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Assessment of 12 Metals and Metalloids in Blood of General Populations Living in Wuhan of China by ICP-MS

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Abstract

Assessment of trace element levels in general population from the specific area is of importance for nutritional and occupational monitoring. In the current study, baseline blood levels of 12 toxic and/or essential metals and metalloids, including arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg), chromium (Cr), thallium (Tl), manganese (Mn), copper (Cu), Zinc (Zn), calcium (Ca), iron (Fe), and magnesium (Mg), in general populations ($n = 477$) of Wuhan in central China were investigated by using inductively coupled plasma mass spectrometry (ICP-MS). The geometric means for As, Cd, Pb, Hg, Cr, Tl, Mn, and Cu were measured as 2.25, 0.70, 17.84, 1.90, 0.36, < 0.05, 12.40, and 783.76 μg/L, respectively. The geometric means for Zn, Ca, Fe, and Mg were 5.85, 56.66, 488.98, and 39.44 mg/L, respectively. We found the men had higher blood As, Pb, Hg, Zn, Fe, and Mg levels but had lower blood Cu and Ca levels than the women ($p < 0.05$). Age-related difference were found for blood Cu, Zn, Ca, Mg, Pb, Mn, As, Cd, and Hg levels ($p < 0.05$). Moreover, many metal concentrations were found correlated, with the strongest correlations between the pairs Fe–Mg ($r = 0.57$), Fe–Zn ($r = 0.42$), As–Hg ($r = 0.46$), Ca–Cu ($r = 0.34$), Pb–Hg ($r = 0.36$), Pb–Cd ($r = 0.31$), Pb–As ($r = 0.25$), and Ca–Fe ($r = -0.23$). Compared with reports from other countries, most of our results were consistent, except that As Pb, Hg, Mn, and Cu showed different blood levels with European, Korea, or Beijing areas. Our study would be of importance for nutritional, environmental, and/or occupational monitoring of these metals in human.

Keywords Metals and metalloids · Biomonitoring · Whole blood · ICP-MS

Introduction

Chemicals like metals and metalloids are continually released in the environment via industrial, agricultural, and natural sources in modern society. The general population is exposed to these chemicals via air, water, soil, and consumer products, which made the environmental and occupational exposure becoming a growing concern [1]. The use of biological exposure indicators has developed greatly in the past years and enables an assessment of the exposure to chemical substances whatever their sources and the entries [2]. The levels of the metals and metalloids measured in the populations are not only useful for assessing exposure and following up the levels over the course of time, but also for estimating their impact upon the health, thus contributing to the identification of vulnerable or high exposure risk groups [3].

For specific populations, countries or areas around the world have their own background levels of metals and metalloids. In America and Europe, surveys in countries such as the USA [4], Canada [5, 6], Brazil [7], Germany [8], France [3], Italy [9, 10], and Czech Republic [11, 12] have enabled an estimation of the blood levels of toxic and/or essential metals and metalloids. In Asia, the South Korea [13] has developed their human biomonitoring programs to follow up the long-term time trends in chemical exposure of the population. In China, similar programs for establishing biological monitoring index of reference values for important chemicals in population seem to be in operation [14], but considering the regional inequalities, the reliable reference values among the general population still remained uncertain.

Hao-Long Zeng and Huijun Li contributed equally to this work.

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As the largest city in Central China with a relatively complete industrial economy including steel, energy, and manufacturing, Wuhan is reported of facing heavy ambient particulate matter pollution. The crustal elements (Mg, K, Ca, and Fe) dominated the 20 detected elements in PM 2.5. Ten trace elements (Cu, Ga, Ag, Tl, Ca, As, Zn, Pb, Se, and Cd) were enriched in PM 2.5, especially for Ag, Pb, Se, and Cd [15]. The current study had a unique focus on the general population of Wuhan City in central China and attempts to establish background blood levels of toxic (Pb, Cd, As, Hg, Cr, Tl) and essential (Mn, Cu, Zn, Ca, Fe, Mg) metals and metalloids in the healthy people living in Wuhan area and to compare the values with previous reports from other areas and countries. The results provided a snapshot of blood trace element levels for the populations in central China and shall be of importance for nutritional, environmental, and occupational monitoring of these metals and metalloids in human.

