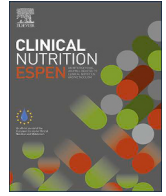




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Original article

Serum reference intervals for essential and toxic trace elements in a healthy population: A basis for nutritional monitoring and clinical intervention

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SUMMARY

Background & aims: Trace elements play a crucial role in human metabolism, and both deficiencies and toxic exposures can have significant clinical implications. This study aimed to establish clinically relevant reference intervals for essential and toxic trace elements in serum from a healthy adult population to support nutritional assessment and public health monitoring.

Methods: A cross-sectional study was conducted in 501 healthy adults. Serum concentrations of essential [cobalt (Co), copper (Cu), iron (Fe), iodine (I), manganese (Mn), molybdenum (Mo), selenium (Se), zinc (Zn)] and toxic [(arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg), nickel (Ni)] trace elements were measured using inductively coupled plasma mass spectrometry (ICP-MS). Reference intervals (2.5th–97.5th percentiles) were calculated following CLSI guidelines, and stratified analyses were performed based on sex and age.

Results: Reference intervals were established for each element, revealing significant sex-related differences. Women exhibited higher serum concentrations of Co, Cu, Mo, Cd, and Hg, and lower Fe compared to men. Age-related differences were also observed for several elements, with distinct sex-specific trends particularly for Cu and Hg. While serum levels of most essential elements were within expected physiological ranges, Se concentrations were suboptimal in a considerable proportion of the population, suggesting potential nutritional inadequacy. Toxic element levels remained generally low, indicative of minimal environmental exposure.

Conclusions: Essential trace element concentrations were generally adequate within the Northern Spain population, with the notable exception of Se, where a significant proportion exhibits low levels, suggesting a potential benefit from dietary supplementation or intervention. Toxic element concentrations were low corresponding to a basal level of environmental exposure; however, observed variations by age and sex highlight the importance of ongoing monitoring.

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1. Introduction

Trace elements, or microminerals, are essential for life, despite the minimal concentrations at which they are present in the body [1]. They perform critical roles in regulating metabolic pathways

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and maintaining homeostasis. Deficiencies in trace elements can lead to weakened immune defence, increased oxidative stress and accelerated cellular aging. Conversely, excessive concentrations of trace elements can result in toxicity, disrupt physiological processes and interfere with the bioavailability of other elements, as observed with toxic elements such as lead (Pb) and mercury (Hg) [2].

Dietary intake is the primary route through which both essential and toxic trace elements enter the body under normal environmental conditions [3]. The amounts of trace elements ingested are strongly influenced by geographical factors [4], as well as other cultural and socioeconomic factors. Regions characterized by specific types of soil may have insufficient or excessive levels of certain elements, such as selenium (Se) and iodine (I). For example, Se-deficient soils can increase rates of immune dysfunction [5], whereas excessive dietary iodine may contribute to thyroid-related disorders [6]. These imbalances, even when subclinical, are recognized to contribute significantly to the development of chronic diseases, including cancer and diabetes, and neurodegenerative conditions such as Alzheimer's disease [7].

While the importance of trace elements in health is undeniable, knowledge about trace element status in human populations is limited [8], especially in comparison with the precise management observed in veterinary science. In animal husbandry, trace element intakes are meticulously adjusted to enhance immune function during critical periods such as weaning, vaccination and reproduction, leading to improved health outcomes, reduced antibiotic usage and enhanced reproductive success [9].

These practices underscore the potential benefits of improving trace element management in human nutrition, in which mineral status is often poorly understood, and there are significant gaps in our ability to design effective interventions [8]. Moreover, unlike the direct measurements used in veterinary science, determining trace element status in humans has traditionally relied on indirect methods such as dietary surveys and use of food composition databases. Although these approaches provide broad estimates, they often fail to capture individual variation due to factors such as age, health conditions and specific dietary habits, and overlook nutrient bioavailability, leading to inaccurate evaluations. Thus, there is an urgent need for direct measurements of trace element concentrations tailored to the unique characteristics of specific human populations and regions.

Compounding this challenge, modern dietary trends are reshaping the intake and absorption of trace elements in human diets. Although animal-based foods remain a primary source of many essential microminerals, the growing shift towards vegan and plant-based diets is raising concerns about potential deficiencies [10]. While these diets are rich in fibre and other beneficial compounds, they may also impair the absorption of critical nutrients. Changes in dietary patterns, such as increased consumption of fibre and the avoidance of animal products, could profoundly impact the micromineral status of populations in the long term [11].

Given the serious implications of trace element imbalances, determining the trace element status of populations is essential. This would enable identification of regional nutritional deficiencies or excesses, monitoring of lifestyle-associated changes in dietary intake, and the creation of targeted nutritional interventions where necessary. Such strategies are crucial for preventing disease and promoting optimal health across diverse populations [12].

Efforts to directly assess trace elements in human populations have begun to emerge in recent years, offering valuable insights and paving the way for establishing reference values for essential trace elements and limiting excessive exposure to toxic elements (e.g. [13–15]). However, comprehensive studies of the trace

element status of the population remain scarce in Spain. To the best of our knowledge, this is the first study to evaluate both essential and toxic trace element concentrations in blood serum in an adult study population in north-west Spain.

Various analytical techniques such as atomic absorption spectrometry (AAS) and inductively coupled plasma optical emission spectrometry (ICP-OES) are available for elemental analysis, although inductively coupled plasma mass spectrometry (ICP-MS) is widely preferred due to its superior sensitivity, low detection limits, and multielement capability, making it particularly suitable for clinical and nutritional studies [13]. Recent biomonitoring studies using ICP-MS have established reference values for trace elements in healthy populations in various European countries, including Switzerland, Slovenia and Serbia, confirming the utility of serum as a matrix and the clinical value of trace element profiling [14–16].

The primary objective of this study was to evaluate the trace and toxic element status in the study population by measuring the concentrations of various elements in blood serum and establishing reference ranges where applicable. This research aims to provide essential data to enable identification of potential deficient or excess levels of specific elements in different sectors of the population. The findings will guide the development of further studies to investigate risk factors (e.g. dietary patterns or lifestyle), examine the influence of trace and toxic element profiles on the onset of certain pathologies and implement targeted nutritional strategies to enhance public health.

2. Materials and methods

2.1. Ethical statement

This study forms part of the first phase of a broader research project aimed at investigating the role of microminerals in oncology patients in the province of Lugo (NW Spain). Ethical approval was granted by the Ethics Committee of Galicia, Spain (code 2022/034) and the study was conducted in accordance with the Declaration of Helsinki and Spanish legislation on clinical research. All data collected were treated confidentially, adhering to Spain's data protection regulations. Participants were verbally informed about the nature of the study, the use of biological samples, and the data obtained. Written informed consent was obtained from all participants.

2.2. Study population

The study participants ($n = 501$, 326 women and 174 men, with one case missing sex information), aged 18–79 years, were voluntarily recruited in the province of Lugo, Galicia, NW Spain. Participants were stratified by age into four groups: 18–33 years ($n = 127$, 81 female/46 male), 34–50 years ($n = 151$, 93/58), 51–65 years ($n = 158$, 108/50), and 66–79 years ($n = 64$, 44/20). The resulting sample distribution reflects typical patterns of voluntary participation in health-related studies, with higher enrolment among women.

Eligibility criteria required participants to be free of nutritional disorders and not have any active oncological conditions. Additionally, individuals were excluded if they presented chronic inflammatory, hepatic or renal diseases, recent infections, or if they were receiving pharmacological or nutritional treatments (including mineral supplementation) that could interfere with trace element metabolism. Only adults in apparent good health and without significant clinical conditions were included in the study.

