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Micronuclei among Individuals from Three Colombian Mining Contexts Exposed to Pesticides and Chemical Element Mixtures

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Abstract: The contexts where there is mining and agriculture activities are potential sources of risk to human health due to contamination by chemical mixtures. This study explored the association between the frequency of micronuclei and pesticides in regions with ferronickel (Montelibano, Córdoba) and gold (Nechí, Antioquia) mining, and a closed native mercury mine (Aranzazu, Caldas). A cross-sectional study was carried out with 247 residents in the mining regions. Sociodemographic, occupational, and toxicological variables were ascertained. Blood and urine samples were taken for pesticide analysis (12 organochlorines, organophosphates, and carbamates), 68 chemical elements were quantified in hair, and micronuclei (MN) were quantified in lymphocytes. The mixtures of chemical elements were grouped through exploratory factor analysis. Prevalence ratios (PR) were estimated with robust variance Poisson regressions to explore associations. The highest concentrations of chemical elements were in the active mines. The potentially most toxic chemical mixture was observed in the ferronickel mine. Pesticides were detected in a low proportion of participants. The frequency of MN was similar in the three mining contexts. There was great heterogeneity in the exposure to pesticides and chemical elements. The "hormetic effect" of selenium is described, in which at low doses it acts as a chelator, and at high doses it can enhance the toxic effects of other elements. It is proposed that future studies in mining contexts include the measurement of chemical mixtures to better assess exposure and potential adverse health effects.

Keywords: pesticides; mining; chemical mixtures; selenium; hormesis; genetic damage; Colombia

1. Introduction

Pesticides are chemical substances used to prevent or control any pest, and their inadequate handling can have adverse effects on the health of agricultural workers, their families, the community, and the environment [1]. Exposure to these substances can be through skin contact, ingestion or inhalation and can cause acute or chronic poisoning. Chronic adverse effects usually appear long after the first exposure, even at low doses, being more frequent among adults [2] since various pesticides accumulate in the body and the environment, causing disease after several years of exposure[3].

There is evidence of the relationship between chronic exposure to some pesticides and an increased occurrence of neurological, respiratory, dermatological, and renal diseases, reproductive disorders, cancer, and endocrine disorders, among others. Many of these disorders are associated with insecticides and herbicides, especially organophosphates, organochlorines, and triazine compounds [4,5]. Considering the availability of susceptibility biomarkers, carcinogenic effects can be seen in early changes in genomic DNA such as DNA-DNA and DNA-protein cross-linking, strand breaks, and DNA adduct formation, generating defective cells [6–9]. For this reason, DNA damage studies may be useful to prevent some adverse effects.

Agriculture for human and animal consumption is also practiced in many places where mining activities are performed. The consequences of mining and agricultural activity in the same place are not very well known; but they undoubtedly have to do with the type of mining, the geochemical of soil, and the absorption capacity of crops. Open-pit mining is often associated with extensive environmental damage, as mining waste can spread via air and surrounding water sources. It contrasts with damage to water sources related with alluvial and underground mining, although it is higher deleterious effects in alluvial activities [6,10]. Concerning the geochemistry of soils and types of vegetation, generalizations are not easy since the chemical mixtures observed in rocks and soils can be highly variable. For this, specific studies are required for each mining-agricultural region.

In Colombia there is a high consumption and market of pesticides, even though the records are inaccurate [11]. In this country, mining regions may coincide with crops cultivated years ago or fumigated to prevent vector-transmitted diseases [12]. When pesticides are persistent in the environment, such as organochlorines, the toxic minerals' presence may propitiate the occurrence of adverse effects not well-known in humans [13]. Although organochlorine pesticides have been forbidden in Colombia, there is evidence that are used to control pests in coca, marijuana, and poppy crop regions [14]. In this regard, there are about 88,629 m^3 estimated of soil contaminated with organochlorines in the country; however, there is a significant underreporting of contaminated sites [15]. Therefore, it is important to start evaluating the potential places of greatest risk to human health.

In these mining-agricultural contexts, multiple and heterogeneous exposures can generate synergistic effects; that lead to greater damage or disease, or antagonistic effects where the adverse effects of exposure are minimized by the presence of other chemical compounds. Perhaps the most notorious case in Colombia may be the presence of very high selenium concentrations in soils [16], which may reduce the risk associated with metals and metalloids' presence given their chelating action [17]. However, the presence of recognized toxics such as mercury, lead, cadmium, and pesticides with elements that are not widely studied in the country, such as arsenic, beryllium, and uranium [18], could have unpredictable effects. Potential adverse effects in humans due to exposure to complex mixtures of chemical substances are chronic diseases [7,8] and genomic damage [19,20]. In this context, this study explored the association between exposure to pesticide mixtures and the occurrence of micronuclei among individuals residing in three mining areas with very different characteristics. It corresponds to a first approximation to possible mining contaminated sites related to agricultural activities that can have important effects on human health.

2. Materials and Methods

2.1. Study design and mining contexts

A cross-sectional study was conducted with three populations potentially exposed to mining and agricultural activities. The populations were from Montelibano (Córdoba), Nechí (Antioquia), and Aranzazu (Caldas). The selection of these municipalities was based on specific characteristics of each place.

Nechí is a municipality where gold is extracted from alluvium, which has led to the use of mercury for decades to separate and extract the precious metal from other rocks. According to previous studies, it is one of the most mercury-contaminated areas in the world [21]. In this region pesticides are used in crops of rice, corn, cassava, banana, and purple yam (*Dioscorea alata*), and to control vector-borne diseases.

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Montelibano is close to one of the largest ferronickel mines in the world. Additionally, there is a coal mine in the region, and gold is exploited in an artisanal way. Exposure to chemical mixtures (Ni, Fe, As, Pb, Cd, mainly) has been described [22,23]. In this region *Plasmodium falciparum* infection is frequent [24], thus insecticides are used; moreover, cassava and purple yam are common crops in this territory.

In Aranzazu there is a geological fault where native mercury presence is important; in fact, the only mercury mine in Colombia existed in this municipality. It was closed in 1975 due to financial problems and neurotoxicity among the workers [25]. Currently, it is a small municipality whose economic activities are related to avocado and coffee cultivation. It is an example of what can happen after a mercury mine is closed.

2.2. Participants and data collection

Potential participants from the three municipalities were selected by non-probabilistic sampling due to the lack of an updated sampling frame and the logistical limitations related to informal work and difficult access to mining regions. In Nechí and Montelibano regions for decades there have been illegal armed groups, which has limited the presence of public and environmental health researchers due to the inherent security risks. Individuals ≥18 years old living in the place for a minimum of six months participated in the study. Those individuals who had presented illnesses that prevented them from adequately answering the questionnaire and who had undergone X-rays, radiotherapy, or chemotherapy during the previous year were excluded. Consequently, participants were formal and informal workers, or individuals dedicated to housework activities. A questionnaire was applied that included sociodemographic variables (age, sex, education, health affiliation), labor, and toxicological (smoker status, alcohol consumption). Additionally, an adaptation of the World Health Organization´s instrument "Guidance for identifying populations at risk from mercury exposure" was applied [26].

