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E-waste and heavy metals: health hazards and environmental impact

Edited by
Baby Tabassum, Mohammad Imran Ansari, Sarfuddin Azmi,
Mohd Kamil Hussain and Mirza Nuhanović

Published in
Frontiers in Public Health
Frontiers in Environmental Science

ISSN 1664-8714
ISBN 978-2-8325-5737-2
DOI 10.3389/978-2-8325-5737-2

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E-waste and heavy metals: health hazards and environmental impact

Topic editors
Baby Tabassum — M. J. P. Rohilkhand University, India
Mohammad Imran Ansari — University of Maryland, Baltimore, United States
Sarfuddin Azmi — Prince Sultan Military Medical City, Saudi Arabia
Mohd Kamil Hussain — Govt. Raza Post Graduate College, Rampur, India
Mirza Nuhanović — University of Sarajevo, Bosnia and Herzegovina

Citation
Tabassum, B., Ansari, M. I., Azmi, S., Hussain, M. K., Nuhanović, M., eds. (2024). *E-waste and heavy metals: health hazards and environmental impact*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-5737-2

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frontiers | Frontiers in Public Health

TYPE Editorial
PUBLISHED 15 November 2024
DOI 10.3389/fpubh.2024.1506438

OPEN ACCESS

EDITED AND REVIEWED BY
Yang Gao,
Chinese Academy of Sciences (CAS), China

*CORRESPONDENCE
Baby Tabassum
dr.btabassum@gmail.com

RECEIVED 05 October 2024
ACCEPTED 01 November 2024
PUBLISHED 15 November 2024

CITATION
Tabassum B (2024) Editorial: E-waste and heavy metals: health hazards and environmental impact.
Front. Public Health 12:1506438.
doi: 10.3389/fpubh.2024.1506438

Editorial: E-waste and heavy metals: health hazards and environmental impact

Baby Tabassum[1,2]*

[1]Department of Zoology, Toxicology Lab, Govt. Raza P.G. College, Rampur, Uttar Pradesh, India,
[2]Mahatma Jyotiba Phule Rohilkhand University, Bareilly, India

KEYWORDS

e-waste, heavy metal, environmental distribution, exposure, human health and disease

Editorial on the Research Topic
E-waste and heavy metals: health hazards and environmental impact

As the topic editor for "*E-Waste and Heavy Metals: Health Hazards and Environmental Impact*," I am honored to present a Research Topic that addresses the urgent global challenge of e-waste management and the health risks posed by heavy metals. In an era of rapid technological growth, e-waste has become a critical environmental concern, threatening both ecosystems and human health due to hazardous metals like lead, mercury, cadmium, and nickel.

This Research Topic integrates diverse studies that analyse the health consequences of heavy metals from a variety of perspectives. A cross-sectional study on the association between anxiety and blood cadmium, lead, and mercury is a notable manuscript that provides valuable perspectives into the neurotoxic effects of heavy metal exposure. Investigations on systemic inflammation due to combined heavy metals and the risk of metabolic disorders like metabolic syndrome and fatty liver disease are equally significant.

Moreover, pioneering investigations into remediation techniques, including the phytoremediation capabilities of earthworms in cadmium-polluted soil and revelations regarding mercury toxicity from conventional Ayurvedic methods, present promising avenues for alleviating the environmental and health consequences of heavy metal exposure. These studies, despite their diverse focus, converge on a singular theme: the pressing necessity for global consciousness and intervention to address waste management and mitigate heavy metal pollution.

Key insights and strategies:

- **Health implications:** the accepted studies demonstrate alarming associations between heavy metal exposure and a variety of health issues, emphasizing the necessity of immediate action.
- **Sustainable practices:** advocating for eco-friendly design and accountable recycling initiatives is crucial to alleviate the effects of e-waste on health and the environment.
- **Collaborative endeavors:** involving stakeholders—from producers to consumers—is essential for formulating efficient e-waste management strategies.

I express my profound gratitude to all authors and reviewers for their significant contributions to this Research Topic. The findings presented here not only deepen our scientific understanding but also emphasize the need for sustainable solutions in the management of e-waste and heavy metals to safeguard both public health and the environment.

I hope this Research Topic will serve as a significant step toward raising awareness, advancing research, and informing policies that can help mitigate the risks posed by these environmental hazards.

Author contributions

BT: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 28 July 2023
DOI 10.3389/fpubh.2023.1241971

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OPEN ACCESS

EDITED BY
Mohd Kamil Hussain,
Govt. Raza Post Graduate College, Rampur, India

REVIEWED BY
Robeena Sarah,
M. J. P. Rohilkhand University, India
Nida Idrees,
Govt. Raza Post Graduate College, Rampur, India

*CORRESPONDENCE
Shuai Zhang
✉ senomega@hotmail.com

RECEIVED 18 June 2023
ACCEPTED 19 July 2023
PUBLISHED 28 July 2023

CITATION
Zhang S, Tang H and Zhou M (2023) Sex-specific associations between nine metal mixtures in urine and urine flow rate in US adults: NHANES 2009–2018.
Front. Public Health 11:1241971.
doi: 10.3389/fpubh.2023.1241971

Sex-specific associations between nine metal mixtures in urine and urine flow rate in US adults: NHANES 2009–2018

Shuai Zhang[1,2]*, Hanhan Tang[3] and Minglian Zhou[1,2]

[1]Department of Male Reproductive Health, Lianyungang Maternal and Child Health Hospital, Lianyungang, China, [2]Clinical Center of Reproductive Medicine, Lianyungang Maternal and Child Health Hospital, Lianyungang, China, [3]Graduate School of Xuzhou Medical University, Xuzhou Medical University, Xuzhou, China

Background: The urinary system serves as a crucial pathway for eliminating metallic substances from the body, making it susceptible to the effects of metal exposure. However, limited research has explored the association between metal mixtures and bladder function. This study aims to investigate the relationship between urinary metal mixtures (specifically barium, cadmium, cobalt, cesium, molybdenum, lead, antimony, thallium, and tungsten) and urine flow rate (UFR) in the general population, utilizing multiple mixture analysis models.

Methods: This study utilizes data obtained from the National Health and Nutrition Examination Survey. After adjusting for relevant covariates, we assessed the correlations between metal mixtures and UFR using three distinct analysis models: weighted quantile sum (WQS), quantile g-computation (qgcomp), and Bayesian kernel machine regression (BKMR). Additionally, a gender-stratified analysis was conducted. Finally, we also performed sensitivity analyses.

Results: A total of 7,733 subjects were included in this study, with 49% being male. The WQS regression model, when fitted in the positive direction, did not yield any significant correlations in the overall population or in the male and female subgroups. However, when analyzed in the negative direction, the WQS index exhibited a negative correlation with UFR in the overall group ($\beta = -0.078$; 95% CI: −0.111, −0.045). Additionally, a significant negative correlation between the WQS index and UFR was observed in the female group ($\beta = -0.108$; 95% CI: −0.158, −0.059), while no significant correlation was found in the male group. The results obtained from the qgcomp regression model were consistent with those of the WQS regression model. Similarly, the BKMR regression model revealed a significant negative correlation trend between metal mixtures and UFR, with cadmium and antimony potentially playing key roles.

Conclusion: Our study revealed a significant negative correlation between urinary metal mixture exposure and mean UFR in US adults, with notable gender differences. Specifically, higher urinary levels of cadmium and antimony were identified as potential key factors contributing to the decrease in mean UFR. These findings significantly contribute to the existing knowledge on the impact of metal mixtures on bladder function and provide valuable insights for safeguarding bladder health and preventing impaired bladder function.

KEYWORDS

urine flow rate, metal mixtures, BKMR, WQS, qgcomp, NHANES, US adults

1. Introduction

Heavy metals pose significant environmental and occupational hazards, being widely prevalent in the environment and capable of entering the human body through various routes and forms (1, 2). These non-essential metal elements are often resistant to degradation, and some of them can undergo redox reactions, leading to the formation of biologically active substances that exhibit metallotoxicity even at low doses. Consequently, they are implicated in the pathogenesis of various diseases, including cancer, cardiovascular diseases, neurological disorders (such as Alzheimer's and Parkinson's diseases), and chronic inflammatory diseases (3).

Urine production and excretion play a crucial role in human metabolism, serving as a primary route for eliminating most metals from the body. Consequently, the urinary system is inevitably influenced by the presence of metal metabolites. Previous research has demonstrated the nephrotoxic effects of several metal elements, including barium, chromium, cadmium, cobalt, copper, lead, mercury, platinum, and uranium (4–6). These metals can induce cellular oxidative stress, resulting in cell swelling and apoptosis (7, 8). However, studies investigating the impact of metallic elements in urine on human bladder function, particularly at low exposure levels, are limited and primarily confined to animal experimentation (9, 10). Additionally, gender-based disparities in the effects of metal exposure have been observed in some studies (11, 12). Nevertheless, it remains unknown whether such differences extend to bladder function. Furthermore, the analysis of metal mixtures in urine and their influence on bladder function is understudied, hindered by methodological limitations.

Urine biomonitoring is a preferred method for assessing chemical elements, metabolite exposures, and nutritional status due to its non-invasive nature and compatibility with modern analytical techniques. It is equally valuable for detecting metal elements. However, urine analyte concentrations are influenced by various factors beyond exposure, including sampling time, variations in toxicant metabolism kinetics, and physiological characteristics such as dilution changes (13). Therefore, when utilizing field urine samples for research purposes, additional data on urine flow rate (UFR) should be collected to ensure accurate interpretation of urine data (14). Since 2009, the National Health and Nutrition Examination Survey (NHANES) has been assessing the mean UFR of participants aged 6 years and older. The UFR is mainly regulated by the strength of contraction of the detrusor muscle and bladder outlet resistance (15–20), indirectly reflecting bladder function and state.

The objective of our study was to investigate the association between low-dose metal mixtures, specifically barium (Ba), cadmium (Cd), cobalt (Co), cesium (Cs), molybdenum (Mo), lead (Pb), antimony (Sb), thallium (Tl), and tungsten (W), and UFR in the general adult population. We conducted this analysis utilizing the NHANES dataset from 2009 to 2018, aiming to identify the metal elements within the mixture that may have the greatest impact on UFR.

2. Materials and methods

2.1. Design and participants

NHANES, organized by the National Center for Health Statistics (NCHS), is a cross-sectional survey research program aimed at evaluating the health and nutritional status of individuals in the United States, including both adults and children. Since 1999, NHANES has been conducted biennially using a complex multistage probability sampling design. The survey results are instrumental in determining the prevalence of major diseases and identifying associated risk factors (21). For this study, we utilized NHANES data from five cycles spanning 2009–2010, 2011–2012, 2013–2014, 2015–2016, and 2017–2018. The NHANES study was conducted under the authorization of the National Center for Health Statistics (NCHS) Ethics Review Committee, and all participants provided informed consent.

All data utilized in this study are publicly available on the official NHANES website: https://www.cdc.gov/nchs/nhanes/index.htm (Last accessed on March 20, 2023). The initial enrollment consisted of 49,693 participants across the five cycles. After screening the data, individuals under the age of 20 were excluded ($N = 20{,}858$). Furthermore, participants with missing key data were excluded (missing urine metal data: $N = 19{,}802$; missing UFR data: $N = 490$), along with those with missing covariate data ($N = 810$). Ultimately, a total of 7,733 adult participants were included in this study.

2.2. Measurement of urine metals

Urine specimens were collected within mobile exam centers (MEC) and subsequently processed, stored, and transported to the Laboratory Sciences Department of the National Center for Environmental Health for analysis. The levels of metals in urine samples were directly measured using inductively coupled plasma mass spectrometry (ICP-MS), with a comprehensive description of the laboratory method provided in the NHANES official instruction document (22). To address values falling below the lower limit of detection (LLOD), the NHANES guidelines recommended replacing them with the LLOD divided by the arithmetic square root of 2 (22). The detection rates for all metal elements exceeded 75%, and a detailed breakdown of the detection rates for each specific metal element can be found in Supplementary Table S1.

2.3. Measurement of UFR

NHANES initiated the collection of UFR data in 2009. Participants were instructed to note the time of their last urination prior to visiting the MEC. Within the MEC, participants provided urine samples and documented the collection time and volume for UFR calculation. The composite urine sample's UFR was determined using the equation: UFR = (total urine volume)/(total duration) (23). To ensure an adequate urine volume for various analyses, each participant was permitted to provide up to three urine samples. Comprehensive guidelines for urine collection and handling can be found in the NHANES Laboratory Procedures Manual (LPM).

2.4. Assessment of covariates

To control for the effect of confounding factors on the study results, covariate adjustment was performed in the data analysis. The following covariates were included: sex, age (continuous), race, educational attainment, BMI (categorical), smoking status (categorical), cardiac history (categorical), systolic blood pressure (continuous), urinary creatinine (continuous), serum glucose (continuous), aspartate aminotransferase (AST, continuous), and estimated glomerular filtration rate (eGFR, continuous). Race categories were Mexican American, non-Hispanic White, non-Hispanic Black, other Hispanic, and other races. Educational attainment categories were less than 9th grade, 9–11th grade, high school graduate/GED or equivalent, some college or AA degree, and college graduate or above. BMI categories were underweight (<18.5 kg/m2), normal (18.5 to <25 kg/m2), overweight (25 to <30 kg/m2), and obese (30 kg/m2 or greater). Smoking status was defined as never smoker, former smoker, and current smoker based on self-reported information. A history of cardiac disease was defined as a history of one or more of congestive heart failure, coronary artery disease, angina pectoris, and heart attack. Serum glucose levels were measured using the DxC800 modular chemistry system with a Beckman Oxygen electrode, while AST levels were measured using the DxC800 enzymatic rate method. eGFR was calculated using the modified 4-variable Modification of Diet in Renal Disease (MDRD) formula: eGFR (mL/min/m2) = $175 \times (\text{Scr})^{-1.154} \times (\text{age})^{-0.203} \times 0.742$ (if female) $\times$ 1.212 (if black), where Scr is the serum creatinine level (mg/dL) and age is expressed in years (24). Creatinine levels were measured using the Roche/Hitachi Modular P Chemistry Analyzer from serum and urine samples.

2.5. Statistical analysis

Since the elemental metal, UFR, and urinary creatinine data were seriously right-skewed, a natural logarithm (ln) transformation was applied to these variables in order to improve their distribution characteristics and minimize the effect of outliers (25). Quantitative data are presented as the Median (interquartile range, IQR), while qualitative data are reported as percentages. Spearman's correlation test was employed to examine the associations between ln-transformed metals.

To assess the relationship between metal mixtures and UFR, we employed three advanced mixture analysis methods: Weighted quantile sum (WQS), Quantile g-computation (qgcomp), and Bayesian kernel machine regression (BKMR).

2.5.1. WQS model

We used a WQS regression model (26, 27) to assess the effect of metal mixtures. This method realizes dimension reduction and solves the collinearity problem by constructing the WQS index, and further tests the association between the WQS index and outcome. The model assigned weights to each exposure variable to determine their relative importance in influencing the outcome and identify potential high-risk factors. The WQS regression assumes by default that all exposed variables are correlated with the outcome in the same direction (positive or negative). Therefore, in the actual number analysis, two runs are required to test for positive and negative correlations. During the model fitting process, the dataset was divided into a 40% training set and a 60% validation set. The training set was utilized for weight estimation, while the validation set was used to test the significance of the WQS index. The final WQS index of this study was averaged from the weights in the 500 bootstrap samples.

2.5.2. Qgcomp model

The qgcomp model is a newly developed approach that integrates WQS regression with basic g calculation (28). By employing quantile g calculation, we can assess the overall effect on the results when all exposures are simultaneously increased by one quartile, irrespective of the direction of correlation between exposures and results. In cases where different metal elements exert distinct directional influences, qgcomp assigns positive or negative weight values to each metal element, which sum up to 1 or − 1.

2.5.3. BKMR model

The BKMR model (29) was employed to investigate the potential nonlinear relationship between each metal element and UFR, as well as the combined impact of metal mixtures on UFR. This method has strong statistical power in the field of mixed contaminant analysis. By fixing all metals simultaneously at a specific percentile (ranging from the 25th to the 75th percentile) compared to when they are fixed at the median, we can obtain the overall effect of the metal mixture on UFR., we can obtain the overall effect of the metal mixture on UFR. By fixing other metal elements at their respective median levels, we examined the nonlinear correlation between exposure and outcome by looking at exposure-response cross-sections between specific metal elements and the outcome. When all other metals are fixed at the 25th percentile, 50th percentile, and 75th percentile, respectively, the individual effects of a single metal are shown by comparing the risk associated with the 75th percentile of a particular metal element to its 25th percentile. Additionally, the model calculates the posterior inclusion probability (PIP) for each metal. In this study, the model was run with 50,000 iterations of the Markov chain Monte Carlo sampler.

Given NHANES' utilization of a complex multistage probability sampling design, we performed multiple linear regression analyses in a weighted setting to check the robustness of the results. We examined the relationship between urinary metallic elements and UFR using both monometallic and polymetallic models.

To assess potential gender differences in the relationship between metallic elements in urine and UFR, we performed a gender-stratified analysis that covered all models.

All the aforementioned analyses incorporated all covariates, including sex, age, race, educational attainment, BMI, smoking status, cardiac history, systolic blood pressure, ln-urine creatinine, serum glucose, AST, and eGFR. For the gender-stratified study, all covariates other than gender were included.

All statistical analyses were performed using R version 4.2.2. The weighted analysis utilized the "survey" package (version 4.1-1). The WQS regression model employed the "gWQS" package (version 3.0.4), the qgcomp model utilized the "qgcomp" package (version 2.10.1), and the BKMR model employed the "bkmr" package (version 0.2.2). For statistical significance, p-values (two-sided) below 0.05 were considered significant.

3. Results

3.1. Participant baseline characteristics

Table 1 presents the essential characteristics of the study population under investigation. The median age of the participants was 47.0 years. Among the included participants, 49% (n = 3,812) were

TABLE 1 Basic characteristics of the population included in this study (*N* = 7,733), NHANES, USA, 2009–2018.

	Overall	Sex group		
Characteristic	Overall, *N* = 7,733 (100%)[a]	Female, *N* = 3,921 (51%)[b]	Male, *N* = 3,812 (49%)[a]	*p*-value[b]
Cycle (n%)				>0.9
2009–2010	1,745 (19%)	886 (19%)	859 (19%)	
2011–2012	1,449 (20%)	714 (20%)	735 (19%)	
2013–2014	1,579 (20%)	808 (20%)	771 (20%)	
2015–2016	1,541 (20%)	787 (21%)	754 (20%)	
2017–2018	1,419 (21%)	726 (20%)	693 (21%)	
Age (years)	47.0 (33.0, 61.0)	48.0 (34.0, 61.0)	46.0 (33.0, 60.0)	0.024
Sex [n (%)]				
Female	3,921 (51%)			
Male	3,812 (49%)			
Race [n (%)]				0.074
Non-Hispanic White	3,100 (66%)	1,559 (66%)	1,541 (67%)	
Non-Hispanic Black	1,595 (11%)	781 (12%)	814 (9.8%)	
Mexican American	1,150 (8.6%)	598 (8.2%)	552 (9.1%)	
Other/multiracial	1,071 (8.3%)	539 (8.5%)	532 (8.1%)	
Other Hispanic	817 (6.1%)	444 (6.1%)	373 (6.1%)	
BMI [n (%)]				<0.001
Underweight (<18.5)	119 (1.5%)	75 (2.0%)	44 (0.9%)	
Normal (18.5 to <25)	2,100 (27%)	1,090 (31%)	1,010 (24%)	
Overweight (25 to <30)	2,521 (33%)	1,098 (28%)	1,423 (37%)	
Obese (30 or greater)	2,993 (39%)	1,658 (39%)	1,335 (38%)	
Smoking status [n (%)]				<0.001
Never smoker	4,355 (56%)	2,572 (63%)	1,783 (48%)	
Former smoker	1,884 (26%)	707 (20%)	1,177 (32%)	
Current smoker	1,494 (18%)	642 (17%)	852 (20%)	
Education attainment [n (%)]				0.034
Less than 9th grade	773 (5.2%)	380 (4.9%)	393 (5.6%)	
9–11th grade	998 (9.5%)	483 (9.1%)	515 (10.0%)	
High school grad/GED	1,756 (23%)	834 (22%)	922 (24%)	
Some college or AA degree	2,345 (32%)	1,298 (34%)	1,047 (30%)	
College graduate or above	1,861 (31%)	926 (31%)	935 (31%)	
Cardiac history [n (%)]				<0.001
Heart attack	588 (6.4%)	225 (5.1%)	363 (7.7%)	
Non heart attack	7,145 (94%)	3,696 (95%)	3,449 (92%)	
Systolic blood pressure (mmHg)	120 (111, 131)	117 (107, 131)	122 (114, 132)	<0.001
Serum glucose (mg/dL)	93 (85, 103)	92 (85, 101)	94 (86, 104)	<0.001
Urine creatinine (mg/dL)	99 (55, 158)	79 (43, 131)	123 (72, 179)	<0.001
Aspartate aminotransferase (U/L)	22 (19, 27)	21 (18, 25)	24 (20, 29)	<0.001
eGFR (mL/min/m2)	87.48 (74.00, 102.65)	87.42 (73.34, 103.73)	87.49 (74.87, 101.67)	>0.9
Urine flow rate (mL/min)	0.87 (0.55, 1.42)	0.83 (0.51, 1.43)	0.90 (0.59, 1.42)	0.001

[a]n (unweighted) (%); Median (IQR).
[b]Chi-squared test with Rao and Scott's second-order correction; Wilcoxon rank-sum test for complex survey samples.
BMI, body mass index; eGFR, estimated glomerular filtration rate.

male, with a median age of 46 years. Baseline comparisons revealed that male participants exhibited higher levels of systolic blood pressure, glucose, AST, urinary creatinine, and mean UFR. Additionally, more of the male participants had a cardiac history and smoking.

3.2. Metal correlation study

The Spearman correlation coefficients (rs) between the ln-transformed metals ranged from 0.21 to 0.77 (see Figure 1), with the strongest correlations being Cs with Tl ($r = 0.77$), and Cs with Mo ($r = 0.61$), Mo with W ($r = 0.6$), and Cs with Co ($r = 0.59$), respectively, with significant correlations for all metals ($p < 0.001$).

3.3. Differences in the distribution of metal elements in different groups

Supplementary Table S2 shows the distribution of metallic elements in urine in general and among different sexes. We revealed significant differences between males and females in the concentrations of several metal elements. Specifically, males exhibited notably higher levels of Ba, Cs, Mo, Pb, Sb, Tl, and W compared to females. Conversely, females displayed higher levels of Cd exposure.

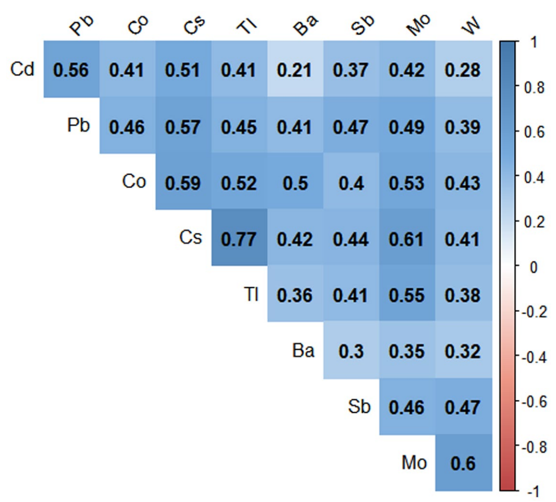

FIGURE 1
Paired Spearman correlations for urinary concentrations of nine metals in the population ($N = 7{,}733$), NHANES, USA, 2009–2018. The blue color represents the positive correlation, all correlations were statistically significant (p value < 0.001).

3.4. WQS regression model and qgcomp model

The WQS regression model was utilized to investigate the correlation between urine metal and UFR in both positive and negative directions. After adjusting for all confounding factors, no significant correlation was observed between the WQS index and UFR in the positive direction. However, in the negative direction, a significant negative correlation was found between the WQS index and UFR in the overall ($\beta = -0.078$; 95% CI: −0.111, −0.045). Subsequent gender-stratified analysis revealed a significant negative correlation between the WQS index and UFR in females ($\beta = -0.108$; 95% CI: −0.158, −0.059), while no significant correlation was observed in males ($\beta = -0.014$; 95% CI: −0.059, 0.032) (see Table 2). Additionally, Figure 2 shows the estimated weights for each WQS index, with Sb and Cd exhibiting the highest negative weights in the overall, and Cd, Co, and Sb showing the highest negative weights in females.

Similar to the results of the WQS model, the results of the qgcomp model showed a similar trend. In the overall, the qgcomp index exhibited a negative correlation with UFR ($\beta = -0.061$; 95% CI: −0.091, −0.031). Regarding single metal weights, urinary Tl (49%) had the highest positive contribution to the overall effect, followed by Ba (45.8%). Conversely, urinary Sb (30.8%) had the most negative weight, followed by Cd (25.1%). Similar to the findings from the WQS model, no significant association between the metal mixture and UFR was observed in males. However, in females, the qgcomp index showed a significant negative correlation with UFR ($\beta = -0.096$; 95% CI: −0.139, −0.053). In terms of single metal weights, urinary Tl (53%) made the largest positive contribution to the overall effect, followed by Ba (44.9%). Urinary Sb (30.8%) had the greatest negative weighting, followed by Cd (29.6%). For detailed results, refer to Table 2 and Figure 3.

3.5. BKMR model to assess the correlation between metal mixture and UFR

Overall association: Figure 4 demonstrates the overall association between the metal mixture and UFR. Upon adjusting for all confounding factors, a consistent decreasing trend was observed in the effect on UFR when the concentrations of all metals were

TABLE 2 Association between urine metal WQS index and qgcomp index and UFR ($N = 7{,}733$), NHANES, USA, 2009–2018.

	Negative WQS		Positive WQS		qgcomp	
	Beta (95%CI)	*p*-value	Beta (95%CI)	*p*-value	Beta (95%CI)	*p*-value
Overall	−0.078 (−0.111, −0.045)	<0.001	0.01 (−0.017, 0.036)	0.47	−0.061(−0.091, −0.031)	<0.001
Male	−0.014 (−0.059, 0.032)	0.563	−0.005 (−0.053, 0.042)	0.823	−0.011 (−0.054, 0.031)	0.601
Female	−0.108 (−0.158, −0.059)	<0.001	−0.023 (−0.061, 0.016)	0.245	−0.096 (−0.139, −0.053)	<0.001

Data are expressed as WQS regression indices (95% confidence intervals) and qgcomp model indices (95% confidence intervals). The model was adjusted for sex, age, race, educational attainment, BMI, smoking status, cardiac history, systolic blood pressure, ln-urine creatinine, serum glucose, AST, and eGFR. In analyses stratified by sex, the full model was adjusted for confounders other than sex. WQS, weighted quantile sum; qgcomp, quantile g-computation; CI, confidence interval.

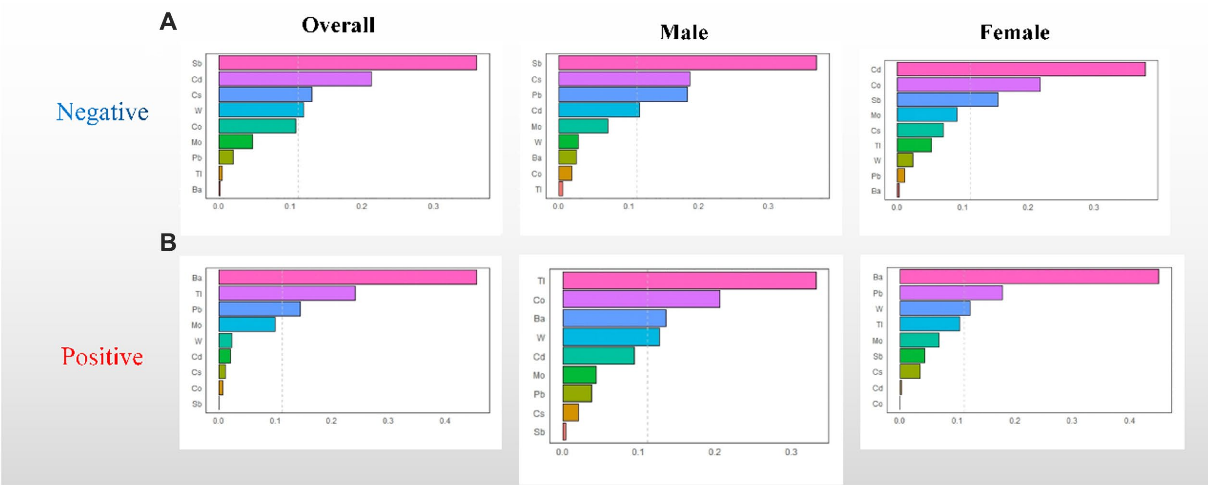

FIGURE 2
WQS regression weights of the urinary metals for UFR. **(A)** Negative WQS regression weights between urinary metals and UFR; **(B)** positive WQS regression weights between urinary metals and UFR. The model adjusted for sex, age, race, educational attainment, BMI, smoking status, cardiac history, systolic blood pressure, ln-urine creatinine, serum glucose, AST, and eGFR. Confounders other than sex were included in the gender-stratified analysis.

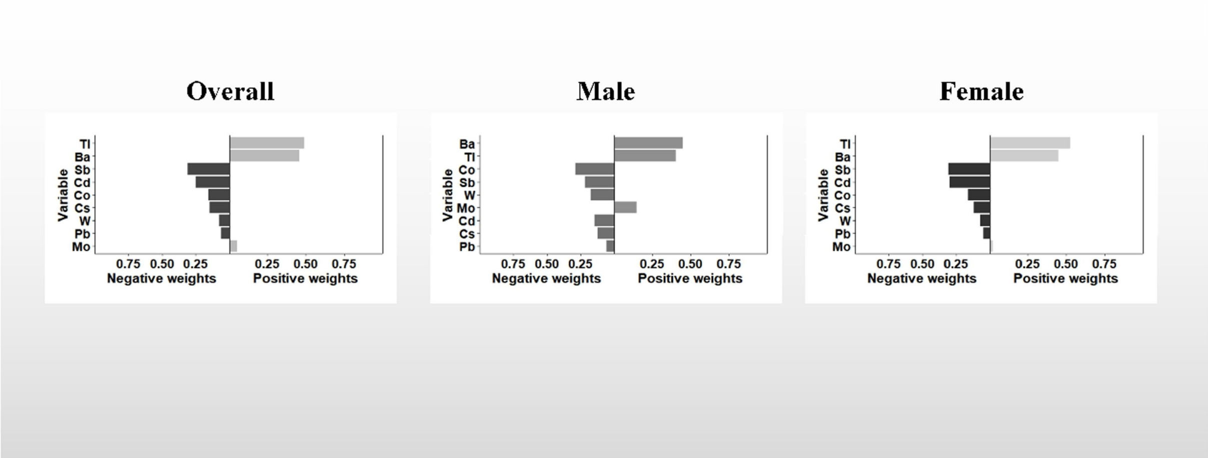

FIGURE 3
The qgcomp model weights of the urinary metal mixture for UFR. The model adjusted for sex, age, race, educational attainment, BMI, smoking status, cardiac history, systolic blood pressure, ln-urine creatinine, serum glucose, AST, and eGFR. Confounding factors other than sex were included in the gender-stratified analysis.

simultaneously fixed at different percentiles (25th-75th percentiles) compared to when they were fixed at the median. Notably, the effect of the urinary metal mixture on UFR reached statistical significance only when all metals were simultaneously fixed at the 60th percentile and above among male participants. This finding indicates a significant negative correlation between urinary metal mixtures and UFR.

Nonlinear exposure-response relationships for single metals: Supplementary Figure S1 presents the nonlinear exposure-response relationships between single metals and UFR, with the concentrations of other metals held constant at their respective median concentrations. Ba exhibited a positive correlation with UFR in both the overall participants and among males. Conversely, Tl showed a positive correlation with UFR in females. Cd, Co, Cs, Sb, and W displayed negative correlations with UFR across all participants. Additionally, Pb demonstrated a negative correlation with UFR specifically among males.

Single metal effect: Figure 5 presents a summary of the risk on UFR when a single metal was increased from the 25th to the 75th percentile, while other metals were fixed at different percentiles (25th, 50th, and 75th). Significant negative associations with UFR were observed for Cd (when other metals were fixed at the 25th, 50th, and 75th percentiles) in all study groups, except for the male group. In the overall participants, a statistically significant negative correlation was found between Sb and UFR (when other metals were fixed at the 50th percentile). The posterior inclusion probability (PIP) analysis indicated that Cd (PIP = 1) and Cs (PIP = 0.9136)

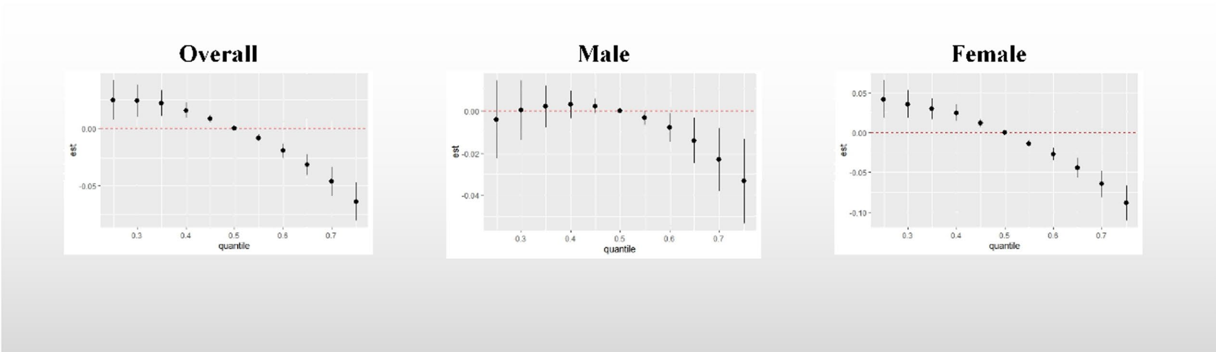

FIGURE 4
Overall association between metal mixtures and UFR. The model adjusted for sex, age, race, educational attainment, BMI, smoking status, cardiac history, systolic blood pressure, ln-urine creatinine, serum glucose, AST, and eGFR. Confounders other than sex were included in the gender-stratified analysis.

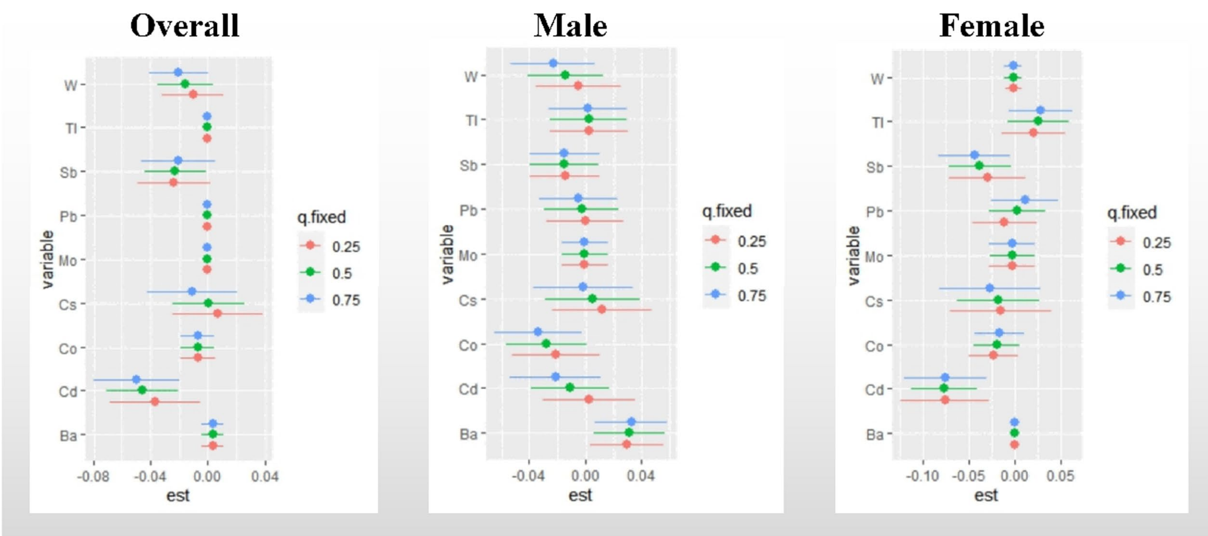

FIGURE 5
Effect of single metal changes on UFR at the 25, 50, and 75% quartiles for single metals (estimates and 95% confidence intervals). The model adjusted for sex, age, race, educational attainment, BMI, smoking status, cardiac history, systolic blood pressure, ln-urine creatinine, serum glucose, AST, and eGFR. in the gender-stratified analysis, confounders other than sex were included. "est" was defined as the association between a single metal element and UFR. 95% confidence intervals excluding any percentile estimate of 0 were considered statistically significant.

contributed the most to the effect on UFR, followed by Sb (PIP = 0.1996). Among males, Ba showed a significant positive correlation with UFR, while Co exhibited a statistically significant negative correlation when other metals were fixed at the 75th percentile. Among females, a statistically significant negative correlation was observed between Sb and UFR when other metals were fixed at the 50th and 75th percentiles. The PIP analysis revealed that Cd (PIP = 0.9994) and Cs (PIP = 0.9663) had the highest contributions to the effect on UFR. Detailed PIP results can be found in Supplementary Table S2.

3.6. Sensitivity analysis

The results of the monometallic and polymetallic weighted linear regression analyses can be seen in Supplementary Figures S2, S3, respectively. After adjusting for all covariates, the monometallic model showed that urine Ba was significantly positively correlated with UFR in the overall participants (β = 0.023, 95% CI: 0.006, 0.041). Conversely, Cd (β = −0.034, 95% CI: −0.056, −0.013), Sb (β = −0.039, 95% CI: −0.062, −0.015), and W (β = −0.019, 95% CI: −0.033, −0.005) were significantly and negatively correlated with UFR. Females exhibited greater sensitivity to the response of metals in urine compared to males, as indicated by the significant negative associations between Cd (β = −0.057, 95% CI: −0.088, −0.027) and Sb (β = −0.082, 95% CI: −0.112, −0.052) with UFR in the female monometallic model.

Similar to the monometallic model, the polymetallic linear regression model, which includes all metals in the regression simultaneously, yielded comparable results. In males, UFR showed no significant associations with any of the metals, while in females, Ba (β = 0.029, 95% CI: 0.005, 0.053) was significantly and positively

TABLE 3 Summary results from different models.

	Overall	Male	Female
BKMR	Cd (−), Sb (−)	Ba (+), Co (−)	Cd (−), Sb (−)
WQS negative maximum weight	Sb (−), Cd (−), Cs (−), W (−)	Sb (−), Cs (−), Pb (−), Cd (−)	Cd (−), Co (−), Sb (−)
WQS positive maximum weight	Ba (+), Tl (+), Pb (+)	Tl (+), Co (+), Ba (+), W (+)	Ba (+), Pb (+), W (+)
Qgcomp negative weight	Sb (−), Cd (−), Co (−), Cs (−), W (−), Pb (−)	Co (−), Sb (−), W (−), Cd (−), Cs (−)	Sb (−), Cd (−), Co (−), Cs (−), W (−), Pb (−)
Qgcomp positive weight	Tl (+), Ba (+), Mo (+)	Ba (+), Tl (+), Mo (+)	Tl (+), Ba (+), Mo (+)
Monometallic weighted linear regression analyses	Ba (+), Cd (−), Sb (−), W (−)	-	Cd (−), Sb (−)
Polymetallic weighted linear regression analyses	Ba (+), Cd (−), Sb (−), W (−)	–	Ba (+), Cd (−), Sb (−)

WQS, weighted quantile sum; qgcomp, quantile g-computation; BKMR: Bayesian kernel machine regression. * (+) means positive weight while (−) means negative weight.

correlated with UFR, while Cd ($\beta = -0.053$, 95% CI: −0.084, −0.023) and Sb ($\beta = -0.078$, 95% CI: −0.111, −0.045) were significantly negatively correlated with UFR. Additionally, all variance inflation factors (VIF) in the polymetallic linear regression model were below 10. A summary of the different models is shown in Table 3.

4. Discussion

To our knowledge, no previous studies have investigated the association between exposure to a mixture of metals and mean UFR. In this study, we examined the relationship between urinary metal elements and average UFR in various populations using multiple mixture analysis models. Overall, the findings from our study were consistent across all the analyzed models. The combined results revealed a significant negative correlation between urinary metal mixture exposure and UFR, with notable gender differences, particularly in females who showed higher sensitivity. It is noteworthy that Cd and Sb appear to be the primary contributors to these results, with Cd exhibiting the highest negative weight. However, it is important to mention that while the BKMR model identified a significant positive correlation between Ba and UFR in males, as well as a significant negative correlation between Co and UFR, these findings were not consistently observed in other models.

Cd is a widely known toxic non-essential metal element that can cause significant effects on the body, even at low doses. It has a low excretion rate and an extended biological half-life, and long-term exposure can have harmful effects on the organs that store the metal. In the United States, the daily dietary intake of Cd primarily comes from cereals and bread (34%) and green leafy vegetables (20%) (30). Additionally, tobacco use is another major source of Cd exposure (31). Cd has been found to inhibit cellular antioxidant enzyme activity, promote lipid peroxidation, and induce oxidative stress responses (32, 33). Studies have demonstrated that even at low concentrations, Cd can bind to cell mitochondria, hindering oxidative phosphorylation and resulting in cell damage and apoptosis (34, 35). Furthermore, Cd exposure can adversely affect the human nervous system (36, 37). Cd can inhibit the release of acetylcholine, which may be a biological effect by interfering with calcium metabolism (38).

Animal studies have demonstrated that exposure to Cd for 3 months adversely affects the neurogenic and myogenic contractile activity of the rat detrusor (39). In a subacute toxicity study involving isolated rat detrusor muscle, Cd was found to decrease contractile activity mediated by electrical field stimulation, acetylcholine (ACh), and adenosine triphosphate (ATP) (10). ACh and ATP are the primary neurotransmitters involved in bladder smooth muscle contraction. The toxicological mechanisms and the results of animal experiments of Cd mentioned above align with our findings. We observed a significant negative linear correlation between Cd and UFR, even at low doses. However, this correlation was not significant in male subjects. This suggests that daily Cd exposure at low doses can also cause abnormal bladder function and affect the contractile activity of the detrusor muscle of the bladder.

Sb is a heavy element widely present in the environment and extensively used in modern industry. Daily life sources of Sb exposure include diet, atmospheric pollution, drugs, and occupational settings (40). High levels of antimony are commonly found in proximity to smelters (41), with waste incineration and fossil fuel combustion also contributing to its presence (42). In addition, the use of plastic products makes food more susceptible to Sb contamination (43). Studies have demonstrated that the inorganic form of Sb exhibits a strong affinity for thiol groups, leading to intracellular glutathione depletion. Furthermore, Sb impairs glutathione peroxidase activity, reducing free glutathione levels and increasing cellular vulnerability to oxidative stress (44). Even at low doses, Sb significantly impacts mitochondrial function, decreasing mitochondrial membrane potential, respiratory enzyme complex activity (I/II/III/IV), ATP/ADP ratio, and ATP concentration (45). Sb exposure also inhibited intracellular pyruvate dehydrogenase activity, causing an increase in anaerobic glycolysis and resulting in a decrease in intracellular ATP levels. These findings suggest that antimony can cause damage to mitochondria. Typically, the respiratory and cardiovascular systems are mainly affected after Sb exposure (40). Experimental studies have revealed that the administration of antimony potassium tartrate induces cardiac fiber degeneration and connective tissue damage, even at low doses (46). In addition, it has been reported that Sb may be neurotoxic and can lead to neuronal apoptosis (47).

To our knowledge, no studies investigating the relationship between Sb and bladder function have been reported. In our study, we observed that the impact of Sb on mean UFR resembled that of Cd. This similarity suggests that Sb may reduce bladder detrusor muscle contractility through mechanisms involving cellular oxidative stress, inhibition of oxidative phosphorylation, apoptosis promotion, and damage to the nervous system. However, further research is required to fully elucidate the exact underlying mechanism.

Significant gender differences were observed in the effects of metal mixtures, as revealed by our study. Multiple mixture analysis models demonstrated a negative correlation between mean UFR and urinary metal mixtures in both males and females, with females displaying greater sensitivity. This finding aligns with previous studies, including research on the differential neurodevelopmental impact of prenatal and/or postnatal exposure to mercury, lead, manganese, cadmium, and arsenic in children (11). Furthermore, gender disparities were identified in the association between blood and urine metal mixtures and cancer mortality, with females exhibiting higher susceptibility to metal exposure (2). Notably, studies have reported a significant relationship between plasma Cu and glycosylated hemoglobin (HbA1c) exclusively in females, while no such association was observed in males (48).

Several potential mechanisms may account for this phenomenon. Firstly, differences in hormone levels between males and females can contribute to variations in metal metabolism (49). Secondly, gender differences in redox homeostasis, characterized by glutathione metabolism, may play a role (50). Additionally, genetic polymorphisms and differences in gene expression between sexes determine the differences in sensitivity to metals (51). Lastly, disparities in diet and behavioral habits between men and women can also have an impact (52). Notably, women's unique physiological mechanisms, such as their propensity to experience greater iron loss, can result in increased metal absorption and subsequent enrichment and accumulation in their bodies (53).

This study possesses several advantages. First, the data were obtained from a large cross-sectional survey research program organized by the CDC, ensuring high data credibility, a large sample size, and generalizability of the findings to the overall population. Second, a range of mixture analysis models, including WQS regression, qgcomp, weighted multiple linear regression, and BKMR models, were utilized to comprehensively assess the relationship between metal mixtures and mean UFR. Traditional linear regression is inadequate for evaluating the combined effects of metal mixtures since their combined effect cannot be simply calculated as the sum of individual effects (54). The WQS model is able to explore the effect of mixed exposure burden on the results in one direction at a time, but since the WQS model takes quantile calculations for exposures, this may lose some of the information about the exposures. Additionally, the WQS regression model needs to satisfy the directional homogeneity assumption and also assumes that individual exposures have linear and additive effects. In contrast, qgcomp allows simultaneous validation of the correlation between exposure and outcome from both directions and calculation of weights for each of the two directions, while allowing for nonlinearity and nonadditivity of the effects of individual exposures and whole mixtures (28). BKMR models are valuable statistical tools for exploring combined mixture effects, offering linear or nonlinear response functions and visualizations for improved identification of key contaminants. However, the BKMR model is limited in assessing the impact of co-exposure patterns of high- and low-level metals. Therefore, these models can complement each other and undergo cross-validation to assess mixture exposure, and the subsequent joint interpretation will also facilitate the determination of specific exposure risks.

This study has a number of limitations. Firstly, it is important to note that it is a cross-sectional study, which means that it only represents the participants' state at the time of testing. Consequently, no causal inferences can be drawn from the analysis results, and further prospective studies are required to support the final conclusions. Secondly, the UFR data available in the NHANES database do not include peak UFR values or the systolic and diastolic values of the detrusor muscle, which directly reflect bladder contractile function. Nonetheless, the mean UFR data provided by NHANES can still serve as a valid reference indicator for assessing bladder function (16, 17, 55).

5. Conclusion

In conclusion, our study revealed significant negative correlations between urinary metal mixture exposure and mean UFR in US adults. Moreover, these associations exhibited notable gender specificity. Higher urinary levels of Cd and Sb were identified as potential key factors contributing to the decrease in mean UFR. These findings underscore the potential detrimental impact of environmental metal exposure on bladder function. Further prospective studies are warranted to elucidate the underlying mechanisms and confirm the observed gender difference.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

NHANES study was conducted under the authorization of the National Center for Health Statistics (NCHS) Ethics Review Committee, and all participants provided informed consent. The patients/participants provided their written informed consent to participate in this study.

Author contributions

SZ contributed to the conception and design of the study, and was responsible for the overall content as guarantor. SZ and HT were responsible for data collection and checking, performed the data analysis, interpretation, and manuscript drafting. MZ supervised the project administration. All authors contributed to the article and approved the submitted version.

Acknowledgments

Thanks to the editors and reviewers for their precious time and constructive comments. The authors thank the participants and staff of NHANES.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1241971/full#supplementary-material

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frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 07 November 2023
DOI 10.3389/fpubh.2023.1277182

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EDITED BY
Baby Tabassum,
M. J. P. Rohilkhand University, India

REVIEWED BY
Priya Bajaj,
Govt. P G College, Noida, India
Kaish Miyan,
Aligarh Muslim University, India

*CORRESPONDENCE
Aziz-Ur-Rahim Bacha
urbaziz17@fudan.edu.cn
Zahid Ullah
2201890048@cug.edu.cn

RECEIVED 14 August 2023
ACCEPTED 23 October 2023
PUBLISHED 07 November 2023

CITATION
Khan K, Room SA, Bacha A-U-R, Nabi I, Ahmad S, Younas M, Ullah Z, Iqbal A, Alrefaei AF, Almutairi MH, Chang J-W and Chi KH (2023) Assessment of heavy metals among auto workers in metropolitan city: a case study.
Front. Public Health 11:1277182.
doi: 10.3389/fpubh.2023.1277182

Assessment of heavy metals among auto workers in metropolitan city: a case study

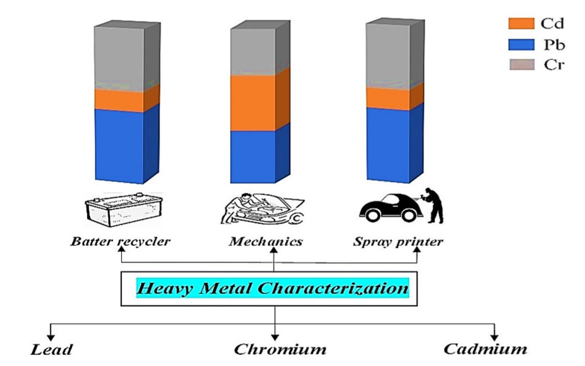
Kaleem Khan[1,2], Shahzada Amani Room[1], Aziz-Ur-Rahim Bacha[3]*, Iqra Nabi[3], Shabir Ahmad[1,2], Muhammad Younas[2,4], Zahid Ullah[5]*, Akhtar Iqbal[6], Abdulwahed Fahad Alrefaei[7], Mikhlid H. Almutairi[7], Jung-Wei Chang[1] and Kai Hsien Chi[1]

[1]Institute of Environmental and Occupational Health Sciences, School of Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan, [2]Department of Environmental Science, Faculty of Basic and Applied Sciences, International Islamic University, Islamabad, Pakistan, [3]State Key Laboratory of Urban Water Resource and Environment, Shenzhen Key Laboratory of Organic Pollution Prevention and Control, School of Civil and Environmental Engineering, Harbin Institute of Technology Shenzhen, Shenzhen, China, [4]College of Resources and Environment, Huazhong Agricultural University, Wuhan, China, [5]State Key Laboratory of Biogeology and Environmental Geology, School of Environmental Studies, China University of Geosciences, Wuhan, China, [6]Department of Environmental Sciences, COMSATS University Islamabad, Abbottabad Campus, Abbottabad, Pakistan, [7]Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia

In recent decades, heavy metals (HMs) have emerged as a global health concern. Unfortunately, in Pakistan, there is a general lack of awareness regarding the potential health risks associated with HMs pollution among automobile workers. Herein, we investigated the concentration of heavy metals such as lead (Pb), cadmium (Cd), and chromium (Cr) among automobile workers who were occupationally exposed in Mingora City, Khyber Pakhtunkhwa, Pakistan. Three different automobile groups, i.e., battery recyclers, spray painters, and mechanics were studied in detail. A total of 40 blood samples were collected from automobile workers groups while 10 blood samples were collected as control individuals from different locations in the study area. We investigated heavy metals concentration with a standard method using an atomic absorption spectrometer AAS (PerkinElmer Analyst 700, United States). Based on our findings, the battery recycling group displayed the most elevated Pb levels ($5.45 \pm 2.11\,\mu g/dL$), exceeding those of both the spray painters' group ($5.12 \pm 1.98\,\mu g/dL$) and the mechanics' group ($3.79 \pm 2.21\,\mu g/dL$). This can be attributed to their higher exposure to Pb pollution resulting from the deterioration, dismantling, grinding, or crushing of old batteries. In the context of chromium (Cr) exposure, a similar trend was observed among the battery recycling group, as well as the spray painters and mechanics groups. However, in the case of cadmium (Cd), the mechanics' group exhibited the highest level of exposure ($4.45 \pm 0.65\,\mu g/dL$), surpassing the battery recycling group ($1.17 \pm 0.45\,\mu g/dL$) and the spray painters' group ($1.35 \pm 0.69\,\mu g/dL$), which was attributed to their greater exposure to welding fumes and other activities in their workplace. We believe that our findings will encourage regulatory measures to improve the health of automobile workers. However, further work is needed to determine various health-related issues associated with heavy metal exposure among automobile workers.

KEYWORDS

heavy metals, autoworkers, mechanics, spray painters, battery recyclers



GRAPHICAL ABSTRACT

1. Introduction

Metals are natural constituents having a high electrical conductivity that occurs in the biological system. They are present everywhere throughout the earth and may also accumulate in living organisms as well. Among 35 natural obtainable metals, 23 metals (lead, cadmium, chromium, mercury, copper, cobalt, arsenic, nickel, platinum, silver, manganese, antimony, cerium, gallium, uranium, iron, tellurium, bismuth, tin, thallium, gold, zinc, and vanadium) have high obvious density with an atomic weight greater than 40.04 are known as heavy metals (1, 2). These metals are not only known for their high density and conductivity but they also have an adverse effect on the biological system (3). In the human body, these metals are present in body tissues, nucleic acids, and proteins which leads to disturbance in working abilities (4). Besides, when these metals are used in industries, a portion of these components are discharged into the air or water bodies as effluents and may indirectly affect humans and other living organisms. Heavy metals (HMs) pollution is a global issue while the associated ecological and health risks are not yet understood due to its vast distribution. Moreover, unspecialized manufacturing and industrial activities leads to an increased discharge into different environmental media (5).

All workplaces have some specific levels of work-related dangers, and each workplace condition is remarkable in nature. Synthetic mixtures are discharged at a workplace from various operations such as residue or splash. It may get into the body either through dermal contact, inhalation, or ingestion (6). Workers commonly eat, drink, and smoke at the workplace due to a lack of knowledge and awareness, and such practices increase their susceptibility to toxic substances (7, 8).

Generally, automobile workshops consist of different groups such as mechanics, spray painters, battery recyclers, and radiator workers for specific tasks. However, these certain activities are the major sources of toxic pollution due to the irresponsible behavior of the occupational workforce. Several studies have suggested that autoworkers are commonly exposed group to toxic metal pollution (9, 10). While others reported that spray painters are at higher risk of cadmium (Cd), chromium (Cr), and lead (Pb) toxicity in automobile paint workshops (11). Conversely, Cd, Cr, and Pb are the most toxic metals that are commonly present in paints (12). Workers in automobile workshops are described as being affected by toxic chemicals like lead fumes, carbon dioxide fumes, chromium, and benzene fumes. It is also reported that lead, chromium, and cadmium are components of spare parts used in the vehicle manufacturing industry, which might influence the levels of these metals in the blood serum of professional workers having regular interaction with them (13, 14). Autoworkers' exposure to these fumes may occur through inhalation during melting processes and absorbed or ingested through the skin when a panel beater regularly uses the metals for repair (13). Although, long-term exposure to Cd may cause several health problems such as dysfunction of the cardiovascular, immune, nervous, respiratory, and endocrine systems. In addition, it may also cause lung cancer in workers (15, 16). Whereas these chemical substances are released in the workplace due to the usage of spray and dust which enters the human body through inhalation, ingestion, as well as dermal contact as a result heavy metals, are a serious concern (17, 18).

The present study was conducted in Mingora city district Swat where automobile workshops are situated in various locations and the reason that these sites are most familiar for different vehicular activities. This study reveals that some automobile workshops were small having 4–5 workers, while others were large having 8–10 working groups. Unfortunately, these automobile workers have no knowledge and awareness about the toxic effects of HMs, nor do they use any protective measures, as a result, they pay slight consideration to protect themselves from the possible impacts due to ingestion and inhalation of these toxic substances. Herein, the evaluations of selected HMs concentrations of Pb, Cd, and Cr were done in exposed autoworkers and a comparison was done with a control group (non-workers).

2. Materials and methods

2.1. Study site

The study area is located in the district Swat of Khyber Pakhtunkhwa (KPK) province, Pakistan, and lies between 34° 46′ 25.1292″ north and 72° 21′ 35.6436″ east. Mingora is the third-largest city in KPK with a high population ratio. This study focused on occupationally exposed autoworkers of Mingora city, district Swat. A total number of 40 autoworkers including mechanics, spray painters, and battery repairing, and 10 non-workers (control group) have participated in this study from different locations including Takhtaband Bypass Rd., Shahdara, and Watkay site, respectively, as shown in Figure 1. The workers' groups were engaged in different activities in workshops, whereas the controls were not directly exposed to such kind of contamination.

All these workers had been working in workshops for 4 to 5 years or more than 10 years, while the workers who had joined workshops for less than 1 year were excluded from this survey. Furthermore, all the workers were interviewed regarding workplace safety precautions. However, it was observed that all the exposed autoworkers had a lack of awareness regarding workplace safety measures.

2.2. Samples collection

Several workshops were visited, and the purpose of the study was discussed with the workers and non-worker groups. The samples were collected from different automobile workshops including mechanics,

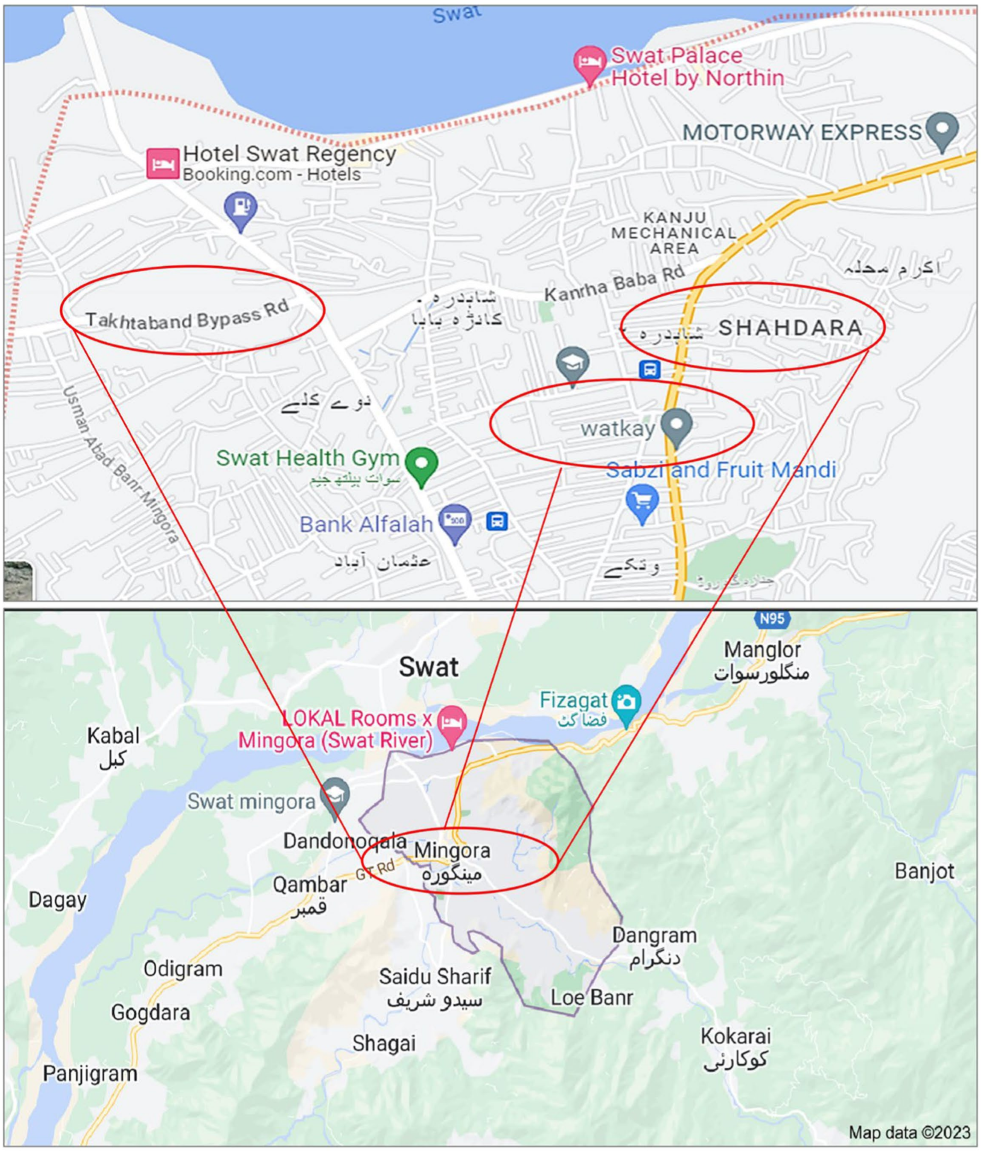


FIGURE 1
Study map of samples collection in district Swat, Khyber Pakhtunkhwa province in Pakistan.

battery recyclers, and spray painters. Each 3 mL blood sample was collected in heparinized tubes using the standard method. Blood was taken with the help of a trained nurse from both autoworkers and non-worker groups. After collection, blood samples were directly subjected to the laboratory for further experimental work.

2.3. Samples preparation and analysis

The sample preparation and analysis were carried out according to the previous method of Ishola et al. (19) with a slight modification. Each blood sample tube was centrifuged at 4,000 rpm for 10 min for the separation of blood supernatant serum. The supernatant serum was kept in a separate tube. The prepared samples were then stored at −200°C for at least 10–12 days before analysis. The levels of lead, cadmium, and chromium were then analyzed by using atomic absorption Spectrometer AAS (PerkinElmer A Analyst 700, United States) respectively. The mean value for each sample was calculated using three measurement readings, and the error estimate was calculated via the standard deviation of the results.

2.4. Statistical analysis

Data were analyzed through the Statistic 9 software. One-way ANOVA was performed considering the ($p < 0.05$). Furthermore, graphs were made by using the graph pad prism version (5.01).

3. Results and discussion

The average concentration of HMs found in the blood serum collected from automobile workers, including mechanics, battery recyclers, spray painters, and the control group. As a result, the battery recyclers group exhibited a higher Pb value (5.45 ± 2.11 μg/dL), surpassing both the spray painters' group (5.12 ± 1.98 μg/dL) and mechanics group (3.79 ± 2.21 μg/dL). The mean values of Pb, Cd, and Cr were recorded higher in the mechanic, battery recyclers, and spray painters' group whereas their mean values were recorded lower in the control group as presented in Table 1 and Figure 2. The result in Figure 2 shows that the mean value of Pb in the battery recyclers group was higher in comparison with Cr and Cd while the concentration of these HMs follows the order Pb > Cr > Cd.

A high concentration of Pb was observed in battery workers as compared to automobile workers in Pakistan Ahmad et al. (20). Meanwhile, a similar phenomenon of Pb concentration was noticed in battery workers in Colombia Restrepo et al. (21). Our findings are consistent with the reported literature. Pb is commonly utilized in various applications, including lead-acid batteries, projectiles, and ammunition (22). The differences in these values were attributed to the higher exposure of battery recyclers to Pb, which includes activities such as deterioration, dismantling, grinding, or crushing of old batteries, respectively. As reported by Gottesfeld and Pokhrel (23), approximately (50%) of the lead production in automotive workshops is attributed to the recycling of lead batteries. In Pakistan, it has been estimated that approximately (95%) of lead-acid batteries are produced by reutilizing old batteries. Moreover, the elevated levels of Pb in battery recyclers can be attributed to inadequate personal hygiene practices, as well as the consumption of contaminated food and water at their workplace (24). Our findings for Pb concentration are in good agreement with the reported literature (9, 25, 26).

Moreover, in Table 1, it was observed that the mechanic's group had the maximum level of Cd (4.45 ± 0.65 μg/dL), while the minimal level was documented in the battery recyclers group (1.17 ± 0.45 μg/dL). On the other hand, the spray painters' group had an intermediate level of Cd (1.35 ± 0.69 μg/dL). The concentration of these HMs in the mechanic's group follows the order Cd > Pb > Cr as shown in Figure 3. Cd is primarily linked with exposure to welding fumes in auto workshops (27). As well as, Cd can also accumulate within the body through various means such as inhalation, consumption of contaminated food, and improper washing practices in the workplace (28). According to Goyal et al. (29), auto workers who were exposed to welding fumes through inhalation within the workplace exhibited higher levels of cadmium in their bodies. Similarly, Ishola et al. (19) have also documented similar findings concerning the levels of Cd in the blood of automobile workers in Benin City, Nigeria. Furthermore, spray painters use high compression to apply paints onto vehicle body parts, causing them to become aerosolized and dispersed into the surrounding environment (30). In addition, during our study, we observed that the spray painters did not use proper respiratory protection such as masks or protective clothing. As a result, they were directly exposed to these toxicants through inhalation, ingestion, and dermal contact while performing their work (31). reported that individuals who work with spray printers face potential exposure to Cr through both inhalation and ingestion.

In the present study, a significant variation was observed in the level of Cr among different groups. The battery recyclers group exhibited a higher mean value of Cr (5.13 ± 2.37 μg/dL), whereas the mechanic's group had a lower mean value (4.38 ± 2.76 μg/dL), however, the spray painters group showed an intermediate level of Cr (4.72 ± 2.27 μg/dL)

TABLE 1 Mean concentrations of metals (μg/dL) in mechanics, battery recyclers, and spray painters group compared to the controls.

Parameters	Controls group	Mechanics group	Battery recyclers group	Spray painters' group	*p*-value
Lead	0.64 ± 0.15	3.79 ± 2.21	5.45 ± 2.11	5.12 ± 1.98	0.001
Cadmium	0.49 ± 0.012	4.45 ± 0.65	1.17 ± 0.45	1.35 ± 0.69	0.001
Chromium	0.87 ± 0.41	4.38 ± 2.76	5.13 ± 2.37	4.72 ± 2.27	0.001

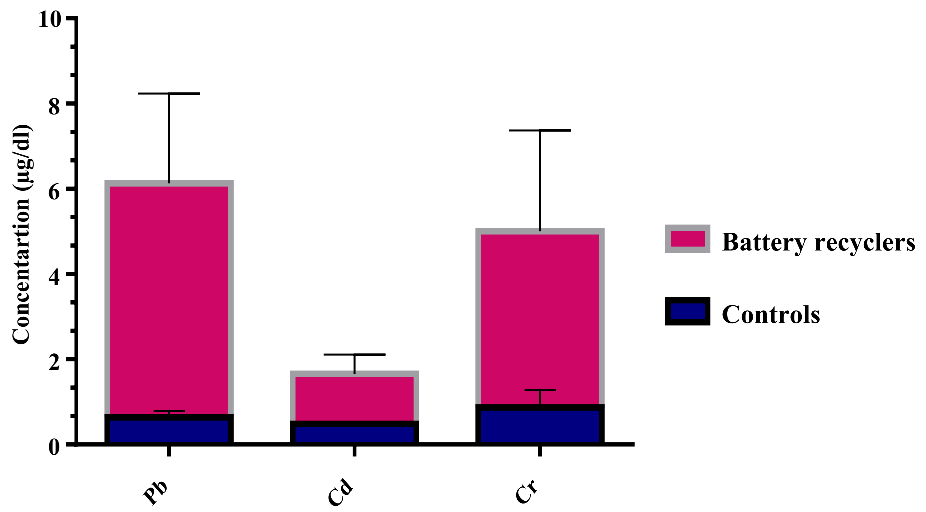


FIGURE 2
Heavy metals concentrations (μg/dL) in battery recyclers automobile group.