Participants signed an informed consent form allowing access to their clinical records if needed and completed a comprehensive questionnaire covering epidemiological, lifestyle and nutritional information. Participants attended the Lucus Augusti University Hospital (HULA), where their blood pressure, weight, height and body composition measurements were recorded, and a blood sample was collected.

2.3. Sample collection and serum preparation

Blood samples were collected after overnight fasting, with 21G butterfly needles and pre-assembled vacuette holders in 6 mL BD Vacutainer™ blood collection tubes for trace element testing: serum clot activator. The samples were centrifuged immediately at 3,500 rpm for 5 min to separate the serum, which was then aliquoted into 1.5 mL portions and immediately stored at -80°C until analysis.

2.3.1. Trace element analysis

ICP-MS was selected due to its high sensitivity, low detection limits, multi-element capability and suitability for small sample volumes, which make it the method of choice for trace element biomonitoring in clinical and nutritional studies.

The serum samples were subjected to microwave-assisted acid digestion (ultraWAVE system, Milestone, Sorisole, Italy) at 240°C and 40–120 bar for 1 h prior to analysis. The individual samples (1 mL) were mixed with 0.75 mL of nitric acid (69 % TMA, Hiperpure, PanReac, Barcelona, Spain) and 0.75 mL of ultrapure water of resistance $18\text{M}\Omega\text{ cm}^{-1}$ (produced using a Milli-Q purification system, Millipore Corp., Bedford, MA). The resulting digests were transferred to polypropylene tubes and diluted with ultrapure water to a final volume of 5 mL. For iodine analysis, a high-temperature alkaline extraction procedure with tetramethylammonium hydroxide (TMAH, 25 % w/v, Sigma Aldrich, Merck, Darmstadt, Germany) was applied following the method described by Luna et al. [17].

The concentrations of fourteen elements were quantified, which include arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iodine (I), iron (Fe), manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), lead (Pb), selenium (Se) and zinc (Zn). Analyses were performed using ICP-MS (Agilent 7900 × ICP-MS system; Agilent Technologies, Tokyo, Japan) in the Research Infrastructures Unit of the University of Santiago de Compostela (Lugo, Spain). The sample introduction system included an autosampler, a dual-step spray chamber with a Peltier cooling system (Agilent Technologies, Tokyo, Japan), a low-flow concentric glass nebulizer (MicroMist, Glass Expansion, West Melbourne, Australia), and a quartz torch (Agilent Technologies, Tokyo, Japan). The operational parameters were as follows: plasma flow rate, 15 L/min; nebulizer flow rate, 1.1 L/min; sample depth, 8 mm; sample speed 0.1 rpm; plasma RF power, 1550 W; and spray chamber temperature, 2°C . Helium (4 L/min) or hydrogen (4.2 L/min) were used to correct for interference. In all cases, the calibration curves were linear, with correlation coefficients >0.999 . All samples were analyzed in triplicate.

2.3.2. Analytical quality control

Analytical blanks were included in all batches to enable calculation of detection limits (defined as three times the standard deviation of the blank divided by the calibration curve slope). The limits of quantification were sufficiently low to enable detection of all elements of interest. Method accuracy was assessed using certified reference materials for human serum (Seronorm™ Trace Elements Serum L-1) and samples spiked at concentrations ranging from 2 to 10 times the expected levels. Both intra-sample

precision (evaluated from ten replicates of the same sample) and inter-assay precision (assessed through ten independent sample preparations on different days) were measured. Results are summarized in Table 1. Precautionary measures aimed at avoiding contamination are applied during all procedural steps.

2.4. Data analysis

Statistical analysis was conducted in accordance with IFCC/CLSI guidelines [18] using IBM SPSS Statistics 27.0.1.0 (IBM Corporation) and MedCalc 11.4.2.0 (MedCalc Software Ltd). For statistical calculations, $\text{LoD}/2$ was assigned for values below the detection limit (LoD). Reference values for each trace element in serum were established by non-parametric methods. Percentiles P2.5 and P97.5 and the corresponding 90 % confidence intervals (CIs) were used to determine lower (LRL) and upper (URL) reference levels. Outliers were excluded using Tukey's method, but only after visual inspection, in compliance with CLSI recommendations for verifying reference intervals and proper handling of outlier data.

The influence of gender (men and women) and age (subjects were divided into groups of age 18–33, 34–50, 51–65 and 66–90 years) on the concentrations of essential trace and toxic elements was evaluated by two-way ANOVA, with gender and age as the main factors. The analysis also explored the interaction between these factors. As the data not fulfil the normality assumptions required for ANOVA, a logarithmic transformation was applied before the analysis. Although the distribution of sexes in our sample was not balanced (326 women and 174 men), the sample size was sufficient to ensure adequate statistical power. Therefore, following CLSI guidelines (EP28-A3c), sex-specific reference intervals were calculated and reported when appropriate.

3. Results

Results of the statistical descriptive analysis of essential trace and toxic element concentrations in the study population in north-west Spain are presented in Table 2. Data are reported as geometric means and medians, with dispersion described by interquartile ranges (P25 and P75) and P5 and P95. Percentiles P2.5 (90 % CI) and P97.5 (90 % CI) were used to establish lower reference levels (LRL) and upper reference levels (URL) for essential trace elements, while only the URL was considered relevant for toxic elements. Gender-specific values are only reported when statistically significant differences between sexes were detected (Table 3). Overall, women exhibited significantly higher serum concentrations of Co ($p = 0.003$), Cu ($p < 0.001$), Mo ($p = 0.006$), Cd ($p < 0.001$) and Hg ($p = 0.016$) and significantly lower serum concentrations of Fe ($p = 0.052$) than men.

The reference limits for essential trace elements were generally within the clinically accepted adequate ranges for Cu (female: 770–2060 $\mu\text{g/L}$, male: 730–1290 $\mu\text{g/L}$), Fe (female: 350–1350 $\mu\text{g/L}$, male: 500–1500 $\mu\text{g/L}$), I (40–92 $\mu\text{g/L}$), Mn (0.5–1.2 $\mu\text{g/L}$), Mo (0.3–2.0 $\mu\text{g/L}$), and Zn (600–1060 $\mu\text{g/L}$) [19]. However, serum Se concentrations in the study population were notably low, with 54.8 % of participants displaying levels below the adequate reference ranges (60 $\mu\text{g/L}$) and 96.0 % below the threshold associated with maximum selenoprotein P expression (90 $\mu\text{g/L}$) [20] (Fig. 1). For other elements for which deficiency reference ranges have not been established, concentrations in the study population were consistent with values obtained in recent similar epidemiological studies involving healthy individuals (Table 4). Toxic element concentrations were generally very low and consistent with levels reported in recent studies of populations of non-contaminated areas and populations not occupationally exposed.

Table 1

Results of the analytical quality programme used in the determination by ICP-MS of the essential trace and toxic elements in serum in the present study.