Materials and Methods

Study Population and Sample Collection

The population in this study comprised 477 participants living in Wuhan of central China, including 262 men and 215 women aged 5–80 years old. They all came for the routine health examination between September 2016 and April 2018 at the Physical Examination Center in Tongji Hospital, Tongji Medical College, Huazhong University of Science & Technology. Four test panels were set for blood element examination, including essential element panel 1 (Cu, Zn, Ca, Fe, Mg), essential element panel 2 (Cu, Zn, Fe), toxic element panel 1 (Pb, Hg, As, Cd, Mn, Tl, Cr), and toxic element panel 2 (Pb, Hg, As, Cd, Mn). Participants could select one essential element panel and/or one toxic element panel for testing.

Approval was received from Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology Review Board in Wuhan, China. All the procedures involving human samples conformed to the principles outlined in the Declaration of Helsinki. Informed consent for the use of the detection results and personal information in this study was obtained from all the adult participants before specimen collection. For the underage participants (age <18 years old), authorization was obtained from parents. The participants had no exposure to metals and were not taking any medication containing metals. At least 2 mL of blood was collected in 6-mL special polyethylene terephthalate vacuum vials, for trace elements, containing 10 mg potassium ethylene diamine tetraacetic acid (BD Vacutainer product reference 368381). Standard stainless steel needles were used (Precision glide, Becton Dickinson). During blood sampling, the trace element vials were the latest ones collected.

Sample Preparation and Analysis

All measurements were carried out in the Department of Laboratory Medicine, Tongji Hospital of Tongji Medical College in Huazhong University of Science & Technology with a quadrupole inductively coupled plasma mass spectrometer (ICP-MS) equipped with a concentric glass nebulizer and a cyclonic spray chamber (7700x ICP-MS system, Agilent Technologies, CA, USA). Analyses were performed in standard/no-gas mode and dynamic reaction cell mode. For cadmium (Cd), mercury (Hg), thallium (Tl), and lead (Pb), the assays were run in dynamic reaction cell mode with high purity helium (He, $>99.995\%$) as the reaction cell gas. For magnesium (Mg), calcium (Ca), copper (Cu), zinc (Zn), iron (Fe), chromium (Cr), manganese (Mn), and arsenic (As), assays were run in standard/no-gas mode. Plasma torch argon purity was higher than $>99.999\%$. The analytical conditions were optimized daily by using the standard tuning solution (Agilent Technologies). All the detailed ICP-MS operating conditions are summarized in Table S1. All sample preparations were accomplished in Clean Bench (ESCO, Singapore).

All reagents were of analytical-reagent grade. Calibrating solutions were prepared every day using the multi-element calibration standard (Agilent Technologies, CA, USA), standard solutions of Hg (Agilent Technologies, CA, USA), and standard solutions of Cu, Zn, Ca, Fe, and Mg (O2si, USA). The subdivision of elements analyzed and the preparation of calibration standards were described in Table S2. Blood samples (200 μl) were diluted 1:20 (*v*/*v*) with an aqueous solution containing 0.1% (*v*/*v*) Triton X-100 (Sigma–Aldrich, France) and 0.1% nitric acid ($\geq 69\%$, Merck, Germany). The samples were vortexed in a table-top vortexer for 1 min. The diluted samples were then quantified by ICP-MS.

According to the recommendations of IUPAC and previous studies [16, 17], the LoD were determined as triple relative standard deviation (RSD) of the sample blank measurements (RSD^b) ($N > 10$), while the LoQ were determined from LoQ = 10 RSD^b C/SBR, C is the concentration of the element in solution and SBR is the signal to background ratio.

Quality Control

Internal quality assessment (IQA) was carried out during analysis by using the traceable Seronorm™ whole blood materials L-2 (ref.:210205) and L-3 (ref.:210305). And another low concentration home-made quality controls L-1 were prepared by diluting the Seronorm™ L-2 five times. For the IQA results, a Z-score was calculated, and the values within the + 2 to − 2 range were satisfactory and indicated analytical trueness. The accuracy of the method was also verified by participation in the National Center for Clinical Laboratories (NCCL) external quality assessment (EQA) scheme.

Data Analysis

The distributions of the elements tested by the Kolmogorov–Smirnov test were mostly not normal. Several metals from the whole blood samples showed skewed distributions even after a log transformation. Thus, the nonparametric tests were used in every analysis. The metal concentrations were described in terms of percentiles (P5, P25, P50, P75, and P90), arithmetic mean (AM), and geometric mean (GM). The Mann–Whitney test was used to compare the influence of Gender. The Kruskal–Wallis test was applied to compare the difference among ages. To assess the correlation between the different element concentrations in blood, the Spearman correlation test and its statistical significance were used. For the determination, individual results below the limit of qualification (LoQ) were replaced by the (LoQ/2) value. The SPSS version 22.0 was used as statistical package (SPSS, Chicago, USA).

