52.9% of whom were female, with a mean age (SD) of 10.2 (3.9) years. The median (IQR) of hair HMs were 294.0 (96.0, 737.0) µg/kg for As, 1.9 (0.7, 5.4) mg/kg for Pb, 157.0 (52.5, 378.5) µg/kg for Cd, and 4.7 (1.5, 12.5) mg/kg for Mn. Seventy-two (18.0%) children had hair As levels over those recommended by the Agency for Toxic Substances and Disease Registry (ATSDR): ≤1,000 µg/kg in hair.[55]

We analyzed the distribution of participant characteristics by hair ln-As quartile (**Table 2**). Mean hair Pb, Cd, and Mn increased across ln-As quartiles, with children in the highest quartile (Q4) exhibiting the highest concentrations of all HM. The age of participants was similar across hair ln-As quartiles. Q4 included a higher proportion of females (73.7%) compared to males. The majority of parents or guardians (64.7%) worked in fishing or agriculture, a pattern consistent across quartiles, while only one participant's parent reported employment in mining. Living in houses made of non-durable materials was more common in Q4. Most children (89.5%) were not exposed to second-hand tobacco smoke, with little variation across quartiles. Wood stove usage was common overall (46.1%), but there was minimal variation in this practice across hair ln-As quartiles. Trash burning was more common in Q4, whereas burning fields was more frequent in Q1 (57.0%). Fish consumption habits, such as fish consumption frequency and fish consumption from the Puyango-Tumbes River, were generally consistent across quartiles.

The mean (SD) of ppFVC was 96.4 (19.2). A restrictive lung function pattern was noted in 12.8% of the participants, whereas 9.8% showed an obstructive pattern. 12.8% of children reported a history of wheezing, and 6.8% reported experiencing dyspnea. A history of coughing at night was reported by 28.3% of participants, whereas 7.0% experienced wheezing during exercise. Nasal symptoms suggestive of allergic rhinitis were reported by 48.4% of participants.

Association models. We first explored the association between ln-As and ppFVC—the primary outcome. **Figure 3** illustrates the graphical distribution of ppFVC by ln-As, which shows no discernible patterns. The crude mixed effects regression model revealed a non-significant positive association between ln-As and ppFVC (β coefficient [95% CI]: 0.32 [-0.94, 1.58]). After adjusting for potential confounders (**Figure 3**), the model demonstrated an inverse association. Specifically, a one-unit increase in ln-As was associated with a 0.09 decrease in ppFVC (β coefficient [95% CI]: -0.09 [-1.30, 1.11]). However, this association was not statistically significant (**Table 3**). Similarly, in the regression model using ln-As quartiles as the exposure, the coefficients for quartiles 2, 3, and 4 (compared to quartile 1) fluctuated without showing a monotonic increase or decrease. Additionally, all 95% CI included the null value (**Table 4**). These findings indicate a lack of a dose-response association between hair ln-As levels and ppFVC.

The crude and adjusted OR of ln-As and spirometric patterns showed no significant positive associations between hair ln-As and spirometry patterns suggestive of restriction (aOR [95% CI]: 1.17 [0.94, 1.46]), obstruction (aOR [95% CI]: 1.13 [0.88, 1.45]), or an abnormal spirometry pattern (aOR [95% CI]: 1.15 [0.97, 1.37]). Similarly, no significant associations were found for respiratory symptoms, including wheezing (aOR [95% CI]: 1.11 [0.90, 1.39]), dyspnea (aOR [95% CI]: 0.90 [0.69, 1.18]), nighttime cough (aOR [95% CI]: 1.04 [0.85, 1.28]), or wheezing with exercise (aOR [95% CI]: 0.98 [0.75, 1.28]). However, a significant positive association was observed between ln-As and nasal symptoms, with an aOR (95% CI) of 1.59 (1.32, 1.91), suggesting a potential link to allergic rhinitis in this population (**Table 5, Figure S2**). To account for clustering of participants within villages, mixed effects models with a random intercept for village were used to compute crude and adjusted ORs for nighttime cough and nasal symptoms.

Mixture analysis. The Pearson correlation analysis revealed that all pairwise correlations among the HM exceeded 0.3, meeting the inclusion criterion for the mixture analysis (**Figure 4).** Each HM was transformed into quartiles and included in a linear model to evaluate the association between the HM mixture and ppFVC, adjusting for confounders using quartile-based G-computation. The mixture slope parameter (Ψ) was estimated as -0.44 (95% CI: -2.33, 1.45), indicating that, on average, a one-quartile increase in the HM mixture exposure was associated with a 0.44-unit reduction in ppFVC. However, the association was not statistically significant, as the 95% CI included the null value of 0. To further explore the mixture, we quantified each HM's contribution to the overall effect (Ψ). Arsenic contributed the most to the negative effect (45.0%), followed by Cd (38.6%) and Mn (16.0%). In contrast, Pb was the only HM with a positive association, contributing 100% to the positive direction (**Figure S3**). Despite these individual contributions, the net effect of the HM mixture on ppFVC was negative but not significant.

Sensitivity analysis. We conducted a sensitivity analysis restricted to children aged 6–17 years old (n=346) to evaluate the association between ln-As and ppFVC, using the Pérez-Padilla reference equations instead of the GLI equations. In this subsample, 52.9% of participants were female, and the mean age was 11.1 (SD 3.4) years. As observed in the main analysis, participant characteristics by hair As quartile (**Table S1**) showed a higher proportion of females (72.1%) and households lacking durable building materials (58.1%) in Q4. While trash burning was more evenly distributed across ln-As quartiles, the highest proportion (32.6%) also occurred in Q4. Similarly, burning fields was most common in Q1 (57.0%). The distributions of age, parent/guardian occupation, second-hand tobacco smoke exposure, stove type, and fish consumption habits were consistent across As quartiles. Using the Pérez-Padilla equations, the mean ppFVC was lower (87.0, SD 15.1) compared to values obtained with the GLI equations (96.4, SD 19.2). A larger proportion of participants (22.5% vs. 12.8%) were classified as having a restrictive lung function pattern, while a smaller proportion (2.9% vs. 9.8%) were classified as obstructive. The crude mixed effects model for the association between ln-As and ppFVC indicated a non-significant positive association (β coefficient [95% CI]: 0.89 [-0.17, 1.95]), which remained non-significant after adjustment (β coefficient [95% CI]: 0.24 [-0.79, 1.28]). Consistent with the main analysis, no dose-response pattern was observed when ln-As quartiles were used as the exposure. Furthermore, adjusted ORs and their 95% CI showed no significant associations between hair ln-As and spirometry patterns suggestive of restriction (aOR [95% CI]: 0.96 [0.81, 1.15]), obstruction (aOR [95% CI]: 1.25 [0.74, 2.12]), or any abnormal spirometry pattern (aOR [95% CI]: 0.98 [0.83, 1.17]).

5. DISCUSSION

5.1. Key observations

Our study results indicate an inverse but non-significant association between hair ln-As levels—both alone and in combination with other HM (Pb, Cd, and Mn)—and lung function, measured as ppFVC. We also observed a positive but non-significant association between hair ln-As levels and abnormal spirometry, including obstructive and restrictive spirometry patterns. Regarding respiratory symptoms, we found a significant positive association between hair ln-As and nasal symptoms indicative of allergic rhinitis (aOR 1.59, 95% CI 1.32, 1.91). Additionally, we observed a non-significant positive association with wheezing and nighttime cough, an inverse but non-significant association with dyspnea, and a null association with exercise-induced wheezing. Sensitivity analyses using reference equations for Latin-American children showed no association between hair ln-As levels and ppFVC or spirometry patterns.

5.2. Consistency with other studies

Our finding of a non-significant inverse association between ln-As and ppFVC aligns with a 2013 Bangladeshi cohort study (n=650), which reported a non-significant inverse association between water As levels and FEV_1 (β = -0.013; 95% CI -0.076, 0.049) and FVC (β = -0.007; 95% CI -0.075, 0.061).[19] However, water As exposure was higher in Bangladesh (10-1512 μg/L) compared to Tumbes (0.02 to 266 μg/L).[56] Additionally, the Bangladeshi study relied on water samples, which may not accurately reflect internalized exposure, whereas our study used hair samples, allowing for individual-level assessments of internalized exposure.

Other studies, however, have measured As in human samples and found significant inverse associations with lung function. The New Hampshire Birth Cohort Study (NHBCS) (n=358) reported an inverse association between total maternal urinary As ($\log_2$-transformed) during pregnancy and children's FVC and FEV_1 z-scores at school age (β = -0.08; 95% CI -0.14, -0.01 and β = -0.10; 95% CI -0.18, -0.02, respectively).[21] Similarly, a 2017 Bangladeshi cohort study (n=540) found an inverse association between total maternal urinary As ($\log_2$-transformed) during pregnancy and both FEV_1 (β = -12; 95% CI -22, -1.9) and FVC (β = -12; 95% CI -22, -1.5) in mL at age 9.[22] However, these studies assessed As in urine rather than hair, used different lung function metrics—z-scores or volumes in mL instead of ppFVC—and did not adjust for dietary As exposure, limiting direct comparability.

Neither the US[21] nor the Bangladeshi[22] studies found an association between urinary As and FEV_1/FVC, suggesting that As exposure is more closely linked to restrictive rather than obstructive spirometric patterns. This aligns with a Mexican study that reported higher mean urinary As levels among children with restrictive patterns compared to those with normal spirometry (150 μg/L vs. 128 μg/L)[23] and with a 2018 meta-analysis of pediatric and adult populations.[45] While our findings of a positive—albeit non-significant—association between hair ln-As and both restrictive and obstructive patterns partially align with these studies, we note that the prevalence of restrictive patterns in our study (12.8%) exceeded population estimates (1.8–7.7%) based on GLI equations.[57] Sensitivity analyses using Latin American-specific reference equations indicated an even higher prevalence (23%), underscoring the importance of using population-specific reference values.

We observed a non-significant inverse association between HM mixtures and ppFVC, with As contributing the most negative weight in quantile-based G-computation. This partially aligns with recent studies identifying inverse associations between HM mixtures and lung function. A 2023 cross-sectional study (n=1227) using quantile-based G-computation found an overall inverse association between a urinary mixture of twelve HM (including As, Pb, and Cd) and lung function (FEV_1, FVC, $FEF_{25\text{-}75\%}$, and PEF), with Pb contributing most to impairment.[33] Similarly, a Mexican cohort study (n=291) using Weighted quantile sum regression (WQSR) found that perinatal exposure to a mixture of seven HM (including As, Cd, Mn, and Pb) measured in deciduous teeth, negatively impacted FVC z-scores, with Cd (41%) and Mn (30%) as the primary contributors.[35] Findings from NHBCS (n=316) showed an inverse association between a maternal urinary HM mixture (including As, Pb, and Cd) and FVC or FEV_1 z-scores using WQSR, with Cu, antimony (Sb), and As contributing most to lung function decline.[34] Differences in study populations, exposure levels, biomarkers of exposure, and statistical modeling approaches likely account for discrepancies with our findings.

Our observed association between hair ln-As and nasal symptoms suggestive of allergic rhinitis is consistent with two recent cohort studies from Taiwan (n=261) and China (n=609), which reported positive associations between maternal urinary inorganic As during pregnancy and allergic rhinitis in childhood (OR = 2.26; 95% CI 1.20, 4.26 in Taiwan;[58] OR=2.04; 95% CI 1.35, 3.07 in China).[59] The positive—although non-significant—association we found between ln-As and wheezing aligns with the 2013 Bangladeshi cohort study, which found a significant association between water As and wheezing (OR 8.41, 95% CI 1.66, 42.6) based on the ISAAC questionnaire,[19,32] and with a US cohort study that found a marginally significant positive association between maternal urinary As and wheezing lasting ≥2 days in the first year of life (RR 1.3, 95% CI 1.0, 1.7).[18] The Bangladeshi study also reported a significant association between water As and nighttime cough (OR 2.5, 95% CI 1.12, 5.69), which is consistent in direction but not in magnitude with our results.[19] Additionally, this study found non-significant positive associations with exercise-induced wheezing (OR 1.91, 95% CI 0.76, 4.79) and dyspnea (OR 3.86, CI 1.09-13.7),[19] which contrasts with our observed null association with exercise-induced wheezing and non-significant inverse association with dyspnea.

5.3. Limitations and strengths

Limitations

Temporality. Temporal variability may affect our findings, as hair samples were collected during the summer, affecting our ability to assess seasonal variations in HM exposure. In Tumbes, the summer overlaps with the rainy season, during which agricultural runoff and changes in water sources may increase exposure to HM, especially through contaminated water and soils. To measure HM exposure, we used the proximal 3 cm of hair, which reflects approximately 3 months of prior exposure, limiting our ability to capture exposure patterns throughout the year. Thus, it is likely that our sampling captured a period of peak exposure, which may limit the generalizability of our results to other seasons. Additionally, because our study is cross-sectional, the exposure and outcomes were measured at the same point in time, raising the potential for reverse causality—i.e., that impaired respiratory health could influence hair HM levels rather than result from them. However, to our knowledge, no physiological mechanism has been described that would support this possibility. Future longitudinal studies are warranted to address these temporal limitations.

Measurement bias. We identified three potential sources of measurement bias. First, while hair As could serve as a useful biomarker for chronic exposure to As,[43,44] especially if we want to isolate the effects of inorganic As,[60] hair is a less reliable biospecimen for other HM (e.g., Pb, Cd, and Mn).[44,60] External contamination from environmental sources or cosmetic products may have led to exposure misclassification.[12,43,60] This concern is particularly relevant given the variable access to proper washing facilities in some communities.[40] However, our standardized washing protocol for hair samples was designed to minimize these effects.

Second, the lack of lung function reference equations for Peruvian children posed a challenge to measuring the main outcome. While GLI race-neutral equations were designed for broader applicability,[49] their development was based on predominantly White populations, limiting generalizability to our study population.[48] Sensitivity analyses using Pérez-Padilla equations[51]—developed for Latin American children but based on a high-altitude population—showed a higher prevalence of restrictive patterns than GLI equations. This suggests that GLI may lead to an underestimation of restrictive patterns because they predict higher lung function values, making it less likely for children to fall below the LLN for FVC. Conversely, Pérez-Padilla may underestimate lung function in our low-altitude population due to altitude-related physiological differences.

Third, respiratory symptoms were assessed using the ISAAC questionnaire, a validated tool for identifying asthmatic and allergic rhinitis symptoms,[32,61,62] which has been widely used in rural Latin American populations.[63,64] However, its reliance on self-reported measures introduces the potential for outcome misclassification. Given that all these potential sources of misclassification are likely non-differential, we theorize they would bias associations towards the null, potentially explaining our non-significant results.

Selection bias. Selection bias may have occurred if families who were both aware of HM exposure and had children with respiratory symptoms were more likely to participate in the study. This could have resulted in the underrepresentation of unexposed individuals with better lung function, potentially biasing the association toward the null. CGH's recruitment strategy led to high participation (>95%), minimizing concerns about selection bias.

Residual confounding. While we accounted for household tobacco smoke exposure, we did not assess participants' smoking status. However, this is likely a minor issue given the pediatric population and low national smoking prevalence.[65] Smoking and air pollution—another unmeasured confounder—may be positively associated with HM exposure and inversely associated with overall health, acting as positive confounders and possibly biasing our results away from the null.

Strengths

Despite these limitations, this study has notable strengths, including the use of standardized approaches to measure hair HM and lung function. It is one of the first to evaluate the impact of As and HM mixtures on the respiratory health of children in Peru. Lung function was measured using different metrics, allowing for a more comprehensive evaluation. Additionally, a systematic approach was used to adjust for confounders, and advanced approaches, like quantile-based G-computation, were used to model multiple HM exposures,[54] effectively estimating causal effects while providing a more robust assessment of their impact on lung function.

5.4. Generalizability

This study's findings are generalizable to children living in the Tumbes region and may also extend to children in non-mining communities of northern Peru. However, the results may not be directly applicable to adults, children living in high-altitude regions, or those in areas with distinct dietary and environmental exposure patterns. Variations in lifestyle factors, co-exposures, and healthcare access between the Tumbes region and other populations could influence the broader generalizability of our findings.