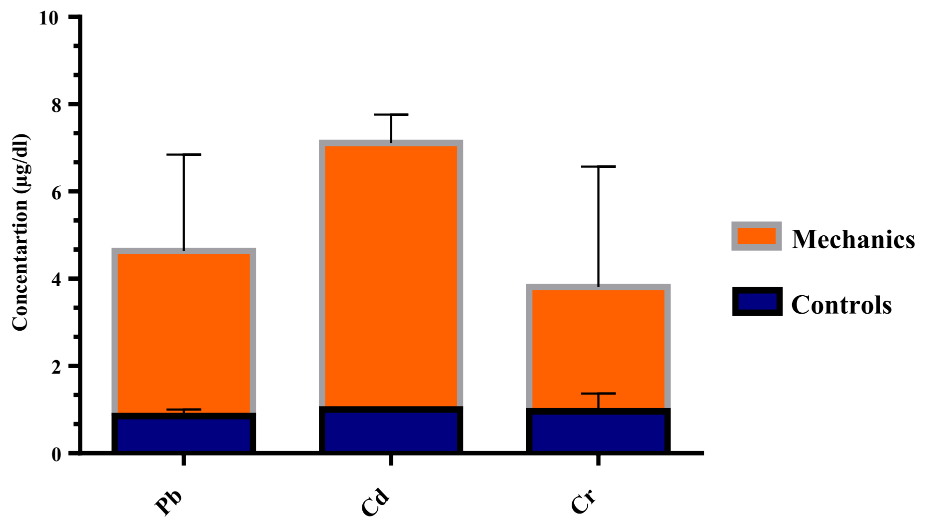


FIGURE 3
Heavy metals concentrations (µg/dL) in mechanics automobile group.

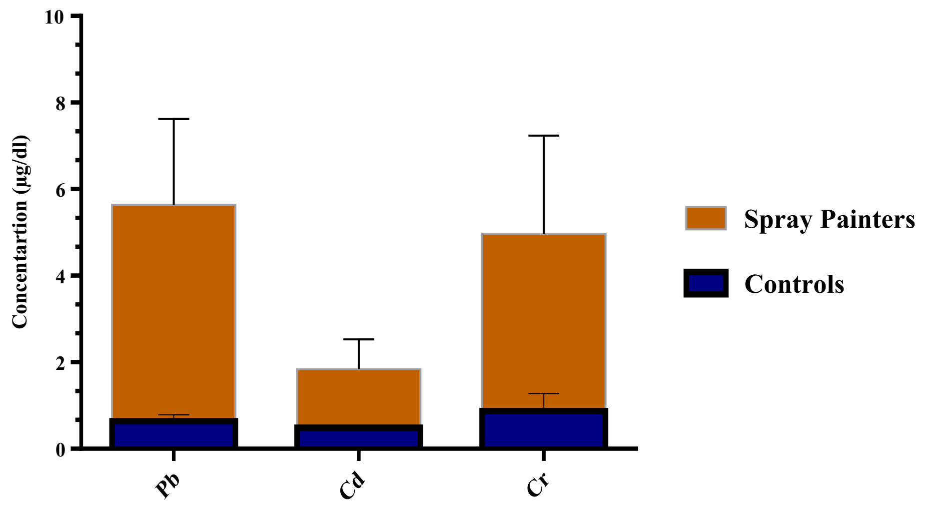


FIGURE 4
Heavy metals concentrations (µg/dL) in spray painters automobile group.

when compared to a control group (0.87 ± 0.41 µg/dL). However, the concentration of these HMs in the spray painters group follows the order Pb > Cr > Cd as shown in Figure 4. Metal-coated welding electrodes, fumes, and lubricants are also the main sources of chromium pollution. Ahmad et al. (32) stated that exposure to Cr typically occurs during various activities such as coating, painting, welding, cutting, and metal treatment. Autoworkers are usually exposed to Cr pollution through (inhalation, ingestion of contaminated food, etc.) and similar results have been reported in the literature (33, 34).

4. Conclusion

In the present work, it is concluded that the levels of lead and chromium in the battery recycling group were significantly higher when compared to both the mechanics and spray printer groups due to their higher exposure to different activities in their work. Moreover, in the case of Cd, the Mechanics groups have the maximum concentrations compared to other groups. Nonetheless, the concentration of all HMs among the autoworker's groups was greater in comparison to control individuals. In the present study, we noticed that workers did not utilize any personal protective equipment and had limited awareness regarding the toxic effects of heavy metals released in their workplaces. However, here are some measures including regular cleanup, safe handling and storage, proper education and awareness, training, regular assessment and improvement, regular health checking, and compliance with regulations might be helpful for workers to reduce potential exposure to heavy metals and other toxic chemicals in workshops.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by the Ethics Committee of the International Islamic University Islamabad (IIUI). Consent was obtained from all the individual participants included in the study. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

KK: Formal analysis, Investigation, Writing – original draft. SR: Writing – review & editing. A-U-RB: Supervision, Writing – original draft, Writing – review & editing. IN: Writing – review & editing. SA: Writing – review & editing. MY: Writing – review & editing. ZU: Supervision, Writing – review & editing. AI: Writing – review & editing. AA: Funding acquisition, Writing – review & editing. MA: Funding acquisition, Writing – review & editing. J-WC: Writing – review & editing. KC: Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. We extend our appreciation to the Researchers Supporting Project (No. RSP2023R191), King Saud University, Riyadh, Saudi Arabia.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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TYPE Original Research
PUBLISHED 09 November 2023
DOI 10.3389/fpubh.2023.1289838

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EDITED BY
Mohd Kamil Hussain,
Govt. Raza Post Graduate College, Rampur, India

REVIEWED BY
Aziz-Ur-Rahim Bacha,
Fudan University, China
Vedat Bulut,
Gazi University, Türkiye

*CORRESPONDENCE
Changfu Hao
✉ haochangfu@126.com
Wu Yao
✉ yaowu@zzu.edu.cn

†These authors have contributed equally to this work

RECEIVED 06 September 2023
ACCEPTED 27 October 2023
PUBLISHED 09 November 2023

CITATION
Qi Y, Si H, Jin X, Guo Y, Xia J, He J, Deng X, Deng M, Yao W and Hao C (2023) Changes in serum TIM-3 and complement C3 expression in workers due to Mn exposure.
Front. Public Health 11:1289838.
doi: 10.3389/fpubh.2023.1289838

Changes in serum TIM-3 and complement C3 expression in workers due to Mn exposure

Yuanmeng Qi[1†], Huifang Si[1,2†], Xiaofei Jin[3], Yonghua Guo[1], Jiarui Xia[1], Jing He[1], Xuedan Deng[1], Meng Deng[1], Wu Yao[1*] and Changfu Hao[4*]

[1]Department of Occupational and Environment Health, College of Public Health, Zhengzhou University, Zhengzhou, Henan, China, [2]Prevention and Infection Control Section, Xi'an Union Hospital, Xi'an, Shanxi, China, [3]Department of Ultrasound, The Third Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China, [4]Department of Child and Adolescence Health, College of Public Health, Zhengzhou University, Zhengzhou, Henan, China

Mn (Manganese, Mn) is an essential trace element involved in various biological processes such as the regulation of immune, nervous and digestive system functions. However, excessive Mn exposure can lead to immune damage. Occupational workers in cement and ferroalloy manufacturing and other related industries are exposed to low levels of Mn for a long time. Mn exposure is one of the important occupational hazards, but the research on the effect of Mn on the immune system of the occupational population is not complete, and there is no reliable biomarker. Therefore, this study aimed to evaluate the immunotoxicity of Mn from the soluble immune checkpoint TIM-3 (T-cell immunoglobulin and mucin containing protein 3, TIM-3) and complement C3. A total of 144 Mn-exposed workers were recruited from a bus manufacturing company and a railroad company in Henan Province. An inductively coupled plasma mass spectrometer was used to detect the concentration of RBC Mn (Red blood cell Mn, RBC Mn), and ELISA kits were used to detect serum complement C3 and TIM-3. Finally, the subjects were statistically analyzed by dividing them into low and high Mn groups based on the median RBC Mn concentration. We found that Mn exposure resulted in elevated serum TIM-3 expression and decreased complement C3 expression in workers; that serum TIM-3 and complement C3 expression showed a dose–response relationship with RBC Mn; and that the mediating effect of complement C3 between RBC Mn and TIM-3 was found to be significant. The above findings indicate that this study has a preliminary understanding of the effect of Mn exposure on the immune system of the occupational population exposed to Mn, and complement C3 and TIM-3 may be biomarkers of Mn exposure, which may provide clues for the prevention and control of Mn occupational hazards.

KEYWORDS

Mn-exposed workers, immunotoxicity, RBC Mn, TIM-3, complement C3

1. Introduction

Mn and its compounds are one of the important occupational hazards. Occupational groups such as welders and ferroalloy makers are susceptible to Mn poisoning when exposed at low concentrations for long periods of time (1), and occupational groups are exposed to Mn mainly through inhalation of Mn-containing fumes (2). A large number of studies have shown that Mn exposure has damaging effects on several systems of the body (3), of which the most widely studied is the neurotoxicity of Mn, but the studies on Mn immunotoxicity are not yet comprehensive enough. However, the immune system, as an important system for recognizing and removing harmful substances and maintaining homeostasis of the internal environment, is highly sensitive to toxicity of poisons (4), and the immunotoxic effects of Mn exposure on the organism should not be ignored. When an injury occurs in the organism, changes in the composition of the blood are often its most intuitive manifestation, and it is so sensitive that any stimulus acting on the organism can cause it to change. In epidemiological surveys of populations, changes in certain immune indicators in the peripheral blood of populations can, to a certain extent, well reflect changes in the immune function of the organism after exposure to toxicants.

Blood parameters are used in clinical and toxicologic studies of numerous inflammatory conditions as important physiologic indicators of systemic function (5). When the body is exposed to a toxicant, an inflammatory response occurs, resulting in the formation of a group of cells such as monocytes, T-cells, and platelets, and ongoing inflammation leads to an increase in the number of macrophages and lymphocytes, both of which can migrate from the bloodstream and accumulate at the site of injury. A Finnish study observed the systemic inflammatory response in 20 male workers after acute exposure to welding fumes and found an increase in the total number of blood leukocytes and neutrophils in the workers after shifts (6). Complement C3 is a central component of the complement system and plays a key role in inflammation and regulation of adaptive immune responses (7). Decreased levels of C3 lead to a reduction in the body's ability to fight infection and a near loss of C3-mediated lysis and clearance of circulating immune complexes. Immune checkpoints are a series of molecules that are expressed primarily on immune cells and regulate the degree of immune activation. Immune checkpoints are capable of generating complex signaling systems that regulate the activation and functional expression of T-lymphocytes, and when they are overexpressed, T-cell immunity is suppressed and tumor cells are able to evade immune killing (8). The immune checkpoint TIM-3, a type I transmembrane protein belonging to the Ig superfamily (9), can be expressed on a wide range of immune cells, including type I helper T cells, Th17 cells, $CD8^+$ T cells, and Tregs, and high expression of TIM-3 can trigger immunosuppression as well as tumor immune escape. However, no reports of its expression in studies related to Mn exposure have been found.

It is well known that obesity, smoking and alcohol consumption are common hazards to human health, capable of inducing a wide range of diseases and promoting their occurrence and development. Studies have shown that cigarettes and cigarette smoke contain a variety of toxic trace elements that accelerate damage and inflammation in the human body (10), and smoking directly or indirectly deteriorates blood flow and tissue oxygenation (11). Kim et al. (12) showed that smoking significantly altered the effects of welding fumes on systemic markers of inflammation such as C-reactive protein, fibrinogen and leukocyte levels in the circulation of workers. Several *in vitro* and *in vivo* studies have shown that alcohol modulates the function of innate immune cells such as monocytes and DCs (dendritic cells, DCs) in a dose- and time-dependent manner, and that acute high-dose alcohol consumption suppresses cytokine production, while chronic alcohol consumption stimulates the production of pro-inflammatory cytokines (13). A range of metabolic abnormalities, oxidative stress, immune dysfunction and chronic inflammation have now been identified in overweight obese organisms (14).

Various studies have shown that excessive Mn exposure can affect important organs, leading to multi-system dysfunction. The cumulative evidence of Mn toxicity hazards and the widespread public concern about it fully illustrate its importance to public health. However, the immune system changes induced by occupational Mn exposure in these organs are not fully understood, and the research on the hazards of Mn toxicity in the occupational environment or its nutritional benefits is far from complete. Therefore, the present study conducted a cross-sectional investigation to observe the expression of immune indicators such as WBCs (white blood cells, WBCs), RBCs, Hb (hemoglobin, Hb), PLT (platelets, PLT), TIM-3 and complement C3 in the peripheral blood of Mn exposed workers. Next, the dose–response relationship between the internal exposure dose of RBC Mn and each of the immune markers was analyzed after adjusting for the confounders of working age, smoking, alcohol consumption and BMI. In order to provide clues for the discovery of biomarkers of organismal immune damage caused by occupational Mn exposure, and to provide a reference basis for the prevention and control of occupational Mn exposure toxicity hazards.

2. Subjects and methods

2.1. Subjects of study

In this study, 144 male Mn-exposed workers, aged 22–53 years old, were recruited from a bus manufacturing company in Henan Province, and their job types were mainly welders. Inclusion criteria: workers aged ≥18 years who had been working in welding or related Mn exposure in the company for more than 1 year, had no other systemic diseases and agreed to participate in the study. Exclusion criteria: those who suffered from significant diseases of the immune system, cardiovascular system, etc. prior to their work related to Mn exposure; those who have recently taken medication that affects their immune function; and workers with a history of other exposures such as benzene and lead. The study complied with medical ethics and was approved by the Life Sciences Ethics Review Committee of Zhengzhou University under the ethical number: ZZUIRB 2022–149. The sample size calculation formula of cross-sectional group design was used in this study:

$$n = \frac{\left(q_1^{-1} + q_2^{-1}\right)\left(Z_{\alpha/2} + Z_\beta\right)^2 S^2}{\delta^2}$$

Sample size: n; proportions of two groups: q_1, q_2; $\alpha = 0.05$; test efficacy: $1\text{-}\beta = 0.9$; standard deviation: $S = 0.34$; tolerable error: $\delta = 0.2$. The calculated sample size was 124 people, but 15% was added to account for low cooperation during the survey process, resulting in $n = 144$.

2.2. Dust collection and detection

According to the Sampling Specification for Monitoring Hazardous Substances in Air in Workplaces GBZ159-2004, the detection of Mn and its inorganic compounds in the environment of the welding operation is carried out by means of fixed-point sampling, selecting representative workplaces, using AKFC-92A and FC-1B dust samplers with microporous membrane collectors, fixed at the height of the human respiratory belt, with a sampling flow rate of 5 L/min and a sampling time of 15 min, and carrying out sampling in the periods of different concentrations of hazardous substances, including the period of time when the concentration of the hazardous substances in the air is the highest in a working day. At the end of sampling, the microporous filter membrane was folded twice and put into the filter membrane bag. The final compositional analysis was carried out by graphite furnace atomic absorption spectrometer, and the results were determined by the concentration of Mn dioxide, and the lowest detectable concentration of Mn dioxide at the fixed point of this method was 0.006 mg/m^3 (based on the collection of 75 L of air samples).

2.3. Occupational health examinations

Basic demographic information of the study population was collected, as well as basic information such as occupational history, smoking history, and alcohol consumption history. The health checkup includes detection of autonomic symptoms such as headache, palpitation and chest tightness, general physical examination such as height and weight, general clinical examination such as medical-surgical examination and neurological examination, and laboratory biochemical examination such as blood and urine routine and liver function.

2.4. Determination of internal exposure dose

Each worker collected 5 mL of biochemical coagulated blood and 2 mL of sodium heparin anticoagulated blood. Blood samples taken on the same day were rested and centrifuged at 1,240 × g for 10 min to retain serum for subsequent detection of soluble immune checkpoints; plasma and blood cells were separated after anticoagulated blood was centrifuged at 600 × g for 5 min. The lower layer of blood cells was washed three times with saline to remove leukocytes, proteins, etc. to obtain red blood cells. Subsequently, RBC Mn content was determined by ICP-MS (Inductively coupled plasma mass spectrometry, ICP-MS).

2.5. Measurement of serum TIM-3 and complement C3 expression by enzyme-linked immunosorbent assay

Two replicates were set up for each experimental standard, and serum TIM-3 was assayed using the stock solution uptake assay, and serum was diluted 10,000-fold for the determination of complement C3. A regression equation for the standard curve was calculated using the concentration of the standard versus the OD value, and the sample concentration was calculated by substituting the OD value of the sample into the equation.

2.6. Statistical analysis

SPSS 21.0 software was used for statistical analysis. Student's *t*-test and Mann–Whitney U-test were used to compare the differences in demographic and immunization parameter values between the two groups based on the normality of the distribution of continuous variables. The chi-square test was used for count data. Analysis of covariance and generalized linear models were used to explore the effects of working age, smoking, alcohol consumption and BMI factors on immunological indicators, respectively. Spearman's correlation coefficient was used to evaluate the correlation between the relevant indicators. After adjusting for working age, smoking, alcohol consumption, and BMI, multiple linear regression and generalized linear models were used to analyze the relationship between internal exposures and each immune parameter. SPSS PROCESS macro was used for mediated effects analyses. The significance level α is 0.05.

3. Results

3.1. Occupational environment survey

The main source of Mn dust from passenger car manufacturing companies is from welding operations. The raw material used for welding is copper-plated wire, which contains 1.80% ~ 2.10% Mn, and the body frame consists of carbon steel and stainless steel, which can also produce Mn fumes during welding. The ambient monitoring results, as shown in Supplementary Table S1, showed that the 8-h time-weighted average (C-TWA) and peak concentrations of Mn and its inorganic compounds in the air at various points in the workshop were 0.001 ~ 0.142 mg/m^3 and 0.010 ~ 0.378 mg/m^3, respectively. The time-weighted average permissible concentration (PC-TWA) for Mn and inorganic compounds (MnO_2) in our country is 0.15 mg/m^3, and the peak concentration is three times that of the PC-TWA, 0.45 mg/m^3.

3.2. General demographic features

A total of 144 Mn-exposed male workers were included in this study and their demographic characteristics are described in Table 1. The study subjects were categorized into a low Mn group ($n = 72$) and a high Mn group ($n = 72$) based on the median exposure dose RBC Mn of 2.76 $\mu g/10^{10}$RBCs. The median age was higher in the low-Mn group than in the high-Mn group, while the overall BMI was greater in the high-Mn group than in the low-Mn group, but none of the differences were statistically significant. The results showed that neither current smoking nor alcohol consumption differed significantly between the two groups; while the distribution of the two groups in different BMIs differed borderline significantly ($p = 0.087$); and the median RBC Mn was higher in the high-Mn group than in the low-Mn group, and the difference was statistically significant ($p < 0.001$).

3.3. Changes in the expression of peripheral blood immunity indicators in the study population

According to the findings shown in Figure 1, a *t*-test analysis revealed that the concentrations of leukocytes and platelets were significantly greater in the high-Mn group compared to the low-Mn group ($p < 0.001$, <0.004, respectively). However, there was no

TABLE 1 Demographic characterization of the study population.

Variables		General population ($n = 144$)	Low Mn group ($n = 72$)	High Mn group ($n = 72$)	χ^2/Z	P
Age (years)		33 (31, 37)	34 (31, 38)	33 (31, 37)	−0.172*	0.863
	≤33	73 (50.7%)	36 (50.0%)	37 (51.4%)	0.028#	0.868
	>33	71 (49.3%)	36 (50.0%)	35 (48.6%)		
Current smoking	Yes	52 (36.1%)	28 (38.9%)	24 (33.3%)	0.482#	0.488
	No	92 (63.9%)	44 (61.1%)	48 (66.7%)		
Drinking history	Yes	11 (7.6%)	5 (6.9%)	6 (8.3%)	0.098#	0.754
	No	133 (92.4%)	67 (93.1%)	66 (91.7%)		
BMI (kg/m^2)		24.5 (22.4, 26.9)	24.1 (21.9, 26.2)	25.0 (23.5, 27.1)	−1.584*	0.113
	18.5 ~ 23.9	58 (40.2%)	35 (48.6%)	23 (31.9%)	5.851#	0.087
	24.0 ~ 27.9	61 (42.4%)	24 (33.3%)	37 (51.4%)		
	≥28.0	25 (17.4%)	13 (18.1%)	12 (16.7%)		
Working age (years)		8 (5, 10)	8 (6, 11)	8 (5, 10)	−0.815*	0.415
	≤8	82 (56.9%)	41 (56.9%)	41 (56.9%)	0.000#	1.000
	>8	62 (43.1%)	31 (43.1%)	31 (43.1%)		
RBC Mn (μg/10^{10} RBCs)		2.76 (1.24, 5.63)	1.26 (0.80, 1.85)	5.63 (3.93, 9.00)	−10.356*	<0.001

*Mann–Whitney U test; #χ^2-test.

statistically significant difference in the two groups' RBC and Hb concentrations. To further explore the changes in human immune levels after Mn exposure, workers' serum TIM-3 and complement C3 levels were measured. We found that TIM-3 was significantly higher in the high-Mn group than in the low-Mn group ($p < 0.001$), with median values of 80.34 and 62.72 pg/mL, respectively. In contrast, complement C3 concentrations were significantly lower in the high Mn group than in the low Mn group, with median values of 0.91 and 1.09 mg/mL, respectively, and the difference between the two groups was significant ($p = 0.001$).

3.4. Relationship between demographic characteristics and indicators of peripheral blood immunity

The association of demographic characteristics with WBCs and RBCs is shown in Supplementary Table S2. By intergroup analysis, it was found that in different subgroups of working age, smoking history, drinking history and BMI, the level of WBCs in the high Mn group was higher than that in the low Mn group, and the differences in all subgroups were statistically significant except for the BMI ≥ 28.0 ($p < 0.05$). However, no differences in the expression levels of RBCs were observed between the two groups with different working age, smoking, alcohol consumption and BMI. Within-group analyses showed that in both groups, the levels of WBCs were significantly higher in smokers and drinkers than in non-smokers and non-drinkers, but the difference was statistically significant only in the high-Mn group ($p < 0.05$). In addition, the effect of different BMI on the concentration of WBCs was significant ($p < 0.05$) in the low Mn group, as demonstrated by the relatively high concentration of WBCs in the subjects with high BMI, but not in the high Mn group. On the contrary, the effect of different BMI on the concentration of RBCs was significant ($p < 0.05$) in the per Mn group, and the effect of different working age, smoking and alcohol consumption on the concentration of RBCs was not observed. Supplementary Table S3 shows the relationship between demographic factors and Hb and PLT. Intergroup analysis revealed that PLT levels were higher in the high Mn group than in the low Mn group in the different subgroups of working age, smoking history, drinking history and BMI, where the differences in the different BMI strata were not statistically significant, and the differences between the two groups of those with ≤8 years of working age and those who were smokers were borderline significant ($p = 0.054$, 0.061), and the differences in all the rest of the subgroups were statistically significant ($p < 0.05$). However, no differences in Hb expression levels were observed between the two groups with different working age, smoking, alcohol consumption and BMI. The results of within-group analysis showed that in both groups, the effects of different working age, smoking history, drinking history and BMI conditions on the expression levels of Hb and PLT were not observed, and the differences were not statistically significant ($p > 0.05$). The correlation between demographic variables and the expression of TIM-3 and complement C3 is presented in Supplementary Table S4. Intergroup analysis showed that TIM-3 levels were higher in the high-Mn group than in the low-Mn group for all subgroups except those who drank alcohol, where the differences were not statistically significant ($p < 0.05$). On the contrary, the levels of complement C3 in the high Mn group were observed to be lower than those in the low Mn group in all factor stratifications except for smoking, alcohol consumption, and obesity, and the difference was statistically significant ($p < 0.05$). Within-group analysis revealed that in the low-Mn group, complement C3 levels were higher in those with >8 years of working age than in those with ≤8 years of working age, with a statistically significant difference ($p < 0.05$), and lower in those who smoked and drank alcohol than in those who did not, with a statistically significant difference in both cases ($p < 0.05$). The effects of different working age, smoking, alcohol consumption and BMI status on TIM-3 concentration were not observed.

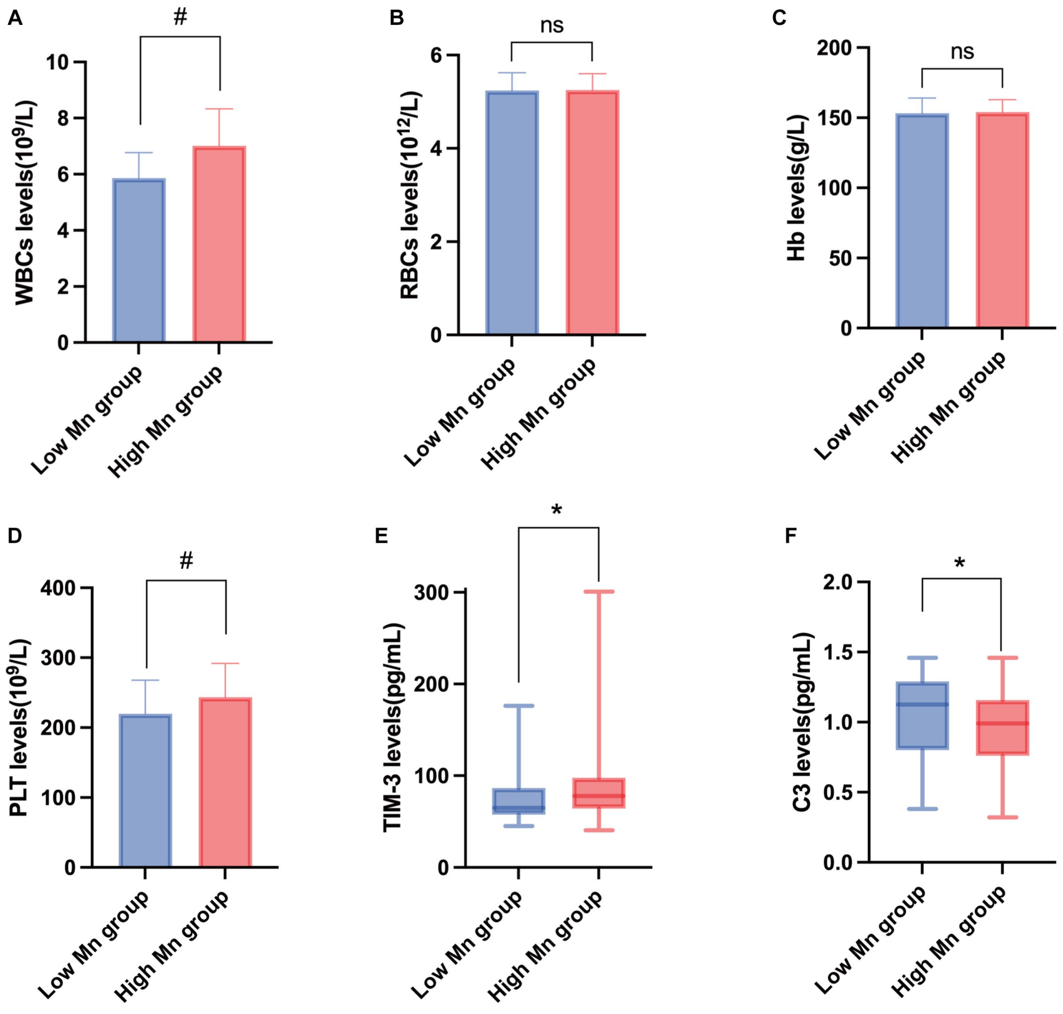

FIGURE 1
Changes in the expression of peripheral blood immunity indicators in the study population. Changes in the expression levels of WBCs **(A)**, RBCs **(B)**, Hb **(C)**, PLT **(D)**, TIM-3 **(E),** and C3 **(F)** in peripheral blood of people exposed to manganese. #*T*-test; *Mann–Whitney U test ($n = 72$).

3.5. Relationship between internal exposure dose and peripheral blood immunity indices

Figure 2 displays the results of the correlation analysis between the internal exposure dose and each peripheral blood immunity index. Spearman's correlation test revealed that WBCs ($r = 0.385$), PLT ($r = 0.190$), and TIM-3 ($r = 0.338$) were positively correlated with the concentration of RBC Mn ($p < 0.05$); whereas complement C3 ($r = -0.302$) was negatively correlated with the concentration of RBCMn ($p < 0.01$); Hb ($r = 0.766$) was positively correlated with the concentration of RBCs, PLT ($r = 0.259$) and WBCs were positively correlated ($p < 0.01$); complement C3 ($r = 0.204$) was positively correlated with Hb ($p < 0.05$); and complement C3 ($r = -0.343$) was negatively correlated with TIM-3 ($p < 0.01$). We then evaluated the dose–response relationship between erythrocyte Mn levels and peripheral blood immunity indices. The participants in the study were categorized into four groups based on the quartiles of RBC Mn. Subsequently, the researchers examined the association between RBC Mn levels and various indices of peripheral blood immunity. To account for potential confounding factors such as working age, history of smoking, history of alcohol consumption, and BMI, appropriate adjustments were made. The results of this analysis can be found in Figure 3. The findings of the study indicate that there was a significant positive association between the concentrations of WBCs, PLT, and TIM-3 with increasing RBC Mn concentration ($P_{trend} < 0.001$, 0.013, and < 0.001, respectively). Conversely, there was a significant negative association between the concentration of complement C3 and increasing RBC Mn concentration ($P_{trend} < 0.001$). Furthermore, no significant dose–response relationship was observed between the concentrations of RBCs, Hb, and RBC Mn. Compared with RBC Mn < 1.24 μg/10^{10} RBC, both WBCs and TIM-3 concentrations were significantly higher when RBC Mn ≥ 2.76 μg/10^{10} RBC ($p < 0.05$), and the differences between other levels were not statistically significant. In addition, compared with RBC Mn < 1.24 μg/10^{10} RBC, complement C3 concentrations were significantly lower at all other RBC Mn concentration levels, and the differences were statistically significant ($p < 0.05$).

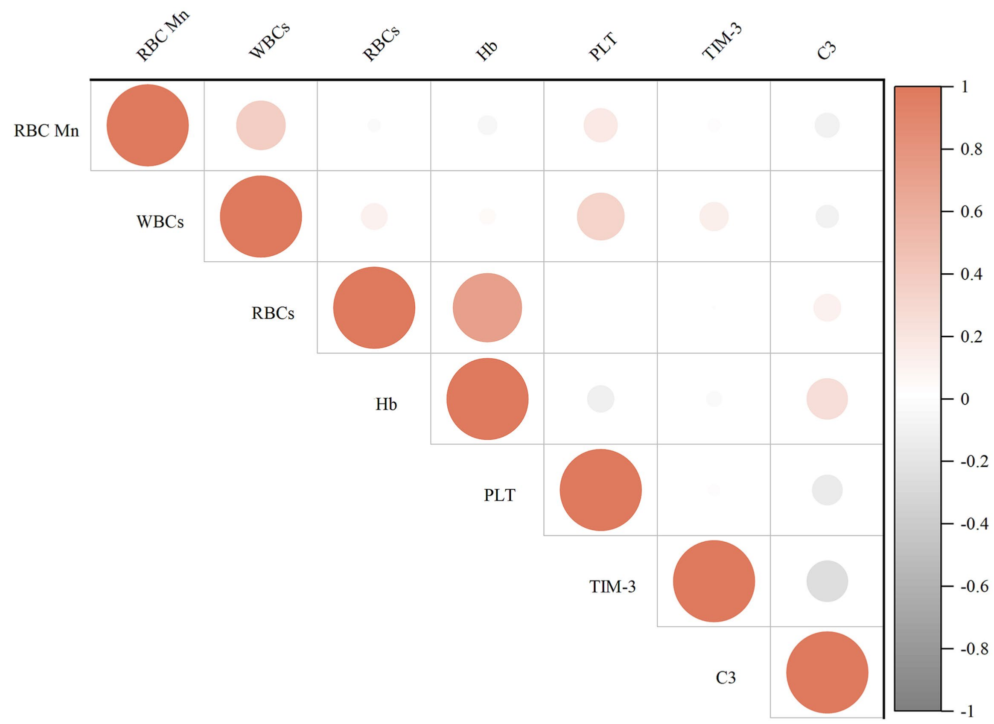

FIGURE 2
Correlation analysis between indicators.

3.6. Mediating effects of complement C3 between RBC Mn and TIM-3

First, the hypothesis was established that complement C3 mediates the effect between RBC Mn exposure levels and changes in TIM3 expression levels, as shown in Figure 4. The PROCESS macro of SPSS was used for mediation effect analysis and the results, as indicated in Supplementary Table S5. The mediating effect was found to be significant for the direct effect of RBC Mn on altered peripheral blood TIM-3 expression levels ($B = 0.267$, $p = 0.01$). After the addition of the intermediate variable complement C3 between RBC Mn and TIM-3, the B of RBC Mn and TIM-3 decreased from 0.267 to 0.205, but remained significant ($p = 0.0034$). The mediating effect was 0.062, and the percentage of mediating effect was 23.2%, demonstrating that the idea that complement C3 partially mediates the interaction between RBC Mn and TIM-3 is correct.

4. Discussion

Currently, in welding, ferroalloy manufacturing, and other related industries, workers are in long-term low-level exposure environments where occupational populations are exposed to Mn primarily through inhalation and dermal contact (15). Studies have shown that Mn exposure can lead to immune damage, and long-term inhalation of Mn-containing fumes can even cause chronic Mn poisoning. Occupational Mn poisoning is one of the legally recognized occupational diseases in China, showing irreversible pathological changes in the late stage, and there is no sensitive diagnostic criterion yet. Therefore, strengthening the assessment and monitoring of the working environment of the relevant occupational groups and actively exploring reliable biomarkers are urgent issues that we need to address.

In recent years, an increasing number of studies have focused on the changes in blood parameters after exposure of the organism to Mn in order to evaluate the toxic effects of Mn. The results of this study showed a significant increase in the number of WBCs and PLTs in the peripheral blood of workers in the high Mn group relative to the low Mn group, but the changes in the number of erythrocytes and hemoglobin were not significant, which is consistent with the results of most studies (16, 17). Chen et al. (18) found that occupational Mn exposure had a significant immunotoxic effect on workers, wherein workers in the high Mn exposure group had increased blood WBCs concentrations relative to those in the low Mn exposure group, although not statistically significant. The above results were also verified in animal experiments, after Mn treatment in acute and subchronic model rats constructed with $MnCl_2$ (manganese chloride, $MnCl_2$), PLTs and WBCs were significantly elevated in the Mn dyed group, in which the number of neutrophils and eosinophils was significantly increased and the number of lymphocytes was decreased (16, 19). More interestingly, the above results were also observed in rats perfused with Mn-containing welding fumes and in fish exposed to Mn (20–22). Taken together, Mn exposure caused an increase in the number of leukocytes, probably due to an increase in neutrophils, which are the main inflammatory cells. Platelets, as one of the indicators of blood inflammation, not only play a hemostatic role, but also produce IgG (Immunoglobulin G, IgG) to play an immune role, while Mn exposure can promote the formation of reactive oxygen species and the consumption of antioxidant elements in the body, which in turn induces the activation of platelets in the body and contributes to the increase of platelet production.

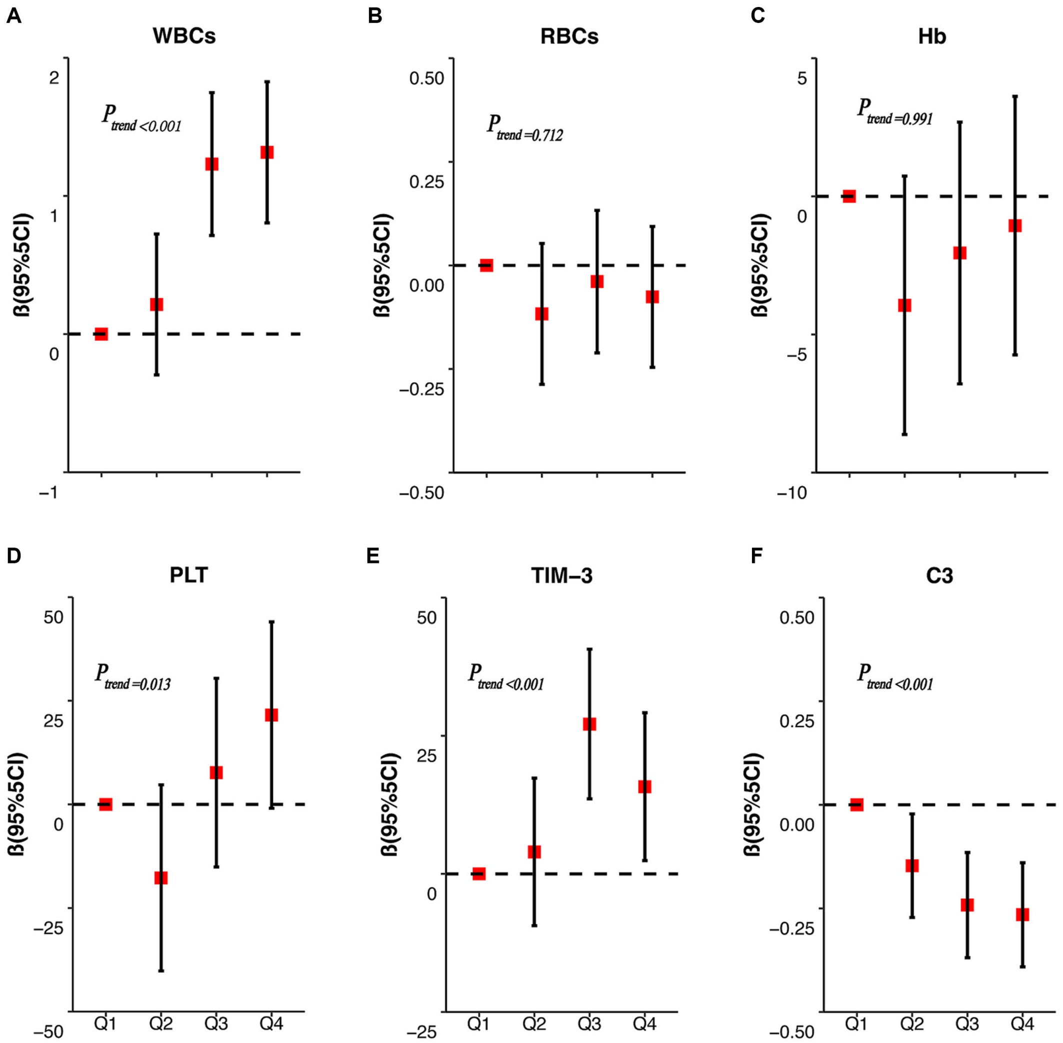

FIGURE 3
RBC Mn dose−response correlations with WBCs, RBCs, Hb, PLT, TIM-3, and complement C3. The dose−response relationships between RBC Mn concentration and peripheral blood WBCs **(A)**, RBCs **(B)**, Hb **(C)**, PLT **(D)**, TIM-3 **(E)** and C3 **(F)** concentrations were analyzed.

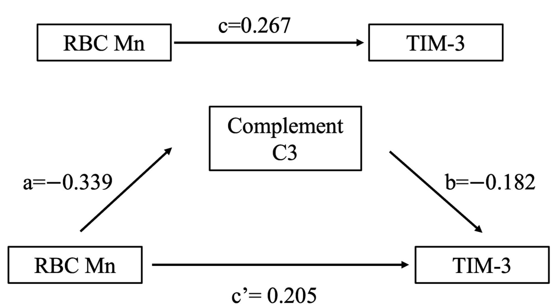

FIGURE 4
Analytical model of the mediating effect of complement C3 between RBC Mn and TIM-3.

With the discovery of aberrant expression of immune checkpoint molecules in a variety of tumors and autoimmune diseases, there is increasing evidence that they play an important role in maintaining homeostasis within the body's immune system. TIM-3 is an inhibitory immune checkpoint receptor structurally expressed on activated T cells that protects healthy cells from excessive inflammatory or autoimmune responses by binding to its ligand Gal-9 (23). Soluble TIM-3 is a form of its presence in the blood that is both responsive to immune changes and easily accessible, making it suitable as an effector marker. At present, domestic and international studies on Mn and immune checkpoints are mainly focused on the field of Mn^{2+} as an interferon gene-stimulating factor agonist combined with immune checkpoint inhibitors for antitumor therapy (24), but no studies on immune checkpoints as markers of Mn exposure effects have been found. Considerably, several studies have demonstrated that high concentrations of TIM-3 can be detected in plasma or serum of cancers such as lung, gastric, liver, and cervical cancers and systemic inflammatory patients such as those with chronic hepatitis B virus infection and transplantation of patients with acute leukemia (25), suggesting that it may be associated with a poor prognosis of the disease, affirming its value as a prognostic or predictive marker for predicting the disease or assessing the response to immunotherapy. In this study, after controlling for smoking, alcohol consumption and obesity influencing factors, the dose–response relationship between Mn internal exposure dose and TIM-3 was analyzed and it was found that the concentration of TIM-3 in the serum of the workers tended to increase with the increase of the internal exposure dose, which indicated that the measured circulating level of TIM-3 may be correlated with the dose of the toxic exposure, suggesting that TIM-3 has a potential value as a marker of the effect of Mn exposure. Complement C3 is a core component of the complement system and plays a crucial role in defense against pathogens, removal of apoptotic cells, enhancement of phagocytosis, inflammation, and regulation of adaptive immune responses (26). Chen et al. administered different doses of $MnSO_4$-H_2O to SD rats by intraperitoneal injection and found that Mn staining showed a biphasic dose–response relationship with complement C3, exhibiting low-dose stimulatory and high-dose inhibitory effects (18). However, it has also been found in animal experiments that the effect of Mn-treated welding fumes on the concentration of complement C3 in the rat circulation was not significant when compared to the control group (20). In population-based epidemiologic surveys, it has been found that male workers in the high Mn-exposed group had slightly lower levels of complement C3, which is consistent with the results of the present study. The results showed that the levels of complement C3 in exposed workers tended to decrease significantly with increasing RBCs Mn content, but the reason for this is not clear. The hepatotoxicity of Mn has been demonstrated in several studies (27, 28), but whether chronic repeated exposure to Mn impairs liver function and thus leads to a decrease in complement C3 values in humans remains to be investigated.

The subjects included in this study were all male welders and there was no difference in working age, smoking and alcohol consumption between the low and high Mn groups, with only a borderline significant difference in the subgroups with different BMIs ($p = 0.087$). Fatma Ates Alkan et al. (29) demonstrated that smoking not only altered hematological indices such as whole blood and plasma viscosity, fibrinogen, WBCs and RBCs, but also Mn trace element levels in smokers. At the same time, elevated serum Mn levels in the body aggravate tissue oxygenation, which in turn leads to disturbances in defense mechanisms and respiratory dysfunction. This study found that smokers had higher leukocyte and platelet concentrations and lower complement C3 concentrations in the high Mn group, suggesting that smoking worsens Mn′s immune system effects. Alcohol consumption is known to affect both cellular and humoral immunity (30, 31), as evidenced by a decrease in the number of lymphocytes, as well as an increase in the number of immunoglobulins. In contrast, the results of the present study showed that in the high Mn group drinkers had significantly higher leukocyte counts than non-drinkers, whereas in the low Mn group drinkers had lower levels of complement C3 than non-drinkers, but the statistical difference was borderline significant ($p = 0.098$). The increase in the number of leukocytes in this case may be due to the fact that alcohol consumption exacerbates the inflammatory effect of Mn on the body, leading to an increase in its main inflammatory cells, the neutrophils, the exact mechanism of which needs to be further investigated. Obesity is now recognized as an important risk factor for cardiovascular disease, immune disorders, and many types of cancer. According to the findings of this study, leukocyte, erythrocyte, and platelet concentrations in the peripheral blood of exposed employees rose with increasing BMI, demonstrating that obesity is a risk factor for Mn exposure. Among them, in the low Mn group, there were significant differences in the number of leukocytes between different BMIs; in the high Mn group, there were significant differences in the number of erythrocytes between different BMIs. Obese individuals have increased metabolism and oxygen consumption in their own tissues and organs due to fat accumulation and excessive weight gain; however, obese individuals tend to be in a state of chronic relative hypoxia, and hypoxia stimulates increased secretion of erythropoietin by renal proximal glomerulonephritis (32), which in turn enhances the function of the bone marrow hematopoietic system, leading to increased erythropoiesis.

The complement system plays a predominantly inhibitory role in T cell-mediated antitumor immunity, and multiple immune checkpoints activated on T cells have been identified and applied to the treatment of tumors and immune-related diseases. In recent years, the use of the complement system in immune checkpoint therapy has received increasing attention from researchers. Many components of the complement system can be involved in tumor immune escape mechanisms by down-regulating T cell activity and immune response through complement-dependent or auto-mechanisms. Understanding the role that complement plays in targeting immune checkpoint proteins is of paramount importance, and may improve therapeutic efficacy as well as reduce drug resistance. One investigator demonstrated that upregulation of checkpoint ligand programmed death ligand 1 in patients with paroxysmal nocturnal hemoglobinuria was explained by proximal complement activation (33). Shao et al. (34) investigators found that inhibition of EGFR up-regulated CD55 and CD59 expression activated the complement system and made lung cancer sensitive to checkpoint blockade. The results of the current study showed that there was a partial mediating effect of complement C3 in the process of RBC Mn affecting the changes in the expression level of TIM-3 at immune checkpoints, and the mediating effect accounted for 23.2%, which suggests that complement C3 is able to modulate the effect of RBC Mn exposure on the changes in the expression level of TIM-3 at immune checkpoints. Therefore, further clarification of the mechanism of interaction between complement C3 and the immune checkpoint TIM-3 may provide a new research strategy for occupational hazards of Mn exposure.

5. Conclusion

In conclusion, the present study showed that Mn exposure could increase the expression of TIM-3 and decrease the expression of complement C3 in the serum of workers, suggesting that Mn has an inhibitory effect on the body's immune system. Smoking, drinking and high BMI can also affect the expression of peripheral blood immune indicators. There is a dose–response relationship between the expression levels of TIM-3 and complement C3 in serum and the level of Mn in red blood cells in workers exposed to Mn, and complement C3 has a significant mediating effect between Mn and TIM-3 in red blood cells, which can provide clues for the discovery of biomarkers of Mn exposure. This study can preliminarily understand the effect of Mn exposure on the body's immune system, and lay the foundation for the study of biomarkers of Mn exposure. However, the research is not comprehensive, and there is no in-depth study on the specific mechanism of changes in the immune system caused by Mn exposure, which needs to be further improved in the future to provide a better basis for the prevention and treatment of Mn immunotoxicity. Therefore, in the future, we will use an animal model of Mn exposure to further explore the immunotoxic effect of Mn, focusing on the changes in histopathology, T cell count, immunoglobulin, related immune checkpoints and cytokines in rats after Mn exposure. To identify the key biomarkers of Mn exposure, use targeted intervention technologies such as small molecule inhibitors and gene editing to intervene the key biomarkers in the Mn exposure model, comprehensively observe the intervention effect, and evaluate the targeted intervention value of related biomarkers.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Life Science Ethics Review Committee of Zhengzhou University/Zhengzhou University. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because We verbally informed the study subjects of the purpose of the survey and obtained their consent.

Author contributions

YQ: Data curation, Investigation, Writing – original draft. HS: Data curation, Formal analysis, Writing – review & editing. XJ: Validation, Writing – original draft. YG: Supervision, Writing – review & editing. JX: Data curation, Writing – review & editing. JH: Validation, Writing – review & editing. XD: Investigation, Writing – review & editing. MD: Investigation, Writing – review & editing. WY: Conceptualization, Supervision, Writing – review & editing. CH: Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was financed by the National Natural Science Foundation of China (no. 82173491).

Acknowledgments

We express our gratitude to all the participants for their valuable contribution.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1289838/full#supplementary-material

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frontiers | Frontiers in Environmental Science

TYPE Original Research
PUBLISHED 13 December 2023
DOI 10.3389/fenvs.2023.1260317

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OPEN ACCESS

EDITED BY
Sarfuddin Azmi,
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REVIEWED BY
Md. Saiful Islam,
Patuakhali Science and Technology University, Bangladesh
Mohd Kamil Hussain,
Govt. Raza Post Graduate College, Rampur, India

*CORRESPONDENCE
Ali Irfan,
raialiirfan@gmail.com
Rab Nawaz,
rab.nawaz@envs.uol.edu.pk
Mohammed Bourhia,
bourhiamohammed@gmail.com

RECEIVED 17 July 2023
ACCEPTED 29 November 2023
PUBLISHED 13 December 2023

CITATION
Ayaz H, Nawaz R, Nasim I, Irshad MA, Irfan A, Khurshid I, Okla MK, Wondmie GF, Ahmed Z and Bourhia M (2023), Comprehensive human health risk assessment of heavy metal contamination in urban soils: insights from selected metropolitan zones. *Front. Environ. Sci.* 11:1260317. doi: 10.3389/fenvs.2023.1260317

Comprehensive human health risk assessment of heavy metal contamination in urban soils: insights from selected metropolitan zones

Harris Ayaz[1], Rab Nawaz[1,2]*, Iqra Nasim[1], Muhammad Atif Irshad[1], Ali Irfan[3]*, Iram Khurshid[1], Mohammad K. Okla[4], Gezahign Fentahun Wondmie[5], Zulkifl Ahmed[6] and Mohammed Bourhia[7]*

[1]Department of Environmental Sciences, The University of Lahore, Lahore, Pakistan, [2]Faculty of Engineering and Quantity Surveying, INTI International University, Nilai, Negeri Sembilan, Malaysia, [3]Department of Chemistry, Government College University Faisalabad, Faisalabad, Pakistan, [4]Botany and Microbiology Department, College of Science, King Saud University, Riyadh, Saudi Arabia, [5]Department of Biology, Bahir Dar University, Bahir Dar, Ethiopia, [6]College of Resource and Civil Engineering, Northeastern University, Shenyang, China, [7]Department of Chemistry and Biochemistry, Faculty of Medicine and Pharmacy, Ibn Zohr University, Laayoune, Morocco

Introduction: This study aims to assess the extent of heavy metal contamination in urban soils in sixteen selected cities of Pakistan, encompassing the elements cadmium (Cd), lead (Pb), cobalt (Co), zinc (Zn), chromium (Cr), nickel (Ni), manganese (Mn), iron (Fe), and copper (Cu).

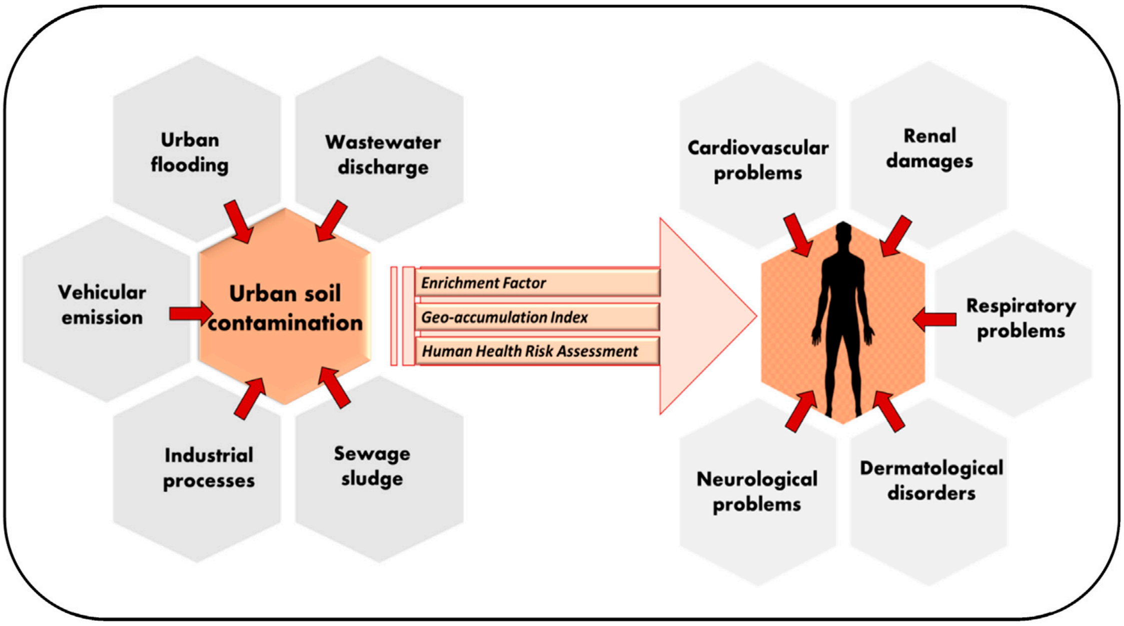
Methods: The data utilized for this study was collected from online literature during the period 2005 to 2019. This study investigated potential threats to human health through a comprehensive analysis, considering standards such as Enrichment Factors (EF), Geo-accumulation Indices (Igeo), and Human Health Risk Assessment (HHRA).

Results: Geo-accumulation Index results indicated varied risk intensities, with Cu, Pb, Co, Mn, and Fe exhibiting "no pollution" levels, while other elements show "moderate to extremely contaminated" values. EF analysis provided evidence of heavy metal presence, revealing a spectrum from "no pollution" to "moderate to extremely high pollution" for Cd, Zn, Cr, Ni, and Cu. The health risk assessment identified both carcinogenic and non-carcinogenic dangers for adults and children.

Discussion: These findings highlighted the substantial contribution of identified sources such as industrial processes, vehicular emissions, sewage sludge, urban flooding, and the production and use of metallic materials that have elevated heavy metal levels in the urban soils. This established the link between urban industrial zones, human health, and long-term economic sustainability. This study provides essential guidance for decision makers to develop effective strategies for soil remediation, enhanced industrial practices, and regulatory measures to address heavy metal contamination in urban areas, ensuring the wellbeing and sustainable environmental quality management in cities.

KEYWORDS

soil contamination, heavy metals, human health, risk assessment, carcinogenic



GRAPHICAL ABSTRACT

1 Introduction

The rapid urbanization and industrial growth in and around urban areas are inherently linked to the accumulation and contamination of heavy metals in the urban soil. These phenomena have significantly affected the urban soil environment (Adimalla, 2020). Urban areas emerge as focal points for environmental hazards across various scales due to increased population, industrial expansion, and heightened vehicular transport. The rapid urbanization and population influx has resulted in human activities disrupting the quality of the urban soil environment and leading to diverse levels of deterioration. Urban soils, functioning as reservoirs for contaminants, serve as dependable indicators of pollution. Soil heavy metal levels play a pivotal role in monitoring the impact of human activities on the soil quality (Tong, 2020; Hayyat et al., 2021). Typically, soil heavy metals are introduced into the urban environment through various pathways, such as urban waste, waste disposal, industrial effluents, vehicle emissions, construction waste, and extensive agrochemical usage (Dong et al., 2019; Sun et al., 2019; Zhao et al., 2019; Adimalla, 2020; Chakraborty et al., 2023). The urban environment is a significant source of trace metals from non-exhaust emissions brought on by the deterioration of vehicle components including the brake, tyre, and clutch. The industries of electroplating, petrochemicals, dyes, pigments, ceramics, tanning, and textiles are some of the industrial sources of the pollution of urban soil with heavy metals (Cu, Pb, Zn, and Cr). Hence, owing to its adverse effects on urban ecology, the contamination of urban soils by heavy metals constitutes a significant issue with ramifications not only at the local and regional scales but also on a global level (Bux et al., 2021). Globally, more than five million sites worldwide are severely contaminated with soil heavy metals (Liu et al., 2018; Sun et al., 2019). Agricultural practices can contribute to the accumulation of heavy metals in soil, posing environmental risks and potential health hazards through both the food chain and soil contact. To address this issue, the utilization of plants (Nawaz et al., 2023a) and ornamentals plants for soil remediation emerges as a viable solution (Ehsan et al., 2016a; 2016b; 2016c). Rashid et al. (2023) found that areas with significant greenery and agricultural land can have elevated heavy metal exposure risks due to the use of metal-based pesticides, fertilizers, and sewage sludge in farming practices.

Due to their toxic effects, long-term persistence, and bio-magnification characteristics, heavy metal pollution has received widespread attention. Heavy metals are recognized as the foremost pollutants among various soil contaminants (Jiang et al., 2019; Xiao et al., 2019). Urban soils, acting as receptors for substantial heavy metal influx from diverse sources, experience simultaneous accumulation from both natural and anthropogenic origins (Keshav Krishna and Rama Mohan, 2016; Zhang et al., 2018; Jiang et al., 2019; Xiao et al., 2019). Diverging significantly from natural soils, urban soils are notably influenced by anthropogenic activities with industrial waste, automobile exhaust, and domestic waste identified as primary contributors to the higher levels of potentially toxic elements (PTEs) such as Pb, Cd, Cu, and Zn (Huang et al., 2018). Consequently, urban soils are more disposed to harboring and accumulating elevated concentrations of heavy metals compared to their natural ones. This accumulation inevitably affects environmental health, leading to contamination in urban soil, water, and crops. Pollutants, entering the human body through the food chain, pose direct or indirect health hazards. Heavy metals are accumulated in human tissues and internal organs can affect the central nervous system and act as cofactors, initiators, or promoters of various diseases. Exposure to mixed metals can result in numerous adverse health effects on humans due to synergistic interactions, even when individual metal concentrations are below their Eco-toxicological benchmark levels. The adverse effects on human health primarily occur through three pathways: ingestion, inhalation, and dermal contact absorption.

Numerous studies highlighted ingestion as the primary exposure pathway for human health risks, with children being especially susceptible to the health risks associated with heavy metal toxicity (Tong et al., 2020).

Globally, there is a severe environmental concern with increased amounts of hazardous metals in urban soil (Yang et al., 2022). Heavy metal contamination in urban soil poses a potential threat to human health, with risks extending beyond the metals themselves. Health risk assessment serves as a valuable technique for gauging the potential harm to human health arising from various contaminants through multiple exposure routes (Tudi et al., 2022; Zhou et al., 2022; Nawaz et al., 2023a). While studies evaluating the health hazards of heavy metal pollution in urban soils have been conducted in selected locations such as Changsha, China (Wang et al., 2010), Sao Paulo, Brazil (Figueiredo et al., 2011), Xiamen, China (Luo et al., 2012), and Belgrade, Serbia (Grzetic and Ghariani, 2008), there is lack of comprehensive assessment of human health risks associated with heavy metals in urban soils of Pakistan.

This study focuses on the issue of higher levels of heavy metals in urban soils within particular metropolitan areas in Pakistan. This concern poses potential health risks, making it imperative to comprehensively address and understand its implications. Urbanization often brings about various human activities that result in the accumulation of heavy metals in soil, and these metals, upon ingestion can pose significant health risks to humans. Despite the gravity of this problem, there is currently a lack of comprehensive knowledge regarding the extent of heavy metal contamination, its associated health risks, and the variations across distinct urban areas in Pakistan. This study endeavors to bridge a significant knowledge gap by conducting a comprehensive assessment of human health risks. The primary focus of this assessment is to delve deeply into the concentrations of heavy metals, pinpoint potential pathways of exposure, and subsequently quantify the health risks that affect both adults and children.

To foster a comprehensive comprehension of the diverse urban landscapes across Pakistan, this research intentionally confines its scope to specific metropolitan zones. The overarching objective is to make a substantial contribution to the formulation of effective policies, the implementation of precise interventions, and the development of informed decision-making methods. This endeavor aims to mitigate and alleviate the risks linked with heavy metal contamination in urban soil environments. The main objectives of this study cover a number of important aspects. These involve calculating the chronic daily consumption amounts of heavy metals for both adults and children, carefully analyzing the potential effects of this intake, and determining the hazard quotient for non-carcinogenic substances. These analytical endeavors are essential for developing a comprehensive understanding of the potential risks connected to heavy metal exposure. The assessment covers a wide range of health risks, encompassing both carcinogenic and non-carcinogenic effects resulting from multiple exposure pathways. The outcomes of this study are anticipated to significantly reduce health risks for urban residents and provide decision-makers with valuable insights for the treatment and appropriate management of contaminated soils. By systematically addressing the health implications of heavy metal contamination and offering actionable data, this research aspires to make a noteworthy contribution to public health and environmental wellbeing in urban settings.

2 Methodology

2.1 Description of study area (selected cities)

Karachi, the largest city in Pakistan, is situated at approximately 24.8607°N latitude and 67.0011°E longitude on the southern coast. Lahore, a significant cultural and economic center, is located in the northeastern part of the country at around 31.5497°N, 74.3436°E. The capital city, Islamabad, and its neighbour city Rawalpindi share coordinates at 33.6844°N, 73.0479°E in the north. Moving towards the northwest, Peshawar is positioned at 34.0151°N, 71.5249°E, while the region of Swat lies at 35.2220°N, 72.4258°E. Faisalabad, an industrial hub, can be found at 31.5497°N, 73.0782°E in the northeast, and Multan is located at 30.1798°N, 71.4580°E in the southern part. Heading southeast, Bahawalpur is situated at 29.3954°N, 71.6728°E. The southwestern city of Quetta has coordinates of 30.1798°N, 66.9750°E. Other notable cities include Gujranwala at 32.1617°N, 74.1883°E, Kasur at 31.1156°N, 74.4465°E, Hyderabad at 25.3969°N, 68.3776°E, Sukkur at 27.7135°N, 68.8480°E, Sahiwal at 30.6717°N, 73.1084°E, and Vehari at 30.0458°N, 72.3422°E (Figure 1). These cities have been chosen due to considerations of population density, economic significance, cultural diversity, and strategic importance within the regional context.

The commonalities among these cities in Pakistan include their integral roles as urban centers contributing to the nation's economic, cultural, and social fabric. They serve as hubs for commerce, industry, and education, influencing regional development. Moreover, these cities often share historical and cultural connections, reflecting Pakistan's diverse heritage. Additionally, their geographic locations across the country contribute to their strategic importance, impacting transportation networks and regional connectivity. Despite unique characteristics, these cities collectively represent the multifaceted dynamics of Pakistan's urban landscape.

2.2 Study data

For this extensive investigation, the secondary data was collected from various reliable and authentic sources for a study period from 2005 to 2019 as shown in Table 3. Target heavy metals include Cd, Pb, Co, Zn, Cr, Ni, Mn, Fe, and Cu for this investigation. Scientific models/equations were used for the estimation of enrichment factors (EFs) and the geo accumulation index (*Igeo*), Average Daily Intake (ADI), Hazard Quotient (HQ), Hazard Index (HI) and Carcinogenic Risk (CR).

2.3 Enrichment factor and geo accumulation index

The quantification of heavy metal pollution levels was determined through the enrichment factors (EFs) and the geo

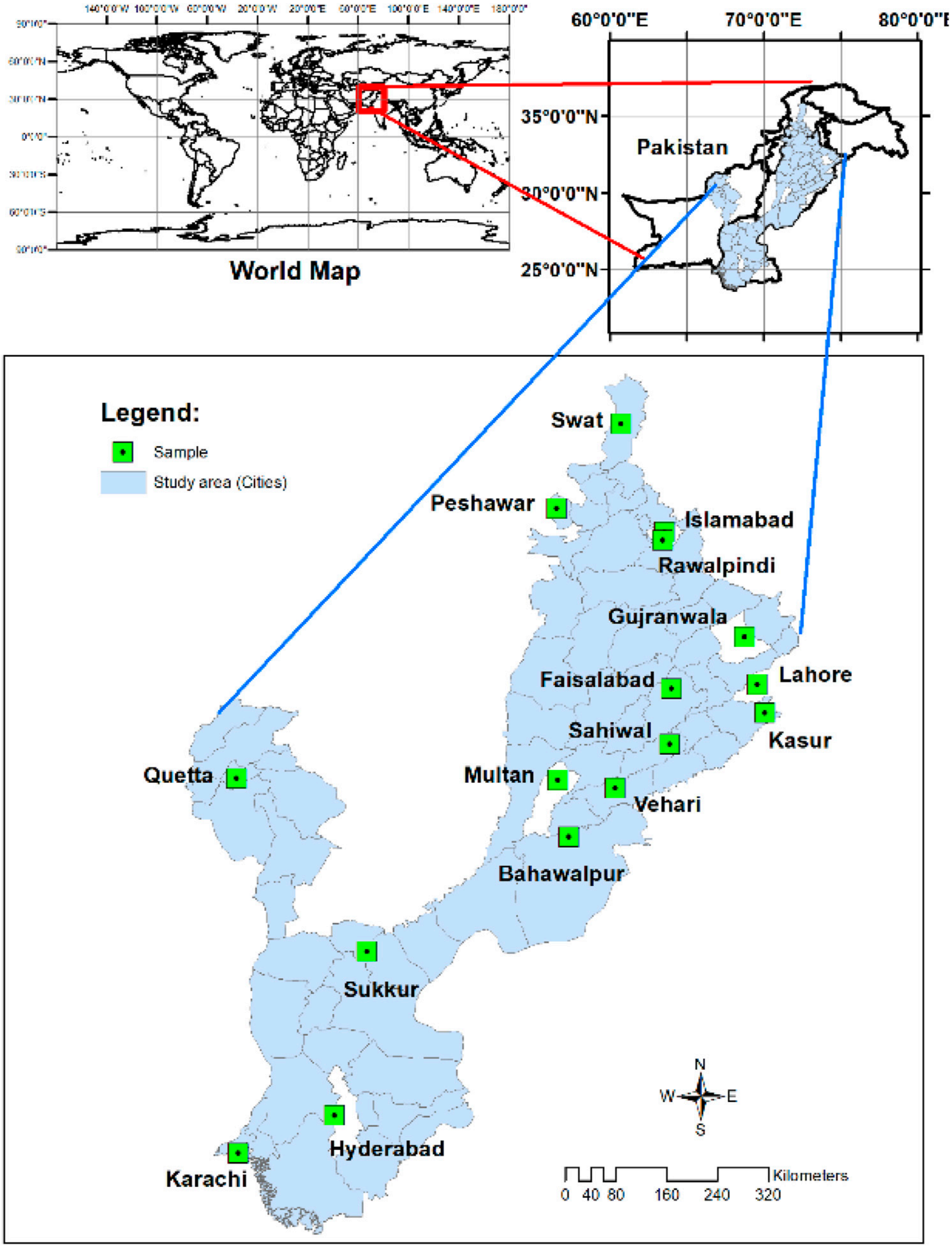


FIGURE 1
GIS map showing study areas (selected cities) of Pakistan.