Results

Characteristics of the Study Population

Of the 477 selected individuals, 262 (56.8%) were men and 215 (43.2%) were women. The mean age was 33.6 years (± 13.0 years) while the mediate age was 33.0 years. All the subjects were grouped based on their age: < 10 age group (3.4%), 10–19 age group (12.6%), 20–29 age group (21.8%), 30–39 age group (28.7%), 40–49 age group (22.4%), 50–59 age group (9.6%), > 59 age group (1.5%) (Table 1).

Table 1 Age and gender of people who participated in this study

Age (years)	Number of subjects		
	Men	Women	Total
0–9	10	6	16
10–19	40	20	60
20–29	55	49	104
30–39	71	66	137
40–49	51	56	107
50–59	30	16	46
≥ 60	5	2	7

Quality Assurance

The analytical performance of the 12 blood trace elements tested by using ICP-MS was shown in Table 2. The detection limits (LoD) ranged from 0.02 μg/L (Cd, Tl) to 1.5 mg/L (Fe), and the quantification limits (LoQ) range from 0.05 μg/L (Tl) to 4.3 mg/L (Fe). For quality assurance, the IQA was implemented by using the traceable Seronorm blood material (SERO, Billingstad, Norway). Following every ten blood samples, Seronorm control samples were tested to ensure quality throughout screening. The Z-score was obtained and interpreted as previous reported [18]. As we can see, the Z-scores for all the three blood levels for the 12 elements were all bellow, which are very satisfactory. The inter-day variable coefficient (CV) of the detection results ($N = 24$) was from 2.37 to 7.73% for level 1, 2.39 to 8.36% for level 2, and 4.06 to 8.91% for level 3, which showed a good precision of the methods.

For EQA, The NCCL certified blood material (#201721, #201725) was tested (Table 3). The measured values for the six elements were all in the acceptable range. The bias was all less than 8%, which indicated the results were satisfactory. The other trace elements (Cr, Hg, Tl, Mn, Cd, As) are not mentioned in Table 3 as they were not proposed in the NCCL controls.

Blood Concentrations of Trace Metals

The measurements of blood trace element levels for selected participants are summarized in Table 4, as a whole and by sex, respectively. In blood, Cu, Zn, Ca, Fe, and Mg were detected over 68% of cases ($N > 450$), while Pb, Mn, As, Cd, and Hg were detected in 39% of cases ($N = 260$) and Cr and Tl were detected in 26% of cases ($N = 176$). The nine elements (Cu, Zn, Ca, Fe, Mg, Pb, Mn, As, Cd) showed 100% of measure values above the LoQ, while the other three elements showed low background levels (7.39% of Cr, 6.54% of Hg, and 63.07% of Tl measured values were below the LoQ). The geometric means for As, Cd, Pb, Hg, Cr, Tl, Mn, and Cu were 2.25, 0.70, 17.84, 1.90, 0.36, < 0.05, 12.40, and 783.76 μg/L, respectively. The geometric means for Zn, Ca, Fe, and Mg were 5.85, 56.66, 488.98, and 39.44 mg/L, respectively.

Correlation Between Metal Concentrations and Age or Gender

For correlations between metal concentrations and gender, as shown in Table 4, the mean levels of Cr, Mn, Cd, and Tl in blood did not differ significantly between men and women ($p > 0.05$). While the mean blood Zn level was significantly higher in men than that in women ($p < 0.001$, GM = 6.06 vs 5.60), as was the mean blood Fe level ($p < 0.001$, GM = 517.38 vs 455.98), mean blood Mg level ($p < 0.001$, GM = 41.00 vs 37.58), mean blood As level ($p = 0.042$, GM = 2.36 vs 2.11), mean blood Pb level (p