5.5. Conclusion

This study indicates a link between hair As exposure and allergic rhinitis in this population, and suggests an inverse association with ppFVC, along with positive associations with abnormal spirometry patterns, wheezing, and nighttime cough. Although exposure levels were lower than those reported in other studies, such as those from Bangladesh, the observed associations suggest that lung function may still be affected at relatively low levels of As and HM exposure. Given that this exposure occurs during a critical developmental period, its effects on lung function could have substantial long-term consequences. This underscores the need for further research to explore these associations longitudinally, considering seasonal variations in exposure, potential dose-response or threshold effects, and the long-term respiratory consequences of chronic HM exposure. Additionally, this population exhibited a higher-than-expected prevalence of restrictive spirometric patterns, surpassing the levels observed in healthy pediatric populations. This highlights the need to investigate the underlying causes of lung function impairment in this group. Furthermore, a substantial difference in the prevalence of restrictive patterns was noted when using the GLI and Pérez-Padilla reference equations, suggesting that future studies should focus on developing population-specific reference equations for Peruvian children living at sea level to ensure more accurate assessments.

6. TABLES AND FIGURES

Table 1. Grading system for FEV_1 and FVC (graded separately)			
Grade	**Number of measurements**	**Repeatability***	
		>6 years old	**≤6 years old**
A	≥3 acceptable	Within 150 ml	Within 100 ml**
B	2 acceptable	Within 150 ml	Within 100 ml**
C	≥2 acceptable	Within 200 ml	Within 150 ml**
D	≥2 acceptable	Within 250 ml	Within 200 ml**
E	≥2 acceptable OR 1 acceptable AND ≥1 usable	>250 ml	>200 ml
U	0 acceptable AND ≥1 usable	NA	NA
F	0 acceptable and 0 usable	NA	NA

* The repeatability criteria are applied to the differences between the two largest FVC values and the two largest FEV_1 values.
** Or 10% of the highest value, whichever is greater; applies for ≤6 years only.
FEV_1: Forced expiratory volume in the first second; FVC: Forced vital capacity
Adapted from Graham et al.[46]

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Table 2. Characteristics of 399 children 4-17 years old living in Tumbes, Peru in 2023 by hair ln-Arsenic quartiles					
	Q1 (n=100)	**Q2** (n=100)	**Q3** (n=100)	**Q4** (n=99)	**Overall** (n=399)
Hair arsenic, µg/kg, mean (SD)	50.5 (26.3)	184 (56.7)	477 (129)	2810 (2990)	874 (1860)
Hair lead, mg/kg, mean (SD)	1.0 (1.3)	2.6 (2.8)	4.5 (5.7)	16.9 (26.1)	6.2 (14.8)
Hair cadmium, µg/kg, mean (SD)	123 (153)	254 (355)	566 (1400)	708 (1290)	412 (994)
Hair manganese, mg/kg, mean (SD)	4.0 (5.7)	7.7 (12.3)	17.1 (57.0)	27.6 (46.5)	14.1 (38.4)
Age, years, mean (SD)	9.3 (3.6)	10.3 (4.2)	10.7 (3.8)	10.5 (3.8)	10.2 (3.9)
Female gender, n (%)	40 (40.0)	43 (43.0)	55 (55.0)	73 (73.7)	211 (52.9)
Parent or guardian's occupation					
Housewife, n (%)	1 (1.0)	3 (3.0)	3 (3.0)	3 (3.0)	10 (2.5)
Service sector, n (%)	27 (27.0)	29 (29.0)	28 (28.0)	29 (29.3)	113 (28.3)
Fishing or Agriculture, n (%)	69 (69.0)	62 (62.0)	64 (64.0)	63 (63.6)	258 (64.7)
Construction or Mining, n (%)	3 (3.0)	6 (6.0)	5 (5.0)	4 (4.0)	18 (4.5)
Non-durable house material, n (%)	47 (47.0)	54 (54.0)	45 (45.0)	60 (60.6)	206 (51.6)
Second-hand tobacco smoke, n (%)	9 (9.0)	9 (9.0)	11 (11.0)	13 (13.1)	42 (10.5)
Wood stove, n (%)	52 (52.0)	37 (37.0)	47 (47.0)	48 (48.5)	184 (46.1)
Burning trash, n (%)	24 (24.0)	28 (28.0)	28 (28.0)	36 (36.4)	116 (29.1)
Burning fields, n (%)	57 (57.0)	49 (49.0)	44 (44.0)	45 (45.5)	195 (48.9)
Fish consumption frequency					
≤ 1 time/week, n (%)	19 (19.0)	18 (18.0)	21 (21.0)	24 (24.2)	82 (20.6)
3 times/week, n (%)	73 (73.0)	67 (67.0)	68 (68.0)	68 (68.7)	276 (69.2)
Daily, n (%)	8 (8.0)	15 (15.0)	11 (11.0)	7 (7.1)	41 (10.3)
Fish consumption from the Puyango-Tumbes River, n (%)	23 (23.0)	29 (29.0)	33 (33.0)	23 (23.2)	108 (27.1)
SD: Standard deviation					

Table 3. Crude and adjusted coefficients for the association between hair ln-Arsenic and percent predicted Forced Vital Capacity (FVC) in 399 children 4-17 years old living in Tumbes, Peru in 2023

	Crude model		**Adjusted model***	
Variable	**Coefficient**	**95% CI**	**Coefficient**	**95% CI**
ln-Arsenic	0.320	-0.94, 1.58	-0.09	-1.30, 1.11
Age	-	-	2.16	1.72, 2.61
Gender				
Female	-	-	Reference	Reference
Male	-	-	-0.95	-4.51, 2.62
Second-hand tobacco smoke				
No	-	-	Reference	Reference
Yes	-	-	-0.77	-6.69, 5.14
Stove type				
Gas	-	-	Reference	Reference
Wood	-	-	-0.69	-4.48, 3.10
Burning fields				
No	-	-	Reference	Reference
Yes	-	-	-0.38	-4.26, 3.50
Burning trash				
No	-	-	Reference	Reference
Yes	-	-	2.21	-1.65, 6.08
Parent of guardian's occupation				
Housewife	-	-	Reference	Reference
Service sector	-	-	-6.31	-17.63, 5.00
Fishing/Agriculture	-	-	-9.54	-20.58, 1.51
Construction/Mining	-	-	-8.32	-21.74, 5.09
House material				
Durable	-	-	Reference	Reference
Not durable	-	-	-1.28	-4.80, 2.25
Fish consumption from the Puyango-Tumbes				
No	-	-	Reference	Reference
Yes	-	-	0.71	-3.32, 4.73
Fish consumption frequency				
≤1 time/week	-	-	Reference	Reference
3 times/week	-	-	3.90	-0.52, 8.33
Daily	-	-	5.61	-1.12, 12.34

*Adjusted for age in years, gender, second-hand tobacco smoke, stove type, burning fields, burning trash, parental occupation, house material, fish consumption from the Puyango-Tumbes River, and fish consumption frequency
CI: Confidence Interval

Table 4. Adjusted coefficients* and 95% confidence intervals (CI) for the association between hair ln-Arsenic (ln-As) quartiles and percent predicted Forced Vital Capacity (FVC) in 399 children 4-17 years old living in Tumbes, Peru in 2023		
Hair ln-As quartiles	**Coefficient**	**95% CI**
Q1	Reference	Reference
Q2	-0.103	-5.07, 4.86
Q3	1.638	-3.33, 6.61
Q4	-0.629	-5.94, 4.68
*Adjusted for age in years, gender, second-hand tobacco smoke, stove type, burning fields, burning trash, parental occupation, house material, fish consumption from the Puyango-Tumbes River, and fish consumption frequency		

Table 5. Crude and adjusted odds ratios (OR) and 95% confidence intervals (CI) for the association between ln-Arsenic and spirometry pattern and respiratory symptoms in 399 children 4-17 years old living in Tumbes, Peru in 2023				
	Crude models		**Adjusted models***	
Outcome for each model	**OR**	**95% CI**	**OR**	**95% CI**
Spirometry pattern**				
Suggestive of restriction	1.06	0.88, 1.29	1.17	0.94, 1.46
Obstruction	1.09	0.88, 1.35	1.13	0.88, 1.45
Abnormal spirometry pattern	1.07	0.92, 1.25	1.15	0.97, 1.37
Wheezing	1.09	0.90, 1.32	1.11	0.90, 1.39
Dyspnea	0.96	0.76, 1.24	0.90	0.69, 1.18
Nighttime cough	1.00	0.83, 1.19	1.04	0.85, 1.28
Wheezing with exercise	1.00	0.79, 1.29	0.98	0.75, 1.28
Nasal symptoms	1.40***	1.19, 1.64	1.59***	1.32, 1.91
*All models were adjusted for age in years, gender, second-hand tobacco smoke, stove type, burning fields, burning trash, parental occupation, house material, fish consumption from the Puyango-Tumbes River, and fish consumption frequency **For this model, the categories "housewife" and "service sector" were merged in the occupation variable due to sparse data in the "housewife" and "restriction" category, which led to unstable estimates ****P*-value <0.05				

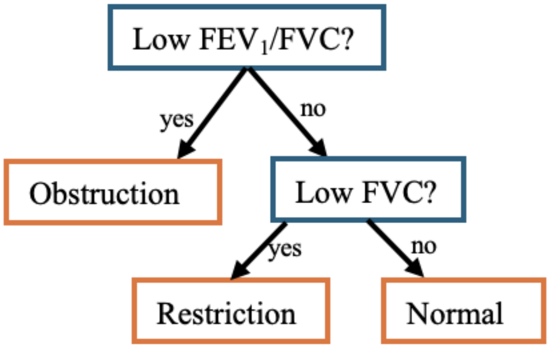

Figure 1 Decision algorithm for classifying spirometry test results. The algorithm first evaluates FEV_1/FVC ratio. If the FEV_1/FVC ratio is low, the test is classified as obstructive; if it is not low, the algorithm proceeds to evaluate FVC. If FVC is low, the spirometry is suggestive of restriction; if FVC is not low, the spirometry is categorized as normal. FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity. Adapted from Vázquez-García and Pérez-Padilla, 2018

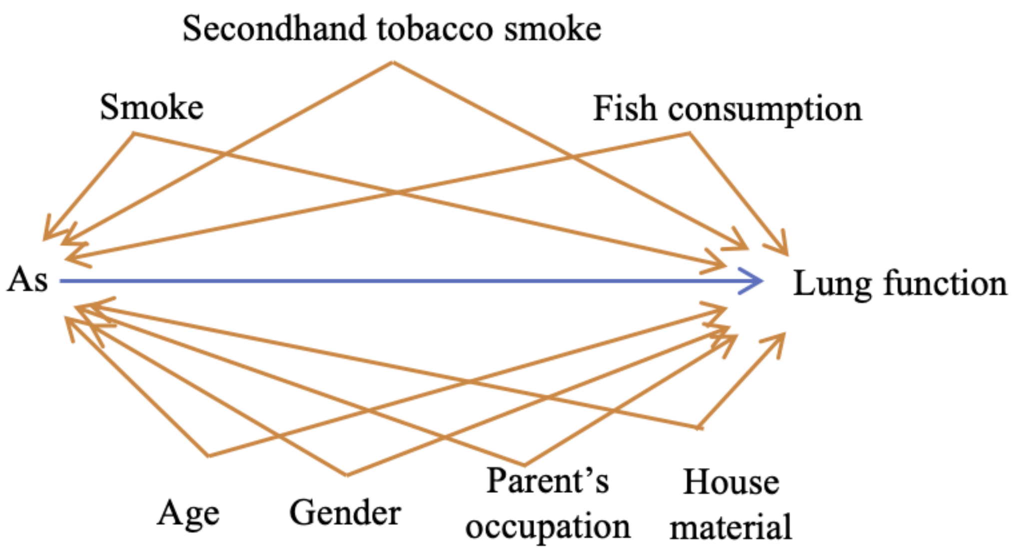

Figure 2 Directed acyclic graph for the association between arsenic (As) and lung function. The blue arrow represents the main association, and the orange arrows represent confounding.

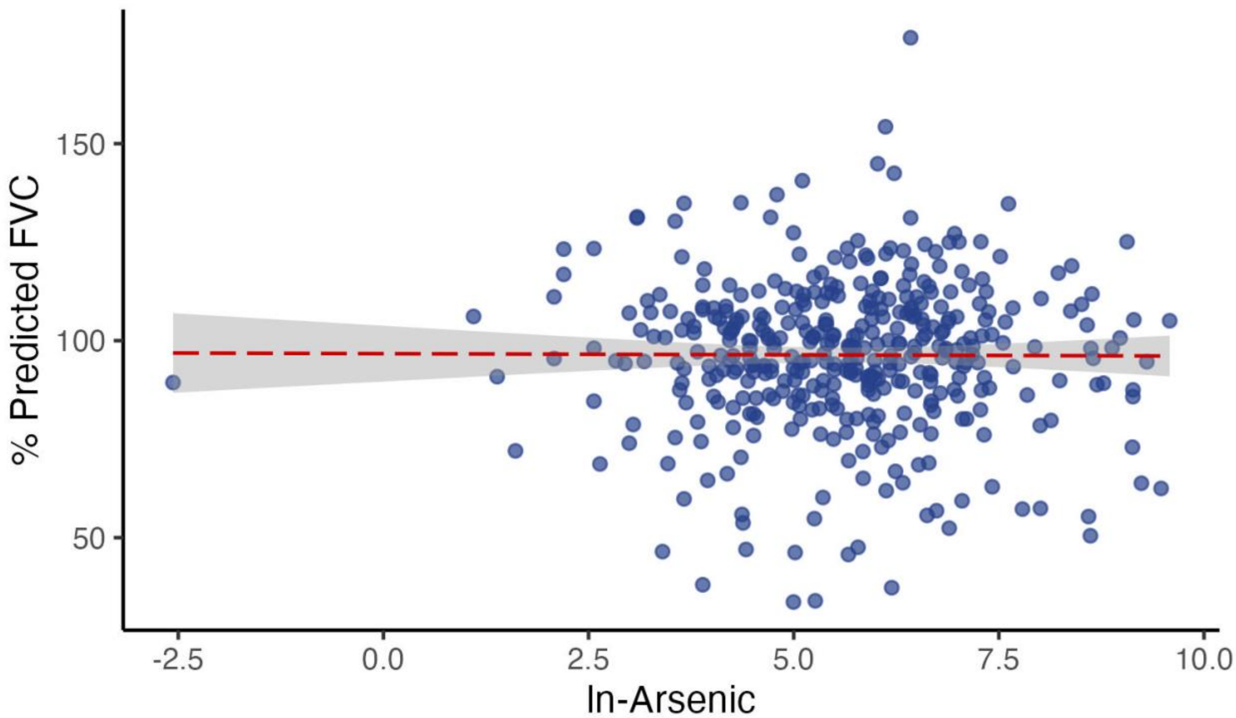

Figure 3 Scatter plot of hair ln-Arsenic vs. % predicted forced vital capacity (FVC) in 399 children 4-17 years old living in Tumbes, Peru in 2023. Each point represents an individual participant. The red dashed line shows linear regression fits with 95% confidence intervals (gray shading).