LoD ($\mu\text{g/L}$)	Seronom TM Trace Elements Serum L-1			Spiked samples				Precision (%CV)	
	Certified (mean \pm SD) ($\mu\text{g/L}$)	Analyzed (mean \pm SD) ($\mu\text{g/L}$)	% Recovery (mean \pm SD)	Unspiked (mean \pm SD) ($\mu\text{g/L}$)	Spike ($\mu\text{g/L}$)	Spiked (mean \pm SD) ($\mu\text{g/L}$)	% Recovery (mean \pm SD)	intrasample	intraassay
As	0.001 (0.2)	0.183 \pm 0.009	91.3 \pm 4.4	0.971 \pm 0.022	5	6.32 \pm 0.20	107 \pm 4	3.01	8.88
Cd	0.001 (0.089)	0.079 \pm 0.007	89.0 \pm 7.7	0.091 \pm 0.007	0.5	0.596 \pm 0.021	101 \pm 4	7.44	11.21
Co	0.002 (1.04 \pm 0.21)	0.969 \pm 0.207	93.2 \pm 6.4	0.356 \pm 0.041	5	5.31 \pm 0.21	99 \pm 4	4.33	7.89
Cr	0.007 (3.02 \pm 0.61)	2.85 \pm 0.60	94.4 \pm 5.1	0.845 \pm 0.045	5	6.20 \pm 0.35	107 \pm 7	2.11	7.11
Cu	0.010 (1050 \pm 211)	1034 \pm 215	98.5 \pm 5.3	1089 \pm 83	5000	5989 \pm 408	98 \pm 8	4.11	5.98
Fe	0.064 (1370 \pm 280)	1480 \pm 339	108 \pm 11	1230 \pm 74	5000	6480 \pm 124	105 \pm 2	2.86	6.52
Hg	0.005 (1.05 \pm 0.32)	0.936 \pm 0.292	89.1 \pm 5.7	0.701 \pm 0.078	5	5.40 \pm 0.31	94 \pm 6	3.11	7.11
I	0.014 (70)	68.7 \pm 5.2	98.1 \pm 7.4	86.8 \pm 11.4	500	612 \pm 42	105 \pm 8	2.44	5.87
Mn	0.029 (10.8 \pm 2.0)	10.2 \pm 2.0	94.3 \pm 4.9	1.21 \pm 0.07	5	6.06 \pm 0.17	97 \pm 3	3.81	8.81
Mo	0.011 (0.67)	0.647 \pm 0.015	96.6 \pm 2.3	0.987 \pm 0.009	5	5.69 \pm 0.15	94 \pm 3	4.81	11.49
Ni	0.009 (5.28 \pm 1.06)	5.60 \pm 1.32	106 \pm 13	0.908 \pm 0.017	5	6.31 \pm 0.25	108 \pm 5	4.11	11.02
Pb	0.006 (0.76)	0.821 \pm 0.122	108 \pm 16	0.666 \pm 0.047	5	5.87 \pm 0.35	104 \pm 7	5.41	8.55
Se	0.013 (84 \pm 17)	88.2 \pm 18.1	105 \pm 3.6	70.5 \pm 8.2	500	611 \pm 26	108 \pm 5	3.77	7.52
Zn	0.088 (1390 \pm 280)	1346 \pm 287	96.8 \pm 6.6	1215 \pm 111	5000	6115 \pm 229	98 \pm 4	2.55	4.21

In brackets only indicative values.

Spiked samples: Recovery was tested by adding known amounts of elements to serum samples at 2 to 10 times the expected levels. Recovery was calculated by comparing the measured and added concentrations.

Table 2Descriptive statistics of essential trace and toxic element concentrations in serum samples (in $\mu\text{g/L}$) from the study population ($n = 501$). Data are reported as geometric mean (GM), median, interquartile range (P25–P75), and percentiles P2.5 and P97.5 with 90 % confidence intervals (CI). Reference intervals were calculated using non-parametric methods, following CLSI guidelines. Data are presented for the whole study population, except in those cases where statistically significant gender differences were observed (see Table 3).

	Participants	GM	Median	P25	P75	P5	P95	P2.5	CI 2.5 %	P97.5	CI 97.5 %
Co	All	0.324	0.325	0.215	0.503	0.136	0.840	0.105	(0.100–0.110)	0.881	(0.836–0.924)
	Female	0.359	0.365	0.235	0.545	0.154	0.865	0.125	(0.119–0.132)	0.895	(0.850–0.940)
	Male	0.274	0.255	0.191	0.405	0.107	0.688	0.093	(0.088–0.097)	0.782	(0.743–0.821)
Cu	All	1074	1051	899	1244	726	1604	684	(649–718)	1668	(1585–1752)
	Female	1153	1151	994	1316	759	1676	712	(676–747)	1714	(1628–1799)
	Male	940	932	836	1036	689	1306	637	(605–668)	1385	(1315–1454)
Fe	All	1191	1188	931	1543	627	2097	546	(519–574)	2177	(2068–2285)
	Female	1154	1132	910	1503	563	2072	521	(495–547)	2172	(2063–2280)
	Male	1261	1294	956	1625	713	2144	658	(625–691)	2209	(2099–2320)
I	All	82.8	82.6	71.3	94.7	59.5	120.0	55.1	(52.0–57.3)	128	(124–131)
Mn	All	1.410	1.440	0.975	1.925	0.684	3.063	0.622	(0.558–0.660)	3.560	(3.27–3.70)
Mo	All	0.877	0.913	0.681	1.160	0.438	1.586	0.378	(0.359–0.397)	1.670	(1.586–1.753)
Se	All	56.8	57.6	50.0	65.4	39.1	78.9	36.8	(35.9–37.7)	83.5	(80.2–85.9)
	Female	0.924	0.990	0.735	1.195	0.447	1.605	0.380	(0.361–0.399)	1.700	(1.615–1.785)
	Male	0.803	0.830	0.615	1.055	0.424	1.565	0.378	(0.359–0.397)	1.594	(1.514–1.673)
Zn	All	1138	1133	995	1309	831	1556	800	(775–827)	1625	(1575–1679)
As	All	1.747	1.825	0.946	3.593	0.375	6.540	0.288	(0.250–0.335)	7.666	(7.15–8.06)
	Female	0.048	0.073	0.010	0.168	0.002	0.491	0.003	(0.002–0.003)	0.546	(0.518–0.573)
	Male	0.059	0.080	0.035	0.255	0.002	0.524	0.003	(0.002–0.003)	0.570	(0.541–0.598)
Cr	All	0.027	0.055	0.003	0.105	0.002	0.417	0.003	(0.002–0.003)	0.482	(0.458–0.506)
	Female	0.821	0.840	0.520	1.645	0.130	3.365	0.042	(0.042–0.118)	3.840	(3.42–4.12)
	Male	0.682	0.775	0.501	1.239	0.160	1.982	0.056	(0.053–0.059)	2.227	(2.115–2.338)
Hg	All	0.710	0.775	0.525	1.240	0.195	2.045	0.115	(0.111–0.121)	2.305	(2.189–2.420)
	Female	0.638	0.775	0.435	1.235	0.107	1.864	0.056	(0.053–0.059)	2.064	(1.961–2.167)
	Male	0.895	0.960	0.625	1.375	0.298	2.082	0.198	(0.171–0.260)	2.417	(2.23–2.51)
Pb	All	0.328	0.340	0.214	0.531	0.094	1.117	0.069	(0.045–0.085)	1.239	(1.414–1.284)

GM: geometric mean; CI: confidence interval.

Age had a significant influence on the concentration of approximately half of the elements considered (Table 3). Except for Cu and Hg, for which significant sex-by-age interactions were observed, both genders showed similar age-related patterns of trace element concentrations. For the essential trace elements (Fig. 2), Se and Zn levels were higher in individuals aged 34–65 years but were significantly lower in the oldest age group. For Cu, age-related differences were observed only in men, with higher levels in individuals aged 34–65 years, mirroring the trends observed for Se and Zn. By contrast, I concentrations were higher in the youngest group (18–33 years) than in the group aged 51–65 years. Considering the toxic elements (Fig. 3), the As and Cd

concentrations increased with age in both genders. However, Cd levels were unexpectedly low in the oldest group. For Hg, age-related patterns differed by sex: thus, while no significant differences were observed across age groups in women, in men the pattern was similar to that observed for Cd, with a gradual increase in levels with age followed by a decline in the oldest group.