Table 2 Whole blood ICP-MS analytical performance

Elements	Unit	LoD[a]	LoQ[b]	Seronorm target value			Measured mean			Z-score			CV (%), $N = 24$		
				L1[c]	L2	L3	L1	L2	L3	L1	L2	L3	L1	L2	L3
Fe	mg/L	1.50	4.30	66.40	332.00	383.00	68.26	326.29	359.97	0.09	− 0.10	− 0.35	3.06	5.17	4.92
Ca	mg/L	0.13	0.37	3.00	15.00	14.20	3.17	16.38	14.23	1.70	0.46	0.01	6.21	4.42	4.51
Mg	mg/L	0.01	0.03	2.88	14.40	17.20	3.01	15.03	16.70	0.14	0.14	− 0.10	2.37	3.83	5.01
Zn	mg/L	0.001	0.003	1.42	7.10	8.97	1.63	8.14	9.67	0.76	0.74	0.39	5.17	4.79	4.62
Cu	mg/L	0.001	0.002	0.27	1.34	2.47	0.27	1.25	2.61	− 0.10	− 0.32	0.57	6.75	5.01	6.19
Pb	μg/L	0.25	0.95	67.40	337.00	447.00	56.51	314.77	416.77	− 0.80	− 0.33	− 0.66	5.85	3.39	6.68
Mn	μg/L	0.23	0.85	6.28	31.40	47.30	6.23	32.02	49.26	− 0.04	0.10	0.21	4.39	2.39	4.06
As	μg/L	0.08	0.23	2.82	14.10	30.40	2.57	15.98	37.15	− 0.41	0.67	0.92	7.73	8.36	6.52
Hg	μg/L	0.18	1.15	3.40	17.00	37.10	3.16	19.93	37.33	− 0.35	0.86	0.03	7.53	8.30	8.91
Cd	μg/L	0.02	0.06	1.00	5.01	12.10	0.93	5.16	11.35	− 0.35	0.15	− 0.57	6.04	4.76	7.49
Cr	μg/L	0.14	0.50	2.14	10.70	23.20	2.00	10.38	22.74	− 0.32	− 0.14	− 0.10	6.73	4.01	4.74
Tl	μg/L	0.02	0.05	2.04	10.20	34.10	1.98	10.44	31.93	− 0.13	0.11	− 0.31	6.34	3.56	5.65

[a] Low limit of detection (LoD)

[b] Low limit of quantification (LoQ)

[c] Level 1 (L1), level 2 (L2), level 3 (L3)

< 0.001, GM = 20.32 vs 14.81), and mean blood Hg level ($p < 0.001$, GM = 2.21 vs 1.53). And in women, the mean blood levels were significantly higher for Cu ($p < 0.001$, GM = 806.25 vs 768.83) and Ca ($p < 0.001$, GM = 58.78 vs 55.02).

For correlations between metal concentrations and age groups, the concentrations of Cu, Zn, Ca, Mg, Pb, Mn, As, Cd, and Hg in the blood were significantly different among the different age groups (Table 5). Mean levels of Zn, Pb, As, Cd, and Hg increased significantly according to the age categories ($p < 0.05$). Mean levels of Cu, Ca, Mg, and Mn were higher in the young age group (< 20 years old) than those in other age groups.

Correlation Between Two Metal Concentrations

Individual correlation analysis resulted in several pairs of metals with statistically significant correlations between their concentrations ($p < 0.05$) (Table 6). We obtained a total of 20 significant Spearman correlations, most of which were positive and only four (Ca–Fe ($r = -0.23$, $p < 0.001$), Ca–Zn ($r = -0.16$, $p < 0.001$), Cd–Mg ($r = -0.31$, $p < 0.05$), Cd–Mn ($r = -0.15$, $p < 0.05$)) were negative. Mg and Pb were the elements with the highest number of significant correlations (6 and 5, respectively). The most significant correlations were found in pairs Fe–Mg ($r = 0.57$, $p < 0.001$), Fe–Zn ($r = 0.42$, $p < 0.001$), As–Hg ($r = 0.46$, $p < 0.001$), and Pb–Hg ($r = 0.34$, $p < 0.001$).