TABLE 1 Values of input parameters for the calculation of average daily intake in age groups.

Sr. #	Parameters	Abbreviation	Unit	Adults	Children	Reference
1	Concentration of metals	C	mgkg^{-1}	—	—	—
2	Ingestion rate	IR	mgkg^{-1}	100	200	USEPA (2002)
3	Exposure frequency	EF	Day/year	350		USEPA (2002)
4	Exposure duration	ED	Year	30	6	USEPA (2002)
5	Body weight	BW	kg	70	20	USEPA (2002)

TABLE 2 Average time (AT) for the calculation of carcinogenic and non-carcinogenic evaluation.

Sr. No	Parameters	Abbreviation	Unit	For non-carcinogens	For carcinogens	Reference
1	Averaging time	AT	—	ED × 365 days	70 × 365 days	USEPA (2002)

TABLE 3 Concentrations of heavy metals in soils of selected cities.

Cities	Cd (mg/kg)	Pb (mg/kg)	Co (mg/kg)	Zn (mg/kg)	Cr (mg/kg)	Ni (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Cu (mg/kg)	Year	Reference
Karachi	0.25	42.1	19.5	99.5	9.6	9.4	6.6	908.4	33.3	2013	Karim and Qureshi (2014)
Lahore	1.03	4.54	2.4	10.8	6.4	6.61	13.82	—	10.19	2013	Mahmood and Malik (2014)
Islamabad	0.048	1.045	0.162	0.163	0.175	—	0.122	7.127	0.057	2011	Rafique et al. (2011)
Rawalpindi	164	15.72	33.37	543	295.28	236	—	—	336	2019	Tahir and Yasmin. (2019)
Peshawar	0.11	0.4	—	40.94	1.65	10.54	—	44.3	20.84	2018	Saddique et al. (2018)
Swat	3	—	—	48	863	—	9.9	400.5	63	2018	Saddique et al. (2018)
Faisalabad	—	21.44	—	48.57	—	21.44	—	—	24.08	2015	Parveen et al. (2015)
Multan	0.23	0.61	0.05	—	—	0.083	0.17	34.2	0.191	2014	Randhawa et al. (2014)
Bahawalpur	0.31	13	—	—	8	8.1	—	—	—	2016	Iqbal et al. (2016)
Quetta	0.29	1.38	—	19.45	0.03	0.74	3.11	—	0.86	2005	Kakar et al. (2005)
Gujranwala	1.8	89	—	18	159.4	104.7	6.5	44	169.5	2007	Bostan et al. (2007)
Kasur	26.3	18.21	8.9	14.3	244.3	—	9.42	—	—	2013	Afzal et al. (2014)
Hyderabad	1.2	30	13.73	—	49.9	55.1	90	70.5	37.7	2021	Bux et al. (2021)
Sukkur	0.04	1.1	15.5	13.83	—	6.43	2	2.7	5.26	2011	Khan et al. (2011)
Sahiwal	0.003	1.48	—	0.49	9	0.09	32	—	—	2019	Ur Rehman et al. (2019)
Vehari	1.6	1.1	17.6	102.2	59.9	1.9	9	87.6	40.3	2019	Sarwar et al. (2020)

accumulation index (Igeo). According to Sutherland (2000), the following equation was used to calculate EF values.

$$EF = \frac{Ci}{CB} \tag{1}$$

Where Ci is the quantity of heavy metal (in mg/kg) in the soils, and CB is the background level of heavy metal (in mg/kg) in the soils, there are six recognized categories of contamination based on the enrichment factors:

- EF less than or equal to 1 = no pollution.
- Greater than 1 EF Less than 2 = slight pollution.
- Greater than or equal to 2 EF Less than 5 = moderate pollution.
- Greater than or equal to 5 EF Less than 20 = significant pollution.
- Greater than or equal to 20 EF Less than 40 = strong pollution.
- EF Greater than and equal to 40 = extremely strong pollution.

Muller (1969) developed the *Igeo*, a geochemical criterion for assessing soil tainting by contrasting the distinctions in contemporary and preindustrial focuses. Not at all like other contamination assessment methods, *Igeo* considers the natural digenesis process, making the evaluations more feasible. The

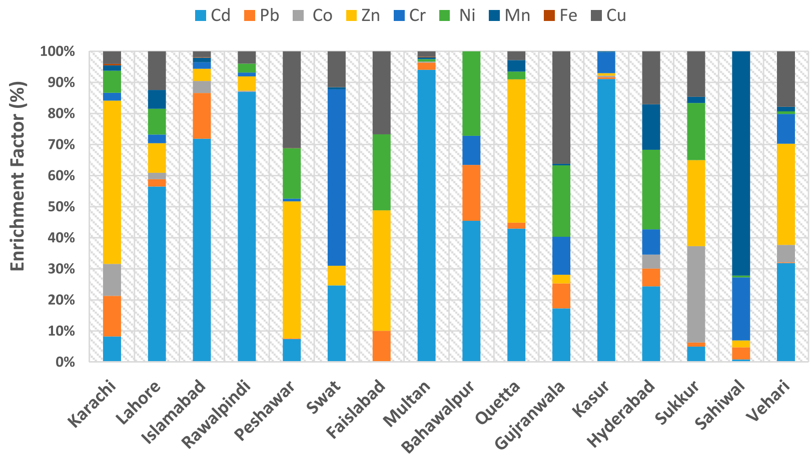


FIGURE 2
Enrichment factor values for heavy metals in soils of selected cities of Pakistan.

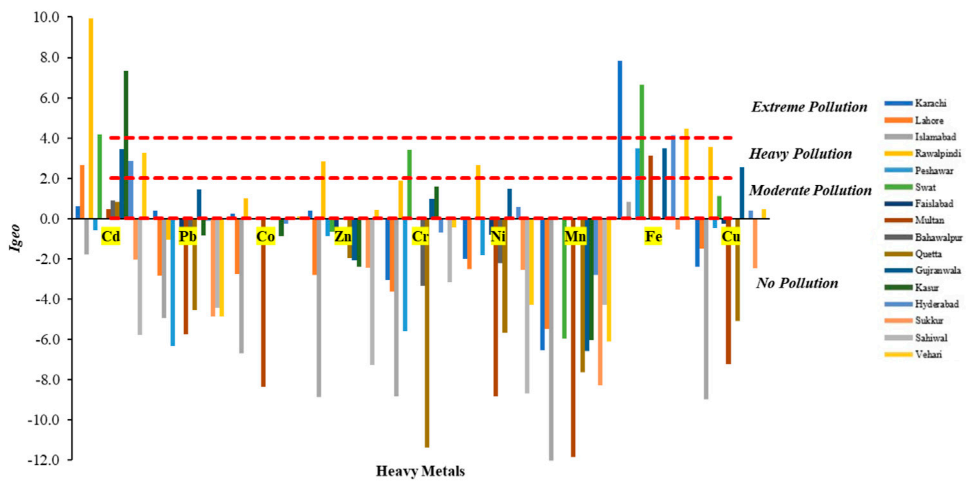


FIGURE 3
Evaluation of heavy metals in soils of selected cities of Pakistan using Igeo analysis.

subsequent equation was employed for the calculation of the Geo-accumulation Index (*Igeo*):

$$Igeo = Log2\frac{Cn}{1.5} \times Bn \tag{2}$$

In this equation, Cn denotes the measured concentration of the heavy metal in soil (mg kg^{-1}), while Bn signifies the corresponding geochemical baseline value for the heavy metals (Zhang et al., 2023), and the coefficient 1.5 is used to account for any changes in the baseline data (Solgi et al., 2012). The *Igeo* were categorized into seven groups by Muller (1969). Following are the correlations between *Igeo* and pollution levels:

- Unpolluted (Igeo $\leq$ 0),
- Moderately polluted (0 $\leq$ Igeo $\leq$ 2)
- Heavily polluted (2 $\leq$ Igeo $\leq$ 4)
- Extremely polluted (4 $\leq$ Igeo $\leq$ 5)

Heavy metal levels found in different metropolitan cities were arranged according to their mean, most severe, least, and standard deviations.

2.4 Human health risk assessment

In this study, the Human Health Risk Assessment (HHRA) was divided into two distinct categories: carcinogenic and non-carcinogenic. This categorization was based on the evaluation of risks associated with exposure to metals or metalloids. Present study specifically aimed to ascertain the degree of heavy metal intake into the human body through the consumption of crops grown in soil contaminated with these pollutants. Guidelines of United States Environmental Protection Agency (USEPA) were followed to conduct health risk assessment. To gauge this risk effectively, critical parameters were computed, including the Chronic Daily

TABLE 4 Non-carcinogenic risk assessment based on average daily intake (ADI) in adults and children.

Average daily intake (ADI)																		
Heavy metals	Pb		Co		Zn		Mn		Fe		Cu		Cd		Cr		Ni	
Cities	Adult	Children	Adult	Children	Adult	Children	Adult	Children	Adult	Children	Adult	Children	Adult	Children	Adult	Children	Adult	Children
Karachi	2.3E-07	8.1E-05	2.7E-05	3.7E-05	1.4E-04	1.9E-04	9.0E-06	1.3E-05	1.2E-03	1.7E-03	7.5E-06	1.1E-05	3.4E-07	4.8E-07	5.6E-06	7.9E-06	5.5E-06	7.7E-06
Lahore	6.2E-06	8.7E-06	3.3E-06	4.6E-06	1.5E-05	2.1E-05	1.9E-05	2.7E-05	—	—	1.4E-05	2.0E-05	1.4E-06	2.0E-06	8.8E-06	5.3E-06	9.1E-06	5.4E-06
Islamabad	1.4E-06	2.0E-06	2.2E-07	3.1E-07	2.2E-07	3.1E-07	1.7E-07	2.3E-07	9.8E-06	1.4E-05	7.8E-08	1.1E-07	6.6E-08	9.2E-08	1.0E-07	1.4E-07	—	—
Rawalpindi	2.2E-05	3.0E-05	4.6E-05	6.4E-05	7.4E-04	1.0E-03	—	—	—	—	4.6E-04	6.4E-04	2.2E-04	3.1E-04	1.7E-04	2.4E-04	1.4E-04	1.9E-04
Peshawar	5.5E-07	7.7E-07	—	—	5.6E-05	7.9E-05	—	—	6.1E-05	8.5E-05	2.9E-05	4.0E-05	1.5E-07	2.1E-07	9.7E-07	1.4E-06	6.2E-06	8.7E-06
Swat	—	—	—	—	6.6E-05	9.2E-05	1.4E-05	1.9E-05	5.5E-04	7.7E-04	8.6E-05	1.2E-04	4.1E-06	5.8E-06	5.1E-04	1.7E-03	—	—
Faisalabad	2.9E-05	4.1E-05	—	—	6.7E-05	9.3E-05	—	—	2.9E-05	4.1E-05	—	—	—	—	—	—	1.4E-05	2.0E-05
Multan	8.4E-07	1.2E-06	6.8E-08	9.6E-08	—	—	2.3E-07	3.3E-07	4.7E-05	6.6E-05	2.6E-07	3.7E-07	3.2E-07	4.4E-07	—	—	4.9E-08	6.8E-08
Bahawalpur	1.8E-05	2.5E-05	—	—	—	—	—	—	—	—	—	—	4.2E-07	5.9E-07	4.7E-06	6.6E-06	4.8E-06	6.7E-06
Quetta	1.8E-05	2.6E-06	—	—	0.0E+00	3.7E-05	0.0E+00	6.0E-06	—		0.0E+00	1.6E-06	4.2E-07	5.6E-07	4.7E-06	2.5E-08	4.8E-06	6.1E-07
Gujranwala	1.2E-04	1.7E-04	—	—	2.5E-05	3.5E-05	8.9E-06	1.2E-05	6.0E-05	8.4E-05	2.3E-04	3.3E-04	2.5E-06	3.5E-06	9.4E-05	1.3E-04	6.1E-05	8.6E-05
Kasur	2.5E-05	3.5E-05	1.2E-05	1.7E-05	2.0E-05	2.7E-05	1.3E-05	1.8E-05	—	—		—	3.6E-05	5.0E-05	1.4E-04	2.0E-04	—	—
Hyderabad	4.1E-05	5.8E-05	1.9E-05	2.6E-05	—	—	1.2E-04	1.7E-04	9.7E-05	1.4E-04	5.2E-05	7.2E-05	1.6E-06	2.3E-06	2.9E-05	4.1E-05	3.2E-05	4.5E-05
Sukkur	1.5E-06	2.1E-06	2.1E-05	3.0E-05	1.9E-05	2.7E-05	2.7E-06	3.8E-06	3.7E-06	5.2E-06	7.2E-06	1.0E-05	5.5E-08	7.7E-08	—	—	3.8E-06	5.3E-06
Sahiwal	2.0E-06	2.8E-06	—	—	6.7E-07	9.4E-07	4.4E-05	6.1E-05	—	—	—	—	4.1E-09	5.8E-09	5.3E-06	7.4E-06	5.3E-08	7.4E-08
Vehari	1.5E-06	2.1E-06	2.4E-05	3.4E-05	1.4E-04	2.0E-04	1.2E-05	1.7E-05	1.2E-04	1.7E-04	5.5E-05	7.7E-05	2.2E-06	3.1E-06	3.5E-05	4.9E-05	1.1E-06	1.6E-06

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TABLE 5 Non-carcinogenic risk assessment via hazard quotient (HQ) and hazard index (HI) for adults and children.

HQ																		
Heavy metals	Pb		Co		Zn		Mn		Fe		Cu		Cd		Cr		Ni	
Cities	Adult	Children	Adult	Children	Adult	Children	Adult	Children	Adult	Children	Adult	Children	Adult	Children	Adult	Children	Adult	Children
Karachi	4.2E-02	5.9E-02	9.0E-02	1.3E-01	3.5E-03	4.8E-03	7.1E-05	9.7E-05	1.8E-03	2.5E-03	3.8E-04	5.3E-04	1.2E-04	1.7E-04	9.9E-09	1.4E-08	4.8E-09	6.8E-09
Lahore	4.6E-03	6.4E-03	1.1E-02	1.5E-02	3.7E-04	5.2E-04	1.5E-04	2.0E-04	—	—	7.1E-04	9.9E-04	4.9E-04	6.9E-04	6.6E-09	9.2E-09	3.4E-09	4.8E-09
Islamabad	1.1E-03	1.5E-03	7.5E-04	1.0E-03	5.7E-06	7.9E-06	1.3E-06	1.8E-06	1.4E-05	2.0E-05	4.0E-06	5.5E-06	2.3E-05	3.2E-05	1.8E-10	2.5E-10	—	—
Rawalpindi	1.6E-02	2.2E-02	1.5E-01	2.1E-01	1.9E-02	2.6E-02	—	—	—	—	2.3E-02	3.3E-02	7.8E-02	1.1E-01	3.0E-07	4.2E-07	1.2E-07	1.7E-07
Peshawar	4.0E-04	5.6E-04	—	—	—	—	—	—	8.7E-05	4.1E-07	1.4E-03	2.0E-03	5.2E-05	7.3E-05	1.7E-09	2.4E-09	5.4E-09	7.6E-09
Swat	—	—	—	—	—	—	1.1E-04	1.5E-04	7.9E-04	1.1E-03	4.4E-03	6.1E-03	1.4E-03	2.0E-03	8.9E-07	1.2E-06	—	—
Faisalabad	2.2E-02	3.0E-02		—	—	—	—	—	4.2E-05	5.9E-05		—	—	—	—	—	1.2E-08	1.7E-08
Multan	6.1E-04	8.6E-04	2.3E-04	3.2E-04	—	—	1.8E-06	2.5E-06	6.7E-05	9.4E-05	1.3E-05	1.9E-05	1.1E-04	1.5E-04	—	—	4.3E-11	6.0E-11
Bahawalpur	1.3E-02	1.8E-02	—	—	—	—	—	—	—	—		—	1.5E-04	2.1E-04	8.2E-09	1.2E-08	4.2E-09	5.8E-09
Quetta	1.3E-02	4.8E-05	—	—	8.9E-06	9.4E-04	3.0E-06	4.6E-05	—	—	8.7E-07	8.3E-05	1.5E-04	1.9E-04	3.1E-11	4.3E-11	3.8E-10	5.3E-10
Gujranwala	9.0E-02	1.3E-01	—	—	6.2E-04	8.7E-04	7.0E-05	9.5E-05	8.6E-05	1.2E-04	1.2E-02	1.6E-02	8.6E-04	1.2E-03	1.6E-07	3.5E-07	5.4E-08	7.5E-08
Kasur	1.8E-02	2.6E-02	4.1E-02	5.7E-02	5.0E-04	6.9E-04	1.0E-04	1.4E-04	—	—	—	—	1.3E-02	1.8E-02	2.5E-07	3.5E-07	—	—
Hyderabad	3.0E-02	4.2E-02	6.3E-02	8.8E-02	—	—	9.7E-04	1.3E-03	1.4E-04	1.9E-04	2.6E-03	3.7E-03	5.7E-04	8.0E-04	5.1E-08	7.2E-08	2.8E-08	4.0E-08
Sukkur	1.1E-03	1.5E-03	7.1E-02	1.0E-01	4.8E-04	6.7E-04	2.2E-05	2.9E-05	5.3E-06	7.4E-06	3.7E-04	5.1E-04	1.9E-05	2.7E-05	—	—	3.3E-09	4.6E-09
Sahiwal	1.5E-03	2.1E-03	—	—	1.7E-05	2.4E-05	3.4E-04	4.7E-04	—	—	—	—	1.4E-06	2.0E-06	9.3E-09	1.3E-08	4.6E-11	6.5E-11
Vehari	1.1E-03	1.5E-03	8.1E-02	1.1E-01	3.5E-03	4.9E-03	9.7E-05	1.3E-04	1.7E-04	2.4E-04	2.8E-03	3.9E-03	7.6E-04	1.1E-03	6.2E-08	8.6E-08	9.8E-10	1.4E-09
HI	2.5E-01	3.4E-01	5.1E-01	7.2E-01	2.8E-02	4.0E-02	1.9E-03	2.7E-03	3.2E-03	4.3E-03	4.8E-02	6.7E-02	9.5E-02	1.3E-01	1.8E-06	2.6E-06	2.4E-07	3.3E-07

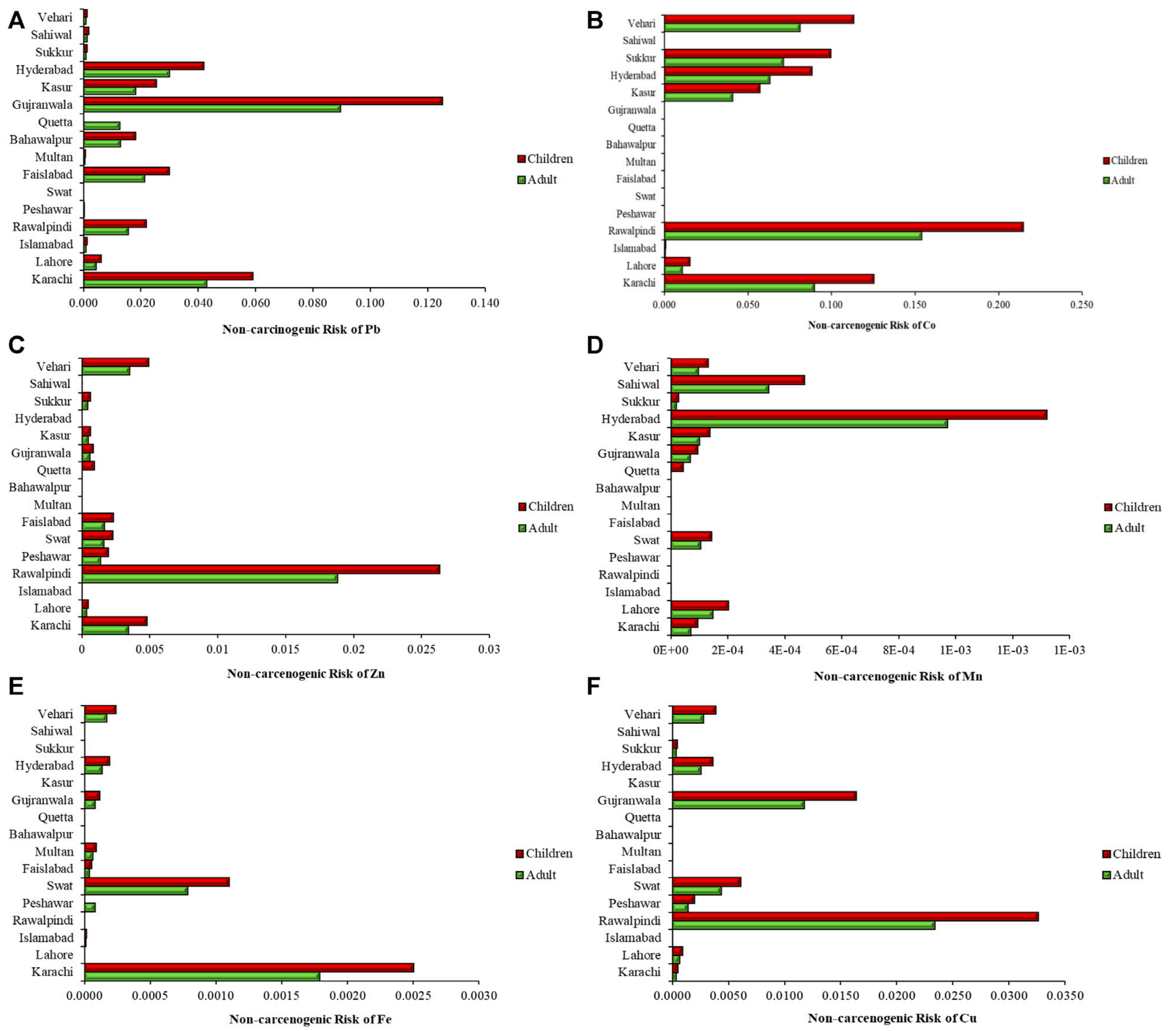

FIGURE 4
Non-carcinogenic risk assessment for Pb **(A)**, Co **(B)**, Zn **(C)**, Mn **(D)**, Fe **(E)**, and Cu **(F)** in urban soils of selected cities of Pakistan using mean parameter values for HQ evaluation.

Intake (CDI), Hazard Quotient (HQ), Hazard Index (HI), and Carcinogenic Risk (CR).

2.4.1 Non-carcinogenic risk assessment

In assessing the possible adverse effects of non-carcinogenic exposure to heavy metals, this study followed the guidelines for toxicant assessment outlined by the United States Environmental Protection Agency (USEPA). Average Daily Intake (ADI) and Hazard Quotient (HQ) calculations were used as part of our assessment as shown in Tables 1, 2. The essential parameter, Average Daily Intake (ADI), was computed using the following equation:

$$\boldsymbol{ADI} = \frac{\boldsymbol{C \times IR \times EF \times ED}}{\boldsymbol{BW \times AT}} \times \mathbf{10^{-6}} \tag{3}$$

The subsequent equation was utilized for the calculation of the Hazard Quotient (HQ):

$$\mathrm{HQ} = \frac{\boldsymbol{ADI}}{\boldsymbol{RfDi}} \tag{4}$$

Where RfD is the heavy metal reference dosage (mg/kg^1/day^1). This is the quantity of heavy metal that may be present without endangering human health. The RfD (reference portion by non-journal ingestion for weighty metals (mg/kg^1/day^1), esteem both for kids and adults in soil was viewed as in this investigation. Since there are no reference portions for estimating dermal ingestion openness to synthetic substances, the USEPA (2002) presents a procedure for evaluating dermal gamble, which includes expanding the dirt admission reference dose by a gastrointestinal retention proportion.

To assess the overall non-carcinogenic effects posed by a combination of various chemicals, the Hazard Index (HI) was determined by the summation of individual HQ values. This

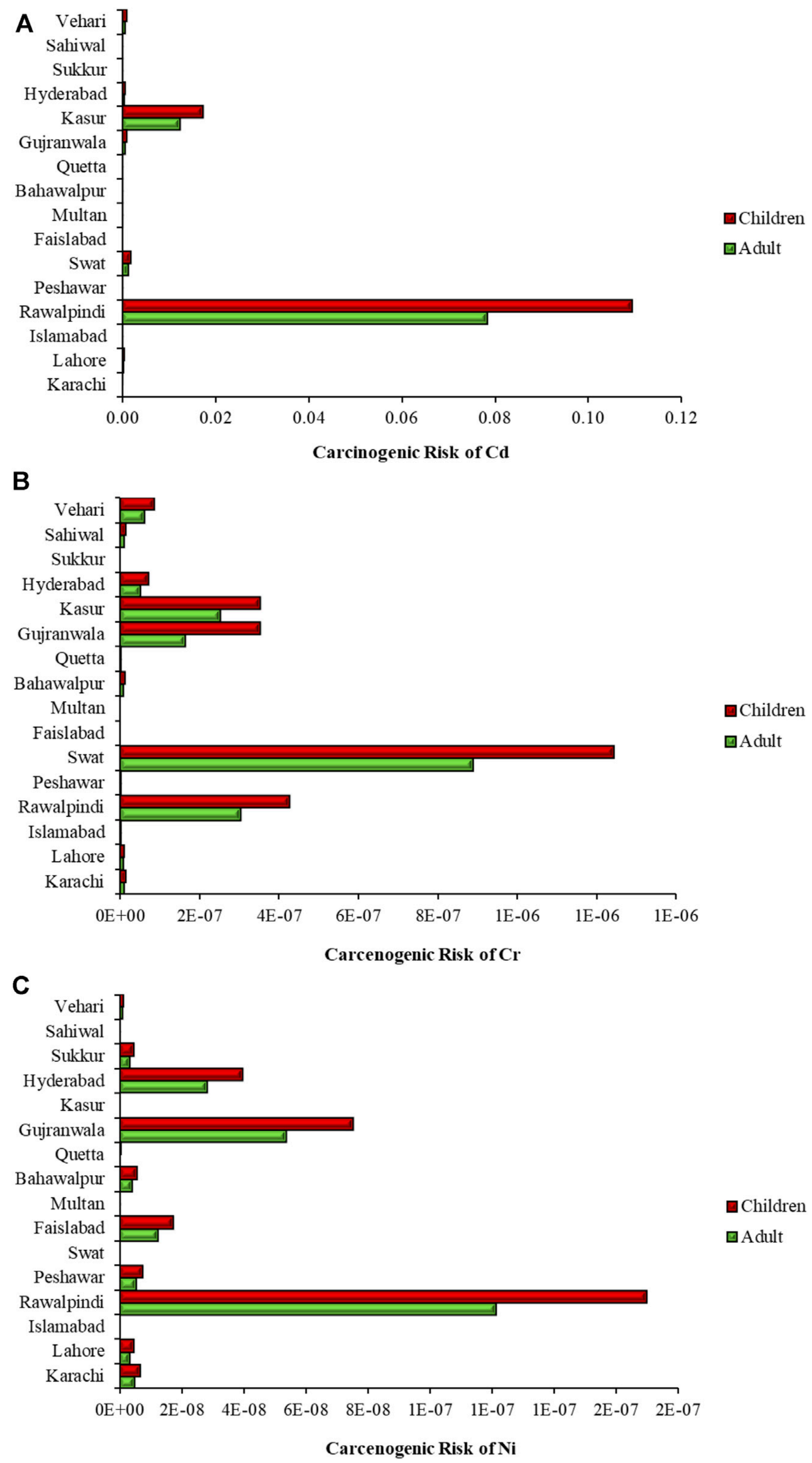

FIGURE 5
Carcinogenic risk assessment for Cd **(A)**, Cr **(B)**, and Ni **(C)** in urban soils of selected cities of Pakistan using mean parameter values for CR estimation.

comprehensive approach helps in evaluating the combined risk of exposure to multiple substances.

$$\boldsymbol{HI} = \Sigma\,\mathbf{HQi} = \Sigma\,\frac{\boldsymbol{ADI}}{\boldsymbol{RfDi}} \tag{5}$$

Hazard Quotient (HQ) values provide insight into the extent of non-carcinogenic health impacts, with values below 1 indicating no significant impact and values above 1 signifying considerable health concerns. Similarly, when assessing the Hazard Index (HI), an HI value below 1 suggests minimal or negligible risks to non-cancer health, whereas an HI value exceeding 1 indicates a substantial risk (USEPA, 1989).

2.4.2 Carcinogenic risk assessment

The assessment of Carcinogenic Risk (CR) assumes a pivotal role in estimating an individual's lifetime risk of developing cancer as a consequence of exposure to carcinogenic substances. This risk estimation is carried out by multiplying the Slope Factor (SF) linked to heavy metals recognized for their carcinogenic potential by the Average Daily Intake (ADI), as delineated in the subsequent equation (USEPA, 2011):

$$\boldsymbol{CR} = \boldsymbol{ADI} \times \boldsymbol{SF} \tag{6}$$

The Carcinogenicity Slope Factor (SF) is a critical parameter expressed in units of milligrams per kilogram per day (mg/kg/day). When multiple cancer-causing agents are present, the cumulative cancer risk resulting from various combinations and exposure pathways is aggregated. The resulting CR is categorized on a scale ranging from very low (less than 1×10^{-6}) to very high (greater than 1×10^{-3}) according to the study by Nawaz et al. (2023b).

3 Results

3.1 Heavy metals' concentration in soils

Table 3 displays the concentrations of several heavy metals (Cd, Pb, Co, Zn, Cr, Ni, Mn, Fe, and Cu), each of which has varying effects on both human health and the environment. These concentrations are observed in the topsoil of urban areas within major cities across Pakistan. Notably, Rawalpindi exhibits the highest count of heavy metals in its topsoil among the cities surveyed. A distinctive feature was found in Vehari, the sole city in Pakistan where each of the analyzed heavy metals was present in its topsoil. The selection of cities for this analysis spans different provinces of Pakistan, aiming to discern patterns in heavy metal distribution within urban soils. Each of the four provinces contributes cities to this study, facilitating an investigation into the relationship between urbanization and its influence on soil composition.

3.2 Enrichment factor and geo accumulation index

The Enrichment Factor (EF) functions as a valuable instrument for evaluating geochemical patterns and distinguishing whether the origins of heavy metal sources are lithogenic or anthropogenic in nature. According to some researchers, heavy metals with EF values below 2 are considered not to be significant contaminant concerns (Almasoud et al., 2015). In this study, the EF values for Cd in selected locations were as Rawalpindi (20.5), Swat (3.75), Gujranwala (2.25), Kasur (32.88), and Vehari (2.0). These values indicate pollution levels ranging from "moderate to extremely severe" in the corresponding soils. Zn displayed EF values of 2.04 and 10.86 in Vehari and Rawalpindi soils, reflecting "moderate to significant pollution." Cr exhibited EF values of 2.44, 2.95, and 8.63 in Kasur, Rawalpindi, and Swat soils, signifying "moderate to significant pollution." Ni showcased EF values of 2.99 and 6.74 in Gujranwala and Rawalpindi soils, indicating "moderate to significant pollution." Cu revealed EF values of 4.71 and 9.33 in Gujranwala and Rawalpindi soils, suggesting "moderate to significant pollution." In contrast, Lead (Pb), Cobalt (Co), Manganese (Mn), and Iron (Fe) exhibited EF values below 1 in the soils of the selected cities, implying "no pollution" as shown in Figure 2. These findings emphasize the diverse levels of heavy metal contamination across the selected areas, offering valuable insights into the potential sources and environmental repercussions of these pollutants. These findings align with the outcomes presented by Rezapour et al. (2022). The Enrichment Factor (EF) indicates a notable elevation of Cd, Zn, Cr, Ni, and Cu in urban soil, transitioning from minimal enrichment (EF < 2) in control soils to moderate enrichment (2 ≤ EF < 5) in urban soils.

The selection of an appropriate evaluation parameter is crucial for accurately assessing environmental pollution, and geochemical baseline values serve as a reliable metric in this context. The geo-accumulation index, introduced by Muller in 1969, is a quantitative parameter used to gauge the pollution of heavy metal elements. In this research, prior heavy metal baseline values were employed as evaluation criteria. The outcomes of this assessment, utilizing the geo-accumulation index (*Igeo*), provide insights into soil pollution across Pakistani cities (Figure 3).

Observations from Figure 3 indicate that the prevalence of heavy metals in the soils of Pakistani cities mostly falls below 0 on the *Igeo* scale. However, specific urban soil samples from selected cities exhibit *Igeo* values between 0 and 2, signifying moderate pollution levels for heavy metals such as Cd, Pb, Zn, Cr, Ni, Fe, and Cu. A limited subset of soils from certain cities demonstrates higher pollution levels, particularly involving Cd, Zn, Cr, Fe, and Cu. Notably, the soils of Rawalpindi, Kasur, Swat and Vehari stand out as the most severely contaminated, particularly with Cd and Fe. This emphasizes the need for targeted interventions and remediation efforts in these areas to address the elevated pollution levels and ensure the environmental health of these urban locations. Similar findings were disclosed by Kumar (2023), where the Geo-accumulation index indicated high contamination of Cu, Zn, As, and Pb attributed to industrial activities.

3.3 Human health risk assessment

3.3.1 Non-carcinogenic risk assessment

Potential health risk assessment associated with non-carcinogenic agents is a pivotal responsibility in safeguarding public wellbeing. This comprehensive study involves evaluating

TABLE 6 Comparison of heavy metals concentration among different cities.

Cities	Cd (mg/kg)	Pb (mg/kg)	Co (mg/kg)	Zn (mg/kg)	Cr (mg/kg)	Ni (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Cu (mg/kg)	References
Southeast China	0.19	30.74	—	85.86	67.37	27.77	—	—	25.81	Yuan et al. (2021)
Chhatak	0.392	3.38	—	1.993	—	188.9	—	—	2.984	Das et al. (2023)
Kushtia	0.28	32.5	—	66.8	29.6	8.2	—	—	58.6	Kabir et al. (2022)
Beijing	—	36.43	—	145.7	63.57	27.12	—	—	35.49	Liu et al. (2020)
Baoji	0.588	37.95	—	97.5	68.2	36.6	—	—	29.1	Zhang et al. (2020)
Rawalpindi	164	15.72	33.37	543	295.28	236	—	—	336	Tahir and Yasmin. (2019)
Peshawar	0.11	0.4	—	40.94	1.65	10.54	—	44.3	20.84	Saddique et al. (2018)
Swat	3	—	—	48	863		9.9	400.5	63	Saddique et al. (2018)
Faisalabad	—	21.44	—	48.57	—	21.44	—	—	24.08	Parveen et al. (2015)
Multan	0.23	0.61	0.05	—	—	0.083	0.17	34.2	0.191	Randhawa et al. (2014)
Bahawalpur	0.31	13	—	—	8	8.1	—	—	—	Rasheed et al. (2014)
Quetta	0.29	1.38	—	19.45	0.03	0.74	3.11	—	0.86	Kakar et al. (2005)
Gujranwala	1.8	89	-	18	159.4	104.7	6.5	44	169.5	Bostan et al. (2007)
Kasur	26.3	18.21	8.9	14.3	244.3		9.42	—	—	Afzal et al. (2013)
Hyderabad	1.2	30	13.73	—	49.9	55.1	90	70.5	37.7	Bux et al. (2021)
Sukkur	0.04	1.1	15.5	13.83	—	6.43	2	2.7	5.26	Khan et al. (2011)
Sahiwal	0.003	1.48	—	0.49	9	0.09	32	—	—	Ur Rehman et al. (2019)
Vehari	1.6	1.1	17.6	102.2	59.9	1.9	9	87.6	40.3	Sarwar et al. (2020)

the exposure levels of individuals to selected heavy metals present in the soil. By determining the potential adverse effects of these substances and comparing them to established safety benchmarks. This study exclusively concentrated on assessing the non-carcinogenic risks (CDI, HQ, and HI) through the ingestion pathway, as presented in Tables 4, 5. Figure 4A visually depicts the diverse risk levels associated with lead contamination in the topsoil of major cities in Pakistan. The figure presents the outcomes of the risk assessment for Lead across different cities in the country, taking into account their respective concentrations and associated impacts. It is evident that Gujranwala stands out with the highest risk levels recorded at 0.090 for adults and 0.125 for children, surpassing all other cities. Conversely, Peshawar demonstrates the lowest level of risk exposure, registering mere values of 0.00 for adults and 0.001 for children. Figure 4B depicts the varying levels of risk associated with Cobalt (Co) in the urban topsoil of main cities in Pakistan. The findings presented underscore that Rawalpindi faces the most significant correlated risk, impacting both the environment and human health. Findings reveal the values of 0.154 for adults and 0.215 for children in this regard. Following closely to Karachi, with respective values of 0.090 for adults and 0.125 for children. Both cities are densely populated and host a multitude of ongoing economic activities. Contrastingly, certain cities such as Multan, Faisalabad, and Gujranwala pose no discernible Co risk, as the element is absent from their soil composition. Depicted in Figure 4C were the levels of risk associated with Zinc (Zn) in the urban topsoil of major cities in Pakistan. As revealed by the findings, the most heightened risk linked to Zn was observed in Rawalpindi. The recorded high values for Rawalpindi were 0.019 for adults and 0.026 for children. In contrast, the lowest measurements were recorded at 0.000 for adults and 0.001 for children. It is noteworthy that data for Multan and Bahawalpur were not available for assessment at that time. This area, characterized by a dense population and encompassing agricultural land and green spaces, was susceptible to elevated Zn levels. Factors such as economic activities and inadequate sewage systems contributed to the accumulation of Zinc in the topsoil. Following Figure 4D shows the level of risk from Manganese in the urban topsoil of Pakistani main cities. The results show the risk exposure from Mn in different cities of Pakistan. There is an overall less or no concentrations found in different cities so that's why the level of risk is certainly on the lower side. Only Hyderabad has a high value 0.0001 in adults and children and Sukkur have lower value 0.00002. Illustrated in Figure 4E are the levels of risk associated with Iron (Fe) in the urban topsoil of major cities in Pakistan. Based on the presented graph, it becomes evident that Karachi exhibits the most significant risk level for Fe. Notably, Karachi recorded the highest values, with a measure of 0.0018 for adults and 0.0025 for children. On the other end of the spectrum, the lowest risk levels were observed in Islamabad, both for adults and children, with a value of 0.0. This outcome comes as no surprise, considering Karachi's status as a prominent industrial and commercial hub within Pakistan, accommodating a substantial population. The levels of risk associated with Copper (Cu) in the urban topsoil of major cities in Pakistan was shown by the results, Rawalpindi exhibited a high value of 0.02 for adults and 0.03 for children. Conversely, cities such as Lahore (0.0007 and 0.0010), Karachi (0.0004 and 0.0005), Islamabad (0.00 and 0.00), and Sukkur (0.0004 and 0.0005) recorded lower values for both adults and children as depicted in Figure 4F. The outcomes underscore that Rawalpindi, as a densely populated urban center, presented the highest level of Cu-related risk. Notably, cities like Multan and Faisalabad, functioning as significant industrial and agricultural hubs, registered no discernible risk from Cu. These findings reflect the historical state of risk from copper in the topsoil of these cities. This assessment employs a multidisciplinary approach that combines elements of toxicology, epidemiology, and exposure science to offer a holistic comprehension of the potential health implications associated with non-carcinogenic agents.

3.3.2 Carcinogenic risk assessment

Evaluating the carcinogenic risks to human health is a crucial undertaking focused on comprehending and addressing the possible dangers presented by heavy metals capable of inducing cancer. This study involves a meticulous evaluation of heavy metals (Cd, Cr and Ni) exposure pathways to determine the likelihood and magnitude of cancer development due to exposure of these heavy metals. Figure 5A illustrates the varying degrees of risk posed by Cadmium in the urban topsoil of major cities in Pakistan. The data in Table 3 provides insights into the levels of Cadmium-related risk in different Pakistani cities. The results unmistakably indicate that, among all the cities, Rawalpindi exhibits the highest risk levels, with a Cadmium value of 0.078 in adults and 0.109 in children. This phenomenon can be attributed primarily to rapid urbanization and the establishment of numerous projects and factories within the city limits. The concentration of traffic congestion and high-density housing projects within a limited radius has significantly impacted the city's topsoil quality, consequently affecting the health of its residents, both adults and children. Notably, Swat exhibited the highest recorded value, with 9×10^{-7} for adults and 6×10^{-6} for children. Conversely, Lahore displayed the lowest values, measuring 7×10^{-9} for adults and 9×10^{-9} for children as shown in Figure 5B. The provided figure presents the outcomes concerning the exposure risk linked to the carcinogenic agent Cr across different cities within Pakistan. Despite the minimal level of risk and concentration of Cr in urban soil, the inherent carcinogenic nature of this agent underscores its potentially lethal effects. Of particular note, the highest level of risk was identified in Swat. These findings reflect the historical state of risk from Chromium in the topsoil of these cities. Depicted in Figure 5C are the levels of risk attributed to Nickel (Ni) in the urban topsoil of major cities in Pakistan. The results illustrate that Nickel is present in minute quantities in the considered cities. Specifically, Rawalpindi registered the highest value, measuring $1 \times 10^{-07}$for adults and $2 \times 10^{-07}$for children. In contrast, Vehari displayed lower values of 1×10^{-09} for adults and children. It is worth noting that despite the minimal presence of Nickel, the fact that it is a carcinogenic agent underscores the potential danger associated with continuous and prolonged exposure, both to the environment and humans. These findings reflect the historical state of risk from Nickel in the topsoil of these cities.

4 Discussion

Rawalpindi city exhibited the highest cadmium concentration among selected cities in Pakistan, reaching 164 mg/kg, while the average concentration across cities was 13.35 mg/kg, surpassing both the WHO Standard (0.8 mg/kg) and PAK-EPA standards (3 mg/kg). Despite the WHO soil quality standard aiming to protect humans, plants, and animals by considering multiple exposure pathways, about 50% of the studied soils in Pakistani cities exceeded the WHO target value for cadmium, raising concerns for potential risks to ecosystems. Various sources contribute to increased cadmium levels in soils, including industrial activities, metal processing, atmospheric emissions, and the prevalence of cadmium-plated items. Natural sources, like volcanic activity and rock weathering, along with human activities such as mining, introduce cadmium into the environment. This persistent element can be transported by air and water as nanoparticles. Elevated cadmium concentrations in the atmosphere can compromise lung health. Prolonged exposure, especially through sources like air, food, water, and cigarette smoke, can lead to cadmium accumulation in the kidneys, resulting in renal and bone diseases, gastrointestinal irritation, and respiratory problems. The carcinogenic properties of cadmium further amplify health concerns, contributing to bone demineralization, cardiovascular effects, osteoporosis, and lung damage (Tahir and Yasmin, 2019).

Gujranwala, with a soil lead concentration reaching 89.0 mg/kg, exhibited the highest levels among the cities studied. The heightened Pb concentration in Gujranwala is attributed to factors such as industrial operations, traffic emissions, improper waste disposal, agricultural practices, and potentially natural geological processes that release lead into the environment. Despite this, the mean Pb concentration of 16.08 mg/kg in the study falls below the WHO and PAK-EPA soil quality standard of 85 mg/kg, indicating a relatively lower level of lead contamination. Comparisons with studies in Bangladesh and other countries show variability in lead concentrations. If soils in Pakistani cities surpass the WHO's target value for lead, there are concerns about potential health risks, including anemia, paralysis, renal problems, and brain damage. Severe consequences, especially during pregnancy, involve neurological damage, developmental disorders, cognitive impairments, reduced IQ, behavioral problems, and kidney damage associated with high lead exposure (Bostan et al., 2009).

Rawalpindi, among the studied cities, exhibits the highest cobalt concentration at 33.37 mg/kg, while the overall average across selected cities in Pakistan is 12.36 mg/kg. Despite the essential role of cobalt, particularly as a component of the vitamin B12 complex, cobalt mining poses environmental concerns, contributing to pollution and impacting eutrophication and global warming through activities like blasting and electricity consumption. This mining process generates significant amounts of carbon dioxide and nitrogen dioxide, highlighting the need for addressing these environmental effects. While the average cobalt concentration remains well below the WHO and PAK-EPA standards of 50 mg/kg, it is crucial to monitor and manage cobalt levels. Excessive oral intake of cobalt can result in adverse effects on humans, terrestrial and aquatic ecosystems, plants, and animals. Toxic effects include increased red blood cell counts (polycythemia), cardiomyopathy, and adverse impacts on the male reproductive system. Exposure to cobalt is also associated with discomfort in the skin, eyes, nose, and throat, along with respiratory problems and potential effects on the heart and thyroid. Future exposure may lead to symptoms such as chest tightness, wheezing, coughing, and shortness of breath, with various organs like the thyroid, liver, kidneys, and heart being susceptible to cobalt-related harm.

Rawalpindi, among the selected Pakistani cities, exhibits the highest zinc concentration at 543 mg/kg, with an average concentration of 73.79 mg/kg across cities. This average exceeds the globally recognized thresholds set by the WHO and PAK-EPA, advocating for a maximum of 50 mg/kg. Previous research by Milam et al. (2017) indicated a broader range of zinc concentrations in soil samples, surpassing the values found in this investigation. Fosu-Mensah et al. (2017) observed similarities in iron (Fe) levels, while Awokunmi et al. (2010) documented considerably higher zinc levels in soil samples, diverging significantly from the present study's results. Soil containing zinc concentrations between 70 and 400 mg/kg is considered highly toxic for plant growth. Despite zinc's recognized benefits for health, such as mitigating inflammation and supporting immunological wellbeing, excessive exposure can lead to gastrointestinal disturbances. It is crucial to note that fatal doses of zinc range from 10 to 30 g, emphasizing the need for moderation. Topical zinc application is generally safe, but on wounded skin, it may provoke sensations of burning, stinging, itching, and tingling. The substantial presence of zinc in soils, not necessarily in toxic waste sites, has the potential to contaminate groundwater. Additionally, industries releasing dust with elevated zinc concentrations into the atmosphere can contribute to soil and waterway contamination.

Hyderabad, among the selected cities in Pakistan, records the highest manganese concentration at 90 mg/kg, while the average across cities is 15.2 mg/kg. Despite the essential role of manganese in various metabolic processes, including the metabolism of amino acids, cholesterol, glucose, and carbohydrates, the average concentration remains comfortably below the globally recognized standards of 100 mg/kg set by the WHO and PAK-EPA. The WHO's comprehensive soil quality guideline, considering various exposure pathways, ensures that manganese levels in the selected Pakistani cities are within acceptable limits, safeguarding human health. Manganese also contributes to vital functions such as bone development, blood coagulation, and inflammation regulation. However, it is important to note that excessive exposure to high levels of manganese has been linked to neurological symptoms resembling those seen in Parkinson's disease. Additionally, plants exhibit distinct responses to manganese in both toxic and insufficient conditions, with insufficiency more frequent in soils with lower pH levels.

Karachi records the highest iron concentration among selected cities in Pakistan, reaching 908 mg/kg, while the average concentration across cities is 177.70 mg/kg. Despite the essential role of iron in the body's growth and development processes, the average falls well below the WHO and PAK-EPA standard of 50,000 mg/kg. The WHO's comprehensive soil quality standard, considering diverse exposure pathways, ensures that iron levels in the selected Pakistani cities remain uncontaminated. The elevated iron concentration in Karachi is attributed to inadequate sanitary and sewage systems, along with limited access to clean drinking water, exacerbated by the city's high level of urbanization. Natural sources of iron in soils, as highlighted by studies like Eddy et al. (2006), contribute substantially to environmental iron concentrations, not solely waste materials. Iron is crucial for proteins like myoglobin and hemoglobin, facilitating oxygen distribution in the body. Excessive iron exposure, while necessary to some extent, can lead to organ damage. The importance of removing excess iron is emphasized in curbing contaminant proliferation in the environment, especially since various pollutants, including uranium, can bond with iron and influence their distribution.

Rawalpindi's soil exhibits an elevated copper concentration, ranging from 0.06 to 336.0 mg/kg, possibly attributed to unregulated industrial emissions and waste incineration activities observed by Kormoker et al. (2021). The collective mean copper concentration across all studied cities is 54.88 mg/kg, surpassing WHO and PAK-EPA standards of 36 mg/kg, indicating considerable copper contamination in major city soils. Hasnine et al. (2017) reported an average copper concentration of 91.06 ± 152.70 mg/kg in surface agricultural soil at DEPZA. Elevated copper concentrations can have detrimental effects on plants, especially when enriched liquid dairy waste is used for irrigation in agricultural lands. This study underscores the potential threat posed by copper to plants, emphasizing its toxicity to specific microorganisms, as highlighted by Hasnine et al. (2017). Copper, while essential for the human body in aiding red blood cell production and contributing to overall health, can lead to gastrointestinal discomfort and copper buildup in the liver and brain in instances of Wilson's disease. The negative impact of copper extends to soil microorganisms and insects, causing disruptions in organic material decomposition, and may pose risks to livestock ingesting hazardous copper levels in agricultural fields tainted with copper.

Swat city in Pakistan registers the highest average chromium concentration at 863.8 mg/kg in this study, potentially attributed to industrial activities like metal processing and leather tanning. The sources of elevated chromium levels in Swat city include tanneries releasing chromium-laden waste into water bodies and soil, along with mining activities such as chromite extraction (Saddique et al., 2018). The average chromium concentration across all selected cities is 131.23 mg/kg, surpassing both the WHO Soil Quality Standard and PAK EPA threshold of 100 mg/kg. This exceeds levels reported in other investigations conducted across different countries. Chromium, released into agricultural areas adjacent to industrial zones, leads to soil contamination, impacting plant growth and essential metabolic processes (Hasnine et al., 2017). Exposure to chromium through crops has been linked to an increased incidence of skin allergies and respiratory issues (Shakir et al., 2017). Studies highlight chromium's toxic threat, affecting seed quality, yield, and the quality of vegetables and wheat. Therefore, a comprehensive monitoring approach is deemed necessary for water, soil, and agricultural production systems (Sharma et al., 2020).

Rawalpindi, in this study, exhibits the highest nickel concentration at 236.0 mg/kg, attributed to various factors, including industrial operations like metal processing and electroplating, traffic-related pollution, inappropriate waste disposal, and natural geological conditions. The wide range of nickel concentrations throughout the research, from 0.08 mg/kg in Multan to the peak in Rawalpindi, underscores the impact of these variables. The average nickel concentration across the investigated cities is 35.47 mg/kg, slightly exceeding both WHO and PAK-EPA standards recommending a maximum nickel concentration of 35 mg/kg in soil. This surpasses levels reported in similar research conducted in Bangladesh and several other countries. Human exposure to nickel, associated with health issues such as dermatitis, lung fibrosis, cardiovascular and kidney diseases, and respiratory tract cancer, emphasizes the health risks posed by elevated nickel levels. Nickel, entering the body through various pathways, including skin contact, ingestion, and inhalation, can result in harmful health effects with prolonged or severe exposure, as noted by Genchi et al. (2020) and sensitization of the skin, triggering respiratory ailments (Shakir et al., 2017). Additionally, nickel's environmental impact extends to greenhouse gas emissions, biodiversity loss, and pollution of air, water, and soil, given its prevalence in low-grade ores, necessitating resource-intensive extraction and refining processes (Shahzad et al., 2018). A comparative analysis of among selected heavy metal concentrations in soil, as presented in Table 6.

5 Conclusion

The research undertook a thorough examination of enrichment factors, geo-accumulation indices, and human health risk assessments concerning the presence of heavy metals in urban soils across diverse cities in Pakistan. The geo accumulation index outcomes revealed a spectrum of risk levels, spanning from "no pollution" for Pb, Co, Mn, and Fe to "moderate to extremely contaminated" for Cd, Zn, Cr, Ni, and Cu. Employing EF analysis, we found that the heavy metal presence in the study area was considerable concern, with risk levels varying from "moderate to extremely strong pollution" for Cd, Zn, Cr, Ni, and Cu, to "no pollution" for Pb, Co, Mn, and Fe. When evaluating health risks, both non-carcinogenic and carcinogenic risks were notably present for both children and adults. Various contributors, including industries, vehicular emissions, urbanization, and agricultural activities, were identified as substantial factors contributing to the heightened levels of heavy metals in the analyzed urban soil environments. It became evident that urban industrial zones within these metropolises are intricately linked to both human health and long-term economic viability. The findings from this study can be immensely valuable for decision-makers seeking to formulate more effective strategies to reduce exposure and efficiently manage soil pollution.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Author contributions

HA: Writing–original draft. RN: Writing–original draft. IN: Writing–review and editing. MI: Writing–review and editing. AI: Writing–original draft. IK: Writing–original draft. MO: Writing–review and editing. GW: Writing–review and editing. ZA: Writing–review and editing. MB: Writing–review and editing.

Funding

The authors declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The authors extend their appreciation to the Researchers Supporting Project number (RSP2023R374) King Saud University, Riyadh, Saud Arabia.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2023.1260317/full#supplementary-material

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frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 16 February 2024
DOI 10.3389/fpubh.2024.1280163

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EDITED BY
Mohammad Imran Ansari,
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United States

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China
Brigitte Le Magueresse-Battistoni,
INSERM U1060 Laboratoire de Recherche en
Cardiovasculaire, Métabolisme, diabétologie
et Nutrition, France

*CORRESPONDENCE
Jiandong Su
✉ stumsjd@163.com

†These authors have contributed equally to
this work

RECEIVED 19 August 2023
ACCEPTED 07 February 2024
PUBLISHED 16 February 2024

CITATION
Tang S, Luo S, Wu Z and Su J (2024) Association between blood heavy metal exposure levels and risk of metabolic dysfunction associated fatty liver disease in adults: 2015–2020 NHANES large cross-sectional study. *Front. Public Health* 12:1280163. doi: 10.3389/fpubh.2024.1280163

Association between blood heavy metal exposure levels and risk of metabolic dysfunction associated fatty liver disease in adults: 2015–2020 NHANES large cross-sectional study

Song Tang[1†], Simin Luo[2†], Zhendong Wu[3†] and Jiandong Su[3]*

[1]Department of Gastrointestinal Surgery, The First Affiliated Hospital of Guangdong Pharmaceutical University, Guangzhou, China, [2]Breast Tumor Center, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, China, [3]Department of Gastroenterology, Dongguan Songshan Lake Tungwah Hospital, Dongguan, China

Background: The relationships between heavy metals and fatty liver, especially the threshold values, have not been fully elucidated. The objective of this research was to further investigate the correlation between blood heavy metal exposures and the risk of Metabolic dysfunction Associated Fatty Liver Disease (MAFLD) in adults.

Methods: Laboratory data on blood metal exposure levels were obtained from National Health and Nutrition Examination Survey (NHANES) data for the period 2015 to 2020 for a cross-sectional study in adults. Associations between blood levels of common heavy metals and the risk of MAFLD in adults were analyzed using multifactorial logistic regression and ranked for heavy metal importance using a random forest model. Finally, thresholds for important heavy metals were calculated using piecewise linear regression model.

Results: In a multifactorial logistic regression model, we found that elevated levels of selenium (Se) and manganese (Mn) blood exposure were strongly associated with the risk of MAFLD in adults. The random forest model importance ranking also found that Se and Mn blood exposure levels were in the top two positions of importance for the risk of disease in adults. The restricted cubic spline suggested a non-linear relationship between Se and Mn blood exposure and adult risk of disease. The OR (95% CI) for MAFLD prevalence was 3.936 (2.631–5.887) for every 1 unit increase in Log Mn until serum Mn levels rose to the turning point (Log Mn = 1.10, Mn = 12.61 μg/L). This correlation was not significant ($p > 0.05$) after serum Mn levels rose to the turning point. A similar phenomenon was observed for serum Se levels, with a turning point of (Log Se = 2.30, Se = 199.55 μg/L).

Conclusion: Blood heavy metals, especially Se and Mn, are significantly associated with MAFLD in adults. They have a non-linear relationship with a clear threshold.

KEYWORDS

MAFLD, heavy metal, NHANES, manganese, selenium

Introduction

MAFLD, is the most common cause of chronic liver disease worldwide (1). Originally referred to as non-alcoholic fatty liver disease (NAFLD), but proposed to be redefined by an international expert group in 2020 (2). MAFLD is a condition of liver fat accumulation with metabolic dysfunction in the form of overweight or obesity and insulin resistance (3). MAFLD is closely related to diabetes, chronic kidney disease, cardiovascular disease (4) and is also prevalent in patients with hepatocellular carcinoma (5, 6), posing a significant potential economic burden to society.

The relationship between heavy metals and liver diseases has been confirmed in previous studies. One study showed that liver function was significantly correlated with blood heavy metals, and different heavy metals had different effects on various indicators of liver function (7). Study has also shown a positive association between blood Se and NAFLD (8). In addition, blood Mn and Se are associated with hepatic steatosis and fibrosis (9). However, previous studies have mostly focused on the effects of heavy metals on the liver, lacking of description of the thresholds for the relationship between heavy metal exposure levels and the risk of developing MAFLD in adults. Given the importance of thresholds in describing relationship, the relationship between heavy metal exposures and the risk of MAFLD in adults must be further explored.

This study retrieved the data of the NHANES from 2015 to 2020, used multi-factor logistics regression to analyze the association between blood levels of common heavy metals and the risk of MAFLD in adults, and ranked the importance of heavy metals by random forest model. Furthermore, the piecewise linear regression model was used to calculate the threshold values of the two most significantly correlated heavy metals, which is important for understanding the relationship between exposure to heavy metals and MAFLD in adults.

Methods

Study population

The NHANES is a cross-sectional survey administered by the National Center for Health Statistics (NCHS) and the Centers for Disease Control and Prevention with data from the civilian population of the United States and is nationally representative. The data collection protocol was approved by the NCHS Ethics Review Committee and all survey participants provided informed consent prior to being interviewed and examined. Because laboratory data on blood metal exposure levels were complete for the period 2015 to 2020 compared to other years, the NHANES public data file for that cycle was used to construct the dataset for this study, and the study population consisted of all NHANES respondents.

Exposure

The exposure variable in this study was blood heavy metal levels. Blood levels of 10 different metals [plumbum (Pb), hydrargyrum (Hg), cadmium (Cd), Mn, Se, chromium (Cr), cobalt (Co), inorganic hydrargyrum (InHg), methyl hydrargyrum (MeHg) and ethyl hydrargyrum (EtHg)] were obtained by direct extraction of participant laboratory data. The test method is described in detail in the NHANES database[1] and focuses on the direct measurement of heavy metals in whole blood samples using mass spectrometry after a simple dilution sample preparation procedure.

Outcome

The primary outcome of this study was the presence or absence of MAFLD, which was defined as the combination of steatosis, irrespective of the gradation, and metabolic dysfunction. This was characterized by either overweight (Body mass index (BMI) $\geq$25 kg/m^2), type 2 diabetes mellitus defined as antidiabetic drug use, fasting plasma glucose $\geq$7.0 mmol/L, HbA1c > 6.4% or based on oral glucose tolerance test (OGTT), or a combination of at least two of the following metabolic abnormalities: (1) waist circumference > 102 cm for male and > 88 cm for female; (2) blood pressure $\geq$ 130/85 mmHg or antihypertensive drug use; (3) plasma triglycerides $\geq$1.70 mmol/L or lipid-lowering drug treatment; (4) high-density lipoprotein cholesterol (HDL-C) <1.0 mmol/L for men and < 1.3 mmol/L for women or lipid-lowering drug treatment; (5) prediabetes defined as fasting plasma glucose 5.6–6.9 mmol/L, HbA1c 5.7–6.4% or matching OGTT; (6) homeostatic model assessment of insulin resistance (HOMA-IR) of $\geq$2.5; (7) or C-reactive protein (CRP) level > 2 mg/L (10). Those who met the diagnostic criteria were identified as MAFLD patients, those who did not were identified as controls, and those with missing diagnosis-related data were removed.

Baseline data on the study population

Demographic characteristics, such as age, gender and ethnicity were included. Social factors, including education level, marital status, ratio of household income to poverty level and health insurance coverage were included. Data on everyday health-related behaviors were collected for smoking and alcohol consumption. Variables related to medical comorbidities, such as liver and kidney function, BMI, diabetes, hypertension and cancer, are collected.

Data analysis

The quantitative data met a normal distribution by selecting a t-test or analysis of variance (ANOVA), or a rank sum test if they did not meet a normal distribution. Data on categorical variables were analyzed for differences in baseline characteristics and blood heavy metal levels between outcome groups using the χ^2 test. We used Spearman correlation matrices to identify correlations for the 10 heavy metals. Subsequently, multifactorial logistic regression models were used to identify exposure factors associated with the screening outcome variables for the preliminary analysis. A random forest (RF) model was used to determine the importance of the variables. Feature importance was assessed based on the out-of-bag (OOB) error rate, reflecting the level of contribution of each variable when classifying the metabolic dysfunction-related fatty liver with the control population.

To explore the non-linear relationship between blood heavy metal concentrations and the risk of MAFLD, as well as the threshold effect,

1 https://www.cdc.gov/nchs/nhanes/index.htm

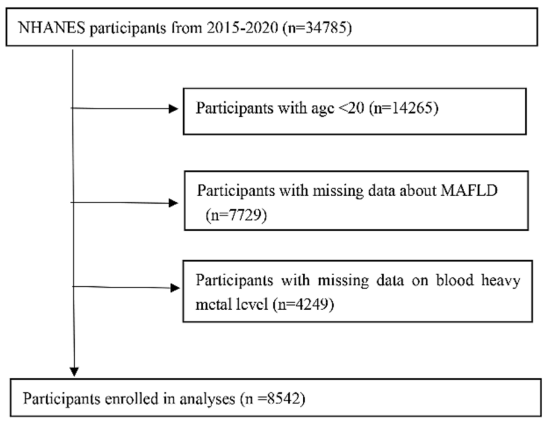

FIGURE 1
Flow diagram of the screening and selection process.

serum heavy metal concentrations were transformed on a Log10 scale. We used the Restrictive Cubic Spline (RCS) function to analyze the odds ratio (OR) relationship between blood heavy metal concentrations and the risk of MAFLD, as serum heavy metal concentrations showed a skewed distribution. Additionally, a piecewise linear regression model was applied to examine the threshold effect of serum heavy metal concentrations on the risk of MAFLD using a smoothing function. Threshold levels (i.e., turning points) were determined by iterative trials involving the selection of turning points along predefined intervals, followed by the selection of turning points that gave the maximum model likelihood.

All data analyzes were conducted using R.3.5.2/R4.2.2.[2] Sample sizes were based on available data and no ex ante sample size calculations were performed. Significance was tested for all descriptive analyzes by two-sided tests at the $p < 0.05$ level of significance.

Results

Baseline characteristics of the study population

Survey data were collected from 34,785 participants. After excluding those lacking MAFLD, heavy metal blood level data (with the exceptions mentioned above), 8,542 adult participants (age ≥ 20 years) were included in the analysis (Figure 1). The distribution of baseline characteristics stratified by outcome is shown in Table 1. In the preliminary analysis, the MAFLD group participants, 53.14% male, were significantly higher than the Non-MAFLD group, in addition the age distribution of participants in the MAFLD group was not significantly different from the Non-MAFLD group. In terms of social factors, the proportion of Married or living with partner was higher in the MAFLD group than in the Non-MAFLD group, and there was no significant difference in the education level of the two groups. For comorbidities, BMI was significantly higher in the MAFLD group than in the Non-MAFLD group (33.05 ± 6.84 vs. 26.97 ± 5.56, $p < 0.001$). estimated glomerular filtration rate (eGFR), alanine transaminase(ALT) and aspartate transaminase (AST) were in the normal range in both groups, and HbA1c was in the normal range in the Non-MAFLD group, while HbA1c was above the normal range in the MAFLD group. The MAFLD group had a higher proportion of combined hypertension and diabetes than Non-MAFLD, and the difference was statistically significant ($p < 0.05$).

Blood levels of heavy metal exposure in the study population

In the preliminary analysis, the 10 heavy metal blood exposure levels were skewed data. Comparing the participants in the Non-MAFLD group, there were differences in the distribution of heavy metal blood levels in Pb, Hg, Mn, Se, MeHg, InHg, Cd and Co among the participants in the MAFLD group, with Mn and Se levels being high and the differences being statistically significant ($p < 0.05$) (Table 2).

Influence of blood heavy metal exposure levels on the risk of MAFLD

Firstly, Spearman's correlation matrix analysis (Figure 2) was performed for the correlation of 10 environmental heavy metals. There was a significant correlation between MeHg and Hg (correlation value of 0.951, $p<0.001$). Therefore, MeHg should be eliminated in the next Poisson review. Exploratory factor analysis confirmed highly correlated covariates, with the other nine metals having limited correlation with each other.

Analysis using a multifactorial logistic regression model found that high blood Mn and Se exposure levels were associated with an increased risk of disease in patients in the MAFLD group compared to the Non-MAFLD group [OR (95% CI): Mn 1.028 (1.015–1.04); Se 1.006 (1.004–1.008)] (Figure 3A). The random forest model determined the importance of the variables, with the top 2 rankings of characteristic importance Se and Mn, which matched the multi-factor logistic regression model (Figure 3B).

Threshold effect of blood Mn and Se exposure levels on the risk of MAFLD prevalence

The RCS plot (Figure 4) shows a non-linear relationship between serum Mn and Se exposure levels and the risk of MAFLD. The OR (95% CI) for MAFLD prevalence (Table 3) was 3.936 (2.631–5.887) for every 1 unit increase in Log Mn until serum Mn levels rose to the turning point (Log Mn = 1.10, Mn = 12.61 μg/L). This correlation was not significant ($p > 0.05$) after serum Mn levels rose to the turning point. A similar phenomenon was observed for serum Se levels, with a turning point of (Log Se = 2.30, Se = 199.55 μg/L).

Discussion

MAFLD, the most common cause of chronic liver disease (1), poses a huge potential economic burden on society. The etiology of

2 http://www.R-project.org

TABLE 1 Characteristics of participants enrolled in study.

Characteristic	Non-MAFLD (*N* = 4,172)	MAFLD (*N* = 4,370)	*p*-value
Age (y)	60 (50, 69)	60 (51, 68)	0.377
Male sex	1899 (45.52)	2,322 (53.14)	<0.001
Race			<0.001
Hispanic	756 (18.12)	1,103 (25.24)	
Non-Hispanic white	1,441 (34.54)	1,644 (37.62)	
Non-Hispanic black	1,182 (28.33)	936 (21.42)	
Other	793 (19.01)	687 (15.72)	
Education beyond high school	3,322 (79.72)	3,435 (78.82)	0.305
Marital status			0.011
Never married	135 (8.63)	147 (8.65)	
Married or living with partner	931 (59.49)	1,090 (64.16)	
Divorced, separated, or widowed	499 (31.88)	462 (27.19)	
BMI (kg/m2)	26.1 (23.3, 29.7)	31.7 (28.1, 36.4)	<0.001
eGFR, ml/min per 1.73 m2	86.93 (71.87, 100.04)	88.48 (72.52, 100.95)	0.037
ALT (U/L)	16 (12, 22)	20 (15, 28)	<0.001
AST (U/L)	19 (16,23)	20 (16,25)	0.001
HbA1c	5.6 (5.4, 5.9)	5.9 (5.5, 6.6)	<0.001
Alcohol user	2,535 (86.28)	2,725 (87.62)	0.123
Smoker	1855 (44.48)	1995 (45.66)	0.274
Hypertension	1916 (45.93)	2,644 (60.50)	<0.001
Cancer	531 (12.73)	600 (13.74)	0.172
Diabetes	709 (16.99)	1705 (39.02)	<0.001

All values are displayed as *n* (%) or median and IQR. χ^2 analysis is used to test significance between groups for categorical variables.
BMI, body mass index; eGFR, estimated glomerular filtration rate; HbA1c, glycosylated Hemoglobin, type A1C; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

TABLE 2 Level of blood heavy metals in Non-MAFLD and MAFLD subjects.

