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Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Micronuclei frequency and exposure to chemical mixtures in three Colombian mining populations

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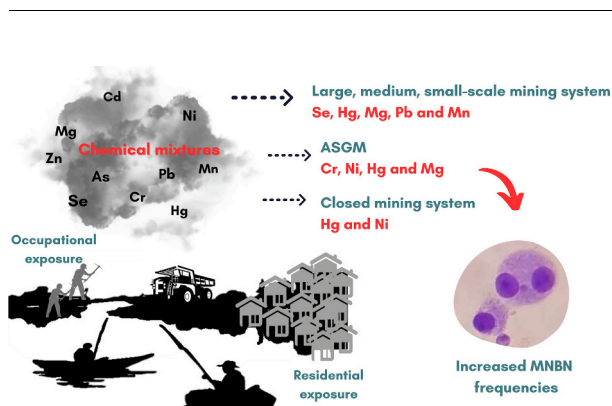
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HIGHLIGHTS

- MNBN frequencies in exposed populations were significantly higher compared to reference area.
- In large, medium, and small-scale mining system, combined exposure to Se, Hg, Mn, Pb, and Mg were associated with increased MNBN frequencies.
- In ASGM systems, Cr, Ni, Hg, Se, and Mg were the primary contributors to elevated frequencies of MNBN.
- In closed mining system a combination of Hg and Ni played a role in increasing MNBN.
- Se consistently correlated with increased MNBN frequencies across all active mining areas.

GRAPHICAL ABSTRACT



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<https://doi.org/10.1016/j.scitotenv.2023.165789>

Received 2 May 2023; Received in revised form 12 July 2023; Accepted 23 July 2023

Available online 25 July 2023

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ARTICLE INFO

Guest Editor: Guilherme Malafaia

Keywords:

Complex mixtures
Montelibano
Nechí
Aranzazu
Micronuclei frequencies
Mining systems

ABSTRACT

The Colombian mining industry has witnessed significant growth. Depending on the scale and mineral extracted, complex chemical mixtures are generated, impacting the health of occupationally exposed populations and communities near mining projects. Increasing evidence suggests that chromosomal instability (CIN) is an important link between the development of certain diseases and exposure to complex mixtures. To better understand the effects of exposure to complex mixtures we performed a biomonitoring study on 407 healthy individuals from four areas: three located in municipalities exploiting different-scale mining systems and a reference area with no mining activity. Large, medium, and small-scale mining systems were analyzed in Montelibano (Córdoba), artisanal and small-scale mining (ASGM) in Nechí (Antioquia), and a closed mining system in Aranzazu (Caldas). The reference area with no mining activity was established in Montería (Córdoba). ICP-MS measured multi-elemental exposure in hair, and CIN was evaluated using the cytokinesis-block micronucleus technique (MNBN). Exposure to mixtures of chemical elements was comparable in workers and residents of the mining areas but significantly higher compared to reference individuals. In Montelibano, increased MNBN frequencies were associated with combined exposure to Se, Hg, Mn, Pb, and Mg. This distinct pattern significantly differed from other areas. Specifically, in Nechí, Cr, Ni, Hg, Se, and Mg emerged as the primary contributors to elevated frequencies of MNBN. In contrast, a combination of Hg and Ni played a role in increasing MNBN in Aranzazu. Interestingly, Se consistently correlated with increased MNBN frequencies across all active mining areas. Chemical elements in Montelibano exhibit a broader range compared to other mining zones, reflecting the characteristics of the high-impact and large-scale mining in the area. This research provides valuable insights into the effects of exposure to chemical mixtures, underscoring the importance of employing this approach in the risk assessment of communities, especially those from residential areas.

1. Introduction

Propelled by state policies, the Colombian mining sector, particularly coal, nickel, and gold extraction, has experienced an exponential increase, placing the country as the largest coal producer in Latin America, the fourth in nickel, and the sixth in gold (ANM, 2019).

Depending on the mineral and the scale of the mining system, extractive activities are characterized by the generation of particulate matter (PM) consisting of a complex mixture of toxic elements such as chromium (Cr), cadmium (Cd), titanium (Ti), manganese (Mn), nickel (Ni), lead (Pb), arsenic (As), zinc (Zn), hydrocarbons and nanoparticles (Oliveira et al., 2022); or the release of mercury (Hg) and cyanide into water bodies (Enamorado-Montes et al., 2021). Large-scale mining typically involves industrial-scale operations with advanced technologies and significant environmental and human impacts (Qudrat-Ullah and Panthallor, 2021). Artisanal mining, on the other hand, is characterized by small-scale, often informal operations with rudimentary tools and practices (Espinoza et al., 2020).

The environmental and health impact of mine residues can persist even after they are closed, as they have the potential to contaminate air, water, soil, and wetland sediments through dispersed tailings. Additionally, discharged leachate can lead to groundwater pollution if proper remediation measures are not undertaken (Aslibekian and Moles, 2003; Kim et al., 2008).

Human exposure to mining residues can occur in these areas through occupational and residential routes. Occupational exposure can occur during mining and milling operations (Sepadi et al., 2020) through inhalation (da Silva-Rêgo et al., 2022), ingestion and dermal contact (Newman et al., 2004); residential exposure, on the other hand, may occur at greater distances from the mines through house dust, which is an important route of exposure, particularly for children (Braga et al., 2007; Entwistle et al., 2019), and the consumption of contaminated water or food (Anyanwu et al., 2018).

Exposure to metals generated during mining operations has been associated with increased morbidity from respiratory diseases (Tikhonova et al., 2020; Weichenthal et al., 2013), cardiopulmonary diseases (Hendryx and Ahern, 2008), decreased kidney function (Rodríguez-Villamizar et al., 2023) and cancer (Giri and Singh, 2017; Kurth et al., 2015).

Although the biological mechanisms behind these associations have not been fully established, increasing evidence has pointed out that chromosome instability (CIN) caused by environmental exposure to

genotoxins like metal mixtures could be the common link between exposure and disease (Fenech et al., 2021). CIN is defined as the gain and/or loss of whole chromosomes or chromosomal segments (de Cárcer et al., 2018) induced by alterations during DNA replication, errors during mitosis, DNA double-strand breaks (DSBs), and exogenous or endogenous sources of DNA damage. CIN may also generate chromosomal rearrangements and is considered the main hallmark of cancer (Simonetti et al., 2019; Yurov et al., 2019). One of the best-validated biomarkers of CIN is the frequency of micronuclei in binucleated cells (MNBN), produced due to the missegregation of chromosomal fragments and/or whole chromosomes during mitosis (Soto et al., 2018). Thus, the presence of MNBN is the hallmark of CIN (Casta et al., 2012).

MNBN frequencies have been previously used in assessing exposure to metals in populations with occupational (León-Mejía et al., 2011) and residential exposure to coal (Espitia-Perez et al., 2018a; Espitia-Perez et al., 2018c) and gold mining residues (Cruz-Esquivel et al., 2019; Galeano-Páez et al., 2021) in mining regions of Colombia, demonstrating the importance of these biomarkers in establishing the risk for public interventions and health surveillance policies (Tique Ortiz, 2022). In these studies, populations occupationally exposed to coal mining residues also showed elevated concentrations of As and Si in their blood (León-Mejía et al., 2014). In contrast, populations with environmental exposure exhibited higher levels of Cr, Ni, Mn, and Br (Espitia-Perez et al., 2018b). Individuals in areas exposed to ASGM (artisanal and small-scale gold mines) residues presented high Hg concentrations in their hair (Galeano-Páez et al., 2021) and Hg and As in their blood (Cruz-Esquivel et al., 2019). However, most studies have focused on the effects of individual metals (García-Villarino et al., 2022) and specific mining practices; thus, there is limited evidence on the toxicity of exposure to mixtures of chemicals (Wallace and Buha Djordjevic, 2020). This research extends the scope by simultaneously investigating multiple mining systems within the same geographic region and examining the differences in exposure types and associated health risks in relation to CIN. This study included four sampling areas: three located in municipalities with the exploitation of different-scale mining systems (large, medium-scale, artisanal and closed mining systems) and a reference area with no mining activity.

In Colombia, although mining activities are distributed throughout the country, they converge particularly in three territories: the departments of Córdoba, Caldas, and Antioquia (Fedesarrollo, 2012). These departments overlap one of the largest open-pit ferronickel mines in the world, along with open-pit coal mines, medium-scale, ASGM, and

inactive Hg mines. Consequently, these areas constitute unique models for evaluating the effects of chemical mixtures on MNBN frequencies and CIN, with approximately 60 thousand workers and 200 thousand individuals living near mining facilities (Baena, 2017).

The municipality of Montelibano in the department of Córdoba includes in the same geographic area large-scale ferronickel mining systems, medium-scale coal mining systems, and ASGM; on the other hand, Nechí in the department of Antioquia includes one of the most important ASGM districts in Colombia, while Aranzazu in the department of Caldas includes a Hg mine active until 1974. Even when the mine was closed in 1974 due to continuing concerns about high levels of occupational exposure, poor ventilation conditions and tunnel collapses, the mine closure failed to eliminate the ongoing health issues and concerns among the communities of Aranzazu (Restrepo, 2016). Finally, a reference area was established in Montería, a city also located in the department of Córdoba, 155 Km away from the nearest mining district but with similar sociodemographic, economic, and genetic characteristics to populations of the mining areas.

By assessing chemical mixture exposure and health effects across diverse mining systems, this approach allows for a comprehensive evaluation of the risks associated with different mining practices, including identifying specific chemical components present in each system and their potential interactions and cumulative effects. It also enables a comparison of the health impacts experienced by workers and communities in various mining contexts, considering factors such as time of exposure, age, sex, and other modulators factors. This study is the first approach at the regional and national level on the effects of elemental chemical mixtures and constitutes an important source of information for decision-making by Colombian government agencies.

2. Materials and methods

2.1. Sampling sites

Considering that each mining system may result in different types and levels of chemical mixtures, as well as distinct exposure pathways for workers and nearby communities, we studied four sampling areas: three located in municipalities with the exploitation of different-scale mining systems (large, medium-scale, ASGM and closed mining systems) and a reference area with no mining activity (Table 1).

The first area located at the Montelibano mining district, also known as the southern mining district of Córdoba, includes a mixture of large-scale ferronickel extraction, medium-scale coal deposits, and ASGM (Fig. 1). This area consists of the largest open-pit ferronickel mine in Latin America (Díaz et al., 2015; UPME, 2005), which is also the second-largest mine in the world (Cruz et al., 2006). This deposit contributes 10 % of Colombian and 3 % of world nickel production (Leon-Medina et al., 2020). In contrast, coal is extracted by medium-scale, technified companies and is consumed in domestic markets because of its high sulfur content (Ingeominas, 2005). The gold mining in this area is mainly artisanal, small-scale, family-operated and for subsistence purposes (González Martínez et al., 2020; Idrovo et al., 2017). In this particular area, we could consider occupational multisource exposure due to labor mobility, particularly among workers in coal-gold mining systems and

certain production characteristics of ferronickel. In ferronickel extraction, coal is used for the pre-reduction of nickel-containing ores through rotatory kiln treatment (Sahajwalla et al., 2004) and electric ARC furnace ferronickel refining (Lis and Nowacki, 2012; Tsubouchi et al., 2010). Simultaneously, traditional ASGM activities serve as a source of secondary economic income in the region, potentially resulting in varying exposure among workers in other mining settings (Bernal and Castelblanco, 2019).

A second area was the gold mining district of El Bagre-Nechí, one of the most important in the country, exploited since pre-Hispanic times and located between the Bajo Cauca-Antioquia and “La Mojana” regions (Leserri and Chaverra Suárez, 2021). Gold is extracted from tributaries and streams of the Cauca River in ASGM systems. Most individuals in this activity operate in illegal systems without government control and surveillance (Gómez-Rodríguez et al., 2017).

The third area was constituted by a closed Hg mine in Aranzazu, located north of the department of Caldas on the central mountain range (Fig. 1). The mining activity was concentrated on Hg extraction at the La Esperanza mine, which was productive from 1948 to 1974 (Escobar, 2002). Although the mine is no longer active, the persistence of Hg in environmental matrices could suggest the presence of residual environmental exposure in this population (Gutiérrez-Mosquera et al., 2021). This comparative approach allows us to understand the extent of environmental contamination and assess its long-term effects on human health.