Discussion

Understanding the effects of trace metals and metalloids on human health is complex and fascinating [19]. The importance of environmental, occupational, and nutritional monitoring of

Table 3 Whole blood results obtained with the NCCL certified reference material

Elements	Unit	Target value (μg/L)		Measured (μg/L)		Bas(%)	
		NCCL 201721	NCCL 201725	NCCL 201721	NCCL 201725	NCCL 201721	NCCL 201725
Fe	mmol/L	6.12 ± 1.22	4.42 ± 0.88	5.95	4.3	− 2.77	− 2.71
Ca	mmol/L	1.18 ± 0.25	1.35 ± 0.25	1.24	1.43	5.08	5.93
Mg	mmol/L	0.50 ± 0.12	0.69 ± 0.17	0.48	0.7	− 4.00	− 1.45
Zn	μmol/L	24.7 ± 6.2	82.7 ± 20.7	23.6	78.6	− 4.45	− 4.96
Cu	μmol/L	46.36 ± 11.59	92.95 ± 23.24	49.2	89.55	6.13	− 3.16
Pb	mmol/L	80.3 ± 40	330.6 ± 40	79.3	325.2	− 1.25	− 1.63

Table 4 Blood metal levels of populations of Wuhan in central China

Elements[c]	Populations	N	AM[a]	GM[b]	% <LoQ[d]	Percentiles					*p*
						P5	P25	P50	P75	P95	
Fe (mg/L)	Total	461	492.11	488.98	0	406.45	452.55	490.13	532.78	576.69	
	Gender										< 0.001
	Women	206	458.01	455.98	0	395.37	432.01	454.29	480.00	532.36	
	Men	455	519.66	517.38	0	434.96	489.90	519.26	551.77	594.39	
Ca (mg/L)	Total	455	57.01	56.66	0	46.91	52.70	56.95	61.05	66.79	
	Gender										< 0.001
	Women	202	59.12	58.78	0	48.75	55.70	58.98	63.45	68.84	
	Men	253	55.32	55.02	0	45.23	51.59	55.12	58.93	65.06	
Mg (mg/L)	Total	455	39.73	39.44	0	31.89	36.53	39.75	42.77	48.04	
	Gender										< 0.001
	Women	202	37.83	37.58	0	31.2885	34.63	37.73	40.65	46.01	
	Men	253	41.26	41.00	0	34.16	38.39	41.08	44.11	48.58	
Zn (mg/L)	Total	461	5.95	5.85	0	4.32	5.26	5.88	6.61	7.75	
	Gender										< 0.001
	Women	206	5.69	5.60	0	4.04	5.04	5.67	6.16	7.22	
	Men	255	6.16	6.06	0	4.41	5.52	6.22	6.81	7.89	
Cu (μg/L)	Total	461	791.39	783.76	0	634.11	713.31	774.27	855.13	999.40	
	Gender										< 0.001
	Women	206	810.80	802.65	0	643.58	729.47	786.62	878.61	1044.61	
	Men	255	775.72	768.83	0	633.90	702.17	761.49	838.34	960.07	
Pb (μg/L)	Total	260	21.86	17.84	0	8.69	12.42	16.67	22.79	48.14	
	Gender										<0.001
	Women	107	18.48	14.81	0	8.22	11.03	13.91	18.02	30.49	
	Men	153	24.22	20.32	0	10.04	14.05	19.52	25.46	51.82	
Mn (μg/L)	Total	260	12.93	12.40	0	8.09	10.12	12.33	14.90	18.49	
	Gender										0.186
	Women	107	13.21	12.74	0	8.292	10.78	12.39	15.07	18.41	
	Men	153	12.73	12.17	0	7.81	9.79	12.22	14.77	18.49	
As (μg/L)	Total	260	3.03	2.25	0	0.93	1.47	1.96	3.30	8.14	
	Gender										0.042
	Women	107	3.10	2.11	0	0.815	1.32	1.77	3.12	8.51	
	Men	153	2.98	2.36	0	1.02	1.54	2.09	3.41	7.46	
Hg (μg/L)	Total	260	2.57	1.90	6.54	<LoQ	1.18	1.93	3.20	5.97	
	Gender										< 0.001
	Women	107	2.19	1.53	10.28	<LoQ	1.00	1.63	2.39	4.97	
	Men	153	2.84	2.21	3.92	0.77	1.29	2.51	3.64	6.03	
Cd (μg/L)	Total	260	1.27	0.70	0	0.22	0.35	0.58	1.02	6.44	
	Gender										0.667
	Women	107	0.82	0.64	0	0.226	0.42	0.62	0.92	2.06	
	Men	153	1.58	0.76	0	0.22	0.32	0.54	1.56	6.92	
Cr (μg/L)	Total	176	0.41	0.36	7.39	<LoQ	0.28	0.39	0.52	0.76	
	Gender										0.124
	Women	77	0.43	0.37	7.79	<LoQ	0.31	0.40	0.56	0.76	
	Men	99	0.39	0.34	7.07	<LoQ	0.27	0.36	0.48	0.76	
Tl (μg/L)	Total	176	<LoQ	<LoQ	63.07	<LoQ	<LoQ	<LoQ	0.06	0.09	
	Gender										0.053
	Women	77	<LoQ	<LoQ	55.84	<LoQ	<LoQ	<LoQ	0.06	0.09	
	Men	99	<LoQ	<LoQ	68.69	<LoQ	<LoQ	<LoQ	0.05	0.07	