2.3. Pesticides in blood and urine

A 5 mL venous blood sample and a 50 ml urine sample were extracted from each participant, which remained refrigerated until processing and analysis to determine pesticides. Concentrations (ppb) of carbamate insecticides: aldicarb, carbofuran, and propoxur; the organochlorines: α-endosulfan, β-endosulfan, endosulfan sulfate, and hexachlorobenzene, and the organophosphorus: malathion, ethyl paraoxon, methyl paraoxon, ethyl parathion, and methyl parathion, were determined in blood and urine. They were selected because they are widely used in Colombia and have high acute toxicity. The analyses were performed through Quechers extraction and high-performance liquid chromatography (HPLC) analyses with single quadrupole (SQ) LC/MSD. We rely on the method published by Sciex (equipment supplier) for environmental applications [27].

2.4. Element mixtures in hair

Hair (in high proportion keratin rich in sulfur) was selected as an internal dose biomarker given the interest in quantifying metals and metalloids; the way of entering the body is via ingestion, although the concentration of some elements may be due to external contamination or compounds included in cosmetics [28]. Hair samples were taken from the occipital region of the scalp (~3 cm long) of all participants. The 68 chemical elements included in the analysis were: lithium (Li), beryllium (Be), boron (B), sodium (Na), magnesium (Mg), aluminum (Al), silicon (Si), phosphorus (P), sulfur (S), potassium (K), calcium (Ca), scandium (Sc), titanium (Ti), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), gallium (Ga), germanium (Ge), arsenic (As), bromine (Br), selenium (Se), rubidium (Rb), strontium (Sr), yttrium (Y), zirconium (Zr), niobium (Nb), molybdenum (Mo), ruthenium (Ru), rhodium (Rh), palladium (Pd), silver (Ag), cadmium (Cd), indium (In), tin (Sn), antimony (Sb), iodine (I), cesium (Cs), barium (Ba), lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd), samarium (Sm), europium (Eu), gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm),

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ytterbium (Yb), lutetium (Lu), tantalum (Ta), tungsten (W), rhenium (Re), platinum (Pt), gold (Au), mercury (Hg), thallium (Tl), lead (Pb), bismuth (Bi), thorium (Th), and uranium (U). Note that several elements (or related compounds) listed by IARC as carcinogenic (group 1), probable (2A), or possible (2B) carcinogenic to humans were included in the analysis. In group 1 are As, Be, Cd, Cr, Sr, Ni, and Th. In Group 2A are Sb, Co, In, Mg, Mn, and Si. In group 2B are Hg, Mo, Pb, K, Na, Ti, and V.

The preparation and the sample analysis were carried out by the ICP-MS and ICP OES Laboratory (LABSPECTRO) of the Chemistry Department of the Pontificia Universidade Católica do Rio de Janeiro, in Brazil. Over there the samples were cleaned with deionized water and acetone for three repetitions, with each solution inside an ultrasound bath to eliminate exogenous elements. Hair samples were oven-dried at 60 ºC and weighed. Samples were then acidified with 2,50 ml of HNO3 in 50 ml polypropylene tubes and digested at 70 °C for 4 hours. Finally, they were diluted for elemental determination. Hair element concentrations were evaluated by inductively coupled plasma mass spectrometry using an Elan DRCII ICP-MS spectrometer (Analyst 200, PerkinElmer, Sciex, Norwalk, CT, USA) [29]. Each curve calibration point (blank, reagent blank, and sample) was analyzed with the internal rhodium standard. Argon was used as carrier gas at a flow rate of 50 ml min-1.

2.5. Citogenetic analysis

Fenech´s methodology was used to explore genetic instability [30]. First, heparinized whole blood (0.5 mL) was added to 4.5 mL of RPMI 1640 medium (Sigma R8758, St. Louis, MO, USA) supplemented with 2 mM L-glutamine (Sigma A5955, St. Louis, MO, USA), 10% fetal bovine fetal serum (Gibco 15000-044, Brazil), 100 µL/mL antibiotic-antimycotic (Sigma A5955, St. Louis, MO, USA), and 2% phytohemagglutinin (Sigma L8754, St. Louis, MO, USA). Cultures were incubated at 37 ◦C for 44 h under 5% CO2. After this incubation period, 6 µg/mL cytochalasin B (Sigma C6762, St. Louis, MO, USA) was added. After incubation, lymphocytes were harvested by centrifugation at 1200 rpm for 8 min, centrifuged again, fixed in 25:1 (v/v) methanol/acetic acid, placed on a clean microscope slide, and stained with Diff-Quik stain (Medion diagnostics; 726443. Düdingen. CH). For each participant´s blood sample, 2000 binucleated (BN) cells (1000 from each of two slides prepared from duplicate cultures) were evaluated for the presence of DNA damage indices—micronuclei (MN) using bright-field optical microscopy at 200–1000× magnification. All slides were coded for blinded analysis according to the criteria proposed by Fenech.

2.6. Statistical methods

The databases were created using Epi Info 7.0, and the statistical analyzes were performed with the Stata 17 program. Values reported by the laboratory as not detected or below the detection limit were considered zero in the statistical analyses. First, description of participants with proportions or measures of central tendency and dispersion were used, according to the observed distribution. Comparison between the three population groups were made with x^2 or Kruskal-Wallis tests. Then, a detailed exploration of possible chemical element mixtures suggested that in each mining site there are different distribution of elements. For this reason, the following analysis was performed for each mine independently.

To identify the mixtures of chemical elements in each mining site, exploratory factor analyses with the 68 element concentrations in hair were carried out. It was useful because factor analysis is a mathematical model that explains covariance or correlation among element concentrations in hair in terms of unobserved latent variables [31], in this case element mixtures. One *a priori* criterion to consider a factor as relevant was that they exhibited a proportional contribution higher than 5%. In Montelibano two common factors (FC1M and FC2M) were extracted after a varimax rotation (Figure 1). With the same method, in Nechí (FC1N, FC2N and FC3N) (see Figure 2) and Aranzazu (FC1A and FC2A) (see Figure 3) were extracted three and two factors, respectively. In Montelibano, the factors explained 46.08% of the variance, whereas in Nechí and Aranzazu were 41.43% and 35.15%, respectively. In addition, factor scores were calculated for every state, and after these scores were normalized. Results are available in Appendix A.