Characteristic	Non-MAFLD (*N* = 4,172)	MAFLD (*N* = 4,370)	*p*-value
Co (μg/L)	0.15 [0.12, 0.19]	0.15 [0.12, 0.18]	<0.001
Cr (μg/L)	0.29 [0.29, 0.29]	0.29 [0.29, 0.29]	0.015
EtHg (μg/L)	0.05 [0.05, 0.05]	0.05 [0.05, 0.05]	0.835
MeHg (μg/L)	0.59 [0.18, 1.42]	0.51 [0.18, 1.25]	<0.001
InHg (μg/L)	0.15 [0.15, 0.23]	0.15 [0.15, 0.22]	0.008
Se (μg/L)	183.52 [169.09, 201.47]	188.11 [172.50, 204.48]	<0.001
Mn (μg/L)	8.92 [7.00, 11.21]	9.18 [7.500, 11.38]	<0.001
Cd (μg/L)	0.38 [0.23, 0.66]	0.30 [0.190, 0.50]	<0.001
Hg (μg/L)	0.79 [0.30, 1.70]	0.69 [0.360, 1.50]	<0.001
Pb (μg/L)	1.17 [0.77, 1.77]	0.99 [0.67, 1.51]	<0.001

All values are displayed as Median (Quartile1-Quartile 3).

MAFLD has not been fully elucidated, with oxidative stress and metabolic imbalance of fatty acids playing a crucial role (11).

Due to the continuous development of human industry, mineral resources have been extensively exploited, leading to severe heavy metal pollution of water and soil, which in turn poses a threat to human health. With the increase in heavy metal pollution, the relationship between heavy metal exposure and metabolic diseases in adults has attracted widespread attention. Studies have shown that the liver is one of the major accumulation organs of heavy metals, especially plumbum, cadmium, nickel and chromium, which accumulate in large quantities in the liver (12). The toxic effects of heavy metals occur mainly through oxidative stress induced by the production of reactive oxygen species (ROS) in cells, which may cause liver damage and lead to the development of fatty liver (13, 14). Previous studies have focused on the effects of heavy metals on the liver, and there is a lack of description of thresholds for the relationship between heavy metal exposures and the risk of developing MAFLD in adults, so more research is needed.

We obtained blood level data for 10 different metals at NHANES from 2015 to 2020 and obtained data on demographic characteristics, social factors, daily health-related behaviors and variables related to medical comorbidities. First, we compared the blood levels of heavy metals between participants in the Non-MAFLD and MAFLD groups and found differences in the distribution of heavy metal blood levels in the MAFLD group for Pb, Hg, Mn, Se, MeHg, InHg, Cd, and Co, with statistically significant differences ($p < 0.05$) for Mn and Se levels. We then analyzed the effect of heavy metal exposure levels on the risk of MAFLD prevalence using a multifactorial logistic regression model and found that high blood Mn and Se exposure levels were associated with an increased risk of prevalence in patients in the MAFLD group.

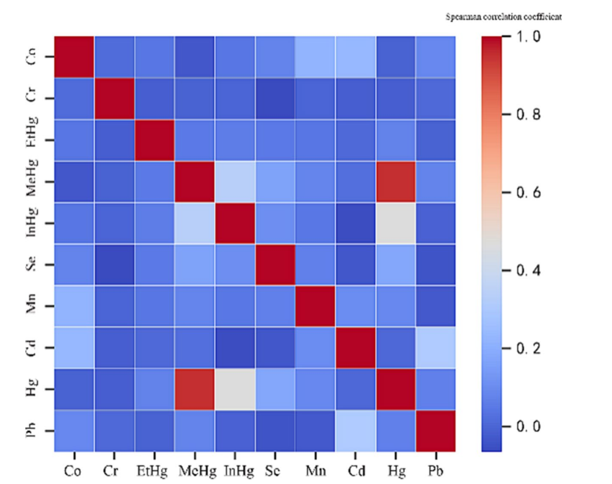

FIGURE 2
Heat maps of correlations between 10 environmental heavy metals.

The importance of the variables in the random forest model also indicated that Se and Mn ranked in the top 2, which was consistent with the multi-factor logistic regression model. We then used the RCS model to find a non-linear relationship between serum Mn and Se exposure levels and the risk of MAFLD. Finally, based on this non-linear relationship, we used a piecewise linear regression model to derive a turning point of Log Mn = 1.10 for serum Mn = 12.61 μg/L and Log Se = 2.30 for serum Se = 199.55 μg/L.

Both Mn and Se are controversial and contradictory in influencing the risk of MAFLD. Mn is an essential element for humans and Mn-containing superoxide dismutase (MnSOD) is highly expressed in differentiated organs containing large numbers of mitochondria, such as the heart, liver and kidney, and is the main antioxidant enzyme in mitochondria, playing a key role in the detoxification of superoxide radicals and protecting cells from oxidative stress (15). A clinical study found that there may be sex differences in the association between blood Mn and MAFLD, higher blood Mn may be a potential protective factor for MAFLD in males, but there is no significant association in females (16). The sex difference may be related to the fact that Mn can act as an endocrine disrupter. Study have shown that Mn can increase the level of serum testosterone (17). The association between testosterone and MAFLD differs by sex, with higher testosterone levels decreasing the odds of MAFLD in men and increasing the odds of NAFLD in women (18, 19). In addition, some clinical studies have shown that higher levels of Mn exposure are positively associated with MAFLD and hepatic steatosis degeneration and liver fibrosis (8, 9, 20). However, hepatic steatosis, fibrosis, and hepatocellular carcinoma all occurred more frequently in men than in women (21, 22). This suggests that there is a sex difference in liver disease itself. Perhaps, Mn may have magnified this difference, which requires more study. Mn also has an effect on liver function. In a cross-sectional study of Chinese manganese miners, ALT, AST and direct bilirubin (DBIL) were found to be significantly elevated in workers exposed to high levels of Mn (23). Additional studies have also shown an association between Mn and elevated liver enzyme levels and mortality associated

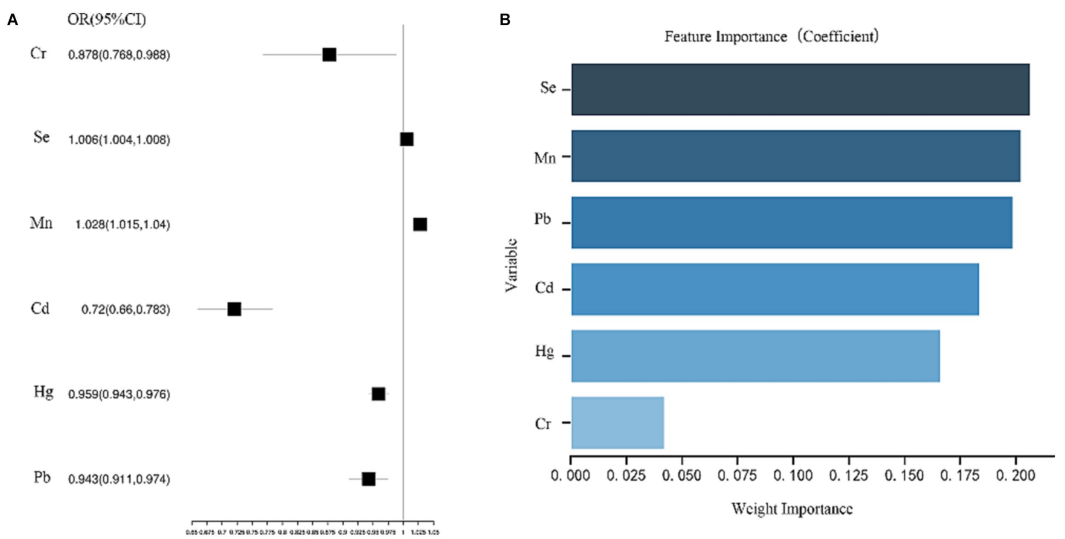

FIGURE 3
(A) Analysis results of multi-factor logistics regression model. **(B)** The order of importance of variables.

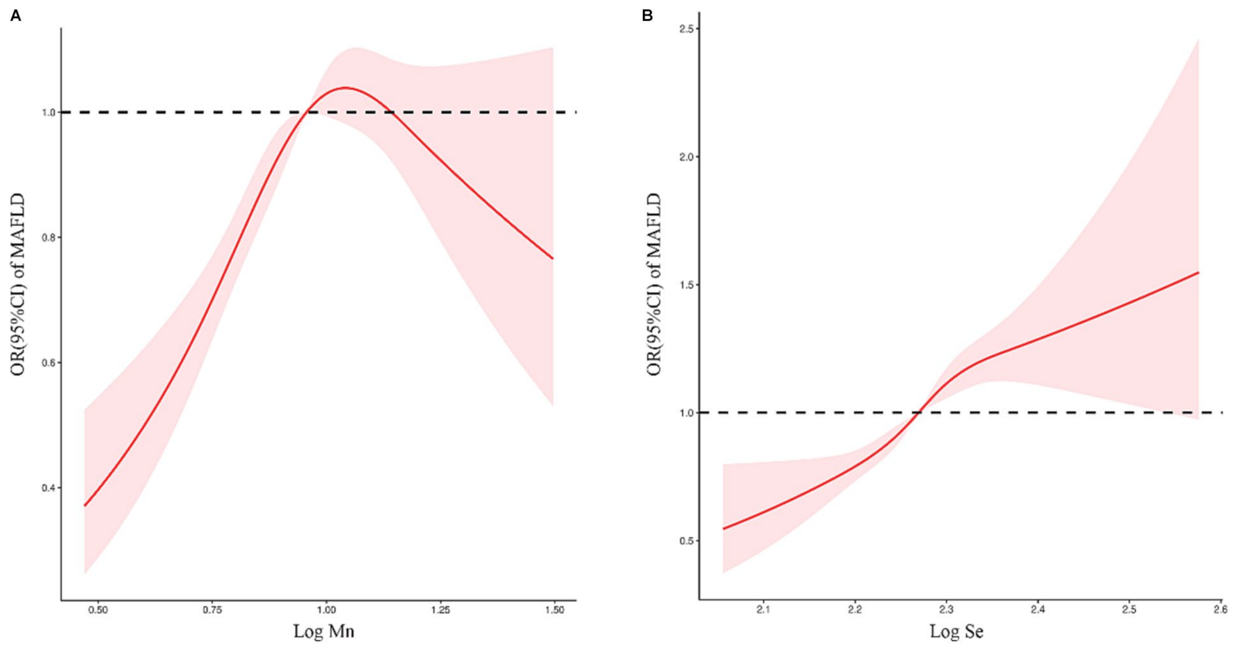

FIGURE 4
(A) Relationship between serum Mn exposure level and risk of MAFLD. **(B)** Relationship between serum Se exposure level and risk of MAFLD.

TABLE 3 Threshold effect analysis of blood heavy metals on MAFLD using piecewise linear regression.

	OR (95% CI)	*p*-value
Se		
Log Se < 2.30	36.438 (10.744–123.576)	<0.001
Log Se ≥ 2.30	5.845 (0.893–38.235)	0.065
Mn		
Log Mn < 1.10	3.936 (2.631–5.887)	<0.001
Log Mn ≥ 1.10	0.458 (0.121–1.726)	0.248

with chronic liver disease (24), with the most significant association between Mn and ALT (7). Animal studies have also shown that Mn exposure can cause liver damage through oxidative stress, mitochondrial damage and thus elevated liver enzymes (25, 26).

We generally believe that the antioxidant effect of Se may attenuate the development of metabolic diseases including MAFLD (27–29). In an animal study, dietary selenium was shown to promote selenoprotein P1 (SEPP1) synthesis and modulate the Kelch-like ECH-associated protein 1 (KEAP1)/NF-E2-related factor 2 (NRF2) pathway to protect against hepatocyte oxidative stress (30). However, several previous clinical studies, including ours, have shown that high levels of selenium are associated with the risk of developing MAFLD, positively correlated with hepatic steatosis (8, 9, 31–33) and positively correlated with changes in liver function, with Se being most significantly associated with changes in ALT (7). We suggest that this may be related to the fact that high dietary selenium intake increases MAFLD risk by modulating dysregulation of insulin biosynthesis and secretion as well as stimulating glucagon secretion, insulin resistance and dyslipidemia (33, 34). It has also been proposed that MAFLD and liver fibrosis are caused by an imbalance in selenium homeostasis rather than by dietary selenium intake (8).

Although our study confirmed the association of Mn and Se with the risk of MAFLD prevalence and also calculated their thresholds precisely, there are still some unavoidable limitations and shortcomings. On the one hand, our study is a retrospective study, based on published data for analysis, and has inherent shortcomings compared to prospective studies. On the other hand, the mechanisms underlying the effects of Mn and Se on MAFLD are not fully understood, and previous studies have had conflicting findings. Therefore, more studies, especially prospective studies and laboratory studies, are needed to further clarify the mechanisms of the effects of Mn and Se on MAFLD.

Conclusion

Our study further clarified the relationship between blood heavy metal exposure levels and MAFLD in adults. Notably, Se and Mn exhibited the most significant influence on MAFLD. Although higher exposure levels of Se and Mn were linked to an elevated risk of MAFLD, this association demonstrated a non-linear pattern with clear thresholds. Furthermore, the exact mechanism behind the impact of heavy metals on MAFLD remains incompletely understood based on current research, necessitating future prospective and laboratory investigations.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

Ethical approval was not required for the studies involving humans because Institutional Review Board approval was not required as the NHANES represents an adequately de-identifed and publicly available dataset. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements because the NHANES represents an adequately de-identifed and publicly available dataset.

Author contributions

ST: Data curation, Formal analysis, Methodology, Project administration, Software, Validation, Visualization, Writing – review & editing. SL: Writing – original draft. ZW: Writing – review & editing. JS: Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by Dongguan Songshan Lake Tungwah Hospital.

Acknowledgments

We thank those people and staff who contributed data to NHANES.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2024.1280163/full#supplementary-material

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frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 05 March 2024
DOI 10.3389/fpubh.2024.1336188

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OPEN ACCESS

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Mohd Kamil Hussain,
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REVIEWED BY
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Harbin Institute of Technology, Shenzhen,
China
Shakir Ahamad,
Aligarh Muslim University, India

*CORRESPONDENCE
Yuhui Dang
✉ dangyh@lzu.edu.cn

RECEIVED 10 November 2023
ACCEPTED 22 February 2024
PUBLISHED 05 March 2024

CITATION
He J, Pu Y, Du Y, Liu H, Wang X, He S, Ai S and Dang Y (2024) An exploratory study on the association of multiple metals in serum with preeclampsia.
Front. Public Health 12:1336188.
doi: 10.3389/fpubh.2024.1336188

An exploratory study on the association of multiple metals in serum with preeclampsia

Jie He[1], Yudong Pu[2], Yue Du[1], Haixia Liu[1], Xiaoxue Wang[1], Shuzhen He[2], Shiwei Ai[1] and Yuhui Dang[1]*

[1]Institute of Maternal, Child and Adolescent Health, School of Public Health, Lanzhou University, Lanzhou, China, [2]Songshan Lake Central Hospital of Dongguan City, Dongguan, China

Background: Individual metal levels are potential risk factors for the development of preeclampsia (PE). However, understanding of relationship between multiple metals and PE remains elusive.

Purpose: The purpose of this study was to explore whether eight metals [zinc (Zn), manganese (Mn), copper (Cu), nickel (Ni), lead (Pb), arsenic (As), cadmium (Cd), and mercury (Hg)] in serum had a certain relationship with PE.

Methods: A study was conducted in Dongguan, China. The concentrations of metals in maternal serum were assessed using inductively coupled plasma mass spectrometry (ICP-MS). Data on various factors were collected through a face-to-face interview and hospital electronic medical records. The unconditional logistic regression model, principal component analysis (PCA) and Bayesian Kernel Machine Regression (BKMR) were applied in our study.

Results: The logistic regression model revealed that the elevated levels of Cu, Pb, and Hg were associated with an increased risk of PE. According to PCA, principal component 1 (PC1) was predominated by Hg, Pb, Mn, Ni, Cu, and As, and PC1 was associated with an increased risk of PE, while PC2 was predominated by Cd and Zn. The results of BKMR indicated a significant positive cumulative effect of serum metals on PE risk, with Ni and Cu exhibiting a significant positive effect. Moreover, BKMR results also revealed the nonlinear effects of Ni and Cd.

Conclusion: The investigation suggests a potential positive cumulative impact of serum metals on the occurrence of PE, with a particular emphasis on Cu as a potential risk factor for the onset and exacerbation of PE. These findings offer valuable insights for guiding future studies on this concern.

KEYWORDS

preeclampsia, metal, copper, logistic regression model, principal component analysis, Bayesian kernel machine regression

1 Introduction

Preeclampsia (PE) is a pregnancy-specific complication with significant morbidity and mortality (1), and it stands out as one of the primary factors associated with maternal and perinatal death (2). Affecting 5–7% of all pregnant women, PE causes over 70,000 maternal deaths and 500,000 fetal deaths worldwide annually (1). Women with PE are usually at higher risk of placental abruption and intrauterine fetal death, as well as at higher risk of liver, kidney, brain, lungs and other organ diseases, which may further develop into eclampsia, cardiovascular and cerebrovascular diseases (3, 4). Furthermore, PE may also associated with adverse neonatal outcomes, including respiratory distress syndrome, retinopathy of prematurity, necrotizing enterocolitis, neurodevelopmental delay, and fetal or neonatal death (5). Characterized by abnormal vascular remodeling in the spiral arteries starting in the first trimester of pregnancy, PE results in placental hypoperfusion and release of various deleterious factors, which may trigger systemic endothelial response (6, 7). Despite this, the etiology of PE remains incompletely defined (8). Currently, there is no effective method to prevent or treat PE, and the primary recourse is abortion or delivery (9). In light of the above, more research is needed to unravel the etiology of PE to provide a foundation for prevention and novel treatment strategies. In view of industrial development, women are increasingly exposed to environmental toxicants, including a variety of metals, recognized as a significant risk factor for adverse pregnancy outcomes, such as spontaneous preterm birth and preeclampsia (10, 11).

In order to mitigate the impact of metal pollution on human health, numerous studies have been conducted to further investigate metals, including the exploration of novel adsorbents for the removal of metal ions from contaminated water (12), and the therapeutic potential of metal dithiocarbamate complexes in certain diseases (13). Expanding new ideas for mitigating metal health hazards and the application of metals in health, however, it is crucial not to overlook the hazards posed by metal exposure. Elucidating the health effects resulting from such exposure remains a pivotal area of research. The term "heavy metal" is a general classification for metals and metalloids with relatively high density and are considered toxic to living organisms and the environment at certain concentrations (14, 15). Lead (Pb), arsenic (As), cadmium (Cd), and mercury (Hg) are some examples of toxic heavy metals. Pb exposures has been shown to affect reproductive, hepatic, endocrine, immune and gastrointestinal systems (16). As is a recognized neurotoxin and is classified as human carcinogen, causing reproductive and developmental problems, as well as damages to the skin, digestive and respiratory systems (17). Cd can accumulate in kidney, liver, bones and other organs, causing damage to the target organs (18). Hg may cause damages to the brain, gut lining, kidneys, lungs and other vital organs, while low-grade chronic exposures to Hg may also induce subtler symptoms and clinical findings (19). Zinc (Zn), manganese (Mn), copper (Cu), and nickel (Ni) are essential trace metals vital for many physiological functions. Severe Zn deficiency may result in pustular dermatitis, alopecia, diarrhea and other symptoms (20), whereas, Mn serve as co-factor for many enzymes such as arginase, glutamine synthetase, manganese superoxide dismutase enzymes, etc. (21). Cu is also required for the catalytic function of several crucial cellular enzymes (22). However, higher levels of Zn can lead to Cu deficiency or anemia (20), while Mn is toxic to humans when exposed to certain concentrations (21). Excessive Cu exposures could also harm cells as it potentially catalyze the generation of toxic reactive oxygen species (ROS) (22). Ni is widely present in nature (23), and Ni exposures can cause a variety of adverse effects to human health, such as allergy, cardiovascular and kidney diseases, lung fibrosis, lung and nasal cancer (24, 25).

Previous studies have indicated significant relationships between the Cu (26) and Pb (27) levels in the maternal circulation and PE. Higher Mn (28, 29) and Zn (30, 31) levels were associated with lower risk of PE, whereas, elevated Cd (32), As (33) and Hg (34) levels, on the other hand, could potentially increase the risk of PE. Most of previous studies have focused to explore relationships between individual metal exposure and PE, however, the aforementioned toxic heavy metals concurrently present in environment and pregnant women are generally exposed to a variety of these metals simultaneously. Therefore, it is essential to explore the relationship between multiple metals and PE. Few studies have reported the association of multiple metals with PE (35, 36), however the results vary significantly and more research is needed to elucidate exact nature of this relationship.

Current study was conducted in Dongguan city, situated southeast of Guangdong Province, China. It is one of the world's largest electronics manufacturing centers, with severe water, air and soil pollution. Excessive industrial emissions in the past few decades have resulted in elevated levels of heavy metals in soil (37, 38). Several studies have reported low to high level pollution of Cd, Cu, Hg, Ni, Pb, and Zn, and local children are facing a slight threat from As and chromium (Cr) mainly through oral ingestion of soil particles (39). Therefore, this research selected pregnant women who lived in Dongguan city for more than 1 year, and determined the concentrations of eight metals [zinc (Zn), manganese (Mn), copper (Cu), nickel (Ni), lead (Pb), arsenic (As), cadmium (Cd), and mercury (Hg)] in their peripheral venous blood to explore whether these metals were related to the occurrence of PE.

Previous studies have predominantly investigated the correlation between individual metals and PE (32–34, 40–42). Furthermore, investigations exploring multiple metals have infrequently delved into their interactions (36, 43), and the results have been inconsistent. In our study, pregnant women were recruited from a typical metal-contaminated area to scrutinize the relationship between multiple metal exposures and PE. We employed more appropriate and diverse methods to assess the prominence of specific metals in relation to PE and to explore potential interactions among multiple metals, thereby providing additional evidence on the mechanisms underlying the impact of metals on PE, offering novel insights for the prevention and treatment of PE.

2 Materials and methods

2.1 Study population

The study population was sourced from pregnant women attending Songshan Lake Central Hospital of Dongguan City in Guangdong Province, China, during the period from January 1, 2017 to December 31, 2017, specifically those who were at the hospital for delivery. Pregnant women aged ≥18 years, without diagnosed mental illness, and living in Dongguan city for more than 1 year were eligible to participate in this study. Women were diagnosed with PE based on the following criteria (2013): new-onset hypertension (SBP ≥ 140 mmHg, or DSP ≥ 90 mmHg) on two occasions at least 4 h apart and proteinuria (≥300 mg/24 h) after 20 weeks of gestation. PE cases were categorized into mild PE or severe PE, women appeared one of the following characteristics were included in the severe PE group: blood pressure (SBP ≥ 160 mmHg, or DBP ≥ 110 mmHg), proteinuria (5,000 mg/24 h), thrombocytopenia, liver dysfunction, renal insufficiency, pulmonary edema, visual impairment, new type headache and no response to drugs. A total of 271 pregnant women were willing to participate in our study, of whom 97 were diagnosed with mild PE, 64 with severe PE, and 110 were normotensive healthy pregnant women. Pregnancies resulting from *in vitro* fertilization, or women with preexistent hypertension, diabetes mellitus, kidney disease, cancer, severe anemia or other endocrine disorders were excluded. Women who did not donate blood samples or had missing data on crucial parameters were also excluded. Finally, our research comprised 28 cases of mild PE, 28 cases of severe PE and 28 normotensive healthy pregnant women. Written informed consent was obtained from all eligible participants. This study was approved by the Ethics Committee of the Songshan Lake Central Hospital of Dongguan City and the Ethics Committee of the School of Public Health of Lanzhou University.

2.2 Sample collection

The demographic characteristics of participants were obtained through face-to-face interview covering details such as age, occupational status, education, marital status and blood type. Information on physician diagnoses, gravidity, parity, gestational age and other diseases history were extracted from hospital electronic medical records at the hospital. Fasting peripheral venous blood samples were collected within 24 h before delivery, and then serum extracted from these samples were collected and stored at −80°C for further analysis. Blood samples were digested by the microwave digestion system (PreeKem, TOPEX+, China). Briefly, 0.5 mL blood sample and 3 mL HNO_3 were added into the digestion tank, with the following temperature–time regimen: 100°C-3 min, 130°C-3 min, 160°C-3 min, and 190°C-20 min. Following digestion, concentrations of metals in blood samples were determined by inductively coupled plasma-mass spectrometry (ICP-MS) (44). Quality control measures were taken into consideration by using blanks, three parallel samples and a standard reference material [GBW (E) 080067]. The relative standard deviations of the parallel samples were <10%, and the recovery of standard reference material was 94%.

2.3 Calculation

Continuous variables were presented as mean ($\bar{x}$) ± standard deviation (*SD*), while categorical variables were expressed by number (*N*) and percentage (%). To compare categorical variables, Pearson chi-square test and Fisher exact test were used. The Kruskal–Wallis *H* test was utilized for the comparison of non-normally distributed continuous variables. The Spearman's rank correlation was used to probe the associations of metal concentrations.

Unconditional logistic regression models were employed to calculate the odds ratios (ORs) and 95% confidence intervals (95% CIs). A univariate model explored the associations between single metals and PE, while a multivariate model examined the associations between multiple metals and PE. Each model was adjusted for potential confounders, specifically maternal education and gestational age, given the comparison of these factors were was statistically significant ($p < 0.05$) between different groups. Associations between PE and an overall measure of exposure to eight metals was derived by summing the quartile category score (1–4, with 1 representing the lowest quartile) for each metal to create an overall score with values ranging from 8 to 32 were estimated (45).

Principal component analysis (PCA) was employed to investigate the collective effects of multiple metals (46). PCA was intended to select clusters and characterized similar metals into new composite variables, called principal components (PCs). Loading factors that indicate the importance of the metals in a specific PC were estimated through varimax rotation, and the eigenvalues and the scree test determined the suitable number of PCs. The relationship between these PCs and PE was investigated using multivariate logistic regression.

Bayesian kernel machine regression (BKMR) was utilized to determine potential nonlinear effects of metals on PE and interactions among metals. Concentrations of metal were log-transformed to address skewed data after scaling. In the study, the BKMR model follows: $Y_i = h\,(Zn, Mn, Ni, Cu, Pb, As, Cd, Hg) + \beta^T Z_i + e_i$, whereas the function h() is a dose–response function, and Z1, …, Zp are p potential confounders. The cumulative and single effects of the eight metals were plotted by comparing the estimated value of the exposure-response function when all of the other metals were at a particular quantile. In addition, a dose–response relationship of each metal with PE was plotted while fixing the rest of metals at their 50th percentile to show the nonlinear relationship. The bivariate exposure-response function for two metals was also visualized, with all of the other metals fixed at their median value, indicating potential interaction between the two metals. Furthermore, a hierarchical variable selection method was applied, and metals were divided into two groups due to the results of PCA.

The statistical analyses were performed using SPSS (version 26) and R studio software (R version 4.3.0). A two-sided *p*-value of less than 0.05 was considered statistically significant, indicating a significant difference between variables.

3 Results

3.1 Participant characteristics

The results indicated statistically significant differences in maternal education and gestational age among three groups ($p < 0.05$). There was significant difference between the control group and the mild PE group in the middle school stratification ($p < 0.05$), with women in the mild PE group having lower education level than controls. In the high school or above stratification, significant differences were observed between the control group and both the mild and severe PE groups ($p < 0.05$), with women in the control group being more educated than those with mild or severe PE. Overall, women who experienced PE were found to be less educated as compared to those without PE. Gestational age showed statistically significant differences between the control or mild PE group and the severe PE group ($p < 0.01$), with women in severe PE group having shorter gestational ages. No statistically significant differences were observed among the three groups in maternal age, occupational status, marital status, blood type, gravidity and parity. Detailed demographic characteristics of the participants are shown in Table 1.

3.2 Concentrations of metal in serum of participants

Table 2 shows concentrations of metal measured in maternal serum. There were statistically significant differences in the concentrations of metals among the three groups ($p < 0.01$). Specifically concentrations of Mn, Ni, Cu, Pb, As, and Hg gradually

TABLE 1 Demographic characteristics of participants.

Characteristics	Controls (*N* = 28)	Mild PE (*N* = 28)	Severe PE (*N* = 28)	*P*-value
Maternal age, years ($\bar{x}$ ±*SD*)	30.54 ± 5.11	29.07 ± 5.76	30.46 ± 7.00	0.342[3]
Occupational status, *n* (%)				
Unemployment	15 (53.60)	20 (71.40)	19 (67.90)	0.337[1]
Employment	13 (46.40)	8 (28.60)	9 (32.10)	
Marital status, *n* (%)				
Unmarried	1 (3.60)	3 (10.70)	4 (14.30)	0.520[2]
Married	27 (96.40)	25 (89.30)	24 (85.70)	
Maternal education, *n* (%)				
Primary school or below	2 (7.10)[a]	2 (7.10)[a]	5 (17.90)[a]	0.028[2]
Middle school	12 (42.90)a	21 (75.00)[b]	18 (64.30)[a,b]	
High school or above	14 (50.00)[a]	5 (17.90)[b]	5 (17.90)[b]	
Blood type, *n* (%)				
A	12 (42.90)	12 (42.90)	8 (28.60)	0.505[2]
B	4 (14.30)	4 (14.30)	6 (21.40)	
O	8 (28.60)	8 (28.60)	13 (46.40)	
AB	4 (14.30)	4 (14.30)	1 (3.60)	
Gravidity, *n* (%)				
1	7 (25.00)	11 (39.30)	12 (42.90)	0.445[1]
≥2	21 (75.00)	17 (60.70)	16 (57.10)	
Parity, *n* (%)				
Primiparous	11 (39.30)	17 (60.70)	17 (60.70)	0.233[1]
Multiparous	17 (60.70)	11 (39.30)	11 (39.30)	
Gestational age, weeks ($\bar{x}$±SD)	38.53 ± 1.37[a]	38.82 ± 1.61[a]	36.67 ± 2.62[b]	0.001[3]

The same letter indicates no statistical difference between different groups. On the contrary, different letters indicate that the comparisons between different groups were statistically significant (such as a, b, and c). $\bar{x}$, mean; SD, standard deviation. [1]Pearson chi-square test. [2]Fisher exact test. [3]Kruskal–Wallis *H* test.

increased from the control group to the severe PE group. However, concentrations of Zn and Cd in the severe PE group were lower than the those in control and the mild PE groups. Figure 1 illustrates the correlation analysis results between concentrations of metal in maternal serum. The Spearman correlation between blood concentrations of Hg, Mn, Ni, Cu, and Pb was highly significant with each other ($p < 0.01$). Additionally, blood concentrations of As correlated with Hg, Mn, Ni, Cu, Pb, and Cd ($p < 0.05$), while Zn correlated with Cd ($p < 0.01$).

3.3 Unconditional logistic regression analyses

The ORs and 95% CIs for PE in relationship to concentrations of single metal after adjustment for potential confounders are presented in Table 3. The results indicated that elevated concentrations of Mn, Cu, Pb, and Hg were associated with the risk and severity of PE. Specifically, the increased concentration of Zn was associated with an increased risk of PE in the mild PE group (OR = 6.66, 95% CI: 1.82, 24.40 for the high vs. low group), while, elevated concentration of As was associated with an increased risk of PE in the severe PE group (OR = 15.79, 95% CI: 3.51, 70.98 for the high vs. low group). The elevated concentration of Cd was associated with an increased risk of PE in the mild PE group (OR = 24.16, 95% CI: 3.84, 151.82 for the high vs. low group), however, in the severe PE group, the elevated concentration of Cd was associated with lower risk of PE (OR = 0.08, 95% CI: 0.01, 0.47 for the high vs. low group). The overall score, derived from the cumulative effects of eight metals, was associated with the risk and severity of PE after adjustment ($p < 0.01$) (Table 3). The high overall score was associated with an increased risk of PE (OR = 11.00, 95% CI: 2.71, 44.66 and 19.99, 4.20, 95.21 for the high vs. low group, in the mild PE group and the severe PE group, respectively).

TABLE 2 Concentrations of metal among three groups.

Metals (μg/L)	Controls	Mild PE	Severe PE	*P*-value
	Median (IQR)	Median (IQR)	Median (IQR)	
Zn	5794.85 (4747.16, 6940.79)[a]	7502.32 (6822.97, 9390.16)[a]	3956.40 (2808.63, 6051.72)[b]	0.000
Mn	4176.40 (3812.12, 4696.05)[a]	4980.18 (4565.15, 5163.88)[b]	8118.92 (5347.99, 9418.99)[c]	0.000
Ni	2893.60 (76.65, 3453.47)[a]	3897.13 (3667.93, 4038.01)[b]	6440.99 (4475.77, 7777.19)[c]	0.000
Cu	1363.93 (1283.23, 1509.60)[a]	1939.52 (1768.27, 2006.59)[b]	2840.72 (2536.13, 3131.70)[c]	0.000
Pb	122.38 (72.73, 152.61)[a]	162.44 (153.10, 182.31)[b]	344.45 (249.50, 452.75)[c]	0.000
As	10.21 (4.93, 12.28)[a]	12.53 (10.91, 14.52)[b]	18.42 (12.70, 24.13)[c]	0.000
Cd	5.97 (2.31, 8.57)[a]	10.11 (8.85, 12.38)[b]	2.29 (1.74, 4.13)[a]	0.000
Hg	5.05 (2.30, 6.15)[a]	6.58 (6.21, 6.95)[b]	13.20 (9.35, 16.07)[c]	0.000

The same letter indicates no statistical difference between different groups. On the contrary, different letters indicate that the comparisons between different groups were statistically significant (such as a, b, and c; IQR, interquartile range; *P*-value by Kruskal–Wallis *H* test.).

Table 4 presents the ORs and 95% CIs for PE in relationship to concentrations of multiple metals after adjustment for potential confounders. An increased risk of PE was associated with elevated concentrations of Cu (OR = 45.37, 95% CI: 3.11, 661.15 and 159.09, 4.53, 5590.15 for the high vs. low group, in the mild PE group and the severe PE group, respectively), and Pb (OR = 8.82, 95% CI: 1.08, 71.90 and 1052.89, 17.57, 63106.99 for the high vs. low group, in the mild PE group and the severe PE group, respectively). Additionally, the elevated concentration of Hg (OR = 172.07, 95% CI: 2.96, 10021.03 for

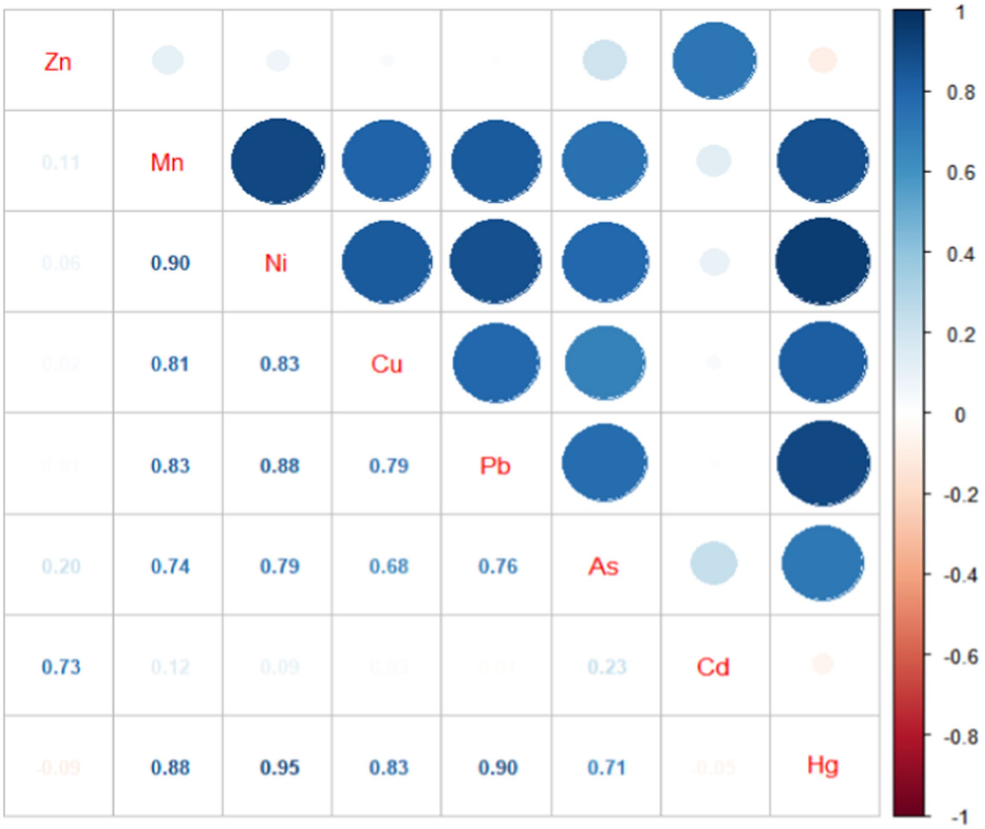

FIGURE 1
Correlation map of the serum metals.

the high vs. low) was associated with an increased risk of PE in the severe PE group.

3.4 Principal component analyses

As shown in Table 5, two PCs were identified through PCA. The first PC (PC1) was predominated by Hg, Pb, Mn, Ni, Cu, and As, while the second PC (PC2) was predominated by Cd and Zn. The ORs and 95% CIs for PE in relationship to PCs, both before and after adjustment for potential confounders, are shown in Table 6. After adjustment, PC1 was associated with an increased risk of PE (OR = 47.97, 95% CI: 4.64, 496.26 and 432.69, 28.17, 6645.03 for the high vs. low, in the mild and severe PE group, respectively), while PC2 was associated with an increased risk of PE (OR = 21.72, 95% CI: 3.61,130.75 for the high vs. low) in the mild PE group, and with lower risk of PE (OR = 0.05, 95% CI: 0.01, 0.46 for the high vs. low) in the severe PE group.

3.5 Bayesian kernel machine regression analyses

A BKMR model was employed to assess the effect of combined of metals exposure on PE, with adjustments made for the maternal education and gestational age. Posterior inclusion probabilities (PIPs) of metals in the BKMR model are presented in Table 7. Notably, Cu and Cd have larger PIPs, indicating the greater their relative greater importance in influencing PE. Based on the results of PCA, Mn, Ni, Cu, Pb, As, and Hg were grouped together, while Cd and Zn formed another group. The group PIPs surpassed 0.5 among all groups, whereas Cd in group 1 and Hg in group 2 had larger conditional PIPs.

The visualization of the BKMR model is depicted in Figure 2A. Cumulative toxic effect of metals is shown in Figure 2A, indicating a statistically significant overall effect when all metals were above their 50th percentile compared to when all metals were at their median values. Thus, elevated exposure could be associated with an increased risk of PE. The single effect of metals was explored by estimating the change in the association of a single metal with PE when it is positioned at the 25th and 75th percentiles, while the other metals were placed at the 25th, 50th, and 75th percentiles, respectively (Figure 2B). Notably, Ni and Cu exhibit a significant positive effect, with concentrations from the 25th to the 75th percentile associated with a significant increase in the risk of PE. To investigate the potential nonlinear relationship, exposure-response cross-sections for single metals were plotted, while fixing the levels of other metals at the median (Figure 2C). The plot suggested nonlinear effects of Ni and Cd, whereas, it showed a linear effect of Cu, while an increase in Cu levels is significantly associated with an increased risk of PE. To further investigate the potential relationship between metals, the bivariate exposure-response curve was plotted (Figure 2D). The curve illustrated the exposure-response relationship of one metal when the level of another metal fixed at the 10th, 50th and 90th percentiles,

TABLE 3 Associations between single metals and PE.

Metals (μg/L)	Mild PE		Severe PE	
	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value
Zn				
Low (<6111.84)	1.00	–	1.00	–
High (≥6111.84)	6.66(1.82,24.40)	0.004	0.53(0.15,1.89)	0.325
Mn				
Low (<4991.59)	1.00	–	1.00	–
High (≥4991.59)	5.03(1.23,20.61)	0.025	40.39(6.97,234.06)	0.000
Ni				
Low (<3804.87)	1.00	–	1.00	–
High (≥3804.87)	9.86E+08(2.10E+08,4.63E+09)	0.000	3.53E+09(3.53E+09,3.53E+09)	–
Cu				
Low (<1926.55)	1.00	–	1.00	–
High (≥1926.55)	34.96(3.94,310.61)	0.001	227.64(19.06,2718.40)	0.000
Pb				
Low (<165.15)	1.00	–	1.00	–
High (≥165.15)	7.07(1.53,32.72)	0.012	334.13(24.27,4601.10)	0.000
As				
Low (<12.40)	1.00	–	1.00	–
High (≥12.40)	2.50(0.72,8.68)	0.148	15.79(3.51,70.98)	0.000
Cd				
Low (<6.55)	1.00	–	1.00	–
High (≥6.55)	24.16(3.84,151.82)	0.001	0.08(0.01,0.47)	0.005
Hg				
Low (<6.58)	1.00	–	1.00	–
High (≥6.58)	11.33(2.13,60.19)	0.004	278.87(24.38,3189.41)	0.000
Overall score				
Low (<21.00)	1.00	–	1.00	–
High (≥21.00)	11.00(2.71,44.66)	0.001	19.99(4.20,95.21)	0.000

Adjusted for maternal education and gestational age.

while remaining 6 metals were all fixed at the median. The plot indicates potential interaction between Cd and Ni as well as Cu and Ni. No evidence of interaction between other metals was observed based on parallel exposure-response relationships.

4 Discussion

In this study, we initially employed traditional unconditional logistic regression model to analyzed the relationship between multiple metals and PE. The associations between individual metals and PE showed that elevated serum levels of Mn, Cu, Pb, and Hg were associated with the risk and severity of PE, while elevated serum levels of Zn and As were associated with the risk of mild and severe PE, respectively. Our results are in-line with a number of previous studies which have reported the relationship between individual metals including Cu (26, 41), Pb (47), Hg (34), and As (33) and their association with PE. In contrary, there a study has reported an inverse dose–response relationship between Mn and PE (28), and a meta-analysis found significantly lower serum levels of Zn in PE patients (30). These discrepancies might be attributed to variations in the study population, region, presence of other metals and influencing factors, thus further analysis is needed. The logistic regression results indicated a dissimilar connection between Cd and mild or severe PE, so we hypothesized that there might be a nonlinear relationship between Cd and PE, which was explored in subsequent BKMR analyses.

Considering the current situation of metal pollution in Dongguan, we hypothesized that people are not simply exposed to a single metal, but to multiple metals at the same time. Therefore, we investigated the relationship between the overall effect of multiple metals and PE, and our findings revealed that an elevated overall score of metals was associated with the risk and severity of PE, indicating an association between exposure to multiple metals and PE. A study conducted in Taiyuan, China provided supporting evidence for similar results (35). The results from multivariate logistic regression showed that elevated concentrations of Cu and Pb were associated with the risk and severity of PE, while elevated concentration of Hg was associated with risk of

TABLE 4 Associations between multiple metals and PE.

Metals (μg/L)	Mild PE		Severe PE	
	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value
Mn				
Low (<4991.59)	1.00	–	1.00	–
High (≥4991.59)	0.49(0.06,4.20)	0.515	1.40(0.09,21.12)	0.809
Cu				
Low (<1926.55)	1.00	–	1.00	–
High (≥1926.55)	45.37(3.11,661.15)	0.005	159.09(4.53,5590.15)	0.005
Pb				
Low (<165.15)	1.00	–	1.00	–
High (≥165.15)	8.82(1.08,71.90)	0.042	1052.89(17.57,63106.99)	0.001
Hg				
Low (<6.58)	1.00	–	1.00	–
High (≥6.58)	8.43(0.87,82.08)	0.066	172.07(2.96,10021.03)	0.013

Adjusted for maternal education and gestational age.

TABLE 5 Principal component analysis results of metals.

Metals	PC 1	PC 2
Hg	0.991	0.046
Pb	0.990	0.040
Mn	0.965	0.124
Ni	0.955	0.143
Cu	0.943	0.090
As	0.894	0.304
Cd	0.107	0.908
Zn	0.101	0.894
Characteristic root	5.515	1.764
Percentage of total variance	68.941	22.051
Cumulative contribution rate	68.941	90.992

Used the rotated component matrix. PC, principal component.

severe PE. However, existing studies on the effect of Cu on PE have yielded inconsistent. Some studies align with our results, suggesting a relationship between Cu and PE (26, 41). Moreover, maternal blood pressure was positively correlated with the concentration of Cu (48). In contrast, a study in Bangladesh reported significantly reduced concentration of Cu in PE patients (49), and a study in Saudi Arabia proposed that the reduction of Cu might be one of the causes of PE (50). Regarding the association of Pb (51) and Hg (34) with PE, our results are consistent with some studies while conflicting with others that found null associations between Pb, Hg, and PE (36, 43). Notably, most available studies have utilized traditional statistical analyses, such as logistic regression and linear regression, the outcomes of which may be influenced by sample size and interactions among exposure factors.

The Spearman correlation analysis revealed highly significant correlations between serum concentrations of various metals. Subsequently, we utilized PCA for dimensionality reduction of metals and found that PC1 was predominated by Hg, Pb, Mn, Ni, Cu, and As, while, PC2 was predominated by Cd and Zn. Our study supported the association of PC1 with increased risk of PE, suggesting that pregnant women exposed to a combination of these metals might face an increased risk of developing PE. Correlations between combination of metals have also been reported in previous studies, with one study reporting that levels of Cu, Zn, Mg, and Mn were positively associated with Pb and Cd (52). It is known that toxic heavy metals such as Hg, Pb, and Cd might interfere and compete metabolically with essential metals such as Cu, Zn (53, 54), and Zn has been recognized for its protective role against Cd toxicity (55). Thus, when multiple metals are exposed simultaneously, there may be antagonistic, competitive, and promoting relationships between them, rather than a singular metal effecting the human body (56, 57). Pregnancy represents a unique physiological period, characterized by distinct sensitivity and adaptability compared to non-pregnant individuals, thus the effect of metals during this period may be more complicate. Establishment of inter-metal relationships may suggest a disturbance in element homeostasis among PE patients, potentially operating through shared pathways. More research is needed to explore the mechanism of interaction among metals in pregnant women and their role in PE.

In this study, we also employed a novel nonparametric BKMR model to further investigate the relationship between multiple metals and PE. The BKMR model unveiled a significant positive cumulative effect of serum metals on prevalence of PE when concentrations exceeded the 50th percentile. It is known that, if exposure surpasses a critical level, both essential and non-essential metals could exert a wide range of toxic effects on living systems (14, 58), including immune system dysfunction (59), reproductive performance (60), multifunction of neuronal systems (61), cancers (62), and induction of oxidative stress (63, 64), which may explain the cumulative effect of metals on the development of PE. The bivariate cross-sections of exposure-response functions indicated potential interactions between Cd and Ni, as well as Cu and Ni. Previous studies have suggested that certain metals, such as Cd, Cu and Ni can act as endocrine disruptors by mimicking the action of estrogens, thus metal ions with "estrogenic activity" are also termed as metalloestrogens (MEs) (65, 66). MEs may influence estrogen receptor function by binding to cellular estrogen

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TABLE 6 Associations between PCs and PE.

Model	PCs	Mild PE		Severe PE	
		OR (95%CI)	*P*-value	OR (95%CI)	*P*-value
Model 1	PC 1				
	<−0.77	1.00	–	1.00	–
	≥−0.77	36.00(4.27,303.44)	0.001	225.00(21.94,2307.07)	0.000
	PC 2				
	<−0.08	1.00	–	1.00	–
	≥−0.08	15.00(2.97,75.70)	0.001	0.09(0.02,0.45)	0.003
Model 2	PC 1				
	<−0.77	1.00	–	1.00	–
	≥−0.77	47.97(4.64,496.26)	0.001	432.69(28.17,6645.03)	0.000
	PC 2				
	<−0.08	1.00	–	1.00	–
	≥−0.08	21.72(3.61,130.75)	0.001	0.05(0.01,0.46)	0.009

Model 1 was an unadjusted model. Model 2 was adjusted for maternal education and gestational age.

TABLE 7 Posterior inclusion probabilities of metals in BKMR model.

Metals	PIPs	Group	Group PIPs	Conditional PIPs
Zn	0.51	1	1	0.00
Mn	0.37	2	1	0.00
Ni	0.96	2	1	0.25
Cu	1.00	2	1	0.00
Pb	0.54	2	1	0.00
As	0.21	2	1	0.00
Cd	1.00	1	1	1.00
Hg	0.59	2	1	0.75

PIPs, posterior inclusion probabilities.

receptors, thereby mimicking the action of physiological estrogens. This modulation of the hormonal status in the organism by MEs can induce disturbances in human organism homeostasis (67–69). The shared mechanism of action may explain the observed interactions between metals.

The strength of BKMR lies in its capability to address both the cumulative mixture effect and the dose–response impact of individual metal when other metals fixed a particular percentile (70). Our study identified a significant positive single effect of Cu and Ni on the risk of PE. It is suggested that the association between PE and serum Cu and Ni is stable and not affected by other metals. The findings for Cu in the BKMR model were consistent with the traditional logistic regression, both indicating that Cu exposure increases the risk of developing preeclampsia. A meta-analysis also supported the notion that plasma or serum Cu level in PE patients was significantly higher than that in healthy pregnant women (71). However, a systematic review indicated that Cu was associated with PE, but the levels of Cu leading to increased risk of PE varied across regions and economic development (72). Cu is an essential trace element that is involved in many biochemical processes and the function of several cuproenzymes and also acts as a powerful antioxidant to protect cells from damage. However, an excess of Cu can harm cells due to its potential to catalyze the generation of toxic reactive oxygen species (22). In conclusion, we propose the existence of a safe dose range of Cu in pregnant women, emphasizing that elevated levels of Cu are associated with the development of PE. The BKMR model indicated nonlinear effects of Ni and Cd, providing an explanation and correction of anomalous results in logistic regression. A study in South Africa showed no significant differences in the hair and serum levels of Ni between the PE group and the control group (42). Ni as a metalloestrogen, may impact on PE by modulating the hormonal status, however, further research is needed to explore to potential relationship. A study has found that elevated Cd levels in maternal circulation could potentially increase the risk of PE (32), whiles another study indicated that there were no significant differences in the plasma concentrations of Cd between different concentration groups (43). An *in vivo* study even reported Cd-induced immune abnormalities, possibly contributing to PE pathogenesis and offering insights into treatment strategies (73).

A significant strength of our study lies in its focus on exploring the association of multiple metals with PE, aligning more closely with the natural exposure of pregnant women to these metals. Moreover, we employed novel and flexible statistical methods (BKMR), allowing us to quantify and visualize the cumulative effect of the serum metals, investigation of dose–response relationship, and overcome the limitations associated with traditional analyses including challenges of high degree of correlation between compounds. While most of the current studies on metal toxicity predominantly focus on occupational groups, our research emphasizes the potential harm of metal exposure to the general population in heavily polluted areas. Our study was conducted in an electronics manufacturing city with varying degrees of metal pollution, local pregnant women may have a higher exposure to metals. Therefore, our study shed lights on the health effects of regional metal pollution on the pregnant women living in the area. Since our study is pre-exploratory and the sample size was relatively small, limiting our ability to assess subgroup effects. However, these preliminary findings provide valuable insights and encourage us to establish a cohort study in the later stage to study this issue further with expanded sample size. Moreover, subsequent research is needed

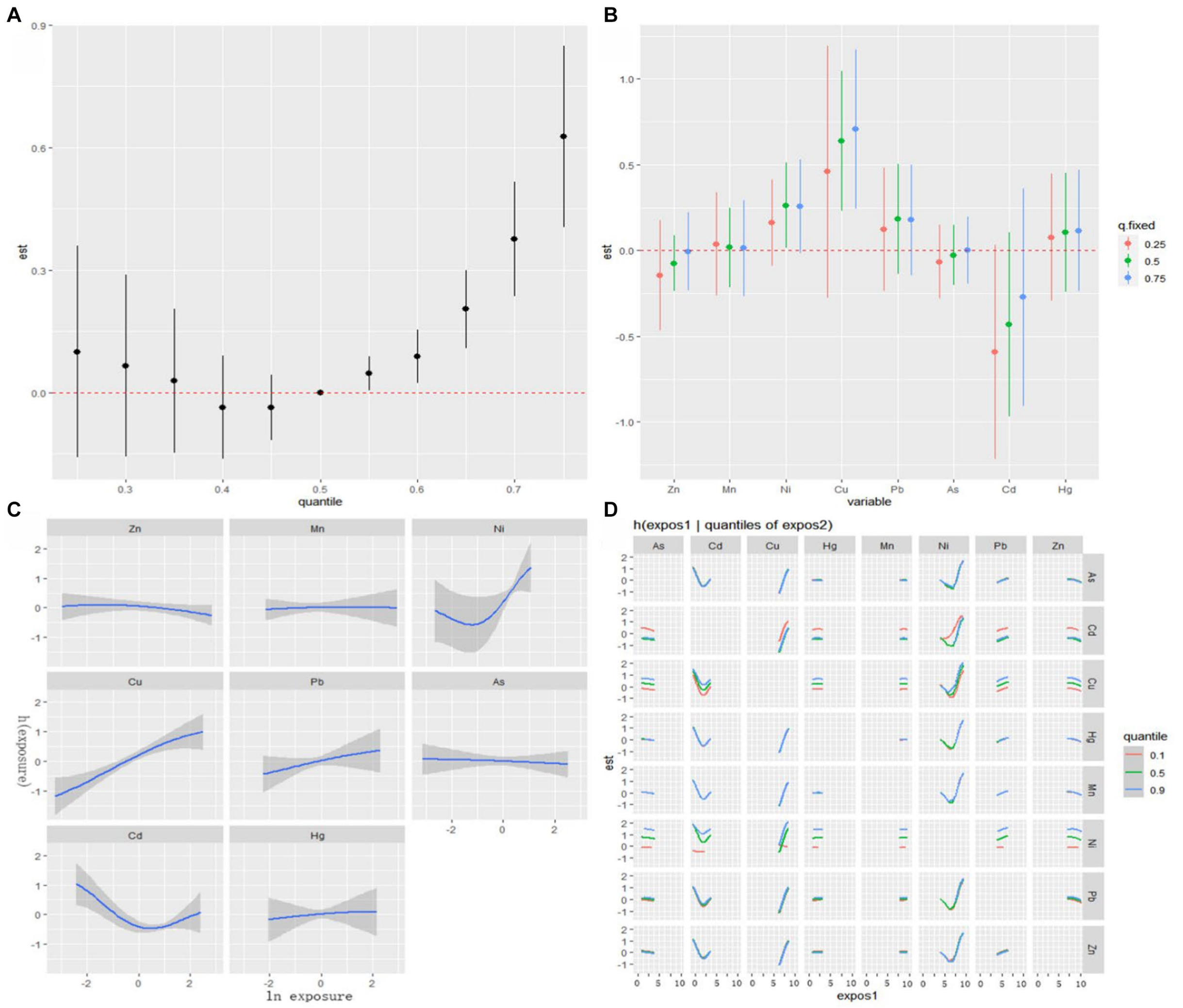

FIGURE 2
Associations between serum metals and PE among the study population by BKMR model. The model is adjusted for maternal education and gestational age. **(A)** The cumulative effect of the serum metals (estimates and 95% CIs). **(B)** The single-exposure effect (estimates and 95% CIs). **(C)** Univariate exposure-response functions and 95% confidence bands for each metal with the other metals fixed at the median. **(D)** Bivariate exposure-response functions.

to investigating the primary intake routes and sources of metals in pregnant women, contributing to the management of metal exposure and the prevention of PE.

5 Conclusion

This study unveiled a potential positive cumulative effect of serum metal levels on the risk of PE, with a particular emphasis on Cu as a potential risk factor for the onset and exacerbation of PE. Our findings suggest that pregnant women should maintain vigilance regarding the combined exposure to multiple metals, especially concerning elevated levels of Cu, Pb, Hg, and Ni. The results of this study offer valuable insights for directing future research on this issue and provide an additional foundation for preventing and treating PE. Nevertheless, larger cohort studies and experimental studies are required to investigate the risk of PE associated with exposure to multiple heavy metals. Furthermore, these studies should delve into potential interactions among different metals and elucidate the underlying mechanisms influencing the effects of metals on PE.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Ethics Committee of the Songshan Lake Central Hospital of Dongguan City and the Ethics Committee of the School of Public Health of Lanzhou University (audit no. IRB18010101). The studies were conducted in accordance with the local legislation and institutional requirements.

The participants provided their written informed consent to participate in this study.

Author contributions

JH: Conceptualization, Data curation, Investigation, Writing – original draft, Writing – review & editing. YP: Conceptualization, Investigation, Writing – review & editing. YDu: Data curation, Investigation, Writing – review & editing. HL: Investigation, Writing – review & editing. XW: Investigation, Writing – review & editing. SH: Investigation, Writing – review & editing. SA: Investigation, Writing – review & editing. YDa: Conceptualization, Funding acquisition, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by the Social Science and Technology Development (Key) Fund of Dongguan City of China (grant no. 2015108101033) and the Natural Science Foundation of Gansu Province (grant no. 21JR11RA090).

Acknowledgments

We would like to thank all the women who participated in the study and the obstetricians and nurses of the Songshan Lake Central Hospital of Dongguan City for their invaluable contributions to this research. We would also like to thank the graduate students Ke Wang, Ruiping Zhang, Linyan Wei, Xia Gao, and others for their help in data collection.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Public Health

TYPE Review
PUBLISHED 08 March 2024
DOI 10.3389/fpubh.2024.1327611

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OPEN ACCESS

EDITED BY
Mohiuddin Md. Taimur Khan,
Washington State University Tri-Cities,
United States

REVIEWED BY
Keith Dana Thomsen,
Lawrence Livermore National Laboratory (DOE), United States
Sandeep Kondakala,
National Center for Toxicological Research (FDA), United States

*CORRESPONDENCE
Baby Tabassum
✉ dr.btabassum@gmail.com
Hussain Arif
✉ arifkap@gmail.com

RECEIVED 25 October 2023
ACCEPTED 19 February 2024
PUBLISHED 08 March 2024

CITATION
Hashim M, Arif H, Tabassum B, Rehman S, Bajaj P, Sirohi R and Khan MFA (2024) An overview of the ameliorative efficacy of *Catharanthus roseus* extract against Cd^{2+} toxicity: implications for human health and remediation strategies.
Front. Public Health 12:1327611.
doi: 10.3389/fpubh.2024.1327611

An overview of the ameliorative efficacy of *Catharanthus roseus* extract against Cd^{2+} toxicity: implications for human health and remediation strategies

Mohammad Hashim[1,2], Hussain Arif[3]*, Baby Tabassum[2]*, Shahnawaz Rehman[4], Priya Bajaj[5], Rekha Sirohi[1] and Mohd Faizan Ali Khan[6]

[1]Department of Biochemistry, S. S. Faculty of Science, Mohammad Ali Jauhar University, Rampur, UP, India, [2]Toxicology Laboratory, Department of Zoology, Govt. Raza P. G. College, Rampur, UP, India, [3]Department of Biochemistry, Faculty of Life Sciences, Aligarh Muslim University, Aligarh, UP, India, [4]IIRC-1, Department of Biosciences, Integral University, Lucknow, UP, India, [5]Department of Zoology, Govt. P. G. College Noida, Noida, India, [6]Environmental Engineering Laboratory, Department of Civil Engineering, Aligarh Muslim University, Aligarh, India

Rapid industrialization has led to an increase in cadmium pollution, a dangerously toxic heavy metal. Cadmium (Cd) is released into the environment through industrial processes and can contaminate air, water, and soil. This pollution poses a significant risk to human health and has become a pressing concern in many industrialized areas. Due to its extended half-life, it leads to a range of health problems, including hepato-nephritic toxicity, brain damage, and degenerative bone disorders. Intoxication alters various intracellular parameters, leading to inflammation, tissue injury, and oxidative stress within cells, which disrupts normal cellular functions and can eventually result in cell death. It has also been linked to the development of bone diseases such as osteoporosis. These adverse effects highlight the urgent need to address cadmium pollution and find effective solutions to mitigate its impact on human health. This article highlights the Cd-induced risks and the role of *Catharanthus roseus* (*C. roseus*) extract as a source of alternative medicine in alleviating the symptoms. Numerous herbal remedies often contain certain bioactive substances, such as polyphenols and alkaloids, which have the power to mitigate these adverse effects by acting as antioxidants and lowering oxidative cell damage. Research conducted in the field of alternative medicine has revealed its enormous potential to meet demands that may be effectively used in safeguarding humans and their environment. The point of this review is to investigate whether *C. roseus* extract, known for its bioactive substances, is being investigated for its potential to mitigate the harmful effects of cadmium on health. Further investigation is needed to fully understand its effectiveness. Moreover, it is important to explore the potential environmental benefits of using *C. roseus* extract to reduce the negative effects of Cd. This review conducted in the field of alternative medicine has revealed its enormous potential to meet demands that could have significant implications for both human health and environmental sustainability.

KEYWORDS

cadmium, toxicity, exposure, human health, oxidative stress, *Catharanthus roseus*

1 Introduction

Heavy metals pose a major environmental concern due to their toxicity, inability to biodegrade, ability to accumulate biologically, and carcinogenic properties. These factors constitute a serious hazard to the environment and human health (1). In contrast to organic pollutants, heavy metals exhibit non-biodegradable characteristics and have a propensity to accumulate within vegetation, animals, and human beings subsequent to their discharge into the environment. This accumulation can have detrimental effects on the overall well-being of all organisms (1–3).

Exposure to heavy metals in the workplace and other settings causes both short-term and long-term health problems, making them a serious environmental concern. Epidemiological research has shown that exposure to low concentrations of harmful metals in the environment leads to the development of numerous disorders (4). Therefore, knowledge of the molecular processes, physiological adjustments, and physio pathological changes brought on by exposure to heavy metals is becoming increasingly important. The Agency for Toxic Substances and Disease Registry (ATSDR) ranks Cd among the top five most harmful environmental pollutants, as it is a non-essential metal (5). Many people, including governments and scientists, have been concerned about the toxicity of Cd ever since the Itai-itai sickness first appeared in Japan in 1912 (6). According to Wang et al. (7), industrial activity in many developing nations continues to pose a challenge to the reduction of mercury pollution, despite the fact that several countries have implemented various measures to that effect.

Moreover, Cd ion is a highly toxic heavy metal that is commonly found in the environment due to industrial activities; approximately 45,000 tons of Cd are released into the environment annually through various causes, including volcanic emissions, the combustion of fossil fuels, and the erosion of sedimentary materials. Such contamination affects landowners, lakes, and rivers, the quality of the air, and aquatic life (8). According to the United States Geological Survey Mineral Yearbook, which contains valuable insights into worldwide Cd output, the following countries supplied the most significant amounts between 2017 and 2020: Australia (29%), China (20%), Germany (19%), Peru (11%), and others (29%) (4). It can accumulate in various organs of the body, such as the liver and kidneys, causing various detrimental effects on human health. Understanding these diverse sources and regional variations is crucial for developing effective strategies to mitigate Cd exposure and protect public health. Therefore, it is crucial to investigate potential remedies that can mitigate the negative consequences of Cd2+ toxicity. The study of *C. roseus* extract is significant because it possesses bioactive compounds that have shown potential for combating heavy metal toxicity and its associated health problems.

In recent years, researchers and environmentalists have explored various avenues to mitigate the adverse effects of Cd toxicity. One promising avenue is the utilization of plant-based remediation strategies, where certain plant species exhibit the ability to accumulate and detoxify heavy metals from the soil. *Catharanthus roseus*, commonly known as Madagascar periwinkle or *Vinca rosea*, has gained attention for its potential in phytoremediation due to its unique ability to hyperaccumulate heavy metals, including Cd. Moreover, *C. roseus* is well-known for its rich repertoire of bioactive compounds (5), making it a subject of interest not only for environmental remediation but also for its potential implications in human health.

The bioactive compounds found in *C. roseus*, such as alkaloids, flavonoids, and terpenoids, have been reported to possess antioxidant and chelating properties (9, 10). These properties suggest that *C. roseus* extracts may play a pivotal role in ameliorating Cd-induced oxidative stress and cellular damage (5). Furthermore, the potential use of *C. roseus* as a dietary supplement holds promise for preventing or mitigating Cd toxicity in humans, thereby contributing to the development of novel strategies for human health protection (11, 12).

This review aims to provide a comprehensive overview of the existing literature on the ameliorative efficacy of *C. roseus* extract against Cd2+ toxicity. We will delve into the biochemical and molecular mechanisms underlying the protective effects of *C. roseus* against Cd-induced toxicity, emphasizing its role in mitigating oxidative stress, inflammation, and cellular damage. Additionally, we will explore the implications of *C. roseus* in human health, considering its potential as a dietary supplement to counteract Cd toxicity.

To provide a holistic perspective, this review will incorporate findings from both *in vitro* and *in vivo* studies, highlighting the diverse experimental approaches employed to evaluate the efficacy of *C. roseus* in mitigating Cd toxicity. Moreover, we aim to contribute valuable insights to the fields of environmental science, pharmacology, and human health. Through a synthesis of existing knowledge, this review seeks to stimulate further research and innovation in the development of sustainable remediation strategies and health interventions in the face of Cd contamination.

2 Adverse effects on a population caused by heavy metals

In recent years, there has been growing concern about the impact of environmental toxins, particularly heavy metals, on human health and the ecosystem. Rapid industrialization has led to widespread pollution, and heavy metals, such as cadmium (Cd), play a significant role in these environmental issues (13). According to the United Nations Environment Program/Global Program of Action (UNEP/GPA) (14), World Health Organization (WHO), and the European Parliamentary Council, heavy metals can originate from either natural or artificial sources. Agricultural and geological effluents, electronic waste, cosmetic waste, pharmaceutical waste, industrial waste, and domestic waste contribute to the contamination (15–17).