[a] Arithmetic mean (AM)

[b] Geometric mean (GM)

[c] Element units in bracket are for AM, GM, and percentile columns

[d] Percentage of values below the LoQ

trace elements necessitate the establishment of background levels for trace metals in the general population to promote public health [20]. In the current study, we have determined the blood concentrations of 12 essential and/or toxic metals and metalloids among 477 subjects of all ages living in Wuhan City of central China (Table 1). Cu, Zn, Ca, Fe, Mg, and Mn are classified as essential trace elements that are important for human metabolism even though their excessive accumulation in the body can be harmful. In contrast, Pb, Cd, Hg, As, Cr, and Tl are classified as nonessential or toxic trace elements.

Table 5 Blood metal levels in the population of Wuhan according to age categories (years)

Elements[c]	Age	N	AM[a]	GM[b]	Percentiles					*p*
					P5	P25	P50	P75	P95	
Fe (mg/L)										0.553
	0–19	73	487.22	484.93	413.47	461.26	486.87	506.31	571.54	
	20–29	99	494.84	491.38	407.70	449.60	499.67	535.06	591.04	
	30–39	134	488.37	485.38	404.95	445.35	488.95	527.41	568.63	
	> 40	155	495.90	492.52	412.25	458.75	490.90	538.44	576.25	
Ca (mg/L)										< 0.001
	0–19	73	60.40	60.13	52.36	56.00	60.53	65.04	68.10	
	20–29	99	56.07	55.78	48.58	51.95	57.12	59.21	65.69	
	30–39	134	55.93	55.57	46.173	52.01	55.44	59.91	66.43	
	> 40	149	56.93	56.58	46.30	52.89	57.16	60.80	66.28	
Mg (mg/L)										0.001
	0–19	73	41.75	41.51	35.40	38.31	41.33	45.10	49.28	
	20–29	99	39.08	38.78	31.82	35.68	38.59	42.03	47.90	
	30–39	134	39.10	38.83	31.74	35.91	39.04	41.93	46.16	
	> 40	149	39.75	39.46	31.80	36.84	40.22	42.42	47.64	
Zn (mg/L)										< 0.001
	0–19	73	5.36	5.27	3.74	4.75	5.30	6.13	6.68	
	20–29	99	6.02	5.91	4.16	5.42	5.84	6.73	7.75	
	30–39	134	6.00	5.91	4.53	5.30	5.91	6.58	7.86	
	> 40	155	6.16	6.05	4.69	5.54	6.10	6.82	7.82	
Cu (μg/L)										0.011
	0–19	73	828.77	817.01	667.64	717.07	794.32	881.53	1141.71	
	20–29	99	764.43	758.58	635.83	695.34	752.70	814.81	915.75	
	30–39	134	782.96	776.95	644.94	714.91	770.27	845.39	949.98	
	> 40	155	798.31	790.71	619.17	728.89	786.59	861.81	977.49	
Pb (μg/L)										< 0.001
	0–19	71	14.17	13.26	8.23	10.22	12.61	16.12	23.60	
	20–29	46	15.53	14.78	9.71	11.70	14.81	18.72	22.41	
	30–39	41	22.17	19.09	9.25	13.21	18.64	24.31	44.41	
	> 40	102	29.94	23.23	11.02	15.94	21.22	30.14	89.17	
Mn (μg/L)										0.008
	0–19	71	13.95	13.47	9.08	11.53	13.24	16.12	20.48	
	20–29	46	13.60	12.86	8.10	10.80	12.24	15.65	18.04	
	30–39	41	11.76	11.46	8.60	9.59	11.25	13.51	17.07	
	> 40	102	12.38	11.89	7.41	9.49	12.15	14.53	18.72	
As (μg/L)										0.001
	0–19	71	2.42	1.92	0.85	1.25	1.71	2.66	7.81	
	20–29	46	3.25	1.97	0.83	1.33	1.66	2.66	5.45	
	30–39	41	2.63	2.31	1.2	1.55	2.13	3.14	5.28	
	> 40	102	3.51	2.66	1.08	1.59	2.3	3.95	10.88	
Hg (μg/L)										< 0.001
	0–19	71	1.60	1.39	0.72	1.02	1.35	1.76	3.49	
	20–29	46	1.65	1.31	<LoQ	0.81	1.59	2.01	3.65	
	30–39	41	2.93	2.24	<LoQ	1.43	2.79	3.80	5.90	
	> 40	102	3.52	2.62	<LoQ	1.88	2.73	4.32	7.91	
Cd (μg/L)										< 0.001
	0–19	71	0.52	0.40	0.18	0.28	0.34	0.54	1.06	
	20–29	46	1.05	0.68	0.24	0.44	0.57	0.86	4.82	
	30–39	41	1.78	0.90	0.27	0.45	0.65	1.12	7.32	
	> 40	102	1.67	0.96	0.24	0.47	0.85	1.75	6.71	
Cr (μg/L)										0.66
	0–19	10	0.38	0.31	<LoQ	0.24	0.31	0.41	0.82	
	20–29	31	0.41	0.37	0.18	0.29	0.38	0.52	0.73	
	30–39	40	0.40	0.34	<LoQ	0.26	0.35	0.50	0.84	
	> 40	95	0.42	0.36	<LoQ	0.30	0.40	0.54	0.75	
Tl (μg/L)										0.764
	0–19	10	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	0.04	0.08	
	20–29	31	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	0.05	0.09	
	30–39	40	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	0.05	0.07	
	> 40	95	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	0.06	0.10	