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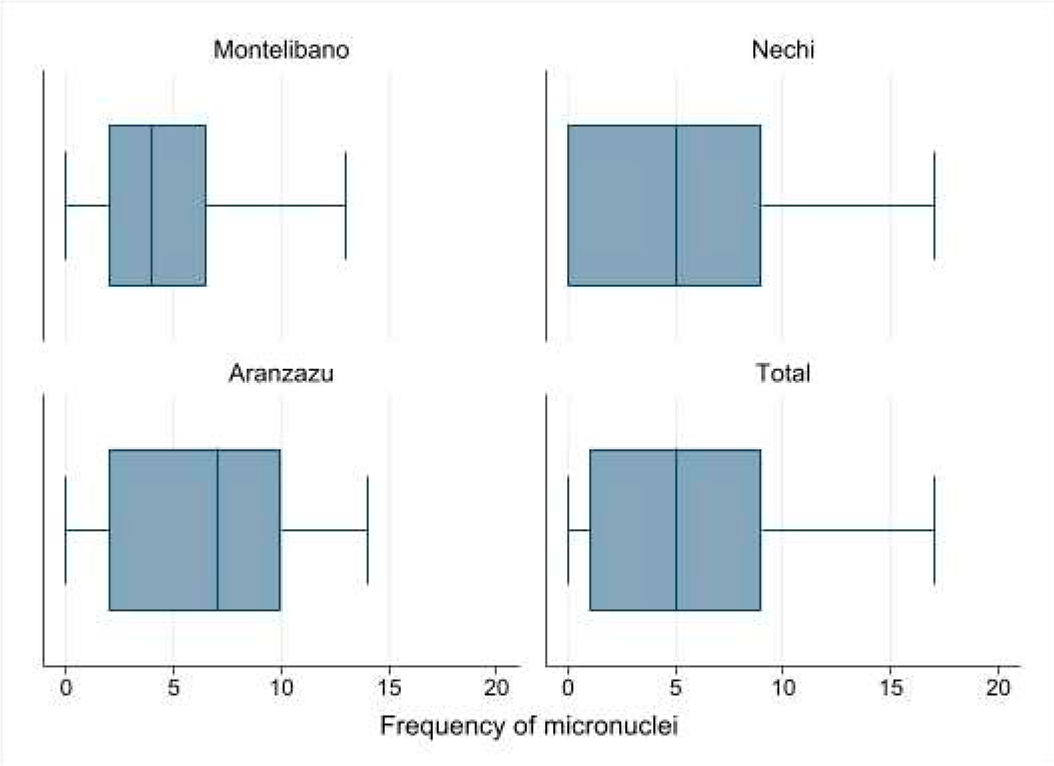

Figure 1. Frequency of micronuclei in the three mining contexts.

To explore the associations with the frequency of micronuclei, prevalence ratios (PR) with 95% confidence intervals (95% CI) were estimated for each mining site. They were first estimated bivariate and then multiple, using Poisson regression models with robust variance [32]. In the analysis, special care was taken with some chemical elements that could have adverse or antagonistic effects. It was considered whether it belonged to the chemical mixtures included in the analyses; if they were not included, they were analyzed independently in each mining site. Interactions between selenium and other elements were explored in Nechí and Aranzazu because of its potential protective effect [33].

3. Results

3.1. Mean characteristics of participants

Information from 306 individuals were collected, but after excluding those with missing data, information from 247 individuals were analyzed. Of these, 30.77% were from Montelibano (n=76), 34.01% from Nechí (n=84) and 35.22% from Aranzazu (n=87). It was evident that participants differed between the three locations (see Table 1). There was a high participation of women, much higher in Montelibano and lower in Aranzazu. Age was also higher in Montelibano and lower in Aranzazu. There were many more married or cohabiting in Montelibano and more single in Nechí. Regarding education, it is evident that the lowest levels were found in Nechí, including a very high proportion of illiterates, which contrasts with the highest educational levels in Montelibano. As expected, there were more individuals carrying out mining-related activities in Montelibano and none in Aranzazu since the mercury mine has been closed since 1975; however, in Aranzazu it is where more individuals were engaged in work related to agriculture.

Cigarette consumption was higher in Aranzazu and lower in Montelibano, while alcoholic beverage consumption was higher in Montelibano and lower in Aranzazu. Regarding food consumption, it was striking that the only difference is in the consumption of fish, which was higher in Montelibano and lower in Aranzazu. In relation to the chemical elements in the hair, it was evident that the highest concentrations are found in places where there are active mines; these include elements that in some of their chemical forms are carcinogenic or probably carcinogenic for humans

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according to the IARC. Selenium, with its potential chelating effect, had a higher concentration in Nechí and a lower one in Aranzazu.

Table 1. Mean characteristics of individuals participating in the study (n=247).

Site (Minerals)	**Montelibano (Fe/Ni)**	**Nechí (Au)**	**Aranzazu (Closed Hg)**	
Variables	(n=76) % / median (min-max)	(n=84) % / median (min-max)	(n=87) % / median (min-max)	**P value**
Sex (female)	80.26	72.62	60.92	0.023
Age (years)	54 (21 – 81)	48 (21 – 83)	40 (19 – 83)	<0.001[a]
Civil status				
Married / Free union	85.53	65.48	70.11	0.029
Divorced / Widower	2.63	7.14	10.34	
Single	11.84	27.38	19.54	
Education				
Illiteracy	2.63	11.90	5.75	<0.001
Elementary (partial or full)	13.16	39.29	39.08	
Secondary (partial or full)	31.58	22.62	37.93	
Technic (partial or full)	35.53	19.05	4.60	
University (partial or full)	17.11	7.14	12.64	
Occupation				
In mining activities	52.63	44.05	0	<0.001
In agricultural activities	6.58	22.62	45.98	<0.001
Cigarette consumption (any moment)	21.05	39.29	43.68	0.007
Alcohol consumption (any moment)	61.84	44.05	40.23	0.015
Food consumption				
Fish	97.37	91.67	71.26	<0.001
Canned food	56.58	48.81	60.92	0.274
White meat	97.37	92.86	96.55	0.328
Red meat	94.74	94.05	96.55	0.734
Fruits	96.05	94.05	94.25	0.825
Vegetables	98.68	97.62	94.25	0.242
Carcinogenic elements* (IARC 1)				
Arsenic	0.14 (0.05 – 4.28)	0.18 (0.06 – 1.64)	0.12 (0.05 – 0.36)	<0.001[a]
Beryllium	0 (0 – 16.06)	0 (0 – 0.07)	0 (0 – 0.02)	<0.001[a]
Cadmium	0.05 (0 – 1.69)	0.04 (0.01 – 0.48)	0.05 (0.01 – 0.94)	0.395[a]
Chromium	0.51 (0.18 – 19.06)	0.33 (0.18 – 7.25)	0.33 (0.20 – 3.53)	<0.001[a]
Strontium	2.09 (0.1 – 55.42)	3.49 (0.33 – 46.21)	2.97 (0.19 – 46.13)	0.011[a]
Nickel	0.45 (0.05 – 17.77)	0.25 (0.05 – 2.07)	0.14 (0.02 – 3.92)	<0.001[a]
Thorium	0 (0 – 0.27)	0 (0 – 0.04)	0 (0 – 0.01)	<0.001[a]
Probably carcinogenic* (IARC 2A)				
Antimony	0.03 (0.00 – 0.35)	0.02 (0.00 – 0.35)	0.01 (0.00 – 0.29)	0.040[a]
Cobalt	0.04 (0.00 – 4.46)	0.03 (0.01 – 1.14)	0.01 (0.00 – 0.13)	<0.001[a]
Indium	0 (0 – 0.002)	0	0 (0 – 0.004)	0.3484
Magnesium	77.90 (12.97 – 1449)	105.81 (19.44 – 2131)	49.73 (9.51 – 608.74)	<0.001[a]
Manganese	1.66 (0.06 – 92.63)	3.09 (0.15 – 46.54)	0.80 (0.10 – 11.50)	<0.001[a]