Epidemiological studies, as reported by Agency for Toxic Substances and Disease Registry (ATSDR) (5), indicate that over 5 million people are exposed to dangerous metals like Cd annually, known for their toxic properties and cumulative effects (18). Hashim et al. (17) note that toxicity from mercury (Hg) still affects 40,000–80,000 people worldwide, 200 million from lead (Pb), and approximately 250 million from Cd. Also, according to the WHO, 90% of the world population breathes polluted air, which is responsible for the deaths of approximately 7 million people each year (19). A 2020 research study by United Nations Children's Fund (UNICEF) revealed that a majority of the 800 million children affected by lead worldwide reside in India. It is estimated that air pollution causes the death of approximately one out of eight people worldwide, and this rate is increasing in regions where population density is higher and thus air pollution is higher (20).

2.1 Economic consequences associated with Cd exposure

The global prevalence of trace metal problems, particularly Cd, continues to escalate significantly despite considerable financial investments in developing cutting-edge technologies and services to remove these contaminants from soils (21). This persistent issue is primarily attributed to inadequate awareness and economic limitations, especially in developing nations (22). Chronic exposure to Cd has been associated with a spectrum of health issues, including kidney dysfunction (5), bone disorders (18), and an increased risk of cancer (23). These health impacts impose significant economic burdens, as medical expenses and productivity losses contribute to the overall cost (5). The economic consequences extend beyond healthcare, impacting resources that could otherwise be directed toward development and improving living conditions.

The economic burden stemming from Cd exposure is substantial, with the costs associated with treating illnesses and rehabilitating affected individuals placing a strain on financial resources (5). Urgent action and stricter regulations are imperative to mitigate the detrimental effects of heavy metal exposure in both India and Europe.

Cadmium, one of the most common heavy metals, has a historical context as it was used in World War I as a tin substitute and in the paint industry as a pigment. It accumulates in the human body through various sources, primarily ores of zinc, copper, or lead. The extraction and processing of these ores release large amounts of Cd into the hydrosphere, atmosphere, soil, and food chain (24). As a result, Cd pollution has broader environmental consequences, affecting ecosystems and biodiversity. Industries reliant on natural resources, such as fisheries and forestry, experience declines in productivity, and revenue due to Cd-induced environmental degradation.

There are over 50 elements classified as heavy metals, with 17 considered particularly toxic. Cd stands out as abundant, pervasive, non-essential, and the most harmful divalent industrial and environmental heavy metal (24). Recognizing the widespread impact of Cd pollution on both human health and the environment, comprehensive strategies, including stricter regulations and effective remediation methods, are essential to address the economic repercussions associated with Cd exposure.

2.2 Toxicological mechanism associated with Cd exposure

Cadmium (Cd) is ubiquitously present in environmental matrices such as air, water, soil, and biological tissues, existing predominantly in bound forms rather than in its elemental state. Its classification as a Group 1 carcinogen by the International Agency for Research on Cancer underscores its profound implications for human health (25). Cd toxicity poses a formidable challenge due to the absence of a specific, safe, and efficacious detoxification protocol. Furthermore, its protracted elimination half-life and wide-ranging organ toxicity augment its hazardous nature, making it one of the most poisonous heavy metals in the environment. (17). Unsal et al. (26) found that Cd reduces the levels of total glutathione and protein-bound sulfhydryl groups in various cell lines. This reduction leads to an increase in reactive oxygen species (ROS) like hydroxyl radicals, hydrogen peroxide, and superoxide ions. Elevated ROS levels are associated with enhanced lipid metabolism, high lipid peroxidation, modification of cellular oxidation states, DNA breakage, changes in gene expression, or even cell death (17, 27–29). Although Cd does not undergo redox reactions like copper, it can induce DNA damage through the generation of ROS in the presence of hydrogen peroxide. Previous studies have demonstrated Cd's ability to harm plasmid DNA by interacting with nitrogenous bases and enzymes involved in their function (30). Moreover, Cd exposure is known to cause oxidative stress, leading to DNA damage, emphasizing the importance of understanding the mechanisms through which Cd induces such harm for the development of effective protective strategies.

2.3 Cadmium toxicity and its detrimental impact on human health

Cadmium-induced itai-itai disease causes a variety of symptoms, including reduced bone mineralization, a high incidence of injuries, an elevated risk of osteoarthritis, and severe bone discomfort. In Japan, an explosion of the illness was reported (31, 32). Severe abdominal discomfort, nausea, vomiting, diarrhea, headache, and/or vertigo are all symptoms of acute Cd toxicity, which can result in death within 24 h or up to 2 weeks later due to liver and kidney damage. Bone illness, including osteoporosis and spontaneous bone fracture, is also associated with chronic Cd exposure, along with respiratory issues and renal dysfunction (18, 23). Other effects of Cd exposure include psychological issues, damage to the central nervous system, diarrhea, abdominal discomfort, severe vomiting, reproductive problems, infertility, and DNA damage (33, 34).

Cadmium is not an essential element, so it does not enter the body through a specific transport system (35). Instead, it can affect hormones and breathing systems in humans and invertebrates. Cd can also cause oxidative stress, which can lead to kidney damage and liver problems. This is because Cd can bind to estrogen receptors in cells and mimic the effects of estrogens. There is also some evidence to suggest that being exposed to Cd may increase one's risk of developing prostate cancer (36, 37). However, some grazing animals are born with a low amount of Cd in their bodies, but it still builds up gradually. It has a known biological purpose, although it imitates the effects of similar divalent metals, which are necessary for a wide range of biological activities, according to Peana et al. (38). In addition to the testicles and ovaries, the liver and kidneys are the main tissues, which have multiple cytotoxic and metabolic effects because of Cd buildup or overdose. The liver accumulates most of the Cd that is easily collected. It also makes less Cd available to organ systems like the kidneys and testicles, which are more sensitive to its harmful effects (31, 39). Although most domesticated animals are born with a small concentration of Cd, the two organs listed above accumulate 40–80% of the body's overburden of Cd over the course of a lifetime. Low-level exposures, like those found in the environment, cause the kidneys to store about half of the body's Cd. The cortex has concentrations that are 1.25 times higher than the rest of the kidney, and the amount of Cd that is not linked to MTs is directly related to the amount of tubular damage (40).

The concentration of Cd in the livers of individuals who are not occupationally exposed grows steadily with age. Additionally, the concentration grows in the renal cortex until the age of 50–60 years, after which it levels out or even declines (23, 41, 42). In the human kidney, it usually builds up in the segment one section of the proximal tubule, which also causes phosphate reabsorption issues (Fanconi), amino acid, protein, and bicarbonate by damaging transport proteins and mitochondria, which may cause tubular cells to die (43–45). Cells contain almost 90% of the Cd in the blood. Blood Cd levels in persons who have never been exposed to the metal in the workplace are typically below 0.5 μg/100 mL. The amount of Cd in one's blood during Cd exposure is a good predictor of the amount absorbed over the preceding months (41). People who have been exposed to a lot of Cd in the past, like retired workers, may have a blood concentration that is mostly affected by the amount of Cd in their bodies if the amount of Cd released from storage sites is higher than the amount that is being absorbed at the moment. Some research suggests that zinc and iron can mitigate Cd toxicity (4, 46, 47).

Cadmium-induced cell death is caused by the release of harmful molecules, the increase in calcium levels, a certain protein being turned on, a protective protein being turned off, and the absence of another protein that regulates cell growth. The newest evidence also shows that Cd arsenite can lower the viability of yeast cells by blocking the unfolding of new proteins. This has a big impact on many diseases, such as neurological diseases, age-related diseases, and neurodegenerative disorders like Parkinson's and Alzheimer's (31). Furthermore, Cd exhibition is considered a possible hazardous ingredient for bone density, even though crucial levels of exposure and accurate processes remain unclear. Also, it has been established that prenatal exposure to Cd influences a child's brain and kidneys (48). Disruption of the blood-testis barrier disrupts testosterone synthesis, which in turn leads to diminished germ cell adhesion, germ cell death, decreased sperm count, and either subfertility or infertility (49–51).

Some antioxidant enzymes, like CAT, manganese SOD, glutathione, GPx, glutathione reductase, and copper-zinc superoxide dismutase, cannot work as well when Cd is present (17, 27, 52). MT is a protein that can scavenge free radicals and concentrates the mineral Zn. In cells, the presence of MTs confers resistance to the harmful effects of Cd, but the inability to produce MTs makes cells more susceptible to the metal's effects (53). In cases of Cd-induced toxicity, the expression of MTs is what determines whether apoptosis or necrosis will take place (54). The consequences of Cd hitting mitochondria include oxidative stress, the production of ROS, the start of apoptosis, the mutation of MTs-DNA, the modification of gene expression, the inhibition of respiratory chain complexes, the reduction of ATP synthesis, and the modification of endo-mitochondrial conductivity. Such mitochondrial effects allow the evaluation of a wide range of human illnesses (55, 56). Cd's toxic effects and accumulation locations in the body are illustrated in Figure 1.

This review mentioned that cadmium exposure is a global concern, with millions of people being exposed to this toxic heavy metal annually (17). The long-term accumulation of cadmium in the body and its association with various health issues, such as organ toxicity and degenerative disorders, highlights the importance of understanding cadmium toxicity and finding effective remediation strategies.

3 Therapeutic efficacy of medicinal plants in mitigating cadmium-induced toxicity

Several cancer statistics reports, including Cancer Statistics (American Cancer Society 2012), showed an estimated 1.64 million new cases of cancer detected and an alarming number of approximately 577,000 deaths caused by cancer in the United States of America (57), while India estimated 14.61427 million new cases of cancer detected alone in 2022 (58). It has been demonstrated that cancer is a disease that can be prevented to some extent, and it is thought that dietary components may be able to modulate cancer risk. This is despite the fact that cancer is one of the key causes of death across the globe.

Natural resources, such as herbal plants, comprise an extensive array of phytochemicals that hold promise as conventional remedies for chronic and infectious ailments. In contrast to synthetic agents, these resources are regarded as secure and efficacious alternatives that exhibit a reduced incidence of adverse effects (59). It has been estimated that appropriate dietary modifications can prevent 30–50 percent of all cancers. Dietary patterns, foods, nutrients, and other nutritional components are intimately related to the possibility of several kinds of cancer (60). According to several epidemiological and animal studies, a diet rich in fruits and vegetables can lower your chances of developing many illnesses (60). This indicates that specific dietary elements may be useful in preventing cancer (61). Some nutrients, dietary supplements, and naturally occurring substances found in nutraceuticals (62, 63) may help fight some diseases by reducing oxidative stress. These substances can be found in herbal medicines, vitamins, amino acids, and processed foods and drinks like cereals, drinks, gums, and turmeric. Flavonoids and terpenoid indole alkaloids are the primary nutraceutical components of plants (TIAs) (64). There was a favorable effect on Alzheimer's disease symptoms in the research involving plant extracts such as *C. roseus*, *Zizyphus jujube,* and *Lavandula officinalis* (64, 65).

3.1 Significance of using medicinal plants and herbs as alternative medicines

Many of the phytochemicals included in foods like fruits, seeds, herbs, plants, and grains have been linked to lowering cancer hazards. Plants produce polyphenols, which are secondary metabolites used in defense against microbial, insect, and herbivore attacks. Dietary nutrients derived from plants have been linked to acting as active ingredients in some herbal and traditional medications (29). Researchers have found that polyphenols can help fight different types of cancer, protect the heart, brain, and joints, lower blood sugar, stop cancer from spreading, and protect the kidneys and liver (29, 66, 67). Bioactive compounds are classified as alkaloids, flavonoids, and polyphenols. These are composed of heterocyclic nitrogen, which is generally basic, which makes them extremely therapeutic. Indole

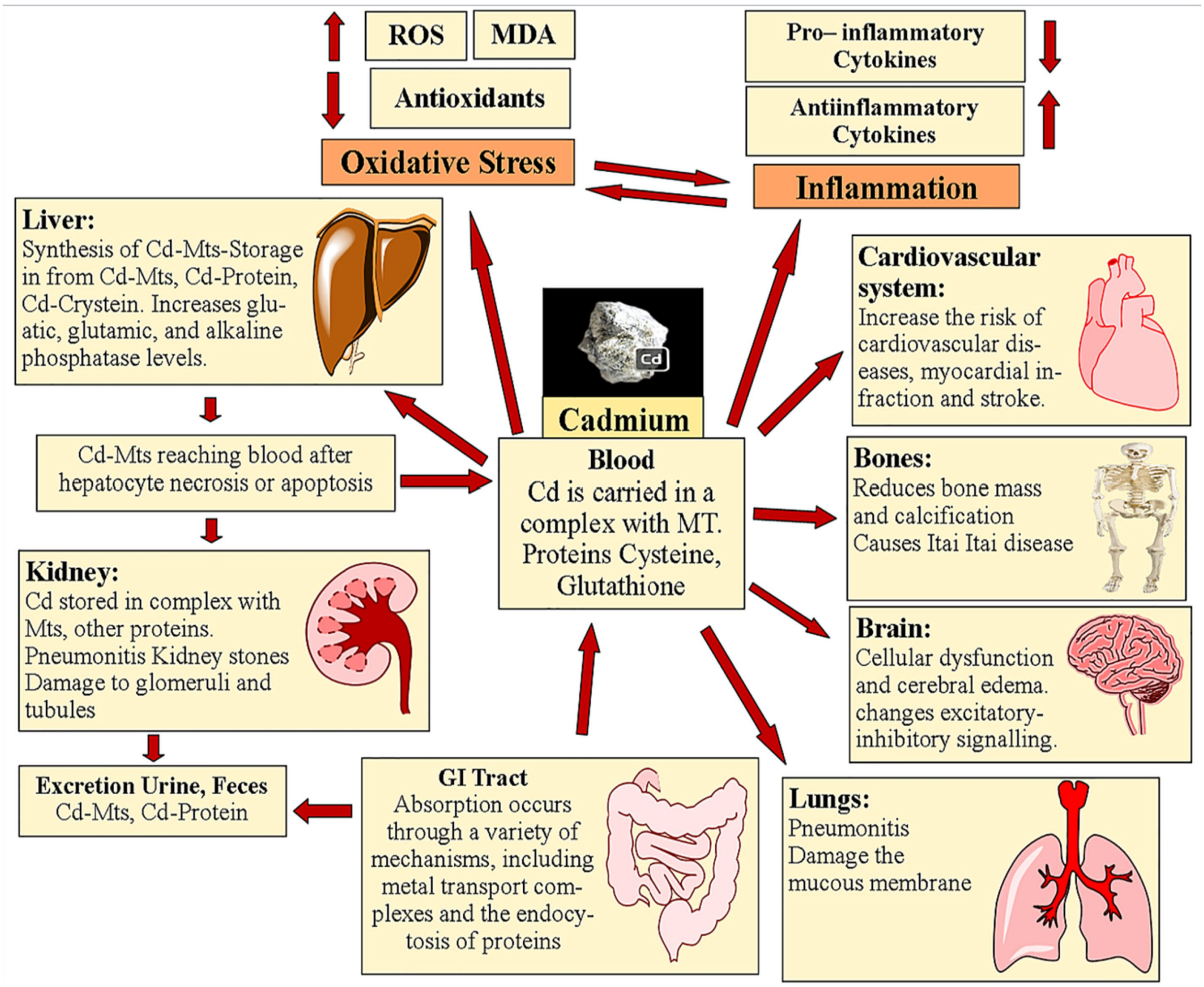

FIGURE 1
Toxicity of Cd and its dispersion inside the body.

alkaloids and polyphenols are widely spread and abundantly present in different plant families (68). A lot of different phenolic compounds, flavonoids, and secondary metabolites can be found in plants that have aromatic rings with at least one hydroxyl group (69). Herbal remedies typically contain phytochemical compounds. It has been observed that flavonoids and several alkaloids can act as antioxidants, anticancer agents, antibacterial agents, anti-inflammatory agents, and interesting applicants for pharmaceutical and medical applications (70, 71). The Indian subcontinent is home to the ancestors of some of the plants and herbs. These plants and herbs contain apparent probable therapeutic characteristics that are acknowledged in Ayurvedic and Unani, the traditional medical systems of India and worldwide, respectively. In a variety of research models, it was shown that a significant number of traditional medicines possessed a feature that defended them against the poisonous effects of heavy metals.

Ginger, *Sesamum indicum*, has natural antioxidants that greatly reduce lipid peroxidation and heavy metal toxicity (72, 73). It has been shown that medicines like *Albizia amara, Allium sativum, Ocimum sanctum, Datura stramonium, Teucrium polium L., Crataeva nurvala Buch-Ham, Urtica dioica L.,* and *Dracocephalum molsdavica L.* can fight lipid peroxidation and a number of illnesses (74–76). Vitamin E can also shield the cell membrane from the deteriorating results of oxidative stress and lipid peroxidation (77). Studies done on living things have shown that alpha-tocopherol's antioxidant properties can stop damage caused by Cd and lower oxidative stress (78). Polyphenol curcumin, found in turmeric, is known to prevent lipid peroxidation (43). Coenzyme Q10 (CoQ10), unlike vitamins, is generated from phenylalanine and mevalonic acid endogenously in the body. An instrumental function helps in avoiding lipid peroxidation from commencing and harming biomolecules (79). It helps to lessen the lipid peroxidation that Cd toxicity causes (80). N-acetyl cysteine, an amino acid found in nature, blocks lipid peroxidation by an autocatalytic mechanism (81). Quercetin is a flavonoid that can be found in onions and tomatoes. It has been shown to protect rats' brains from Cd-induced neurotoxicity by blocking the ion of lipid peroxide (82, 83).

The antioxidant activities of apple polyphenol extract have been shown to have a protective impact against oxidative stress in the liver (28). Several of the damaging effects of Cd on organs, including the liver and kidney, can be prevented or slowed by taking vitamins A, C, E, and selenium (23). Herbal medicines like *A. sativum L, S. marianum, Z. officinale, W. somnifera, O. sanctum, E. officinalis, R. officinalis,* *P. ginseng, and C. roseus* (17, 84) reduced the amount of Cd that built up in the liver and kidneys. Antioxidant properties can be found in alpha-lipoic acid (ALA). Reportedly, ALA causes regulating effects on Cd toxicity and slows down the detrimental effects of Cd, which results in significant decreases in Cd residues in the liver and kidneys (17, 85). Resveratrol and antioxidant quercetin reduced Cd-induced oxidative damage to the kidneys, oxidative stress, and liver injury in rats (86).

3.2 Overview of *Catharanthus roseus* (L.)

The Indian subcontinent is home to a diverse range of medicinal plants, many of which have not been researched to their full potential. The evergreen herbaceous plant *C. roseus* is also known as rose *Catharanthus.* In common parlance, periwinkle is referred to as "Nayantara" or "Sadabhar." The name "*Catharanthus roseus*" comes from the Greek language and literally translates to "perfect flower." The word "roseus" can also indicate red, rose, or rosy. It has been discovered that this plant is critically endangered in its natural habitat, and the principal reason for their dwindling numbers is the destruction of their natural habitat by agricultural practices that involve slashing and burning (9, 87).

This plant has a long history of medicinal applications, and it has been used to cure a wide variety of illnesses. For ages, people in Europe used it as a home cure to treat diabetes (88). Wasp stings were alleviated using the juice extracted from the leaves in India. Poultices used to staunch bleeding were traditionally made in Hawaii by boiling the plant. In China, people used it as a treatment for coughs as well as for astringent and diuretic uses (9). In Central and South America, it was used as a home medicine for the common cold to relieve symptoms of inflammation. Oral administration of a humid extract of dehydrated leaves is used to treat menorrhagia and diabetes in Australia, and oral administration of an extract of root bark is used to treat fever (9, 88).

3.3 Phytochemical constituents found in *Catharanthus roseus*

Several studies have shown that terpenoids and phenylpropanoids, along with sugars, flavonoids, saponins, and even alkaloids, make up the main parts of *C. roseus* (17). Alkaloids are the *C. roseus* chemical ingredient with the greatest potential for biological activity. In terms of chemicals, the plant has more than 200 different types of alkaloids, such as catharanthine, actineoplastidemeric, reserpine, raubasine, ajmalicine, Vinceine, vinneamine, raubasine, and more (89, 90). These alkaloids are used in a variety of industries, including pharmaceuticals, agro-chemicals, flavoring and perfume, components, artificial additives, etc.

The roots, leaves, and basal stem of this plant contain various alkaloids, including the well-known chemically used anti-carcinogenic alkaloids vinblastine, vindoline, vindolidine, vindolicine, vindolinine, vindogentianine, and vincristine (9). The flower of the *C. roseus* plant contains an anthocyanin pigment known as rosindin (91). These compound groups' antioxidant properties are well documented and also aid in compensating for the oxidative damage that heavy metals cause (17).

Important by-products of this plant include anhydrovinblastine, vindoline, catharanthine, ajmalicine, and serpentine. *Catharanthus roseus* has a lot of bisindole alkaloids, which are about 40 different constituents, and a lot of them have a vindoline and catharanthine moiety. A few alkaloids found in *C. roseus* are used in the pharmaceutical industry (92, 93). Vincristine, also known as leucocristine and Oncovin R, was first developed in 1963. It is an oxidized version of the drug vinblastine. Even though these compounds can be extracted from the leaves of the plant, the herb itself excludes large amounts of them while it is still living. Vindoline and catharanthine are two of the precursor alkaloids that are found in plants (in the waxy coating of the leaves). It is impossible to synthesize specific compounds for use in cancer treatment without first combining these precursors (9, 92, 94).

Terpenoids such as monoterpenes and carotenoids, along with polyphenols like quercetin and other flavonoids, encompass essential phytochemicals known for their wide array of antioxidant effects (95). Phenolic compounds are, found in various plants, share a common feature of having one or more hydroxyl substituents attached to aromatic or benzene rings, and they can be grouped into phenolic acids, flavonoids, stilbenoids, and lignans. These compounds exhibit a wide array of therapeutic properties, including anticancer, anti-inflammatory, and antioxidant effects, with their antioxidant potential dependent on the number and position of hydroxyl groups in their structures (96). Terpenoids, like menthol (Figure 2), play a vital role in maintaining fruit quality by preserving levels of sugars, organic acids, anthocyanins, and antioxidant capacity in fruits like strawberries and tomatoes. Carotenoids, used widely in the food industry, are divided into hydrocarbon carotenoids (carotenes) and oxygenated derivatives (xanthophylls) and serve as both dyes and secondary antioxidants, effectively scavenging reactive oxygen species due to their conjugated double bonds, thus helping prevent oxidative damage (94, 98). Quercetin having higher no of hydroxyl group in both ring a and b tends to have a higher efficacy in protecting lipid membrane in plants. This property is attributed to its higher antioxidants' potential depending on the str activity study (96).

3.4 Therapeutic properties of *Catharanthus roseus*

Catharanthus roseus, recognized as a significant pharmaceutical plant source among over 21,000 plants and herbs, has been historically employed to treat various health issues, ranging from hyperglycemia and sore mouth to leukemia, cancer, and diabetes (10). People have reported using this plant for diverse ailments, including memory loss, high blood pressure, wasp sting pain, gum bleeding, mouth ulcers, and nosebleeds. Since ancient times, *C. roseus* has been utilized in diabetes treatment and hypertension due to the belief that it either stimulates insulin synthesis or enhances the body's ability to utilize sugar (99). Studies on animal have demonstrated that the ethanolic extract, derived from leaves and flowers, can lower blood glucose levels and inhibit the formation of new metarterioles, crucial for preventing tumor growth (93).

Catharanthus roseus exhibits a broad range of pharmaceutical effects, with phytoconstituents used to address various ailments such as loose bowels, Alzheimer's disease, asthma, throat infections, digestive issues, toothache, skin conditions, and more (88, 92, 93). The herb serves as a vital source of therapeutic compounds directly related to herbalism, toxicology,

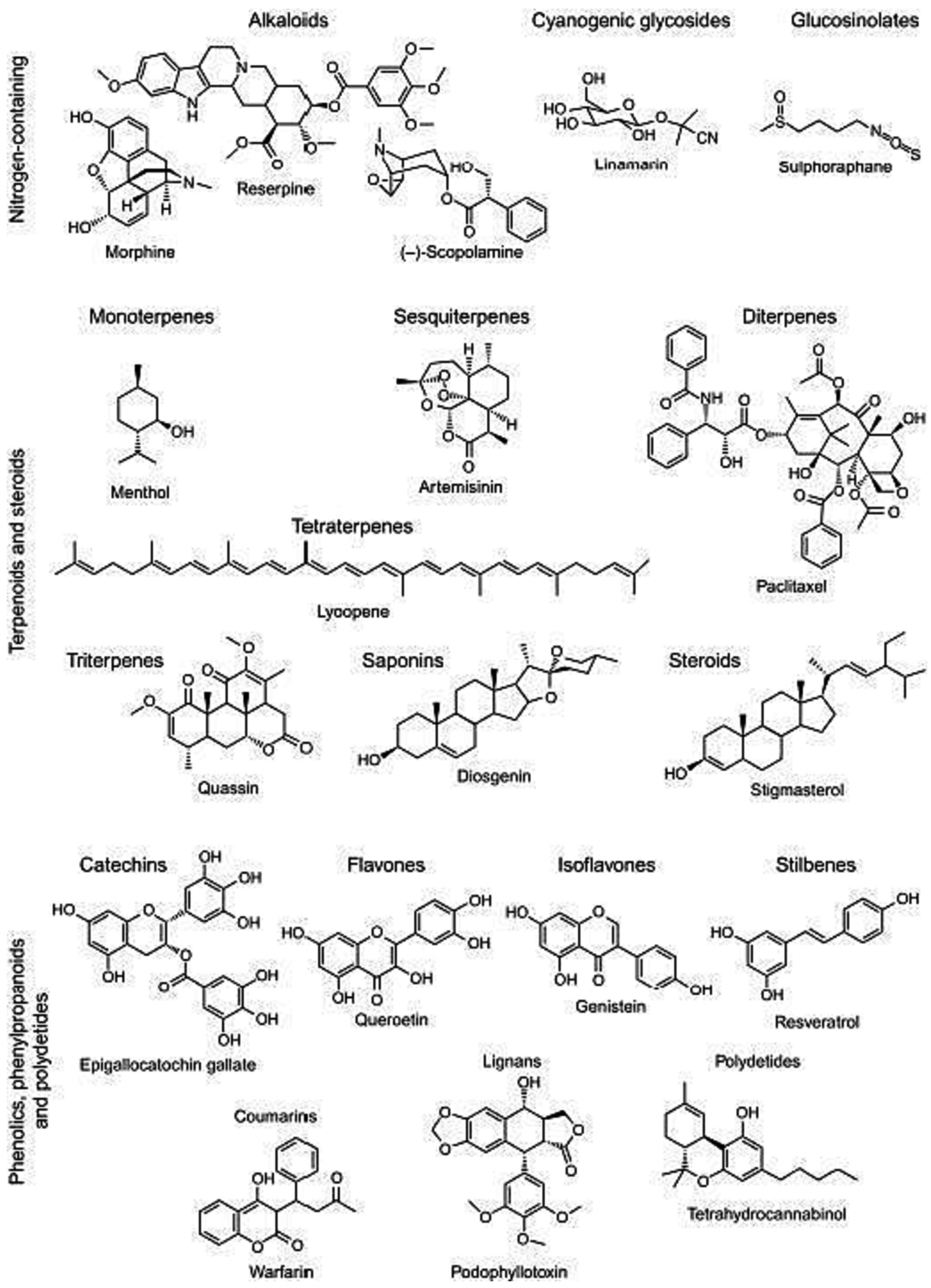

FIGURE 2
Main phytoconstituent of *Catharanthus roseus* extract source (94, 97).

pharmacognosy, and organic chemotherapy drugs and therapies. However, several studies suggested that the aqueous or alcoholic dried leaf extract might not have any negative effects on myeloid tissue, immune system, GI tract, kidney, or liver (17, 100). In addition to its usefulness as an ornament, it possesses a variety of pharmaceutical qualities, such as antibacterial, antioxidant, antidiarrheal, anti-inflammatory, antioxidant, and anti-carcinogenic activities (17, 101, 102). Alkaloids, the primary phytochemical element of *C. roseus*, are utilized in a wide variety of therapeutic applications. Alkaloids are a type of plant molecule that has a pungent taste and is primarily composed of nitrogen. Research has shown that many alkaloids have qualities that alleviate pain. *C. roseus* contains cancer-fighting alkaloids, including vinblastine and vincristine, which are important in the prevention of the disease (93, 100, 101).

Furthermore, it should be noted that three recently discovered dimeric indole alkaloids namely 17-deacetoxycyclovinblastine, 14′,15′-didehydrocyclovinblastine, and 17-deacetoxyvinamidine along with five established compounds (vinamidine, leurosine, catharine, cycloleurosine, and leurosidine) exhibited *in vitro* viability inhibition against the human breast cancer cell line MDA-MB-231 with an inhibitory concentration ranging from 0.73 to 10.67 μM (44). Significantly, cathachunine, a novel bisindole alkaloid from *C. roseus*, decreased the activity of leukemia cells while having considerably lesser cytotoxicity against normal human endothelium cells, showing that its activity was inhibited specifically toward leukemia cells (93).

In addition to research on the anticancer effects of individual alkaloids from *C. roseus*, research was also done on the whole crude extract on different types of cancer cells. Researchers recently discovered that a product from the roots and stems of *C. roseus* was very good at killing several cancer cell lines in the lab (93). Fernández-Pérez et al. (103) also discovered that the strong anticancer effect of the indole alkaloid-rich extract from *C. roseus* cell cultures was not caused by a single compound but by the action of several bioactive compounds working together. Together, these results show that the bioactive parts of *C. roseus* work well together to kill cancer cells. This effect has been seen in other plant products (104, 105) and is being thought of as a way to treat cancer (106). This phenomenon can be explained by compounds that have little to no activity, helping the main active component reach its target through mechanisms of action that complement each other, reverse resistance, improve bioavailability, reduce metabolism and excretion, and modulate side effects (93). This study underscores the potential of *C. roseus* extract as a natural remedy to counteract the harmful effects of cadmium and promote overall organ health.

3.5 The significance ameliorative efficacy of *Catharanthus roseus* extract on Cd-induced toxicity

Several reports have observed that plant-based medications and their ingredients are fast being employed to treat a variety of harmful disorders in humans, either explicitly or implicitly (107). Herbal medicines, vegetables, and fruits that are high in polyphenols, such as terpene indole alkaloids, flavonoids, vitamins, and minerals, can fight oxidative stress in a number of ways. They may even be able to help with the bad effects of too many toxic metals. Moreover, the accessibility and characteristics of these plant-based elements play a significant role in indicating preservation. In this investigation, we attempted to demonstrate that exogenously administered *C. roseus* extracts can provide protection against metal cytotoxicity. According to Hashim et al. (17), *C. roseus* extract contains bioactive substances, including polyphenols acting as antioxidants. The protective effect of *C. roseus* extract on the kidney and liver from Cd toxicity is crucial, as oxidative cell damage can lead to various health issues, including kidney and liver diseases. *Catharanthus roseus* extract contains bioactive substances, including polyphenols acting as antioxidants. Moreover, significantly reduced glomerulosclerosis, vascular endothelium, and tubular serious injuries, with "considerable preservation of serum waste substances, DNA breakage, and normalization of protein profiles, hematological parameters, MDA concentration, and antioxidant enzyme levels" (6, 17). This made chemotherapeutic medicine less harmful, increased people's lifespans, and made it easier for better health care systems to develop.

4 Conclusion

In conclusion, the study into how *C. roseus* extract protects against cadmium-induced toxicity in animal models has led to useful findings that could have therapeutic implications. Cd, a highly hazardous heavy metal, poses severe health risks to both humans and animals due to its pervasive environmental contamination. This review indicates that the administration of *C. roseus* extract exhibits a substantial capacity to mitigate the adverse consequences of Cd exposure. Important considerations when utilizing *C. roseus* extract to lessen the negative effects of Cd include its impact on the economy and the environment. The economic implications include the cost of production, extraction, and implementation, as well as the potential impact on market prices for other remediation strategies. This analysis is crucial in determining the feasibility and sustainability of using *C. roseus* extract as a remediation strategy for Cd toxicity. Ongoing research efforts aim to address these concerns and identify ways to improve the economic and ecological efficiency of implementing *C. roseus* extract.

Catharanthus roseus extract possesses potent antioxidant properties that are crucial for squelching free radicals and reducing the oxidative stress that Cd causes. The extract also has chelating properties that allow it to bind to Cd ions and make it easier for the body to get rid of them. These mechanisms collectively contribute to the alleviation of Cd-induced damage to various organs and physiological systems, encompassing the liver, kidneys, and reproductive system. Additionally, the study underscores that the extract has the potential to rectify histopathological abnormalities and restore normal biochemical parameters perturbed by cadmium toxicity. By bringing these parameters back to their normal levels, the *C. roseus* extract shows that it is effective at protecting against Cd-induced toxicity, which highlights its potential as a natural medicine. The implications of these research findings extend to the sphere of both human and animal health, considering the persistent global concern regarding cadmium exposure. The extract of *C. roseus* shows promise as a way to prevent and treat Cd toxicity, which would lower the health risks that come with it. Nevertheless, it is imperative to conduct further investigations, including clinical trials, to ascertain the safety, efficacy, and optimal dosage of the extract in human subjects.

Author contributions

MH: Conceptualization, Investigation, Writing – original draft, Writing – review & editing, Formal analysis, Resources, Visualization. HA: Supervision, Visualization, Writing – review & editing. BT: Resources, Supervision, Writing – review & editing. SR: Writing – review & editing. PB: Writing – review & editing. RS: Writing – review & editing. MK: Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The authors would like to express their sincere gratitude to Ilya Raskin for granting permission to reproduce Figure 2 in this article. Their generosity in allowing the use of this figure, significantly enhances the quality of the research. The authors would also like to thank Aisha Farhana for her valuable contributions to the review process.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 15 April 2024
DOI 10.3389/fpubh.2024.1378041

The relationship between heavy metals and metabolic syndrome using machine learning

Jun Yao[1†], Zhilin Du[2†], Fuyue Yang[3†], Ran Duan[4,5]* and Tong Feng[6]*

[1]Department of Respiratory and Critical Care, Guangyuan Central Hospital, Guangyuan, Sichuan, China, [2]Department of Oncology, Chengdu Seventh People's Hospital (Affliated Cancer Hospital of Chengdu Medical College), Chengdu, Sichuan, China, [3]Department of Rheumatology and Immunology, Chengdu Fifth People's Hospital, Chengdu, Sichuan, China, [4]Clinical Medical College, Chengdu Medical College, Chengdu, Sichuan, China, [5]Department of Oncology, The First Aliated Hospital of Chengdu Medical College, Chengdu, Sichuan, China, [6]The Second School of Clinical Medicine, Southern Medical University, Guangzhou, China

OPEN ACCESS

EDITED BY
Sarfuddin Azmi,
Prince Sultan Military Medical City,
Saudi Arabia

REVIEWED BY
Orazio Valerio Giannico,
Local Health Authority of Taranto, Italy
Aziz-Ur-Rahim Bacha,
Harbin Institute of Technology,
China

*CORRESPONDENCE
Ran Duan
✉ 1345295687@qq.com
Tong Feng
✉ 543051181@qq.com

†These authors have contributed equally to this work

RECEIVED 29 January 2024
ACCEPTED 02 April 2024
PUBLISHED 15 April 2024

CITATION
Yao J, Du Z, Yang F, Duan R and Feng T (2024) The relationship between heavy metals and metabolic syndrome using machine learning. *Front. Public Health* 12:1378041.
doi: 10.3389/fpubh.2024.1378041

Background: Exposure to high levels of heavy metals has been widely recognized as an important risk factor for metabolic syndrome (MetS). The main purpose of this study is to assess the associations between the level of heavy metal exposure and Mets using machine learning (ML) method.

Methods: The data used in this study are from the national health and nutrition examination survey 2003–2018. According to the demographic information and heavy metal exposure level of participants, a total of 22 variables were included. Lasso was used to screen out the key variables, and 9 commonly used ML models were selected to establish the associations with the 5-fold cross validation method. Finally, we choose the SHapley Additive exPlanations (SHAP) method to explain the prediction results of Adaboost model.

Results: 11,667 eligible individuals were randomly divided into two groups to train and verify the prediction model. Through lasso, characteristic variables were selected from 24 variables as predictors. The AUC (area under curve) of the models selected in this study were all greater than 0.7, and AdaBoost was the best model. The AUC value of AdaBoost was 0.807, the accuracy was 0.720, and the sensitivity was 0.792. It is noteworthy that higher levels of cadmium, body mass index, cesium, being female, and increasing age were associated with an increased probability of MetS. Conversely, lower levels of cobalt and molybdenum were linked to a decrease in the estimated probability of MetS.

Conclusion: Our study highlights the AdaBoost model proved to be highly effective, precise, and resilient in detecting a correlation between exposure to heavy metals and MetS. Through the use of interpretable methods, we identified cadmium, molybdenum, cobalt, cesium, uranium, and barium as prominent contributors within the predictive model.

KEYWORDS

metabolic syndrome, NHANES (National Health and Nutrition Examination Survey), machine learning, heavy metals, SHapley additive exPlanations (SHAP)

1 Introduction

Metabolic syndrome (MetS) constitutes a conglomerate of disorders related to energy imbalance and metabolic dysfunction, predisposing individuals to cardiovascular diseases, diabetes, and subsequent complications, thereby elevating all-cause mortality rates and presenting a significant challenge to public health and socio-economic stability. By 2012, MetS had affected approximately one-third of the adult population in the united states (US), with a prevalence rate of 35% (1). The primary underlying mechanisms of MetS, attributed to an array of adverse lifestyle factors—including genetic predispositions, imbalanced nutritional intake, sedentarism, tobacco use, and alcohol consumption—entail the disruption of energy metabolism and the pathological accumulation of visceral fat (2–4). Moreover, environmental pollution has garnered considerable attention in recent research.

Growing epidemiological evidence suggests a link between heavy metal exposure and the risk of MetS and its components in the general population. For example, studies in Korean adults have shown that elevated blood levels of cadmium and lead correlate with a higher MetS risk, hinting at possible cumulative or synergistic effects among various heavy metals (5, 6). Nonetheless, some research has identified a negative or inverse relationship between heavy metal concentrations and MetS prevalence (7, 8). Compared to the general populace, individuals residing in heavy metal-contaminated areas—whether due to occupational or environmental factors—are at an increased risk of exposure through diverse pathways, which could affect the correlation between metal exposure and MetS risk. In Taiwan, particularly in industrial regions, there is a noted increase in MetS prevalence and blood glucose levels with rising arsenic exposure (9). Furthermore, MetS patients in areas endemic with arsenic-related diseases often report a history of consuming arsenic-laden water, and early arsenic exposure is linked to higher rates of hypertension and dyslipidemia (10, 11). While many studies have established a robust connection between chronic heavy metal exposure and Mets, the complex, nonlinear relationship between heavy metals and MetS complicates the use of traditional linear statistical methods. Moreover, epidemiological research frequently focuses on the effects of individual metals, neglecting the potential interplay among multiple metals and the common scenario of simultaneous exposure, which limits the ability to interpret complex health outcomes effectively.

To address this challenge, epidemiologists are increasingly adopting machine learning (ML) techniques known for their interpretability. Unlike traditional logistic regression, ML approaches offer several key advantages in the realms of medical research and healthcare applications (12). First, ML algorithms exhibit remarkable adaptability, adeptly managing complex, non-linear relationships among variables (13). This adaptability enables the detection of nuanced patterns and interactions within the data, enhancing prediction accuracy and overall model efficacy. Second, ML techniques generally exhibit greater resilience to outliers compared to logistic regression, managing extreme values with higher efficiency and less susceptibility to bias (14). Lastly, ML approaches enhance modeling of complex relationships in medical research and healthcare through their inherent flexibility, automation, and robustness (15).

In this study, we aimed to explore the associations between heavy metals and MetS using data from the National Health and Nutrition Examination Survey (NHANES, 2003–2018). We assessed nine ML models for their efficacy in detecting MetS from heavy metal exposure levels. Furthermore, we employed an advanced ML technique involving SHapley Additive exPlanations (SHAP) to shed light on the contribution of individual heavy metals to MetS detection. This methodology seeks to: (1) quantify the impact of each heavy metal on the ML models' predictions; (2) explore the total effect of different heavy metals on metabolic syndrome and (3) enhance the development of early detection and intervention strategies specific to heavy metal exposure.

2 Materials and methods

2.1 Study population

The NHANES initiated in the early 1960s, is a comprehensive program aimed at evaluating the health and nutritional status of adults and children in the US. As a key initiative of the National Center for Health Statistics (NCHS), which is under the Centers for Disease Control and Prevention (CDC), NHANES uniquely integrates interviews with physical examinations to generate essential health statistics for the nation. Since 1999, NHANES has operated continuously, adapting its focus to address evolving health and nutrition issues and examining approximately 5,000 nationally representative individuals annually across various counties, with 15 counties selected each year (16).

The survey's methodology includes both a detailed interview, covering demographic, socioeconomic, dietary, and health-related aspects, and a comprehensive examination that entails medical, dental, and physiological assessments, along with laboratory tests, conducted by skilled medical professionals. The selection process for NHANES is designed to reflect the demographic composition of the U.S. population, with particular emphasis on over-sampling older adults, African Americans, and Hispanics to ensure accurate and representative data.

Participants undergo a thorough examination by a physician, which includes dietary assessments and body measurements for all, blood sampling and dental screenings for most, and age-dependent tests and procedures. Data collection occurs in participants' homes and in state-of-the-art mobile examination centers that are equipped to travel nationwide. The NHANES team, comprising physicians, technicians, and interviewers, utilizes advanced technology for data collection and processing, significantly reducing reliance on paper forms and manual coding.

Ethical approval for NHANES protocols was granted by the National Center for Health Statistics' research ethics review committee, with all participants providing written informed consent.

For this analysis, we compiled NHANES data from 2003 to 2018, focusing on blood and urine metal levels and pertinent covariates, initially involving 80,312 participants. Exclusions were made for pregnant women or individuals under 20 years of age (32,723), those lacking heavy metal level data (33,120), and subjects with incomplete MetS information (3). Ultimately, 11,667 adults aged 20–79 were selected for the study. The selection process is illustrated in Supplementary Figure S1.

2.2 Data collection

2.2.1 Participant demographic data

Basic characteristics such as age, race, gender, family poverty to income ratio (PIR), education level, smoking and alcohol consumption were obtained through questionnaire surveys (17).

2.2.2 Analysis of heavy metals

In this study, 18 kinds of heavy metals in urine and blood were analyzed. All samples were collected during laboratory examination, and blood and urine samples were stored under appropriate freezing (−30 ° C) conditions until the day of detection. The whole blood and urine concentrations of heavy metals were determined by inductively coupled plasma mass spectrometry (ICP-MS) (16).

During the sample preparation phase of study, researchers subjected whole blood specimens to vortexing to achieve homogeneous distribution of cellular elements, followed by the extraction of a precise volume for metal concentration analysis. This procedure is pivotal, especially for metals predominantly located in red blood cells, such as lead, to ensure the representation of the specimen's mean metal content. The addition of anticoagulants, notably ethylene diamine tetraacetic acid, is critical to prevent coagulation and preserve the uniformity of the sample, as coagulation can hinder the accurate sampling from the bulk specimen.

The dilution protocol preceding the analysis entails a standardized mixture of the sample with water and a specific diluent. This diluent comprises agents that liberate metals from red blood cells to facilitate ionization, mitigate ionization suppression, avert blockages due to biological matter, and incorporate internal standards to enhance analytical precision. Key diluent components, including Tetramethylammonium hydroxide and Triton X-100™, are instrumental in dissolving blood constituents and safeguarding the analytical instruments from contamination.

For the analytical phase, researchers employ an ICP-MS, which transforms liquid samples into aerosols, subsequently ionized within a plasma field, before their admission into the mass spectrometer. This stage demands meticulous temperature regulation and the application of internal standards to compensate for variations in the instrument's performance. The spectrometer's Dynamic Reaction Cell (DRC) is capable of operating in distinct modes, thereby amplifying specificity and sensitivity by diminishing interference, a crucial feature for analyzing elements like manganese, mercury, and selenium.

When the concentration of biomarkers is lower than the detection limit, the limit is divided by the square root of 2 according to NHANES scheme. See NHANES website for detailed determination methods. The NHANES quality assurance and quality control protocol meets the requirements of the clinical laboratory improvement act of 1988.

2.2.3 Ascertainment of outcomes

Metabolic syndrome can be diagnosed if the following ≥3 items are met (18):

1. Hypertension is systolic blood pressure ≥ 130 mmHg, diastolic blood pressure ≥ 85 mmHg, or has been diagnosed with hypertension and treated;
2. Fasting triglyceride ≥150 mg/dL, or the current use of drugs to treat high triglyceride;
3. Female HDL-c < 50 mg/dL, male hdl-c < 40 mg/dL, or the current use of drugs to reduce HDL;
4. Female waist circumference ≥ 88 cm, male waist circumference ≥ 102 cm;
5. Hyperglycemia is defined as fasting blood glucose ≥100 mg/dL or diabetes mellitus diagnosed and treated.

2.3 Data preprocessing and feature filtering

The data set was initially composed of 24 variables, called features in ML. It can be seen from Supplementary Tables S1, S2 that most of the data in this study sample are complete, and the missing data are less than 10%. According to the type of missing values, random forest filling method is used to deal with the missing data and the abnormal value is handled (Supplementary Tables S3, S4). The distribution of interpolated data is similar to the observed data (Supplementary Figure S2). In order to ensure that the data follow the normal distribution in the subsequent analysis, we performed logarithmic transformation on metal variables (Supplementary Table S5). Collinearity makes the parameter estimation of the model inaccurate, resulting in the model being too complex and over fitting the training data. We performed Pearson correlation analysis to test the relationship between these metals (Supplementary Figure S3). Using the least absolute shrinkage and selection operator (LASSO), the regularization term is introduced into the loss function to punish the model parameters and reduce the influence of collinearity. Variance inflation factor (VIF) is used to help identify the high correlation between independent variables, where VIF value below 10 indicates that there is no multicollinearity.

2.4 ML model strategies

The purpose of the multi-model classification approach is to identify the optimal model type, rather than to directly construct the final model. It employs a resampling mechanism (similar to k-fold) for training/validation to deduce the performance of each model (e.g., average AUC scores and variance) across multiple training sessions, focusing on the overall performance of each model category within the dataset. Then we will employ the best machine learning method for classification, with a total dataset sample size of $N = 11{,}667$, containing the following class information: Class (0): $N = 7{,}827$, Class (1): $N = 3{,}840$. From the total sample, a test set of $N = 2{,}333$ (20.00%) is randomly drawn, with the remaining samples used as a training set for 5-fold cross-validation. In this study, we used nine ML algorithms, namely extreme gradient boosting (xgboost) algorithm, logistic regression (LR), random forest (RF), AdaBoost, gaussian Nb (GNB), complementnb (CNB), multi-layer perceptron (MLP), and support vector machine (SVM) and k-nearest neighbor machine (KNN) learning models. See Supplementary Table S6 for specific parameters of each model. In order to further evaluate the predictive ability of the model for the final treatment results, the model was evaluated by area under the curve (AUC) value, accuracy, kappa coefficient, sensitivity, specificity, accuracy, recall rate, F1 and other indicators of the test data set (19).

SHAP additivity analysis is a method to explain individual prediction. It was originally evolved from the best Shapley value in game theory. The goal is to explain the prediction of instance X by calculating the contribution of each feature to prediction X. In this study, the algorithm is mainly used to sort according to the importance of variable characteristics in the model established by xgboost classifier, and screen out the top ten predictive factors in tb-dm patients. SHAP can reasonably explain the contribution value of each variable to the model, and avoid the long-standing "black box" theory in ML. Therefore, clinicians can make more optimal judgments when formulating treatment plans for patients (20).

2.5 Statistical analysis

In this study, continuous variables were expressed as mean ± standard deviation or interquartile interval (IQR; 25–75%). T test was used for normal continuous variables, while Mann Whitney U test was used for non-normal continuous variables. Categorical variables are described as percentages (%). Chi square or Fisher exact probability test is used for constituent ratio comparison. All statistical analyses were conducted using Python (version 3.10.9) and R software (version 4.2.3), with the Lasso R package glmnet at version 4.1.8, XGBoost in Python at xgboost = 2.0.1, and other methods in Python using scikit-learn = 1.1.3.The overall design of the paper is shown in Figure 1.

3 Results

3.1 Baseline data comparison

Table 1 presents the demographic characteristics of the study participants. A total of 11,667 individuals were included in the analysis, with 48.99% being male and an average age of 48.0 (interquartile range, 34.0–64.0). Out of the participants, 3,840 were diagnosed with MetS. Individuals with MetS tended to be older, have a higher body weight, be non-Hispanic white, and have a higher average family income (all $p < 0.05$). The content distribution of heavy metals in blood or urine in each two cycles from 2003 to 2018 is shown in Table 2.

3.2 Feature selection

16 variables were selected using LASSO regression analysis based on their non-zero coefficients, as shown in Figure 2. These selected variables were blood cadmium, blood lead, blood mercury total, arsenous acid, arsenobetaine, arsenocholine, dimethylarsonic acid, barium, cadmium, cesium, lead, antimony, thallium, tungsten, uranium, molybdenum. The evaluation of multicollinearity among the various chosen metals and covariates using VIFs revealed no evidence of multicollinearity (Supplementary Table S7).

3.3 Evaluation and comparison of the model

The AdaBoost model exhibited superior performance compared to other models, achieving a larger AUC as depicted in Figure 3 and Supplementary Table S8. Precision-recall curve are shown in Figure 4. A forest plot of the AUC score for the multiple models based on the AUC of the nine models was created (Figure 5), with the AdaBoost algorithm demonstrating the best predictive performance, achieving an AUC of 0.807. Since the performance of the validation set under the AUC index does not exceed the test set or the exceeding ratio is less than 10%, it can be considered that the fitting is successful (Figure 6). Consequently, the AdaBoost algorithm was chosen for further analyses. The training dataset values are presented in Table 3, and the validation set values are available in Table 4. Supplementary Figure S4 displays the confusion matrix for the nine ML algorithms used.

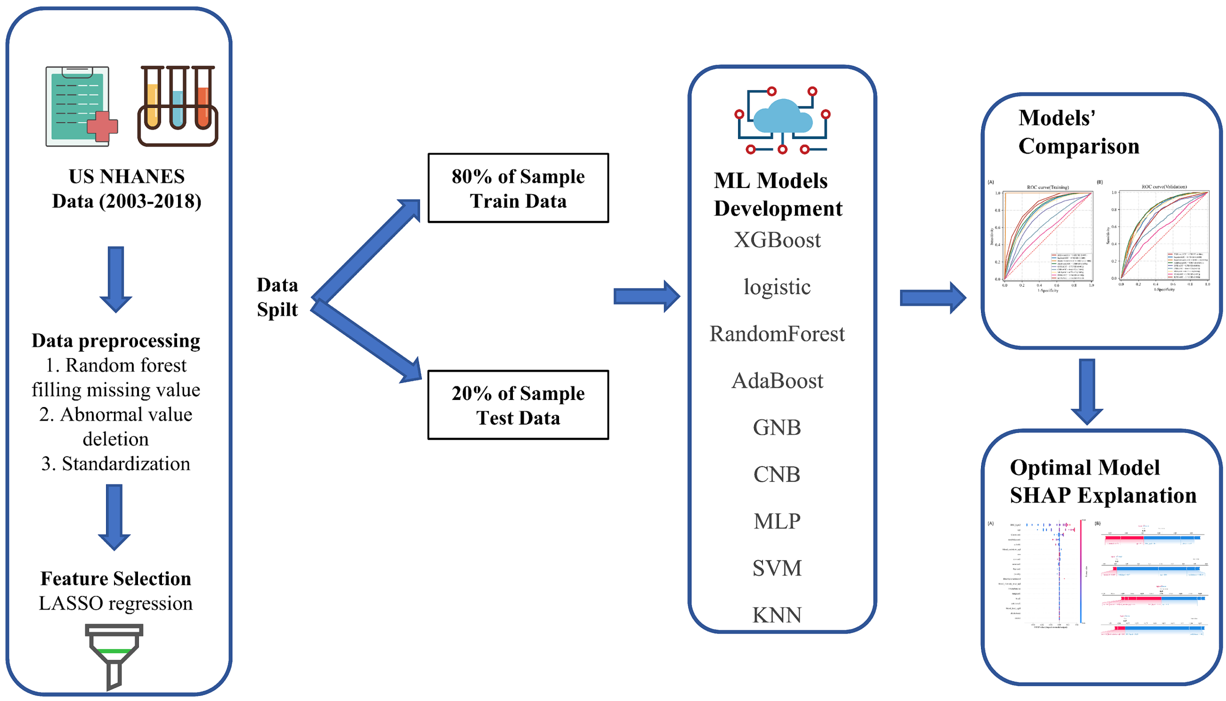

FIGURE 1
Model-making process and article framework. This figure shows how the data were obtained from electronic medical record systems, and the collection of data on all study variables, including demographic characteristics, laboratory indicators. Data on a total of 24 variables were collected, 22 of which were selected. The 22 variables were used to establish the machine learning models.

TABLE 1 Characteristics of the study participants with and without MetS form 2003–2018 in US NHANES.

Characteristics	Total (n = 11,667)	Without MetS (n = 7,827)	With MetS (n = 3,840)	p
Education Level, n (%)				<0.001
Less than high school	1,410 (12.085)	839 (10.719)	571 (14.870)	
High school or equivalent	4,359 (37.362)	2,865 (36.604)	1,494 (38.906)	
College or above	5,898 (50.553)	4,123 (52.677)	1775 (46.224)	
Alcohol user, n (%)				<0.001
No	1,634 (14.005)	1,052 (13.441)	582 (15.156)	
Former	2,237 (19.174)	1,358 (17.350)	879 (22.891)	
Mild	3,834 (32.862)	2,513 (32.107)	1,321 (34.401)	
Moderate	1756 (15.051)	1,286 (16.430)	470 (12.240)	
Heavy	2,206 (18.908)	1,618 (20.672)	588 (15.313)	
Smoke, n (%)				<0.001
No	6,367 (54.573)	4,415 (56.407)	1952 (50.833)	
Former	2,908 (24.925)	1,655 (21.145)	1,253 (32.630)	
Current	2,392 (20.502)	1757 (22.448)	635 (16.536)	
Race, n (%)				<0.001
Non-Hispanic Black	2,402 (20.588)	1,559 (19.918)	843 (21.953)	
Other Hispanic	1,039 (8.905)	702 (8.969)	337 (8.776)	
Non-Hispanic White	5,188 (44.467)	3,403 (43.478)	1785 (46.484)	
Mexican American	1957 (16.774)	1,319 (16.852)	638 (16.615)	
Other race – including multi-racial	1,081 (9.265)	844 (10.783)	237 (6.172)	
Sex, n (%)				0.078
Male	5,716 (48.993)	3,790 (48.422)	1926 (50.156)	
Female	5,951 (51.007)	4,037 (51.578)	1914 (49.844)	
Age	48.000[34.000,64.000]	42.000[30.000,58.000]	61.000 [48.000,71.000]	<0.001
Poverty	2.110 [1.130,3.920]	2.100 [1.100,3.930]	2.131 [1.180,3.900]	0.049
BMI	27.810 [24.290,32.290]	26.410 [23.250,30.620]	30.440 [27.290,34.600]	<0.001

3.4 Visualization of feature importance

After the above analysis, we use the SHAP method to explain the model established by AdaBoost. Figures 7, 8 shows the contribution of each screened feature to the model obtained by the SHAP method. Figure 8 offers an illustration of the assessment of MetS risk, showing the influence of features such as cadmium, body mass index (BMI), cesium, gender, and age on the estimated probability of MetS.

4 Discussion

In our study, we utilized a ML approach, complemented by an intuitive process, to investigate the relationships between heavy metal exposure levels and Mets. We developed nine ML models to detect MetS, achieving noteworthy predictive accuracy and interpretability from heavy metal datasets. The AdaBoost model, in particular, exhibited outstanding performance, pinpointing cadmium, molybdenum, cobalt, cesium, uranium, and barium as critical contributors to MetS detection. This ML model holds promise for supporting the creation of tailored healthcare strategies for individuals, based on their specific heavy metal exposure profiles.

This study extends previous work that employed ML algorithms for disease prediction, as demonstrated in several key studies (21–23). These investigations have underscored the capability of sophisticated classification algorithms to enhance prediction accuracy. In recent years, ML algorithms have increasingly contributed valuable insights to clinical decision-making processes. Leveraging vast clinical datasets, ML has rapidly advanced and proven its efficacy in forecasting the outcomes of various diseases (14). ML algorithms excel at synthesizing and analyzing large volumes of diverse data, a task that poses significant challenges for human analysts. Nonetheless, interpreting the outputs of ML algorithms remains complex (24). To address this complexity, we utilized SHAP values within the AdaBoost model to facilitate optimal interpretation and elucidate their influence on the predictive outcomes. In the context of the 2003–2018 NHANES survey, a positive SHAP value suggests that certain features elevate MetS risk, whereas a negative SHAP value indicates a lower risk.

This research revealed significant correlations between MetS and exposure to certain heavy metals, emphasizing the importance of

TABLE 2 Geometric means and geometric standard deviations of heavy metals by each cycle of US NHANES (2003–2018).

Heavy metal	Year 2003–2004 (*n* = 1,488)	Year 2005–2006 (*n* = 1,457)	Year 2007–2008 (*n* = 1714)	Year 2009–2010 (*n* = 1934)	Year 2011–2012 (*n* = 1,622)	Year 2013–2014 (*n* = 1741)	Year 2015–2016 (*n* = 1711)	*p*
Blood cadmium	0.40[0.20,0.60]	0.34[0.21,0.60]	0.35 [0.23,0.62]	0.35 [0.22,0.62]	0.33 [0.21,0.61]	0.30 [0.18,0.58]	0.30 [0.18,0.53]	<0.001
Blood lead	1.70 [1.10,2.60]	1.48 [0.92,2.37]	1.41 [0.96,2.24]	1.29 [0.84,2.00]	1.08 [0.72,1.71]	1.00 [0.64,1.56]	0.97 [0.60,1.59]	<0.001
Blood mercury total	0.90 [0.50,1.70]	0.94 [0.50,1.73]	0.87 [0.48,1.58]	0.93 [0.50,1.85]	0.88 [0.44,1.88]	0.79 [0.42,1.63]	0.77 [0.42,1.55]	<0.001
Arsenous acid	0.80 [0.80,0.80]	0.85 [0.85,0.85]	0.85 [0.85,0.85]	0.85 [0.85,0.85]	0.34 [0.34,0.56]	0.46 [0.08,0.73]	0.14 [0.08,0.56]	<0.001
Arsenic acid	0.70 [0.70,0.70]	0.71 [0.71,0.71]	0.71 [0.71,0.71]	0.71 [0.71,0.71]	0.62 [0.62,0.62]	0.56 [0.56,0.56]	0.56 [0.56,0.56]	<0.001
Arsenobetaine	1.40 [0.30,6.10]	1.85 [0.40,7.42]	0.98 [0.28,5.07]	1.27 [0.28,7.02]	1.40 [0.84,7.39]	1.17 [0.82,4.85]	0.82 [0.82,5.64]	<0.001
Arsenocholine	0.40 [0.40,0.40]	0.42 [0.42,0.42]	0.42 [0.42,0.42]	0.42 [0.42,0.42]	0.20 [0.20,0.20]	0.08 [0.08,0.08]	0.08 [0.08,0.08]	<0.001
Dimethylarsonic acid	4.00 [2.00,6.00]	3.96 [2.39,6.46]	3.70 [2.28,6.42]	3.69 [2.06,6.59]	3.99 [2.16,7.44]	3.33 [1.35,5.66]	3.35 [1.35,5.87]	<0.001
Barium	1.35 [0.66,2.44]	1.31 [0.68,2.57]	1.32 [0.66,2.46]	1.30 [0.66,2.48]	1.08 [0.54,2.17]	0.93 [0.48,1.93]	1.03 [0.51,2.03]	<0.001
Cadmium	0.30 [0.15,0.56]	0.27 [0.13,0.52]	0.27 [0.14,0.52]	0.26 [0.13,0.48]	0.22 [0.11,0.44]	0.18 [0.08,0.38]	0.20 [0.09,0.42]	<0.001
Cobalt	0.31 [0.19,0.49]	0.36 [0.23,0.58]	0.34 [0.21,0.53]	0.34 [0.20,0.54]	0.30 [0.18,0.48]	0.38 [0.22,0.64]	0.40 [0.24,0.63]	<0.001
Cesium	4.84 [2.84,7.30]	5.06 [3.07,7.59]	4.72 [2.95,6.92]	4.37 [2.76,6.50]	4.19 [2.56,6.36]	4.09 [2.47,6.46]	4.45 [2.68,6.53]	<0.001
Lead	0.70 [0.40,1.17]	0.67 [0.37,1.15]	0.59 [0.32,0.97]	0.53 [0.31,0.90]	0.41 [0.24,0.73]	0.33 [0.18,0.57]	0.35 [0.19,0.62]	<0.001
Antimony	0.07 [0.05,0.12]	0.07 [0.04,0.12]	0.05 [0.03,0.09]	0.05 [0.02,0.09]	0.04 [0.02,0.07]	0.04 [0.01,0.07]	0.04 [0.02,0.08]	<0.001
Thallium	0.16 [0.09,0.25]	0.16 [0.10,0.25]	0.14 [0.09,0.23]	0.15 [0.08,0.23]	0.16 [0.09,0.25]	0.15 [0.08,0.23]	0.16 [0.09,0.25]	<0.001
Tungsten	0.06 [0.03,0.11]	0.07 [0.03,0.14]	0.08 [0.04,0.17]	0.07 [0.03,0.13]	0.06 [0.03,0.13]	0.05 [0.02,0.11]	0.05 [0.02,0.11]	<0.001
Uranium	0.007 [0.004,0.012]	0.005 [0.003,0.010]	0.007 [0.004,0.013]	0.007 [0.004,0.014]	0.005 [0.002,0.011]	0.005 [0.002,0.011]	0.005 [0.003,0.010]	<0.001
Molybdenum	41.50 [22.60,71.0]	46.70 [25.70,75.10]	45.60 [23.80,79.10]	42.20 [23.90,73.50]	40.20 [21.30,67.50]	34.46 [17.72,62.15]	38.50[19.44,66.70]	<0.001

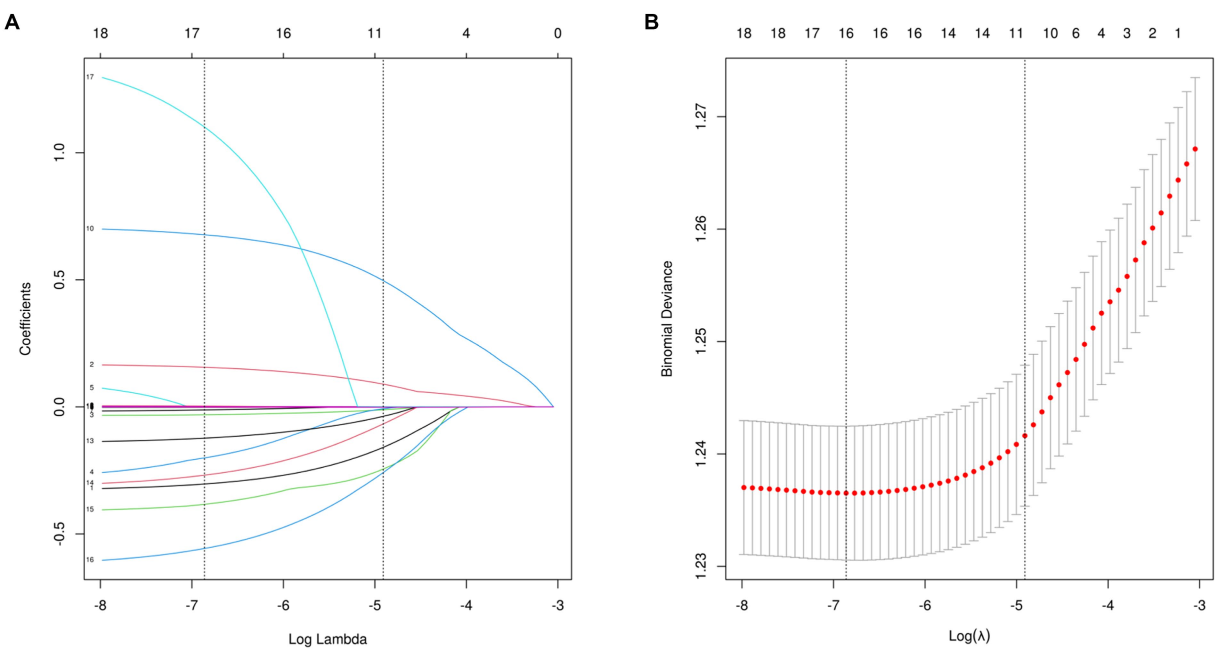

FIGURE 2
Features selection used the LASSO regression. **(A)** A coefficient profile plot is created against the logarithmic (lambda) sequence. In this study, the selection of predictors is based on the minimum criteria (indicated by the left dotted line), resulting in the selection of 16 nonzero coefficients using the LASSO regression model. **(B)** The tuning parameter (lambda) selection is based on the deviance in the LASSO regression, using both the minimum criteria (indicated by the left dotted line) and the 1-SE criteria (indicated by the right dotted line). LASSO, least absolute shrinkage and selection operator; SE, standard error.

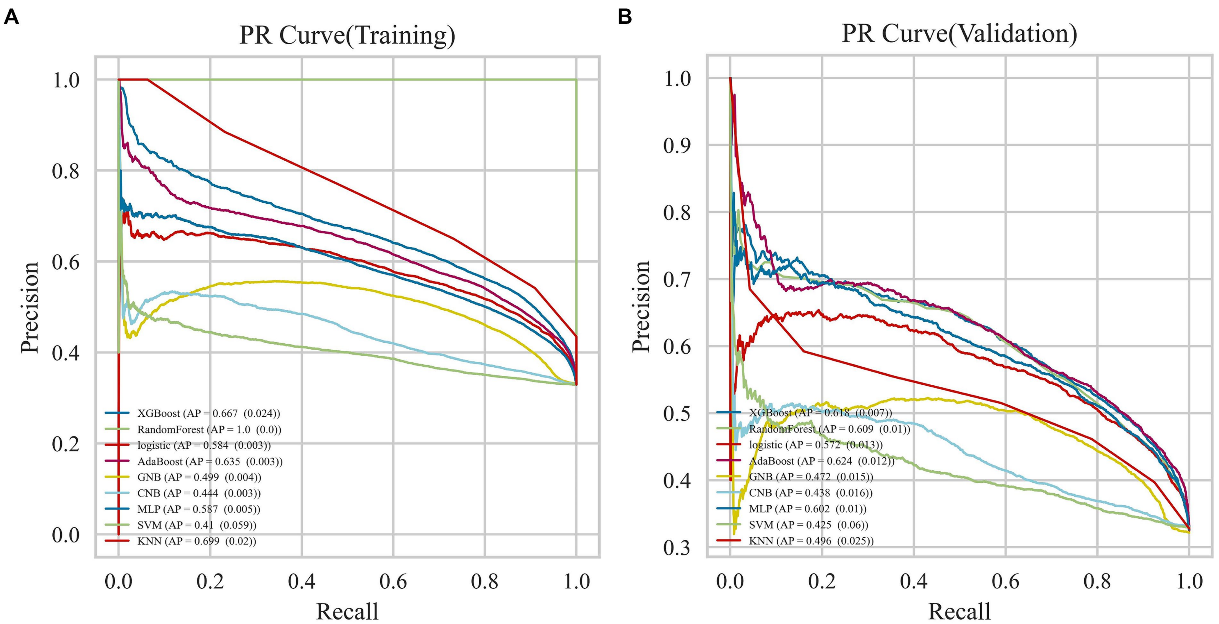

FIGURE 3
AUC curves for nine machine learning models. The AdaBoost model achieved a larger (better) AUC compared with the other models. **(A)** Training dataset **(B)** Validation dataset.