Finally, the municipality of Montería, established as the reference zone, is the capital of the department of Córdoba (Fig. 1). The selection of a reference area like Montería, located 155 Km away from the nearest mining district in Montelibano, allows us to establish a zone outside the influence of the mining systems, which guarantees that the reference individuals are not exposed to gold, coal, or ferronickel mining residues, but with sufficient similarity to allow for comparison. Montería is part of one of the agro-productive clusters of the Colombian caribbean region together with Montelibano and Nechí (Ávila Castillo, 2016), and thus, shares common sociodemographic, economic, and genetic characteristics with populations of the mining areas. Regarding sociodemographic characteristics, exposed and reference communities typically mix of urban and rural settings, with cultural practices influenced by a blend of indigenous, African, and European traditions. Even when Montería is considered an intermediate city, its economy is based on a minimally technified agricultural industry, cattle breeding, and a flourishing commercial district, a characteristic that shares with Montelibano, Nechí, and Aranzazu (Echeverri Uribe, 2021). Additionally, the study populations' genetic backgrounds are similar, constituted by diverse populations with a predominant mestizo ethnic composition (Ibarra et al., 2014). This trace was corroborated by the sociodemographic questionnaires, where all participants self-identified their ethnicity as mestizo, and there was no recorded participation of minority ethnic communities.

2.2. Participants

In the exposed areas, the participants included workers and permanent residents around the areas of influence of mining systems

Table 1
Main exposure characteristics of the mining areas.

Sampling areas	Municipalities	Mining types	Mining systems	Exposure	Potential exposure pathways
Exposed	Montelibano	Ferronickel Coal	Large-scale systems Medium-scale systems	Occupational and residential	Inhalation, ingestion and dermal contact with PM and contaminated soil, and water
	Nechí	Gold Gold	ASGM ASGM	Occupational and residential	Inhalation, ingestion and dermal contact with PM and contaminated soil, and water
	Aranzazu	Mercury	Closed mine	Residential	Legacy contamination

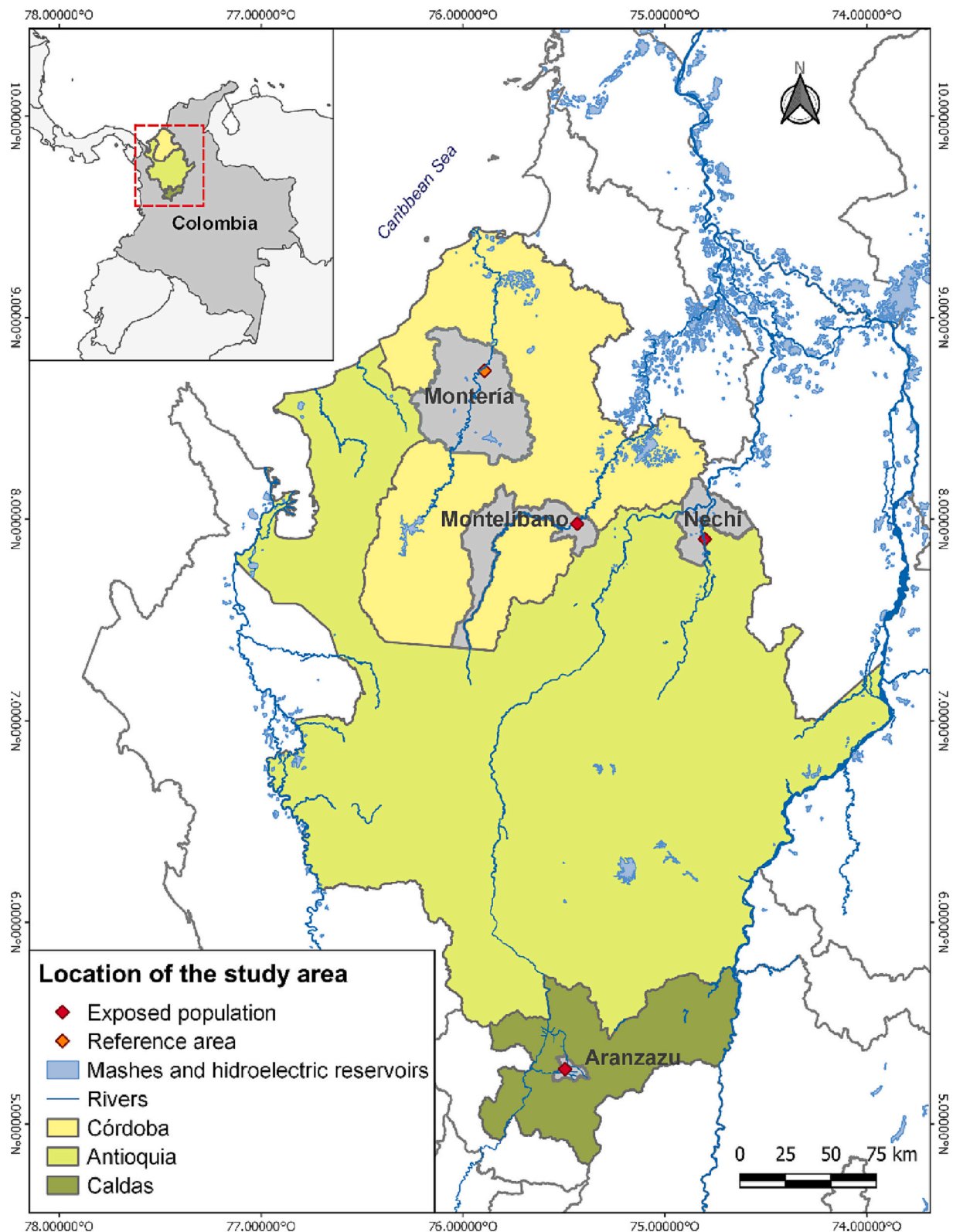


Fig. 1. Sampling sites in Colombia.

(ferronickel mining, gold, coal and Hg).

The Committees on Research Ethics of each institution approved the study. Two questionnaires were used to collect sociodemographic information such as age, time of residence in the area (time of exposure), exposure history, dietary habits (fish and vegetable consumption/kinds and frequency), health and family cancer history, amalgams, smoking

and alcohol consumption habits, and use of specific medications. Questionnaires were elaborated according to the protocol published by The International Commission for Protection against Environmental Mutagens and Carcinogens (Carrano and Natarajan, 1988) and adjusted from the guidance for identifying populations at risk from Hg exposure to evaluate clinical manifestations related to the toxic effect of metals

(WHO, 2016).

Exposed individuals were selected with the following inclusion criteria: volunteer acceptance, no diagnosis of disease, occupational or residential exposure to mining residues (nickel, gold, or coal) for at least a year, age between 18 and 85 years, no smoking habits, no medical treatment in the last three months and no recent exposure to X-rays. For non-exposed individuals, the criteria included: volunteer acceptance, absence of any disease diagnosis, employment as an office worker, university professor, and/or living in areas with no proximity to mining systems, no history of recent exposure to hydrocarbons, solvents, industrial fumes, etc., no smoking habits and no recent exposure to X-rays. All data was organized and recorded in databases. No significant differences were identified regarding the social-economic status or dietary habits between exposed and non-exposed. Exposed and non-exposed control individuals were matched by age (± 5 years), sex, social-economic status, and ethnicity. Alcohol consumers were classified as low, moderate, or heavy drinkers based on the NIAAA (National Institute of Alcohol Abuse and Alcoholism) classification (Espitia-Pérez et al., 2018). Briefly, low drinking is no more than three drinks daily and no more than seven drinks per week. For men, it is defined as no more than four drinks on any single day and no more than 14 drinks per week. Moderate drinking is up to one drink per day for women and up to two drinks per day for men and heavy drinking as five or more drinks on the same occasion on each of 5 or more days in the past 30 days. Fish consumption was established in three intervals previously established as Low, Medium and High: 1–2 days per week (Low), 3–4 days per week (Medium) and 5–7 days per week (High) (Galeano-Páez et al., 2021).

2.3. Blood sample collection

Prior to sample collection, written informed consent was obtained from each individual. Blood collection was performed on 407 healthy individuals: 99 from Montelibano, 103 from Aranzazu and 104 from Nechí (50 % occupationally exposed and 50 % with residential exposure where possible), and 101 from the reference zone. The sample size was determined using the Open Epi application considering a descriptive cross-sectional design. The calculation considered the total population size of each municipality based on the National Administrative Department of Statistics (DANE) census (485700 inhabitants) (DANE, 2005), an anticipated frequency of the study factor set at 30.3 %, a margin of error of 5 %, and a confidence level of 95 %. To account for potential follow-up loss, an additional 20 % of samples were included in the computed sample size.

Peripheral blood (4 mL) was collected from each participant in tubes with heparin (Becton Dickinson, vacutainer) to perform the cytokinesis-block micronucleus technique. Each tube was tagged using a unique barcode, preserved at 4 °C, and transported to the laboratory for processing within 24 h of collection. Simultaneously with the collection of blood samples from exposed and non-exposed individuals, additional whole blood samples from the research staff were collected, transported, and processed under the same conditions. These samples were used as internal controls to detect confounders caused by sample manipulation or transportation to the laboratory.

2.4. Cytokinesis-block micronucleus assay (CBMN)

The CBMN assay was performed following the methodology previously described by Fenech, 2007. To summarize, in duplicated cultures, heparinized whole blood (0.5 mL) was added to 4.5 mL of RPMI 1640 medium (Sigma R8758, USA) supplemented with 2 mM L-glutamine (Sigma A5955, USA), 10 % fetal bovine serum (Gibco/Invitrogen 15000-044, Brazil), 100 μ L/mL antibiotic-antimycotic (Sigma A5955, USA) and 2 % phytohemagglutinin (Sigma L8754, USA). Cultures were incubated at 37 °C in the dark for 44 h under 5 % CO₂. At 44 h of incubation, 6 μ g/mL cytochalasin B (Sigma, C6762) was added. Following incubation, lymphocytes were collected by centrifugation at 1200 rpm for 8 min,

centrifuged again, fixed in 25:1 (v/v) methanol/acetic acid, placed on a clean microscope, and stained with Diff-Quik stain (Lab-Aids; LP64851). For each blood sample, 2000 binucleated (BN) cells (i.e., 1000 from each of two slides prepared from the duplicate cultures) were scored for the presence of MNBN by bright-field light microscopy at 200–1000 \times magnification. All samples were analyzed in a single-blinded fashion according to the criteria proposed by Fenech, 2007.

2.5. Determination of pesticides metabolites in blood samples

The analysis of pesticide levels in blood samples was carried out using Quechers extraction and high-performance liquid chromatography with a triple quadrupole detector (HPLC/Ms-Ms) (Shin et al., 2019). The determined pesticide levels were compared with the levels reported by the German Commission for Human Biomonitoring (Apel et al., 2017) and with the values of the Association of Industrial Hygienists of the United States (ACGIH, 2022). All values found in the samples were considered positive if the permissible limit values were not reported on the abovementioned bases.

Limits of detection (LOD) and quantification (LOQ) To establish the minimum concentration of pesticides, a mixture of pesticides was added to the sample matrix below the first level of the curve (0.0375, 0.01 ng/mL for curve 1 and 1, 0.5, and 0.25 for curve 2). An extraction procedure was performed, and the LOD was identified at the concentration at which a chromatographic peak with a signal-to-noise ratio (S/N) was more significant than three, and its corresponding spectrum was found. The sample was analyzed five times. The LOQ was determined using level 1 of the curve and calculating the percent coefficient of variation (CV%) and percent bias (Bias%). The data are reported in units of parts per billion (ppb).

2.6. Multi-elemental analysis in hair samples by ICP-MS

Considering that human hair has become a commonly used method for monitoring metal toxicity and conducting human health risk assessments (Kim et al., 2008), we use it to biomonitoring heavy metals to estimate environmental exposure levels. The protocol described by Galeano-Páez et al. (2021) was used to evaluate heavy metals in hair. Briefly, hair samples were taken from the occipital region of the scalp (3 cm long). The total hair length was between 4 and 5 cm (approximately 500 mg). Samples were stored and codified accordingly for multi-element analysis.