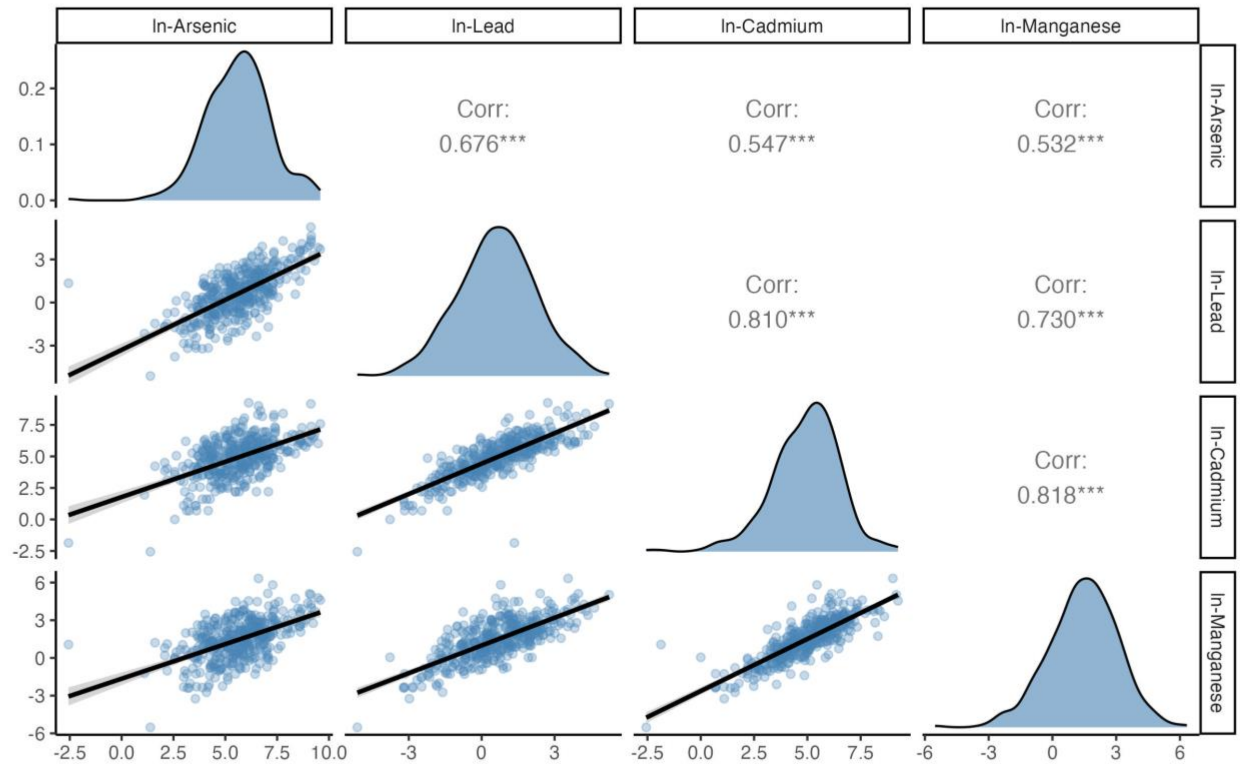

Figure 4 Pairwise correlations between log-transformed hair arsenic, lead, cadmium, and manganese in 399 children 4-17 years old living in Tumbes, Peru in 2023. The upper diagonal shows Pearson correlation coefficients with significance levels ($^{***}p<0.001$). Lower diagonal displays corresponding scatter plots with fitted regression lines and 95% confidence intervals (blue shading). The diagonal shows density distributions of each HM concentration.

7. SUPPLEMENTAL TABLES AND FIGURES

Table S1. Characteristics of 346 children 6-17 years old living in Tumbes, Peru in 2023 by hair ln-Arsenic quartiles

	Q1 (n=87)	**Q2** (n=87)	**Q3** n=86)	**Q4** (n=86)	**Overall** (n=346)
Hair Arsenic, µg/kg, mean (SD)	50.2 (26.7)	186 (54.5)	492 (132)	2720 (2960)	859 (1830)
Hair Lead, mg/kg, mean (SD)	0.91 (1.2)	2.56 (2.8)	4.49 (5.8)	15.70 (20.3)	5.90 (12.1)
Hair Cadmium, µg/kg, mean (SD)	104 (126)	242 (309)	482 (1050)	644 (983)	367 (761)
Hair Manganese, mg/kg, mean (SD)	3.4 (4.5)	8.1 (12.9)	16.8 (60.7)	26.6 (45.1)	13.7 (39.2)
Age, years, mean (SD)	10.2 (3.2)	11.0 (3.8)	11.6 (3.1)	11.5 (3.2)	11.1 (3.4)
Female gender, n (%)	34 (39.1%)	42 (48.3%)	45 (52.3%)	62 (72.1%)	183 (52.9%)
Parent or guardian's occupation					
Housewife	1 (1.1%)	3 (3.4%)	3 (3.5%)	2 (2.3%)	9 (2.6%)
Service sector	24 (27.6%)	25 (28.7%)	24 (27.9%)	27 (31.4%)	100 (28.9%)
Fishing or Agriculture	59 (67.8%)	54 (62.1%)	56 (65.1%)	53 (61.6%)	222 (64.2%)
Construction or Mining	3 (3.4%)	5 (5.7%)	3 (3.5%)	4 (4.7%)	15 (4.3%)
Non-durable house material, n (%)	38 (43.7%)	46 (52.9%)	38 (44.2%)	50 (58.1%)	172 (49.7%)
Second-hand tobacco smoke, n (%)	7 (8.0%)	6 (6.9%)	9 (10.5%)	12 (14.0%)	34 (9.8%)
Wood stove, n (%)	47 (54.0%)	32 (36.8%)	42 (48.8%)	41 (47.7%)	162 (46.8%)
Burning trash, n (%)	21 (24.1%)	25 (28.7%)	27 (31.4%)	28 (32.6%)	101 (29.2%)
Burning fields, n (%)	50 (57.5%)	43 (49.4%)	37 (43.0%)	42 (48.8%)	172 (49.7%)
Fish consumption frequency					
≤1 time/week	16 (18.4%)	18 (20.7%)	19 (22.1%)	21 (24.4%)	74 (21.4%)
3 times/week	62 (71.3%)	56 (64.4%)	57 (66.3%)	59 (68.6%)	234 (67.6%)
Daily	9 (10.3%)	13 (14.9%)	10 (11.6%)	6 (7.0%)	38 (11.0%)
Fish consumption from the Puyango-Tumbes River, n (%)	20 (23.0%)	24 (27.6%)	27 (31.4%)	20 (23.3%)	91 (26.3%)
SD: Standard deviation					

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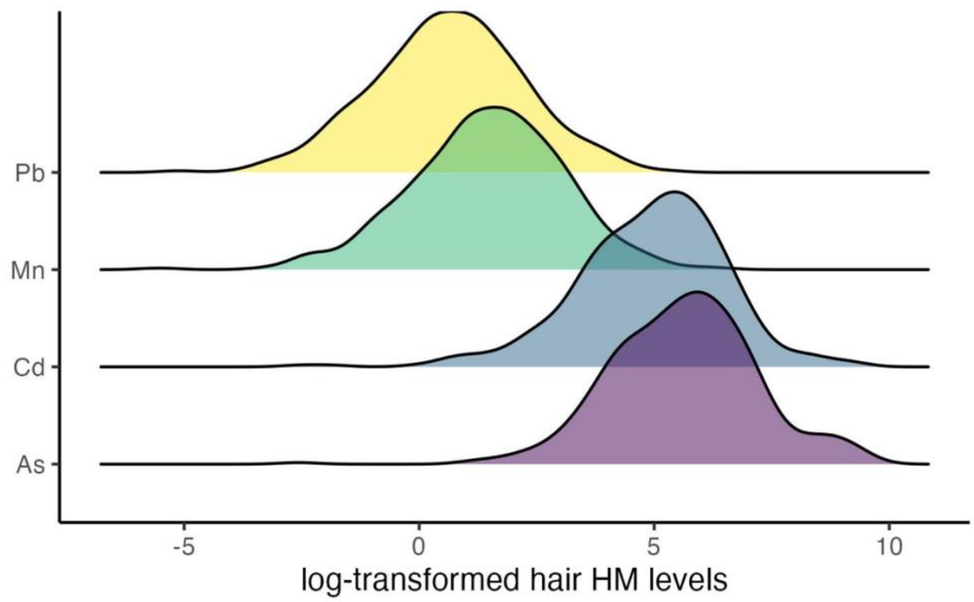

Figure S1. Density plots of the distribution of log-transformed hair heavy metals (HM) in 399 children, 4-17 years old, living in Tumbes, Peru, in 2023.

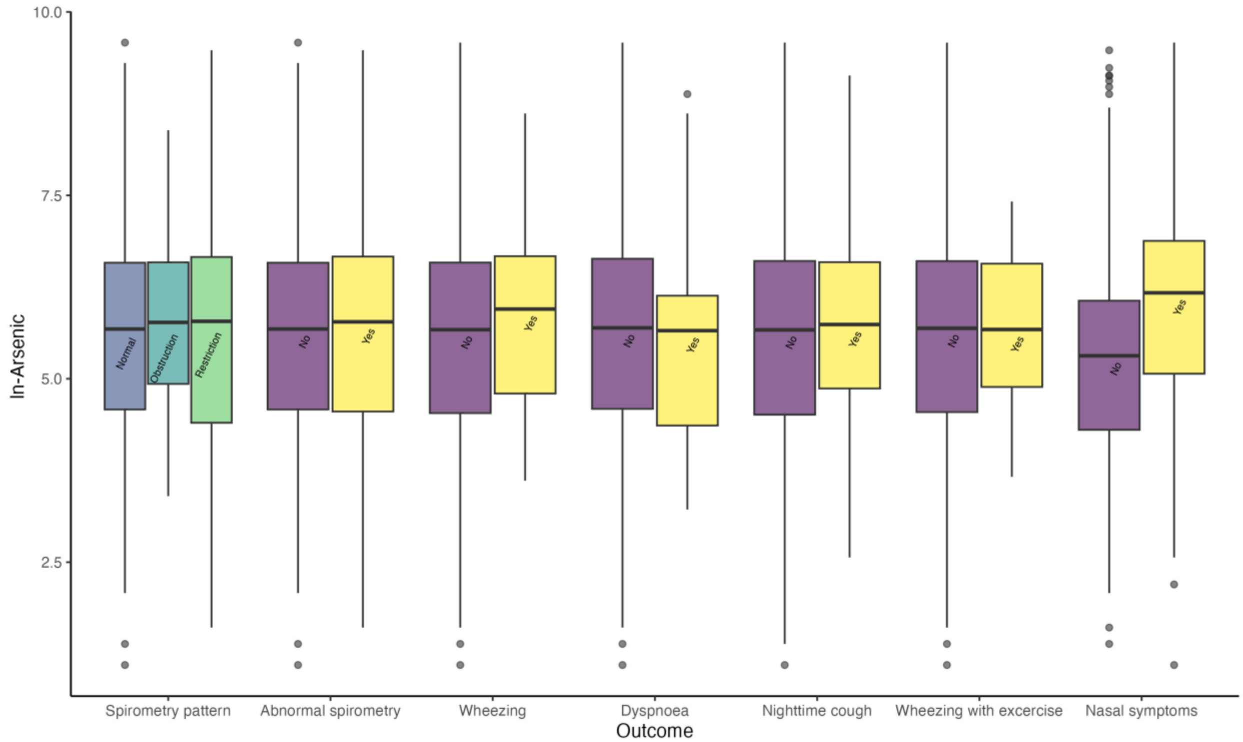

Figure S2. Box plots showing distributions of log-transformed hair arsenic concentrations (ln-As) by spirometry patterns and respiratory symptoms in 399 children aged 4-17 years from Tumbes, Peru (2023). Each box shows the interquartile range (IQR) with the lower and upper edges indicating the 25th and 75th percentiles, respectively. The line inside the box represents the median. Whiskers extend to the smallest and largest values within 1.5 times the IQR from the lower and upper quartiles, and gray dots represent individual observations that fall outside this range. Note: One extremely low ln-As observation (<-2) was excluded to improve figure readability.

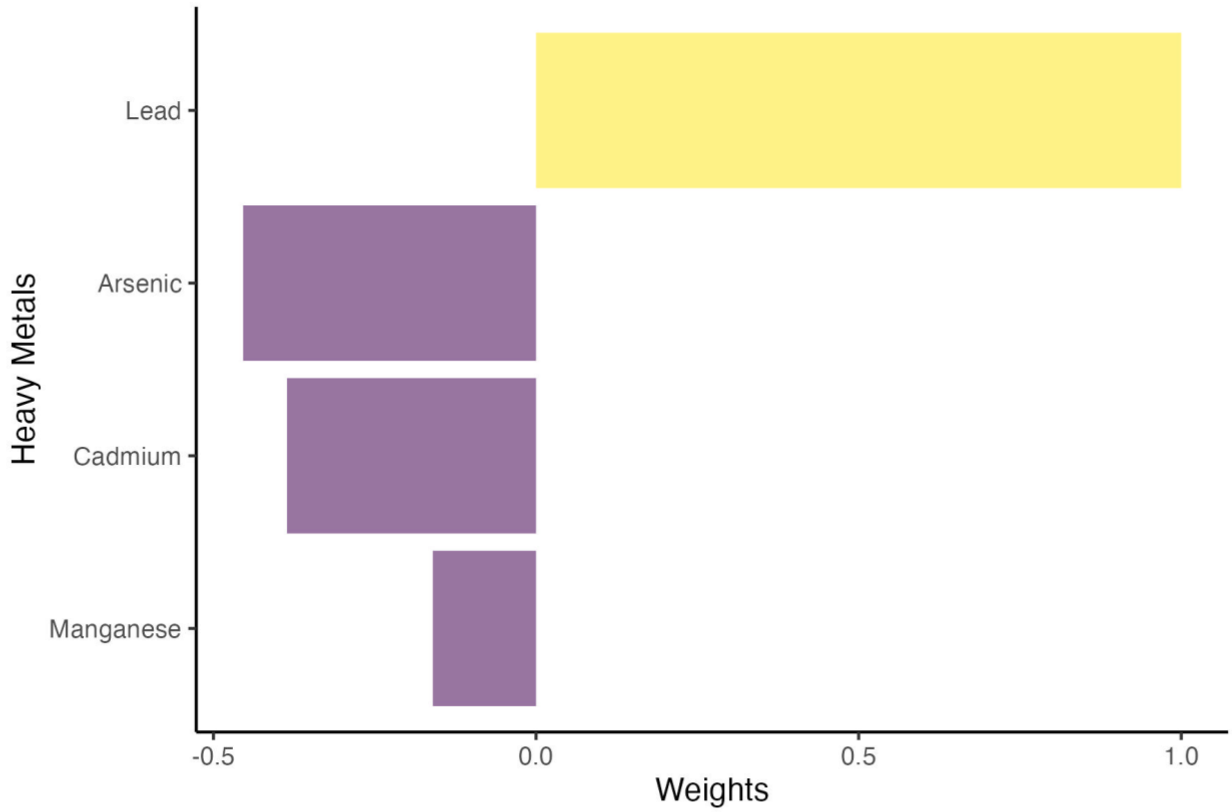

Figure S3 Effect size estimates from quantile-based g-computation modeling the association between a mixture of heavy metals (arsenic, lead, cadmium, and manganese) and percent predicted forced vital capacity (ppFVC) in 399 children 4-17 years living in Tumbes, Peru (2023) adjusting for age, gender, second-hand tobacco smoke exposure, stove type, burning fields, burning trash, parental occupation, house material, fish consumption from the Puyango-Tumbes River, and fish consumption frequency. The figure displays the relative contribution (weights) of each metal to the overall effect of the mixture on ppFVC. Positive weights are displayed as yellow bars, while negative weights are displayed as purple bars.

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CHAPTER 5. RESEARCH PAPER #3

Association of biomarker-derived heavy metal exposures with childhood anemia in Tumbes, Peru

1. ABSTRACT

Background: Heavy metal (HM) exposure is a global health concern, particularly in low- and middle-income countries (LMICs) affected by gold mining pollution. In underregulated regions, HM contamination of water and food threatens children's health, who are particularly vulnerable due to ongoing organ development. The Puyango-Tumbes River, Tumbes, Peru's main freshwater source, often exceeds the Environmental Protection Agency's thresholds for multiple HM, including lead (Pb), arsenic (As), and cadmium (Cd), potentially from upstream Ecuadorian gold mining. Pb disrupts hemoglobin (Hb) synthesis and iron metabolism, contributing to anemia. However, the combined impact of Pb and other HM is understudied. Lower ferritin levels previously observed among children near the Puyango-Tumbes River suggest impaired iron storage may mediate the Pb-anemia association. This cross-sectional study examined the association of Pb and other HM exposures from hair samples, both individually and as a mixture, with clinically assessed anemia in Tumbes children and assessed the causal mediation effects of ferritin in the Pb-anemia association.

Methods: We analyzed data from 404 children (4–17 years) from 14 Tumbes villages (January-March 2023). Questionnaires, hair, and blood samples were collected. Hair samples were analyzed using Inductively Coupled Plasma Mass Spectrometry to assess internalized Pb, As, Cd, and manganese exposure (~3-month window). Anemia status was assessed using blood Hb levels and further classified by red blood cell indices and serum biomarkers (iron, ferritin, folic acid, and vitamin B12). Associations between Pb and HM exposures and anemia were modeled using linear regression models and quantile-based G-computation, adjusting for sociodemographic and behavioral confounders. Causal mediation analysis using natural effects models tested whether ferritin mediated the Pb–anemia association.