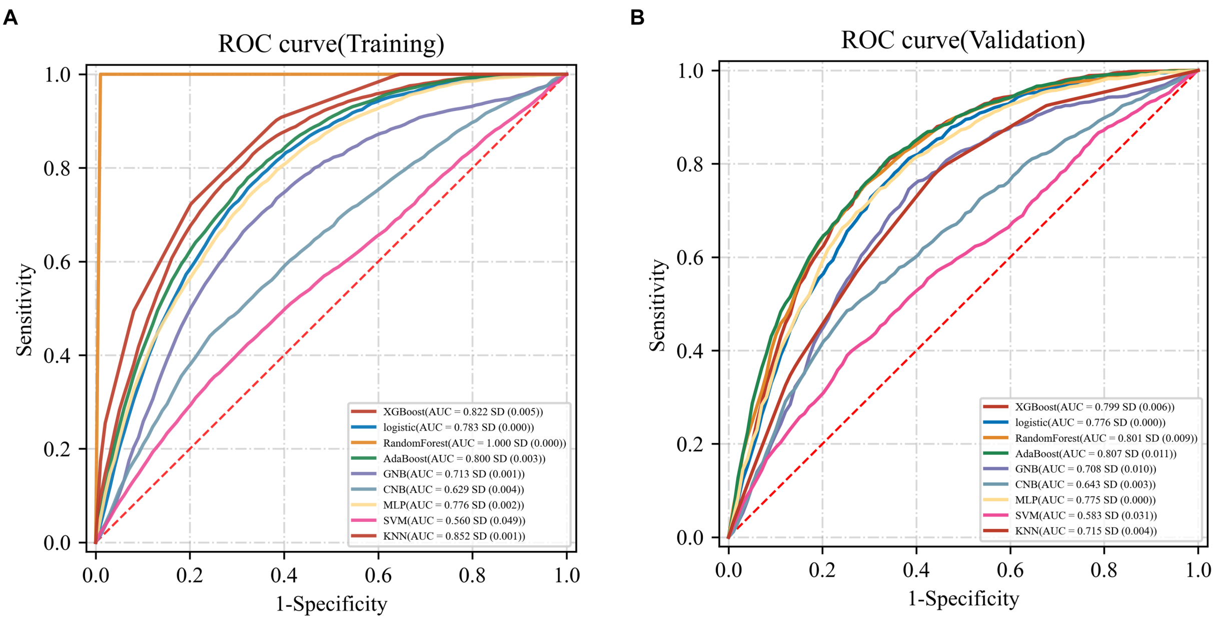

FIGURE 4
Precision-recall curve curves for nine machine learning models. **(A)** Training dataset **(B)** Validation dataset.

recognizing the various dietary pathways through which these metals can be ingested. For instance, cadmium, commonly found in cereals, leafy vegetables, and shellfish, and chromium, present in meat, whole grains, and fruits, represent notable sources of exposure (25). Additionally, the consumption of fatty fish, dairy products, and meats may lead to exposure to Persistent Organic Pollutants (POPs), such as polychlorinated biphenyls (PCBs) and dioxins, which accumulate in the food chain and are associated with various negative health outcomes, including metabolic disorders (26–28). The identification of these heavy metals as factors contributing to MetS highlights the urgent need for comprehensive public health measures to mitigate exposure to these detrimental elements. Such measures could encompass policy-level initiatives to enforce stricter controls on industrial discharges and agricultural chemicals, alongside community and individual initiatives to enhance awareness of heavy metal and POP sources. Public health campaigns and dietary guidelines could also be instrumental in reducing consumption of contaminated foods. Furthermore, healthcare professionals have a pivotal role in screening

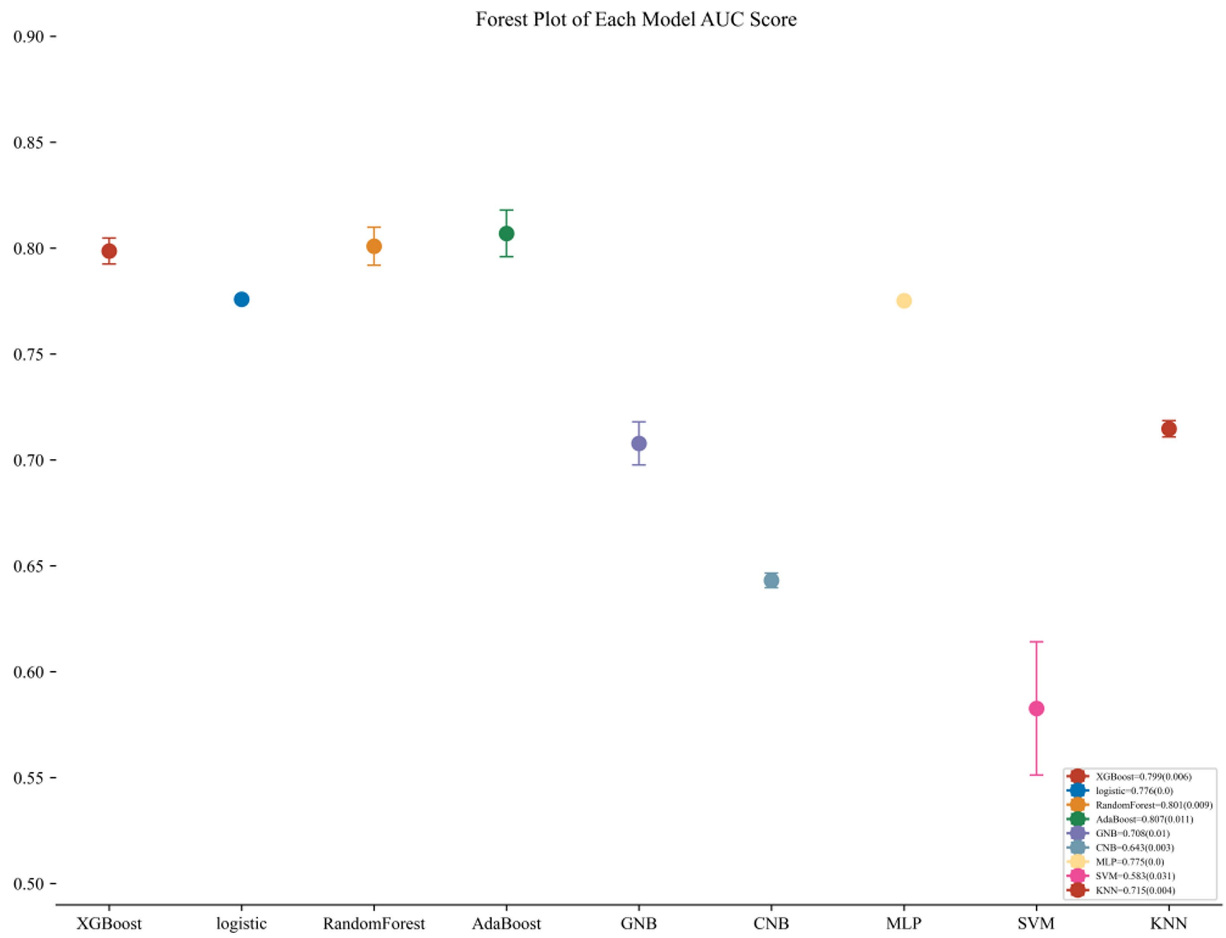

FIGURE 5
Forest plot of the AUC Score of the nine models. The AdaBoost model achieved a smaller (better) standard deviation (SD) compared with the other models.

for heavy metal exposure among susceptible groups and advising on dietary modifications to reduce such exposure.

The findings derived from SHAP analysis align with prior research outcomes. Specifically, Lee and Kim identified a notable positive association between blood cadmium levels and MetS risk in Korean males, utilizing data from the Korean National Health and Nutrition Examination Survey for the periods 2005–2010 and 2008–2012 (5, 29). Similarly, an Iranian study reported elevated urine cadmium levels in individuals with MetS (30). Additionally, experimental evidence revealed that serum chromium concentrations were lower in the diabetes group ($0.0205 \pm 0.0012\,\mu g/mg$) compared to the control group ($0.0267 \pm 0.0009\,\mu g/mg$). This finding was corroborated by Flores et al., who reported serum chromium levels in healthy individuals and diabetes patients to be 1.44 μg/L and 0.66 μg/L, respectively, further substantiating the observed disparity (31). From a mechanistic perspective, chromium enhances insulin sensitivity by activating insulin receptor kinase and facilitating insulin's interaction with its cellular receptors, thus amplifying its biological efficacy.

Metals exhibit either additive or synergistic effects due to shared exposure routes and mechanisms of action, with oxidative stress being a principal shared pathway (32). Chronic exposure to heavy metals such as cadmium and lead results in glutathione depletion and the binding to sulfhydryl groups in proteins (33). The oxidation of Arsenite (As III) to Arsenate (As V) leads to the formation of hydrogen peroxide and the interaction with critical thiol groups (34). This cascade triggers an extensive production of free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS), disrupting the balance within the antioxidant/oxidant system. The ensuing oxidative stress culminates in lipid peroxidation, Deoxyribo Nucleic Acid (DNA) and cellular membrane damage, protein alterations, and other detrimental effects, ultimately contributing to the onset of chronic conditions such as diabetes and cardiovascular diseases (35).

The AdaBoost model is distinguished by several key features. It leverages existing demographic, laboratory, and questionnaire data from the US NHANES, obviating the need for new data collection. This model integrates data from various sources to pinpoint the top 10 variables crucial for ML applications. Furthermore, between 2009 and 2013, heightened attention by the US government on heavy metal exposure, particularly in the context of environmental health, led to the initiation of numerous environmental governance initiatives (36). These initiatives resulted in reduced heavy metal levels and diverse MetS occurrences. Our ML models were developed and assessed using detailed participant data on blood heavy metal concentrations. Despite a general decline in heavy metal levels during 2009–2013, the NHANES dataset represents a single-time participation for individuals, implying that the heavy metal data does not reflect annual

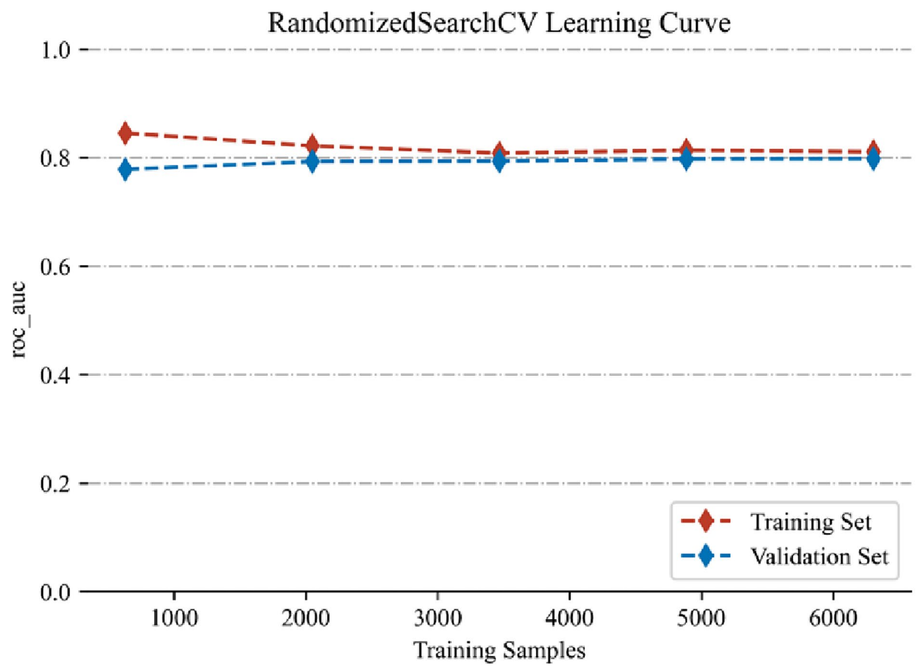

FIGURE 6
AUC performance of training set and validation set.

TABLE 3 Performance metrics for nine models in the training dataset.

Characteristics	AUC	Cutoff	Accuracy	Sensitivity/ Recall	Specificity	PPV	NPV	F1 score	Kappa
XGB	0.822 (0.005)	0.357 (0.038)	0.730 (0.011)	0.808 (0.040)	0.690 (0.037)	0.571 (0.016)	0.877 (0.017)	0.668 (0.003)	0.452 (0.008)
logistic	0.783 (0.000)	0.313 (0.009)	0.698 (0.006)	0.777 (0.014)	0.659 (0.016)	0.533 (0.005)	0.855 (0.003)	0.632 (0.001)	0.390 (0.005)
RF	1.000 (0.000)	0.535 (0.015)	1.000 (0.000)	1.000 (0.000)	1.000 (0.000)	1.000 (0.000)	1.000 (0.000)	1.000 (0.000)	1.000 (0.000)
AB	0.800 (0.003)	0.475 (0.012)	0.711 (0.001)	0.783 (0.003)	0.674 (0.001)	0.552 (0.000)	0.858 (0.003)	0.647 (0.001)	0.414 (0.002)
GNB	0.713 (0.001)	0.923 (0.023)	0.664 (0.007)	0.715 (0.022)	0.638 (0.021)	0.500 (0.010)	0.815 (0.009)	0.589 (0.001)	0.319 (0.004)
CNB	0.629 (0.004)	0.986 (0.006)	0.649 (0.002)	0.453 (0.006)	0.748 (0.008)	0.476 (0.008)	0.730 (0.003)	0.464 (0.001)	0.203 (0.004)
MLP	0.776 (0.002)	0.329 (0.023)	0.692 (0.005)	0.757 (0.013)	0.660 (0.014)	0.530 (0.007)	0.843 (0.005)	0.623 (0.001)	0.377 (0.005)
SVM	0.560 (0.049)	0.437 (0.039)	0.536 (0.111)	0.627 (0.178)	0.488 (0.260)	0.413 (0.063)	0.710 (0.017)	0.475 (0.013)	0.113 (0.088)
KNN	0.852 (0.001)	0.500 (0.000)	0.776 (0.001)	0.723 (0.002)	0.798 (0.001)	0.758 (0.001)	0.781 (0.001)	0.740 (0.001)	0.451 (0.004)

XGB, extreme gradient boosting; RF, random forest; AB, AdaBoost; SVC, support vector classification; NB, Naive Bayes; MLP, multilayer perceptron; SVM, support vector machine; KNN, K-Nearest Neighbor; AUC, area under the receiver operator curve; FPR, false positive rate; FNR, false negative rate; PPV, positive predictive value; NPV, negative predictive value.

average exposure levels. However, this did not compromise the models' stability, with the AdaBoost model demonstrating consistent reliability, indicated by an average AUC of 0.807. In addition to AdaBoost, we explored nine other ML techniques to detect MetS based on heavy metal exposure, drawing from recent cardiovascular disease research for enhanced insights. Certain models showed greater robustness and predictive accuracy with the incorporation of more granular data (37). An exhaustive evaluation of the ML models'

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TABLE 4 Performance metrics for nine models in the validation dataset.

Characteristics	AUC	Cutoff	Accuracy	Sensitivity/ Recall	Specificity	PPV	NPV	F1 score	Kappa
XGB	0.799 (0.006)	0.357 (0.038)	0.713 (0.010)	0.778 (0.034)	0.694 (0.034)	0.555 (0.017)	0.863 (0.010)	0.647 (0.000)	0.420 (0.012)
logistic	0.776 (0.000)	0.313 (0.009)	0.694 (0.005)	0.773 (0.039)	0.658 (0.033)	0.548 (0.015)	0.841 (0.015)	0.640 (0.003)	0.388 (0.004)
RF	0.801 (0.009)	0.535 (0.015)	0.736 (0.003)	0.758 (0.005)	0.706 (0.012)	0.667 (0.018)	0.754 (0.004)	0.710 (0.012)	0.339 (0.012)
AB	0.807 (0.011)	0.475 (0.012)	0.720 (0.009)	0.792 (0.016)	0.686 (0.031)	0.556 (0.016)	0.862 (0.001)	0.653 (0.005)	0.426 (0.017)
GNB	0.708 (0.010)	0.923 (0.023)	0.658 (0.006)	0.755 (0.015)	0.609 (0.004)	0.500 (0.017)	0.815 (0.006)	0.602 (0.017)	0.315 (0.020)
CNB	0.643 (0.003)	0.986 (0.006)	0.658 (0.006)	0.488 (0.006)	0.746 (0.010)	0.508 (0.011)	0.730 (0.012)	0.498 (0.008)	0.234 (0.002)
MLP	0.775 (0.000)	0.329 (0.023)	0.697 (0.006)	0.697 (0.025)	0.733 (0.023)	0.545 (0.004)	0.835 (0.004)	0.611 (0.007)	0.385 (0.003)
SVM	0.583 (0.031)	0.437 (0.039)	0.539 (0.105)	0.649 (0.206)	0.495 (0.256)	0.412 (0.063)	0.744 (0.026)	0.477 (0.019)	0.125 (0.062)
KNN	0.715 (0.004)	0.500 (0.000)	0.692 (0.009)	0.790 (0.002)	0.551 (0.011)	0.578 (0.035)	0.722 (0.003)	0.667 (0.024)	0.245 (0.029)

XGB, extreme gradient boosting; RF, random forest; AB, AdaBoost; SVC, support vector classification; NB, Naive Bayes; MLP, multilayer perceptron; SVM, support vector machine; KNN, K-Nearest Neighbor; AUC, area under the receiver operator curve; FPR, false positive rate; FNR, false negative rate; PPV, positive predictive value; NPV, negative predictive value.

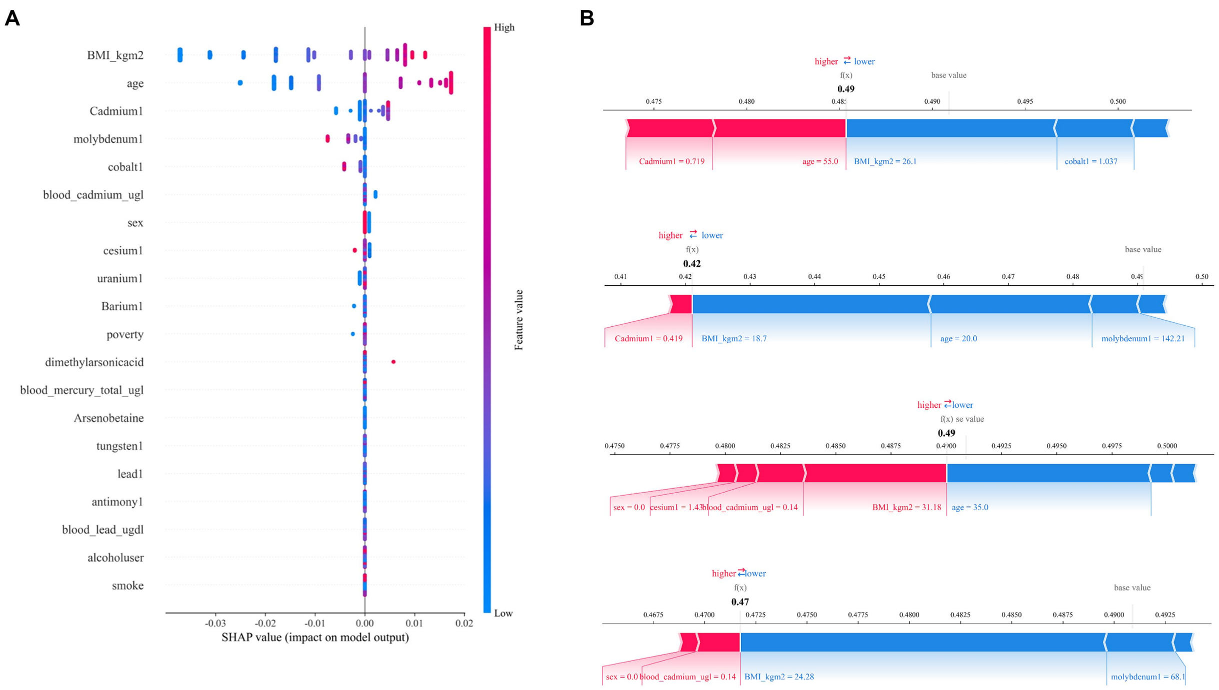

FIGURE 7
SHAP analysis of the AdaBoost model. **(A)** A visual representation of each feature of the AdaBoost model, showing the relationship between the importance of each feature. The color represents the value of the variable, with red representing the larger value and blue representing the smaller value. **(B)** The contributing variables are arranged in the horizontal line, sorted by the absolute value of their impact.

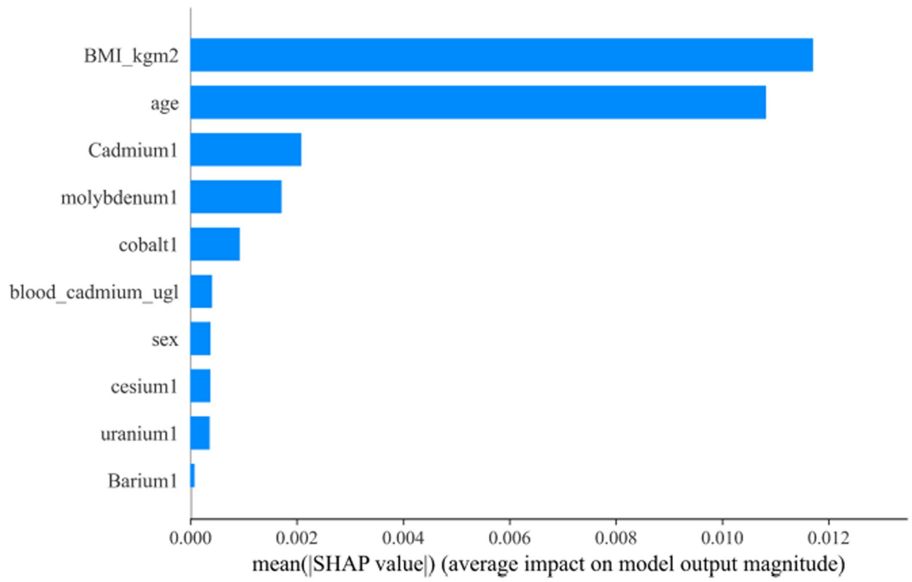

FIGURE 8
"SHAP" package to explain the importance of key variables to the model.

predictive capabilities revealed that the AdaBoost model stood out for its superior classification performance, underscored by nine distinguishing features.

This research presents novel insights, yet it is constrained by certain limitations that warrant acknowledgment. The cross-sectional nature of this study curtails the ability to establish causality or temporal sequences. Although the presence of heavy metals in biological specimens might indicate a connection with MetS or its constituents, the singular biomarker assessments in this study provide only a snapshot of exposure, with blood lead levels particularly reflecting recent exposures. Moreover, the dependence on self-reported data for MetS diagnosis in the US NHANES survey introduces a potential for information bias, including inaccuracies related to memory, which could affect the precision of the ML models in pinpointing MetS. The lack of an external validation group within our study design also limits the possibility of further substantiating the model's reliability and its generalizability to a wider population.

Future research should emphasize the need for longitudinal studies to elucidate the causal connections between exposure to heavy metals and POPs and MetS. Exploring alternative biological matrices such as toenails or hair could provide more dependable indicators of long-term metal exposure, albeit with potential challenges related to measurement precision and varying growth rates. Subsequent inquiries should delve into the underlying mechanisms by which such exposures influence metabolic health, potentially through pathways like oxidative stress, inflammation, or hormonal disruption. A comprehensive understanding of the combined impacts of various metals and pollutants is crucial for developing effective prevention and intervention strategies. Expanding research to encompass diverse populations and settings will enhance the relevance and applicability of the findings, thereby informing public health initiatives tailored to the unique needs and risks of different communities. Furthermore, continuous analysis and interpretation of critical features will empower professionals with the insights necessary for informed decision-making, transcending mere dependency on algorithmic outputs. Efforts should also focus on validating the performance of models by enlarging the database and refining the interface between healthcare providers and ML models to improve their interpretability and practicality in clinical contexts.

5 Conclusion

In our study, the AdaBoost model exhibited exceptional effectiveness, precision, and resilience in identifying the association between heavy metal exposure and the incidence of MetS in participants from the US NHANES spanning 2003 to 2018. Employing transparent methods, we identified cadmium, molybdenum, cobalt, cesium, uranium, and barium as key contributors to the model's predictive performance. Our results highlight the benefits of integrating machine learning approaches with SHAP techniques to explore the intricate impact of environmental exposures on health. Additionally, the predictive framework established by this research holds promise for devising targeted interventions to prevent and control MetS.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The ethics review board of the National Center for Health Statistics approved all NHANES protocols and written informed consents were obtained from all participants or their proxies (< 18 years). The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Bioethics Committee of Southern Medical University reviewed our study and have waived the need for ethical approval.

Author contributions

JY: Writing – original draft. ZD: Investigation, Software, Writing – original draft. FY: Formal analysis, Methodology, Writing – original draft. RD: Writing – review & editing. TF: Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

Thanks to Jing Zhang (Shanghai Tongren Hospital) for his work on the NHANES database. His outstanding work, nhanesR package and webpage, makes it easier for us to explore the NHANES database.

The manuscript has utilized written content generated or edited using a generative AI technology named "ChatGPT," based on the GPT-4 architecture. GPT-4 stands for "Generative Pre-trained Transformer," an advanced natural language processing model developed by OpenAI.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2024.1378041/full#supplementary-material

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frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 09 May 2024
DOI 10.3389/fpubh.2024.1368112

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EDITED BY
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Peter James Franklin,
University of Western Australia, Australia
Mohammad Hashim,
Mohammad Ali Jauhar University, India

*CORRESPONDENCE
Zoey Laskaris
laskaris@umich.edu

RECEIVED 09 January 2024
ACCEPTED 15 April 2024
PUBLISHED 09 May 2024

CITATION
Laskaris Z, O'Neill MS, Batterman SA, Mukherjee B, Fobil JN and Robins TG (2024) Cross-shift changes in pulmonary function and occupational exposure to particulate matter among e-waste workers in Ghana. *Front. Public Health* 12:1368112. doi: 10.3389/fpubh.2024.1368112

Cross-shift changes in pulmonary function and occupational exposure to particulate matter among e-waste workers in Ghana

Zoey Laskaris[1]*, Marie S. O'Neill[2], Stuart A. Batterman[3], Bhramar Mukherjee[4], Julius N. Fobil[5] and Thomas G. Robins[3]

[1]Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor, MI, United States, [2]Department of Epidemiology, Department of Environmental Health Sciences, University of Michigan School of Public Health, Ann Arbor, MI, United States, [3]Department of Environmental Health Sciences, University of Michigan School of Public Health, Ann Arbor, MI, United States, [4]Department of Biostatistics, University of Michigan School of Public Health, Ann Arbor, MI, United States, [5]Department of Biological, Environmental, and Occupational Health Sciences, University of Ghana School of Public Health, Accra, Ghana

Introduction: Little is known on the association between cross-shift changes in pulmonary function and personal inhalation exposure to particulate matter (PM) among informal electronic-waste (e-waste) recovery workers who have substantial occupational exposure to airborne pollutants from burning e-waste.

Methods: Using a cross-shift design, pre- and post-shift pulmonary function assessments and accompanying personal inhalation exposure to PM (sizes $\leq$1, $\leq$2.5 μm, and the coarse fraction, 2.5–10 μm in aerodynamic diameter) were measured among e-waste workers (n = 142) at the Agbogbloshie e-waste site and a comparison population (n = 65) in Accra, Ghana during 2017 and 2018. Linear mixed models estimated associations between percent changes in pulmonary function and personal PM.

Results: Declines in forced expiratory volume in one second (FEV1) and forced vital capacity (FVC) per hour were not significantly associated with increases in PM (all sizes) among either study population, despite breathing zone concentrations of PM (all sizes) that exceeded health-based guidelines in both populations. E-waste workers who worked "yesterday" did, however, have larger cross-shift declines in FVC [−2.4% (95%CI: −4.04%, −0.81%)] in comparison to those who did not work "yesterday," suggesting a possible role of cumulative exposure.

Discussion: Overall, short-term respiratory-related health effects related to PM exposure among e-waste workers were not seen in this sample. Selection bias due to the "healthy worker" effect, short shift duration, and inability to capture a true "pre-shift" pulmonary function test among workers who live at the worksite may explain results and suggest the need to adapt cross-shift studies for informal settings.

KEYWORDS

electronic-waste, respiratory health, informal sector, air pollution, particulate matter, personal inhalation, pulmonary function, Ghana

1 Introduction

Reductions in occupational and environmental health risks associated with the recovery of valuable metals and plastics from used electronic and electrical equipment waste (e-waste) are urgently needed around the globe (1, 2). In informal e-waste recycling sectors common in low- and middle-income (LMIC) countries (e.g., Nigeria, Ghana, Thailand, Argentina), occupational and environmental health and safety regulations are often unenforced, putting workers and nearby communities at risk of exposure to a multitude of physical and chemical pollutants (3–5). Despite a growing body of evidence documenting the occupational and community-level health effects of exposure to e-waste associated pollutants [e.g., lead, chromium, cadmium, flame retardants, dioxins, furans, polycyclic aromatic hydrocarbons (PAHs), particulate matter (PM)] (6–8), little is known about the respiratory health effects among workers associated with air pollution generated from e-waste recovery practices (9–14).

Burning e-waste in open surface fires is a commonly used technique for efficiently eliminating plastic coatings from valuable metals in the informal sector. Measures of PM air pollution from burning e-waste can reach high concentrations ($>500\,\mu g\,m^{-3}$) and the PM can be comprised of a high fraction of toxic constituents (e.g., heavy metals, PAHs and flame retardants), posing risks to workers and surrounding communities (5, 10, 11, 13, 15–20). Other techniques used to process e-waste, such as manual dismantling of generators, cathode ray tubes, and fluorescent lighting, for example, present additional inhalation hazards including metal-contaminated dust and vapors (21).

Few occupational studies have measured acute responses to PM. Using data from wildland firefighters, Gaughan et al. found a cross-shift decline in pulmonary function [forced expiratory volume in one second (FEV1)] associated with increased exposure to levoglucosan, a byproduct of biomass burning measured in PM ($<10\,\mu m$) (22). Similarly, among firefighters responding to a controlled burn, Slaughter et al. found a measured decline in pre- and post-shift FEV1; however, the decline was not significantly associated with accompanying exposure to PM ($<3.5\,\mu m$) (23). And in non-occupational settings, emerging evidence established an association between acute respiratory effects, including pulmonary function declines and reduced exercise performance, and short-term exposure to diesel exhaust and PM (≤ 1 and $\leq 2.5\,\mu m$) in healthy individuals and in those with asthma, chronic obstructive pulmonary disease, and ischemic heart disease (24–29). Based on this literature, we expect that e-waste workers exposed to high concentrations of PM and co-occurring inhalation hazards [e.g., carbon monoxide (CO), PAHs, metals] are likely to exhibit accelerated declines in pulmonary function. The severity of declines may differ by specific work activities.

Using data collected at the Agbogbloshie informal e-waste recovery site and a reference community in Accra, Ghana, this study evaluates whether acute changes in pulmonary function are associated with personal PM using a highly sensitive cross-shift study design. Cross-shift studies enable each study participant to serve as their own referent, reducing the impact of confounding, and do not require a long-term follow up. The first aim is to evaluate the association between cross-shift changes in pulmonary function and personal exposures to PM_1 ($<1\,\mu m$), $PM_{2.5}$ ($<2.5\,\mu m$), coarse fraction PM (2.5–10 μm), and self-reported pre-shift exposures among e-waste recovery workers and a reference population. The second aim is, among e-waste workers only, to evaluate the association between cross-shift changes in pulmonary function and activities performed during the work shift. The results will contribute to the limited epidemiologic evidence on acute respiratory health effects associated with unusually high personal PM concentrations among e-waste workers in Accra, Ghana.

2 Materials and methods

2.1 Study sample

Study participants were enrolled in the West Africa-Michigan CHARTER II for GeoHealth (GeoHealth) ($N = 207$), a longitudinal cohort study with four waves of data collection designed to assess environmental and occupational health among e-waste recovery workers at the Agbogbloshie e-waste site in Accra, Ghana. Eligible participants ($N = 131$) included male e-waste recovery workers from the Agbogbloshie e-waste site ($N = 81$) and male residents from a reference population ($N = 50$) living in the Madina Zongo (MZ) district of Accra who completed personal shift sampling during the second (August 2017–October 2017) and/or the third wave (January-April 2018) of data collection.

Details on the geographic setting and participant recruitment at Agbogbloshie have been described previously (18, 19). An attempt to enroll an inception cohort of e-waste workers at Agbogbloshie was unsuccessful, as information on when or if a new worker arrived to the site was unavailable. The MZ community members were selected as an appropriate reference population based on their geographic separation from e-waste associated pollutants and similar religion and region of origin to the e-waste worker population. In this study, the role of the reference population is to provide otherwise unavailable background levels of personal PM inhalation exposure and respiratory health of Accra residents with similar socio-, cultural- and economic characteristics of e-waste workers. MZ is comprised of housing structures and small-scale businesses serving community needs and is surrounded by a high traffic four-lane road (N4). A sufficient number of individuals in both study locations volunteered to participate. Compensation at each wave included 30 Ghana Cedis (~7 USD), lunch and a t-shirt. Informed consent was obtained and all study questionnaires were administered by trained, local interpreters in the preferred language of the participant. Institutional Review Board approval was obtained from the University of Ghana and the University of Michigan.

2.2 Data collection

2.2.1 Baseline health interviews

A baseline health survey was completed for each participant at their initial study visit (wave I or wave II). The survey included questions on socio-demographics, tobacco use and indoor cooking habits adapted from the Ghana Demographic Health Survey (30).

Standard respiratory symptoms (see Table 1) were derived from the Medical Research Council questionnaire (MRCQ) on respiratory symptoms (31). Symptoms included: usual cough; usual phlegm production; phlegm production longer than 3 months; chronic bronchitis (defined as cough longer than 3 months and phlegm production longer than 3 months); breathlessness when walking; severe breathlessness when walking; wheezing; chest tightness; and shortness of breath.

2.2.2 Personal inhalation exposure to particulate matter

Personal inhalation exposure to size-specific PM was estimated using measurements from a 5-channel optical particle counter (Aerocet 831, Met One Instruments, Oregon, USA) worn in a sampling backpack by each participant during a partial work-shift (e-waste and reference population) or, among reference participants who did not go to work, during completion of daily activities. Specific details on the sampling protocol and how the device works have been described previously (18). The device continuously measures (once every minute) particle counts (sizes $\leq$1, $\leq$2.5, $\leq$4, and $\leq$10 µm in aerodynamic diameter) from the participant's breathing zone and converts them into size-specific mass measurements (as µg m^{-3}). Measures of PM_{10} exceeding 2000 µg m^{-3} (0.3% of the data, $n = 369$ min) were censored to avoid potential bias from coincidence error (i.e., when multiple small particles appear as one larger particle resulting in an overestimate of large particles). $PM_{2.5-10}$ was derived by subtracting $PM_{2.5}$ from PM_{10}. Shift averages for PM_1, $PM_{2.5}$ and $PM_{2.5-10}$ were derived for each participant. Shift peak concentrations were defined as the maximum 5-min means for PM_1, $PM_{2.5}$ or $PM_{2.5-10}$ concentrations for each participant.

Deployment of the personal sampling backpacks for both study groups occurred between 8 and 11 AM and retrieval occurred between 12 and 3 PM. Participants were initially asked to wear the sampling backpacks for a minimum of 6 h. The sampling time was initially reduced to 4 h during wave 2 after learning that the majority of workers stopped working after 4 h; reducing the sampling period limited potential confounding from PM exposure caused by the performance of non-e-waste related activities while having the added benefit of reducing participant burden. A subsequent reduction in sampling duration from 4 to 2 h occurred during the Harmattan season (wave 3) when winds from the Saharan Desert transported sand and dust across the region between November and February as the high PM levels could compromise measurements obtained by some of the equipment in the sampling backpacks. The Harmattan winds were expected to impact the personal sampling equipment and inhalation exposure concentrations of both the e-waste and reference populations; studies have shown 4-fold increases in $PM_{2.5}$ concentrations across the Greater Accra Metropolis during Harmattan season in comparison to non-Harmattan seasons (32).

2.2.3 E-waste recovery activities

Image-derived time-activity data were generated for a sub-cohort of e-waste worker participants ($n = 50$) during their work-shifts. Time-lapse images (one per minute) were taken using

TABLE 1 Socio-demographics of the GeoHealth cohort with valid cross-shift pulmonary function tests ($N = 120$; 73 E-waste workers and 47 members of a reference population), Accra, Ghana, 2017–2018.

Characteristic		E-waste	Reference	p-value
Sex (%)	Male	100	100	NA
Age (years) [mean (SD)]		26.5 (6.6)	30.7 (9.2)	<0.01
Country of origin (%)	Ghana	100	97.8	0.39
	Other	0	2.2	
Region of origin (%)	Northern	100	33.3	<0.01
	Other	0	44.4	
	Accra	0	22.2	
Daily income[a] (%)	<= GHS 20	15.1	18.6	0.23
	GHS 21–60	65.8	53.5	
	GHS 61–200	13.7	11.6	
	>200 GHS	5.5	16.3	
Religion (%)	No religion	2.7	0	0.010
	Other	2.7	17.0	
	Muslim	94.5	83.0	
Marital status (%)	Single	45.2	70.2	0.009
	Married	54.8	29.8	
Education (%)	No education	27.4	19.1	0.010
	Less than secondary	60.3	44.7	
	Secondary	12.3	29.8	
	Higher	0	6.4	
Home type (%)	Rented room	27.4	40.4	<0.01
	Rented/owned Kiosk	43.8	6.4	
	Outdoors/ mosque	1.4	0	
	Own home	27.4	53.2	
Use of indoor cooking (%)	Yes	16.4	51.1	<0.01
	No	83.6	48.9	
Method of cooking (%)	Open fire	0	2.2	<0.01
	Stove/Coal pot WITH vent	5.5	8.7	
	Stove/Coal pot WITHOUT vent	0	4.3	
	LPG cook stove	2.7	23.9	

(Continued)

TABLE 1 (Continued)

Characteristic		E-waste	Reference	p-value
	Electricity	6.8	10.9	
	Do not cook indoors	84.9	50.0	
Sleep in same room as cooking (%)	Yes	9.9	17.4	0.36
	No	90.1	82.6	
Tobacco smoke status (%)	Current	25.0	6.5	0.014
	Former	1.4	2.2	
	Never	73.6	91.3	

[a]1 USD was equivalent to ~4.42 GHS at the time of the study.

a wide-angle GoPro Hero4© camera mounted to the shoulder strap of the personal sampling backpack. Details on how the images were processed to derive time-activity data have been described previously (18). Activity categories included: burning e-waste, dismantling e-waste, sorting/loading e-waste, buying/selling e-waste, transporting e-waste and scrap materials, other e-waste activities, other work activities unrelated to e-waste recovery, use of a motorcycle or car, walking, bicycling, smoking or in the presence of tobacco smoke, and not actively working (i.e., sitting, eating or drinking, cell phone use, prayer, and communicating with others).

2.2.4 Pre-and post-shift interviews and self-reported pre-shift exposures

Pre-shift and post-shift interviews were performed for each participant prior to the deployment of the personal sampling backpack (between 8 and 11 AM) and after backpack retrieval (between 12 and 3 PM). The pre-shift survey included questions on current respiratory symptoms and pre-shift exposures. Current respiratory symptoms included: irritation or burning of the eyes, nose or throat; cough; wheezing or whistling sound in chest; shortness of breath, difficulty catching your breath, or a smothering feeling; and chest tightness. Pre-shift exposures include working "yesterday" (the day before the pulmonary function test) and working prior to the pre-shift pulmonary function test (same day). The post-shift survey included the same questions on current respiratory symptoms and tobacco use during the shift. Incident respiratory symptoms were defined as those reported on the post-shift questionnaire and not on the pre-shift questionnaire.

2.2.5 Cross-shift pulmonary function

Pre-shift and post-shift pulmonary function tests were performed at the same time as the pre- and post-shift interviews, i.e., prior to the deployment of the personal sampling backpack (between 8AM and 11AM) and after backpack retrieval (between 12PM and 3PM). Pulmonary function was assessed using the handheld EasyOne Diagnositic spirometry device (NDD Medical Technologies, Andover, MA) following the guidelines of the American Thoracic Society (ATS) (33). Two examiners, a local physician and emergency medical technician, were trained on how to use the device and administer the test. Before beginning the test, age, height and weight were recorded and the maneuver was demonstrated. Participants were coached to take a maximal inspiration and then blast the air out of their lungs into the device as hard, fast and as long (minimum 6 s) as they could. Participants performed a maximum of six maneuvers, and were asked to stop after performing three maneuvers that were considered adequate by both the examiner and an automated quality grade. The device stored the best three maneuvers for each participant.

Pulmonary function parameters of interest included cross-shift changes in forced vital capacity (FVC), forced expiratory volume in one second (FEV1) and the FEV1/FVC ratio. Before calculating cross-shift measures, two trained reviewers graded the acceptability of each of these parameters for each maneuver following the acceptability criteria of the ATS (33). The duration of the exhalation had to be $\geq$4 seconds with a plateau on the volume-time curve showing no change in volume for at least 1 s. A third reviewer was consulted in the event of a discrepancy. As per ATS criteria, the best FEV1 and best FVC values were used even if from two different maneuvers and, when possible, the FEV1/FVC ratio was calculated from the curve with the largest sum FEV1 plus FVC. FEV1 and FVC values were graded for between-maneuver repeatability criteria (i.e., a difference -$\leq$ 0.15 L between the two largest of each values). However, repeatability was not a basis for exclusion as this has been shown in prior research to produce selection bias (34). "Valid" measures are those that met acceptability criteria, and "reproducible" measures are valid measures that also met repeatability criteria. Cross-shift change in FEV1, FVC and FEV1/FVC ratio were calculated for all participants with valid paired pre- and post-shift FEV1, FVC or FEV1/FVC ratio measures. A cross-shift change is defined as the percent change in FEV1, FVC and FEV1/FVC ratio per hour and calculated as: [(post-shift value – pre shift value) / pre-shift value * 100] / shift length (hours)].

Valid test results were expressed as the percentage of the predicted values expected for a "normal" population of the same sex, age, height and race using equations derived from a population-based study of 7, 429 asymptomatic, non-smoking participants of the National Health and Nutrition Examination Survey (NHANES)-III (35). While no established predicted values exist for the African continent or Ghanaians in particular (36), African-Americans are expected to share substantial common ancestry. The NHANES-III sample used to create the predicted values includes 2, 508 African-American participants out of a total of 7, 429 (35).

2.3 Statistical methods

Study groups (e-waste and reference population) were compared across socio-demographic characteristics, baseline respiratory health status, baseline pulmonary function (absolute values and percent predicted), personal inhalational exposure to PM_1, $PM_{2.5}$, $PM_{2.5-10}$, pre-shift exposures and incident respiratory symptoms. The primary health outcomes included cross-shift changes in pulmonary function measures (FEV1, FVC, and FEV1/FVC ratio). The distributions of PM measures were log-normal. A binary log transformation was used; a one-unit change in PM represented a two-fold or doubling effect of PM exposure on the outcome. Associations between cross-shift changes and exposures were estimated using linear mixed effects (LME) models. LME models include a random intercept for participant to account for correlated outcomes among participants in more than one wave of the study. In cases when the random effect for subject was ∼0, linear regression was used to avoid overfitting the model. The main effects for study group, PM_1, $PM_{2.5}$ and $PM_{2.5-10}$ (shift mean and peak concentrations), and pre-shift exposures are presented for each of the primary health outcomes. All models were adjusted for *a priori* confounders including age, height, the use of cigarettes during the shift, study wave, and day of week. Participants with a history of asthma ($n = 2$) were excluded from the regression analyses. No participants reported a history of tuberculosis. Differences in the effects of personal PM and pre-shift exposures on cross-shift change in pulmonary function between study groups were tested using both interaction terms added to the fully adjusted models and stratified models. Among e-waste workers only, linear regression models adjusted for age, height and smoking cigarettes during the shift were used to estimate the associations between activities performed during the shift and cross-shift changes in pulmonary function. Activity was parameterized as both a binary variable (performed the activity or not) and a count variable (number of minutes spent performing the activity). All analyses were accomplished using the statistical software R (37).

TABLE 2 Self-reported respiratory health by study groups among the GeoHealth cohort ($N = 120$; 73 E-waste workers and 47 members of a reference population), Accra, Ghana, 2017–2018.

Self-reported respiratory health	E-waste	Reference	p-value
Age (years) [mean (SD)]	26.5 (6.6)	30.7 (9.2)	<0.01
Height (cm) [mean (SD)]	171.4 (6.7)	173.8 (7.2)	0.06
Weight (kg) [mean (SD)]	70.9 (9.7)	73.2 (13.2)	0.28
Body mass index [mean (SD)]	24.1 (2.6)	24.2 (3.7)	0.91
Asthma, ever (%)	1.4	2.2	1.00
TB, confirmed by doctor (%)	0	0	NA
Usual Cough (%)	30.1	21.3	0.39
Cough, longer than 3 months (%)	13.7	12.8	1.00
Usual phlegm production (%)	25	21.3	0.67
Phlegm production, longer than 3 months (%)	13.7	10.6	0.83
Chronic bronchitis (%)	6.8	4.3	0.70
Breathlessness when walking (%)	8.3	2.1	0.24
Severe breathlessness when walking (%)	6.9	2.1	0.40
Wheezing (%)	23.6	8.5	0.049
Chest tightness (%)	32.9	17.0	0.089
Shortness of Breath (%)	15.1	6.4	0.24

3 Results

3.1 Sample

Personal sampling was conducted during 175 monitored shifts (from 131 unique participants; 81 e-waste workers and 50 members of the reference population). Complete data sets (including personal PM and a valid cross-shift FEV1 and/or FVC) were available for 156 shifts (120 unique participants; 73 e-waste workers and 47 members of the reference population). More e-waste worker participants (9%) than reference population participants (6%) were removed from the analysis due to the lack of a cross-shift pulmonary function measures that met ATS acceptability criteria. Socio-demographic characteristics of participants with complete data did not differ significantly from the full cohort (data not shown).

Unexpectedly, the population of MZ residents chosen as the reference population for this study differed from the e-waste worker population across the majority of socio-demographic characteristics (Table 1). In comparison to the reference population, e-waste workers were younger, with lower incomes and education, had a higher prevalence of current cigarette smokers (25% vs. 6%) and lived less frequently in an abode where indoor cooking routinely took place (16% vs. 51%). Among the reference population that did cook indoors, liquid petroleum (23.9%) or electric stoves (10.9%) were most common followed by (non-electric) stove or coal pots with (8.7%) and without vents (4.3%). The majority of both populations were Muslim in a majority Christian city and country. Among the 70% of those currently employed in the reference population, "current" jobs included traders ($n = 15$), skilled workers (e.g., tailors, electricians) ($n = 14$), *tro-tro* (public van) drivers and driver assistants ($n = 7$) and other ($n = 9$).

3.2 Baseline respiratory health status

There were no self-reported cases of tuberculosis and only two cases of asthma confirmed by a doctor among the sub-cohort (Table 2). E-waste workers reported a higher prevalence of all of the 10 respiratory symptoms queried in comparison to the reference population, but only wheezing (23.6% vs. 8.5%, *p*-value: 0.049) met statistical significance at the 0.05 alpha level.

3.3 Exposure to particulate matter

The average duration of monitored shifts was longer for the reference population (265.9, range 171–399 min) than the e-waste workers (230.3, range: 148–370 min); 18% of e-waste workers had a shift length $\leq$ 3 h in comparison to 3% of the reference population. Mean and peak personal PM_1, $PM_{2.5}$ and $PM_{2.5-10}$ concentrations for each participant's

TABLE 3 Measured and self-reported exposures during the work-shift and prior to the pre-shift pulmonary function test (PFT) by study group, $N = 120$ unique participants and $N = 156$ work-shifts in the GeoHealth cohort, Accra, Ghana, 2017–2018.

		Total	E-waste	Reference	*p*-value
N work-shifts		156	92	64	
Personal inhalation exposure ($\mu g\ m^{-3}$)					
PM1, shift mean	Median (IQR)	38.2 (33.8)	51.4 (32.2)	26.3 (12.8)	<0.001
	Mean (SD)	46.2 (27.3)	57.6 (26.4)	29.6 (18.6)	<0.001
PM_1, shift peak	Median (IQR)	104.4 (103.7)	136.7 (102.0)	54.5 (52.8)	<0.001
	Mean (SD)	123.9 (86.0)	156.1 (84.6)	76.5 (63.3)	<0.001
$PM_{2.5}$, shift mean	Median (IQR)	51.3 (37.7)	63.8 (28.9)	32.9 (10.7)	<0.001
	Mean (SD)	55.8 (27.7)	69.6 (24.8)	35.6 (17.4)	<0.001
$PM_{2.5}$, shift peak	Median (IQR)	142.1 (144.6)	173.0 (111.3)	68.5 (83.5)	<0.001
	Mean (SD)	159.5 (102.4)	195.7 (93.9)	106.6 (91.2)	<0.001
$PM_{2.5-10}$, shift mean	Median (IQR)	66.4 (51.7)	77.4 (65.5)	47.4 (34.9)	<0.001
	Mean (SD)	86.6 (75.4)	101.1 (76.9)	65.3 (68.4)	<0.001
$PM_{2.5-10}$, shift peak	Median (IQR)	221.3 (254.8)	257.5 (256.6)	170.4 (199.9)	0.004
	Mean (SD)	314.1 (283.8)	331.6 (249.0)	288.5 (328.8)	0.004
Smoked cigarettes during the shift, self-report	Yes	28	24 (85.7)	4 (14.3)	0.001
Pre-shift exposures					
Worked "yesterday" (day prior to PFT)	Yes	113	78 (69.0)	35 (31.0)	<0.001
Worked prior to pre-shift PFT (same day)	Yes	73	54 (74.0)	19 (26.0)	0.001

shift are summarized by study group in Table 3. Mean and peak personal PM_1, $PM_{2.5}$ and $PM_{2.5-10}$ concentrations were significantly higher ($p < 0.001$) among the e-waste workers in comparison to the reference population (Table 3). The prevalence of tobacco use during the work shift was also higher among e-waste workers than the reference population (86% vs. 14%, $p < 0.001$). A significantly larger proportion of e-waste workers in comparison to the reference population reported working the day before (69% vs. 31%, $p < 0.001$) and working prior to the pre-shift pulmonary function test (74% vs. 26%, $p < 0.001$) (Table 3). Less than half of the reference participants (39%) reported working *during* the shift; among those that did, work activities associated with the highest tertiles of PM_1, $PM_{2.5}$ and $PM_{2.5-10}$ concentrations included selling marijuana, "trading," "digging" and "tiling."

3.4 Cross-shift change in pulmonary function

Among the 156 monitored shifts with complete data, a total of 153, 123 and 120 cross-shift measures of FEV1, FVC and FEV1/FVC ratio were obtained. Of the 156 eligible sessions, 46% ($n = 72$) were reproducible (i.e., a difference $\leq$0.15 L between the two largest of each values) (33) (Supplementary Table 1). The proportion of reproducible pulmonary function maneuvers among e-waste (56%) and reference population (54%) participants were similar.

Pre-shift (baseline) pulmonary function measures for the total cohort and by study group are described in Table 4. Average pre-shift FEV1 and FVC measures for the whole cohort were 86% and 90% of the predicted value for a normal population of the same age, height and race, respectively (Table 4). When comparing study groups, pre-shift FEV1 and FEV1/FVC ratio were lower among e-waste workers (Table 4). Pre-shift FVC averages and percent predicted values were modestly higher among e-waste workers in comparison to the reference population. These results were replicated when using only reproducible values (Supplementary Table 1).

Unadjusted cross-shift changes in FEV1 and FVC were negative for both study groups, indicating a decrease in pulmonary function throughout the work-shift (Table 4). Cross-shift changes in FEV1/FVC ratios were not observed in either study group. E-waste workers had larger cross-shift changes in FEV1 (−2.2 + 9.4%) and FVC (−1.2 + 7.1%) than the reference population (−1.5 + 6.4% and −0.8 + 6.0% respectively); however, the differences did not reach statistical significance. More e-waste workers (18.7%) than the reference population (7.8%) had a post-shift FEV1 percent predicted below 70% (p-value: 0.094). When using only reproducible results, the reference population had greater decreases in both FEV1 and FVC, however, the differences between groups did not reach statistical significance (Supplementary Table 1).

TABLE 4 Cross-shift pulmonary function (PF) by study group among the GeoHealth cohort ($N = 120$ unique participants), Accra, Ghana 2017–2018.

		Overall[a]	E-waste	Reference	*p*-value[b]
	N matched pre- and post-shift PF tests	156	92	64	
	Age (years) [mean (SD)]	28.5 (7.9)	26.7 (6.4)	31.0 (9.1)	0.01
	Height (cm) [mean (SD)]	171.6 (7.0)	170.7 (6.9)	172.9 (7.0)	0.05
	Weight (kg) [mean (SD)]	71.1 (11.3)	70.3 (10.1)	72.4 (12.8)	0.25
Pre-shift PF	FEV1, pre-shift [mean (SD)]	3.1 (0.5)	3.0 (0.5)	3.2 (0.5)	0.06
	FEV1 % predicted [mean (SD)]	86.3 (12.3)	84.9 (13.5)	88.4 (10.2)	0.08
	FEV1 % predicted <70 = yes (%)	12 (7.8)	9 (9.9)	3 (4.8)	0.39
	Best FVC, pre-shift [mean (SD)]	3.8 (0.6)	3.8 (0.5)	3.7 (0.6)	0.77
	FVC % predicted [mean (SD)]	90.2 (12.9)	91.6 (13.4)	88.3 (11.9)	0.15
	FVC % predicted <70 = 1 (%)	7 (5.4)	3 (3.9)	4 (7.5)	0.62
	FEV1/FVC Ratio [mean (SD)]	0.8 (0.1)	0.8 (0.1)	0.8 (0.1)	0.01
	Ratio <0.7 = yes (%)	8 (6.3)	8 (10.7)	0 (0.0)	0.04
Post-shift PF	FEV1, post-shift [mean (SD)]	3.0 (0.5)	2.9 (0.5)	3.1 (0.5)	0.04
	FEV1 % predicted [mean (SD)]	84.5 (13.1)	82.8 (14.2)	86.9 (10.9)	0.06
	FEV1 % predicted <70 = 1 (%)	22 (14.2)	17 (18.7)	5 (7.8)	0.09
	Best FVC, post-shift [mean (SD)]	3.7 (0.6)	3.7 (0.6)	3.7 (0.6)	0.83
	FVC % predicted [mean (SD)]	88.2 (13.6)	89.1 (15.4)	86.8 (10.4)	0.34
	FVC % predicted <70 = yes (%)	8 (5.8)	5 (6.1)	3 (5.5)	1.00
	FEV1/FVC Ratio [mean (SD)]	0.8 (0.1)	0.8 (0.1)	0.8 (0.1)	0.01
	Ratio <0.7 = yes (%)	10 (7.4)	10 (12.3)	0 (0.0)	0.02
Cross-shift change	% Change in FEV1 [mean (SD)]	−1.9 (8.2)	−2.2 (9.4)	−1.5 (6.4)	0.61
	% Change in FVC [mean (SD)]	−1.0 (6.7)	−1.2 (7.1)	−0.8 (6.0)	0.77
	% Change in FEV1/FVC ratio [mean (SD)]	−0.5 (4.7)	−0.8 (5.2)	−0.1 (3.9)	0.41

[a]Number of matched sessions with a valid pre and post-shift FEV1, FVC and FEV1/FVC ratio were 153, 123 and 120, respectively; [b]T-test p-values are comparing exposed and reference populations.

3.5 Association between PM and cross-shift change in pulmonary function

Measures of association between personal inhalation exposure to PM_1, $PM_{2.5}$ or $PM_{2.5-10}$ and percent change per hour in FEV1, FVC, and FEV1/FVC ratio adjusted for age, height, the use of cigarettes during the shift, study wave, and day of week for the full sample and stratified by study group are summarized in Figure 1 and Supplementary Table 2. As a whole, the results show no signal that increasing levels of PM were associated with a decrement in pulmonary function throughout the work-shift in either study population. Contrary to our expectations, the directions of the estimated risk ratios were overwhelmingly positive; however, the 95% confidence intervals for all tested associations crossed zero, indicating no effect of PM on pulmonary function. When using only reproducible pulmonary function values, we still did not see any negative associations between PM exposure concentrations and cross-shift change in PF. We did, however, observe a positive association between a doubling of mean and peak PM_1 concentrations throughout the work-shift with FEV1 and FVC in the full cohort after adjusting for age, height and smoking during the shift (Supplementary Figure 1).

3.6 Association between pre-shift exposures and cross-shift change in pulmonary function

Working "yesterday" was associated with a 1.22% decrease in FVC per hour (95% CI: −2.18, −0.27) after adjusting for age, height, use of cigarettes during the shift, wave of data collection and day of week (Supplementary Table 2). When stratified by study group, e-waste worker participants who reported working "yesterday" had an average 2.4% (95% CI: −4.04, −0.81) and 1.2% (95% CI: −3.07, 0.69) cross-shift decrement in FVC and FEV1, respectively, while essentially no association was observed among the reference population (Supplementary Figure 2, Supplementary Table 3). When using only reproducible values, the same trend was observed (Supplementary Figure 3). Working prior to the pre-shift pulmonary function test was not associated

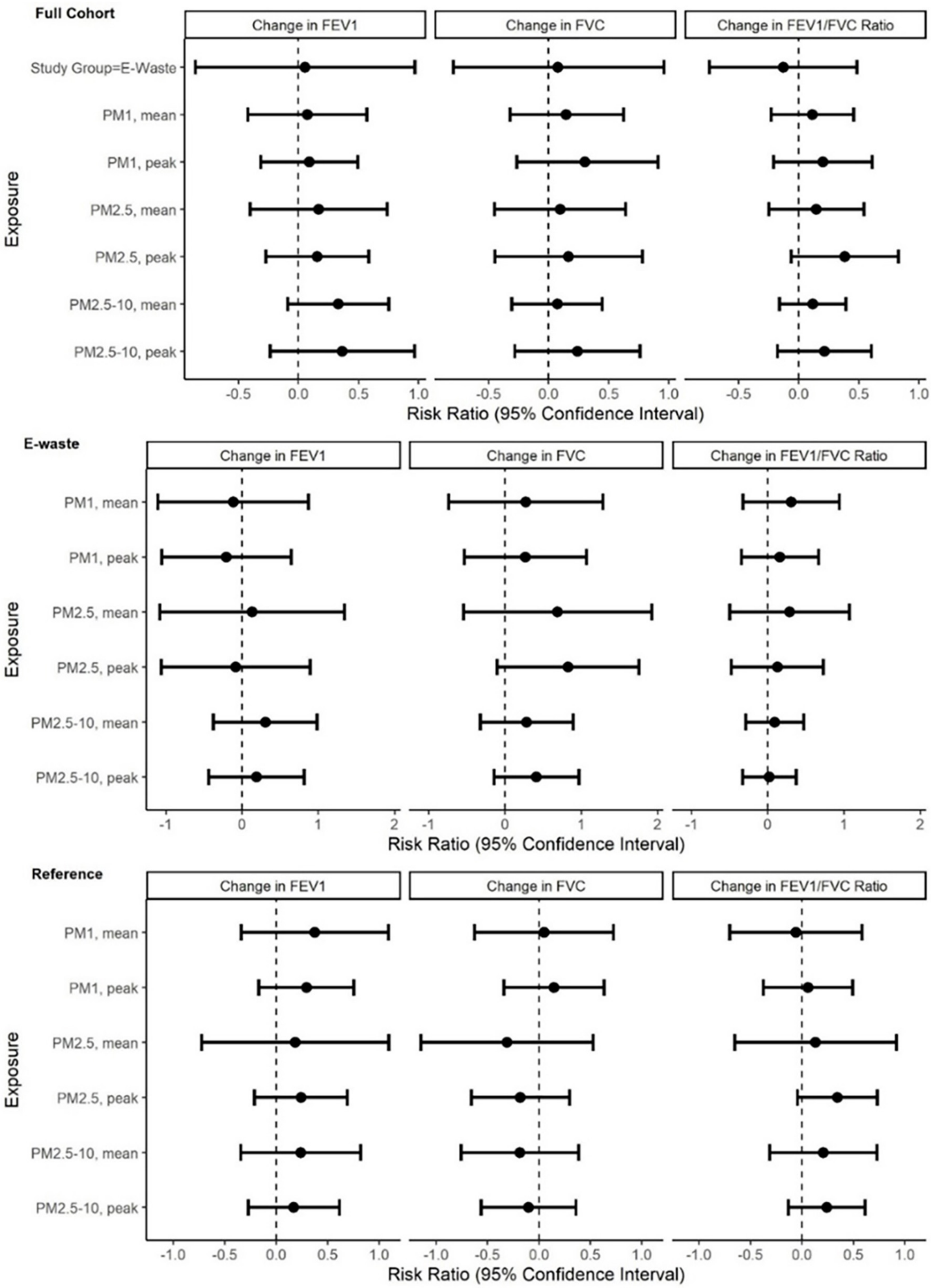

FIGURE 1
Associations between percent change in pulmonary function outcome per doubling of personal inhalation exposure to PM_1, $PM_{2.5}$ and $PM_{2.5-10}$ for the full cohort ($N = 156$) and stratified by study group ($n = 92$ E-waste; $n = 64$ Reference participants) in the GeoHealth cohort at Agbogbloshie, Accra, Ghana, 2017–2018. Effect estimates and 95% confidence intervals were derived using linear mixed effects models with a random intercept for participant to account for correlated outcomes among participants in more than one wave of the study. Models were adjusted for age, height, the use of cigarettes during the shift, study wave, and day of week.

TABLE 5 Descriptive statistics on image-derived activities performed by e-waste worker participants during their monitored work shift at Agbogbloshie, Accra, Ghana, 2017–2018.

Activity	Mean duration in minutes (range)	Number of participants who performed the activity
Burning e-waste	69.6 (7, 147)	5
Dismantling e-waste	78.4 (3, 238)	8
Buying, selling e-waste	25.5 (16, 35)	2
Transporting materials	28.0 (4, 88)	8
Sorting, loading e-waste	59.7 (5, 170)	10
Motorcycle or car use	32.6 (4, 129)	17
Bicycling	29.5 (17, 37)	4
Walking	31.4 (5, 113)	45
Not actively working	105.2 (9, 225)	43
Presence of tobacco smoke	48.0 (23, 67)	3
Other, e-waste related work	69.7 (16, 116)	3
Other, non-e-waste related work	6.0 (4, 8)	2

with a measured increase or decrease in FEV1, FVC or the ratio in either study group.

3.7 Incident symptoms and pulmonary function

E-waste workers had a higher incidence of all symptoms in comparison to the reference population (Supplementary Table 4). More than twice as many e-waste workers than reference population reported incident chest tightness (9.8% vs. 3.1%) cough (17.4% vs. 11.1%), shortness of breath (9.8% vs. 6.2%) and wheezing (16.3% vs. 7.8%). However, the total numbers were small and the difference did not reach statistical significance at the 0.05 alpha level. No statistical associations were observed between incident symptoms and cross-shift changes in pulmonary function (data not shown).

3.8 Activities performed by e-waste workers and pulmonary function

The average length and range of time for each activity performed by the e-waste worker participants with image-derived data ($n = 50$) is summarized in Table 5. The most common activities performed among the e-waste workers included not actively working (e.g., sitting, cell phone use, communicating), walking, motorcycle or car use, sorting/ loading and dismantling e-waste. Very few participants ($n = 5$) performed burning e-waste during the monitored work shift.

Among the activities performed by five or more participants, comparisons between unadjusted cross-shift changes in FEV1, FVC and FEV1/FVC ratio for those who performed the activity to those who did not showed no measurable differences (data not shown). In models adjusted for age, height and smoking during the shift, associations between all of the activities and percent changes in FEV1, FVC or FEV1/FVC were all close to zero (Figure 2). A modestly protective effect of burning e-waste and dismantling e-waste was found; workers who burned and dismantled e-waste at least once during the work-shift had a 0.38 (−1.84, 2.59) and a 0.69 (−1.11, 2.50) percent increase in FEV1 per hour, respectively, in comparison to those who did not perform those activities at all. Interestingly, not actively working was associated with a −1.05 (−2.88, 0.78) percent decrease in FEV1 per hour in comparison to those who did not perform that activity (i.e., were not ever "not actively working") at all. When activity was parameterized as a count variable (length of time performing the activity), similar results were found with one exception: an increase in the number of minutes a worker performed dismantling was associated with an improvement in FVC per hour (Supplementary Figure 4).

4 Discussion

The aim of this study was to examine the effect of personal inhalation exposure to PM on pulmonary function among e-waste recovery workers and a reference population in Accra, Ghana using a cross-shift study design. Personal inhalation exposure to PM_1, $PM_{2.5}$ and $PM_{2.5-10}$ among e-waste workers at the Agbogbloshie e-waste site were nearly double the levels experienced by the comparison group, Accra residents who live and work near a heavily trafficked road. Both populations, however, experienced mean concentrations of personal $PM_{2.5}$ that far exceeded the WHO's ambient Air Quality Guideline recommendations (24-h mean: 15 $\mu g\ m^{-3}$) (38). Although the mean PM concentrations for the e-waste workers in this study would fall below the general particulate occupational standard used by U.S. Department of Labor Occupational Safety and Health Administration (8-h time-weighted average: 5000 $\mu g\ m^{-3}$), it is not protective in an informal setting given the likelihood of toxic metals and organic compounds in the PM (20, 39), and because there is nothing approaching the hierarchy of controls at the worksite that is intended to reduce exposure to as low as practicable when toxic substances are present (40). Following an average monitored shift of 4 hours ($\pm$44 min), declines in pulmonary function (FEV1 and FVC) were observed among e-waste workers and the reference population. Although declines in FEV1 and FVC were largest among e-waste workers, they did differ significantly from those observed among the reference population. Exposure to personal PM_1, $PM_{2.5}$ and $PM_{2.5-10}$ concentrations was not associated with cross-shift declines in pulmonary function in either study group. Cross-shift changes in pulmonary function were also not significantly associated with e-waste recovery activities performed during the shift.