Silica	0 (0 -7598)	0 (0 – 4495)	1201 (0 – 3913)	<0.001[a]
Possibly carcinogenic* (IARC 2B)				
Mercury	1.89 (0.06 – 12.29)	2.48 (0.17 – 17.14)	0.08 (0 – 1.63)	<0.001[a]
Molybdenum	0.05 (0.01 – 1.40)	0.06 (0.02 – 0.36)	0.04 (0.02 – 0.24)	<0.001[a]
Lead	0.92 (0.04 – 89.01)	0.64 (0.08 – 75.17)	0.55 (0.06 – 163.37)	0.761[a]
Potassium	1.83 (0 – 155.47)	3.98 (0 – 106.10)	1.08 (0 – 129.28)	<0.001[a]
Sodium	3.12 (0.47 – 238.26)	3.94 (0.49 – 701.25)	1.44 (0.30 – 379.54)	<0.001[a]
Titanium	0.13 (0 – 3.16)	0.24 (0.09 – 1.24)	0.18 (0.08 – 0.60)	<0.001[a]
Vanadium	0.05 (0.00 – 1.41)	0.12 (0.03 – 0.85)	0.05 (0.02 – 0.45)	<0.001[a]
Other elements				
Phosphorus	219 (66 – 2883)	218 (111 – 342)	205 (148 – 297)	0.114[a]
Selenium	1.35 (0.23 – 14.70)	1.40 (0.46 – 60.69)	1.01 (0.41 – 1.44)	<0.001[a]

3.2. Pesticide exposure

In relation to the quantified pesticides (Table 2), the most relevant finding is that they were detected in only a low proportion of the participants, which suggests a low number of individuals with recent exposure. In the blood samples, were not detected aldicarb, alpha-endosulfan, betha-endosulfan, endosulfan-sulfate, malathion, hexachlorobenzene, parathion-methyl, propoxur and carbofuran. In the urine samples were not detect aldicarb, alpha-endosulfan, betha-endosulfan, endosulfan-sulfate, malathion, hexachlorobenzene, and carbofuran. In Montelibano more pesticides were detected, being paraoxon-methyl in blood the most frequent, whereas in Aranzazu the most common was paraoxon-ethyl in blood.

Table 2. Concentrations of selected pesticides in blood and urine among inhabitants near to three mines in Colombia.

Montelibano (Fe/Ni open-pit mine)		**Positives**	**Concentrations (ppm)**					
			Percentiles					
Pesticide	**Sample**	**%**	**Min**	**25**	**90**	**95**	**99**	**Max**
Paraoxon-methyl	Blood	27.55	ND	ND	0.24	0.29	37.26	37.26
Parathion-ethyl	Blood	1.02	ND	ND	ND	ND	ND	1.02
Paraoxon-ethyl	Urine	2.04	ND	ND	ND	ND	0.06	0.06
Parathion-ethyl	Urine	2.04	ND	ND	ND	ND	0.07	0.07
Parathion-methyl	Urine	2.04	ND	ND	ND	ND	0.07	0.07
Propoxur	Urine	2.04	ND	ND	ND	ND	0.13	0.44

Nechí (Au fluvial mines)		**Positives**	**Concentrations (ppm)**					
			Percentiles					
Pesticide	**Sample**	**%**	**Min**	**25**	**90**	**95**	**99**	**Max**
Paraoxon-ethyl	Blood	0.99	ND	ND	ND	ND	ND	0.75
Parathion-ethyl	Urine	0.99	ND	ND	ND	ND	ND	0.05

Aranzazu (Hg closed mine)		**Positives**	**Concentrations (ppm)**					
			Percentiles					
Pesticide	**Sample**	**%**	**Min**	**25**	**90**	**95**	**99**	**Max**
Paraoxon-ethyl	Blood	18.81	ND	ND	0.08	0.09	0.10	0.13
Paraoxon-methyl	Urine	0.99	ND	ND	ND	ND	ND	0.38

3.2. Chemical element mixtures in hair

There were very different mixtures of elements in the populations residing in the surroundings of the three mines (see Figures A1, A2 y A3 in Appendix). In the FC1M mixture in Montelibano the most important elements were P, S, Cu, As, Se, Mo, Li, Mg, Ca, V, Cr, Mn, Fe, Ni, Zn, Ge, Sr, Pd, Cd, Sb, Re, and Tl. This factor included the elements with economic interest (Fe/Ni) and recognized toxics like As, Cd, Mn, Ta and Cr, and Se as potential chelator. This mixture is highly dangerous to the health of those who are exposed, as it was described previously [23]. FC2M included Dy, Tb, Y, Er, Tm, Lu, Sm, Yb, Ho, Gd, Eu, La, and Ce. This mixture included rare earth elements (REEs) related with modern products such as electronics, chemicals, medical, aviation and defense technologies, and more recently in agriculture [34]. Some studies suggest that REEs are associated with nephrotoxic, neurotoxic, and reproductive disorders, fibrotic tissue injury, oxidative stress, pneumoconiosis, and cytotoxicity [35]. Maybe REEs are consequence of the smelting processes in the ferronickel mine. Note that Be, Hg and Pb have not important participation in these mixtures.

In Nechí, FC1N included Dy, Er, Tm, Yb, Y, Lu, Tb, Eu, Ho, Gd, Sm, La, Pd, Be, and Co. This mixture has elements few frequent with Be and Co. FC2N in Nechí included Ru, Sr, Mg, Ca, Ba, Ga, Ni, Na, Pd, K, Zn, and Mn, being the more toxic Ni y Mn. FC3N in Nechi included Cd, Ti, As, Al, V, Li, Sb, Mo, Ge, Th, B, Cs, Cr, Mn, Rh, and Ag. This is the most toxic mixture in Nechí. Note that Hg, Se and Pb have not important participation in these mixtures.