2.6.1. Hair sample preparation

Hair sample preparation was performed accordingly to a previously reported protocol (Miekeley et al., 1998). First, collected hair materials were aliquoted to approximately 300 mg, stored in 50 mL Falcon tubes, and weighed to obtain the wet weight. Next, the samples were subjected to pretreatment consisting of washes with water and reagent-grade acetone to remove impurities. The washes were performed in three cycles per solvent using an ultrasonic bath for 15 min. Finally, washed samples were dried overnight in an oven at 50 °C to obtain the dry weight.

Once the dry samples were obtained, they were subjected to digestion with a mixture of nitric acid, hydrogen peroxide, and water, whose volumes were corrected to aliquoted hair weight. This process was carried out at 70–80 °C in a fume hood and a total digestion time of 4 h. The digestion with strong oxidizing agents allows the destruction of the protein and fatty material of the hair samples. After digestion, the samples were equilibrated at room temperature and neutralized with MilliQ water to obtain a stable solution of elemental analytes, which were taken for analysis. This water dilution was accordingly to hair aliquot weight.

2.6.2. Elemental analysis of hair samples

The elemental contents of each sample were analyzed according to

the protocol described by Miekeley et al. (1998) using inductively coupled plasma mass spectrometry (ICP-MS). The solubilized hair samples were analyzed on the Perkin Elmer ICP Mass Spectrometer NexION 300x. Argon was used as carrier gas at a flow rate of 50 mL min⁻¹. The samples were run with the internal Rhodium (Rh) standard prepared at 40 mg L⁻¹ from a stock solution of 1000 mg L⁻¹ (Merck). Toxic elements analyzed were Pb, Hg, As, Cd, and Ni, and the essential elements were Cr, Mg, Mn, Se and Zn contents were determined.

The limits of detection (LOD) expressed as 3 s for all analyzed elements were the following: Pb (0,0009 mg L⁻¹); Hg (0,003 mg L⁻¹); As (0,003 mg L⁻¹); Cd (0,001 mg L⁻¹); Ni (0,003 mg L⁻¹); Cr (0,003 mg L⁻¹); Mg (0,04 mg L⁻¹); Mn (0,002 mg L⁻¹); Se (0,03 mg L⁻¹), and Zn (0,02 mg L⁻¹). Quality assurance (QA) and quality control (QC) were implemented for the multi-elemental analysis. The QA and QC of the method consisted of using method blank, certified reference materials (CRM), and an internal reference sample included in the same analytical run. The two CRM used were: NCS DC73347a (Human hair, China National Analysis Center for Iron and Steel) and ERM DB001 (Joint Research Center, European Commission). Data are expressed in parts per million (ppm).

2.7. Statistical methods

Individual data were organized in databases using the Microsoft Excel program for Windows. A data quality control process was implemented to assess and manage records with errors, missing data, and outliers. This quality control procedure was conducted on 100 % of the records in the database. It involved descriptive analysis of each variable, data cleaning, and identification of missing, omitted, and blank values.

Categorical variables were expressed as percentages, while continuous variables were summarized using central tendency and dispersion measures. Given the skewed distribution of metal concentrations, quartiles were used to summarize the data. The Mann-Whitney test was employed to compare the types of exposure (residential and occupational) in Montelibano and Nechí and the median concentrations of each area against the reference area.

Poisson regression analysis assessed the association between

exposure to different metals, sociodemographic variables, and micronucleus count in three exposure groups and one control group. For each group, univariate and multivariate analyses were performed, selecting variables based on the minimum Akaike's Information Criterion (AIC) to identify the optimal combination of sociodemographic and metal variables that best explained the variability of the micronucleus count within each group. The results of the Poisson regression were interpreted by examining the prevalence ratio and its corresponding 95 % confidence intervals, providing insights into the strength and direction of the associations between the variables. Correlation graphs were generated to study the dynamics of the relationships between metals at each site. Principal component analyses (PCA) were conducted at each exposure site to identify patterns in metal concentrations. In Montelibano, where there was a strong correlation between metal concentrations, a new multivariate Poisson regression analysis was performed, incorporating the first principal component (PC-1) as an explanatory variable alongside sociodemographic variables.

All statistical tests were two-sided, with a significance level set at 0.05. The statistical analyses were performed using R version 3.3.0 (R Core Team, 2020).

3. Results and discussion

3.1. Sociodemographic characteristics

The main demographic characteristics of sampled populations are depicted in Table 2. For all the studied areas, most participants were male, with a median of 49.0 years, while women represented 25.8 %, with a median age of 45.0 years. There was no significant difference in average age, socioeconomic status, or dietary habits between exposed and reference populations. The matching ratio between exposed and reference individuals was 3.0. Considering that mining is the primary economic activity in Montelibano and Nechí, it is not surprising that the population in these areas was predominantly occupationally exposed. Conversely, in Aranzazu, the entire population was classified as residentially exposed since the local Hg mine ceased operations in 1974. Therefore, the population residing near the Aranzazu mine site was

Table 2
Main characteristics for occupational and residential exposed and reference individuals.

Group	Sampling areas				Total
	Exposed			Reference area	
	Montelibano	Nechí	Aranzazu	Montería	
Number of participants	99	104	103	101	407
Gender					
Women (%)	16 (16.2)	25 (24.0)	37 (35.9)	27 (26.7)	105 (25.8)
Age Median (P ₂₅ - P ₇₅)	41.5 (32–52)	43 (36–50)	43 (27–54)	52 (42–68)	45 (34–57)
Men (%)	83 (83.8)	79 (76.0)	66 (64.1)	74 (73.3)	302 (74.2)
Age Median (P ₂₅ - P ₇₅)	57 (49–61)	52 (32–60)	40 (28–51)	38 (32–54)	49 (36–59)
Exposure characteristics					
Occupational exposure (%)	63 (63.6)	62 (59.6)	–	–	125 (30.7)
Time of work in years Median (P ₂₅ - P ₇₅)	39.3 (27.7–48)	35.5 (21.8–52.6)	–	–	37.4 (24.6–50.3)
Residential exposure (%)	36 (36.4)	42 (40.4)	103 (100)	101 (100)	282 (62.3)
Time of residence in years Median (P ₂₅ - P ₇₅)	18 (2.4–30)	14 (5–26.6)	2.1 (1–11.3)	12 (4–25)	11.5 (3.1–23.2)
Consumption habits					
Alcohol (%)	64 (64.6)	47 (45.2)	44 (42.7)	65 (64.4)	220 (54.1)
Frequency ^a					
Low (%)	63 (98.4)	41 (87.2)	42 (95.5)	58 (89.2)	204 (93.1)
Moderate (%)	1 (1.6)	4 (8.5)	–	–	5 (2.3)
Heavy (%)	0	2 (4.3)	2 (4.5)	7 (10.2)	10 (4.6)
Non-alcohol consumers (%)	35 (35.4)	57 (54.8)	59 (57.3)	36 (35.6)	184 (45.2)
Tobacco (%)	21 (21.2)	30 (29.0)	25 (24.3)	23 (22.8)	99 (24.3)
Non-tobacco smokers (%)	78 (78.8)	74 (71.0)	78 (75.7)	78 (77.2)	308 (75.7)
Fish intake days/week (%)					
1–2 (Low)	83 (86.5)	76 (80.8)	63 (85.1)	67 (74.4)	289 (81.6)
3–4 (Medium)	4 (4.2)	9 (9.6)	2 (2.8)	2 (2.2)	17 (4.8)
5–7 (High)	9 (9.3)	9 (9.6)	9 (12.2)	21 (24.4)	48 (13.6)

^a According to NIAAA (National Institute of Alcohol Abuse and Alcoholism).

selected for analysis to investigate the potential existence of chronic residual Hg contamination in the general population following the mine's closure.

Individuals with occupational exposure had an average work time of 37.4 years, while those with residential exposure reported a period of residence in the mining areas of almost 11.5 years. Concerning alcohol consumption, 45.2 % of exposed and reference individuals were declared non-drinkers. Most participants who declared themselves drinkers reported low consumption, while a small proportion were classified as moderate and heavy drinkers. Regarding the participants' smoking habits, our findings show that 75.7 % were non-smokers, while the remaining participants reported smoking an average of 1 to 2 cigarettes per week.

Considering that the economic dynamics of the studied populations also include agricultural and fishing activities, additional sources of chemical elements exposure, such as pesticide exposure and fish consumption, were also evaluated in the exposed and reference populations.

The blood levels of 12 pesticides, including Aldicarb, Propoxur, Carbofuran, Endosulfan alpha, Endosulfan beta, Endosulfan sulfate, Malathion, Hexachlorobenzene, Ethyl paraoxon, Methyl paraoxon, Ethyl parathion, and Methyl parathion, were assessed to determine the extent of exposure to pesticides. However, the levels of these pesticides were found to be below the limit of quantification (BLOQ) in 87.5 % of the individuals and were therefore considered undetectable (Table S1). Low concentrations of three pesticides were detected in the remaining 12.5 % of the population: Malathion, Hexachlorobenzene, and Ethyl paraoxon. Malathion was detected in all the exposed areas in blood, while Hexachlorobenzene and Ethyl paraoxon only were quantified in Montelibano.

Analysis of the exposure characteristics of the individuals with detectable levels of pesticides showed that all individuals with Malathion in their blood had residential exposure, something that can be anticipated considering that Malathion is frequently employed to manage mosquitoes and diverse insects, and it is also present in other pesticide products used indoors and on pets (Tchounwou et al., 2015). However, interestingly Hexachlorobenzene was detected mainly in individuals from the mining sector ($n = 18$) and residents with residential exposure ($n = 9$) (Table S1).

Hexachlorobenzene, also known as HCB, is utilized as a seed treatment fungicide to manage fungal diseases but also can be formed as a byproduct of specific industrial processes, including the production of certain chemicals and the combustion of fuels (Thakur and Pathania, 2020). Even when prolonged exposure to Malathion and HCB in humans may cause liver disease, skin lesions, thyroid, and bone-related complications, and chronic exposure may also cause hair loss, embryo lethality, and teratogenic effects (Jiang et al., 2018), concentrations quantified in exposed populations are well below the reference dose (RD) and are not likely to pose a significant risk to human health and neither a primary source of metals in these populations. In individuals from the control area, all evaluated pesticides were BLOQ.

In regard to the fish consumption, to ensure that the exposure period represented by the hair biomarker (T-Hg levels in hair) was accurately captured, participants were requested to report their fish intake patterns during the last 1 and 3 months preceding the survey. Of all the individuals, 81.6 % reported consuming fish only 1 to 2 times per week, indicating a low frequency of fish intake. Individuals from Monteria's reference area showed the highest fish consumption frequency (24.4 %).

In Montelibano and Nechí, the fish consumption survey showed that most consumed species were Bocachico (*Prochilodus magdalenae*) with a consumption frequency of 39.31 %, Bagre (*Pseudoplatystoma magdaleniatum*) with a consumption frequency of 2.46 % and Doncella (*Ageneiosus pardalis*) with 1 %. Even though in this study we did not analyze metals concentration in fish muscle, for these particular species in these areas, significant heavy metal contamination, primarily from ASGM, has been extensively investigated (Carranza-Lopez et al., 2019; Marrugo-Negrete et al., 2014). Previous studies warned about the risk of

bio-accessibility of Hg from fish intake in the zone and reported total mercury (Hg-T) concentrations that exceed the permissible limits established by the WHO (0.5 mg/g) (Marrugo-Negrete et al., 2020).