The lack of a significant association between cross-shift pulmonary function and PM among e-waste workers may be attributable to health-related job selection or the "healthy worker" effect (41–43). Current workers selected for the study may still be

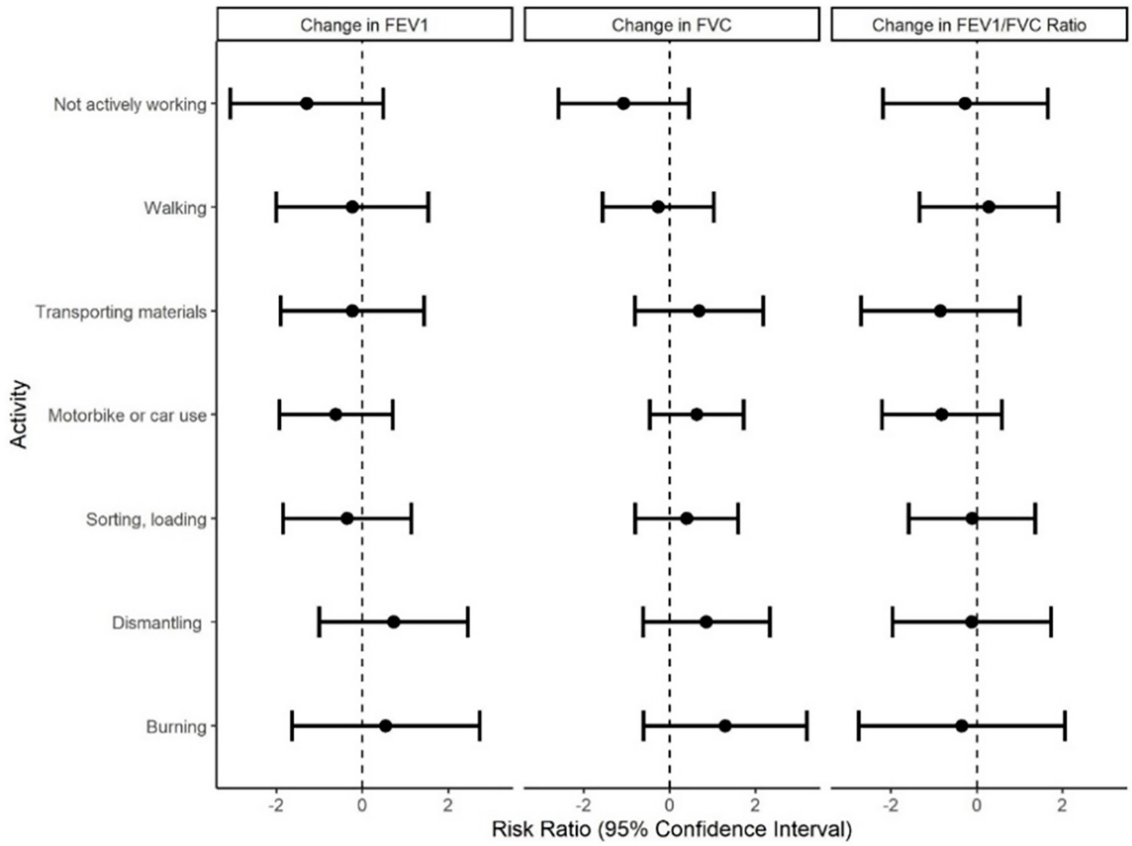

FIGURE 2
Associations between percent change in pulmonary function outcomes and performance of activities among e-waste recovery workers ($n = 50$) in the GeoHealth cohort at Agbogbloshie, Accra, Ghana, 2017–2018. Effect estimates and 95% confidence intervals were derived using linear regression adjusted for age, height and smoking cigarettes during the shift.

able to tolerate high levels of PM exposure and be more resistant to short-term respiratory effects than the workers who have left the job due to health complications (44). In previous studies, E-waste recovery workers have reported leaving their jobs to return to their family homes in other regions of Ghana when health complications arise (45).

There are, however, alternative explanations to our study findings. First, E-waste workers may have experienced a decline in pulmonary function associated with e-waste-related exposures, but only during their initial months of employment and prior to our study. Several studies have observed differential effects of metal working fluids and respirable dust among machinists and coal miners, respectively, according to years of employment; the dose-response curve flattens out with increasing years of employment suggesting a threshold effect (46–48). In our sample, the average length of employment at Agbogbloshie among e-waste participants (8.8 $\pm$ 6.6 years) was too long to measure initial reductions in pulmonary function (48, 49). Second, we may not have captured a true pre-shift measure of pulmonary function, due to the fact that most e-waste workers reported living at the e-waste site (89%) and having already worked prior to the pre-shift test (74%). Lastly, any cross-shift changes may have been diluted by the diurnal variation in pulmonary function. A limitation of using pulmonary function measures without a resting control is that we do not know how much of the variation is due to natural diurnal variation (49). Given the lack of a true pre-shift pulmonary function assessment and the diurnal variation in pulmonary function, a shift duration of 4 h ($\pm$44 min) from morning to mid-afternoon may have been too short to capture a measurable change in lung function due to occupational exposures.

The steeper decline in pulmonary function among e-waste workers who reported working the day before the cross-shift pulmonary function assessments, in comparison to those who did not, provides some evidence of respiratory health effects from e-waste associated PM pollution occurring in the range of 24–96 h before the measurements took place. Time-series studies using distributed lag models to examine the association between ambient air pollution and health outcomes, such as mortality, asthma and hospitalizations for myocardial infarction, have found larger effect sizes for 1 to 6 day lag exposures in comparison to same-day exposure (50–53).

The high incidence of respiratory symptoms, particularly cough (17%) and wheeze (16%), reported by e-waste workers following an average of 4 h of work is notable. Prior studies have observed a high prevalence of self-reported respiratory symptoms among e-waste workers (45) and children living in the vicinity of an e-waste site (15). Together, these findings provide evidence of an acute respiratory response to e-waste associated pollutants that was undetected by spirometry in our study. In research among World Trade Center responders exposed to toxic dust from the collapse of the World Trade Center in 2001, the frequency and severity of respiratory symptoms were found to be associated with small airways abnormalities that were initially undetected using spirometry (54, 55). In other words, the use of routine spirometry may not have been sensitive enough to detect abnormalities in lung function among symptomatic e-waste workers.

Given the high variability in pulmonary function outcomes, spirometry, as performed in this study, may have had low levels of accuracy. The standard deviations in valid cross-shift FEV1 and FVC response variables for the whole cohort, $\pm$8.25 and $\pm$6.67, respectively, were high. In a sensitivity analysis, we compared the residual variance for cross-shift FEV1 and FVC response variables between study groups using a distributional model adjusted for age, height and the use of cigarette smoking during the shift ("brms" package). We found that the residual variance was significantly higher for FEV1 (95% CI: 2.33, 3.14) and FVC (95% CI: 1.88, 2.60) among the e-waste study group in comparison to the reference population. Unequal variance may be a result of field conditions, unmeasured factors, or challenges in eliciting a valid pulmonary function test among e-waste workers. There is some evidence that repeated pulmonary function maneuvers exacerbate airflow narrowing among individuals with prevalent obstructive lung diseases, including asthma, making it harder to achieve valid test results (56). When using only reproducible pulmonary function values, the main results did not change; however, the reproducible models had limited power and may be excluding participants with accelerated declines in pulmonary function rather than those with measurement error (34) .

The application of a gold standard, cross-shift study design, in an informal occupational setting is a strength of this study. The combination of spirometry to assess pre- and post-shift pulmonary function with continuous measures of personal inhalation exposure to three PM sizes (<1, <2.5 and 2.5–10 μm) provided a rich dataset from which causal evidence can be generated, in addition to contributing to the limited available evidence on respiratory health among residents of Accra, Ghana. Continuous PM concentrations allowed us to examine the effects of both daily mean and the very high peak PM concentrations which are unique to e-waste recovery, on pulmonary function. Lessons related to the measurement of cross-shift pulmonary function among informal e-waste workers can inform future studies in other non-traditional occupational settings that are grappling with similar challenges (e.g., the lack of separation between work and life activities).

This work has several limitations. Breathing zone concentrations of PM were estimated using optical measurements rather than gravimetric mass measurements (considered the reference approach), which may be associated with measurement error. Based on simulations and experiments described in our previous work, it was concluded that the optical-based measurements underestimated the true PM concentrations and that particle losses were greatest among the larger sized particles (i.e., $PM_{2.5-10}$) (19, 39). Pre-shift pulmonary function measurements were limited by the fact that most e-waste worker participants reported living and sleeping on or near to the worksite. The shift duration may have been too short to capture significant changes in pulmonary function considering the lack of a true "pre-shift" assessment. Limited statistical power, particularly among the reference population, impeded our ability to make comparisons that we would have liked to make (i.e., between e-waste workers who did not work yesterday *and* did not work prior to the pre-shift assessment with the same subcategories among the reference population). The absence of an inception cohort limited our ability to observe possible early decrements in lung function experienced among new workers in comparison to seasoned workers. The high degree of variability in the pulmonary function data may be indicative of inaccuracies; such uncertainty in the data is hard to overcome. The sample size of e-waste worker participants with image-derived activity data was too small to establish reliable results.

Future studies in informal settings where workers commonly live and work in the same vicinity should aim to monitor participants for a longer period, possibly over the course of multiple, consecutive workdays starting with the lightest exposure day. With participant cooperation, performing spirometry at multiple time-intervals could help distinguish between diurnal variation and exposure-related change in pulmonary function. We also recommend the use of a stratified recruitment strategy to include workers with varying lengths of employment and, if possible, a cohort of former e-waste recovery workers. If possible, it would be helpful to perform spirometry or alternative techniques for observing distal airway function in a health center using equipment that allows medical staff to review the results immediately to avoid uncertainty in the data. Alternative longitudinal study designs that estimate total or cumulative exposure and account for exposure mixtures are also needed.

5 Conclusions

E-waste recovery is associated with high concentrations of PM pollution (13, 18, 57). The short- and long-term respiratory-related occupational health burden due to e-waste associated PM pollution is unknown, but likely to be substantial. In using a cross-shift study design that combined morning and afternoon pulmonary function assessments with personal monitoring of PM pollution, we contributed to a limited knowledge base on acute respiratory health effects from e-waste recovery work. In this sample, cross-shift declines in pulmonary function were not associated with linked PM_1, $PM_{2.5}$ and $PM_{2.5-10}$ breathing zone concentrations. The limitations we encountered in conducting the study, including the inability to capture a true pre-shift pulmonary function assessment among e-waste workers who sleep at the site, an average shift length of <4 h, and uncertainty in the pulmonary function data, are plausible explanations for the null findings, which should be interpreted with caution. The challenges encountered in this study highlight how social and economic disparities that underlie the growth of informal economies contribute to occupational hazards themselves (58). In informal sectors, where workers live and work in the same vicinity, ensuring a safe place to sleep goes hand in hand with having a safe place to work.

Data availability statement

The datasets presented in this article are not readily available because of privacy and ethical restrictions. Requests to access the datasets should be directed to jfobil@ug.edu.gh.

Ethics statement

This study involving humans was approved by the University of Ghana and the University of Michigan. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

ZL: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. MO'N: Conceptualization, Funding acquisition, Methodology, Supervision, Writing – original draft, Writing – review & editing. SB: Writing – original draft, Writing – review & editing, Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Supervision. BM: Formal analysis, Methodology, Writing – original draft, Writing – review & editing. JF: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. TR: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. ZL was funded by the Rackham Predoctoral Fellowship, the National Institute of Occupational Safety and Health (T42 OH008455), and the Dow Chemical Company Foundation through the Dow Sustainability Fellows Program at the University of Michigan. The parent study was supported by the ½ West Africa-Michigan CHARTER in GEOHealth with funding from the United States National Institutes of Health/Fogarty International Center (US NIH/FIC) (paired grant #: 1U2RTW010110-01/5U01TW010101) and Canada's International Development Research Center (IDRC) grant #: 108121-001. We also acknowledge support from the National Institute for Environmental Health Sciences (P30 ES017885, R01ES016932, and R01ES017022).

Acknowledgments

We acknowledge the workers at the Agbogbloshie e-waste site and the residents of Madina Zongo who dedicated their time to participate in this research study; our colleagues and the field team at the University of Ghana School of Public Health, especially Afua Amoabeng, who helped conduct and review pulmonary function tests; and the Consulting For Statistics, Computing and Analytics Research (CSCAR) staff, especially Joseph Dickens, at the University of Michigan.

Conflict of interest

The authors declare that this study received funding from the Dow Chemical Company Foundation through the Dow Sustainability Fellows Program at the University of Michigan. The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2024.1368112/full#supplementary-material

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TYPE Original Research
PUBLISHED 24 May 2024
DOI 10.3389/fpubh.2024.1401347

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OPEN ACCESS

EDITED BY
Mohd Kamil Hussain,
Govt. Raza Post Graduate College, Rampur, India

REVIEWED BY
Aziz-Ur-Rahim Bacha,
Harbin Institute of Technology, Shenzhen, China
Shakir Ahamad,
Aligarh Muslim University, India

*CORRESPONDENCE
Yu Zuo
✉ 86761568@qq.com

†These authors have contributed equally to this work

RECEIVED 18 March 2024
ACCEPTED 07 May 2024
PUBLISHED 24 May 2024

CITATION
Cheng Z, Kong Y, Yang W, Xu H, Tang D and Zuo Y (2024) Association between serum copper and blood glucose: a mediation analysis of inflammation indicators in the NHANES (2011–2016).
Front. Public Health 12:1401347.
doi: 10.3389/fpubh.2024.1401347

Association between serum copper and blood glucose: a mediation analysis of inflammation indicators in the NHANES (2011–2016)

Zijing Cheng[1†], Yuzhe Kong[1†], Wenqi Yang[1], Haitao Xu[1], Decheng Tang[2] and Yu Zuo[3]*

[1]Xiangya School of Medicine, Central South University, Changsha, Hunan, China, [2]Department of Management Science, School of Management, Fudan University, Shanghai, China, [3]Third Xiangya Hospital, Central South University, Changsha, China

Background: The rising prevalence of diabetes underscores the need for identifying effective prevention strategies. Recent research suggests environmental factors, particularly heavy metals like copper, significantly influence health outcomes, including diabetes, through mechanisms involving inflammation and oxidative stress. This study aims to explore how serum copper levels affect blood glucose, employing NHANES data from 2011 to 2016, to provide insights into environmental health's role in diabetes prevention and management.

Methods: The study analyzed data from 2,318 NHANES participants across three cycles (2011–2016), focusing on those with available data on serum copper, inflammatory markers, and blood glucose levels. We utilized principal component analysis for selecting inflammatory markers, mediation analysis to examine direct and indirect effects, multiple linear regression for assessing relationships between markers and glucose levels, and weighted quantile sum regression for evaluating individual and collective marker effects, adjusting for demographic variables and serum copper.

Results: Participants averaged 42.70 years of age, with a near-even split between genders. Average serum copper was 119.50 µg/dL, white blood cell count 6.82 × 109/L, and fasting blood glucose 107.10 mg/dL. Analyses identified significant mediation by inflammatory markers (especially white blood cells: 39.78%) in the copper-blood glucose relationship. Regression analyses highlighted a positive correlation between white blood cells (estimate: 1.077, 95% CI: 0.432 to 2.490, $p = 0.013$) and copper levels and a negative correlation for monocyte percentage (estimate: −1.573, 95% CI: 0.520 to −3.025, $p = 0.003$). Neutrophil percentage was notably influential in glucose levels. Sensitive analyses confirmed the study's findings.

Conclusion: Serum copper levels significantly impact blood glucose through inflammatory marker mediation, highlighting the importance of considering environmental factors in diabetes management and prevention. These findings advocate for public health interventions and policies targeting environmental monitoring and heavy metal exposure reduction, emphasizing the potential of environmental health measures in combating diabetes incidence.

KEYWORDS

serum copper, inflammatory factor, blood glucose, National Health and Nutrition Examination Survey, mediation analysis

1 Introduction

High blood glucose, indicative of diabetes, leads to a significant and often irreversible decline in health, contributing to cardiovascular diseases, kidney failure, and vision loss (1–4). It represents a major cause of disability worldwide, with a growing prevalence especially alarming among the older adult (5). These highlight the urgency in identifying effective primary prevention measures for managing high blood glucose (BG) levels (6, 7).

One of the myriad factors influencing BG levels is the concentration of heavy metals in serum. Beyond dietary influences, the prevalence of diabetes mellitus is notably exacerbated by exposure to various environmental contaminants, including heavy metals, whose presence in the atmosphere has surged alongside industrialization (8–10). Research indicates a substantial positive correlation between the concentration of heavy metals in the blood and their atmospheric levels, suggesting that the accumulation of specific heavy metals in the human body impacts BG concentration (11). For instance, cadmium (Cd) accumulation in insulin-producing β-cells can diminish insulin release and elevate BG levels, while lead (Pb) has been linked to increased insulin resistance and a higher risk of diabetes mellitus (12, 13).

Inflammation also plays a critical role in regulating BG levels. Insights from the Framingham Offspring Study reveal a direct correlation between insulin resistance and elevated markers of oxidative stress. This oxidative stress compromises the ability of muscle and adipose tissues to absorb glucose, as well as impairing pancreatic islet cells' insulin secretion capacity, thereby contributing to higher BG concentrations (14). Reactive oxygen species generated within the body inflict damage on cellular DNA, membranes, lipids, and proteins, further inducing the expression of inflammatory genes. Such inflammation disrupts insulin-mediated metabolic pathways, culminating in insulin resistance (15).

Serum heavy metal concentrations are mainly related to the external environment, with heavy metals entering the body through inhalation, ingestion, and dermal contact, while inflammation and oxidative stress are usually caused by other *in vivo* abnormalities (16). Relevant studies have shown that heavy metal exposure induces systemic inflammatory responses, and disruption of metal ion homeostasis can also lead to oxidative stress, for example, Cu induces oxidative stress through two pathways, namely, catalyzing the formation of ROS via a related reaction as well as decreasing glutathione levels, and zinc deficiency increase oxidative damage levels to some degree, thus it is envisioned that serum heavy metals affect BG levels as being mediated by inflammation and oxidative stress *in vivo* (16–18).

However, although existing researches have highlighted the roles of diet and genetic factors in the onset of diabetes, in-depth studies into the impact of environmental factors, particularly heavy metals, on diabetes were scarce. As environmental exposure to heavy metals such as Cd has been confirmed to increase the risk of diabetes, there still remains a significant research gap regarding how copper, a common environmental metal, affects blood glucose levels through internal biological processes. Moreover, although inflammation was widely considered key pathways in the progression of diabetes, systematic studies on how these pathways mediate the interaction between copper and blood glucose levels are extremely limited. Current research tends to focus on individual biomarkers, overlooking the complexities of the combined effects of multiple markers.

Therefore, the present study was to analyze the correlation between Cu and the concentration of BG and the mediating role of inflammatory factors therein by means of a large-scale cross-sectional study based on the NHANES database.

2 Materials and methods

2.1 Study population

Led by the National Center for Health Statistics (NCHS) at the Centers for Disease Control and Prevention (CDC), NHANES is a biannual program of studies designed to assess the health and nutritional status of adults and children in the United States. NHANES is designed as a multiyear, stratified, clustered four-stage sample of non-institutionalized civilians with fixed sample-size targets for sampling domains defined by age, sex, race and ethnicity, and socioeconomic status, with data released in 2-y cycles.

Participants gave informed consent of the survey process and their rights as a participant, and the survey was approved by the NCHS Review Board.27 Questionnaires were administered in-home followed by standardized health examinations in specially equipped mobile examination centers. Publicly available, de-identified, and detailed health data sets are available on the NHANES website.[1] We acquired all data from the NHANES database that measured Cu levels in serum, including 3 2-y cycles (2011–2016) and all of the cycles with available data on blood and urinary metals and detailed drug use, to create a larger and more geographically diverse sample.

2.2 Exclusion criteria

Data from 3 cycles of NHANES from 2011 to 2016 were used in this study. First, a total of 29,902 individuals participated in the cross-sectional study. After excluding participants without serum copper concentration, inflammatory markers, and BG concentration data types, 2,318 participants were included in this study. In addition to performing PCA by substituting missing values using mean or median for missing values, we excluded participants with missing data, and

1 https://wwwn.cdc.gov/nchs/nhanes/

2,162 participants were included in subsequent statistical analyses. Finally, for sensitivity analyses (when adjusted for demographic variables and metal concentrations), we excluded participants with missing data on demographic variables, and a total of 1873 participants were included (Figure 1).

The NHANES database was approved by the National Center for Health Statistics and was directly accessible to researchers who met eligibility requirements. All participants in the database signed an informed consent form.

2.3 Measurement of serum Cu

Serum specimens were processed and stored under appropriate frozen (−70°C) conditions until they were shipped to the National Center for Environmental Health for analysis. Serum Cu concentrations were measured by inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS)—a multi-element analytical technique capable of trace-level elemental analysis. Liquid samples were introduced into the ICP through a nebulizer and spray chamber carried by a flowing argon stream. Radio-frequency power was coupled into flowing argon to form a plasma. The sample passed through a region of the plasma, and the thermal energy atomized the sample and then ionized the atoms. The ions, along with the argon, entered the mass spectrometer through an interface that separated the ICP from the mass spectrometer. The ions passed through a focusing region, dynamic reaction cell, and the quadrupole mass filter, and finally, were counted in rapid sequence at the detector allowing individual isotopes of an element to be determined. The isotopes measured by this method included Zn (m/z 64), Cu (m/z 65), and Se (m/z 78), as well as the internal standard, gallium (m/z 71). Serum samples were diluted 1 + 1 + 28 with water and diluent containing gallium (Ga) for multi-internal standardization.

2.4 Measurement of inflammation biomarkers

Fasting blood sample of the participants for the laboratory tests was collected by the mobile examination center phlebotomist. The NHANES laboratory manual provides the reference ranges on laboratory parameters in the form of lower and upper limits. Analysis for the complete blood count was done in the mobile examination center, and refrigerated or frozen blood samples were transported and analyzed in the central laboratories for the other parameters.

The DxC800 with lactate dehydrogenase (LDH) reagent (using lactate as substrate) utilizes an enzymatic rate method to measure LDH activity in biological fluids. The DxC600i system or DxC800 system uses a kinetic rate method using a 2-Amino-2-Methyl-1-Propanol (AMP) buffer to measure alkaline phosphatase (ALP)

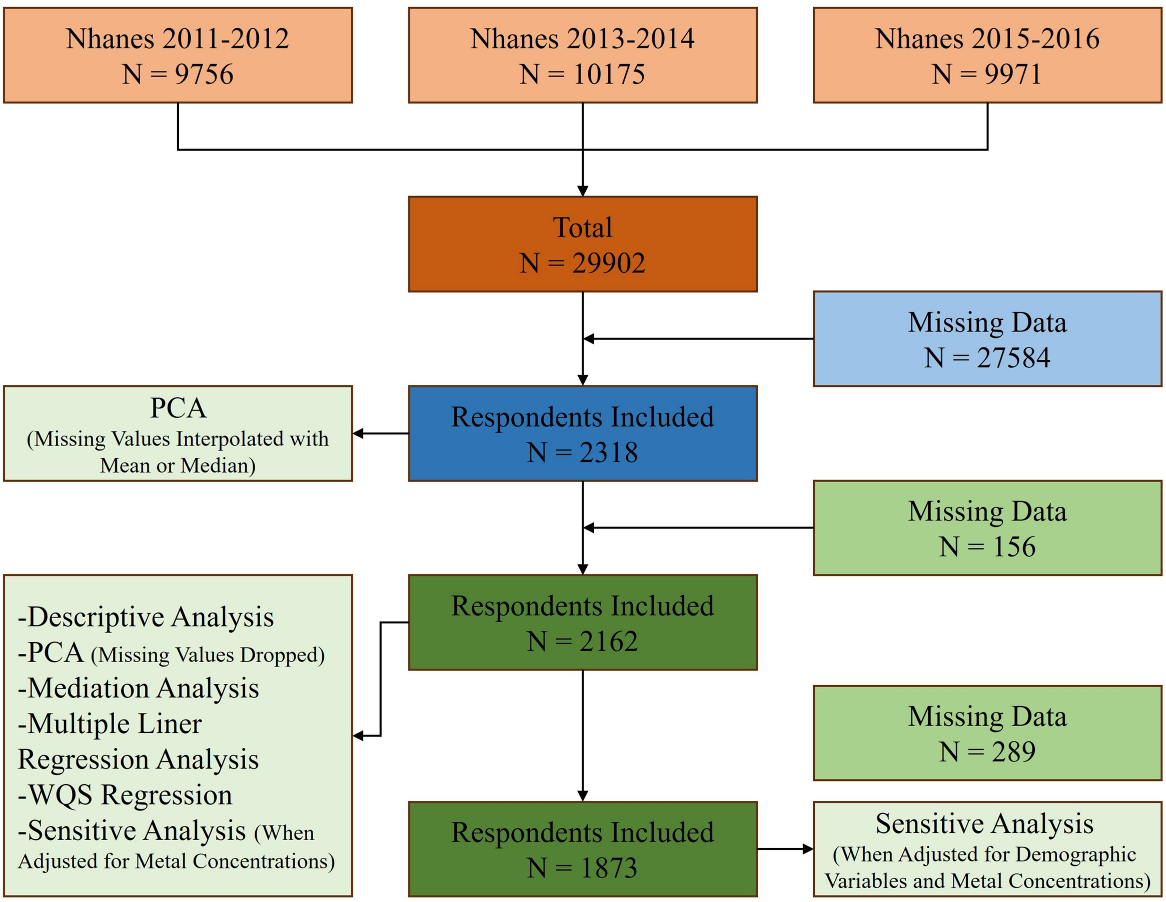

FIGURE 1
Study flowchart.

activity in serum or plasma. For the processing of CRP, latex-enhanced nephelometry with particle-enhanced assays was used for quantitation. These assays were performed on a Behring Nephelometer for quantitative CRP determination. The methods used to derive complete blood count (CBC) parameters [white blood cell (WBC), segmented neutrophils (Nsg), lymphocyte (Lym), monocyte count (Mono), eosinophils (Eos) and basophils (Baso)] are based on the Beckman Coulter method of counting and sizing, in combination with an automatic diluting and mixing device for sample processing. The Beckman Coulter MAXM instrument in the Mobile Examination Centers (MECs) produces a CBC on blood specimens.

2.5 Measurement of BG

The method for measuring BG levels involves using the Roche Cobas C311 system, which first requires the patient to be fasting or to undergo an oral glucose tolerance test. Blood is collected via venipuncture, and plasma samples are collected in fluoride-containing gray top tubes, with a minimum volume of 200 microliters. To reduce sugar decomposition, the collected samples must be immediately placed in an ice water bath, and plasma and cells must be separated within 30 min. If testing is not conducted immediately, the samples are then frozen and stored at −70°C. The measurement principle is based on the reaction catalyzed by hexokinase between glucose and ATP to produce glucose-6-phosphate (G-6-P) and ADP, then G-6-P is further oxidized by glucose-6-phosphate dehydrogenase, simultaneously generating NADH directly proportional to the glucose concentration, which is measured by spectrophotometry at 340 nanometers. System calibration and quality control are performed before testing to ensure the accuracy and repeatability of the results. The Roche Cobas C311 automatically calculates the glucose concentration, and the results are reported in mg/dL or mmol/L.

2.6 Covariates

Age, sex, race and ethnicity, education, marriage, and PIR were acquired from self-reported questionnaires on demographic information. Race and ethnicity are classified as Mexican American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black, and Other Race (including Multiple Races) based on self-identification originally designated by NHANES. “Other Race” encompasses all other races, including Non-Hispanic Asians and individuals reporting multiple races. Common exposures to other metals (e.g., dietary) may vary by race and ethnicity, hence are adjusted for in our models. Education is reclassified into Less than 9th grade, 9–11th grade (Includes 12th grade with no diploma), High school graduate/GED or equivalent, Some college or AA degree, and College graduate or above. Marital status is divided into married, widowed, divorced, separated, never married, living with partner, refused, and missing.

2.7 Statistical analysis

First, we utilized PCA to determine the combination of inflammatory markers adopted in this study. Second, we conducted a descriptive analysis of the serum copper (Cu), inflammatory markers, and BG concentration in the included population, reporting mean, standard deviation, median, and quartiles.

Third, we performed mediation analysis to explore the direct and indirect relationships and the extent of mediation effect using non-parametric bootstrapping ($n = 1{,}000$).

Fourth, we conducted linear regression analysis to investigate the mixed effects of different inflammatory markers on glucose concentration. Fifth, we performed WQS regression analysis to assess the comprehensive and individual effects of inflammatory markers on BG concentration by calculating weighted linear indices and assigning corresponding weights. In this study, 10,000 bootstrap iterations were used to construct positive and negative WQS indices. When the WQS index was significant, the corresponding weights were examined to determine the relative contribution of each heavy metal in the index to glucose concentration. The dataset was randomly split, with 40% allocated to the training set and the remaining 60% as the validation set.

Finally, we conducted the sensitivity analysis. We adjusted for demographic variables in mediation analyses and for serum copper concentrations as well as demographic variables in MLRA, WQS regression analyses. We also performed sensitivity analyses of PCA using different missing value treatments to determine the optimal combination of inflammatory indicators for inclusion in the study.

Adjusted for demographic factors, weighted data was not used. All analyses, including WQS, MLRA (Multiple Linear Regression Analysis), and mediation analysis, were conducted using R software. A p-value of <0.05 was considered statistically significant.

3 Results

3.1 General information

This study involved 2,318 participants (Table 1), evenly split between males (50.17%, $n = 1{,}163$) and females (49.83%, $n = 1{,}155$), with an average age of 42.70 years ($SD = 15.34$). The population was diverse, including Mexican American (14.41%), other Hispanic (12.21%), non-Hispanic White (33.69%), non-Hispanic Black (22.30%), and other races (17.39%). Educational levels ranged from less than 9th grade (8.02%) to college graduate or above (26.92%). Marital status varied, with 47.63% married and 21.05% never married. The median poverty income ratio (PIR) was 1.94 (IQR = 0.97–3.81).

3.2 Principal component analysis

First, we conducted a principal component analysis (PCA) to explore the combination of inflammatory markers in the study. We calculated the standard deviation (S.D.) and explained variance (VER) for each marker, resulting in the cumulative explained variance (CVER) across the components.

After imputing missing values with the mean, the analysis indicated that WBC count, Lym percentage, Mono percentage, Nsg percentage, Eos percentage and Baso percentage exhibited higher variances, with their cumulative variance accounting for a substantial majority of data variability (Table 2). Similarly, imputing missing values with the median or removing missing values altogether yielded comparable results.

TABLE 1 Baseline characteristics of participants included in the study.

Item		Data		
		n (Mean)	% (*SD*)	Median (Q1, Q3)
Population		2,318	/	/
Gender	Male	1,163	50.17	/
	Female	1,155	49.83	/
Age		42.70	15.34	42.00 (29.00, 56.00)
Race	Mexican American	334	14.41	/
	Other Hispanic	283	12.21	/
	Non-Hispanic White	781	33.69	/
	Non-Hispanic Black	517	22.30	/
	Other Race – Including Multi-Racial	403	17.39	/
Education level	Less than 9th grade	186	8.02	/
	9–11th grade (Includes 12th grade with no diploma)	332	14.32	/
	High school graduate/ GED or equivalent	512	22.09	/
	Some college or AA degree	664	28.65	/
	College graduate or above	624	26.92	/
	Missing	0	0.00	/
Marital status	Married	1,104	47.63	/
	Widowed	64	2.76	/
	Divorced	231	9.97	/
	Separated	69	2.98	/
	Never married	488	21.05	/
	Living with partner	206	8.89	/
	Refused	2	0.09	/
	Missing	154	6.64	/
PIR		2.37	1.64	1.94 (0.97, 3.81)

PIR stands for "People Income Ratio".

3.3 Level of serum Cu, inflammation markers and BG

Then, we analyzed the baseline levels for serum Cu, WBC count, Lym percentage, Mono percentage, Nsg percentage, Eos percentage, Baso percentage, and BG (Table 3). The mean serum Cu level was found to be 119.50 μg/dL, with a standard deviation (SD) of 32.04, highlighting a moderate variability among individuals. WBC count averaged at 6.82×10^9/L. Lym percentage, Mono percentage, Nsg percentage, Eos percentage, and Baso percentage demonstrated diverse immune cell distribution, with mean values of 31.67, 7.89, 56.77, 2.98, and 0.76%, respectively. The mean BG level was noted at 107.10 mg/dL ($SD = 36.62$).

3.4 Mediation analysis

Then, we conducted the mediation analysis investigating the role of inflammatory factors in the relationship between serum Cu levels and BG, we focused on four intermediary variables: WBC count, Lym percentage, Mono percentage, Nsg percentage (Table 4). The analysis revealed significant mediation effects, with WBC count showing a substantial mediation proportion of 39.78% ($p = 0.0004$), indicating a strong mediator role. Lym percentage displayed a moderate mediation effect with a proportion of 10.16% ($p = 0.0421$), while Mono percentage also demonstrated significant mediation, with a proportion of 33.37% ($p = 0.0006$). Nsg percentage contributed notably as well, with a mediation proportion of 20.82% ($p = 0.0076$).

3.5 Multiple linger regression analysis

Building on the results from the mediation analysis, we further investigated the influence of significant mediatory variables on the relationship between serum Cu levels and BG through multiple linear regression analysis.

The WBC presented a positive and significant association with serum Cu (estimate: 1.077, 95% CI: 0.432 to 2.490, $p = 0.013$), suggesting higher WBC counts correspond with increased serum Cu levels. Mono showed a notable negative relationship (estimate: −1.573, 95% CI: 0.520 to −3.025, $p = 0.003$), indicating that higher Mono is associated with decreased serum Cu levels. Lym and Nsg did not demonstrate statistically significant associations (p-values of 0.126 and 0.210, respectively) (Table 5).

3.6 WQS regression models

Subsequently, we employed WQS regression analysis to further explore the weighted impact of inflammatory factors on BG. The WQS regression results showed that the overall model estimate was 4.7167, with statistical significance (p-value = 0.0216), indicating a significant effect of the combination of inflammatory factors on BG. Among the inflammatory factors, Nsg had the highest weight coefficient (0.4472). WBC and Lym had weight coefficients of 0.3558 and 0.1954, respectively. Meanwhile, the weight coefficient for Mono was 0.0015, indicating its relatively minor impact on BG.

3.7 Sensitive analysis

We conducted several sensitivity analyses to verify the stability of our research results. Initially, after adjusting for demographic variables in the mediation analysis, the results showed a significant increase in the mediation effects of WBC and Mono ($p < 0.005$), while the mediation effect of Lym became non-significant ($p = 0.7203$) (Supplementary Table S1). Secondly, upon adjusting for serum Cu levels and then for serum Cu levels plus demographic variables in the MLRA, the negative correlation between Mono and serum Cu levels remained significant across all models ($P <= 0.003$), whereas the correlation of WBC became non-significant after including

TABLE 2 Factor loadings of 11 inflammation indicators based on principal component analysis.

	WBC	LymPCT	MonoPCT	NsgPCT	EosPCT	BasoPCT	LymC	MonoC	NsgC	EosC	BasoC
Model I											
S.D.	1.8627	1.5654	1.3369	1.2126	1.2050	0.4465	0.2698	0.2377	0.2028	0.0230	0.0062
VER	0.3154	0.2228	0.1625	0.1337	0.1320	0.0181	0.0066	0.0051	0.0037	0.0001	0.0000
CVER	0.3154	0.5382	0.7007	0.8343	0.9663	0.9845	0.9911	0.9962	1.0000	1.0000	1.0000
Model II											
S.D.	1.8618	1.5671	1.3359	1.2086	1.2047	0.4540	0.2757	0.2377	0.2035	0.0310	0.0147
VER	0.3151	0.2233	0.1622	0.1328	0.1319	0.0187	0.0069	0.0051	0.0038	0.0001	0.0000
CVER	0.3151	0.5384	0.7006	0.8334	0.9653	0.9841	0.9910	0.9961	0.9999	1.0000	1.0000
Model III											
S.D.	1.8627	1.5654	1.3369	1.2126	1.2050	0.4465	0.2697	0.2377	0.2028	0.0204	0.0062
VER	0.3154	0.2228	0.1625	0.1337	0.1320	0.0181	0.0066	0.0051	0.0037	0.0000	0.0000
CVER	0.3154	0.5382	0.7007	0.8343	0.9663	0.9845	0.9911	0.9962	1.0000	1.0000	1.0000

LymPCT, Lym percentage; MonoPCT, mono percentage; NsgPCT, Nsg percentage; EosPCT, Eos percentage; BasoPCT, Baso percentage; LymC, Lym count; MonoC, Mono count; NsgC, Nsg count; EosC, Eos count; BasoC, Baso count. Model I used the overall mean to fill in the missing values, Model II used the overall median to fill in the missing values, and Model III removed the missing values.

TABLE 3 Level of serum Cu, inflammation markers and blood glucose among the participants included in the study.

	Mean	*SD*	Median	Q1	Q3		Mean	*SD*	Median	Q1	Q3
Serum Cu	119.50	32.04	113.80	98.00	133.98	NsgPCT	56.77	9.46	57.10	50.50	63.30
WBC	6.82	2.05	6.50	5.40	7.90	EosPCT	2.98	2.21	2.40	1.60	3.70
LymPCT	31.67	8.52	31.20	25.80	36.80	BasoPCT	0.76	0.45	0.70	0.50	0.90
MonoPCT	7.89	2.20	7.60	6.40	9.10	BG	107.10	36.62	99.00	92.00	108.00

LymPCT, Lym percentage; MonoPCT, Mono percentage; NsgPCT, Nsg percentage; EosPCT, Eos percentage; BasoPCT, Baso percentage.

demographic variables ($p = 0.072$) (Supplementary Table S2). Additionally, in the WQS regression model, after adjusting for serum Cu levels and for serum Cu levels plus demographic variables, the overall estimate of the model significantly increased, with the *p*-value decreasing from 0.0216 to 0.0002, indicating an enhanced significance of the model under different adjustments (Supplementary Table S3).

4 Discussion

Long-term hyperglycemia can lead to serious complications, including cardiovascular diseases, diabetic neuropathy, nephropathy, and retinopathy (19–23). These conditions significantly increase morbidity and mortality among diabetic patients. Recent studies have also indicated that heavy metals are key factors in the pathogenesis of several diseases, including obesity, metabolic syndrome, and hypertension (24–26). Environmental pollution caused by heavy metals has become an ongoing concern worldwide (27, 28). In recent years, the impact of exposure to individual heavy metals on blood glucose levels has garnered widespread attention. These metals, by interfering with the body's normal metabolic functions, may increase the risk of diabetes. Research covering various heavy metals, including manganese (29), nickel (30), mercury (31), cadmium (32), and lead (33), has indicated that they disrupt glucose metabolism and insulin sensitivity through different mechanisms, thereby affecting blood glucose levels. However, all of the above studies did not include copper exposure, so it remains uncertain whether copper exposure is associated with abnormal changes in blood glucose in heavy metal mixtures.

In this study, we investigated the serum copper levels, inflammatory markers, and fasting blood glucose levels among 2,318 participants, uncovering a range of meaningful results. The average serum Cu level was found to be 119.50 µg/dL, indicating moderate variability among individuals. Through PCA, we identified that WBC, Lym, Mono, and Nsg as inflammatory markers occupied significant positions in the variability of the data. Mediation analysis further revealed the important mediating roles of WBC, Lym, Mono, and Nsg in the relationship between serum Cu levels and BG, with WBC showing the highest mediation proportion at 39.78%, and Nsg showing the lowest at 20.82%. During MLRA, we observed a positive correlation between WBC and serum Cu levels, while Mono showed a negative correlation with serum Cu levels. Additionally, using the WQS regression model, we explored the cumulative impact of inflammatory factors on BG and found a significant effect of the combination of inflammatory markers on BG levels. Sensitivity analysis confirmed the robustness of these findings, with the estimates of mediation effects and the WQS regression model being strengthened after adjusting for demographic variables.

Accumulating evidence supports the role of inflammation in the abnormal changes in blood glucose (34). Lin et al. (35) revealed the relationship between excessive intake of sugary drinks and abdominal obesity with diabetes and the elevation of C-reactive protein (CRP) levels, emphasizing the positive correlation between sugar intake and CRP levels in adults with prediabetes. Moreover, Mi et al. (36) found

TABLE 4 The mediating effects of the relationship between serum Cu and BG.

Intermediary Variable	Indirect effects β (95% CI)			Direct effects β (95% CI)			Total effects β (95% CI)			Mediated proportion	p-value
	Estimate	CI lower	CI upper	Estimate	CI lower	CI upper	Estimate	CI lower	CI upper		
WBC	0.0234	−0.0193	0.0661	0.0155	0.0069	0.0240	0.0389	−0.0032	0.0810	0.3978	0.0004
LymPCT	0.0350	−0.0072	0.0771	0.0040	0.0001	0.0078	0.0389	−0.0032	0.0810	0.1016	0.0421
MonoPCT	0.0259	−0.0166	0.0684	0.0130	0.0055	0.0204	0.0389	−0.0032	0.0810	0.3337	0.0006
NsgPCT	0.0308	−0.0116	0.0732	0.0081	0.0022	0.0141	0.0389	−0.0032	0.0810	0.2082	0.0076

LymPCT, Lym percentage; MonoPCT, Mono percentage; NsgPCT, Nsg percentage.

TABLE 5 Association between potential mediators and BG determined by MLRA.

Variable	Unadjusted model				Variable	Unadjusted model			
	Estimate	Lower CI	Upper CI	p-value		Estimate	Lower CI	Upper CI	p-value
WBC	1.0769	0.4325	2.4902	0.0128	MonoPCT	−1.5743	−2.5988	−0.5498	0.0026
LymPCT	−0.5403	0.3529	−1.5309	0.1259	NsgPCT	−0.4527	−1.1208	0.2154	0.1840

LymPCT, Lym percentage; MonoPCT, Mono percentage; NsgPCT, Nsg percentage.

that the Dietary Inflammatory Index (DII) is positively associated with insulin resistance in adults of normal and healthy weight, highlighting the potential value of anti-inflammatory diets in the prevention or management of insulin resistance. Furthermore, Yuan et al. (37) explored the relationship between the DII and long-term all-cause and cardiovascular mortality, pointing out that high DII scores are associated with an increased risk of long-term all-cause and cardiovascular mortality in patients with diabetes.

Given this common pathogenesis, it is reasonable to investigate whether Cu exposure leads to abnormal changes in BG through inflammation. In this study, we found that WBC, Lym, Mono, and Nsg were involved in the positive correlation between serum Cu concentration and changes in BG concentration, accounting for 39.78, 10.16, 33.37, and 20.82%, respectively, in the mediation analysis. Therefore, we hypothesized that excessive Cu exposure promotes inflammation and thus increases blood glucose concentration.

The results of our sensitivity analysis underscore the stability of our findings when considering the effects of serum copper levels and demographic variables. Notably, the negative correlation between Mono and serum Cu levels remained consistent across various model adjustments, highlighting the potential of Mono as a robust indicator of serum Cu levels. However, the association between WBC and serum Cu levels became non-significant after adjusting for demographic variables, suggesting the need to consider a broader range of demographic and biological factors when evaluating the relationship between biomarkers and environmental exposure (38, 39). Finally, the results of the WQS model further support the importance of the relationship between serum Cu levels and health outcomes under different adjustments.

Our study possesses several strengths. First, this research constitutes the inaugural investigation into the impact of Cu exposure on BG concentration via inflammatory factors. Second, we utilized a variety of statistical methodologies and adjusted for potential confounding variables to enhance the robustness and reliability of our findings. Third, all data were sourced from a large population database and adhered to stringent quality control measures. However, this study also has certain limitations. First, due to its cross-sectional design, we cannot ascertain the causality between copper exposure and alterations in blood glucose levels. Second, the NHANES database lacks data on uncontrollable factors, such as exposure to wastewater and cosmetics, which might affect the accuracy of our results. Third, not considering the cumulative amount of copper exposure could impact the outcomes.

As for the future direction and suggestion, it is necessary to conduct long-term longitudinal studies to determine the causal relationship between Cu exposure and BG levels, and explore its molecular mechanisms, particularly how Cu affects BG regulation through inflammatory pathways. Moreover, we should further investigate how environmental Cu exposure interacted with lifestyle factors such as diet and physical activity to collectively influenced the development of abnormal BG concentration. Most importantly, for populations living in areas of high heavy metal exposure, healthcare providers should consider regular testing for serum Cu and other relevant biomarkers as part of routine health screenings. In addition, specific nutritional and medical advice should be provided to these populations to reduce the potential health risks of heavy metal exposure.

5 Conclusion

This research with 2,318 NHANES participants conclusively demonstrated that serum Cu levels significantly influenced BG concentrations through specific inflammatory markers, suggesting reducing heavy metal exposure in the environment could lower the risk of developing diabetes. Mediation analyses revealed the impact of inflammatory markers on BG, emphasizing the role of public health measures to reduce heavy metal exposure and consider environmental factors in reducing diabetes.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://wwwn.cdc.gov/nchs/nhanes/Default.aspx.

Ethics statement

The studies involving humans were approved by NCHS Ethics Review Board Protocol #2011–17. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

ZC: Visualization, Writing – original draft. YK: Formal analysis, Project administration, Writing – original draft, Writing – review & editing. WY: Methodology, Project administration, Software, Writing – review & editing. HX: Investigation, Resources, Writing – review & editing. DT: Investigation, Methodology, Supervision, Writing – review & editing. YZ: Conceptualization, Investigation, Methodology, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

We acknowledge the members of the National Center for Health Statistics of the Centers for Disease Control and Prevention and all participants of the National Health and Nutrition Examination Survey. We would also like to thank Shuping Yang from the School of Mathematics and Statistics, Central South University, for providing initial guidance on statistical analysis.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2024.1401347/full#supplementary-material

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frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 12 August 2024
DOI 10.3389/fpubh.2024.1402715

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OPEN ACCESS

EDITED BY
Mohammad Imran Ansari,
University of Maryland, Baltimore,
United States

REVIEWED BY
Damaris Albores-Garcia,
Cinvestav, Mexico
Haesoo Kim,
Keimyung University, Republic of Korea

*CORRESPONDENCE
Zongliang Wen
wenzl@xzhmu.edu.cn
Yan Zhu
zhuyan@xzhmu.edu.cn

RECEIVED 18 March 2024
ACCEPTED 22 July 2024
PUBLISHED 12 August 2024

CITATION
Bai L, Wen Z, Zhu Y, Jama HA, Sawmadal JD and Chen J (2024) Association of blood cadmium, lead, and mercury with anxiety: a cross-sectional study from NHANES 2007–2012. *Front. Public Health* 12:1402715. doi: 10.3389/fpubh.2024.1402715

Association of blood cadmium, lead, and mercury with anxiety: a cross-sectional study from NHANES 2007–2012

Long Bai[1], Zongliang Wen[1,2,3]*, Yan Zhu[4]*, Hamdi Abdirizak Jama[2], Joyce D. Sawmadal[2] and Jialin Chen[2]

[1]School of Public Health, Xuzhou Medical University, Xuzhou, China, [2]School of Management, Xuzhou Medical University, Xuzhou, China, [3]Affiliated Hospital of Xuzhou Medical University, Xuzhou, China, [4]School of Medical Information and Engineering, Xuzhou Medical University, Xuzhou, China

Objectives: The purpose of this paper is to explore the relationship between blood levels of cadmium, lead, and mercury and anxiety in American adults.

Methods: Blood metals and self-reported anxiety days were extracted from laboratory data and questionnaire data, respectively, using NHANES data from 2007–2012. Weighted logistic regression was used to assess the relationship between cadmium, lead and mercury with anxiety. Restricted cubic spline was used to visualize the non-linear relationship between metal concentrations and anxiety. Weighted quantile sum (WQS) regression was used to investigate the effect of combined exposure to the three metals on anxiety.

Results: The prevalence of anxiety in adults was 26.0%. After adjusting for potential confounding variables, cadmium levels in the highest quartile (Q4) were associated with a higher risk of anxiety compared to the lowest quartile (Q1) (OR = 1.279, 95% CI: 1.113–1.471, $p < 0.01$). Restricted cubic spline analysis indicated a positive association between blood cadmium levels and anxiety. Furthermore, co-exposure to multiple heavy metals was positively associated with anxiety risk (WQS positive: OR = 1.068, 95% CI: 1.016–1.160, $p < 0.05$), with cadmium contributing the most to the overall mixture effect. Compared to the Light RPA, the Vigorous/Moderate RPA group had a relatively low risk of anxiety after cadmium exposure.

Conclusion: High levels of blood cadmium are positively associated with the development of anxiety disorders, which needs to be further verified in future studies.

KEYWORDS

cadmium, lead, mercury, anxiety, restricted cubic spline, NHANES

1 Introduction

Concerns about mental health-related issues, particularly anxiety disorders, have been increasingly expressed by the public. Anxiety is one of the manifestations of a wide range of mental disorders, characterized by acute, overwhelming, and persistent worry and fear that can peak within minutes (1). Anxiety is a common disorder that can have a detrimental effect on quality of life (QOL), especially when untreated (2). A study on the global burden of anxiety disorders from 1990 to 2019 showed a 50% increase in the absolute number of anxiety disorders compared to 1990 (3). Excessive anxiety constitutes the most common psychiatric complaint (4). The causes of anxiety are complex and varied, including life stress, illness, and exposure to environmental pollutants (5, 6). The relationship between environmental chemicals, such as heavy metals, and the etiology of mental disorders has garnered widespread attention (7).

Heavy metals, defined as metals with a density >5 g/cm^3 (e.g., mercury [Hg], lead [Pb], and cadmium [Cd]), are non-essential and highly toxic to humans (8). Metals are present in almost all environmental media in everyday life, and people are often exposed to many types of metals at once. Cadmium is a major contaminant in agricultural soils worldwide and its toxicity and persistence in the environment has become a matter of concern (9, 10). Lead is widely used in the weapons, paint and battery industries, and was previously widely used in plumbing and food packaging, and is released from sources such as batteries (11). Mercury is typically found in elemental form and as methylmercury, the latter being highly toxic (12). Prolonged exposure to these environmental heavy metals, even if they are essential elements required by the human body, can have adverse effects on human health when certain threshold levels are exceeded (13). It has been shown that heavy metal pollution can negatively impact mental health (14). An epidemiological study has linked living in areas with high concentrations of heavy metals and metalloids in the soil to an increased likelihood of developing mental disorders. In this study, compared to the lowest metal concentration level quartile, the odds ratios (OR) for the second, third and fourth quartiles for lead were 1.29, 1.37 and 1.51, respectively; and for cadmium, the OR for the fourth quartile was 1.84 (15). Heavy metals are known to be severely neurotoxic, causing brain damage by interfering with the release of neurotransmitters and neurotrophic proteins, generating neuroinflammation and oxidative stress, and leading to necrosis and apoptosis of neurons and glial cells, which may be a potential mechanism for psychiatric disorders (16–18), and which are often dependent on the relevance of the dose and exposure window.

Many reports have demonstrated the relationship between heavy metal exposure and mental disorders. It has been noted that joint exposure to metals is associated with elevated anxiety symptoms (OR_{WQS} series = 1.56, 95% CI: 1.24, 1.96); Cd (61.8%), Cr (14.7%), and Cs (12.7%) contributed most to the mixed effect (19). A study based on data from the National Health and Nutrition Examination Survey (NHANES) showed a positive correlation between higher levels of cadmium and depression (20). Lead exposure is also positively associated with the development of anxiety and depression (21). Despite evidence that heavy metal exposure increases the risk of psychiatric disorders, only a few studies have shown that heavy metal exposure may increase anxiety (7, 19, 22). In a study examining the effect of whole blood lead and cadmium levels on the prevalence of anxiety and depressive symptoms in postmenopausal women, a relationship was found between lead levels and the severity of anxiety states. Those without anxiety had the highest whole blood lead concentration (22.84 ± 9.79 μg/L), while those with anxiety had the lowest whole blood lead concentration (17.20 ± 7.52 μg/L), a difference that was statistically significant (22). However, some meta-analyses have found no evidence of a correlation between lead exposure (assessed as blood lead levels) and anxiety (23). A recent study exploring the relationship between heavy metals and anxiety found a significant association with urinary metals (7). However, the relationship between blood heavy metal levels and anxiety remains to be fully explored.

Given these conflicting findings, we aimed to investigate whether blood concentrations of three heavy metals (lead, mercury, and cadmium) are associated with anxiety in the American population. We examined cross-sectional associations between anxiety and blood concentrations of these heavy metals among participants in the National Health and Nutrition Examination Survey (NHANES), a randomly selected, non-institutionalized sample of Americans.

Abbreviations: Cd, Cadmium; Pb, Lead; Hg, Mercury; NHANES, National health and nutrition examination survey; RPA, recreational physical activity; BMI, body mass index; PIR, poverty income ratio.

2 Method

2.1 Study population

The National Health and Nutrition Examination Survey (NHANES) is a series of cross-sectional health and nutrition surveys conducted by the National Centre for Health Statistics. NHANES employs a stratified multistage sampling design. The survey has received approval from the Ethical Review Board of the National Center for Disease Control and Prevention (NCDC), and informed consent was obtained from all participants. Detailed information about the NHANES procedures is available on the official website (https://www.cdc.gov/nchs/nhanes/index.htm). For this study, we used data from the NHANES 2007–2012 survey cycles. We excluded participants younger than 20 years old, those without information on anxiety status, and those with unreliable data. Additionally, individuals with missing information on blood cadmium, lead, and mercury levels were excluded. The specific procedure is illustrated in Figure 1.

2.2 Definition of anxiety

During the personal interview, anxiety status was assessed by the following question, “During the past 30 days, about how many days did you feel worried, nervous, or anxious?” This assessment is based on the 14-item Healthy Days measure developed by the CDC, which is part of the Health-Related Quality of Life (HRQoL) assessment. The Reliability of HRQoL monitoring questions ranges from moderate to excellent (24). Anxiety states were categorized as follows: “no” (feeling anxious 0 to 6 days per month) and “yes” (feeling anxious 7–30 days per month) (25).

2.3 Measurement of blood metals

Whole blood specimens were processed, stored, and shipped to the Division of Laboratory Sciences, National Center for Environmental Health, and Centers for Disease Control and Prevention for analysis. Detailed instructions on specimen collection and processing can be found in the NHANES Laboratory/Medical Technologists Procedures

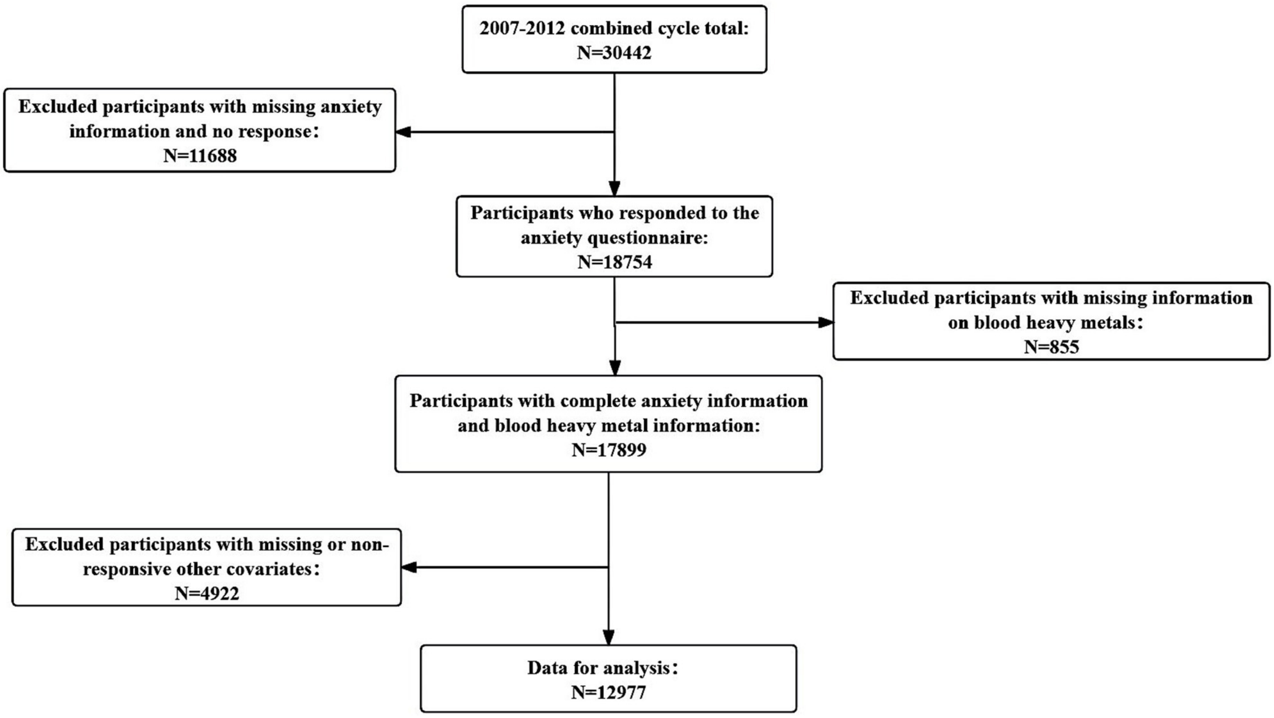

FIGURE 1
Flowchart for selecting study participants.

Manual (LPM). Whole blood lead (Pb), cadmium (Cd), and total mercury (THg) concentrations are determined using inductively coupled plasma mass spectrometry. This multi-element analytical technique is based on quadrupole ICP-MS technology. Produced by ion detection signal is processed into digital information, used to indicate the intensity of the ion, then the concentration of indicator elements. For values below the detection limit, we followed NHANES analytical guidelines by dividing the detection limit by the square root of two.

2.4 Covariates

The following variables were included in the statistical analyses: gender (male/female), age (20–39, 40–59, $\geq$ 60), race (Mexican American, Other Hispanic, non-Hispanic white, non-Hispanic black, or other race), marital status (not living alone/living alone), and education (<high school, high school, or >high school), and poverty income ratio (<1.50, 1.50–3.49, $\geq$3.50). Other confounding variables included smoking status (defined as having smoked at least 100 cigarettes in a lifetime) and drinking status (defined as having had at least 12 drinks in a year). Hypertension and diabetes were derived from self-reported physician diagnoses (yes/no). Body mass index was obtained from examination data (<25.0,25.0–29.99, $\geq$30.0). Physical activity was classified as light RPA and vigorous/moderate RPA.

2.5 Statistical analyses

Considering the complex sampling of NHANES, appropriate weights were used in the statistical analyses, with a sample weight of 1/3 * WTMEC2YR for the years 2007–2012, the formula for this weight can be found on this website (https://wwwn.cdc.gov/nchs/nhanes/tutorials/Weighting.aspx). Covariates were transformed into categorical variables and expressed as observations and weighted percentages. *P*-values were tested using the weighted chi-square test. Logistic regression was used to examine the association of cadmium, lead and mercury with anxiety. In the crude model, no variables were adjusted; model 1 was adjusted for gender, age, and race; model 2 was adjusted for gender, age, race, education, marital status, BMI, poverty income ratio, smoking status, drinking status, diabetes, hypertension, and physical activity. A restricted cubic spline was used to explore the non-linear relationship between heavy metals and anxiety. Weighted quantile sum (WQS) regression was employed to assess the combined effect of heavy metal exposure on anxiety. The data was divided into 40% training dataset and 60% validation dataset and the positive and negative effects of WQS index on anxiety risk were analyzed by WQS regression. Subgroup analyses based on different characteristic differences (e.g., gender and age) were conducted to assess the effects of heavy metals on anxiety across different demographics. Data were analyzed using SPSS 27.0, Empowerstats (http://www.empowerstats.com), and R version 4.2.2. The R package "gWQS" was used for WQS. A two-sided p-value $<$ 0.05 was considered statistically significant.

TABLE 1 Characteristics of participants by anxiety status, NHANES 2007–2012, weighted.

	Total	No	Yes	*P* value
N	12,977 (100%)	9,617 (74.0%)	3,360 (26.0%)	
Gender, %				<0.001
Male	6,449 (48.8)	5,095 (51.5)	1,354 (41.1)	
Female	6,528 (51.2)	4,522 (48.5)	2,006 (58.9)	
Age, years, %				<0.001
20–39	4,402 (36.8)	3,192 (36.2)	1,210 (38.8)	
40–59	4,250 (38.6)	2,924 (36.8)	1,326 (43.8)	
≥60	4,325 (24.5)	3,501 (27.1)	824 (17.4)	
Race, %				0.018
Mexican American	1,925 (7.7)	1,436 (7.9)	489 (7.2)	
Other Hispanic	1,289 (5.1)	904 (4.8)	385 (5.9)	
Non-Hispanic White	6,123 (70.7)	4,460 (70.5)	1,663 (71.5)	
Non-Hispanic Black	2,639 (10.3)	2,011 (10.4)	628 (10.1)	
Other Races	1,001 (6.2)	806 (6.5)	195 (5.3)	
Education, %				<0.001
<High school	3,362 (17.2)	2,384 (16.1)	978 (20.5)	
High school	2,978 (22.5)	2,264 (22.8)	714 (21.4)	
>High school	6,637 (60.3)	4,969 (61.1)	1,668 (58.1)	
Marital status, %				<0.001
Not living alone	7,711 (63.5)	5,854 (64.7)	1,857 (60.0)	
Living alone	5,266 (36.5)	3,763 (35.3)	1,503 (40.0)	
Smoking status, %				<0.001
Smoker	5,985 (45.4)	4,256 (43.4)	1,729 (51.1)	
Never smoker	6,992 (54.6)	5,361 (56.6)	1,631 (48.9)	
Drinking status, %				0.060
Yes	9,493 (78.2)	6,972 (77.7)	2,521 (79.8)	
No	3,484 (21.8)	2,645 (22.3)	839 (20.2)	
Diabetes, %				0.373
Yes	1,605 (8.8)	1,146 (8.6)	459 (9.3)	
No	11,372 (91.2)	8,471 (91.4)	2,901 (90.7)	
Hypertension, %				0.128
Yes	4,586 (30.6)	3,337 (30.1)	1,249 (32.0)	
No	8,391 (69.4)	6,280 (69.9)	2,111 (68.0)	
Body mass index, %				0.009
<25.0	3,805 (30.9)	2,835 (30.7)	970 (31.5)	
25.0–29.99	4,353 (33.9)	3,350 (34.8)	1,003 (31.5)	
≥30	4,819 (35.2)	3,432 (34.5)	1,387 (37.0)	
Poverty income ratio, %				<0.001
<1.50	4,956 (25.9)	3,406 (23.5)	1,550 (32.8)	
1.50–3.49	4,028 (30.5)	3,041 (30.5)	987 (30.7)	
≥3.50	3,993 (43.5)	3,170 (46.0)	823 (36.6)	
Physical activity, %				<0.001
Light RPA	6,832 (45.6)	4,856 (43.4)	1,976 (52.1)	
Vigorous/Moderate RPA	6,145 (54.4)	4,761 (56.6)	1,384 (47.9)	

For categorical variables: (N-observe) survey-weighted percentage, P-value was by survey-weighted Chi-square test; RPA: Recreational Physical Activity.

3 Results

3.1 Basic characteristics of participants

Table 1 summarizes the general characteristics of the study participants, including 12,977 individuals, with 6,449 (48.8%) males and 6,528 (51.2%) females. All participants were adults aged ≥20 years: 4,402 (36.8%) were aged 20–39 years, 4,250 (38.6%) were aged 40–59 years, and 4,325 (24.5%) were aged ≥60 years. Among the participants, 4,586 (30.6%) had hypertension, and 1,605 (8.8%) had diabetes. Anxiety was present in 3,360 (26.0%) individuals, of whom 1,354 (41.1%) were male and 2,006 (58.9%) were female. In the anxious population, 1,210 (38.8%) were aged 20–39 years, 1,326 (43.8%) were aged 40–59 years, and 824 (17.4%) were aged ≥60 years. Additionally, 1,503 (40.0%) were living alone, 1,976 (52.1%) engaged in light recreational physical activity (RPA), and 1,384 (47.9%) engaged in vigorous/moderate RPA. All characteristics, except for alcohol drinking status, diabetes, and hypertension, showed statistically significant differences between the anxious and non-anxious groups.

3.2 Relationship between blood metals and anxiety

Table 2 shows the relationship between blood levels of cadmium, lead and mercury with anxiety, categorizing participants according to the interquartile range (IQR) of heavy metal concentrations (Q1: 0–25%; Q2: >25%-50%; Q3: >50%-75%; and Q4: >75%-100%), as shown in Supplementary Table S1. Three logistic regression models were developed, and in the crude model, blood cadmium was positively associated with the risk of anxiety in the Q4 group (OR =1.631, 95% CI: 1.458–1.825, $P < 0.001$), while blood lead in the Q4 group and blood mercury in the Q4 group were negatively associated with the risk of anxiety. After adjusting for gender, age, and race in model 1, the association between blood cadmium and anxiety remained strong (OR = 1.691, 95%CI: 1.501–1.904, $p < 0.001$), and Q2 (OR = 1.172, 95%CI: 1.014–1.356, $p < 0.05$) and Q4 (OR = 1.223, 95%CI: 1.047–1.428, $p < 0.05$) of blood lead were associated with the risk of anxiety. An association between Q4 and anxiety remained for blood mercury (OR = 0.812, 95% CI: 0.700–0.942, $p < 0.01$). In Model 2, after adjusting for gender, age, race, education, marital status, smoking status, drinking status, hypertension, diabetes, body mass index, poverty income ratio, and physical activity, the association between blood cadmium and anxiety persisted (OR = 1.279, 95% CI: 1.113–1.471, $p < 0.01$), while the associations with blood lead and mercury were not statistically significant.

Figure 2 illustrates the non-linear relationship between blood cadmium and anxiety derived from the restricted cubic spline model analysis, adjusted for gender, age, race, education, marital status, smoking status, drinking status, hypertension, diabetes, body mass index, poverty income ratio, and physical activity. The analysis showed that high levels of cadmium in the blood were positively associated with anxiety risk.

TABLE 2 Relationship between blood metals and anxiety, 2007–2012, weighted.