4. Discussion

The results of the present study provide new insights into the trace element status of a healthy adult population in north-west Spain. The discussion below explores the biological, clinical and

Table 3

Summary of the general linear model used to evaluate the influence of gender (male and female) and age (18–33, 34–50, 51–65, and 66–90 years old) as main factors in essential trace and toxic element concentrations in serum from the study population. Statistical analysis was performed using two-way ANOVA after logarithmic transformation of the data.

	Sex		Age		Sex*Age		R2
	F	P	F	P	F	P	
Co	$F_{1,491} = 8.935$	0.003	$F_{1,491} = 0.667$	0.573	$F_{1,491} = 3.578$	0.014	0.066
Cu	$F_{1,491} = 83.165$	<0.001	$F_{1,491} = 3.210$	0.023	$F_{1,491} = 3.124$	0.026	0.198
Fe	$F_{1,491} = 3.795$	0.052	$F_{1,491} = 0.693$	0.557	$F_{1,491} = 0.078$	0.972	0.013
I	$F_{1,491} = 1.233$	0.267	$F_{1,491} = 5.617$	<0.001	$F_{1,491} = 1.431$	0.233	0.039
Mn	$F_{1,491} = 0.017$	0.896	$F_{1,491} = 1.791$	0.148	$F_{1,491} = 2.503$	0.059	0.024
Mo	$F_{1,491} = 7.576$	0.006	$F_{1,491} = 0.286$	0.836	$F_{1,491} = 0.185$	0.907	0.020
Se	$F_{1,491} = 0.444$	0.505	$F_{1,491} = 6.198$	<0.001	$F_{1,491} = 1.863$	0.135	0.049
Zn	$F_{1,491} = 0.214$	0.644	$F_{1,491} = 3.112$	0.026	$F_{1,491} = 1.675$	0.171	0.032
As	$F_{1,491} = 0.017$	0.896	$F_{1,491} = 5.783$	<0.001	$F_{1,491} = 0.659$	0.578	0.036
Cd	$F_{1,491} = 11.300$	<0.001	$F_{1,491} = 6.145$	<0.001	$F_{1,491} = 0.836$	0.475	0.069
Cr	$F_{1,491} = 1.024$	0.312	$F_{1,491} = 0.803$	0.493	$F_{1,491} = 4.118$	0.007	0.030
Hg	$F_{1,491} = 5.883$	0.016	$F_{1,491} = 8.090$	<0.001	$F_{1,491} = 6.391$	<0.001	0.073
Ni	$F_{1,491} = 1.025$	0.312	$F_{1,491} = 1.142$	0.331	$F_{1,491} = 0.326$	0.806	0.018
Pb	$F_{1,491} = 0.818$	0.366	$F_{1,491} = 1.551$	0.201	$F_{1,491} = 1.519$	0.209	0.022

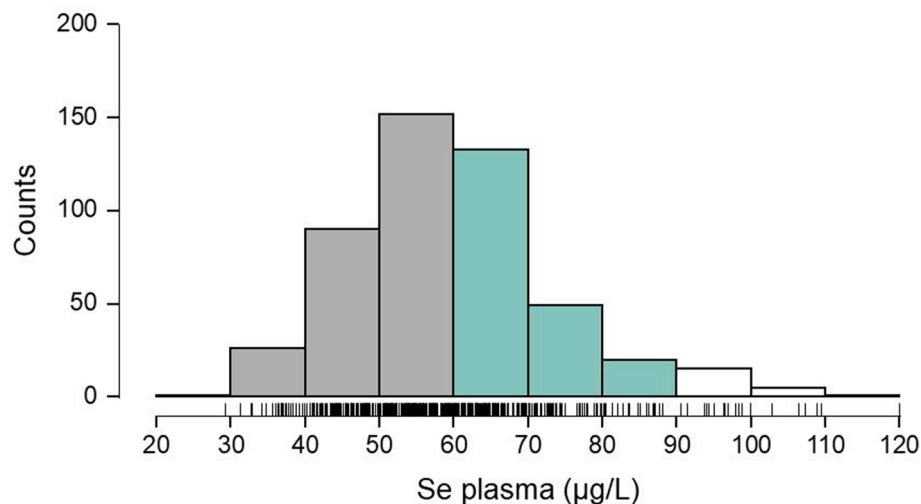


Fig. 1. Histogram of serum Se concentrations measured in the study population. Grey bars represent individuals with Se concentrations below adequate levels (60 µg/L), while green bars represent individuals with Se concentrations below the maximum expression of the SelenoP protein (90 µg/L), and white bars represent individuals with optimal Se concentrations.

public health relevance of the findings, including sex- and age-related differences, and places them in the context of recent international research.

4.1. Trace element status

The results of the present study emphasize the critical role of Se among the essential trace elements evaluated in the study population in north-west Spain. Selenium is a key micronutrient involved in numerous physiological processes, particularly antioxidant defence and immune function, and deficiency is a significant global concern [20]. The findings of the present study are consistent with observations in other European regions [21,22], where Se deficiency is widespread due to factors such as low soil Se content and soil acidity [23], as well as lack of tradition of Se supplementation. For instance, studies in Eastern Europe have consistently reported extensive Se deficiency, with large segments of the population falling below adequate levels, negatively impacting health outcomes. By contrast, variable Se levels have been observed in the population of Central Europe, often at the lower end of the traditionally accepted adequate plasma range

(60 µg Se/L serum). There is broad agreement that traditional thresholds for Se status may not fully reflect optimal levels [24,25]. However, it is well known that higher Se levels, from 90 to 140 µg/L, are required for the full expression of the Se-dependent enzymes, such as glutathione peroxidase and selenoprotein P, which are essential for protecting cells from oxidative damage [26]. Remarkably, in the aforementioned study, although less than half of the general population approached the lower limit of adequacy for Se intake, almost the entire population (96 %) fell short of the levels required for optimal antioxidant enzyme activity. This indicates that a significant proportion of the population would benefit from increased Se intake.

For decades, Nordic countries, where Se deficiency was found to be particularly pronounced, have implemented targeted policies to improve Se content in soils through fertilization programs. These efforts have significantly enhanced the Se status of the population, demonstrating the effectiveness of such interventions [27]. Additionally, the production of Se-enriched foods, such as eggs, onions and mushrooms, has shown promise in addressing this nutritional gap [28], although these strategies have not yet been widely implemented in practice. Selenium

Table 4

Essential trace and toxic element concentrations in serum determined in other recent studies. Values are presented as medians, P5–P95 values, lower reference limits (LRL) and upper reference limits (URL). When these are not available, data are expressed as arithmetic means (AM), geometric means (GM) or ranges (R).