FC1A in Aranzazu included Er, Y, Dy, Yb, Tb, V, Tm, Al, Sm, Lu, Cd, Fe, Be, La, Nb, Ti, and Ce. In this mixture the most toxic elements were Cd and Be. FC2A in Aranzazu included Mg, Ru, Sr, Na, K, Ba, Ga, Ca, Rb, Gd, Eu, Ge, Cs, Zr, and Pd. This element mixture only included Mg as recognized toxic. Note that Hg, Se, As, Cr, Mn, P and Pb have not important participation in these mixtures.

3.3. Micronuclei frequency

The distribution of micronuclei observed in study participants by municipality, and in total, is shown in Figure 1. As can be seen, in Montelibano the median was 4 micronuclei with 25th and 75th percentiles of 2 and 6.5; in Nechí the median was 5 micronuclei with 25th and 75th percentiles of 0 and 9, and in Aranzazu the median was 7 micronuclei with 25th and 75th percentiles of 2 and 10. However, there were no statistically significant differences between the three mining contexts (p= 0.1298, Kruskal–Wallis test).

3.4. Bivariate analysis

A first bivariate exploration (Table 3) of the possible associations with the number of micronuclei allowed us to identify that being a woman is protective in Montelibano, suggesting a differential occupational effect compared to men. Higher educational levels were protective, with their most noticeable effect being in Montelibano. Food consumption seems to mark routes of intake of toxic elements; since the consumption of fish, white meat and vegetables were risk factors in Montelibano, while the consumption of fruits and vegetables were protective in Nechí and Aranzazu, respectively. In relation to the mixtures of elements in hair, it was striking that no mixture showed an association in Montelibano, while in Nechí a mixture (FC1N) with a protective effect was observed, and in Aranzazu there was a mixture (FC1A) that was a risk factor. Mercury in hair was a risk factor in Aranzazu, which was where the lowest concentration was observed among its inhabitants. Contrary to expectations, selenium in hair was a risk factor in Nechí, where the highest values of selenium were. Something similar happened with lead in hair, which was a protective factor in Nechí. Another unexpected finding is that beryllium in hair was a protective factor in Montelibano.

Table 3. Crude prevalence ratios (PR) between pesticide and element exposure and micronuclei occurrence.

Site	**Montelibano (n=76)**		**Nechí (n=84)**		**Aranzazu (n=87)**	
(Type of mine)	**Fe/Ni**		**Au**		Closed Hg	
Variables	**PR**	**95% CI**	**PR**	**95% CI**	**PR**	**95% CI**
Sex (female)	0.68	0.47 – 0.99	0.82	0.56 – 1.21	0.86	0.63 – 1.17
Age (years)	1.01	0.99 – 1.02	1.00	0.99 – 1.01	1.00	0.99 – 1.01

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Civil status						
Married / Free union	1		1		1	
Divorced / Widower	1.26	0.51 – 3.11	1.31	0.64 – 2.68	1.14	0.68 – 1.91
Single	1.17	0.68 – 2.02	1.26	0.85 -1.87	1.01	0.70 – 1.45
Education						
Illiteracy	1		1		1	
Elementary (partial or full)	0.38	0.24 – 0.60	1.02	0.56 – 1.86	0.63	0.42 – 0.94
Secondary (partial or full)	0.48	0.34 – 0.66	0.85	0.46 -1.59	0.67	0.43 – 1.03
Technic (partial or full)	0.41	0.31 – 0.53	1.00	0.53 – 1.89	0.78	0.40 – 1.54
University (partial or full)	0.45	0.31 – 0.64	1.29	0.65 – 2.55	0.78	0.50 – 1.22
Occupation						
In mining activities	0.90	0.66 – 1.23	1.12	0.77 – 1.64	NA	
In agricultural activities	1.13	0.71 – 1.79	0.58	0.33 – 1.02	0.99	0.72 – 1.35
Consumption (any moment)						
Cigarette	1.33	0.94 – 1.87	0.88	0.58 – 1.33	1.15	0.84 – 1.57
Alcohol	0.81	0.59 – 1.12	1.18	0.81 – 1.71	0.86	0.62 – 1.17
Food consumption						
Fish	2.27	1.11 – 4.64	1.74	0.53 – 5.74	1.33	0.91 – 1.97
Canned food	1.14	0.84 – 1.54	1.44	0.99 – 2.08	1.09	0.78 – 1.54
White meat	1.29	1.00 – 1.66	1.93	0.80 – 4.65	1.05	0.67 – 1.64
Red meat	0.89	0.62 – 1.26	1.34	0.45 – 4.01	0.84	0.35 – 2.04
Fruits	1.12	0.38 – 3.30	0.48	0.32 – 0.71	0.73	0.52 – 1.01
Vegetables	1.50	1.28 – 1.75	0.52	0.19 – 1.39	0.75	0.58 – 0.96
Pesticides (ppb)						
Paraoxon-methyl in blood	1.00	0.99 – 1.01				
Paraoxon-ethyl in blood					18.43	0.71 – 476.02
Element mixtures in hair						
FC1	0.97	0.94 – 1.00	0.90	0.83 – 0.98	1.04	1.00 – 1.07
FC2	0.98	0.94 – 1.03	1.04	0.99 – 1.09	1.02	0.99 – 1.05
FC3	NA		0.96	0.90 – 1.03	NA	
Selected elements in hair						
Mercury	0.97	0.93 – 1.02	0.98	0.92 – 1.03	1.76	1.06 – 2.93
Selenium	NA		1.02	1.01 – 1.02	0.58	0.25 – 1.34
Lead	1.01	1.00 – 1.01	0.94	0.89 – 0.98	1.00	0.99 – 1.00
Berylium	0.77	0.66 – 0.89	NA		NA	

3.5. Multiple analysis

Table 4 shows the most relevant variables associated with the number of micronuclei, obtained in the multiple regression models. In Montelibano it turned out that the consumption of fish and lead in hair were risk factors; as well as, the pesticides parathion ethyl in blood and paraoxon ethyl in urine. The mixture of FC1M elements, beryllium in hair and the consumption of vegetables and red meat were protective factors. Due to its importance, it should be clear that selenium is part of the FC1M mix.

In Nechí, the FC2N mixture (which includes Mg, Mn and Ni), lead and selenium were identified as risk factors. FC1N (with beryllium and very rare elements) and fruit consumption appeared as protective factors. Additionally, an interaction between lead and selenium was identified. In Aranzazu, the main risk factor is mercury, followed by nickel. Selenium and fruit consumption were identified as a protective factor. A strong interaction between mercury and selenium was identified.