Parallel studies also indicated the presence of Hg in other environmental matrices, including soil, water, and even crops in the area; a specific evaluation focusing on the spatial distribution of Hg accumulation in La Mojana region found significant concentrations of Hg-T in the roots of *Eichhornia crassipes*, reaching values of $0.191 \pm 0.017 \mu\text{g/g}$. These concentrations showed a significant correlation with sediment values obtained in the same study ($r = 0.77$; $p < 0.05$), leading the researchers to conclude that the permeability of this metal in the biogeochemical cycles and dynamics of the ecosystems in this region is notable (Marrugo Negrete et al., 2018). Furthermore, a recent study discovered the accumulation of Hg in soils of different commercial varieties of rice, *Oryza sativa L.*, with one of the evaluated varieties exceeding the permissible levels ($20 \mu\text{g Hg kg}^{-1}$) (Enamorado-Montes et al., 2021).

On the other hand, elevated concentrations of Pb, Cd, and As, among other toxic elements, have been detected in the blood of residents in the region. The results revealed that 100 % of the exposed individuals had As levels exceeding the permissible limit ($1 \mu\text{g/L}$) set by the ATSDR (Calao Ramos et al., 2023). These findings were attributed to the consumption of water and food contaminated with mining residues, including fish and rice, which could potentially induce DNA damage in the population. These observations align with similar findings reported by Galeano et al. in 2021.

3.2. MNBN frequencies in the studied populations

Individuals from Aranzazu presented the highest MNBN frequencies (5.90 ± 4.53), followed by Nechí (5.09 ± 4.51) and Montelibano (4.62 ± 3.09) (Table 3). Considering that sex and age represent one of the main factors contributing to differences in MNBN frequencies, we also evaluated the influence of both confounding factors and exposure status as predictors of this parameter. The MNBN frequencies did not show any statistically significant differences between males and females in the study areas, indicating a similar response to the exposure conditions among both genders. However, in the bivariate model MNBN frequencies were significantly influenced by age in Montelibano, the environmentally exposed population of Nechí and reference area population. In these populations, age contributed significantly to increased MNBN frequencies, while gender was found to be a protective variable, with female participants presenting higher frequencies than male participants.

To identify whether the type of exposure modulated the MNBN frequencies, we evaluated the differences between values obtained for occupational and residential exposed individuals. Results showed that occupational and residential exposure is very similar. Even when in general occupational exposure tends to be more intense and prolonged compared to residential exposure, it's important to note that workers in occupational settings may have access to personal protective equipment (PPE) and training to reduce their exposure to mining residues. Additionally, regulatory standards may be in place to protect workers' health in occupational settings, while residential exposure may not be subject to the same level of regulation. In our specific case, the survey conducted in the work areas revealed that 66.6 % of individuals with occupational exposure in Montelibano and 37 % in Nechí reported wearing work clothes while working. Additionally, other PPE such as gloves (60 % in Montelibano; 29 % in Nechí) and face masks (45 % in Montelibano; 20 % in Nechí) was also used. These protective measures are specifically designed to minimize the inhalation of particles and reduce skin contact with materials generated during mining activities.

Accordingly, multi-element levels in both exposures, did not show significant statistical differences between occupational and residential exposures, except for some values of Pb and Hg in women from Nechí (Table S2).

Table 3
Comparison of MNBN frequencies among studied sites and gender of participants.

Parameter	Montelibano		Nechí		Aranzazu	Montería
	Occupational exposure	Residential exposure	Occupational exposure	Residential exposure	Residential exposure	Reference area
MNBN ^a	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Women	5.67 ± 2.52	5.85 ± 4.41	3.00 ± 4.36 ^a	6.05 ± 4.48 ^a	5.68 ± 4.49	2.33 ± 3.11
Men	4.65 ± 2.64	3.70 ± 3.27	5.24 ± 4.53	3.90 ± 4.48	5.68 ± 4.64	1.70 ± 3.14
Total	4.70 ± 2.63	4.47 ± 3.81	5.13 ± 4.51	5.02 ± 4.56	5.90 ± 4.53	1.83 ± 3.13
Total population	4.62 ± 3.09		5.09 ± 4.51		5.90 ± 4.53	1.83 ± 3.13

Bold for statistically significant differences compared to reference individuals (Mann-Whitney test).

^a Frequency calculated in 2000 binucleated cells.

^a For statistically significant differences between individuals with occupational and residential exposure within the same area.

These findings significantly contribute to raising awareness regarding the potential risks associated with residential exposure to mining residues and underscore the importance of government authorities taking proactive measures to establish mitigation strategies for protecting the health of communities residing near mining areas.

A multivariate Poisson regression was used to estimate adjusted prevalence ratios (PRs) and 95 % confidence intervals (CIs) for the association between sociodemographic characteristics, individual hair metals levels and MNBN frequencies (Table 4). Even when only a small percentage of exposed individuals were classified as heavy or moderate drinkers, heavy alcohol intake had a statistical significance influence on increased MNBN frequencies in the total population (PR = 1.842; 95 % CI: 1.18–2.86) and individuals from Nechí (PR = 2.629; 95 % CI: 1.43–4.83). The alcohol content (%) of the most popular types of alcohol consumed by both exposed and reference populations were beer (4.7 %), rum (39 %), and whiskey (36 %) being the most frequently consumed. As with high alcohol consumption, cigarette smoking was also a risk factor for an increase in the frequency of MNBN in Nechí (PR = 1.470; 95 % CI: 1.094–1.975). Between the exposed areas, Aranzazu showed the highest fish consumption levels (5/7 days per week), but only Nechí showed a significant influence of fish consumption on the increase in the frequency of MNBN within the exposed areas (PR = 1.783; 95 % CI: 1.142–2.785). In the general population a significant influence of the frequency of fish consumption on MNBN levels was also demonstrated (PR = 1.42; 95 % CI: 1.192–1.700). The internal control values indicated that transportation conditions were ideal and did not negatively impact the results obtained.

3.3. Large-scale, medium-scale mining and ASGM in Montelibano

3.3.1. Toxic and essential trace elements concentrations in hair samples

Montelibano includes large-scale open-pit ferronickel mining, along with coal and gold mining activities. Multi-elemental ICP-MS analysis of hair samples showed elevated concentrations of toxic elements such as Hg, followed by Ni, and Cr, supporting our hypothesis that mining activity could be an important source of environmental contamination. The latter results are summarized in Table S2, for total population and differentiated by gender and type of exposure. Interestingly, Ni and Cr hair contents in exposed individuals from Montelibano were among the highest found compared to other exposed areas from this study (Fig. 2), which might be related to the type of exposure. To support this, we performed comparisons between occupational and residential exposures. In concordance, as showed in Table S2, Ni contents in men occupationally exposed from Montelibano were higher than the reference individuals ($p < 0.0001$), and this measured Ni showed the highest values from all exposed areas ($p < 0.0001$, when comparing men from Nechí and Aranzazu). In addition, hair Cr levels increased significantly in men occupationally exposed from Montelibano ($p = 0.018$). Finally, other elements like Cd, Pb, and Zn did not show significant differences when compared to reference or other exposed areas (Fig. S1).

3.3.2. Chemical mixtures and possible exposure sources

For assessing hallmarks and potential exposure sources in multi-elemental hair analysis, correlations between toxic and essential elements were performed in exposed populations (Jursa et al., 2018). Spearman correlation heatmaps are depicted in Fig. 3 for total populations (reference and exposed areas) and differentiated by occupational and residential exposure in Fig. 4. Herein, positively stronger correlations are indicated as red-colored areas, and the negatively inverse correlations are represented as bluish-colored areas. As shown in Fig. 4, results summarize only Montelibano and Nechí correlations due to the presence of occupational and residential exposure types in these areas.

The highly clustered behavior of hair samples from Montelibano is evidenced in Fig. 3, showing that significant positive correlations were encountered almost exclusively for occupationally exposed individuals (Fig. 4). In the total population, significant correlations were As-Se, As-Zn, and Se-Zn. Current scientific investigations suggest that Se represents a contaminant that can be discharged into the environment during the pyrometallurgical extraction of metals, including Cu, Zn, Pb, and Ni (Desai et al., 2016; Wu et al., 2021). However, Ni, Mn, and Mg clusters increase in significance when assessing occupational exposure, similarly to other elements such as As and Cr. Exposure hallmarks in Montelibano may indicate a global atmospheric deposition of mining material, potentially spreading to residential areas due to open-pit ferronickel ore extraction and processing. In fact, there is previous evidence of Ni, Cd, and Zn atmospheric deposition in Montelibano as a mining-related consequence (Marrugo-Negrete et al., 2014). In concordance, the most abundant and strongest correlations were found for individuals with residential exposure, when we found a robust Cd-Ni correlation, followed by Cr-As in smaller proportion (Fig. 4).

The obtained correlations and their relationship with the elemental pattern of exposure in the exposed population of Montelibano are described in more detail in Section 3.6: Particularities of the exposure to metal mixtures and MNBN frequencies in large-scale mining areas.

3.3.3. MNBN frequencies and their association with chemical element mixtures

A univariate linear regression analysis was used to identify possible toxic agents in the metal mixture and evaluate the possible independent effects of each mixture member on the MNBN frequencies (Table S3). Most of the single activity of metals on MNBN frequency in Montelibano was represented by Pb (PR = 1.009; 95 % CI: 1.033–1.015) and Zn (PR = 1.000; 95 % CI: 0.999–1.000).

The subsequent analysis involved identifying interactions between members of the chemical mixtures and identifying possible patterns of exposure (due to common sources) by using a step-by-step multivariate analysis with a model selection according to the minimum AIC (Table 5).

This comprehensive examination in Montelibano revealed a significant association between high frequencies of MNBN and the presence of Se (PR = 1.374; 95 % CI: 1.073–1.759) (Table 5). The potential health risks associated with exposure to Se and other toxic metals have recently garnered significant attention (Cui et al., 2017; Zhao et al., 2023). In

Table 4
Multivariate Poisson regression model* for the influence of sociodemographic variables and metal levels on the frequency of MNBN.

Total population				Aranzazu			Reference area		
Variables	PR	IC 2.5 %-97.5 %	p-value	PR	IC 2.5 %-97.5 %	p-value	PR	IC 2.5 %-97.5 %	p-value
Aranzazu	3.672	2.952–4.567	0.00	–	–	–	–	–	–
Montelibano	2.645	2.114–3.310	0.00	–	–	–	–	–	–
Nechí	2.909	2.317–3.652	0.00	–	–	–	–	–	–
Heavy alcohol consumption	1.842	1.186–2.862	0.00	–	–	–	–	–	–
Fish consumption	1.424	1.192–1.700	0.00	–	–	–	–	–	–
Age	1.004	1.000–1.008	0.03	0.995	0.989–1.001	0.09	1.031	1.018–1.044	0.00
Sex	0.865	0.764–0.98	0.02	–	–	–	–	–	–
Hg	0.991	0.977–1.006	0.25	1.960	1.399–2.746	0.0	0.99	0.974–1.006	0.23
Ni	1.063	0.997–1.133	0.06	1.251	1.116–1.402	0.0	–	–	–
Cr	0.915	0.863–0.969	0.00	–	–	–	–	–	–
Mg	1.000	1.000–1.001	0.00	–	–	–	1.001	1.000–1.002	0.00
Mn	0.989	0.981–0.996	0.00	0.936	0.883–0.993	0.02	–	–	–
Se	1.025	1.016–1.034	0.00	0.624	0.353–1.101	0.10	1.088	1.034–1.144	0.00
Pb	–	–	–	–	–	–	1.110	1.023–1.205	0.01
Cd	–	–	–	–	–	–	1.033	1.006–1.060	0.01

Montelibano				Occupational exposure			Residential exposure		
Total population									
Variables	PR	IC 2.5 %-97.5 %	p-value	PR	IC 2.5 %-97.5 %	p-value	PR	IC 2.5 %-97.5 %	p-value
Low alcohol consumption	–	–	–	0.636	0.442–0.916	0.01	–	–	–
Hg	–	–	–	0.94	0.875–1.010	0.09	1.327	1.146–1536	0.00
Age	1.014	1.004–1.025	0.00	–	–	–	–	–	–
Sex	0.671	0.489–0.923	0.01	–	–	–	–	–	–
Pb	1.005	0.999–1.012	0.12	–	–	–	–	–	–
Cr	0.749	0.614–0.914	0.00	0.65	0.469–0.902	0.01	–	–	–
Mn	–	–	–	1.000	1.000–1.000	0.00	1.020	1.002–1.039	0.02
Zn	–	–	–	–	–	–	0.999	0.998–1.000	0.03
Mg	–	–	–	1.001	1.000–1003	0.00	0.996	0.994–0.998	0.00
Se	1.374	1.073–1.759	0.01	1.632	1.071–2.487	0.00	1.563	1065–2.263	0.02
Pb	–	–	–	–	–	–	1.012	1.004–1.019	0.00