	Belgium [77]	Denmark [78]	France [79]	Germany [13]	Serbia [16]	Slovenia [14]	Switzerland [15]	USA [80]	Australia [57]
Co									
Median	<LoD	1	0.3	0.12 ^{GM}		0.221	0.104		0.47 ^{AM}
P5–P95			0.24–0.59	0.06–0.387		0.14–0.544	0.064–0.413		
LRL–URL	a–0.8	0.065–0.572		0.04–0.77 ^R		0.10–1.00	0.059–0.636		0.21–1.3 ^R
Cu									
Median	948	1226	952	1146 ^{GM}	729	837	952	1130	1100
P5–P95			627–1659	795–1949		619–1176	650–1713	a–1690	
LRL–URL	520–2100	794–2173		560–2280 ^R	683–774	580–1750	577–1990		670–2490 ^R
Fe									
Median		804							
P5–P95									
LRL–URL		313–1675							
I									
Median				56 ^{GM}			57.6		
P5–P95				43–72			13.5–104.3		
LRL–URL				39–118 ^R			8.68–121.5		
Mn									
Median	<LoD	1.4	0.65	0.47 ^{GM}	1.25	0.493	0.529		1
P5–P95			0.35–1.08	0.39–0.6		<LoD –0.792	0.329–0.862		
LRL–URL	a–0.9	0.461–10.37		0.29–0.63 ^R	1.36–2.09	a–1.00	0.300–1.074		<1–3.1 ^R
Mo									
Median			0.61	0.62 ^{GM}		0.873	0.878		0.91
P5–P95			0.36–1.15	0.35–1.12		0.50–1.58	0.486–1.524		
LRL–URL				0.21–2.7 ^R		0.45–2.00	0.430–1.886		0.26–3 ^R
Se									
Median	93.7	110	92	87 ^{GM}	65.7	86.8	114.8	127	130
P5–P95			73–110	70–109		67–114	86.3–151.7	a–155	
LRL–URL	65–125	79–150		63–123 ^R	62.3–70.6	63–120	80.5–162.4		82–180 ^R
Zn									
Median	762	706	660	903 ^{GM}	529	825	784	809	1150
P5–P95			510–809	733–1110		639–1339	619–1010	a–1060	
LRL–URL	480–1150	517–1053		605–1348 ^R	534–608	600–1450	588–1067		820–1660 ^R
As									
Median		1.21	2.19	0.21 ^{GM}		0.172	0.463		
P5–P95			1.55–7.58	0.03–1.73		0.030–1.160	0.078–5.494		
LRL–URL		0.247–12.5		0.02–6.20 ^R		a–1.4	a–7.07		
Cd									
Median			<0.03	<LoD		<LoD	0.053		
P5–P95			<0.03–0.04	<0.009–0.017		0.070–0.221	0.029–0.096		
LRL–URL				<0.009–0.033 ^R		a–0.50	0.024–0.106		
Cr									
Median		0.276	<0.29	0.044 ^{GM}		<LoD	0.332		<LoD
P5–P95			<0.29–0.79	<0.03–0.080		0.248–0.777	<LoD		
LRL–URL		0.121–0.552		<0.03–0.20 ^R		a–0.80	a–1.274		
Hg									
Median			0.36	0.12 ^{GM}		0.304	0.267		
P5–P95			0.09–1.01	<0.02–0.56		0.030–2.570	0.096–0.802		
LRL–URL				<0.02–1.10 ^R		a–4.0	0.077–0.993		
Ni									
Median	0.62	0.63	0.84	0.28 ^{GM}		<LoD	1.085		
P5–P95			0.44–1.26	0.19–0.40		0.330–1.620	<LoD–2.613		
LRL–URL	a–1.2	0.34–1.74		0.17–0.48 ^R		a–3.00	a–2.857		
Pb									
Median				0.033 ^{GM}		<LoD	1.066		
P5–P95				0.01–0.10		0.122–0.644	0.387–3.381		
LRL–URL				<0.006–0.13 ^R		a–0.85	0.304–4.031		

LoD: limit of detection; a: LRL below the LoD.

deficiency is not limited to humans and also poses substantial challenges for rearing livestock in Se-deficient regions, particularly when locally produced feed is used [16]. In Galicia, Se deficiency is particularly prevalent in extensive livestock systems and organic farming [29] with serious impacts on animal production. Intensive systems routinely address Se deficiencies by supplementing animal diets, effectively compensating for the low Se content in raw feed ingredients or local forage [30]. Selenium supplementation is especially critical during periods of stress, enhancing immunity at key moments such as weaning and vaccination or during heat stress [9,31]. Moreover, the inclusion of Se in animal diets not only improves animal health but also

enriches Se levels in animal-derived products such as meat, eggs and milk [32]. This is facilitated by the high intestinal bioavailability of Se and renal regulatory mechanisms, similar to I. Selenium has a narrow safety margin, and its supplementation is tightly regulated in the EU due to concerns about toxicity at high concentrations. However, recent EFSA evaluations suggest that the Se levels reached in animal products and, consequently, in human populations, remain far below those observed in countries like the US or Canada, where natural soil Se levels are sufficient [26]. This disparity highlights the need to reconsider current regulatory limits to address the suboptimal Se status observed across much of Europe.