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Table 4. Adjusted prevalence ratios (adj. PR) between pesticide and element exposure and micronuclei occurrence.

Site (Type of mine)	**Montelibano (n=76) (Open-pit Fe/Ni)**		**Nechí (n=84) (Fluvial Au)**		**Aranzazu (n=87)** (Closed Hg)	
Variables	**Adj. PR**	**95% CI**	**Adj. PR**	**95% CI**	**Adj. PR**	**95% CI**
Pesticides (ppb)						
Parathion ethyl (blood)	0.04	0.01 – 0.14				
Paraoxon ethyl (urine)	0.01	0.00 – 0.04				
Element mixtures in hair (scores)						
Factor 1	0.97	0.94 – 0.99	0.88	0.79 – 0.98		
Factor 2			1.05	1.00 – 1.09		
Hair concentration (ppm)						
Beryllium	0.67	0.59 – 0.76				
Lead	1.01	1.00 – 1.01	1.37	1.06 – 1.77		
Mercury					36.02	2.69 – 380.55
Selenium			1.05	1.03 – 1.08	0.86	0.32 – 2.33
Nickel					1.28	1.13 – 1.45
Interactions						
Selenium*Lead			0.78	0.64 – 0.95		
Selenium*Mercury					0.11	0.02 – 0.77
Diet consumption						
Fish	4.73	3.94 – 5.68				
Vegetables	0.47	0.38 – 0.59				
Red meat	0.67	0.54 – 0.83				
Fruits			0.55	0.38 – 0.80	0.66	0.46 – 0.95

These findings may seem strange at first approximation but can be better understood by considering the different exposure pathways involved in each type of mine. In Montelibano the exposure can be food and aerial, while in Nechí it is mainly food in the context of occupational and para-occupational aerial exposure to mercury. In Aranzazu the exposure is mainly for food.

4. Discussion

The most important finding of this study is the great heterogeneity in the exposure to pesticides and chemical elements in the three contexts that combine mining and agriculture. Within the complexity it was clear that where there is recent mining activity is where more pesticides and chemical elements were detected, and higher concentrations were observed. This is logical since mining mobilizes large amounts of rocks and soils, facilitating the entry of various elements to humans; these mainly through food and water, although the respiratory tract is likely in places with open pit mining. In fact, chemical mixtures were associated with the number of micronuclei only in Montelibano and Nechí, where there are active mines. In Aranzazu the associations are between isolated elements or in interaction with another element.

In addition to this expected finding, selenium showed two different faces. On the one hand, selenium acted as a protective factor and on the other, as a risk factor framed in the context of statistical interaction. This seems to be strong evidence of the "hormetic response"[36], in which selenium at low doses is beneficial (Aranzazu case) or acts synergistically with other chemicals (Nechí case) when it is in high concentrations, increasing the adverse effect. In the case of Montelibano, the presence of selenium in a mixture with many toxic elements seems to be related to the mitigation of the adverse effect. Note that the observed concentrations of selenium in hair in Montelibano were intermediate between Nechí and Aranzazu. To the best of our knowledge, this is the first time that the "hormetic effect" of selenium in humans is reported under non-experimental conditions.

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The findings related to selenium may be studied in future studies. It is important to identify the concentrations at which selenium acts as a protective factor; when it changes to act as a risk factor for the occurrence of different outcomes. Since Colombia has regions with some of the highest underground selenium concentrations in the world [16], its presence may explain the contradiction between the highest global exposure to mercury [21] and the low occurrence of diseases among exposed individuals.

In Aranzazu there is a health issue that must be pointed out due to the possible implications it has on the results, and that unfortunately could not be addressed in the present study. Among the inhabitants of Aranzazu, endogamy has been common for several generations; in fact, the occurrence of neuropsychiatric disorders, such as mood bipolar disorder, is so high that studies have been carried out that report its association with the genetic factor. In this case, the question arises as to whether these genetic changes have an influence on the number of micronuclei observed, like that of the other mining sites.

This study should be interpreted considering the limitations of the study. First, the population was not representative, so the findings should be understood as exploratory of important environmental health problems. Interest in the study was the main motivation of the participants, which explains the greater participation of women; men tend to be more cautious in places where there are illegal armed groups, as occurred in Montelibano and Nechí. Colombia is one of the countries with more socio-ecological conflicts [37], and where more assassinations of environmental leaders occur [38], which is evidence of the difficulties of carrying out environmental health studies in places with the greatest contamination.

Perhaps no pesticides were found because it is easier to find them in those who directly carry out fumigation activities, which by habit is an activity associated with men. Given that the miners could be the ones most exposed to chemical elements, it is possible that their low participation has the effect that their results are higher than reported. An important issue to note is that, due to the high number of chemical elements included, several of these do not have certified standards. However, due to the exploratory and novel nature of the study approach, it was decided to include all the results. If there was a measurement error, we have no evidence that it was differential.

In conclusion, the results show the complex exposures that occur in mining contexts where there is also agricultural activity. Each place is so different from the others that it is difficult to extrapolate the findings to other similar areas[39]. In the Colombian case, it is a difficult task because the data on the use of pesticides do not have adequate information; it is even more complicated in places where illegal activities are carried out, such as coca, marijuana, or poppy cultivation [14]. In addition, soil geochemistry data is scarce, and much of what exists is only available to mining companies that collect data as part of their economic activities. For this reason, studies such as this one are essential to understand the effects of large extractive activities, especially if they were carried out before and after starting work[40].

Additionally, this study identified the region where the open pit ferronickel mine is located as the most polluted, and which demands urgent attention from the environmental and health authorities. Since this may be happening at other large open pit mines, it is important to perform similar studies in regions with coal in La Guajira [41] and Cesar departments [42], and gold mining in La Colosa in Cajamarca, Tolima [43]. Periodic monitoring of environmental agents, mainly in the most polluted sites, is essential to have ~~an~~ adequate vigilance of the potential adverse effects associated with chemical agents. Without a doubt, it is very important that Colombian authorities prioritize toxic chemicals; because there is an exaggerated use of pesticides, and many extractive activities with a high impact on the environment.

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Appendix A

Main chemical element mixtures in each mining site obtained with principal component analysis are in Figures A1, A2, and A3.