Nechí				Occupational exposure			Residential exposure		
Total population									
Variables	PR	IC 2.5 %-97.5 %	p-value	PR	IC 2.5 %-97.5 %	p-value	PR	IC 2.5 %-97.5 %	p-value
Smoking habits	1.470	1.094–1.975	0.01	1.962	1.280–3.008	0.00	2.310	1.362–3.915	0.00
Fish consumption	1.783	1.142–2.785	0.01	–	–	–	–	–	–
Heavy alcohol consumption	2.629	1.431–4.831	0.00	3.244	1.626–6.474	0.00	–	–	–
Low alcohol consumption	1.095	0.889–1.350	0.39	1.138	0.846–1.530	0.39	–	–	–
Age	1.006	0.999–1.013	0.11	0.979	0.967–0.990	0.00	1.021	1.010–1.032	0.00
Pb	0.947	0.915–0.98	0.00	0.930	0.889–0.973	0.00	1.107	0.979–1.252	0.10
Mg	1.001	1.000–1.001	0.00	1.003	1.002–1.004	0.00	1.001	1.000–1.001	0.01
Mn	0.984	0.973–0.994	0.00	0.987	0.971–1.002	0.09	0.961	0.939–0.984	0.00
Cr	–	–	–	–	–	–	6.114	2.650–14.107	0.00
Ni	–	–	–	0.321	0.182–0.566	0.00	1.970	1.262–3.075	0.00
Hg	–	–	–	1.095	1.042–1.150	0.00	0.900	0.822–0.993	0.03
Zn	–	–	–	0.995	0.992–0.998	0.00	0.999	0.998–1.000	0.02
Se	1.022	0.00–0.00	0.00	1.022	1.011–1.033	0.00	–	–	–

Bold for statistically significant differences.

PR: Prevalence ratio.

* To identify the best model for each area, a step-by-step regression was conducted with the selection of the model being based on the minimum Akaike Information Criterion (AIC).

prior research, Se has been found to exhibit toxicity at elevated levels, with acute and chronic effects being observed (Huang et al., 2013).

Although Se is an indispensable trace element for human health, studies have demonstrated that high concentrations of Se can interfere with DNA repair mechanisms and proper chromosome segregation during cell division (Se-associated DNA methylation) (Tian et al., 2020); additionally, the toxicity of Se compounds is also associated with the generation of reactive oxygen species (ROS), which can induce damage to DNA Single-Stranded Binding Proteins (SSBs) and Double-Stranded Binding Proteins (DSBs) (Letavayová et al., 2006; Safarzad et al., 2019).

On the other hand, the antagonistic interplay between Se and toxic metals plays a vital role in modulating their absorption and toxicity

(Bjørklund et al., 2017). For instance, when present in a complex mixture, Pb and Cd can interfere with the absorption and metabolism of Se leading to deficiencies (Balali-Mood et al., 2021; Mousavi et al., 2019). Cd can directly interfere with Se metabolism by binding to specific enzymes, such as glutathione peroxidase, which are required for Se to function properly in the body (Leal et al., 2023).

In the occupational population, MNBN frequencies were modulated by the exposure to Mg (PR = 1.001; 95 % CI: 1.000–1.003), Mn (PR = 1.000; 95 % CI: 1.00–1.00), and Se (PR = 1.632; 95 % CI: 1.071–2.487).

The genotoxic effects of metals such as Se, Mg and Mn have previously been associated with DNA damage. Several reports indicate that high concentrations of Mg induce conformational changes and

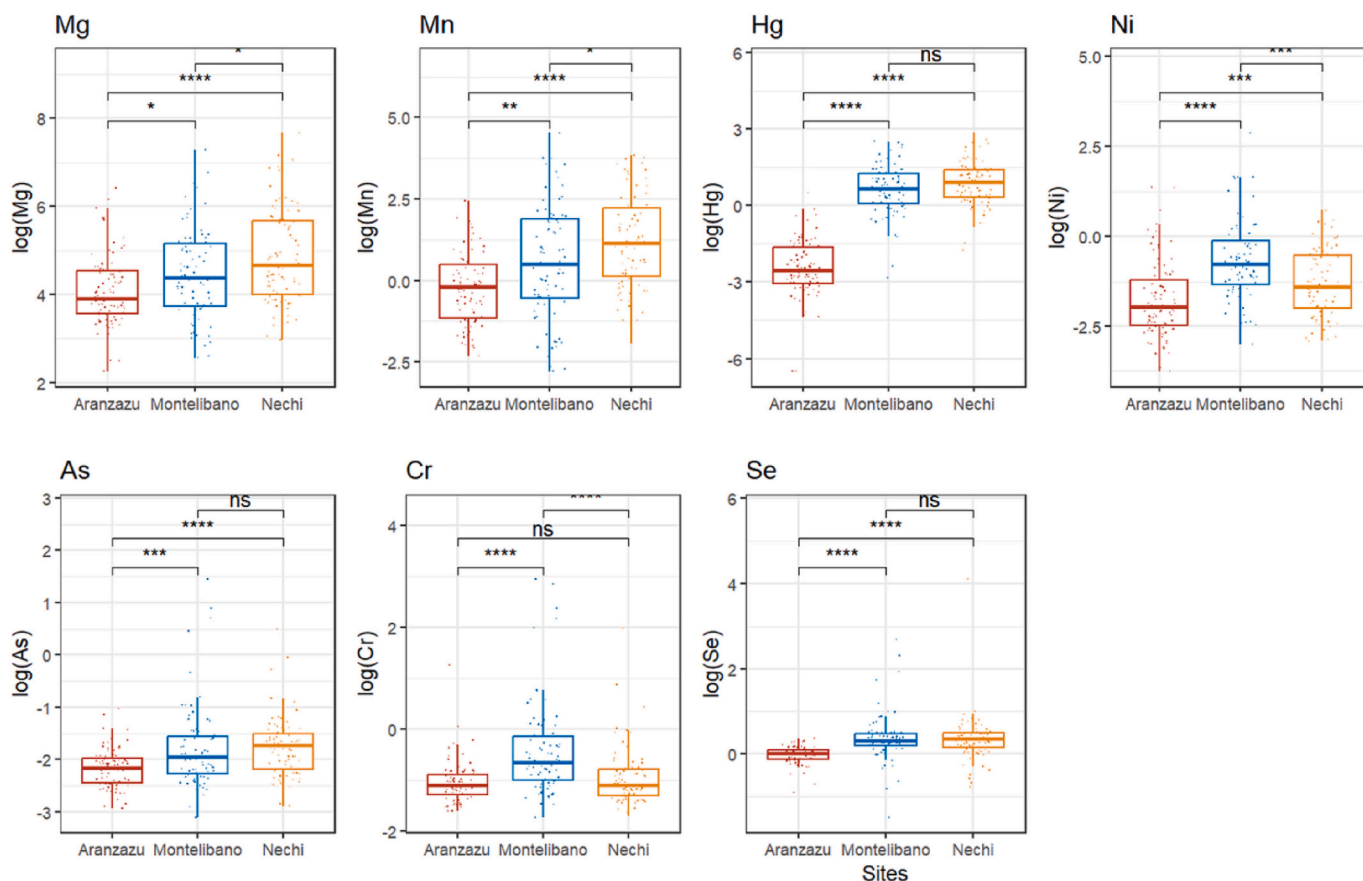


Fig. 2. Box-plots of elemental hair contents described by areas. Data were log normalized. *P* values are represented as follows: * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), **** ($p < 0.0001$).

destabilization of DNA. When Mg binds “covalently” to DNA, it forms a coordinated complex, causing local distortion of the double helix, which can lead to cellular damage. The biological and clinical consequences of this DNA breakage have been associated with disease and carcinogenesis (Anastassopoulou and Theophanides, 2002).

Mn also decreases mitochondrial membrane potential through the elevation of ROS generation (Gugnani et al., 2018; Wang et al., 2017), which can alter DNA methylation levels as demonstrated in cellular and animal models (Tarale et al., 2017), leading to subsequent mitochondrial respiratory dysfunction primarily associated with the neurotoxic effects of Mn (Tinkov et al., 2021).

Additionally, Mn can cause cytogenetic instability by affecting the absorption and metabolism of other metals (Chen et al., 2015; Li and Yang, 2018). Mn competes with Fe transporters by inhibiting the binding of divalent metal transporter 1 (DMT1) to Fe and altering the homeostasis of Cs, Co, Pb, Hg, Ni, and Zn in cells (Chen et al., 2015; Li and Yang, 2018).

In contrast, the residential population showed a higher representation of other metals, such as Pb (PR = 1.012; 95 % CI: 1.004–1.019), Hg (PR = 1.327; 95 % CI: 1.146–1.536), Mn (PR = 1.020; 95 % CI: 1.002–1.039), Se (PR = 1.563; 95 % CI: 1.065–2.293) and Zn PR = 0.999; 95 % CI: 0.998–1.000). Ferronickel mining may be associated with Hg and Pb as contaminants; a previous study found high levels of Hg in the blood and the presence of Cd, Cu, Zn, and Pb in residents near the ferronickel mine (Idrovo et al., 2017).

This particular mixture can cause DNA damage through various mechanisms: Pb and Hg have the ability to directly bind to DNA molecules, causing structural alterations and distortions in the DNA helix, interfere with the DNA repair processes via NER (Calsou et al., 1996), and leading to mutations and DNA damage. Additionally, Pb and Hg can

also cause DNA damage via oxidative stress by interfering with components of antioxidant defense (Xu et al., 2018; Zhang et al., 2014) and other cell signaling pathways (Mitra et al., 2017).

3.4. ASGM system in Nechí

3.4.1. Toxic and essential trace elements concentrations in hair samples

The El Bagre-Nechí mining complex is an important center of ASGM alluvial mining, characterized by informal and rudimentary techniques, often carried out in areas close to residential communities. The results of metal concentrations in hair samples from this population indicate the presence of the highest measured Hg contents from this study (Fig. 2), which were found mostly in men (Table S2). These results are consistent with previous studies in areas with ASGM of La Mojana (Díaz et al., 2018; Díaz et al., 2020). In addition, increased Mn contents were also found in exposed residents, being the largest found in this study (Fig. 2), and particularly encountered in residentially exposed women (Table S2). No differences were found between Cd, Pb and Zn hair contents when comparing Nechí residents with other areas (Fig. S1).

3.4.2. Chemical mixtures and possible exposure sources

The municipality of Nechí shows differential exposure patterns when comparing occupational and residential populations (Fig. 4). In the occupationally exposed population Cr clustered positively and significantly with As, followed by lesser Mn associations comprising Mn-Ni-As, and Mn-Cr-Mg. The residentially exposed population correlations changed since a robust Pb-Cd-As cluster was found, followed by the Cd-As correlation. Exposure hallmarks observed in Nechí may indicate common sources from ASGM, a growing economic activity in most of the exposed areas.