Results: The prevalence of anemia was 11.4%. All children with anemia had iron deficiency but normal or elevated ferritin levels. The adjusted odds of anemia for each unit increase in the natural logarithm of lead (mg/kg) were 1.31 (95% CI 1.05, 1.65). In the HM mixture model assuming additivity, each quartile increase in the combined metals was associated with a 55% increase in the odds of having anemia (OR 1.55; 95% CI 1.02, 2.08). Estimates from models including all pairwise metal-metal interactions were weaker (OR 1.17; 95% CI 0.98, 2.34), likely due to Cd's antagonistic effects on the other metals. We found no evidence supporting ferritin as a mediator of the Pb-anemia association, suggesting alternative biological pathways may be involved.

Conclusions: Exposure to Pb—both alone and as part of a mixture with other HMs—is associated with increased odds of anemia in children from Tumbes, Peru. The mixture effect was stronger than the effect of Pb alone, suggesting that children with simultaneous exposure to multiple HMs may face an increased risk of anemia. The lack of mediation by ferritin indicates that mechanisms beyond iron depletion—such as inflammation or impaired iron transport—may explain this association.

2. INTRODUCTION

Anemia is a widespread global health issue that impairs oxygen delivery throughout the body, contributing to both short- and long-term morbidity and mortality.[1,2] In 2021, an estimated 25% of the global population had anemia,[2] with children under five years old being disproportionately affected.[2] The burden of anemia is especially high in low- and middle-income countries (LMICs), including Peru, where

43.1% of children aged 6–25 months had anemia in 2023,[3] qualifying as a severe public health problem according to the World Health Organization (WHO) criteria.[4]

Iron deficiency is the most common cause of anemia globally,[2] resulting from inadequate intake, impaired gastrointestinal absorption, or increased physiological requirements.[4] However, emerging evidence also points to environmental exposures—particularly to heavy metals (HM)—as an important, underrecognized contributor to anemia.[5–8] HM, such as lead (Pb), arsenic (As), cadmium (Cd), and manganese (Mn), can induce anemia through several mechanisms, including enzyme inactivation (e.g., glutathione peroxidase and reductase), oxidative stress, and weakening of the antioxidant defense system.[9–11] These metals can impair hematologic function by directly damaging the bone marrow, inhibiting enzymes involved in cell division and maturation (erythropoiesis), disrupting hemoglobin (Hb) synthesis, impairing erythrocyte transport, promoting immune-mediated hemolysis, and interfering with iron absorption.[5,12–16]

Pb is one of the most studied HM in this context.[5,17] Although some research from LMICs has not found an association between Pb and anemia,[18,19] multiple studies across diverse populations support this link.[7,20–25] Pb disrupts heme biosynthesis by inhibiting key enzymes such as delta-aminolevulinic acid dehydratase (ALAD) and ferrochelatase,[5,9,10] impairs erythrocyte membrane integrity,[10] promotes oxidative stress through accumulation of aminolaevulinic acid, and induces hemolysis by inhibiting phosphoribosyl transferases.[26]

Importantly, individuals are often co-exposed to multiple HMs,[27] and animal studies suggest that these metals may exert additive hematotoxic effects—where the combined toxicity equals the sum of individual effects—or even synergistic effects, in which the combined toxicity is greater than the sum of individual effects.[28,29] However, the extent to which combined HM exposures contribute to anemia in human populations remains unclear. Studies exploring this association in children are limited, conducted in varied settings, and have produced conflicting results.[30–32]

The Tumbes region of Peru, located downstream of the Puyango-Tumbes River, is an area of concern due to ongoing contamination from upstream gold mining activity in Ecuador.[33] Previous research has shown that children living closer to the river have higher levels of hair Pb, As, and Mn, and lower serum ferritin levels—an iron-storage biomarker—than those living farther away.[34,35] These findings raise concern that chronic environmental exposure to HM may be contributing to deficient iron storage and anemia in this population.[21,36–38] However, the extent to which HM exposure is linked to ferritin depletion and anemia in this population remains unclear.

Accordingly, this study aims to investigate the association between hair concentrations of Pb—alone and in combination with As, Cd, and Mn—and anemia in children living in Tumbes, Peru. We hypothesize that higher concentrations of Pb and HM mixtures will be associated with increased odds of anemia. In addition, this study aims to evaluate whether ferritin levels mediate the association between Pb exposure and anemia. Findings from this research will contribute critical evidence to our understanding of the health effects of HM exposure and support future efforts to reduce exposure and its consequences in children.

3. METHODS

3.1. Study setting and design

The Puyango-Tumbes River, which flows from gold mining areas in Ecuador into northern Peru, is heavily contaminated with HM (As, Pb, Cd), often exceeding the safety limits established by the US Environmental Protection Agency (USEPA) at multiple points.[33,39] This contamination affects water, sediment, and food sources,[40] posing ongoing health risks—especially to children. In the Tumbes region—home to 260,000 people[41] who face substantial socioeconomic challenges[42]—many residents rely on inadequately treated river water,[43] further increasing their vulnerability to HM exposure.

We conducted this cross-sectional study between January to March 2023 in the Tumbes province of Peru, focusing on children under 18 years of age. Fourteen villages were purposively selected from four regions based on their proximity to the Puyango-Tumbes River to capture a range of HM exposure levels—some located within the river's watershed (0.6-3.4 km from the river), and others were farther away (20.5-27.5 km). This study was part of a larger investigation evaluating the association between HM exposure and lung function. Thus, eligible participants were children aged ≥4 years who were able to perform forced spirometry.[44,45] Children were excluded if they were unable to complete spirometry or declined to provide a hair or blood sample.

3.2. Data collection

The Center of Global Health (CGH) research team enrolled a total of 409 children. Data collection took place at participants' homes, where informed consent was obtained prior to participation. CGH staff collected hair samples, recorded household global positioning system (GPS), took blood samples, and administered a structured questionnaire covering demographic, health, and behavioral information. To enhance response rates, the team allowed for up to three follow-up visits if families were not available during the initial contact. Close coordination with local authorities was essential to build trust, facilitate community entry, and encourage participation across the selected sites.

Exposures: Hair lead (Pb) and HM mixture

Hair Pb (mg/kg) served as the primary exposure variable, with additional metals—As (µg/kg), Cd (µg/kg), and Mn (mg/kg)—also measured for the mixture analysis. Trained staff from CGH personnel collected three hair samples (~100 strands each) from the occipital region using stainless steel scissors, cutting as close to the scalp as possible. Samples were stored at room temperature; two were shipped to the Elemental Analysis Core Lab at Oregon Health & Science University (OHSU) for metal analysis, and one was stored at CGH. The proximal 3 cm of hair (~3 months of exposure) was processed.[46] Following standard protocols, samples were washed, then 200 µl of concentrated Nitric acid (HNO_3) was added, and samples were heated to 90°C for 45 minutes. After cooling, 1% HNO_3 was added to reach a total volume of 2000 µl, followed by digestion at room temperature for 12 hours. HM levels were measured using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) with Agilent 8900 triple quad equipped with a Sample Preparation System (SPS) autosampler. Data were quantified using weighed, serial dilutions of a multi-element standard for Mn and Pb, and single-element standards for Cd and As, with a 12-point calibration curve. Measurements were performed in triplicate and averaged. Precision was assessed via the coefficient of variation, while internal standards (scandium, germanium, bismuth) corrected for detector fluctuations and monitored plasma stability. Controls and standards, including National Institute of Standards and Technology (NIST) SRM 1643f, NIST SRM 1683f (8x dilution), and Bovine Liver NIST SRM 1577c, ensured accuracy. Recovery for NIST water and bovine liver SRMs ranged from 83-112%, with spikes and repeats within 5% of expected values, indicating robust analytical performance.

Outcome: anemia

We defined anemia (yes/no) according to WHO criteria as Hb<10.9 g/dL for children <5 years old, <11.4 g/dL for children 5-11 years, <11.9 g/dL for children 12-14 years old and girls ≥15 years old, and <12.9 g/dL for boys ≥15 years old.[47] CGH nursing technicians collected 10 mL of peripheral venous blood from the upper extremity of each participant using a 21-gauge butterfly needle. Two milliliters of this blood were drawn into BD Vacutainer tubes containing K2 ethylenediaminetetraacetic acid (EDTA) anticoagulant, and the remaining 8 mL were drawn into BD Vacutainer serum collection tubes with clot activators. The samples were placed on ice in coolers and transported on the same day to the local health authorities' (DIRESAT) main laboratory, where they were refrigerated until processing. DIRESAT's personnel analyzed the whole blood in the lavender top vial using a semi-automated hematology analyzer (Prokan PE-6100, Guangdong, China) to obtain Hb levels (g/dL), mean corpuscular volume (MCV) in femtoliters (fL), and mean corpuscular Hb concentration (MCHC) in picograms (pg). Blood from the red top vial was centrifuged to separate the serum, which was then analyzed with a biochemical analyzer (iFlash ImmunoAssay Analyzer) to measure ferritin (µg/L) for all participants. Additionally, vitamin B12 (pg/mL), folate (ng/mL), and iron levels (µg/dL) were measured only in those with anemia.

Other covariates

Erythrocyte size was classified as microcytic (MCV<80 fL), normocytic (MCV 80-100 fL), or macrocytic (MCV>100 fL),[48] erythrocyte color was classified as hypochromic (MCHC<32 g/dL), normochromic (MCHC 32-36 g/dL), or hyperchromic (MCHC>36 g/dL),[49] and ferritin levels were classified as low (<12 µg/L for children under 5 years old, and <15 µg/L for children aged 5-17 years), normal (12-150 µg/L for girls under 5 years, 12-200 µg/L for boys under 5 years, 15-150 µg/L for girls 5-17 years, and 15-200 µg/L for boys 5-17 years), or high (>150 µg/L for girls, and >200 µg/L for boys) according to adjusted WHO criteria to permit an exhaustive categorization of all participants.[50] Anemia cases were classified by erythrocyte size and color using the thresholds mentioned above. In addition, we classified cases based on biomarker thresholds: anemia with low serum iron (<50 µg/dL),[51] low serum folate (<2 ng/mL),[52] or low vitamin B12 (<200 pg/mL).[53]

Potential confounders

Based on the literature and using a directed acyclic graph (DAG) (**Figure 1**), we identified potential confounders, which included age (in years), gender (male or female), secondhand smoking (yes or no), smoke exposure from wood stoves (yes or no), field burning (yes or no), and trash burning (yes or no), family occupation (housewife, service sector, fishing/agriculture, and construction/mining) and durable house material (yes or no) as proxies for SES, fish consumption from the river (yes or no), fish consumption frequency (daily, 3 times per week or ≤ 1 time per week), breastfeeding (yes or no), and frequent beetroot and spinach consumption (≥3 or <3 times per week). CGH personnel recorded participants' age and gender using their national identity document (NID) and collected the other variables through a questionnaire administered to the parents or guardians.

3.3 Statistical analysis

Descriptive analysis. In the descriptive analysis, we summarized continuous variables as means and standard deviations (SD) or medians and interquartile ranges (IQR), as appropriate. Categorical variables were summarized as frequencies. Participants with missing values for exposures, outcomes, or confounders were excluded, with missingness below 10% considered acceptable. If missing data were≥10%, we planned to conduct a sensitivity analysis to assess potential selection bias. All HM levels showed a right-skewed distribution and were transformed to their natural logarithm to achieve normal distributions (**Figure S1**). All continuous variables were centered on their mean to improve model stability. Hair Pb was categorized into quartiles to show the distribution of variables across different levels of hair Pb. The mean and SD of Hb, MCV, MCHC, and ferritin, along with the prevalence of anemia, were calculated.

Association models. Crude and adjusted multivariable regression models measured the association between ln-Pb and anemia (dichotomous), yielding odds ratios (OR) and their 95% confidence intervals (CI). We assessed dose-response using multivariable regression models with quartile-categorized ln-Pb to identify monotonic changes in the Pb-anemia association across ln-Pb quartiles. To account for potential clustering at the village level, we assessed the necessity of incorporating random effects by calculating the intraclass correlation coefficient (ICC) and comparing model fit using the Akaike Information Criterion (AIC). When these evaluations indicated a meaningful clustering effect, we included village-level random intercepts in the final models.

Mixture analysis. Pair-wise Pearson correlations among HMs (Pb, As, Cd, and Mn) were calculated to identify those with correlations >0.3 for inclusion in the HM mixture after transforming them into quartiles. We used quantile-based G-computation to estimate the marginal effect of this mixture on anemia, reflecting the change in the probability of anemia per quartile increase in the mixture, while adjusting for confounders. Each HM's contribution to the overall effect was weighted to estimate both the total mixture effect and the individual metal contributions. A nonparametric bootstrap approach was used to calculate the 95% CI.[54,55]

Mediation analysis. To evaluate if the association between Pb and anemia is mediated by ferritin, we conducted a causal mediation analysis to decompose the total effect of Pb exposure into direct and indirect effects. The direct effect (DE) captures the influence of Pb on anemia that is not mediated by ferritin, while the indirect effect (IE) represents the portion of the association that operates through ferritin. We estimated these effects using natural effects models and a weighting-based approach, which allows the mediator (ferritin) to vary naturally, as it would do under different Pb levels. Specifically, we estimated two types of natural direct effects (NDE): the total NDE, defined as the effect of changing the exposure from Pb to Pb^*, while holding ferritin at its natural value under Pb; and the pure NDE, defined as the effect of changing the exposure from Pb to Pb^* while ferritin is held at its natural level under Pb^*. We also estimated two types of natural indirect effects (NIE): the total NIE, which reflects the effect of changing ferritin value from $Ferritin(Pb^*)$ to $Ferritin(Pb)$, while holding the exposure constant at Pb; and the pure NIE to represent the effect of changing ferritin value from $Ferritin(Pb^*)$ to $Ferritin(Pb)$, while holding the exposure constant at Pb^*. Finally, we estimated the total effect (TE) of Pb on anemia **(Table 1)**. The 95% CI was computed using a percentile bootstrap approach.[56] All tests were two-tailed at a significance level of 0.05.

Power. The sample size for this study (n = 404) was originally calculated for a related study using the same population and exposure (HM) but targeting a different outcome (respiratory health). Nevertheless, this sample size provides 85% power to detect a moderate correlation (r = 0.15) between Pb and Hb, indicating that the study is adequately powered to detect a statistically significant association.

Ethics statement

This study was approved by the Institutional Ethics Committee for Humans at the Universidad Peruana Cayetano Heredia and the Institutional Review Board at OHSU.

4. RESULTS

Descriptive analysis. Of the 409 children who met the inclusion criteria and agreed to participate, 4 were excluded due to missing Hb data, and 1 was excluded for missing hair HM data. None of the missing data exceeded 10%. The final analytical sample consisted of 404 children, 52.7% of whom were female, with a mean age (SD) of 10.2 (3.9) years. The median (IQR) of hair HMs were 1.9 (0.6, 5.3) mg/kg for Pb, 291.5 (94.8, 729.0) µg/kg for As, 152.5 (51.8, 376.5) µg/kg for Cd, and 4.5 (1.5, 12.3) mg/kg for Mn. One hundred and five (26.0%) children had hair Pb levels over the recommended levels of 5 mg/kg.[57]

We analyzed the distribution of participant characteristics by hair ln-Pb quartile (**Table 2**). The mean levels of hair HMs (As, Cd, and Mn) increased with higher ln-Pb quartiles. Age distribution was similar across ln-Pb quartiles. The highest ln-Pb quartile (Q4) had the highest proportion of females (73.3%) compared to males. The majority of parents or guardians (64.6%) worked in fishing or agriculture, a pattern consistent across quartiles, while only one participant's parent reported working as a miner. Approximately half (48.3%) of the participants lived in households made of durable materials, 10.4% were exposed to second-hand tobacco smoke, and 46.5% lived in households with wood stoves, with little variation across quartiles for these three variables. Living in houses that burn trash or fields was more common in Q4 (34.7% and 57.4%, respectively). Dietary patterns revealed that fish consumption was frequent across all groups. However, fewer children in Q4 reported consuming fish from the Puyango-Tumbes River compared to Q1 (18.8% vs. 31.7%). Rice consumption was nearly universal, with 98.8% of participants reporting intake at least 3 times per week. Consuming beetroot and spinach three times per week or more was more frequent in Q4 (50.5%). Almost all participants (94%) were breastfed, with little variation across quartiles.