References

1. Kaur, K.; Kaur, R. Occupational Pesticide Exposure, Impaired DNA Repair, and Diseases. Indian J Occup Environ Med 2018, 22, 74, doi:10.4103/ijoem.IJOEM_45_18.
2. Stanaway, J.D.; Afshin, A.; Gakidou, E.; Lim, S.S.; Abate, D.; Abate, K.H.; Abbafati, C.; Abbasi, N.; Abbastabar, H.; Abd-Allah, F.; et al. Global, Regional, and National Comparative Risk Assessment of 84 Behavioural, Environmental and Occupational, and Metabolic Risks or Clusters of Risks for 195 Countries and Territories, 1990–2017: A Systematic Analysis for the Global Burden of Disease Study 2017. The Lancet 2018, 392, 1923–1994, doi:10.1016/S0140-6736(18)32225-6.
3. Damalas, C.; Koutroubas, S. Farmers' Exposure to Pesticides: Toxicity Types and Ways of Prevention. Toxics 2016, 4, 1, doi:10.3390/toxics4010001.
4. Kim, K.-H.; Kabir, E.; Jahan, S.A. Exposure to Pesticides and the Associated Human Health Effects. Sci Total Environ 2017, 575, 525–535, doi:10.1016/j.scitotenv.2016.09.009.
5. Rani, L.; Thapa, K.; Kanojia, N.; Sharma, N.; Singh, S.; Grewal, A.S.; Srivastav, A.L.; Kaushal, J. An Extensive Review on the Consequences of Chemical Pesticides on Human Health and Environment. Journal of Cleaner Production 2021, 283, 124657, doi:10.1016/j.jclepro.2020.124657.
6. Cotton, J.; Edwards, J.; Rahman, M.A.; Brumby, S. Cholinesterase Research Outreach Project (CROP): Point of Care Cholinesterase Measurement in an Australian Agricultural Community. Environ Health 2018, 17, 31, doi:10.1186/s12940-018-0374-1.
7. Imran, I.; Ansari, A.; Saleem, S.; Azhar, A.; Zehra, S. Insights of OPs and PYR Cytotoxic Potential Invitro and Genotoxic Impact on PON1 Genetic Variant among Exposed Workers in Pakistan. Sci Rep 2022, 12, 9498, doi:10.1038/s41598-022-13454-0.
8. Marcelino, A.F.; Wachtel, C.C.; Ghisi, N. de C. Are Our Farm Workers in Danger? Genetic Damage in Farmers Exposed to Pesticides. Int J Environ Res Public Health 2019, 16, 358, doi:10.3390/ijerph16030358.

9. Mostafalou, S.; Abdollahi, M. Pesticides: An Update of Human Exposure and Toxicity. Arch Toxicol 2017, 91, 549–599, doi:10.1007/s00204-016-1849-x.
10. Ofosu, G.; Dittmann, A.; Sarpong, D.; Botchie, D. Socio-Economic and Environmental Implications of Artisanal and Small-Scale Mining (ASM) on Agriculture and Livelihoods. Environmental Science & Policy 2020, 106, 210–220, doi:10.1016/j.envsci.2020.02.005.
11. Valbuena, D.; Cely-Santos, M.; Obregón, D. Agrochemical Pesticide Production, Trade, and Hazard: Narrowing the Information Gap in Colombia. Journal of Environmental Management 2021, 286, 112141, doi:10.1016/j.jenvman.2021.112141.
12. Castellanos, A.; Chaparro-Narváez, P.; Morales-Plaza, C.D.; Alzate, A.; Padilla, J.; Arévalo, M.; Herrera, S. Malaria in Gold-Mining Areas in Colombia. Mem Inst Oswaldo Cruz 2016, 111, 59–66, doi:10.1590/0074-02760150382.
13. Zaragoza-Bastida, A.; Valladares-Carranza, B.; Ortega-Santana, C.; Zamora-Espinosa, J.; Velázquez-Ordoñez, V.; Aparicio-Burgos, J. Repercusiones del uso de los organoclorados sobre el ambiente y salud pública. AbanicoVet 2016, 6, 43–55.
14. Varona, M.E.; Díaz-Criollo, S.M.; Lancheros-Bernal, A.R.; Murcia-Orjuela, A.M.; Henao-Londoño, G.L.; Idrovo, A.J. Organochlorine Pesticide Exposure among Agricultural Workers in Colombian Regions with Illegal Crops: An Exploration in a Hidden and Dangerous World. Int J Environ Health Res 2010, 20, 407–414, doi:10.1080/09603123.2010.491855.
15. García Ubaque, C.A.; García Ubaque, J.C.; Vaca Bohórquez, M.L. Compuestos orgánicos persistentes en Colombia: cuantificacióny diagnóstico para pesticidas organoclorados. RT 2015, 19, 163, doi:10.14483/udistrital.jour.tecnura.2015.1.a11.
16. Ancizar-Sordo, J. Occurrence of Selenium in Soils and Plants of Colombia, South America: Soil Science 1947, 63, 437–438, doi:10.1097/00010694-194706000-00002.
17. Spiller, H.A.; Hays, H.L.; Casavant, M.J. Rethinking Treatment of Mercury Poisoning: The Roles of Selenium, Acetylcysteine, and Thiol Chelators in the Treatment of Mercury Poisoning: A Narrative Review. Toxicology Communications 2021, 5, 19–59, doi:10.1080/24734306.2020.1870077.
18. Rodriguez-Villamizar, L.A.; Medina, O.M.; Flórez-Vargas, O.; Vilanova, E.; Idrovo, A.J.; Araque-Rodriguez, S.A.; Henao, J.A.; Sánchez-Rodríguez, L.H. Chemical Element Mixtures and Kidney Function in Mining and Non-Mining Settings in Northern Colombia. Int J Environ Res Public Health 2023, 20, 2321, doi:10.3390/ijerph20032321.
19. Braun, J.M.; Gennings, C.; Hauser, R.; Webster, T.F. What Can Epidemiological Studies Tell Us about the Impact of Chemical Mixtures on Human Health? Environmental Health Perspectives 2016, 124, A6–A9, doi:10.1289/ehp.1510569.
20. Martin-Reina, J.; Casanova, A.G.; Dahiri, B.; Fernández, I.; Fernández-Palacín, A.; Bautista, J.; Morales, A.I.; Moreno, I. Adverse Health Effects in Women Farmers Indirectly Exposed to Pesticides. Int J Environ Res Public Health 2021, 18, 5909, doi:10.3390/ijerph18115909.
21. Cordy, P.; Veiga, M.M.; Salih, I.; Al-Saadi, S.; Console, S.; Garcia, O.; Mesa, L.A.; Velásquez-López, P.C.; Roeser, M. Mercury Contamination from Artisanal Gold Mining in Antioquia, Colombia: The World's Highest per Capita Mercury Pollution. Science of The Total Environment 2011, 410–411, 154–160, doi:10.1016/j.scitotenv.2011.09.006.
22. Idrovo, A.J.; Rivero-Rubio, C.; Amaya-Castellanos, C.; Idrovo, A.J.; Rivero-Rubio, C.; Amaya-Castellanos, C. Perception of Pollution and Arsenic in Hair of Indigenous Living near a Ferronickel Open-Pit Mine

(Córdoba, Colombia): Public Health Case Report. Revista de la Universidad Industrial de Santander. Salud 2017, 49, 115–123, doi:10.18273/revsal.v49n1-2017011.