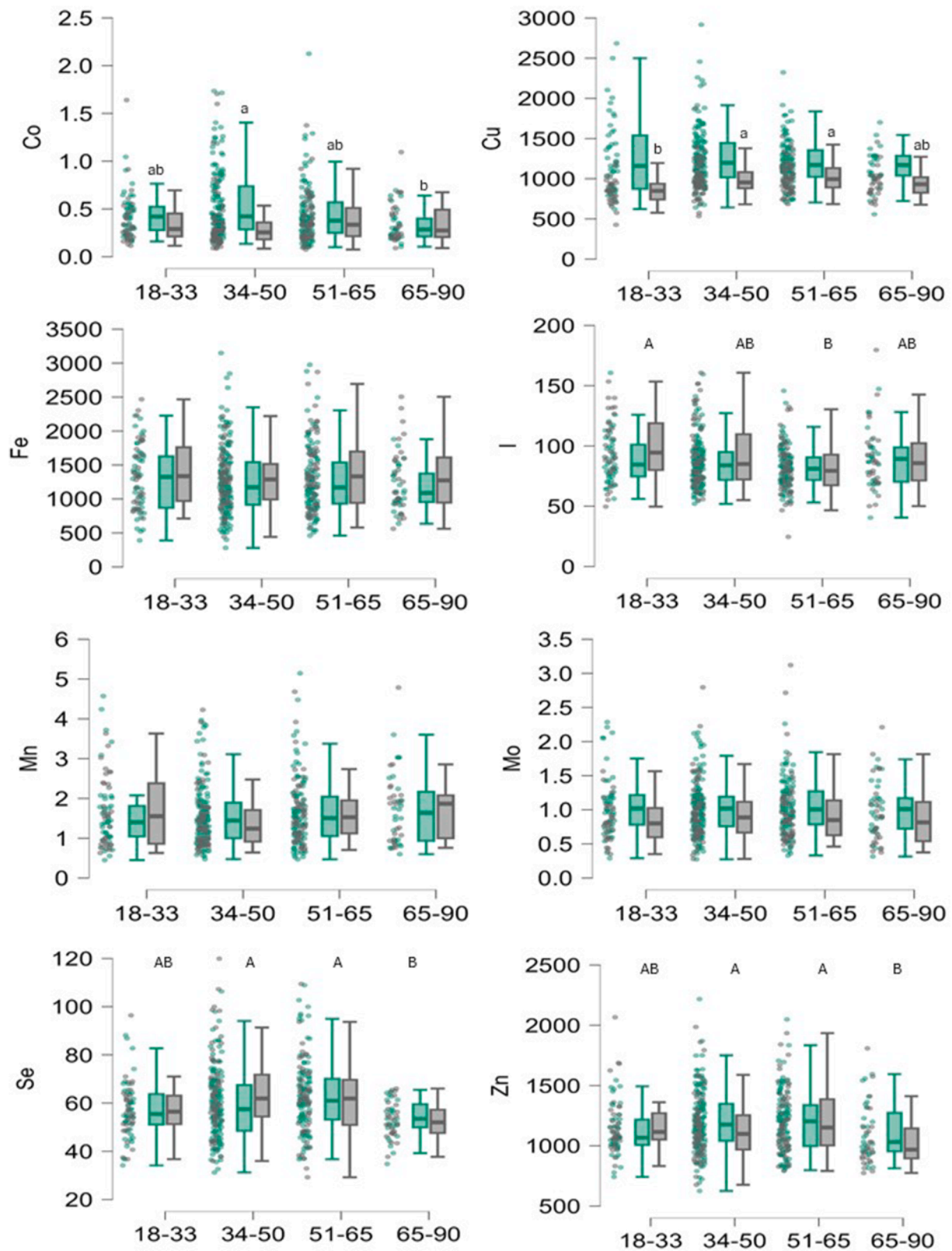


Fig. 2. Box-and-whisker plots of essential trace element concentrations in serum from the study population. Green bars represent females and grey bars males. Uppercase letters indicate statistically significant differences between genders, while lowercase letters indicate statistically significant differences within the gender.

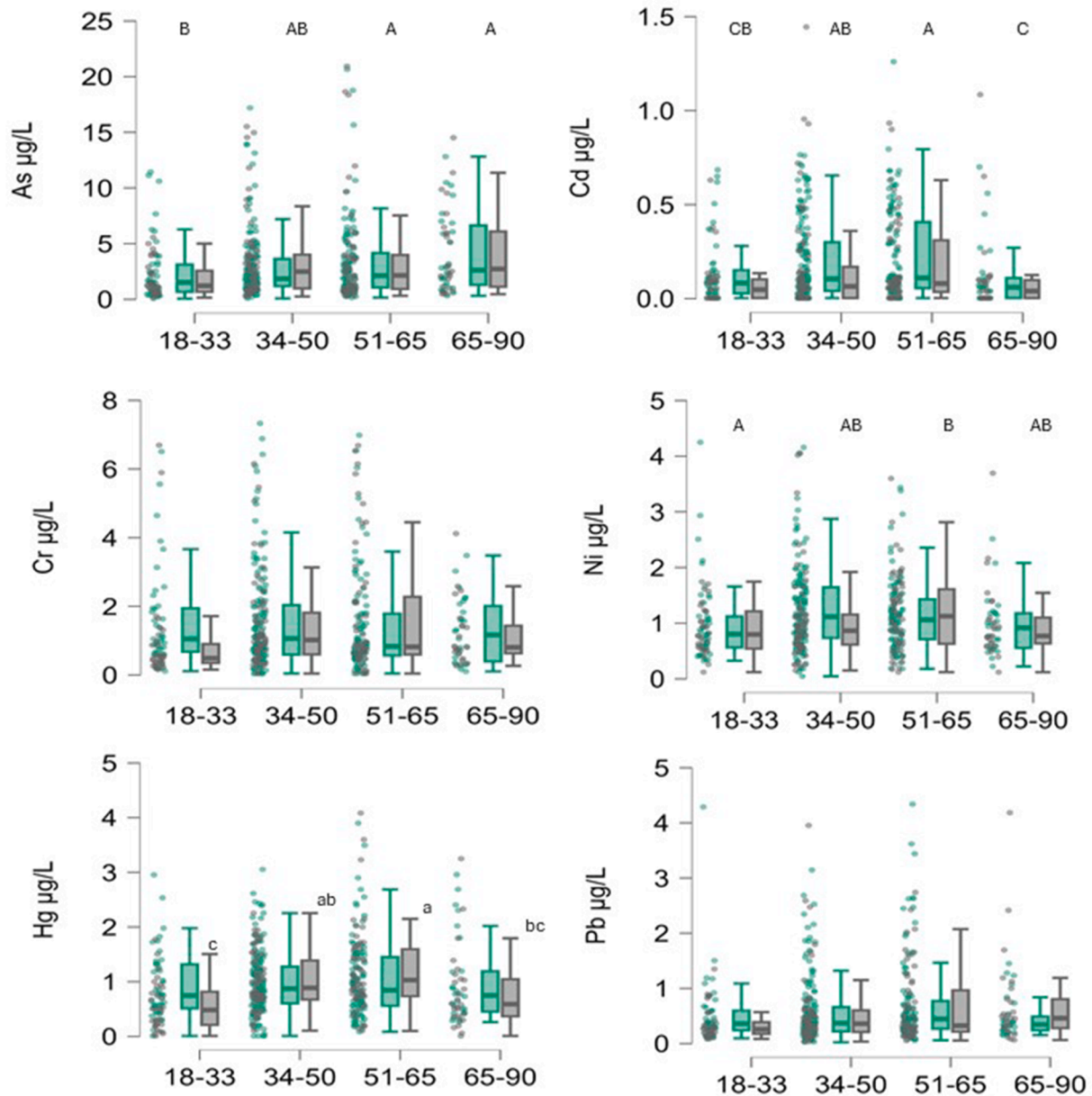


Fig. 3. Box-and-whisker plots of toxic element concentrations in serum from the study population. Green bars represent females and grey bars, males. Uppercase letters indicate statistically significant differences between genders, while lowercase letters indicate statistically significant differences within the gender.

Extensive research has highlighted the critical role of Se in human health, linking its status to various diseases, notably cancer. In vitro and animal studies have demonstrated the involvement of Se in preventing oxidative damage, modulating cell proliferation and regulating apoptosis, immunity and inflammation [33]. Observational cohort studies in humans support this relationship, showing an inverse correlation between Se levels and the incidence of common cancers, including breast, prostate and lung cancer [34]. Recent findings have also highlighted the influence of Se on cancer prognosis, with studies in regions with low Se soil levels demonstrating improved survival rates in breast cancer patients with higher serum Se levels [35,36].

In the present study, Se levels were notably low in older adults, many of whom were included in the deficient range. This finding is particularly concerning as aging populations are more vulnerable to chronic conditions such as heart disease, cancer and cognitive

decline, which are often associated with oxidative stress and immune dysfunction and which can be mitigated by Se. Addressing Se deficiency in elderly populations could yield significant health benefits, including reduced disease incidence, improved quality of life and enhanced longevity. These observations are consistent with the results of a large German cohort study, which demonstrated a strong inverse association between serum selenoprotein P levels and all-cause mortality, as well as mortality from cardiovascular, cancer, respiratory and gastrointestinal diseases [37]. These researchers demonstrated that participants in the lowest selenoprotein P tertile (<4.1 mg/L) exhibited significantly higher mortality risk than those in the top tertile, with men showing a particularly pronounced risk. These findings emphasize the critical role of Se in aging populations and the potential value of interventions, such as targeted Se supplementation in regions with Se-deficient soils, to reduce mortality and improve health outcomes in older adults.