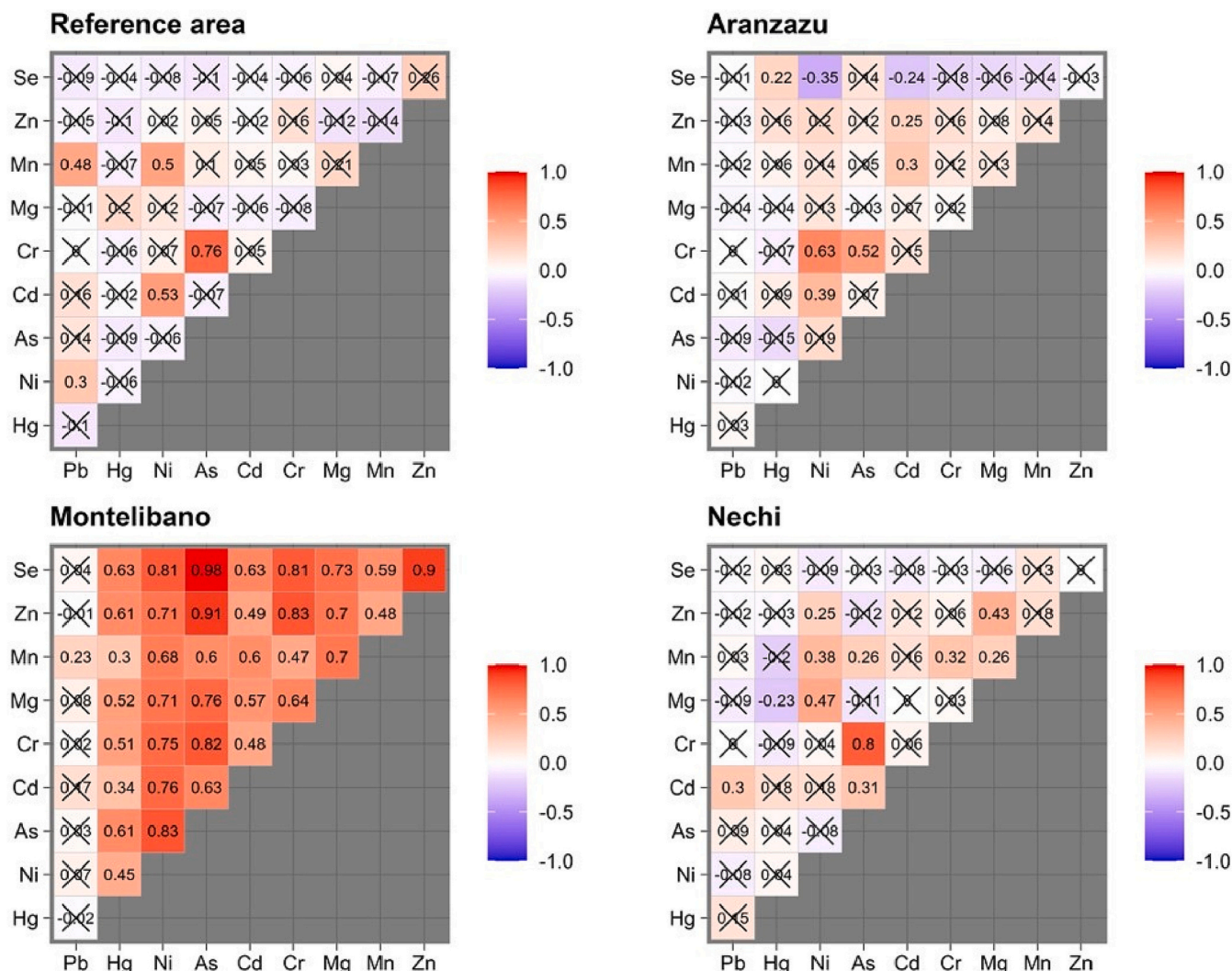


Fig. 3. Correlation heatmap for metal hair contents in each area. Numbers are Spearman's correlation coefficients. Positively correlated variables are painted in a red tone scale, whereas negatively correlated variables are shown in a blue tone scale. Crossed-out numbers represent variables with no statistical correlation.

During the gold amalgamation process, Mn-oxide minerals, galena (PbS), and chalcite (Cu₃AsS₄) are disposed in tailings as undesired elements, increasing their availability in soil surfaces around ASGM operations (Nurcholis et al., 2017). Mn and Cd correlations can be found near ASGM operations due to the deposition of gold mining tailings in nearby areas (Tariq et al., 2019). However, Ni exposure may be related to increased agricultural and ferronickel activities (Marrugo-Negrete et al., 2017).

3.4.3. MNBN frequencies and their association between chemical element mixtures

The El Bagre-Nechí mining complex is an important center for alluvial ASGM mining, characterized by informal and rudimentary techniques, often carried out in areas near residential communities. The results of metal concentrations in hair samples from this population indicate the presence of significant exposure to toxic metals. Hg was found in the highest concentrations, suggesting significant exposure through mining activity. In addition to Hg, elevated Pb, Ni, As, and Cd concentrations were found in hair samples. These metals are associated with various adverse health effects, such as kidney damage, respiratory problems, and neurotoxicity (Gibb and O'Leary, 2014; Lv et al., 2023).

In the study of the total population of Nechí, an association was observed between the frequency of MNBN and the combined presence of Pb, Mg, Mn, and Se (Table 4). In this mining system, the increase in

MNBN frequencies in individuals with occupational exposure was significantly modulated by the mixture of Mg (PR = 1.003; 95 % CI: 1.002–1.004), Hg (PR = 1.095; 95 % CI: 1.042–1.150), and Se (PR = 1.022; 95 % CI: 1.011–1.033). In addition, the combined presence of Pb (PR = 0.979; 95 % CI: 0.889–0.973), Zn (PR = 0.995; 95 % CI: 0.992–0.998), and Ni (PR = 0.321; 95 % CI: 0.182–0.566) was associated with decreased MNBN frequencies (Table 4). Toxic metals like Hg can bind to sulfur-containing proteins in the body, such as glutathione (GSH), and deplete their levels, leading to an imbalance in the cell's redox status, increasing oxidative stress and DNA damage (Taylor et al., 2022). Mg and Se are not typically associated with increased micronuclei frequencies, but high doses can be toxic and cause DNA damage (Shokrzadeh et al., 2013). Nechí is in fact, the area where participants showed the highest levels of Se (1.40 (1.17–1.63)) and Mg (105.81 (54.92–288.18)) in hair. Further investigations are required to examine the impact of geogenic Se on genetic markers, such as MNBN particularly in the context of complex mixtures. Similar results were obtained in the univariate linear regression analysis (Table S3).

On the other hand, metals like Pb and Zn can bind to the same sulfur-containing proteins as Hg and compete with Hg for binding sites, thereby reducing its toxicity (Ajsuvakova et al., 2020; Rubino, 2015). In addition, Zn is also a cofactor for many DNA repair enzymes, which can help to mitigate DNA damage caused by Hg and Se (Ajsuvakova et al., 2020).

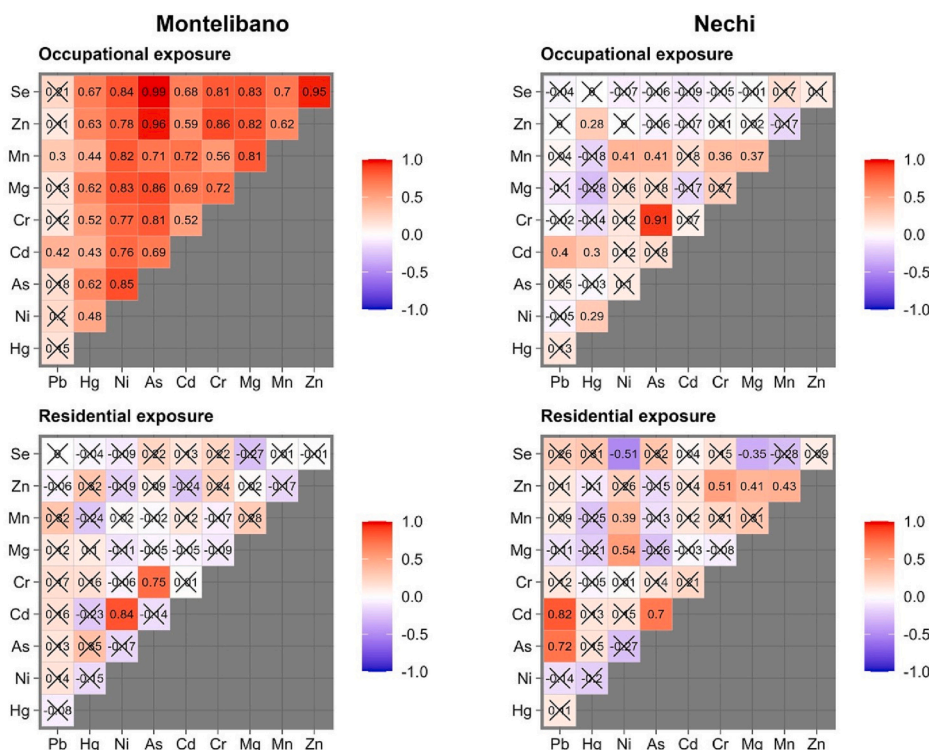


Fig. 4. Correlation heatmap for metal hair contents and type of exposition in Montelibano and Nechí. Numbers are Spearman's correlation coefficients. Positively correlated variables are painted in a red tone scale, whereas negatively correlated variables are showed in a blue tone scale. Crossed-out numbers represent variables with no statistical correlation.

Table 5

Multivariate Poisson regression for MNBN in Montelibano with first principal component as explanatory.

Variables	Montelibano		
	PR	IC 25 %-97.5 %	p-value
Heavy alcohol consumption	0.359	0.088–1.465	0.15
Low alcohol consumption	0.947	0.743–1.207	0.66
Smoking habit	1.055	0.432–2.581	0.90
Residential exposure	0.823	0.64–1.054	0.12
Fish consumption	2.431	0.902–6.551	0.07
Age	1.015	1.005–1.025	0.00
Sex	0.594	0.594–0.428	0.00
PC-1	0.935	0.875–1.000	0.04

Bold for statistically significant effect.

In the population with residential exposure, a chemical signature that includes Cr, Ni and Mg appears as a significant modulator of the high frequencies of MNBN. Cr and Ni have been associated with DNA damage and micronuclei formation (Guo et al., 2019; Issah et al., 2023). In addition, Ni can induce DNA damage through direct DNA binding and ROS stimulation (Guo et al., 2019). While the genotoxic effects of Cr are predominantly the formation of oxidative adducts and apurinic/apyrimidinic lesions, eventually resulting in DNA damage (Torres-Ávila et al., 2020; Vasylykiv et al., 2010). As in the occupationally exposed population, Mg also showed high concentrations in hair, possibly related to its toxicity (88.48 ppm).

ASGM in this area can release some elements present in the mixture such as Hg, Pb, Zn, Ni, and Se (Thiombane et al., 2023) leading to the exposure of occupational and residential communities. However, other sources of metal exposure, such as fish consumption also appeared related to high frequencies of MNBN (PR = 1.783; 95 % CI: 1.142–2.785) in the multivariate model. In this regard, several studies have reported the presence of heavy metals in the muscle of fish from El Bajo Cauca River, particularly Hg and As (Alvarez et al., 2012; Cruz-

Esquivel et al., 2023).

3.5. Closed mining system: environmental liabilities in Aranzazu

3.5.1. Toxic and essential trace elements concentrations in hair samples

Surprisingly, individuals with residential exposure from Aranzazu presented the lowest hair Hg content within our study (Table S2), a tendency that was conserved in the total population from this municipality. Remarkably, elements such as Mg, Mn, Ni, As, Cr and Se were the lowest compared to other exposed areas (Fig. 2). Particularly Ni and As, where significantly lower compared to the reference area (Table S2). Cd, Pb or Zn did not show any differences when compared between exposed areas (Fig. S1).

3.5.2. Chemical mixtures and possible exposure sources

Aranzazu displayed a distinctive source pattern characterized by the presence of Cr-Ni while maintaining the Cd-Ni and Cr-As patterns observed in Nechí. This finding could be attributed to Aranzazu's history of underground mining activities of Hg, widely used in the ASGM operations present in Nechí. Previous studies conducted on water and populations residing in ASGM communities have reported the presence of toxic elements such as Cr, Mn, As, and Pb (Calao and Marrugo, 2015; Long et al., 2015).

3.5.3. MNBN frequencies and their association between chemical element mixtures

Most of the single activity of metals on MNBN frequency in Aranzazu was represented by Hg (PR = 1.763; 95 % CI: 1.312–2.368), Ni (PR = 1.276; 95 % CI: 1.159–1.406), Cr (PR = 1.233; 95 % CI: 1.033–1.471) and Cd (PR = 1.945; 95 % CI: 1.149–3.291) (Table S3). Similarly, the multivariate analysis revealed that the mixture of Hg (PR = 1.960; 95 % CI: 1.399–2.746), Ni (PR = 1.251; 95 % CI: 1.116–1.402), and Mn (PR = 0.936; 95 % CI: 0.883–0.993), had a significant effect on the modulation of MNBN frequency (Table 4).