The mean (SD) Hb concentration was 12.8 (1.1) g/dL. For other blood parameters, the mean (SD) values were 81.8 (5.3) fL for MCV, 33.4 (2.9) g/dL for MCHC, and 204.9 (68.2) µg/L for ferritin. A total of 46 children were classified as having anemia, corresponding to a prevalence of 11.4%. MCV values indicated that 138 children (34.2%) had microcytosis, while the remainder had normal MCV; no cases of macrocytosis were observed. Based on MCHC values, erythrocytes were classified as hypochromic in 30 children (7.4%) and hyperchromic in 31 children (7.7%). None of the participants had low ferritin levels; however, 283 children (70%) had high ferritin concentrations.[50]

When anemia was categorized by erythrocyte size, 31 cases (67.4%) were classified as microcytic and 15 (32.6%) as normocytic. Based on MCHC, anemia was classified as hypochromic in 5 children (10.9%), hyperchromic in 14 (30.4%), and normochromic in 27 (58.7%). All children with anemia had low iron levels, 4 (8.7%) had low folic acid levels, and none had low vitamin B12 or ferritin levels.

Association models. A visual assessment (**Figure 2)** showed higher hair Pb levels among children with anemia compared to those without. The unadjusted OR of anemia was 1.33 (95% CI 1.09, 1.63), indicating a significant positive association. After adjusting for confounders, the OR remained essentially unchanged and statistically significant (OR 1.31, 95% CI 1.05, 1.65), indicating that each one-unit increase in hair ln-Pb was associated with a 31% increase in the odds of anemia. In this multivariable model, age, second-hand tobacco smoke, and use of wood stoves were positively associated with anemia, whereas male gender and frequent consumption of spinach and beetroot were inversely associated with anemia (**Table 3**). In quantile-based G-computation models, the adjusted OR for a quartile increase in hair Pb was 1.36 (95% CI 0.96, 1.79). When we replicated the adjusted model using ln-Pb quartiles as the exposure, the ORs for quartiles 2, 3, and 4—compared to quartile 1—increased monotonically in magnitude, suggesting a possible dose-response pattern (**Table 4** and **Figure 3**). Due to the limited variability in rice consumption and breastfeeding—reported by nearly all participants—these variables were excluded from the multivariable models.

Mixture analysis. The Pearson correlation analysis revealed that all pairwise correlations among the HM exceeded 0.3, meeting the inclusion criterion for the mixture analysis (**Figure S2).** Each HM was transformed into quartiles and included in a linear model to evaluate the association between the HM mixture and anemia, adjusting for confounders using quartile-based G-computation. The estimated OR for the overall mixture effect, assuming additivity, was 1.55 (95% CI 1.02, 2.08), indicating that, on average, each quartile increase in the HM mixture was associated with a 55% increase in the odds of having anemia—a statistically significant association. To further explore the mixture, we quantified each HM's contribution to the overall effect. Mn contributed the most to the positive effect (76.0%), followed by Pb (23.7%). In contrast, Cd was the main contributor to the inverse effect (71.4%), followed by As (28.6%) (**Figure S3**).

We systematically evaluated pairwise interaction terms in the models to explore potential synergistic or antagonistic effects between HM (**Table 5**). Cd appeared to antagonize the effects of Mn and Pb—both of which contributed positively to the mixture effect—reducing the overall mixture OR to 1.19 (95% CI 1.03, 2.12) when interacting with Mn, and to 1.25 (95% CI 1.02, 2.10) when interacting with Pb. When both antagonistic interactions (Mn×Cd and Pb×Cd) were included simultaneously, the mixture OR was further attenuated to 1.15 (95% CI 1.02, 2.14). Including all six pairwise interactions resulted in a non-significant joint OR of 1.17 (95% CI 0.98, 2.34) per quartile increase in the HM mixture.

Mediation analysis. The results of the causal mediation analysis using natural effects models to evaluate whether ferritin mediates the association between hair ln-Pb levels and anemia are presented in **Table 6** and **Figure 4**. We observed a marginally significant positive total effect (TE) of hair ln-Pb on anemia (OR 1.31; 95% CI 1.00, 1.67), consistent with prior findings. The natural direct effects indicated that hair ln-Pb has a marginally significant positive effect on anemia, independent of ferritin—PNDE (OR 1.31; 95% CI 0.99, 1.66) and TNDE (OR 1.33; 95% CI 1.00, 1.69). In contrast, the natural indirect effects through ferritin—PNIE (OR 0.99; 95% CI 0.97, 1.02) and TNIE (OR 1.00; 95% CI 0.98, 1.03)—were close to the null and not statistically significant, suggesting that the mechanism by which Pb exposure increases the odds of anemia does not substantially operate through altered ferritin levels.

5. DISCUSSION

5.1. Key observations

We found a statistically significant positive association between hair ln-Pb levels and anemia (aOR: 1.31; 95% CI: 1.05, 1.65), as well as between a mixture of hair HMs (Pb, As, Cd, and Mn) and anemia under the assumption of additive effects (aOR per one-quantile increase in the mixture: 1.55; 95% CI: 1.02, 2.08). This association was attenuated after including interaction terms with Cd, suggesting a potential antagonistic role. Causal mediation analysis did not support ferritin as a mediator of the association between ln-Pb and anemia.

5.2. Consistency with other studies and interpretation

Pb-anemia association. Compared to other studies that assessed the Pb–anemia association using hair as the biomarker of internalized exposure to Pb, our results, showing a positive association between hair Pb and anemia, align with a 2010 study in Uruguay (n=180), which reported a statistically marginal association between anemia (Hb<10.5 g/dL) and $\log_{10}$-transformed hair Pb concentrations (β=0.28; P<0.1). Notably, the median hair Pb level in the Uruguayan study (13.7 mg/kg) was substantially higher than in our sample (1.9 mg/kg), potentially reflecting a higher level of exposure. Comparability is limited by differences in setting (urban vs. rural), participant age (5–37 months vs. 4–17 years), and the lack of adjustment for behavioral factors and a clearly defined exposure source in the Uruguay study.[20] Conversely, our results contrast with a 2012 study in children aged 6-60 months in Pakistan (n=216), which found no difference in mean hair Pb levels between children with and without anemia (0.17 mg/kg in both groups). The lower mean hair levels (0.16 mg/kg) observed in the Pakistani children compared to our study (6.16 mg/kg), along with differences in study setting (urban vs. rural) and exposure pathways (air and soil vs. water pollution), could explain this difference.[18]

Compared to studies that measured Pb in blood, our findings align with a 2025 study of Argentinian children aged 1-6 years (n=392), which reported a positive association between elevated blood Pb (≥5 µg/dL) and anemia (OR 2.52; 95% CI 1.21, 3.30).[21] Similarly, a 2018 study of Egyptian children 6-15 years old (n=100) reported significantly higher mean blood Pb levels in children with anemia compared to those without (19.59 vs. 5.89 µg/dl, P<0.01).[22] A 2010 study in Pakistan also reported a higher prevalence of anemia among children 1-5 years (n=340) with elevated blood Pb levels (≥10 µg/dL) compared to those with normal Pb levels (OR=10.29, 95% CI 6.04, 17.52).[23] Additionally, our study is consistent in direction with a 2021 study in Peruvian children aged 3-24 months old (n=40), which found a non-significant positive association between blood Pb and anemia (OR 1.27; 95% CI 0.62, 2.12).[24] While our study used hair Pb, and these studies used blood Pb, all suggest that Pb exposure contributes to anemia. In contrast, our findings differ from a 2006 Brazilian study, which found no difference in blood Pb levels between children 2-11 years old (n=136) with and without anemia (2.8 µg/dL vs. 2.6 µg/dL, P=0.98). The inconsistency may be explained by differences in exposure levels (lower for the Brazilian study), lack of adjustment for confounding factors, and differences in exposure pathways (inactive Pb processing site vs. freshwater).[19]

Mixture analysis. Our findings from the mixture analysis are directionally consistent with a 2023 study in Uganda (n=100, age: 6-59 months), which reported a non-significant inverse association between a mixture of blood metals (Pb, Cd, Mn, Cobalt) and Hb using weighted quantile sum regression (WQSR) (estimate=-0.18, $P > 0.05$). A decrease in Hb levels implies a higher risk of anemia, which is consistent with our finding of a positive association between the HM mixture and anemia. Notably, both our study and the Ugandan study identified Mn as the main contributor to the association, accounting for 76% and 60% of the overall weight, respectively.[30] Our results also align with a 2024 Chinese study (n=1,460, age: 2-6 years) which found an inverse association between a mixture of eight urinary HMs (barium, aluminum, uranium, thallium, iron, and tungsten) and Hb using quantile-based g-computation (β=-0.083; 95% CI -0.132, -0.033), with aluminum contributing most to the joint effect (40%).[31] Although the biospecimens and specific metals differed from those in our study, both studies support the idea that HM mixtures may contribute to lowering Hb levels and ultimately lead to anemia.

In contrast, our findings diverge from those of a 2022 US study using NHANES 2017-18 data, which included adolescents 12-17 years old (n=588). That study found a positive association between a mixture of blood metals (Pb, Cd, Mn, and selenium) and Hb using Bayesian kernel machine regression (BKMR), with higher Hb levels observed at the 75th percentile of the mixture compared to the 50th percentile (β=0.20; 95% CI 0.13, 0.27),[32] suggesting a protective effect against anemia. Differences in the composition of the HM mixtures, biospecimens used (blood vs. hair), and population characteristics (US adolescents vs. Peruvian children) may explain these contrasting findings.

In quantile-based G-computation models, the adjusted OR for a quartile increase in hair Pb alone was 1.36 (95% CI 0.96, 1.79). When Pb was evaluated as part of a mixture with As, Cd, and Mn—assuming additive effects—the association with childhood anemia was stronger (OR 1.55; 95% CI 1.02, 2.08), indicating that the odds of anemia are higher among children exposed to multiple HM simultaneously, and supports the relevance of considering combined exposures. The inclusion of interaction terms revealed that some metals—particularly Cd—antagonize the effects of others. Interaction models that included Cd's modulation of Pb and Mn reduced the mixture OR to 1.15–1.25, and the fully interactive model yielded a non-significant effect (OR 1.17; 95% CI 0.98, 2.34). These findings suggest that although additive models are useful for summarizing the overall direction and magnitude of risk, they may mask important antagonistic interactions. Future analyses and policy decisions should account for both the additive burden and potential metal–metal interactions when evaluating the health impact of mixed HM exposures.

Mediation analysis. To our knowledge, no previous studies have evaluated ferritin as a mediator in the Pb-anemia association, making comparisons challenging. Based on prior research showing lower levels of ferritin among children living close to the Puyango-Tumbes River,[35] and inverse associations between Pb and ferritin,[21,36–38] we hypothesized that ferritin, as an iron storage marker, might mediate the association between Pb exposure and anemia, assuming Pb depletes iron stores. Our mediation analysis, however, did not support this hypothesis, as ferritin was not a significant mediator in the Pb-anemia association. These findings support the idea that Pb may contribute to anemia through pathways other than iron depletion, such as inflammation, interference with heme synthesis, increased erythrocyte destruction, or bone marrow suppression.

We observed that anemia in this population was characterized by low serum iron levels and normal to high ferritin concentrations, a pattern that aligns with anemia observed under inflammatory conditions.[34] Inflammatory responses—potentially triggered by Pb exposure—can stimulate the production of hepcidin, a key regulator of iron metabolism. Hepcidin degrades ferroportin—the protein responsible for exporting iron from enterocytes into circulation. This disruption impairs iron transport, potentially resulting in reduced serum iron levels despite adequate iron stores. Importantly, ferritin is not only a marker of iron storage but also an acute-phase reactant that increases in response to systemic inflammation.[34,58] Thus, Pb-induced inflammation may impair iron availability, contributing to anemia even when ferritin levels appear sufficient, explaining both the observed biomarker pattern and the lack of mediation by ferritin in our analysis.

These findings underscore the need for future research to investigate the mechanisms underlying anemia in pediatric populations exposed to environmental toxicants such as Pb and other HM, to better understand its etiology and inform targeted interventions.

5.3. Limitations and strengths

Limitations

Temporality. Hair samples were collected exclusively during the summer, which coincides with the rainy season in Tumbes. While hair reflects approximately three months of exposure and can smooth short-term fluctuations, it may still miss variations occurring across the year. Environmental factors such as increased rainfall, agricultural runoff, and changes in water sources during the rainy season may elevate HM exposure, particularly from contaminated water and soils. As a result, HM levels in our samples may be higher than those in other, drier periods, potentially representing a worst-case scenario in terms of exposure impact. Therefore, while our findings likely reflect a true association under the exposure conditions present during sampling, the magnitude of the association may differ under lower exposure levels. As such, our results may represent a worst-case scenario for exposure impact and may not be generalizable to other seasons. Additionally, the cross-sectional design of this study restricts our ability to establish the temporal sequence between exposure and outcome, raising the possibility of reverse causality—that is, that anemia could lead to higher HM levels rather than result from them. Some evidence suggests that anemia enhances HM absorption by upregulating divalent metal transporter 1 (DMT-1) receptors in the duodenum.[34,59] These receptors facilitate the uptake of divalent metals such as iron—but also Pb, Cd, and Mn—into enterocytes, making it biologically plausible for anemia to favor HM toxicity.[50,59,60] Future longitudinal studies are warranted to address these temporal limitations.

Measurement bias. We used hair samples to measure internalized Pb and other HM. Hair offers several advantages—including non-invasive and painless collection, ease of storage and transport, and the ability to reflect longer-term exposure[61,62]—but its reliability as a biomarker of internal Pb is limited.[46,63] Whole blood Pb is the most commonly used biomarker for assessing Pb exposure; however, due to the rapid exchange of Pb in the bloodstream, it may not accurately reflect chronic exposure.[62,63] Although a single blood measurement can provide meaningful insights under constant exposure conditions, alternative biomarkers may be warranted for assessing long-term exposure.[63] Hair, which eliminates a small fraction of total body Pb,[63] may serve as a useful alternative. However, external contamination from environmental sources or cosmetic products—particularly in distal segments—can introduce high variability in hair Pb levels, even after washing.[46,61,64] This concern is particularly relevant in communities with limited access to adequate washing facilities like Tumbes.[42] To address this, we limited our analysis to the 3 proximal centimeters of hair and employed a standardized hair washing protocol. Nevertheless, the extent of residual contamination—and therefore potential misclassification of exposure—is uncertain. This misclassification is likely non-differential with respect to anemia status and would thus be expected to bias associations towards the null.

Selection bias. Our study may be subject to selection bias, as information about the pollution in the Puyango-Tumbes River is publicly available and a source of concern within the community. As a result, families with known HM exposure or children showing anemia symptoms may have been more likely to participate. This could have led to overrepresentation of exposed, anemic children, reducing exposure contrast, and potentially biasing the association toward the null. However, the CGH recruitment strategy—which included repeated household visits and collaboration with local authorities—achieved high participation: over 95% of the approached families agreed to participate, helping to mitigate concerns about substantial selection bias.

Confounding. Residual confounding may also have influenced our results. Although we adjusted for household tobacco smoke exposure—which we theorize is positively associated with internalized Pb levels and anemia—we did not assess participants' smoking status. This limitation is likely minor given the pediatric population and low national smoking prevalence.[3] Additionally, we did not account for air pollution, which may be positively associated with both hair Pb concentrations and anemia. These unmeasured variables could have acted as positive confounders, possibly biasing our results away from the null.

Strengths

Despite these limitations, this study has notable strengths, including the use of standardized approaches to measure hair HM and anemia and a systematic approach to identify and adjust for confounders. This study is one of the first to evaluate the impact of HM mixtures in this setting, with the extra advantage of using a cutting-edge method—quantile-based G-computation[54]–which effectively estimates the causal effects of mixtures. This study is also one of the first to investigate potential anemia mechanisms in this population using causal mediation analysis.

5.4. Generalizability

The findings of this study are generalizable to children living in the Tumbes region. These results may also be relevant to children in other non-mining communities in northern Peru with similar environmental and socioeconomic conditions. However, the results may not be directly applicable to adults, children living in densely populated urban settings, or populations with different dietary patterns, healthcare access, or exposure routes. Nonetheless, these findings may inform public health interventions in other LMICs facing similar environmental contamination and resource limitations.

5.5. Implications

Given that HM exposure occurs during a critical developmental period, its effects on hematological health may have substantial long-term consequences. Future research should explore these associations longitudinally to better understand the temporal dynamics and long-term outcomes of chronic exposure. Additionally, studies should consider potential dose-response or threshold effects that may not be fully captured in cross-sectional designs. Clinically, relying solely on ferritin as a biomarker of iron status may be misleading in contexts of HM exposure, highlighting the need for a comprehensive iron profile—including transferrin saturation, soluble transferrin receptor, and hepcidin—as well as inflammatory markers to accurately interpret iron metabolism.