Iodine (like Se) has a distinct geographical distribution and a tightly regulated renal excretion mechanism, which plays a crucial role in maintaining balanced levels in the body [38]. Regions with low environmental I availability, known as goitrogenic areas, have historically faced a high prevalence of thyroid disorders. Within Europe, north-west Spain is a notable example of this situation, as the introduction of iodized salt in a public health intervention successfully reduced thyroid-related problems. Similarly, in the United Kingdom, I deficiency was inadvertently addressed during the 1960s when cattle feed was fortified with I to enhance milk production, leading to increased I concentrations in dairy products and subsequent improvements in population I status [39]. Unlike Se, serum I has not traditionally been used as a marker of nutritional status. Instead, urinary I concentration (UIC) has been the preferred indicator, typically measured over 24 h and adjusted for creatinine to account for variations in hydration and renal excretion rates [40]. This choice stems from the fact that urinary I reflects recent intake, whereas serum I levels are influenced by the tightly regulated homeostasis of the thyroid gland. However, recent studies suggest that serum I concentrations may also serve as reliable markers of I status, especially when fasting samples are used. Significant correlations between serum I, urinary I, dietary I intake and thyroid function have been reported, highlighting the potential value of serum I as a biomarker for individual nutritional assessment [41]. The I serum levels determined in the present study fall within the range considered adequate, indicating that I deficiency is currently not a concern in the population of north-west Spain. This reflects the success of I fortification strategies and adequate dietary intake in the region [8]. These findings are consistent with broader trends in Europe and other regions where targeted public health measures have successfully improved I nutrition [42].

The other essential trace minerals, except for Mo, are subject to tight homeostatic regulation at the intestinal level. This means that problems typically arise from deficiencies, when dietary concentrations of these minerals are low or unavailable, rather than from toxicity. Among these minerals, Fe and Zn are of paramount importance in human nutrition [43]. The challenge with these nutrients does not relate to geographical distribution patterns, as seen with Se and I, but rather to the insufficient intake of foods containing bioavailable forms, which are predominantly animal products [11]. This insufficiency is particularly concerning because it leads to widespread deficiencies, especially in low-income regions where access to such animal-derived nutrients is limited. In developed countries, dietary preferences favouring high-fibre foods can inhibit the absorption of Fe and Zn, potentially leading to deficiencies, even in affluent societies [44]. This issue is compounded in vegan diets, where the exclusion of animal products eliminates key sources of these essential minerals [45]. Similarly, the 'Planetary Health Diet', which promotes a substantial reduction in animal product consumption, has been critiqued for potentially exacerbating these nutritional deficiencies [11]. Our study data indicate that the levels of both Zn and Fe in serum were within normal ranges, confirming adequate status of the study population in north-west Spain. Zinc levels in serum are considered an effective marker of Zn status, as supported by EFSA and mirrored in recent European studies [46]. For Fe, while functional markers such as haemoglobin and haematocrit provide a more comprehensive assessment of Fe status, they can be influenced by factors unrelated to dietary intake, such as menstrual blood loss in fertile women, genetic conditions and lifestyle factors like smoking [47]. In the present study, serum Fe levels, measured in fasting conditions to minimize diurnal variation [48], were significantly correlated with haemoglobin concentrations, which were also within normal ranges in most participants (data not shown). This

correlation supports the validity of serum Fe as a practical marker of iron status in this population, particularly when analyzed alongside functional parameters. These findings suggest that iron deficiency is not a major concern in the study population, probably reflecting adequate dietary intake and bioavailability of this essential mineral.

Copper deficiency has classically been considered rare in the human population, unlike in animals. This difference can be attributed to the wide availability of Cu in various foods, the absence of significant dietary antagonists (as seen in ruminants, [49], and the higher efficiency of intestinal Cu absorption in humans [50]. However, recent studies suggest that Cu deficiency may have increased in the past few decades. While genetic conditions such as Wilson's disease are rare, numerous pathological processes, changes in dietary habits or malabsorption following bariatric surgery can influence Cu metabolism [51]. EFSA reports indicate that marginal or subclinical Cu deficiencies may be more widespread than previously assumed and deserve further investigation [52]. Monitoring Cu levels in serum or plasma is an effective way to detect these deficiencies, and establishing reference ranges for normal populations is therefore important. However, interpretation of these levels must account for their sensitivity to inflammatory responses, as ceruloplasmin, the primary Cu-carrying protein in blood, is also an acute-phase reactant [8].

Regarding the other essential elements analyzed -Mn, Co and Mo- these elements have not been identified as of public health concern, after review by international bodies such as EFSA [53,54] and the Nordic Nutrition Recommendations [55,56]. Current scientific evidence suggests that deficiencies of these trace elements are rare and not widespread. For example, Mn is readily available in a variety of plant-based foods, and dietary intakes are generally within recommended levels, with deficiencies observed only under controlled experimental conditions [26]. Similarly, Mo, primarily sourced from cereal products, vegetables and dairy products, has an efficient absorption mechanism, and no clinical signs of dietary deficiency have been reported in healthy individuals [53]. The present study findings are consistent with those of recent studies (Table 4), showing that the levels of Mn, Co and Mo in the study population fall within normal ranges. The findings further indicate that these elements are not a significant concern for this population, supporting the conclusion that dietary intake is adequate.

The present study findings also highlight the impact of sex and age on the serum levels of several essential trace elements, possibly reflecting the interplay of hormonal, metabolic and dietary factors. High plasma Cu levels in women are well documented in the literature [14–16,57] and are associated with hormonal regulation, particularly the role of oestrogens in Cu metabolism through their effect on ceruloplasmin synthesis [58]. Higher Cu levels have also been linked to the use of oral contraceptives and hormone replacement therapy, further reinforcing the influence of hormonal factors on Cu homeostasis [15]. Although there is less information available for Co, other studies [15,59] have also reported higher levels in women. These differences could be associated with sex-specific variations in the metabolism of vitamin B12 [60], with which Co is closely linked. It has been suggested that the higher Co levels in women may reflect differences in B12 absorption, utilization or storage capacity, potentially influenced by hormonal and metabolic factors. In the case of Mo, while the precise mechanisms remain unclear, differences in serum Mo levels may be influenced by factors such as environmental exposure, dietary intake and metabolic processes, which could vary by sex [61]. Conversely, serum Fe levels were lower in women, as commonly observed and often attributed to blood loss in women during their reproductive years, which can

lead to Fe deficiencies and anaemia [62]. However, our study also noted lower Fe levels in postmenopausal women, suggesting that additional mechanisms influencing gender differences in Fe metabolism may be occurring. While not fully elucidated, these differences are probably influenced by metabolic and hormonal factors, including greater body mass and the effects of androgens on haematopoiesis [63].

Age-related effects were evident for some trace elements, with different patterns observed depending on the element. For Cu, an age effect was noted only in men, with progressively higher levels in older age groups. This may reflect the cumulative impact of low-grade inflammation, often associated with aging, which stimulates ceruloplasmin synthesis and thus increases circulating Cu levels [64]. Interestingly, this effect was not observed in women, possibly due to the protective anti-inflammatory properties of oestrogens, even in the postmenopausal phase, when residual oestrogen levels and their long-term effects on metabolic regulation may still exert some influence [65]. For Co, an age-related decline was observed exclusively in women, with lower levels in individuals over 65 than in those aged 34–50. This pattern is probably driven by metabolic changes related to hormonal shifts during the menopausal transition, as well as potential age-related alterations in vitamin B12 metabolism [66]. In these cases, the observed trends seem more likely to be attributed to intrinsic metabolic differences rather than to dietary factors, given the sex-specific nature of the effects.

For Se and Zn, levels declined with age in both sexes, with the lowest levels observed in individuals over 65 years. Such decreases have been described in other studies [15,67] and may result from a combination of increased metabolic demands, such as heightened antioxidant requirements to counteract age-related oxidative stress. Older individuals may consume fewer Se- and Zn-rich foods, such as fish, nuts, meat and legumes, due to dietary restrictions, reduced appetite or changes in food preferences [68]. Similarly, an age-related pattern was observed for I levels, which were highest in younger individuals (18–33 years) and lowest in the 51–65 years age group. This probably reflects differences in dietary habits, particularly the consumption of iodized salt. Younger individuals may consume more processed or prepared foods, which often contain iodized salt [69], whereas older adults may reduce their salt intake for health reasons, such as managing hypertension.