Hg and Ni have been identified as modulators of the frequency of MNBN, with both metals showing potential genotoxic effects, including damage to genetic material and increased cancer risk (Sánchez-Alarcón et al., 2021). As previously discussed, the primary mechanism of Hg, Cd and Ni toxicity is through the depletion of glutathione and binding to sulfhydryl groups of proteins (Valko et al., 2005); Ni and Cd can also activate redox-sensitive transcription factors, such as AP-1, NF-kappaB, and p53, involved in DNA damage response (Valko et al., 2005). Like most metals discussed previously, oxidative stress is also part of these particular metals' genetic damage mechanisms. The frequency of MNBN in Aranzazu was also associated with Mn levels in the mixture (PR = 0.936; 95 % CI: 0.88–0.99) (Table 4). Mn is considered an essential element and is critical in several physiological processes.

In this area, La Esperanza mine could be a potential source of Hg contamination. Even when the mine was closed in 1974 due to continuing concerns about high levels of occupational exposure, poor ventilation conditions and tunnel collapses, the mine closure failed to eliminate the ongoing health issues and concerns among the communities of Aranzazu (Restrepo, 2016). Although no scientific studies have investigated the link between Hg exposure and disease incidence in Aranzazu, reports suggest a potential association with mental illnesses, specifically bipolar disorder (Restrepo, 2016). The inadequate closure of Hg mines has been linked to environmental impacts. Analysis of water sources in Spain reveals that years after mine closures, trace amounts of metals continue to discharge into ecosystems (Jorge et al., 2006). Furthermore, Hg exposure has been associated with numerous health effects, such as neurotoxicity, reduced IQ, and cardiovascular diseases (Delgado and Núñez, 2020).

Inadequate sealing works in mines can cause metal leaks, leading to prolonged exposure. Of particular concern is Hg, which can undergo methylation in the environment, resulting in methylmercury (MeHg) formation. This compound is known for its bioaccumulative properties, making it a potent and hazardous agent, mainly when exposure occurs chronically at low levels (Wallace and Buha Djordjevic, 2020). Although the levels of Hg in Aranzazu are the lowest among the evaluated areas (Fig. 2), the possible association of this metal with cytogenetic damage cannot be ruled out, especially in cases of chronic exposure to low doses. Similar studies conducted in La Mojana region presented similar findings, with a direct correlation between low doses of Hg-T and MeHg and MNBN frequencies (Espitia-Perez et al., 2018b; Galeano-Páez et al., 2021).

Vulnerable populations, including pregnant women, children, and those with underlying health issues, are more vulnerable to the adverse effects of this type of contamination (Pinheiro et al., 2009). Hg is a well-known contaminant associated with tailings from gold mining activities (Rocha-Román et al., 2018). Ni can also be present in gold and nickel deposits and leached during soil removal for gold mining (Kohanpour et al., 2018). In Caldas, where gold deposits are currently exploited by ASGM, Ni discharge from these activities is also a potential source of contamination (SGC, 2020). Additionally, Ni emissions from agricultural activity (Chai and Guo, 2023; Marrugo-Negrete et al., 2017) can contribute to Ni contamination, as Ni is a common component of agrochemicals (Hassan et al., 2019; Kayode et al., 2022).

Analysis of the population in the reference area, revealed a significant association between frequencies of MNBN and the combined effect of Pb (PR = 1.110; 95 % CI: 1.023–1.205), Cd (PR = 1.033; 95 % CI: 1.006–1.060), Se (PR = 1.088; 95 % CI: 1.034–1.144), and Mg (PR = 1.001; 95 % CI: 1.00–1.002) (Table 4).

Like most of the mixtures previously discussed in this article, the combination of Pb, Cd, Se, and Mg is constituted by a blend of toxic and essential metals that can interact synergistically, antagonistically, or additively. In reference area, Cd presented the highest levels among all the studied zones 0.05 (0.02–0.13), while Pb presented higher but intermediate levels (0.72 (0.32–2.35)) when compared to the other zones (Table S2). There is limited information on the specific effects of Cd and Pb on DNA repair. However, some studies suggest that exposure to these

metals can induce DNA damage and affect DNA repair mechanisms (Viau et al., 2021). In addition, Cd and Pb can have synergistic effects, meaning their toxicity is increased when they are present together (Balali-Mood et al., 2021; Mousavi et al., 2019). On the other hand, Se and Mg are essential minerals and play crucial roles in human health, particularly in DNA repair (Hossain et al., 2022) and the maintenance of antioxidant defenses and genomic integrity (Anastassopoulou and Theophanides, 2002; Hartwig, 2001). However, when present in a complex mixture with Pb and Cd, Se and Mg are poorly absorbed, leading to deficiencies (Balali-Mood et al., 2021; Mousavi et al., 2019). Pb can replace Mg in certain enzymes, disrupting their function (Leal et al., 2023; Ostoich et al., 2020). This could partially explain how these two metals considered essential and protective against DNA damage, can act as modulators associated with increased MNBN frequencies.

3.6. Particularities of the exposure to metal mixtures and MNBN frequencies in large-scale mining areas

The correlation dynamics between metals in each area showed a differentiated pattern in Montelibano compared to Nechí and Aranzazu (Fig. 3). After categorizing the patterns according to the type of exposure, we found that elevated levels of metals in individuals with occupational exposure were responsible for the strong correlation dynamics observed in the region (Fig. 4). Thus, what makes the occupational exposure in this area so unique compared to the mining systems of Nechí and Aranzazu?

As previously discussed, Montelibano is the only studied area that involves highly technified and intensive extraction (Díaz et al., 2015; UPME, 2005), with medium-scale coal mining systems, and ASGM in the same geographic area. On the other hand, the mining system of the Bagre-Nechí is associated with systems (UNODC, 2016), while Aranzazu presents an extinct mining operation that we explore as a possible example of residual contamination.

Considering these characteristics, we used a PCA to investigate the interplay among the chemical elements that contribute to the mixture of metals in all three areas (Fig. S2). This first PCA analysis included 95 % confidence ellipses to represent the distribution of the data in terms of their variability and correlation. Recent work used a similar approach to analyze contaminant mixtures (Zuk et al., 2021). The analysis of the figure illustrates how the exposure levels in the mining district of Montelibano encompass and represent the overall variability observed in Nechí and Aranzazu. Chemical elements in Montelibano exhibit a broader range of values and greater diversity than the other mining zones. In contrast, Aranzazu and Nechí show limited correlations between metals, further supporting the hypothesis that Montelibano possesses distinctive characteristics in its chemical element composition. Chemical elements with the highest contributions in PC-1 were As, Se, Ni, Zn, Cr, and Mg, explaining 46.3 % of the total variance in all data. A PC-2, with a 10.6 % variance, was represented by the contributions of Pb, Mn, and Cd.

Next, separate PCAs were conducted for Montelibano, Aranzazu, and Nechí to examine specific patterns and variations within each mining area (Fig. 5). This approach allowed us to identify any shared or distinct patterns among the areas, contributing to a comprehensive understanding of the exposure levels across the different mining zones.

In Montelibano, PC-1 explained 63.5 % of the total variance, while for Aranzazu and Nechí, PC-1 was only 23.7 % and 22 %, respectively (Fig. 6A). In Aranzazu, 3 metals showed the highest contribution to PC-1: Ni, Cr and Cd; in Nechí PC-1 was contributed by 5 main elements as follows: Mn, Cr, As, Ni and Mg.

Since PC-1 in Aranzazu and Nechí accounts only 23.7 % and 22 % of the variance, the further PCA elemental contribution analysis focused on Montelibano. Fig. 6B shows the contribution percentages of each of the elements of PC-1 analyzed for Montelibano. Elements with high contribution can be arranged in decreasing order as As > Se > Ni > Zn > Cr > Mg. This heterogeneous mixture of toxic metals, such as As and Ni,

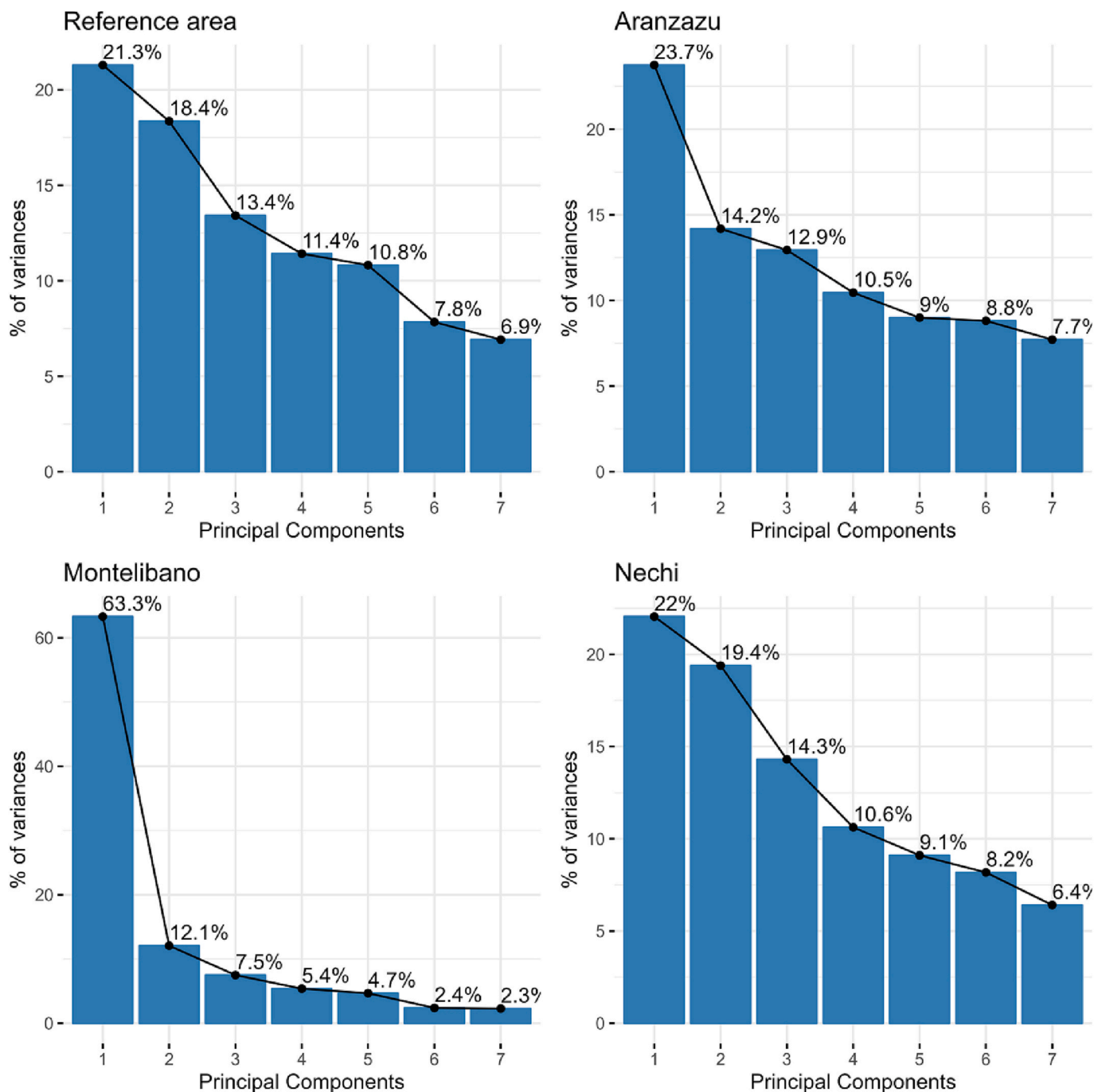


Fig. 5. Percentage of explained variance for each PC discriminated by area.