From a public health perspective, the observed association between Pb and anemia—even at relatively low hair Pb concentrations—underscores the need for screening programs and context-specific interventions in the affected communities. Traditional iron supplementation strategies may be insufficient when anemia stems from disrupted iron metabolism or inflammation due to HM exposure rather than from iron deficiency. The observed health impacts underscore the urgency of developing and enforcing environmental regulations to control HM contamination in water, soil, and food chains in the region, such as through safer mining practices and improved water quality standards. Our findings also call attention to environmental justice issues and the need for regional cooperation to protect child health in settings affected by cross-border environmental exposures.

5.6. Conclusion

In this cross-sectional study of children of Tumbes, Peru, we found evidence that exposure to Pb and a mixture of HM was associated with increasing odds of anemia, supporting the role of environmental HM in pediatric hematologic health. Although our mediation analysis did not identify ferritin as a mediator in the Pb-anemia association, biomarker patterns suggest that inflammation and disrupted iron transport may contribute to anemia in this population. These results emphasize the need to consider both individual Pb and combined HM exposures in addressing pediatric anemia in contaminated settings.

6. TABLES AND FIGURES

Table 1. Definitions of the natural causal mediation effects	
Effect	**Definition**
PNDE	$Y_i(x, M_i(x^*)) - Y_i(x^*, M_i(x^*))$
TNDE	$Y_i(x, M_i(x)) - Y_i(x^*, M_i(x))$
PNIE	$Y_i(x^*, M_i(x)) - Y_i(x^*, M_i(x^*))$
TNIE	$Y_i(x, M_i(x)) - Y_i(x, M_i(x^*))$
TE	$Y_i(x, M_i(x)) - Y_i(x^*, M_i(x^*))$
PNDE, pure natural direct effect; TNDE, total natural direct effect; PNIE, pure natural indirect effect; TNIE, total natural indirect effect; TE, total effect; DE direct effect; IE, indirect effect. Adapted from Rijnhart, et. al. 2021[56]	

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Table 2. Characteristics of 404 children 4-17 years old living in Tumbes, Peru in 2023 by hair ln-Lead quartiles					
	Q1 (n=101)	**Q2** (n=101)	**Q3** (n=101)	**Q4** (n=101)	**Overall** (n=404)
Hair lead, mg/kg, mean (SD)	0.3 (0.2)	1.2 (0.3)	3.2 (0.9)	19.9 (24.7)	6.16 (14.7)
Hair arsenic, μg/kg, mean (SD)	155 (209)	370 (525)	574 (693)	2370 (3150)	866 (1850)
Hair cadmium, μg/kg, mean (SD)	45.7 (50.9)	140 (121)	365 (421)	1080 (1750)	408 (988)
Hair manganese, mg/kg, mean (SD)	2.4 (4.6)	6.6 (12.5)	16.8 (36.7)	29.8 (62.3)	13.9 (38.1)
Age, years, mean (SD)	10.2 (3.7)	10.0 (4.0)	9.9 (4.0)	10.6 (3.9)	10.2 (3.9)
Female gender, n (%)	35 (34.7)	46 (45.5)	58 (57.4)	74 (73.3)	213 (52.7)
Parent or guardian's occupation					
Housewife, n (%)	1 (1.0)	4 (4.0)	3 (3.0)	3 (3.0)	11 (2.7)
Service sector, n (%)	27 (26.7)	29 (28.7)	28 (27.7)	30 (29.7)	114 (28.2)
Fishing or Agriculture, n (%)	69 (68.3)	64 (63.4)	68 (67.3)	60 (59.4)	261 (64.6)
Construction or Mining, n (%)	4 (4.0)	4 (4.0)	2 (2.0)	8 (7.9)	18 (4.5)
Non-durable house material, n (%)	48 (47.5)	57 (56.4)	49 (48.5)	55 (54.5)	209 (51.7)
Second-hand tobacco smoke, n (%)	10 (9.9)	15 (14.9)	12 (11.9)	5 (5.0)	42 (10.4)
Wood stove, n (%)	46 (45.5)	52 (51.5)	45 (44.6)	45 (44.6)	188 (46.5)
Burning trash, n (%)	26 (25.7)	28 (27.7)	28 (27.7)	35 (34.7)	117 (29.0)
Burning fields, n (%)	43 (42.6)	44 (43.6)	52 (51.5)	58 (57.4)	197 (48.8)
Fish consumption frequency					
≤1 time/week, n (%)	15 (14.9)	27 (26.7)	18 (17.8)	23 (22.8)	83 (20.5)
3 times/week, n (%)	78 (77.2)	63 (62.4)	72 (71.3)	67 (66.3)	280 (69.3)
Daily, n (%)	8 (7.9)	11 (10.9)	11 (10.9)	11 (10.9)	41 (10.1)
Fish consumption from the Puyango-Tumbes River, n (%)	32 (31.7)	29 (28.7)	32 (31.7)	19 (18.8)	112 (27.7)
Frequent rice consumption*, n (%)	101 (100)	99 (98.0)	99 (98.0)	100 (99.0)	399 (98.8)
Frequent beetroot & spinach consumption*, n (%)	40 (39.6)	38 (37.6)	44 (43.6)	51 (50.5)	173 (42.8)
Breastfeeding, n (%)	95 (94.1)	91 (90.1)	97 (96.0)	98 (97.0)	381 (94.3)
Ferritin (μg/dL)	200 (62.5)	199 (72.9)	209 (70.9)	212 (66.2)	205 (68.2)

*Frequent consumption: ≥3 times per week
SD: Standard deviation

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Table 3. Crude and adjusted odds ratios (OR) and their 95% confidence intervals (CI) for the association between hair ln-Lead (mg/kg) and anemia in 404 children aged 4-17 years in Tumbes, Peru (2023)

	Crude model		**Adjusted model***	
Variable	**OR**	**95% CI**	**OR**	**95% CI**
ln-Lead	1.33	1.09, 1.63	1.31	1.05, 1.65
Age	-	-	1.12	1.02, 1.23
Gender				
Female	-	-	Reference	
Male	-	-	0.43	0.19, 0.90
Second-hand tobacco smoke				
No	-	-	Reference	
Yes	-	-	3.04	1.18, 7.53
Stove type				
Gas	-	-	Reference	
Wood	-	-	2.39	1.15, 5.18
Burning fields				
No	-	-	Reference	
Yes	-	-	0.68	0.34, 1.36
Burning trash				
No	-	-	Reference	
Yes	-	-	0.98	0.46, 2.02
Parent of guardian's occupation				
Housewife	-	-	Reference	
Service sector	-	-	0.50	0.11, 2.82
Fishing/Agriculture	-	-	0.29	0.06, 1.54
Construction/Mining	-	-	0.35	0.03, 3.02
House material				
Durable	-	-	Reference	
Non-durable	-	-	1.94	0.95, 4.15
Fish consumption from the Puyango-Tumbes				
No	-	-	Reference	
Yes	-	-	0.60	0.26, 1.32
Fish consumption frequency				
≤1 time/week	-	-	Reference	
3 times/week	-	-	0.56	0.26, 1.25
Daily	-	-	0.79	0.22, 2.54
Beetroot or spinach consumption frequency				
Not frequent	-	-	Reference	
Frequent	-	-	0.45	0.20, 0.97

*Adjusted for age in years, gender, second-hand tobacco smoke, stove type, burning fields, burning trash, parental occupation, house material, fish consumption from the Puyango-Tumbes River, fish consumption frequency and beetroot or spinach consumption frequency

Table 4. Adjusted odds ratios (OR)* and 95% confidence intervals (CI) for the association between hair ln-Lead (ln-Pb) quartiles and anemia in 404 children aged 4-17 years in Tumbes, Peru (2023)		
Hair ln-Pb quartiles	**OR**	**95% CI**
Q1	Reference	Reference
Q2	1.26	0.43, 3.88
Q3	1.27	0.42, 3.95
Q4	2.57	0.95, 7.61

*Adjusted for age in years, gender, second-hand tobacco smoke, stove type, burning fields, burning trash, parental occupation, house material, fish consumption from the Puyango-Tumbes River, fish consumption frequency, and beetroot or spinach consumption frequency

Table 5. Adjusted OR* and 95% confidence intervals (CI) for the association between a heavy metal mixture** and anemia, with various interaction terms included in the models, among 404 children aged 4-17 years in Tumbes, Peru (2023)		
Model with interaction term(s)	**OR**	**95% CI*** **
No interactions	1.55	1.02, 2.08
Pb & Mn	1.52	1.02, 2.08
Pb & Cd	1.25	1.02, 2.10
Pb & As	1.70	1.02, 2.10
Mn & Cd	1.19	1.03, 2.12
Mn & As	1.61	1.02, 2.08
Cd & As	1.60	1.02, 2.08
Pb & Mn, and Cd & As	1.56	1.01, 2.10
Mn & Cd, and Pb & Cd	1.15	1.02, 2.14
All pairwise interactions	1.17	0.98, 2.34

*Adjusted for age in years, gender, second-hand tobacco smoke, stove type, burning fields, burning trash, parental occupation, house material, fish consumption from the Puyango-Tumbes River, fish consumption frequency, and beetroot or spinach consumption frequency

** The mixture includes hair lead (Pb), arsenic (As), cadmium (Cd) and manganese (Mn)

***Computed using a bootstrap approach

Table 6. Adjusted odds ratios (OR)* and 95% confidence intervals (CI) for the causal effect of hair ln-Lead (ln-Pb) (mg/kg) on anemia mediated through ferritin among 404 children aged 4-17 years in Tumbes, Peru (2023)			
Effect	**OR**	**95% CI**	**Interpretation**
Natural Direct Effects (NDE)			
Pure NDE	1.31	0.99, 1.66	Effect of changing the individual's hair ln-Pb from *ln-Pb* to *ln-Pb**, holding ferritin fixed at the value it would naturally take under *ln-Pb**
Total NDE	1.33	1.00, 1.69	Effect of changing the individual's hair ln-Pb from *ln-Pb* to *ln-Pb**, holding ferritin fixed at the value it would naturally take under *ln-Pb*
Natural Indirect Effects (NIE)			
Pure NIE	0.99	0.97, 1.02	Effect of changing the individual's ferritin level from the value it would take under *ln-Pb** to the level it would take under *ln-Pb,* while keeping hair ln-Pb fixed at *ln-Pb**
Total NIE	1.00	0.98, 1.03	Effect of changing the individual's ferritin level from the value it would take under *ln-Pb** to the level it would take under *ln-Pb,* while keeping hair ln-Pb fixed at *ln-Pb*
Total effect	1.31	1.00, 1.67	Effect of changing the individual's hair ln-Pb from *ln-Pb* to *ln-Pb**
*Adjusted for age in years, gender, second-hand tobacco smoke, stove type, burning fields, burning trash, parental occupation, house material, fish consumption from the Puyango-Tumbes River, fish consumption frequency and beetroot or spinach consumption frequency			

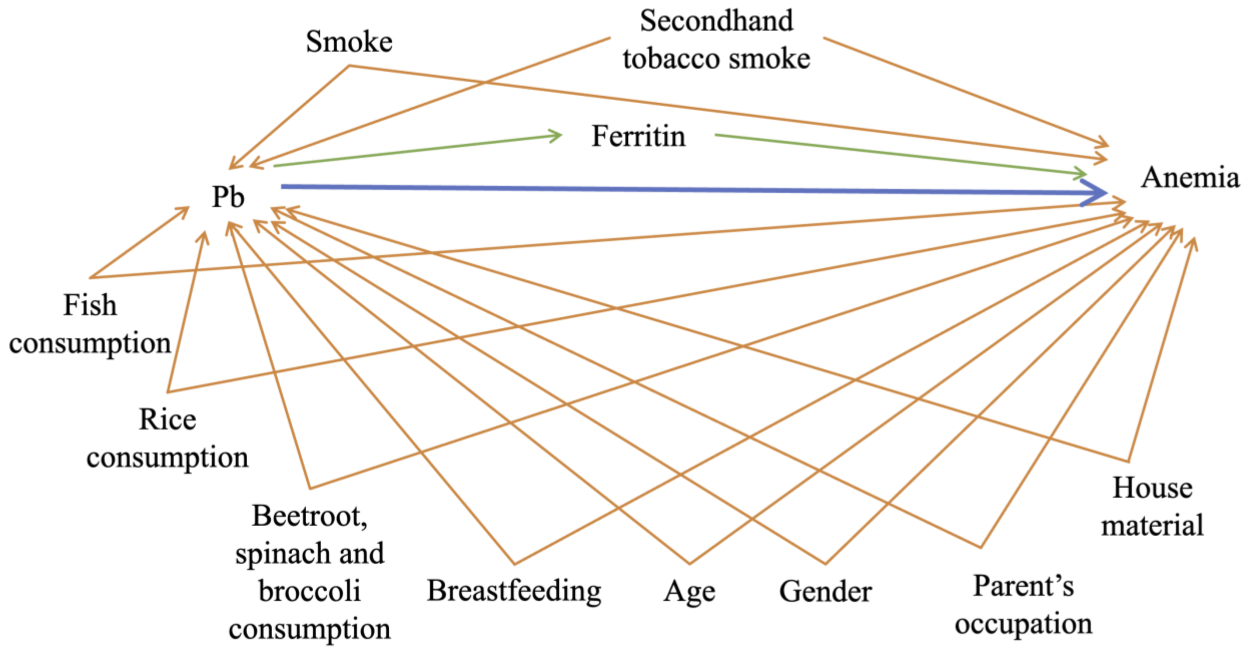

Figure 1 Directed acyclic graph for the association between lead (Pb) and anemia. The blue arrow represents the main association, the orange arrows represent confounding, and the green arrows represent mediation.

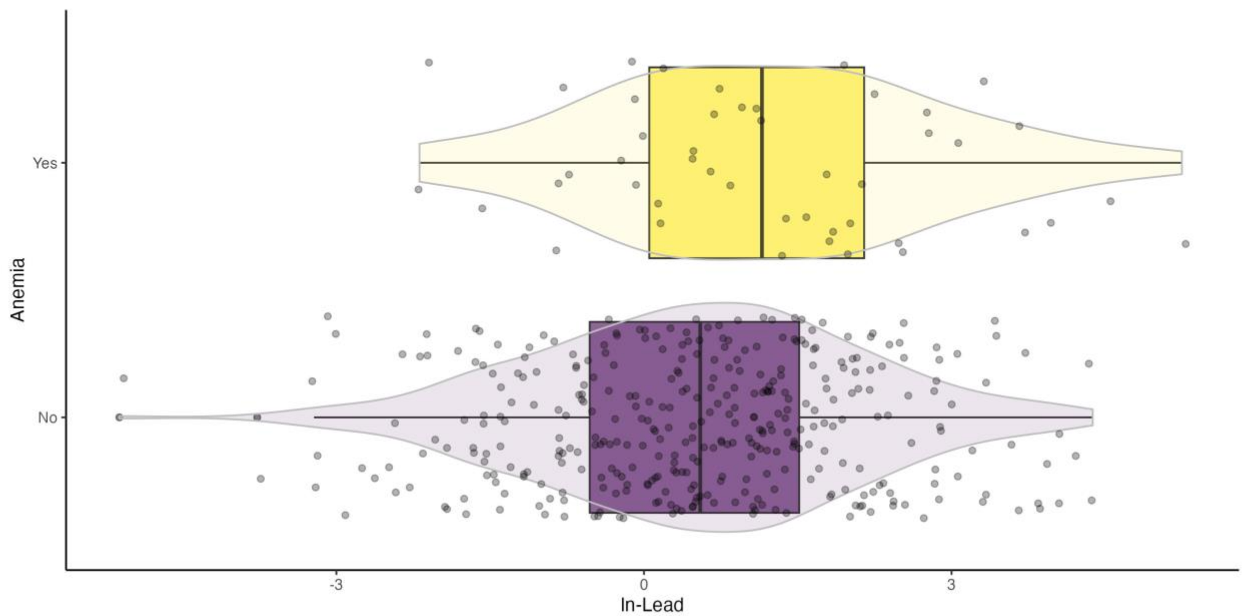

Figure 2 Box and violin plot of hair ln-Lead by anemia status in 404 children aged 4-17 years in Tumbes, Peru (2023). Gray dots represent individual observations. Yellow boxes represent children with anemia, while purple boxes represent children without anemia. Each box shows the interquartile range (IQR) with the left and right edges indicating the 25th and 75th percentiles, respectively. The line inside the box represents the median. Whiskers extend to the smallest and largest values within 1.5 times the IQR from the lower and upper quartiles. The surrounding violin plot displays the kernel density of the data distribution, illustrating the full shape and spread of ln-Lead levels for each group.