23. Idrovo, A.J. Cerro Matoso Mine, Chemical Mixtures, and Environmental Justice in Colombia. The Lancet 2018, 391, 2320, doi:10.1016/S0140-6736(18)30855-9.
24. Fernández-Niño, J.A.; Idrovo, A.J.; Cucunubá, Z.M.; Reyes-Harker, P.; Guerra, Á.P.; Moncada, L.I.; López, M.C.; Barrera, S.M.; Cortés, L.J.; Olivera, M.; et al. Paradoxical Associations between Soil-Transmitted Helminths and Plasmodium Falciparum Infection. Trans R Soc Trop Med Hyg 2012, 106, 701–708, doi:10.1016/j.trstmh.2012.07.012.
25. Bonilla Ayala, S.; Rojas, A.; Rojas Velandia, C.; Figueroa Salamanca, H.; Idrovo, A. Dimercaprol and Penicillamine in Occupational Hydrargyrism in Aranzazu: Birth of Occupational Toxicology in Colombia (Submitted).
26. Word Health Organization Guidance for Identifying Populations at Risk from Mercury Exposure Available online: https://www.who.int/publications/m/item/guidance-for-identifying-populations-at-risk-from-mercury-exposure (accessed on 28 June 2023).
27. Shin, Y.; Lee, J.; Park, E.; Lee, J.; Lee, H.S.; Kim, J.-H. A Quantitative Tandem Mass Spectrometry and Scaled-Down QuEChERS Approach for Simultaneous Analysis of Pesticide Multiresidues in Human Urine. Molecules 2019, 24, 1330, doi:10.3390/molecules24071330.
28. Kales, S.; Christiani, D. Hair and Metal Toxicity. In Hair in Toxicology; Tobin, D.J., Ed.; The Royal Society of Chemistry, 2005; pp. 125–158 ISBN 978-0-85404-587-7.
29. Nunes, J.A.; Batista, B.L.; Rodrigues, J.L.; Caldas, N.M.; Neto, J.A.G.; Barbosa, F. A Simple Method Based on ICP-MS for Estimation of Background Levels of Arsenic, Cadmium, Copper, Manganese, Nickel, Lead, and Selenium in Blood of the Brazilian Population. J Toxicol Environ Health A 2010, 73, 878–887, doi:10.1080/15287391003744807.
30. Fenech, M. Cytokinesis-Block Micronucleus Cytome Assay. Nat Protoc 2007, 2, 1084–1104, doi:10.1038/nprot.2007.77.
31. Widaman, K.F. Common Factor Analysis Versus Principal Component Analysis: Differential Bias in Representing Model Parameters? Multivariate Behav Res 1993, 28, 263–311, doi:10.1207/s15327906mbr2803_1.
32. Barros, A.J.D.; Hirakata, V.N. Alternatives for Logistic Regression in Cross-Sectional Studies: An Empirical Comparison of Models That Directly Estimate the Prevalence Ratio. BMC Med Res Methodol 2003, 3, 21, doi:10.1186/1471-2288-3-21.
33. Stafoggia, M.; Breitner, S.; Hampel, R.; Basagaña, X. Statistical Approaches to Address Multi-Pollutant Mixtures and Multiple Exposures: The State of the Science. Curr Environ Health Rep 2017, 4, 481–490, doi:10.1007/s40572-017-0162-z.
34. Ramos, S.J.; Dinali, G.S.; Oliveira, C.; Martins, G.C.; Moreira, C.G.; Siqueira, J.O.; Guilherme, L.R.G. Rare Earth Elements in the Soil Environment. Curr Pollution Rep 2016, 2, 28–50, doi:10.1007/s40726-016-0026-4.
35. Gwenzi, W.; Mangori, L.; Danha, C.; Chaukura, N.; Dunjana, N.; Sanganyado, E. Sources, Behaviour, and Environmental and Human Health Risks of High-Technology Rare Earth Elements as Emerging Contaminants. Science of The Total Environment 2018, 636, 299–313, doi:10.1016/j.scitotenv.2018.04.235.
36. Calabrese, E.J.; Baldwin, L.A. Inorganics and Hormesis. Critical Reviews in Toxicology 2003, 33, 215–304, doi:10.1080/713611040.
37. Pérez-Rincón, M.P.; Peralta-Ardila, M.; Vélez-Torres, I.; Méndez, F. Conflicto Armado Interno y Ambiente en Colombia: Análisis desde los Conflictos Ecológicos, 1960-2016. J Pol Ecol 2022, 29, 672-703.

38. Albarracín, J.; Milanese, J.P.; Valencia, I.H.; Wolff, J. Local Competitive Authoritarianism and Post-Conflict Violence. An Analysis of the Assassination of Social Leaders in Colombia. Int Interact 2022, 49, 237-267.
39. Bedoya, G.; García, J.; Montoya, P.; Rojas, W.; Amézquita, M.E.; Soto, I.; López, M.C.; Ospina-Duque, J.; Ruiz-Linares, A. Análisis de isonimia entre poblaciones del noroeste de Colombia. Biomedica 2006, 26, 538, doi:10.7705/biomedica.v26i4.323.
40. Bedoya P, S.; García R, A.; Londoño F, Á.L.; Restrepo C, B. Determinación de residuos de plaguicidas organoclorados en suero sanguíneo de trabajadores de cultivo de Café y plátano en el departamento del quindío por gc-μecd. Revista Colombiana de Química 2014, 43, 11–16.
41. Espitia-Pérez, L.; Sosa, M.Q.; Salcedo-Arteaga, S.; León-Mejía, G.; Hoyos-Giraldo, L.S.; Brango, H.; Kvitko, K.; da Silva, J.; Henriques, J.A.P. Polymorphisms in Metabolism and Repair Genes Affects DNA Damage Caused by Open-Cast Coal Mining Exposure. Mutat Res Genet Toxicol Environ Mutagen 2016, 808, 38–51, doi:10.1016/j.mrgentox.2016.08.003.
42. Guerrero-Castilla, A.; Olivero-Verbel, J.; Marrugo-Negrete, J. Heavy Metals in Wild House Mice from Coal-Mining Areas of Colombia and Expression of Genes Related to Oxidative Stress, DNA Damage and Exposure to Metals. Mutat Res Genet Toxicol Environ Mutagen 2014, 762, 24–29, doi:10.1016/j.mrgentox.2013.12.005.
43. Vélez-Torres, I.; Vanegas, D. Contentious Environmental Governance in Polluted Gold Mining Geographies: The Case of La Toma, Colombia. World Development 2022, 157, 105953, doi:10.1016/j.worlddev.2022.105953.