	Crude	Model 1	Model 2
Cadmium			
Q1	Ref	Ref	Ref
Q2	1.051 (0.933, 1.183)	1.063 (0.941, 1.200)	1.047 (0.917, 1.196)
Q3	0.975 (0.853, 1.114)	1.034 (0.898, 1.190)	0.939 (0.820, 1.075)
Q4	1.631 (1.458, 1.825)***	1.691 (1.501, 1.904)***	1.279 (1.113, 1.471)**
P for trend	<0.001	<0.001	<0.001
Lead			
Q1	Ref	Ref	Ref
Q2	1.029 (0.899, 1.176)	1.172 (1.014, 1.356)*	1.101 (0.958, 1.267)
Q3	0.861 (0.737, 1.007)	1.105 (0.930, 1.312)	0.983 (0.825, 1.172)
Q4	0.861 (0.763, 0.972)*	1.223 (1.047, 1.428)*	1.020 (0.885, 1.175)
P for trend	0.005	0.040	0.797
Mercury			
Q1	Ref	Ref	Ref
Q2	0.861 (0.741, 1.001)	0.878 (0.754, 1.022)	0.945 (0.813, 1.099)
Q3	0.887 (0.770, 1.022)	0.891 (0.771, 1.030)	1.014 (0.878, 1.172)
Q4	0.779 (0.673, 0.900)**	0.812 (0.700, 0.942)**	1.011 (0.866, 1.180)
P for trend	0.004	0.023	0.670

*p < 0.05; **p < 0.01; ***p < 0.001.
OR, Odds Ratio; Q, quartile.
Crude: unadjusted.
Model 1: adjusted for gender, age, race.
Model 2: adjusted for gender, age, race, education, marital status, smoking status, drinking status, body mass index, poverty income ratio, hypertension, diabetes, and physical activity.

We also constructed bimetallic and polymetallic models to assess the effects of combined metal exposures, such as cadmium. The results supported our previous findings. After adjusting for covariates in the polymetallic model, blood cadmium remained positively associated with anxiety risk in the Q4 group (Supplementary Table S2).

3.3 Effects of cadmium on anxiety under different physical activities

Weighted logistic regression results in Table 3 indicate that vigorous/moderate RPA reduces the risk of anxiety following cadmium exposure compared to light RPA. After adjusting for other variables, the OR and 95% CI for the risk of anxiety after cadmium exposure for vigorous/moderate RPA was 1.132 (1.006–1.274, $p < 0.05$).

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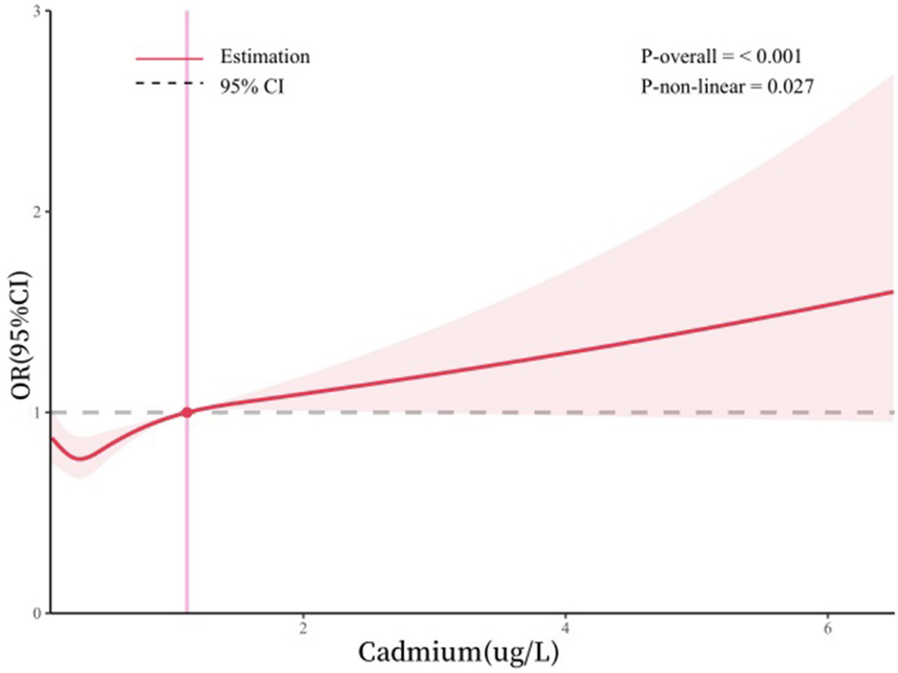

FIGURE 2
Dose-response relationship between blood cadmium and anxiety. Adjusted for gender, age, race, education, marital status, smoking status, drinking status, body mass index, poverty income ratio, hypertension, diabetes, and physical activity.

TABLE 3 Relationship between blood cadmium and anxiety at different levels of physical activity, weighted.

	Crude	Model 1	Model 2
Light RPA	1.380 (1.196,1.592)***	1.361 (1.164,1.590)***	1.174 (1.030,1.337)*
Vigorous/ Moderate RPA	1.245 (1.120,1.384)***	1.237 (1.111,1.377)***	1.132 (1.006,1.274)*

*p < 0.05; ***p < 0.001.
RPA, Recreational Physical Activity.
Cadmium is brought in as a continuous variable.
Crude: unadjusted.
Model 1: adjusted for gender, age, race.
Model 2: adjusted for gender, age, race, education, marital status, smoking status, drinking status, body mass index, poverty income ratio, hypertension, and diabetes.

3.4 The combined effect of the three metals: weighted quantile sum regression (WQS)

As shown in Figure 3, the weighted quantile sum (WQS) regression model indicated that each one-unit increase in the WQS index for the positive effect of all metals was associated with an 8.6% increase in the risk of anxiety (95% CI: 1.016–1.160). Conversely, for the negative effect of all metals, the overall OR for anxiety was 0.959 (0.890, 1.032). Additionally, the weights of blood heavy metals estimated by the WQS model are presented in Supplementary Table S3. Cadmium had the highest estimated weight for anxiety risk (weight = 0.823), followed by mercury (weight = 0.158). We did not find any significant negative correlation between heavy metal mixtures and anxiety risk.

3.5 Subgroup analysis

In the subgroup analyses, stratification was based on gender, age, race, education, marital status, smoking status, drinking status, body mass index, poverty income ratio, hypertension, and diabetes. As shown in Figure 4, participants who were female, 20–39 years old, under high-school education, not living alone, smoking, drinking alcohol, BMI < 25.0 kg/m^2, and PIR < 1.50 were more likely to suffer from anxiety.

4 Discussion

Heavy metal exposure poses a significant risk to human health. We investigated the relationship between cadmium, lead, and mercury and anxiety using NHANES data from 2007 to 2012, revealing a positive association between blood cadmium levels and anxiety. When stratified by physical activity and adjusted for other variables, the risk of cadmium exposure was lower in the Vigorous/Moderate RPA group compared to the light RPA group (OR: 1.132 < 1.174).

Although the neurotoxicity of cadmium is well known (26), limited studies have explored the association between cadmium exposure and anxiety risk. A recent study found a positive correlation between cadmium levels in urine and anxiety risk

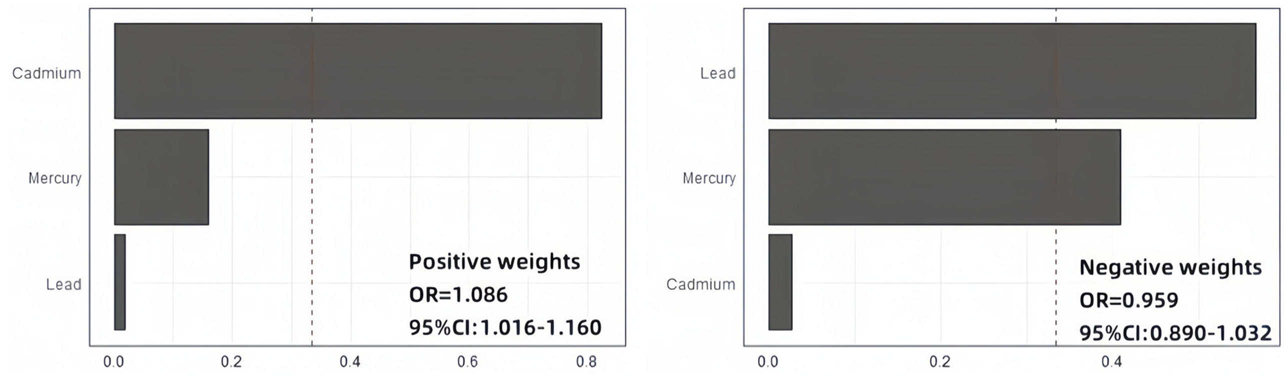

FIGURE 3
WQS regression results. Adjusted for gender, age, race, education, marital status, smoking status, drinking status, body mass index, poverty income ratio, hypertension, diabetes, and physical activity.

(7). Joint exposure to metals was associated with elevated anxiety symptoms, with Cd (61.8%), contributing the most to the mixed effect (19). In another experiment, control rats received intraperitoneal injections of 0.9% NaCl, while test rats were injected with $CdCl_2$ dissolved in physiological solution at doses of 1 mg/kg, 2 mg/kg, and 3 mg/kg, and their behavioral activities were observed. The results showed that acute cadmium administration dose-dependently increased anxiety in rats (27). Contrarily, previous studies have suggested that cadmium is not related to anxiety. A systematic evaluation of observational studies found no association between blood cadmium levels and anxiety (23). In our study, blood lead levels were not associated with anxiety after controlling for all covariates. In a study of the association of metal ions (containing lead) in cerebrospinal fluid with anxiety, depression, and insomnia in smokers, no association between lead and anxiety was found (28). A study on the relationship between blood lead exposure and mental health in pregnant women also found no association between low levels of lead exposure and psychological symptoms (29). However, some studies have shown a positive correlation between lead exposure and the onset of anxiety. Intermittent lead exposure can lead to adverse health effects, including anxiety (30). Mercury is a toxic metal that can cause health problems with prolonged exposure. Studies have shown that metalworkers regularly exposed to mercury are at risk of developing anxiety (31). In a study of mercury exposure in the Terra do Meio region of the Amazon, a high prevalence of symptoms associated with mercury poisoning was observed, with anxiety being one of the symptoms (32). Our study found no association between blood mercury levels and anxiety. Some studies have found no relationship between mercury levels in the body and mental disorders (33). A study on the relationship between exposure to environmental pollutants and behavioral indicators in Inuit preschool children in the Québec Arctic region found no association between mercury and anxiety (34).

We used weighted quantile sum (WQS) regression to explore the effect of combined exposure to the three metals on anxiety and found that a positive WQS index was significantly associated with an increased risk of anxiety. In an analysis of urine metal-anxiety associations in American adults, a positive WQS index was significantly associated with anxiety risk (OR [95% CI]: 1.23 [1.04, 1.39]) (7). Another WQS analysis of the NHANES database examining blood heavy metal exposure and anxiety associations found that mixed metal exposure was positively associated with anxiety [$P = 0.033$, OR (95%): 1.437 (1.031, 2.003)] (33). These findings are consistent with our results.

Current studies have highlighted the positive effects of exercise on anxiety. In our study, the risk of anxiety following cadmium exposure was relatively lower in the vigorous/moderate RPA group compared to the light RPA group. Some studies suggest that physical activity is an effective way to address anxiety symptoms in children and adolescents (35). Research on the relationship between physical activity and anxiety indicates that low physical activity levels are associated with an increased prevalence of anxiety (36). Other studies have shown that physical activity can reduce the risk of anxiety (37, 38).

The mechanism of cadmium-induced anxiety remains unclear. Cadmium is a highly neurotoxic heavy metal that interferes with DNA repair mechanisms by generating reactive oxygen species (39). Animal experiments have shown that cadmium-treated mice have fewer adult cells, fewer adult neurons, and a reduced proportion of adult cells that differentiating into mature neurons in the dentate gyrus granules. This suggests that cadmium exposure from puberty to adulthood is sufficiently high to cause cognitive deficits and impair key processes of hippocampal neurogenesis in mice (18). Additionally, cadmium selenide quantum dot (CdSe QD) exposure may induce neurobehavioral toxicity and alter mRNA levels of dopamine and oxidative stress-related genes in developing animals, as demonstrated in a toxicological assessment of cadmium-containing quantum dots in developing zebrafish (40). Cadmium enters the nervous system and disrupts mitochondrial respiration by decreasing ATP synthesis and increasing the production of reactive oxygen species. It also impairs normal neurotransmission by increasing the asynchrony of neurotransmitter release and disrupting neurotransmitter signaling proteins, damages the blood-brain barrier and alters the regulation of glycogen metabolism (41). These neurotoxicities of cadmium may cause the onset of anxiety.

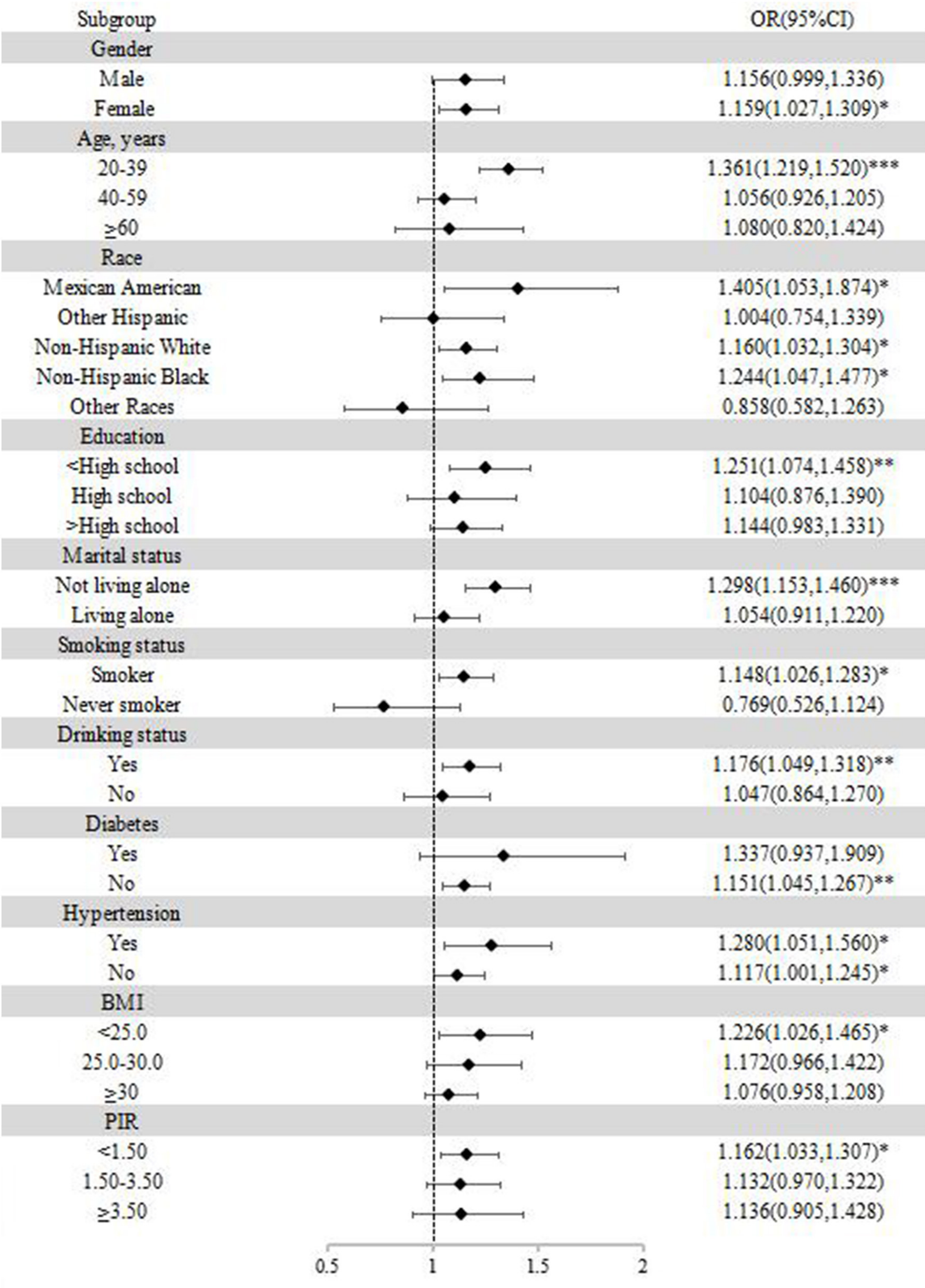

FIGURE 4
Subgroup analysis of the association between blood cadmium and anxiety. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

This study has several strengths. Firstly, the data come from three cycles of NHANES 2007–2012, a reliable data source, with a sufficiently large sample size. Secondly, the complex sampling with NHANES was considered in the statistical analyses, enhancing the credibility of the results. Finally, the use of standardized data collection and reliable information from the NHANES database increases the objectivity and reliability of the findings.

Our article has several limitations. Firstly, NHANES data are cross-sectional, which prevents an in-depth exploration of causality. Future cohort studies are necessary to confirm our conclusions. Secondly, although the confidence level of the dependent variable was high, the measurements were obtained from questionnaires and may be influenced by the subjectivity of respondents. Lastly, as the data were sourced from the United States, caution should be exercised when generalizing the findings to other populations.

5 Conclusion

High levels of blood cadmium are positively associated with the development of anxiety disorders, which needs to be further verified in future studies.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.cdc.gov/nchs/nhanes/index.htm.

Ethics statement

The studies involving humans were approved by National Center for Health Statistics Research Ethics Review Board. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

LB: Writing – original draft, Writing – review & editing. ZW: Writing – review & editing. YZ: Writing – review & editing. HJ: Validation, Writing – review & editing. JS: Writing – review & editing. JC: Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by the Ministry of Humanities and Social Science Education Project (No. 19YJC630182), Jiangsu Province Postdoctoral Research Funding Program (No. 2021K629C), and Scientific Research Foundation for Excellent Talents of Xuzhou Medical University (No. D2019004).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2024.1402715/full#supplementary-material

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frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 29 August 2024
DOI 10.3389/fpubh.2024.1385500

OPEN ACCESS

EDITED BY
Baby Tabassum,
M. J. P. Rohilkhand University, India

REVIEWED BY
Mohammad Javad Mohammadi,
Ahvaz Jundishapur University of Medical Sciences, Iran
Dong-Xing Guan,
Zhejiang University, China
Brigitte Le Magueresse-Battistoni,
INSERM U1060 Laboratoire de Recherche en Cardiovasculaire, Métabolisme, diabétologie et Nutrition, France

*CORRESPONDENCE
Emmanuel Obeng-Gyasi
eobenggyasi@ncat.edu

RECEIVED 12 February 2024
ACCEPTED 12 August 2024
PUBLISHED 29 August 2024

CITATION
Obeng-Gyasi E and Obeng-Gyasi B (2024) Association of combined lead, cadmium, and mercury with systemic inflammation. *Front. Public Health* 12:1385500.
doi: 10.3389/fpubh.2024.1385500

Association of combined lead, cadmium, and mercury with systemic inflammation

Emmanuel Obeng-Gyasi[1,2]* and Barnabas Obeng-Gyasi[3]

[1]Department of Built Environment, North Carolina A&T State University, Greensboro, NC, United States, [2]Environmental Health and Disease Laboratory, North Carolina A&T State University, Greensboro, NC, United States, [3]Indiana University School of Medine, Indianapolis, IN, United States

Background: Exposure to environmental metals has been increasingly associated with systemic inflammation, which is implicated in the pathogenesis of various chronic diseases, including those with neurodegenerative aspects. However, the complexity of exposure and response relationships, particularly for mixtures of metals, has not been fully elucidated.

Objective: This study aims to assess the individual and combined effects of lead, cadmium, and mercury exposure on systemic inflammation as measured by C-reactive protein (CRP) levels, using data from the National Health and Nutrition Examination Survey (NHANES) 2017-2018.

Methods: We employed Bayesian Kernel Machine Regression (BKMR) to analyze the NHANES 2017-2018 data, allowing for the evaluation of non-linear exposure-response functions and interactions between metals. Posterior Inclusion Probabilities (PIP) were calculated to determine the significance of each metal's contribution to CRP levels.

Results: The PIP results highlighted mercury's significant contribution to CRP levels (PIP = 1.000), followed by cadmium (PIP = 0.6456) and lead (PIP = 0.3528). Group PIP values confirmed the importance of considering the metals as a collective group in relation to CRP levels. Our BKMR analysis revealed non-linear relationships between metal exposures and CRP levels. Univariate analysis showed a flat relationship between lead and CRP, with cadmium having a positive relationship. Mercury exhibited a U-shaped association, indicating both low and high exposures as potential risk factors for increased inflammation. Bivariate analysis confirmed this relationship when contaminants were combined with lead and cadmium. Analysis of single-variable effects suggested that cadmium and lead are associated with higher values of the h function, a flexible function that takes multiple metals and combines them in a way that captures the complex and potentially nonlinear relationship between the metals and CRP. The overall exposure effect of all metals on CRP revealed that exposures below the 50th percentile exposure level are associated with an increase in CRP levels, while exposures above the 60th percentile are linked to a decrease in CRP levels.

Conclusions: Our findings suggest that exposure to environmental metals, particularly mercury, is associated with systemic inflammation. These results highlight the need for public health strategies that address the cumulative effects of metal exposure and reinforce the importance of using advanced statistical methods to understand the health impact of environmental contaminants. Future research should focus on the mechanistic pathways of metal-induced inflammation and longitudinal studies to ascertain the long-term effects of these exposures.

KEYWORDS

environmental metals, systemic inflammation, C-reactive protein, Bayesian Kernel Machine Regression, NHANES, posterior inclusion probability

Introduction

Lead, cadmium, and mercury are pervasive environmental contaminants with a well-documented history of toxicity (1–4). Human exposure to these metals can occur through various routes, including inhalation, ingestion of contaminated food and water, and occupational exposure. Additionally, incidental ingestion of soils and household dust, are increasingly recognized as concerning exposure routes (5–7). Despite extensive regulation and efforts to reduce environmental contamination, these metals continue to pose a significant public health challenge due to their persistence in the environment and their potential for bioaccumulation in the human body (8, 9).

The association between metal exposure and systemic inflammation is biologically plausible, given the known mechanisms of metal-induced toxicity (10). These metals can induce oxidative stress by generating reactive oxygen species (ROS) (11, 12), which in turn can activate a range of inflammatory pathways. Additionally, these metals have been shown to disrupt the normal functioning of the immune system, either by directly affecting immune cells or by altering the expression of cytokines, chemokines, and other inflammatory mediators (13, 14).

This research article delves into the intricate relationship between these metals and systemic inflammation, a critical pathophysiological process underlying numerous chronic diseases. Systemic inflammation, characterized by the activation of immune pathways and the release of inflammatory mediators throughout the body, has been implicated in the progression of a variety of conditions, including cardiovascular diseases, neurodegenerative disorders, and certain cancers (15). When the body's immune system detects a threat, such as an infection, injury, or the presence of harmful substances like heavy metals, it triggers an inflammatory response to neutralize the threat and initiate healing processes. While acute inflammation is a protective mechanism, chronic systemic inflammation can become detrimental.

In cardiovascular diseases, systemic inflammation contributes to the development and progression of atherosclerosis, where inflammatory cells and mediators promote the formation of plaques in the arterial walls (16). This can lead to reduced blood flow, increasing the risk of heart attacks and strokes. Inflammatory markers like C-reactive protein (CRP) are often elevated in individuals with cardiovascular conditions, indicating ongoing inflammation that exacerbates these diseases.

Neurodegenerative disorders, such as Alzheimer's disease and Parkinson's disease, are also linked to systemic inflammation (17, 18). Chronic inflammation can lead to the activation of microglia, the immune cells of the brain, which release pro-inflammatory cytokines that damage neurons. This persistent inflammatory state contributes to the progressive loss of neuronal function and structure, leading to cognitive decline and motor impairments.

In the context of cancer, systemic inflammation creates a tumor-promoting environment. Inflammatory mediators can induce genetic mutations, promote tumor growth, and enhance the ability of cancer cells to invade surrounding tissues and metastasize to distant organs (19). Chronic inflammation is associated with increased cancer risk and poorer prognosis, as it supports the hallmarks of cancer, including sustained proliferative signaling, evasion of apoptosis, and angiogenesis.

Moreover, systemic inflammation is linked to metabolic disorders such as obesity and type 2 diabetes (20). Inflammatory cytokines interfere with insulin signaling, leading to insulin resistance, a key feature of type 2 diabetes. In obese individuals, adipose tissue becomes a source of chronic inflammation, contributing to the development of metabolic syndrome and associated complications.

In the context of assessing the impacts of environmental exposures, such as those from heavy metals like lead, cadmium, and mercury, on systemic inflammation, traditional analytical approaches often consider each pollutant in isolation (21). However, in real-world scenarios, individuals are typically exposed to a mixture of pollutants, rather than a single contaminant. This complexity necessitates the use of advanced statistical methods capable of evaluating the health effects of multiple pollutants simultaneously (22). One such method that has gained prominence in environmental health research is Bayesian Kernel Machine Regression (BKMR) (23).

BKMR is a novel statistical approach designed to address the challenges posed by multi-pollutant exposure analysis. This method allows researchers to evaluate the health effects of a mixture of pollutants, considering potential interactions and synergistic effects among the different components of the mixture (24). BKMR is particularly advantageous in its ability to handle highly correlated exposures and to provide insights into the combined and individual effects of each component in the mixture.

BKMR offers a comprehensive and nuanced approach in environmental health research, particularly for studying the effects of metal exposures like lead, cadmium, and mercury on systemic inflammation. This method allows for the evaluation of the collective impact of these metals, providing a holistic understanding of associated health risks (24, 25). The flexibility of BKMR in modeling non-linear relationships and varying sensitivities to different exposure levels is crucial, considering the complex nature of biological responses to toxicants (23, 26). Moreover, this approach not only assesses the joint effect of these metals on inflammation but also distinguishes the specific contribution of each individual metal, enhancing our understanding of their respective roles in systemic inflammation.

We chose to study lead, cadmium, and mercury due to their common co-existence in the environment, significant toxicological effects, and strong links to systemic inflammation. These metals are prevalent in industrial emissions, contaminated food and water, and certain consumer products, leading to higher combined exposure risks. Previous research has shown that they induce oxidative stress, a known pathway for systemic inflammation. Understanding their combined effects can inform risk assessment and the development of targeted strategies to mitigate exposure and protect public health.

Materials and methods

Study cohort and design

Data from the NHANES 2017-2018 was utilized in this investigation. This dataset is a representative sample of non-institutionalized people residing in all 50 U.S. states and the

District of Columbia. The U.S. Centers for Disease Control and Prevention (CDC) collected the data, which are available in two-year cycles and include multi-year, stratified, multi-stage, and clustered samples. The NHANES employs a complex, multistage probability sampling design to ensure that the data is representative of the U.S. civilian non-institutionalized population. The 2017–2018 NHANES cycle includes thousands of participants, providing sufficient power to detect significant associations and enabling subgroup analyses. This large sample size enhances the robustness and credibility of the study results. Additionally, the data is collected by the CDC, ensuring rigorous data collection methods and high-quality standards. The consistency and reliability of the data make it a trusted source for epidemiological studies. The sampling process involves the selection of primary sampling units (PSUs), which are typically counties or groups of counties, followed by the selection of segments within PSUs, households within segments, and finally, individuals within households. Oversampling of certain subgroups, such as Hispanics, non-Hispanic Blacks, and low-income individuals, is conducted to improve the reliability and precision of health status indicator estimates for these groups. Selected individuals in the NHANES undergo a comprehensive physical examination conducted in mobile examination centers (MECs), which include detailed medical, dental, and physiological measurements. In addition to the physical examination, participants complete extensive interviews that collect demographic, socioeconomic, dietary, and health-related information. Blood samples are drawn from participants and sent to laboratories for the measurement of various biomarkers, including metal concentrations and C-reactive protein (CRP) levels. The NHANES dataset includes extensive quality control and quality assurance protocols to ensure the accuracy and reliability of the data. Data collection procedures are standardized, and staff are rigorously trained. The CDC continuously monitors data collection and laboratory procedures to maintain high standards of data quality. On the NHANES website of the CDC, additional descriptions and detailed information about the study design, sampling methodology, data collection procedures, and protocols are provided. Researchers can access comprehensive documentation and resources to understand and utilize the dataset effectively for their investigations (27).

Metals and CRP measurements

Metals measurement

Metals in diluted whole blood were measured using inductively coupled plasma mass spectrometry (ICP-MS). ICP-MS is a validated technique widely recognized for its accuracy and precision in analyzing metals in biological media (28). All metal analytes in the dataset had the same detection limits. For analytes below the lower limit of detection, an imputed fill value was used, calculated as the lower limit of detection divided by the square root of 2. The NHANES Laboratory Procedures Manual provides detailed descriptions of specimen collection and processing. The National Center for Environmental Health (NCEH) within the CDC's Division of Laboratory Sciences performed the metal assays on whole blood samples using the ICP-MS method (Method No. ITB0001A).

CRP measurement

The concentration of C-reactive protein (CRP) in the blood was assessed using a two-reagent immunoturbidimetric approach. In this method, the blood sample was initially mixed with a Tris buffer and allowed to incubate. Following this, latex particles coated with mouse-derived antibodies against human CRP were added. These antibodies bind to CRP present in the sample, forming immune complexes that increase the solution's turbidity. This increase in turbidity, caused by light scattering, is proportional to the CRP concentration in the sample. The degree of light scattering was quantitatively measured at primary and secondary wavelengths of 546 nm and 800 nm, respectively. The resulting light absorbance was compared against a calibrated CRP standard curve to determine the CRP levels in the specimen.

These detailed and standardized procedures ensure the reliability and validity of the metal and CRP measurements in the NHANES dataset.

Statistical analysis

Our study utilized linear regression and Bayesian Kernel Machine Regression (BKMR) analysis to evaluate the relationship between metal exposure and systemic inflammation. To ensure the integrity of our analysis, we addressed missing values in the variables of interest by imputing them with the median value. This approach helped to maintain a complete dataset and reduce potential bias associated with missing information.

Our data analytics approach began with thorough data cleaning to address any inconsistencies, duplicate records, or irrelevant information. This crucial step ensured that our analysis was based on accurate and reliable data. For any missing values within the variables of interest, we used median imputation, replacing missing values with the median value of the observed data. This method preserved the overall distribution of the data and minimized the impact of outliers.

We initially applied linear regression to examine the individual relationships between metal exposures and CRP levels, providing a preliminary understanding of potential associations. To capture the complex and potentially non-linear interactions between multiple metals and CRP levels, we then employed BKMR. This advanced statistical method allows for the evaluation of non-linear exposure-response functions and interactions between multiple exposures simultaneously, providing a more comprehensive analysis of the data.

These steps ensured a robust and thorough analytical process, enabling us to derive meaningful insights from the NHANES 2017-2018 data.

Descriptive statistics

Descriptive statistics are presented to describe the distribution of the exposure and demographic variables in the dataset and stratify them by the c-reactive protein. Spearman correlation was used to assess the relationships among the metal's exposure variables and c-reactive protein.

Bayesian Kernel Machine Regression (BKMR)

In this research, we employed Bayesian Kernel Machine Regression (BKMR) with the Markov Chain Monte Carlo (MCMC) sampling method, following the methodology outlined by Bobb et al. (24). Our process involved conducting 5,000 iterations to ensure robust analysis. The choice of priors in our BKMR model was guided by established Bayesian practices to ensure meaningful inference and computational efficiency. Specifically, we utilized non-informative priors for parameters where prior knowledge was limited, allowing the data to primarily inform the posterior distributions. Convergence diagnostics were meticulously conducted to validate the stability and reliability of our results. This included assessing trace plots, autocorrelation plots, density plots, and the Gelman-Rubin convergence statistics for each parameter, ensuring they exhibited stable and consistent patterns without trends. The Gelman-Rubin statistic was confirmed to be below 1.1, indicating successful convergence. A central component of our BKMR analysis was the use of Posterior Inclusion Probabilities (PIPs). PIPs, which range from 0 to 1, are critical in assessing the impact of individual metals within an environmental mixture. They help quantify the relative importance of each metal, such as lead, cadmium, and mercury, in influencing the outcome of interest. To understand the interaction between these metals and systemic inflammation, we computed high-dimensional exposure-response functions, denoted as h(z), at various intervals. This was done while keeping other influencing variables constant at their median values, allowing us to isolate the effects of each metal. BKMR's graphical interpretation capabilities were particularly valuable in our study. These features enabled us to visually compare the effects—both collective and individual—of metal exposures. Specifically, we could contrast outcomes observed at specific exposure percentiles against those at median exposure levels. This approach highlighted the unique relationships between each metal and the outcome while considering the constant median values of other exposures. We adjusted our analysis for potential confounders, including body mass index (BMI), gender, age, education, and ethnicity. This methodological approach provided a nuanced understanding of the individual and combined effects of metals like lead, cadmium, and mercury on systemic inflammation. The analyses were completed using R (version 4.2.3; R Foundation for Statistical Computing, Vienna, Austria)(29). The significance level was set at 0.05.

TABLE 1 Comparative analysis of study variables by median CRP levels: statistical significance and variations.

Variable	CRP above median	CRP below median	*p*-values
Mercury (mean/SE)	1.04 (0.056)	1.15 (0.069)	0.134
Cadmium (mean/SE)	0.387 (0.017)	0.332 (0.010)	0.006
Lead (mean/SE)	1.01 (0.030)	0.928 (0.021)	0.026
BMI (mean/SE)	30.04 (0.288)	24.23 (0.178)	<0.0001
Age (mean/SE)	40.98 (0.511)	34.89 (0.823)	<0.0001

SE, Standard error.

Results

Comparative analysis of critical study variables: CRP levels and their associations

The mean levels of critical study variables were explored by CRP levels above and below the median (Table 1). The results

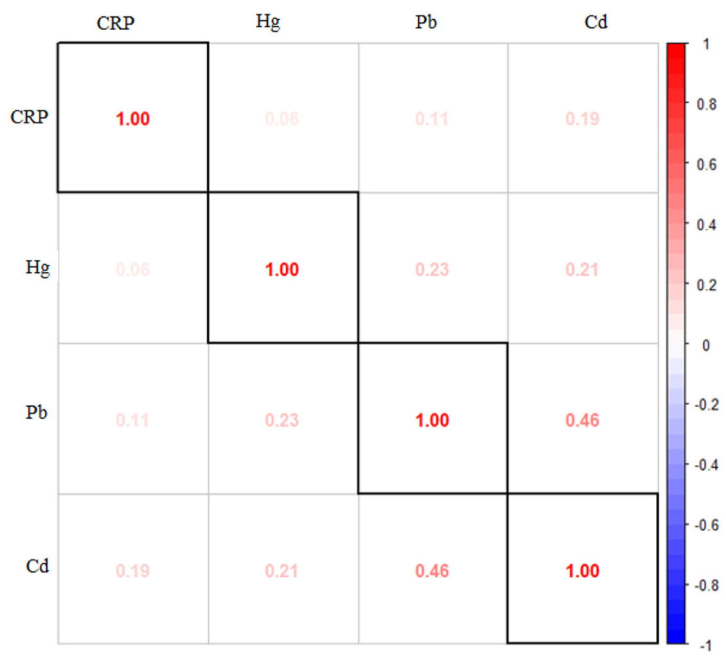

FIGURE 1
Spearman Correlation among variables of interest.

indicated that all variables apart from mercury had a statistically significantly higher level above the median for CRP as compared to below.

Figure 1 presents the Spearman correlation analysis conducted on the study's exposure and outcome variables. The results reveal strong correlations among the metals themselves, indicating inter-metal correlations. Other notable correlations are between CRP levels and cadmium, emphasizing the notable relationship between these two variables. Statistical analysis unveiled notable associations ($p < 0.05$) among various pairs of variables, including significant correlations between CRP and cadmium, lead and cadmium, lead and mercury, and cadmium and mercury.

BKMR results

The significant correlations identified among the variables in our dataset signaled the necessity for employing Bayesian Kernel Machine Regression analysis as opposed to traditional linear regression methods. In traditional linear regression, the assumption of linearity between the independent and dependent variables is fundamental. However, in real-world scenarios like ours, where intricate and potentially nonlinear relationships exist among the variables, these linear methods may not capture the complexity of the data adequately.

BKMR, on the other hand, is a statistical technique that excels in situations where the relationships among variables are intricate and nonlinear. By utilizing flexible kernel functions and Bayesian modeling, BKMR helped to uncover hidden patterns, account for interactions, and capture intricate dependencies that linear regression models might overlook. This adaptability makes BKMR a powerful tool ultimately leading to more accurate and informative insights.

TABLE 2 BKMR analysis of systemic inflammation: group and conditional posterior inclusion probabilities for lead, cadmium, and mercury.

Variable	Group PIP	Conditional PIP
Lead	1	0.01000
Cadmium	1	0.0032
Mercury	1	0.9868

Quantifying metal-related factors in CRP variations: PIP and BKMR analysis

The Posterior Inclusion Probability (PIP) for each metal concerning its relationship with CRP serves as a metric quantifying the likelihood of each contaminant playing a significant role in explaining the variations observed in CRP levels. The PIP values for the influence of lead, cadmium, and mercury on systemic inflammation are 0.3528, 0.6456, and 1.000, respectively.

Table 2 provides hierarchical BKMR analysis for CRP. The analysis categorizes exposure variables into a group and presents the Group PIP and Conditional PIP (values for the group). For CRP,

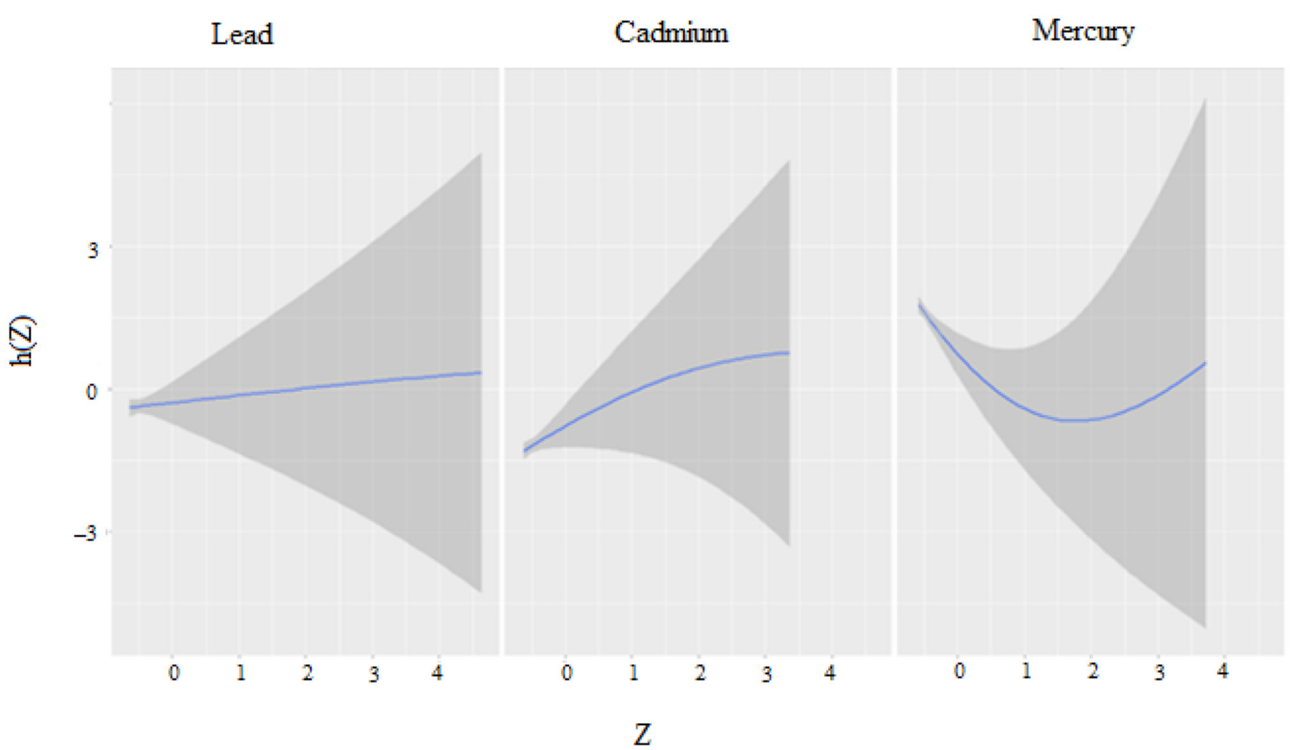

FIGURE 2
Univariate exposure–response functions and 95% credible interval for association between single metal exposure when other metals exposures are fixed at the median.

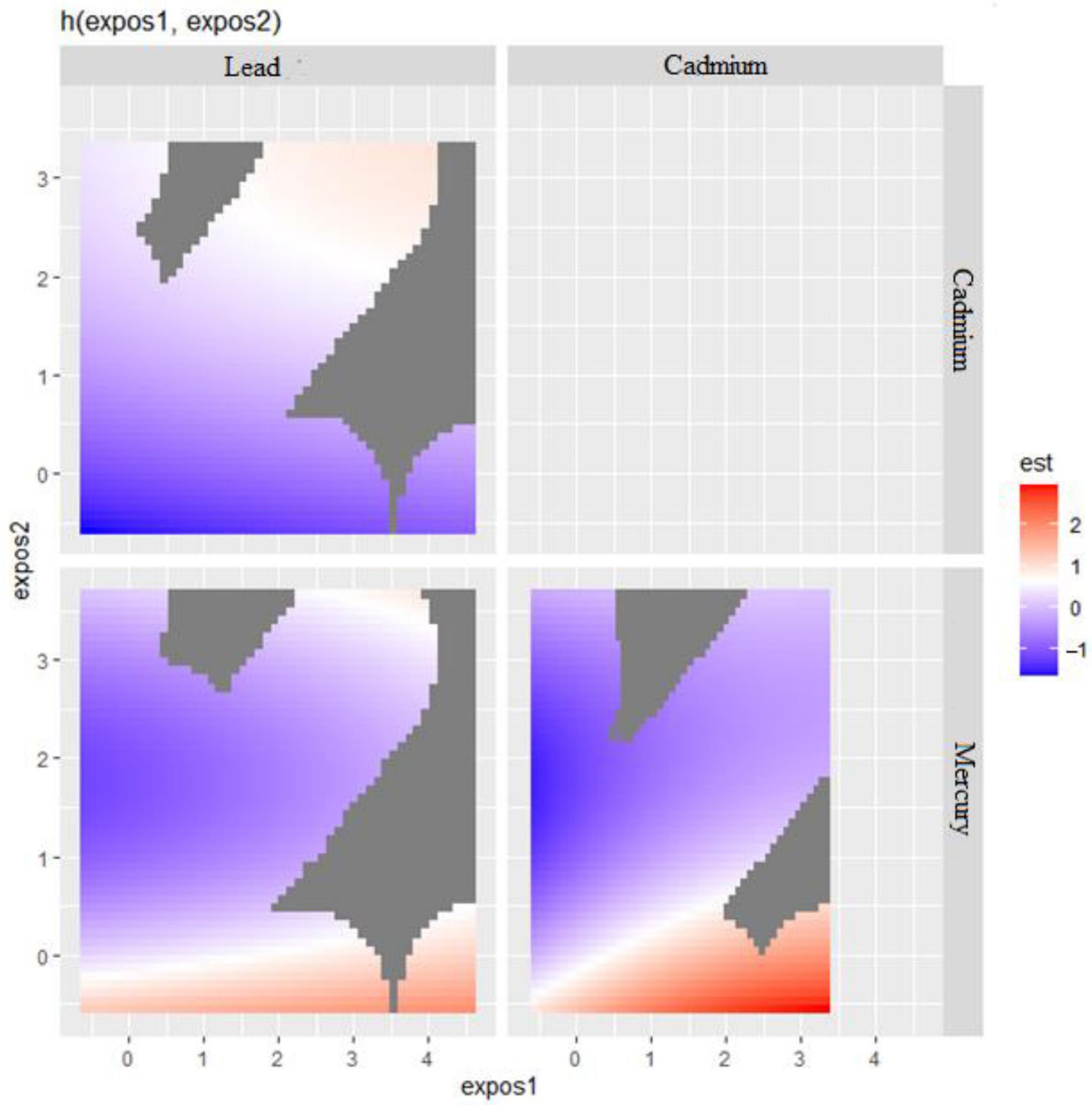

FIGURE 3
Bivariate exposure-response function of metals with CRP.

group 1 includes metals (lead, cadmium, and mercury) all of which have group PIP values of 1 but only mercury has a high condition PIP of 0.9868 suggesting a major influence on CRP.

Univariate analysis: examining the isolated effects of mercury, cadmium, and lead on CRP

The univariate approach visually examines the individual effect of Mercury, Cadmium, and Lead on CRP. Figure 2 shows the impact of each metal on CRP when the other metals are fixed at the median and the covariates are held constant with cadmium and mercury having the largest impact. **Regarding the figure,** the flat curve in the Lead panel suggests that variations in Lead exposure do not significantly affect CRP levels across the range of exposures analyzed. This could mean that Lead, within the study's observed exposure range, might not be a major determinant of CRP levels, or that its effect is overshadowed by other factors not captured in this plot.

The curve for Cadmium rises at lower exposure levels before plateauing, indicating that an increase in Cadmium exposure may be associated with higher CRP levels initially. However, as exposure continues to increase, this effect does not appear to intensify. This might suggest a threshold effect, where below a certain level of exposure, changes in Cadmium concentrations have a more pronounced impact on CRP levels.

The U-shaped curve observed for Mercury implies a non-linear relationship with CRP levels. Low and high levels of Mercury exposure are associated with higher CRP levels, whereas moderate levels correlate with lower CRP. This could be indicative of a complex mechanism by which Mercury affects inflammation, potentially having a hormetic effect where it might exert different biological effects at different concentrations.

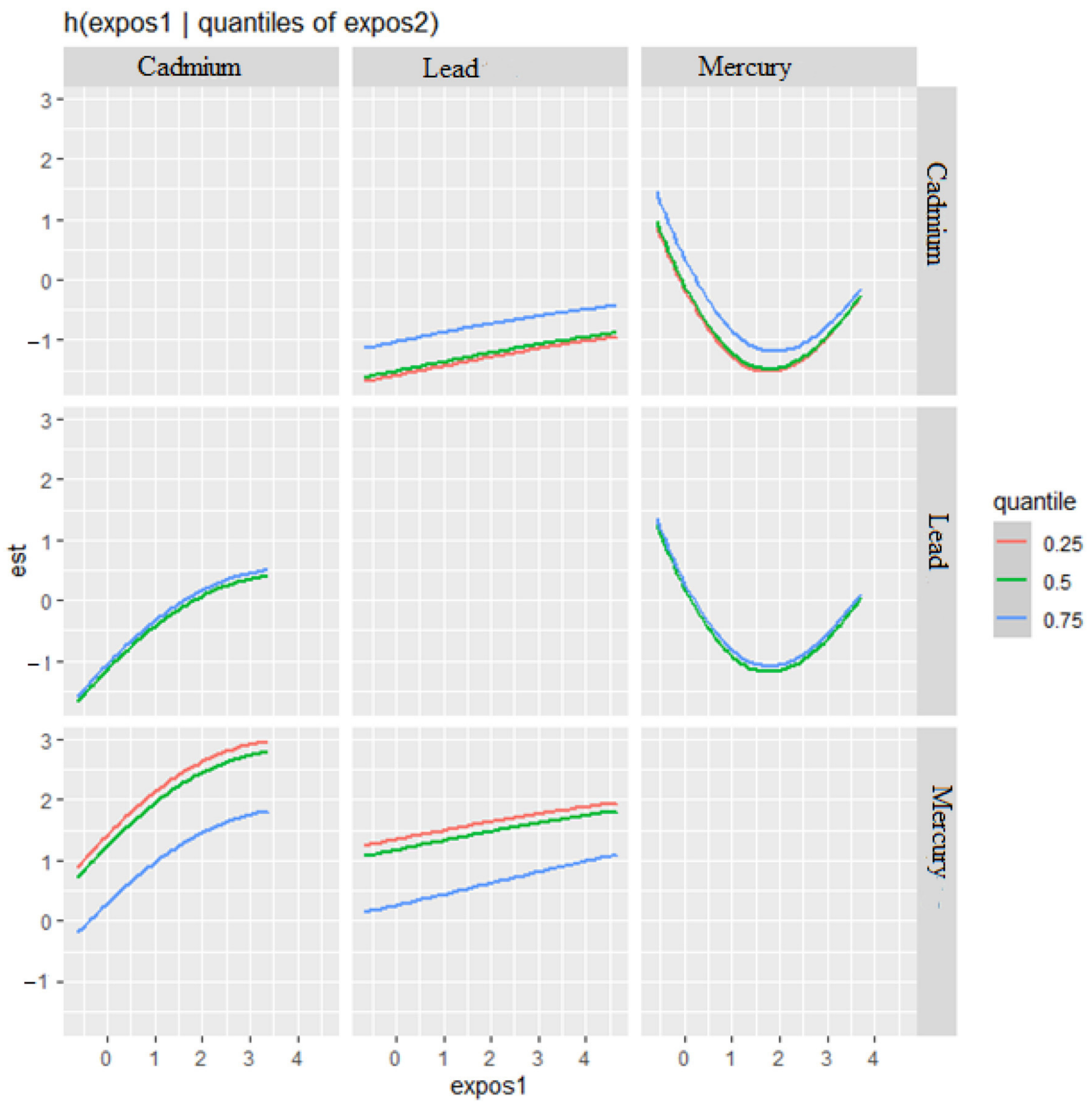

FIGURE 4
Bivariate exposure-response function of metals with CRP–investigating predictor-response function with varying quantiles of the second predictor, while other predictors are fixed.

Visualizing bivariate exposure-response functions with fixed percentile values

Bivariate metals exposure on CRP was explored where two metals of interest effects on CRP were examined while all other predictors were fixed at a particular percentile. In these plots (Figure 3), the color scale (est) represents the estimated effect on the health outcome. In this plot, red indicates a higher positive effect (which means an increased risk of a negative health outcome associated with increasing levels of the biomarker CRP), blue indicates a negative effect, and white or gray indicates no effect. The results seen in Figure 3 suggest that in the 'Lead' vs. 'Cadmium' plot (top left), higher levels of both exposures seem to have no effect on the outcome as indicated by the white and blue regions. In the 'Cadmium' vs. 'Mercury' plot (bottom right), there appears to be a region where increasing levels of both 'Cadmium' and 'Mercury' are associated with a positive effect on the outcome, as indicated by the red area. This happens also with Lead and Mercury but to a lesser extent.

The bivariate relationship was further explored by examining metal pairs. The analysis examined the relationship between individual metals and CRP by fixing the second metal at different quantiles: 25th (red line), 50th (green line), and 75th (blue line), with other metals held at the median (Figure 4). These models were adjusted for the covariates of interest. The x-axis, labeled "expos1", shows the levels of one exposure, while the y-axis, labeled "est", represents the estimated effect on CRP levels. Each row of plots corresponds to a different exposure being considered as "expos1".

Interaction Effects: Each plot shows how the relationship between "expos1" and CRP changes at different quantiles of a second exposure, "expos2". The three lines within each plot correspond to the 25th, 50th, and 75th quantiles of "expos2", as indicated by the color legend.

The interpretation of the plot by each metal is as follows. **Cadmium (as expos1)**: When interacting with Cadmium (top row), the effects on CRP appear relatively flat across all quantiles of Lead and Mercury, suggesting that Cadmium's effect on CRP levels is consistent regardless of the levels of the other metals.

Lead (as expos1): For Lead, the plots show a U-shaped relationship with CRP at different quantiles of Cadmium and Mercury, indicating that both low and high levels of Lead are associated with higher CRP levels, suggesting a non-linear interaction.

Mercury (as expos1): Mercury's interaction plots show a strong U-shaped relationship with CRP at different quantiles of Cadmium and a similar but less pronounced U-shape with Lead. This suggests that Mercury has a non-linear association with CRP levels, potentially indicating a more complex interaction.

Effect of Quantiles: The differences in the shapes of the lines across different quantiles of "expos2" within each plot indicate how the effect of "expos1" on CRP varies with the levels of "expos2". For example, in the bottom left plot (Mercury interacting with Cadmium), the curves for the 25th and 50th quantiles of Cadmium are relatively similar, suggesting consistent effects at lower to mid-levels of Cadmium. However, at the 75th quantile, the curve rises more steeply, suggesting a stronger interaction effect of Mercury on CRP at higher levels of Cadmium.

Overall risk summary of CRP levels in relation to exposure percentiles

Figure 5 measures the total effect of all exposures or mixtures. The exposures are fixed at different quantities starting from the 25th percentile to 75th percentile at increments of 5 using the 50th percentile (median) to compare the exposures. The estimation for all exposures at the 50th percentile shown at zero (dashed line) demonstrates that when comparing all exposures between the 20th and 55th percentile exposure level to the 50th percentile exposure level, the CRP is above zero while after the 60th percentile exposure level CRP falls below zero. This analysis reveals that exposures below the 50th percentile exposure level are associated with an increase in CRP levels, while exposures above the 60th percentile are linked to a decrease in CRP levels.

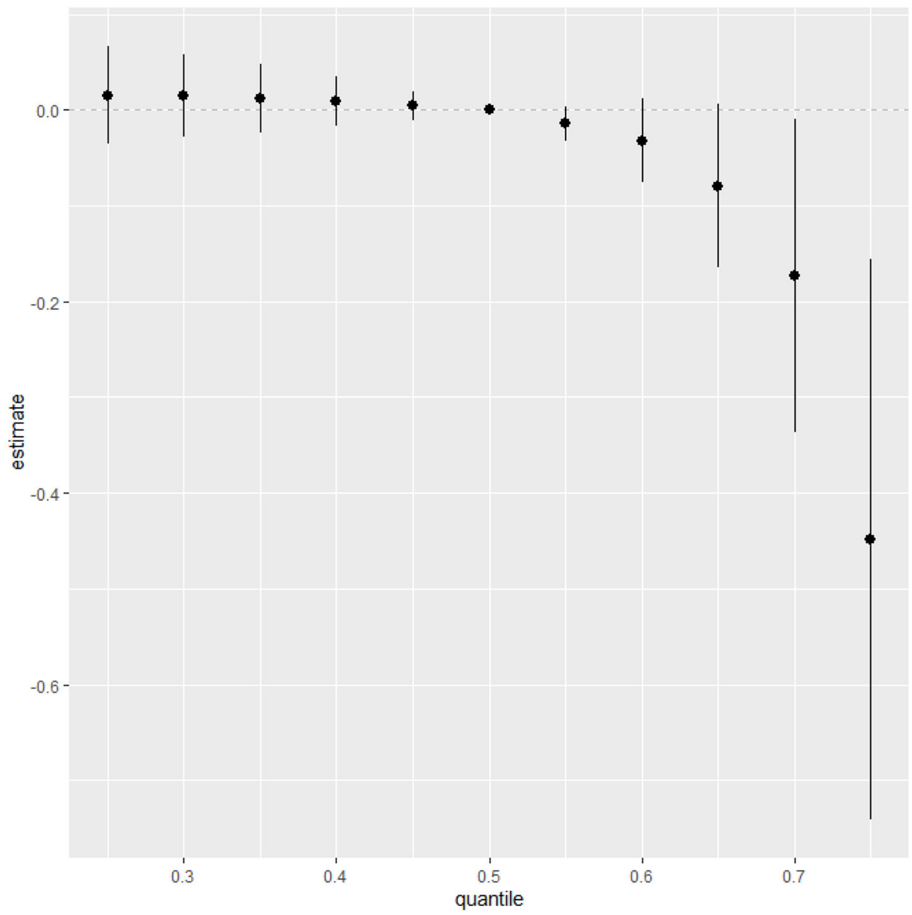

FIGURE 5
Summary of overall health effects of the exposures (multimixers) on the outcome depends on various percentiles (form 25th to 75th percentiles).

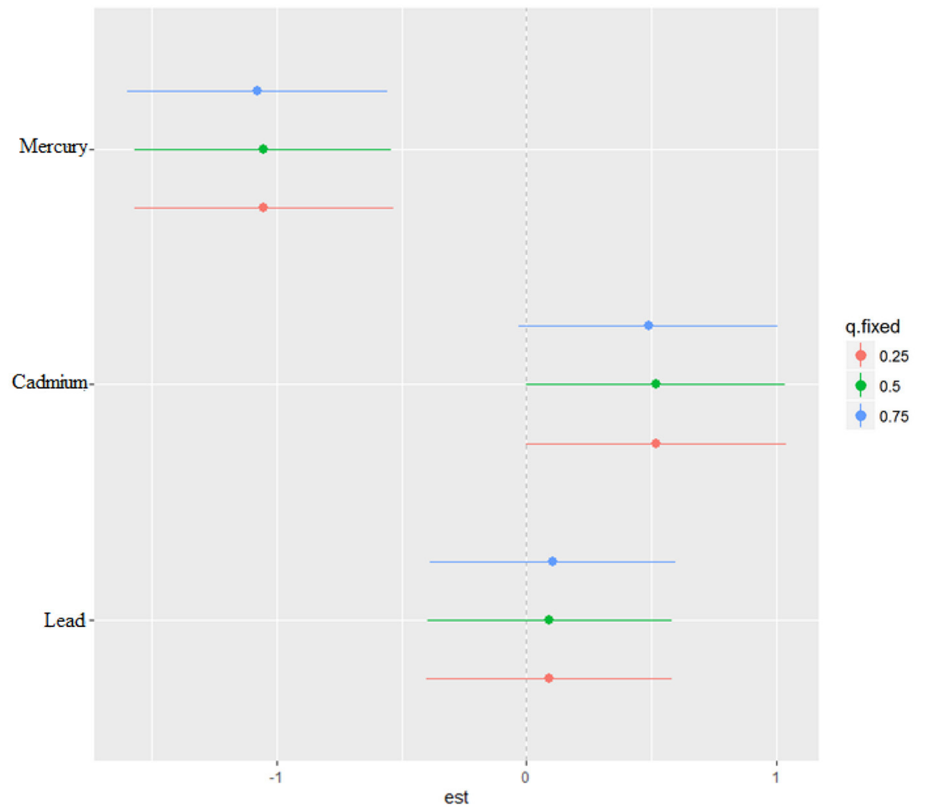

FIGURE 6
Single-variable effect of metals at increasing quartiles for CRP.

Single-variable effects of metals on CRP

The single-variable effect helps to understand the effect of a single predictor at different quantiles giving us the ability to assess their contribution to the overall risk of elevated CRP. Figure 6 demonstrates the single-variable effects of metals on CRP at the 75th (blue), 50th (green), and 25th (red) quantile and suggest cadmium and lead are associated with higher values of the ***h*** function, a flexible function which takes multiple metals and combines them in a way that captures the complex and potentially nonlinear relationship between the metals and CRP. Overall, the plot and the quantiles specifically show how the relationship between each metal and CRP may change across the distribution of the metal exposure.

Discussion

This study embarked on a nuanced exploration of the relationships between exposure to various heavy metals and systemic inflammation, as quantified by C-reactive protein levels, utilizing the robust Bayesian Kernel Machine Regression (BKMR) methodology. Our results substantiate the proposed link between metal-induced oxidative stress and heightened systemic inflammation, drawing attention to the intricate interplay between lead, cadmium, and mercury and their collective influence on CRP. Studying the combined effects of heavy metals like lead, cadmium, and mercury provides a more accurate assessment of health risks. These metals often coexist in the environment, and their interactions can amplify their harmful effects on systemic inflammation and overall health. Understanding the combined impact of these metals can inform more effective public health policies and regulations. Policies can be tailored to address the cumulative risks posed by mixed metal exposures, leading to better protection for at-risk populations. Highlighting the combined effects of heavy metals is crucial for promoting environmental and health justice. Marginalized communities often face higher exposures to multiple pollutants, and this research can support the development of strategies to reduce environmental health disparities and ensure equitable health outcomes for all communities. By recognizing the complex interactions between different heavy metals, healthcare providers can develop more effective interventions and treatments. This knowledge can lead to better screening protocols, preventive measures, and treatment plans for individuals exposed to these harmful substances. Studying the combinations of heavy metals advances scientific understanding of their synergistic effects. This knowledge is essential for developing innovative solutions to mitigate the health impacts of environmental contaminants and improve public health outcomes.

Through the lens of BKMR, we were able to unveil complex non-linear relationships and potential synergistic interactions among metal exposures, phenomena that remain obscured within the confines of conventional linear models. Mercury, in particular, emerged with a pronounced non-linear relationship with CRP levels, a biphasic pattern suggesting that both deficient and excessive exposures bear the potential to exacerbate inflammation. The complexity of Mercury's relationship with health outcomes has been demonstrated in other studies (30). Mercury's effects on inflammation are well known (31), but these findings add to the nuance of how exposure context shapes inflammation-related outcomes. This nuanced understanding has profound clinical implications, especially for populations burdened with high environmental exposure, prompting a shift in public health initiatives to consider the intricate and cumulative effects of metal exposures.

To further dissect these complexities, we leveraged the Posterior Inclusion Probability (PIP) as an analytical compass to gauge the significance of each metal's role in the observed variations in CRP levels. Mercury's unequivocal PIP of 1.000 firmly establishes its significant influence on CRP levels, indicating its strong role in systemic inflammation. Cadmium's substantial, albeit less consistent PIP of 0.6456, along with lead's more modest PIP of 0.3528, paint a more heterogeneous picture of influence, suggesting that their impact on inflammation may be modulated by a confluence of exposure levels (32), biological interactions (33), and other methodological nuances of the model. The substantial impact of cadmium in a mixture have been noted elsewhere (34).

The collective importance of these metals is underscored by Group PIP values of 1, yet it is mercury, with a high conditional PIP of 0.9868, that stands out as a pivotal individual factor in the elevation of CRP levels. This differentiation in the PIP spectrum not only holds clinical weight but also kindles a policy discourse on prioritizing interventions (35) to curtail exposures, with a particular focus on mercury.

Our comparative analysis across critical study variables disclosed a notable divergence, with cadmium and lead exposures correlating with statistically higher CRP levels, an affirmation of their differential impact on inflammation markers. While mercury did not exhibit a similar direct correlation, its U-shaped response curve in the BKMR analysis reveals a potential hormetic effect (36), signifying that varying exposure levels may instigate distinct biological responses. The U-shape may also be due to their mechanism. Specifically, at low levels, mercury exposure might stimulate inflammatory pathways or immune responses, potentially through the activation of oxidative stress or inflammatory signaling pathways. Conversely, at high levels, mercury's toxic effects could overwhelm these pathways, leading to immunosuppression or reduced inflammation. This dual effect could explain the observed U-shaped curve. Additionally, Previous studies have reported similar U-shaped dose-response relationships for other toxicants, suggesting that the effect of mercury on inflammation may not be linear (30). For instance, some research has shown that low-level exposure to certain metals can enhance pro-inflammatory cytokine production (37), while high levels may induce apoptosis or other protective mechanisms that reduce inflammation (38).

Cadmium's threshold effect, with a plateauing of CRP levels in response to increasing exposure, suggests a saturation point in its inflammatory potential, whereas the absence of a pronounced dose-response relationship for lead signals a more intricate or subdued influence on inflammation.

The outcome of the bivariate exposure-response functions further illuminated the potential for synergistic interactions between metals (39), particularly in the dynamic interplay between lead and cadmium and between cadmium and mercury. This synergy, which could amplify inflammation, underscores the need for public health policies (40) to address the multifaceted risk of mixed metal exposures.

Our analysis also highlighted a paradoxical inverse relationship at higher metal exposure percentiles, where increased metal levels were correlated with a decrease in CRP, hinting at possible saturation effects or adaptive biological mechanisms that mitigate inflammation at heightened concentrations of these metals.

The implications of our findings are manifold, extending beyond immediate clinical concerns to inform future research agendas. For example, in the context of our findings on metal-induced oxidative stress and inflammation, the role of CRP as a clinical biomarker gains additional significance. CRP, produced in response to inflammation, serves as a crucial indicator for a range of conditions, including those within the neurosurgical sphere. Elevated CRP levels, linked to an increased risk of neurodegenerative diseases and stroke, underscore the broader implications of metal exposure in systemic inflammation (41). This insight is vital in, for example, neurosurgery, where understanding such inflammatory markers can profoundly impact surgical outcomes and recovery processes (42). Thus, our study's revelation of the nuanced influence of metals like mercury on CRP levels brings to light the critical intersection of environmental health and neurosurgical care.

The need for advanced statistical tools to decipher the labyrinth of complex environmental exposures is clear. Future research should focus on elucidating the mechanistic pathways by which these metals influence inflammation and the progression of related chronic diseases. Longitudinal studies are particularly warranted to unravel the temporal intricacies between metal exposure and inflammation, potentially paving the way for targeted therapeutic and preventive measures. Moreover, public health policies must adapt to address the complex and cumulative risks posed by mixed metal exposures, emphasizing the need for stricter regulations and interventions, particularly concerning mercury. Clinicians and policymakers should collaborate to develop strategies that mitigate metal exposure, enhance environmental safety, and improve health outcomes for affected populations.

One limitation of our study is the lack of specific geographical information in the NHANES dataset, as it is de-identified to protect participant privacy, which precludes analysis of localized environmental exposure risks. Another limitation of our study is that we focused on the end result of systemic inflammation rather than incorporating parameters such as morbid obesity, waist circumference, and other well-known contributors to systemic inflammation and elevated CRP levels, which could provide additional context and enhance the understanding of the relative impact of heavy metal exposures. Additionally, the cross-sectional design of the NHANES dataset limits our ability to infer temporality and causality between heavy metal exposures and systemic inflammation. Longitudinal studies are needed to better understand the temporal relationships and causal pathways involved.

Conclusions

This study highlights the complex interactions between lead, cadmium, and mercury exposures and systemic inflammation, as measured by C-reactive protein (CRP) levels. Utilizing Bayesian Kernel Machine Regression (BKMR) and Posterior Inclusion Probabilities (PIPs), we revealed significant non-linear relationships, particularly noting mercury's pronounced U-shaped association with CRP. The findings underscore the importance of considering combined metal exposures in public health strategies. Future research should focus on the mechanistic pathways and long-term effects of these exposures to better inform policy and therapeutic interventions.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: cdc.gov/nchs/nhanes/index.htm.

Author contributions

EO-G: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. BO-G: Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number R16GM149473.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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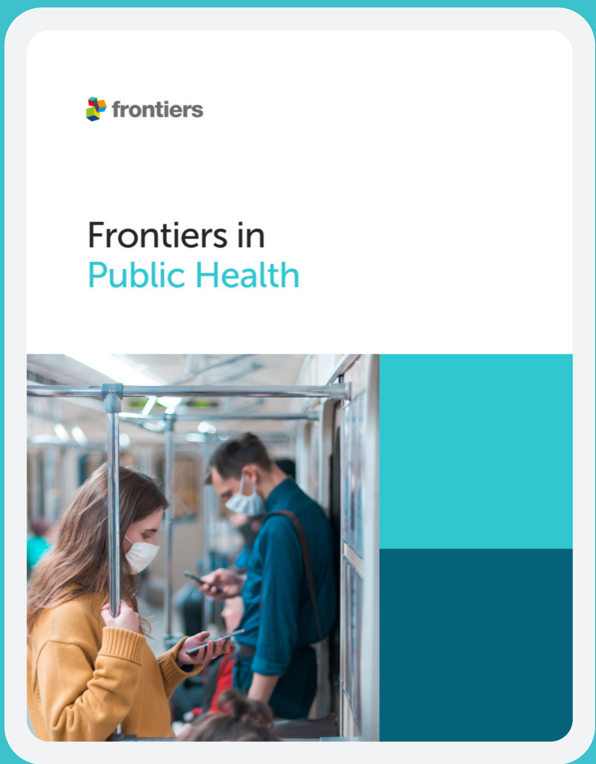

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