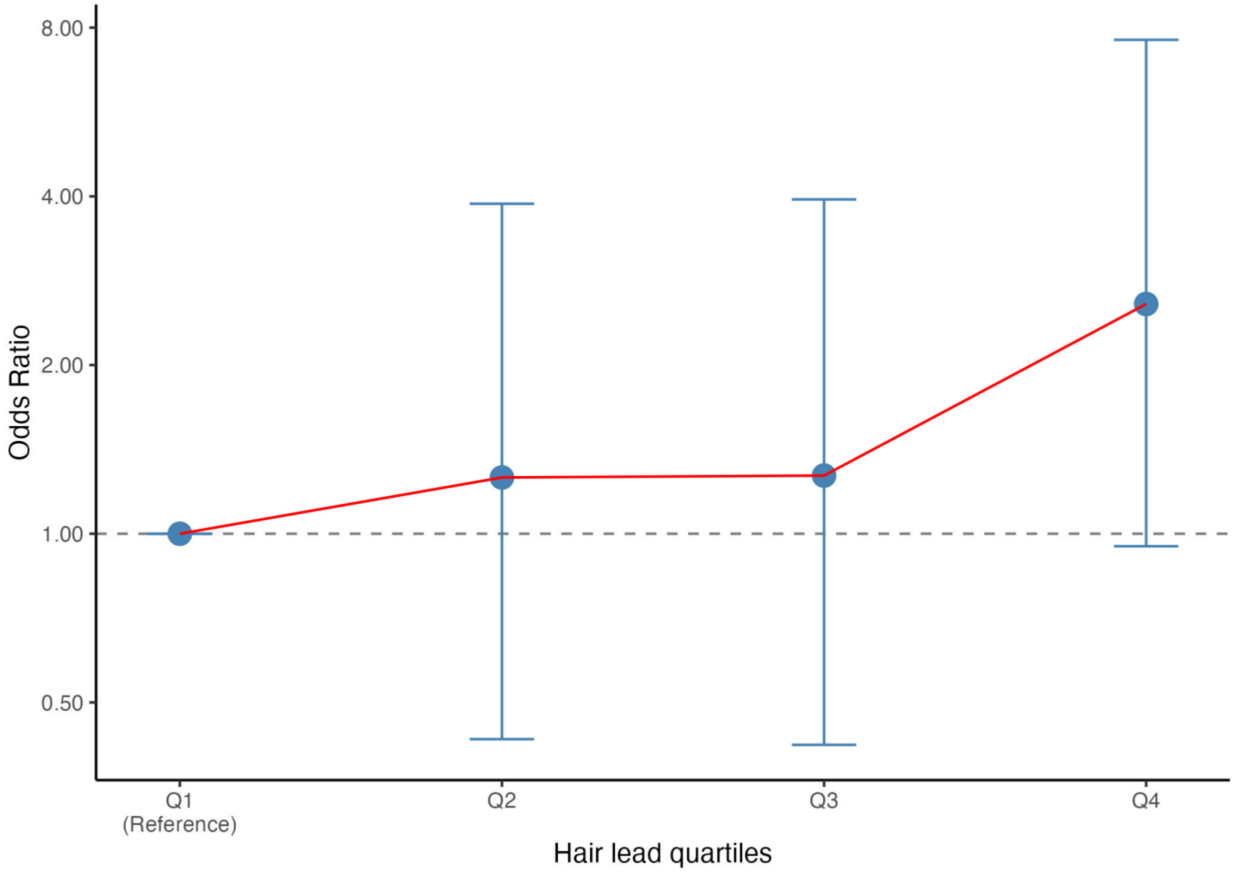

Figure 3 Adjusted odds ratios* (blue dots) and 95% confidence intervals (blue whiskers) for the association between hair ln-Lead quartiles and anemia in 404 children 4-17 years in Tumbes, Peru (2023). The red line indicates the trend in anemia risk across increasing hair lead quartiles, and the dashed gray line represents the null value. *Adjusted for age in years, gender, second-hand tobacco smoke, stove type, burning fields, burning trash, parental occupation, house material, fish consumption from the Puyango-Tumbes River, fish consumption frequency, and beetroot or spinach consumption frequency.

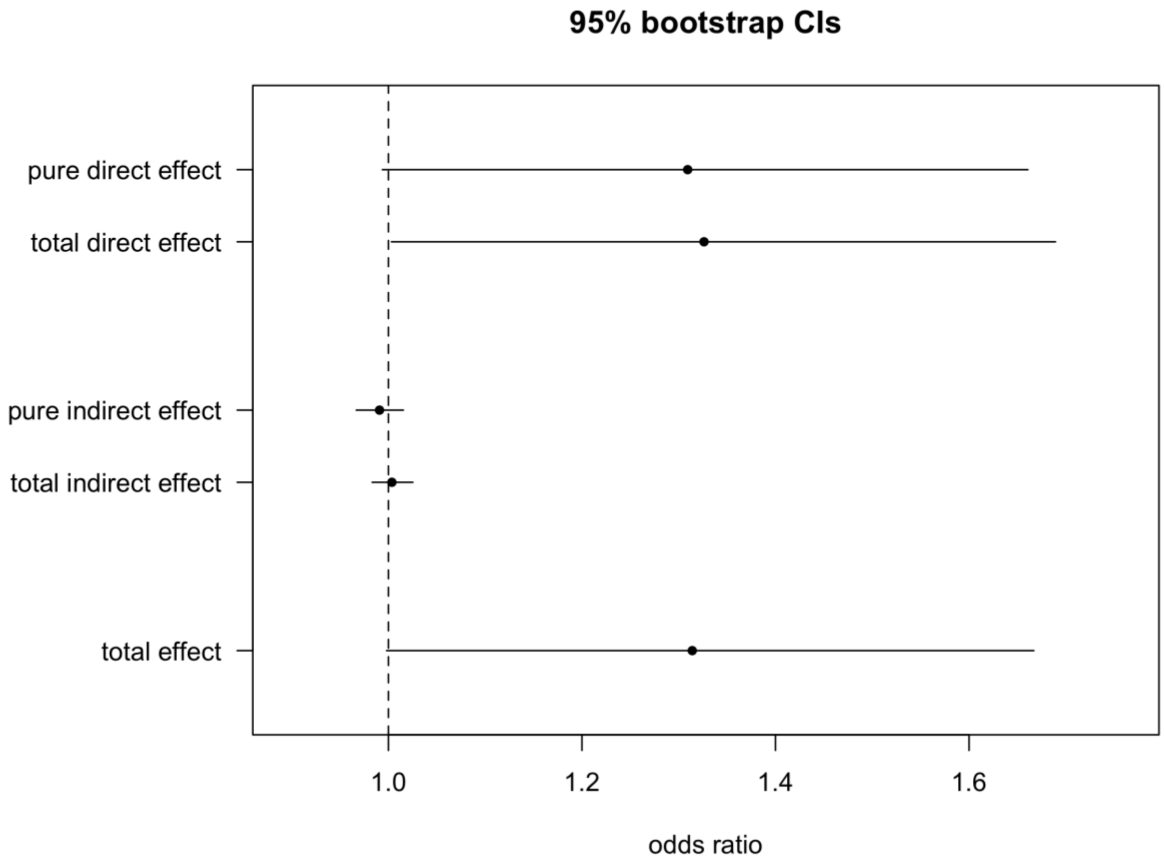

Figure 4 Adjusted odds ratios* and 95% confidence intervals (CI) for the causal effect of hair ln-Lead (mg/kg) on anemia, mediated through ferritin, among 404 children aged 4-17 years in Tumbes, Peru (2023). *Adjusted for age in years, gender, second-hand tobacco smoke, stove type, burning fields, burning trash, parental occupation, house material, fish consumption from the Puyango-Tumbes River, fish consumption frequency, and beetroot or spinach consumption frequency.

7. SUPPLEMENTAL TABLES AND FIGURES

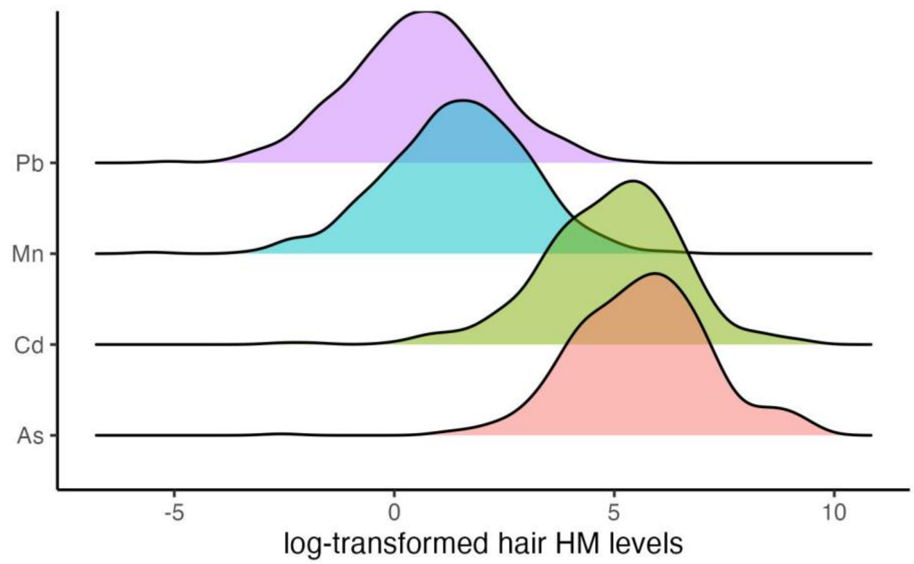

Figure S1 Density plots of the distribution of log-transformed hair heavy metals in 404 children aged 4-17 years in Tumbes, Peru (2023).

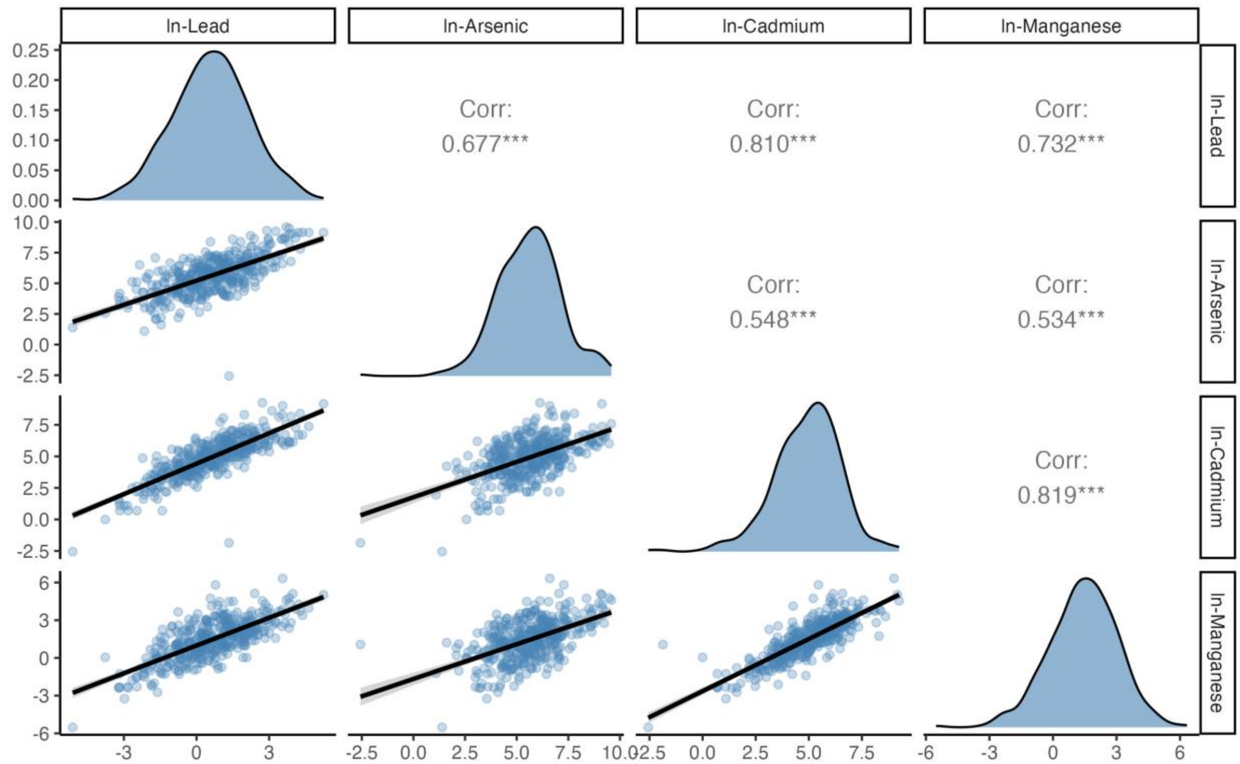

Figure S2 Pairwise correlations between log-transformed hair lead, arsenic, cadmium, and manganese in 404 children aged 4-17 years old in Tumbes, Peru (2023). The upper diagonal shows Pearson correlation coefficients with significance levels ($^{***}p<0.001$). Lower diagonal displays corresponding scatter plots with fitted regression lines and 95% confidence intervals (blue shading). The diagonal shows density distributions of each HM concentration.

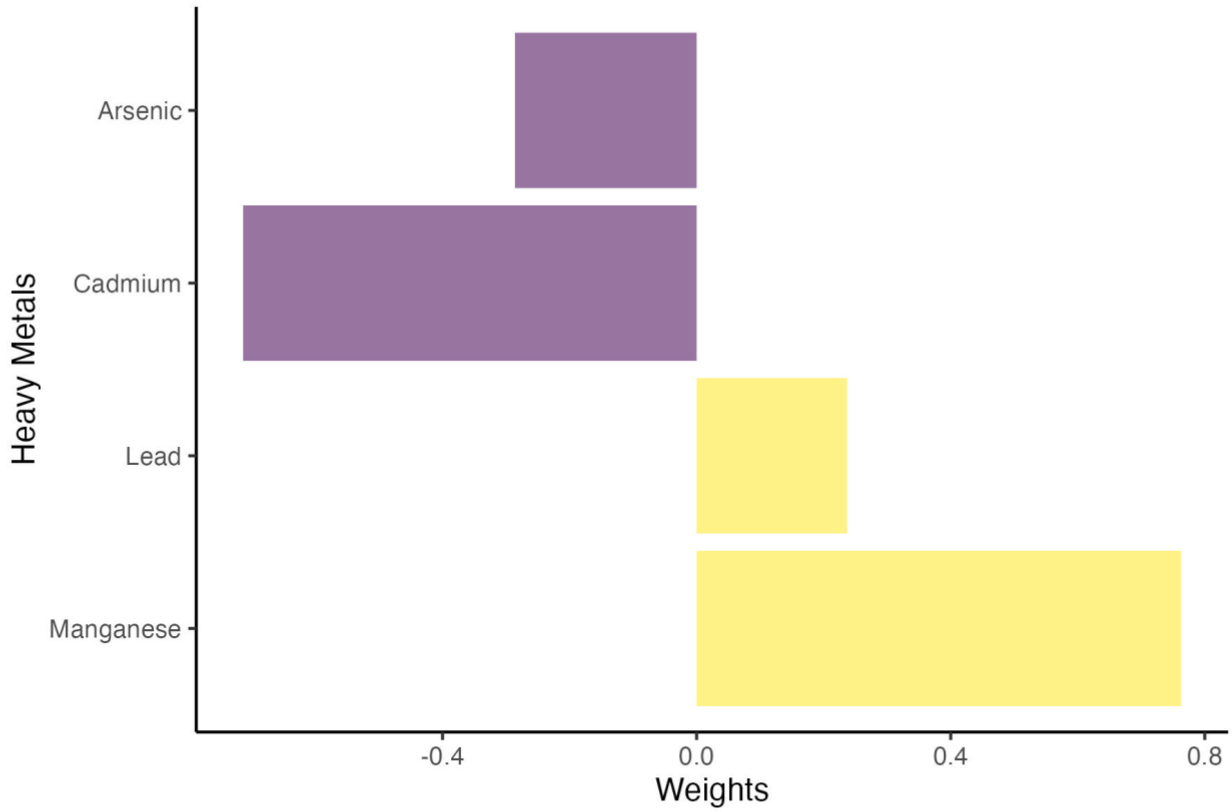

Figure S3 Effect size estimates from quantile-based g-computation modeling the association between a mixture of heavy metals (lead, arsenic, cadmium, and manganese) and the probability of anemia among 404 children aged 4–17 years in Tumbes, Peru (2023). The figure displays the relative contribution (weights) of each metal to the overall effect of the mixture on the odds of anemia, adjusting for age, gender, second-hand tobacco smoking, stove type, burning fields, burning trash, parental occupation, housing material, fish consumption from the Puyango-Tumbes River, fish consumption frequency, and beetroot or spinach consumption frequency. Positive weights are displayed as yellow bars, while negative weights are displayed as purple bars.