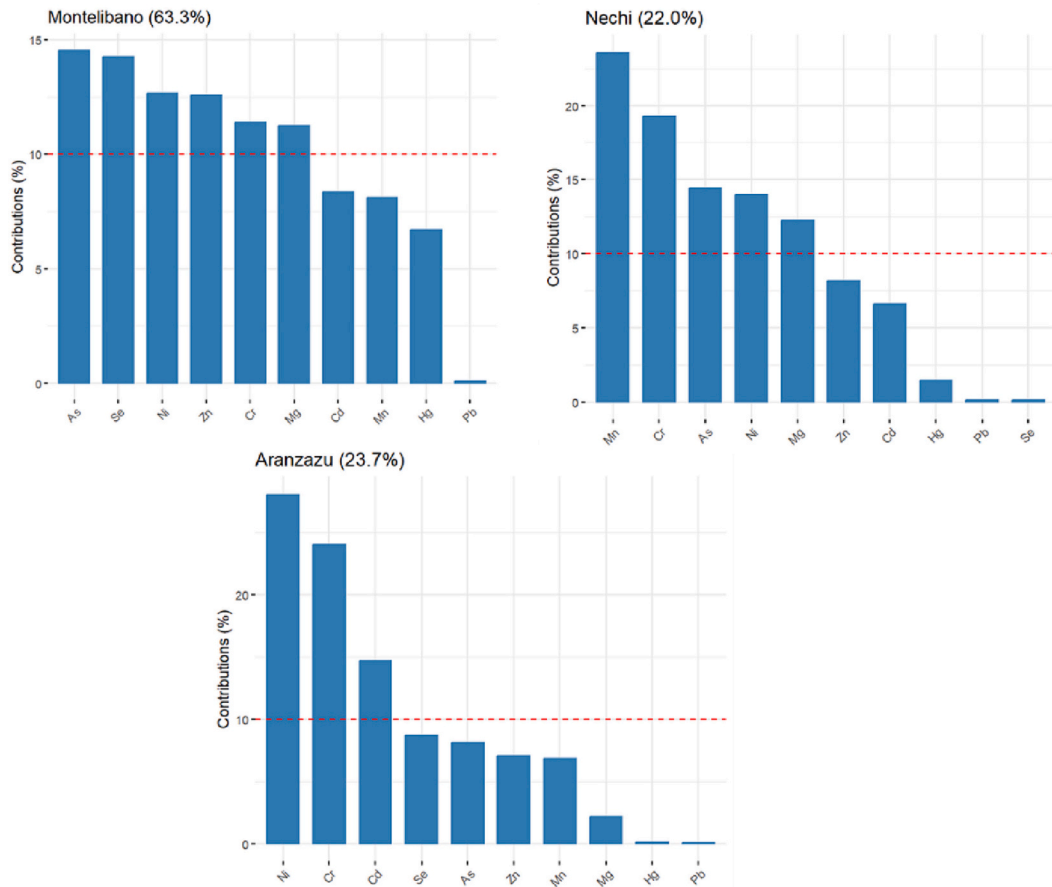
and trace elements, such as Se, Zn, and Cr, suggests an association with metallurgical industry activities in the study area. Montelibano represents an important highly-technified metallurgic system compared to other regions from our study. Our common source data (Fig. 3) concurs with our findings and can be used to explain elemental hallmarks. This Cr cluster of elements was higher in Montelibano occupational exposure.

Although Cr and Ni are naturally enriched elements, this Cr-Ni, one of the most significant correlations, may indicate smelter-related trace deposition via dust deposits in ferronickel processing (Fry et al., 2021). However, more detailed studies are needed since the remaining As-Cd-Pb cluster indicates other mining activities (Sheykhi and Samani, 2020). In addition, the As-Ni-Pb association was previously encountered in a more detailed study of ferronickel smelter atmospheric deposition and was encountered as anthropogenically generated emissions (Baceva

et al., 2012). This emission may be provided by the slag deposition near ferronickel operations (Han and Hong, 2018). The analysis of PC-1 on the frequency of MNB showed a significant relationship in individuals from Montelibano (PR = 0.935; 95 % CI: 0.87–1.00) (Table 5). In addition, age, gender, and lifestyle habits significantly impacted the risk of MNB, compounding the effect of the mixture of metals found in this region. Taken together, the results suggest that the component analyzed has a risk impact on the increase in cytogenetic alterations measured in this study, which could result in a wide range of potential adverse health impact.

According to the Colombian Mining and Energy Planning Unit (UPME), the Montelibano Mining District is located in the southern part of the department of Cordoba, which encompasses the municipalities of Buenavista, La Apartada, Montelibano, Planeta Rica, Pueblo Nuevo, and

A.



B.

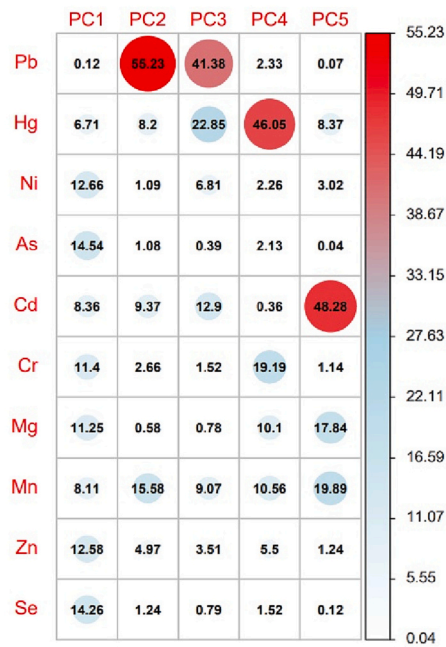


Fig. 6. A) Metals contribution to PC-1 in the different mining zones. B) Individual contribution of metals to PC-1 in Montelibano.

Puerto Libertador. The district is currently extracting coal, ferronickel, gold, and silver, with ferronickel accounting for over 50 % of the mining production (Ponce Muriel, 2005). Classified the Montelibano district under category 2, corresponding to a production scale of fewer than five million tons annually (MINMINAS, 2014). However, it is one of the three districts with a total export component, indicating that mega-mining extractive activities are taking place in the Montelibano Mining District, resulting in increased impacts on health and the environment due to the pollutants produced.

The study has potential limitations that include: 1) the small sample size in each exposed area caused by the confinement restrictions imposed during the COVID19 pandemic that could impact the statistic power specially in models with a great number of variables; 2) the lack of physicochemical data from environmental matrixes that hindered the identification of potential sources of exposure: air, drinking water, contaminated food, indoor dust etc.; 3) the absence of chemical speciation to characterize the chemical species present in hair samples and their interactions. This particular approach should be use when studying elements whose effect depends on the chemical species involved.

4. Future directions

This initial exploration of chemical element mixtures in mining contexts in Colombia has provided a preliminary understanding of environmental health issues. The findings revealed diverse and heterogeneous results, underscoring the situation's complexity. Nonetheless, these results also demonstrate that populations exposed to mining activities, whether residentially or occupationally, face equivalent levels of risk, emphasizing the substantial hazards communities reside near mining sites. Furthermore, this research underscores the necessity for future investigations to focus on the correlation between the observed outcomes and the incidence of prevalent diseases in residential and occupational populations. It is imperative to conduct a comprehensive environmental characterization encompassing speciation analysis of toxic elements and essential nutrients, as well as an evaluation of various environmental matrices, including soil, water, air, flora, and fauna. A comprehensive understanding of pollution and its impact on ecosystem dynamics can be achieved by establishing connections between the concentrations of toxic metals and elements across these matrices.

It is strongly recommended that decision-makers implement robust monitoring programs and adopt mitigation measures to exercise stringent control over the resulting impacts in this region. The research team also suggests actively sharing and disseminating the data and findings with the communities involved. This proactive approach aims to foster awareness and engagement among residents, empowering them to be active participants in identifying and implementing potential solutions. The generation and widespread dissemination of reliable data is vital in raising consciousness, mobilizing the affected population, and facilitating the implementation of effective strategies.

5. Conclusions

Exposure to a mixture of chemical elements in mining areas was associated with high frequencies of MNBN, indicating an effect on CIN in these populations. Results showed a significant increase in MNBN frequencies regardless of the exposure type, indicating that these mixtures effects were similar for workers and residents in mining-affected areas. All the studied mixtures comprised essential and toxic elements, with As, Se, Ni, Cr, Zn, and Mg significantly associated with increased MNBN frequency. These particular metal mixtures can generate DNA damage primarily through different mechanisms, including the induction of oxidative damage, direct binding to DNA, inhibition of DNA repair processes, induction of epigenetic modifications, and activation of DNA damage response signaling pathways.

The correlation dynamics of the elements were more significant and evident in the open-pit large-scale mining areas of Montelibano,

demonstrating the impact of these activities on the biological effects of populations. An interesting finding was the behavior of Se within these mixtures, as it was associated with high MNBN frequencies in all exposed areas. Although contradictory, this result contributes to the discussion on the duality of Se as a contaminant and essential element and poses significant challenges to studying its properties in complex mixtures. Further investigations are required to examine the impact of geogenic selenium on genetic markers such as MNBN, particularly in the presence of complex mixtures. This finding also underscores the significance of chemical speciation in identifying the potential source and properties of chemical elements in complex mixtures. Our research is an important source of information on the effects of exposure to chemical mixtures and highlights the importance of using this approach in the risk assessment of exposed communities.

CRedit authorship contribution statement

Karina Pastor-Sierra: Writing- Original draft preparation, Conceptualization, Methodology; **Lyda Espitia-Pérez:** Conceptualization, Methodology, Writing-Reviewing and Editing. **Pedro Espitia-Pérez:** Writing- Original draft preparation, Methodology. **Ana Peñata-Taborda:** Writing- Original draft preparation. **Hugo Brango:** Data curation, Software and statistical analysis. **Claudia Galeano-Páez:** Writing-Original draft preparation. **Osnamir Elias Bru-Cordero:** Data curation and statistical analysis. **Marien Palma-Parra:** Conceptualization, Investigation. Methodology. Visualization; **Sonia M Díaz:** Visualization, Investigation. Conceptualization. Methodology. **Carlos Trillos:** Visualization, Investigation, Conceptualization, Methodology. **Leonardo Briceño:** Visualization, Investigation. Conceptualization, Methodology. **Álvaro J Idrovo:** Conceptualization, Funding, Methodology, Writing- Reviewing and Editing. **Juan Miranda-Pacheco:** Reviewing and Editing. **Eliana Téllez:** Visualization, Investigation. Conceptualization, Methodology. **Luisa Jiménez-Vidal:** Visualization, Investigation. **Andrés Coneo-Pretelt:** Visualization, Investigation. **Alicia Humanez Álvarez:** Visualization, Investigation. **Gean Arteaga-Arroyo:** Reviewing and Editing. **Dina Ricardo-Caldera:** Visualization, Investigation. **Shirley Salcedo-Arteaga:** Reviewing and Editing. **Alexandra Porras-Ramírez:** Reviewing and Editing. **Marcela Varona-Uribe:** Project management, Investigation, Conceptualization, Methodology, and Supervision.

Funding

This research was funded by MINCIENCIAS (Grant number 905-2019), Universidad del Rosario (UNIROSARIO), Instituto Nacional de Salud (INS), Universidad Industrial de Santander (UIS), and Universidad del Sinú (UNISINU).

Declaration of competing interest

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

Data availability

The data that has been used is confidential.

Acknowledgements

The authors wish to thank all the participants who voluntarily decided to collaborate in the study, the community leaders who generated the channels of approach with the populations, and the local authorities of the municipalities of Aranzazu, Nechí, and Montelibano for their support in carrying out the study. We also thank PUC-Rio's LAB-SPECTRO personnel in Brazil for their assistance in analyzing the hair

samples.

Ethical considerations

Personal information was treated confidentially, preserving the anonymity of each participant, considering the Colombian legislation for handling clinical history and personal data. The study was conducted in accordance with the guidelines of the Declaration of Helsinki, Colombian legislation on health research with human subjects, research during the COVID-19 pandemic. The protocol was approved and supervised by the Ethics and Research Committee of the Universidad del Rosario (Approval No 002 of May 20, 2019).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.165789>.

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