4.2. Toxic element accumulation

Toxic trace elements such as As, Cd, and Pb have no known physiological role in the body and their presence in biological samples reflects exposure rather than nutritional status. Consequently, the reference values proposed in this study should not be interpreted as indicators of adequacy, but rather as indicative ranges of internal exposure in a healthy population. These levels, which result from chronic low-level exposure through environmental, dietary or lifestyle-related sources, serve as useful benchmarks for biomonitoring and public health surveillance.

The levels of toxic elements measured in the study population were low, similar to recent data reported in other countries that reflect low environmental exposure (Table 4). This is a positive indicator of the declining prevalence of significant sources of contamination. For instance, the global phase-out of leaded gasoline, completed in 2021, has significantly reduced Pb levels in the environment, preventing an estimated 1.2 million premature deaths annually and increasing children's IQ levels [70].

Most studies measure toxic elements in whole blood due to the higher concentrations in this matrix [13]. This is because toxic elements tend to accumulate in or bind preferentially to cell components, making whole blood the standard for their detection. However, advances in highly sensitive analytical techniques, such

as ICP-MS, have significantly lowered the limits of quantification. This progress enables the precise and reliable measurement of toxic elements in serum, with most samples showing quantifiable residues. Notably, determining the status of essential elements in serum is more effective, and reference values for key elements (namely Se, Fe, I and Zn) in serum are better standardized and widely recognized. By enabling both toxic and essential element determinations from the same serum sample, the analytical process is greatly simplified. Moreover, serum preparation is less complex than whole blood preparation, reducing both the time and cost of studies while maintaining high analytical accuracy.

In our study the main toxic elements -including As, Cd and Hg- exhibited distinct patterns of accumulation and exposure, shaped by age and sex. The findings suggest that these elements differ notably in their sources of exposure, elimination mechanisms, and the influence of sex-related physiological differences. Age-related trends highlight important differences in how these metals accumulate and are eliminated over time. Arsenic levels increased gradually with age in both men and women. This pattern suggests that exposure to As remains consistent throughout life, primarily through dietary and environmental sources, such as water and seafood [71]. Unlike Cd and Hg, As does not bioaccumulate to a great extent in tissues [72], so that As serum levels reflect ongoing exposure rather than redistribution from long-term storage sites. The continued increase in serum As levels in the oldest individuals may indicate a reduced capacity for elimination, probably due to age-related declines in both renal and hepatic function, compounding the effects of sustained exposure. However, plasma levels of C and Hg declined in the oldest individuals, despite their known cumulative nature. For Cd, levels increased progressively across younger and middle-aged groups in both sexes, peaking before declining in the oldest individuals. This decline can probably be attributed to reduced recent exposure, particularly from tobacco [73], as older individuals are less likely to smoke. Similarly, Hg levels in men increased gradually with age but were lower in the oldest group. These findings suggest that, unlike As, the primary driver for Cd and Hg in serum may be a combination of reduced recent exposure and age-related changes in tissue redistribution. The observed decline in serum levels for these elements at older ages occurs despite a probable reduction in elimination capacity, reflecting differences in their exposure patterns across the lifespan.

Sex-specific patterns further highlight the unique dynamics of these toxic metals. Cadmium levels were significantly higher in women than in men across all age groups, probably due to greater intestinal absorption, which is enhanced by lower iron status [74], a condition more prevalent in women. This consistent difference suggests that sex-specific metabolic factors, alongside cumulative exposure, play a key role in Cd levels. By contrast, there were no significant sex-related differences in As, reflecting shared environmental and dietary sources and the absence of strong sex-specific metabolic influences. However, a distinct sex-related pattern was observed for mercury. Thus, while levels increased gradually in men across younger and middle-aged groups, they remained stable throughout life in women. Sex differences in Hg excretion via the kidney have been reported, with higher urinary levels in women than in men [75]. This suggests that clearance of Hg may occur faster in females than in males [76], which could partly explain the observed stability in Hg levels among women despite cumulative exposure.

4.3. Strengths and limitations

This study has several strengths that highlight its scientific value and potential impact. It is the first biomonitoring study to comprehensively assess serum concentrations of essential and

toxic trace elements in a study population in north-west Spain. The use of validated protocols and cutting-edge analytical techniques, such as ICP-MS, ensures accurate, precise and reliable results. The robust design of the study, including a large sample size and careful participant selection, enables the establishment of population-specific reference values and the identification of patterns of trace element levels influenced by sex and age. Overall, the findings highlight the importance of considering both sex and age when evaluating trace element status. The interplay between hormonal, metabolic and dietary factors is critical in shaping the regulation of these essential elements. This nuanced understanding adds depth to the interpretation of variability in trace element concentrations and indicates areas for further investigation.

While this study proposes plausible hypotheses regarding the role of sex, age and metabolism, as well as their interactions with lifestyle factors, these relationships were not fully explored. However, all relevant data –spanning medical history, dietary habits and lifestyle details–were collected from the participants. Future research will address these limitations by examining the data using advanced statistical approaches, including chemometric techniques, to uncover patterns in the data, and artificial intelligence (AI)-based methods to model and predict complex interactions. These approaches will enable comprehensive exploration of the multifactorial dynamics shaping trace element status, paving the way for more targeted and effective public health strategies.

Taken together, our results align with recent global efforts to better understand micronutrient status in the general population, in line with current One Health strategies and international recommendations from [8,26,71]. Continued biomonitoring across diverse populations is essential to detect nutritional vulnerabilities and guide targeted interventions in response to evolving dietary and environmental trends.

5. Conclusions

The study findings enable a comprehensive evaluation of the serum concentrations of essential and toxic trace elements in a study population in north-west Spain, providing valuable insights into the observed variations and the implications for public health and nutrition. The findings highlight the concern regarding Se deficiency in the region, which affects a substantial portion of the population, particularly older adults, potentially increasing their vulnerability to chronic diseases. This highlights the need for targeted interventions, such as supplementation strategies or the development of Se-enriched foods, to improve Se status and mitigate adverse health outcomes. The adequate levels of I, Fe and Zn observed reflect the success of public health measures and the generally balanced diet consumed in the region. However, continuous monitoring is essential, particularly in light of emerging dietary trends that may compromise the bioavailability of some minerals. Moreover, sex and age play a critical role in the regulation of trace elements, and these factors should be considered in the design of future biomonitoring and nutritional strategies. Regarding toxic elements, the low concentrations detected indicate a reduction in environmental exposure, although some age and sex-related variations warrant further investigation. The ability to measure both essential and toxic elements in serum, enabled by advanced analytical techniques, represents a practical and efficient approach for future biomonitoring studies.

CRedit authorship contribution statement

Inés Rivas: Formal Analysis, Investigation, Writing – original draft. **Marta Miranda:** Conceptualization, Investigation, Writing –

original draft, Writing – review & editing, Funding Acquisition. **Carlos Herrero-Latorre:** Data Curation, Software, Writing – original draft, Supervision. **Rafael Monte-Secades:** Conceptualization, Methodology, Supervision. **Marta López-Alonso:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing, Supervision, Project Administration, Funding Acquisition.

Declaration of competing interest

Although this study was supported by several grants, all authors declare that they have no conflicts of interest.

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