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CHAPTER 6. SYNTHESIS OF RESEARCH

1. Summary

Heavy metal (HM) exposure remains a major yet under-addressed global public health concern, contributing to considerable morbidity and mortality, particularly in low- and middle-income countries (LMICs), where environmental regulations and monitoring are often limited.[1] In the Tumbes region of northern Peru, communities living along the Puyango-Tumbes River and the local authorities have expressed growing concerns about the health impacts of water contamination, especially in children.[2] Although the Tumbes community is not directly involved in mining, poorly regulated upstream gold mining activities in southern Ecuador could contribute to HM contamination in the river, which serves as the region's primary source of freshwater.[3] Additional sources of contamination in the region may include agricultural runoff and inadequate waste disposal.[4] Once released into the environment, these metals can bioaccumulate in food sources, potentially leading to chronic exposure among communities that depend on the river.[5]

Despite these concerns, the extent, distribution, and health consequences of HM exposure in Tumbes have not been well characterized. In addition, some HM, such as arsenic (As),[6,7] are known respiratory toxicants, while others—including lead (Pb), cadmium (Cd), and mercury (Hg)—can disrupt iron metabolism and hemoglobin (Hb) synthesis, potentially leading to anemia.[8,9] Understanding whether and how these metals contribute to respiratory impairment and anemia in this population is essential for identifying at-risk groups and guiding public health interventions. This dissertation aimed to fill these knowledge gaps by evaluating the extent of exposure to HM in children from the Tumbes region (Aim 1) and examining how exposure to As, Pb, Cd, and manganese (Mn) affects respiratory health (Aim 2) and anemia (Aim 3). In collaboration with the Center of Global Health (GCH)-Tumbes, we recruited 409 children aged 4-17 years to measure hair HM concentrations and assess related health outcomes.

To address Aim 1, I conducted a cross-sectional exposure assessment that combined environmental and individual-level data to characterize exposure levels and patterns. I used publicly available data from 40 water samples[10]—which included water quality and HM concentration information—and linked them to hair HM data (As, Pb, Cd, and Mn) and behavioral information from 404 children. A substantial proportion of water samples exceeded national safety thresholds for As (30%), Pb (17.5%), and Mn (25%), particularly in surface water sources. Samples collected near the Puyango-Tumbes River (0.6-3.4 km away) had higher levels of several HMs—including As, Pb, Cd, and Mn—compared to those collected further away (20.5-57.5 km from the river). This pattern was also observed in hair samples—particularly for As and Pb. Mixed-effects models indicated that the geometric mean of hair As among children living closer to the river was 3.8 times that of children living further away (95% confidence interval [CI] 1.16, 6.73), while the corresponding estimate for Pb was 1.77 (95% CI 0.91, 3.43). Correlation analyses showed modest positive associations between water and hair concentrations for As and Mn. Together, these findings suggest that the Puyango-Tumbes River is an important source of HM exposure, particularly As, in this population.

For aim 2, I conducted a cross-sectional study to evaluate the association between As exposure—individually and in combination with Pb, Cd, and Mn—and respiratory health in 399 children. Respiratory health was assessed through spirometry, measured as percent predicted forced vital capacity (ppFVC) and spirometry pattern classification, as well as caregiver-reported respiratory symptoms.[11] I hypothesized that higher hair concentrations of As and HM mixtures would be associated with reduced lung function. Supporting this hypothesis, I observed inverse associations between hair ln-As and ppFVC in multivariable mixed-effects regression models as well as between the HM mixture and ppFVC in a quantile-based G-computation analysis, with As contributing the most to the overall negative effect. An inverse association between ln-As and abnormal spirometry patterns was also observed. However, none of these associations reached statistical significance.

Spirometry reference values were initially calculated using Global Lung Initiative (GLI) reference equations,[12] applying a race-neutral approach.[13] However, because Latin American children were not included in the development of these equations, concerns arose about their generalizability. To address this, I conducted a sensitivity analysis (n=346) using reference equations developed by Pérez-Padilla et al. for Latin American children aged 6 years and older.[14] Although associations remained non-significant, the prevalence of restrictive spirometry patterns was notably higher using the Pérez-Padilla equations (22.5%) compared to the GLI equations (12.8%). Both estimates indicate a higher-than-expected prevalence of restrictive lung function in this population compared to international reference ranges (1.8–7.7%).[15]

I also hypothesized that As exposure would be associated with increased respiratory symptoms. Supporting this hypothesis, I found a statistically significant association between hair ln-As levels and nasal symptoms suggestive of allergic rhinitis (adjusted odds ratio [aOR] 1.59; 95% CI 1.32, 1.91) and a non-significant association with wheezing and nighttime cough.

Aim 3 examined the association between Pb exposure—both alone and as part of a HM mixture including As, Cd, and Mn—and anemia. The hypothesis that higher levels of Pb would be associated with increased odds of anemia was supported by logistic regression models (aOR 1.31; 95% CI 1.05, 1.65). Similarly, quantile-based G-computation models supported the hypothesis that combined HM exposures were associated with higher odds of anemia (aOR 1.55; 95% CI 1.02, 2.08). This association was attenuated when interaction terms involving Cd were included (aOR range: 1.15 to 1.25), likely reflecting Cd's antagonistic effects on Pb and Mn.[16–18] Lastly, a causal mediation analysis using natural effect models showed that ferritin did not mediate the Pb-anemia association, suggesting that alternative biological mechanisms—such as inflammation or disrupted iron transport—may be involved.

2. Limitations

Several limitations and strengths of this dissertation were thoroughly discussed in the individual chapters and are briefly summarized here. The cross-sectional design of all three studies limited the ability to establish temporality, and the timing of data collection—conducted exclusively during the rainy season—may have captured a period of peak HM exposure, reducing generalizability to other times of the year. I selected hair as the primary biospecimen to measure internalized HM exposure because it is non-invasive and easy to store. However, its utility varies by metal, and the potential for external contamination remains, despite efforts to minimize it through standardized washing and proximal segment analysis. This may have introduced non-differential measurement error, likely biasing associations toward the null. Less concerning, though still possible, are selection bias—if families with greater awareness of pollution or symptoms were more likely to participate—and residual confounding from unmeasured variables such as air pollution, socioeconomic status, or participants' own smoking behavior.

Another limitation of this body of research is that I did not include hair mercury (Hg) in my analysis. Previous studies have documented contamination of the Puyango-Tumbes River with Hg and high methylmercury (MeHg) levels in fish from the river and shrimp from the Tumbes estuary.[3,19,20] Given that the river is the region's main freshwater source, it is plausible that it is also used for irrigation, particularly in rice cultivation. This is relevant because rice can translocate MeHg into the grain,[21] and rice is a dietary staple in the region—99% of children in my study consumed it frequently. If river water is used to irrigate rice paddies, the risk of dietary MeHg exposure may be substantial. Supporting this concern, a previous study in the same region found that pregnant individuals living closer to the river had higher levels of hair MeHg than those living farther away.[22] Including Hg in my analysis could have offered valuable insights, particularly given its known associations with impaired lung function and anemia.[23–25] It is possible that Hg exposure contributed, at least in part, to the adverse outcomes I observed.

3. Strengths

This dissertation offers a comprehensive exposure assessment of HM in rural Tumbes by integrating environmental, biomarker, and behavioral data. Using water samples, hair HM measurements, and questionnaires on behaviors and demographics, it identifies potential sources and mechanisms of HM exposure. The study is among the first in Peru to examine the association between As and HM mixtures with children's respiratory health, as well as the impact of Pb and HM mixtures on anemia, applying both conventional and mixture modeling approaches. It also provides novel insights into potential anemia mechanisms through causal mediation analysis.

The study leverages long-term community engagement via the trusted local organization CGH-Tumbes and uses richly contextualized self-report data to better estimate water-related exposure while adjusting for potential confounders. Non-invasive methods, including hair sampling and geospatial water measurements linked to residences, allow for the assessment of internalized HM levels and environmental exposure. Together, these approaches create a multidimensional understanding of HM exposure and its health impacts, while directly addressing community concerns about water contamination and children's health.

4. Public health significance

This dissertation provides evidence to support that children in Tumbes are exposed to HM through water, with surface water identified as a key source. This risk is particularly high among those living near the Puyango-Tumbes River. Although local efforts to ensure water quality are ongoing, they appear insufficient, as some water samples intended for human consumption still exceeded recommended As limits. These findings underscore the need for surface water treatment interventions focused on river-adjacent communities and a re-evaluation of current water treatment protocols to ensure they effectively reduce HM concentrations to safe levels.

Agricultural practices may also contribute to HM exposure. Households using river water for crop cultivation had the highest median As concentrations in nearby water samples, suggesting that irrigation with contaminated surface water may be an important pathway. Bananas were the most frequently grown crop, and previous studies in the region have identified them as potential sources of both As and Pb.[20] Rice poses an additional concern: although few participants reported growing it for self-consumption, rice is the region's main agricultural product[26] and is widely consumed, with 99% of participants reporting frequent intake. Because rice can absorb and translocate As into the grain,[27] contaminated irrigation water may pose a dietary risk not only to local communities but also to consumers in other regions where this rice may be distributed. These findings underscore the need for monitoring HM levels in locally grown produce and ensuring that irrigation water meets safety standards.

This research contributes novel evidence linking environmental contamination to measurable biological and health outcomes in children. I found significantly higher hair As concentrations among children living near the river. Although the inverse association between As and ppFVC was not statistically significant, the direction of the estimates suggests that As exposure may still have respiratory impacts that were not fully captured in this study or may emerge later in life. I also found a statistically significant association between As and symptoms of allergic rhinitis—a condition known to affect quality of life and associated with other atopic conditions, such as asthma.[28,29] These findings reinforce the ongoing call to protect clean water sources and highlight the need to implement interventions for the diagnosis and management of allergic rhinitis in the region.

Additionally, this study identified a higher-than-expected prevalence of restrictive lung function among children in the region. While the origin of this impairment remains unclear, several environmental exposures may contribute: Although respiratory infections are not highly prevalent in this population,[30] the exposure assessment revealed that a substantial proportion of children were regularly exposed to smoke from wood stoves (47%), trash burning (29%), and field burning (49%)—exposures known to cause chronic airway inflammation, trigger immune dysfunction and impair lung function.[31] Notably, the proportion of families reporting trash burning increased across quartiles of As and Pb exposure, while field burning reports increased with higher Pb quartiles, suggesting that these air pollution sources may co-occur with HM exposure and compound respiratory risk factors. These findings highlight the need for early pulmonary health monitoring, including routine respiratory screening, alongside efforts to reduce household and environmental smoke exposure.

Although the association between proximity to the river and hair Pb was not statistically significant, Pb levels were higher among children living closer to the river. This finding suggests the river may be a source of Pb exposure, though further studies are needed to confirm this pathway. Regardless of the source, I found a significant association between Pb and anemia—both alone and as part of a HM mixture. These findings support the need for enhanced anemia screening and mitigation programs in the region. Moreover, the anemia profile observed suggests that iron deficiency alone may not fully explain its prevalence. As such, traditional iron supplementation strategies may be insufficient. Anemia prevention efforts should incorporate broader environmental evaluations, and diagnostic assessments should include a full iron panel and inflammatory biomarkers.

Overall, this research supports the need for public health interventions focused on primary prevention through education. Public health campaigns should raise awareness about the risks of using contaminated surface water for drinking, cooking, and irrigation. Communities should be informed about practical and affordable ways to reduce exposure—such as using treated water, avoiding contaminated irrigation, and limiting open burning. Education should also help individuals recognize early symptoms of HM toxicity and encourage timely medical attention. Community health workers and local clinics should be equipped to provide screening, accessible and culturally appropriate counseling, and referrals for children at risk.

In summary, this dissertation contributes important evidence to inform public health action. By combining environmental, biomarker, and health data, this work directly responds to community concerns and provides a clearer understanding of the pathways and health consequences of HM exposure. The findings support targeted mitigation strategies such as improved water treatment, safer agricultural practices, enhanced respiratory and anemia screening, and long-term health monitoring of children exposed to HM. Hopefully, this research will guide future interventions and promote regional cooperation between Peru and Ecuador to address transboundary environmental pollution and protect child health.

5. Future research directions

Building on the findings of this dissertation, several key areas for future research have emerged. These can be grouped into topic-specific questions related to exposure assessment, respiratory health, and anemia, followed by broader methodological recommendations.

Exposure assessment

In the exposure assessment conducted in this dissertation, I identified As as a particularly important environmental health threat, especially for those living closer to the Puyango-Tumbes River. Given As's established links to cognitive impairment, skin lesions, cardiovascular disease, and certain cancers,[32] further research should explore these and other long-term health outcomes associated with As exposure in this population.

More comprehensive exposure assessments are also needed—particularly those that include Hg measurements in both water and human samples, as well as HM assessments in food sources, especially rice. Hg, which was not included in this dissertation, is a known contaminant in the Puyango-Tumbes River[3,22] and may contribute to anemia and respiratory impairment.[23–25] Additionally, given the high frequency of rice consumption and the ability of rice to accumulate both As and MeHg,[21,27] future studies should assess HM concentrations in locally grown produce and evaluate dietary exposure pathways.

Respiratory health

Respiratory health also warrants further investigation. Although I did not find statistically significant associations between As and ppFVC, the inverse direction of the associations and the high prevalence of restrictive spirometry patterns point to the need for deeper investigation into respiratory impairment in this population. Future research should explore possible causes of restrictive lung function, including environmental, infectious, inflammatory, neuromuscular, and nutritional factors. Validating available spirometry reference equations—or developing new equations specific to Peruvian children, particularly those living at low altitudes—is also a priority to ensure accurate interpretation of lung function data. Additionally, longitudinal evaluations of respiratory health may be warranted to identify which exposures are causally associated with lung function decline.

Anemia and its mechanisms

The mechanisms by which HM exposure leads to anemia also remain unclear. While I found a link between Pb and anemia, ferritin did not mediate this association, indicating that other biological mechanisms—such as inflammation or disrupted iron transport—may be involved. Future studies should use a full iron panel (including hepcidin, soluble transferrin receptor, and transferrin saturation) and inflammatory biomarkers to better understand iron metabolism under HM exposure. Modeling additional hematological indicators (e.g., mean corpuscular volume, mean corpuscular Hb) could also help clarify how Pb affects hematologic function beyond its role in inducing anemia. Longitudinal study designs are also needed to rule out reverse causality and to better understand the timing and progression of HM-related anemia.

Demographic subgroups

Future analyses should also consider stratification by sex—given the higher exposure levels observed among girls compared to boys—and by age, as adolescents may have different dietary patterns, hormonal profiles, or exposure sources (e.g., occupational or behavioral) than younger children, potentially influencing their vulnerability to HM toxicity.

Methodological approaches

Longitudinal studies are needed to establish temporal associations, differentiate chronic versus acute effects, and evaluate how prevention and mitigation strategies affect exposure and health over time. Repeated exposure measurements—ideally paired with repeated outcome assessments—would allow for the evaluation of dose-response and threshold effects, and help clarify how changes in exposure levels influence health outcomes over time.

Studies should also consider critical windows of susceptibility, in line with the Developmental Origins of Health and Disease (DOHaD) framework[33] and evaluate how exposure to HM during critical periods—particularly during the prenatal and early childhood stages—can alter development in ways that increase disease susceptibility later in life.

Advanced statistical approaches— such as mixture modeling and methods that account for time-varying covariates—can further enhance our understanding of complex exposure-outcome associations. Mixture models better reflect real-world scenarios involving combined toxic effects. This dissertation found stronger associations between HM mixtures and anemia than with Pb alone, highlighting the value of mixture analysis and the need for its broader application. Additionally, other advanced methods, such as the parametric g-formula, allow researchers to model associations while accounting for time-varying exposures and covariates, providing more realistic assumptions that better reflect the complexities of real-world exposure dynamics.

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