[a] Arithmetic mean (AM)

[b] Geometric mean (GM)

[c] The measured units are only for AM, GM, and percentile columns

Table 6 Coefficient (above) and *p* value (below) for the Spearman correlation between elements in blood without considering age and sex

	Ca	Mg	Zn	Cu	Pb	Mn	As	Hg	Cd	Cr	Tl
Fe	−0.23	0.57	0.42	−0.08	0.29	−0.15	0.20	0.20	−0.05	−0.14	−0.15
	< 0.0001	< 0.0001	< 0.0001	0.0776	0.0386	0.2944	0.1495	0.1553	0.7222	0.3070	0.2806
Ca		0.16	−0.16	0.34	−0.11	0.23	−0.07	−0.25	0.24	0.06	0.13
		0.0004	0.0006	< 0.0001	0.4179	0.1000	0.6041	0.0681	0.0779	0.6618	0.3483
Mg			0.16	0.10	0.28	0.10	0.15	0.13	−0.31	−0.03	0.14
			0.0009	0.0376	0.0436	0.4661	0.2931	0.3643	0.0241	0.8564	0.3260
Zn				0.04	0.09	−0.03	0.21	0.14	−0.05	0.01	0.05
				0.4361	0.5080	0.8291	0.1311	0.3195	0.7147	0.9602	0.7259
Cu					0.12	0.22	−0.09	0.10	0.04	0.20	0.33
					0.3939	0.1095	0.5291	0.4579	0.7682	0.1450	0.0170
Pb						−0.01	0.25	0.36	0.31	0.02	0.04
						0.8655	< 0.0001	< 0.0001	< 0.0001	0.7701	0.5553
Mn							0.00	−0.05	−0.15	0.15	−0.08
							0.9696	0.4334	0.0168	0.0453	0.3126
As								0.46	0.11	0.02	−0.06
								< 0.0001	0.0839	0.8122	0.4252
Hg									0.23	0.10	−0.14
									0.0002	0.1738	0.0606
Cd										0.05	0.01
										0.4726	0.9327
Cr											0.23
											0.0022

Our results provided geometric means and percentile distribution of the 12 metals and metalloids examined for all the subjects (Table 4) and for the subjects of different genders and age groups, respectively (Tables 4 and 5). Our study revealed a gender-associated difference exists in blood Cu, Zn, Ca, Fe, Mg, Pb, As, and Hg concentrations and the age-related difference in all the 9 metal concentrations (Cu, Zn, Ca, Mg, Pb, As, Mn, Hg, and Cd) except for Fe, Cr, and Tl in the whole blood. Additionally, the inter-element correlation analysis among the blood concentrations of the 12 trace elements for all the subjects showed that 20 pairs of metals showed a significant correlation in the examined populations such as Fe–

Table 7 Comparison of geometry means of blood metals and metalloids in Wuhan residents with people living in other countries

	Chinese (Wuhan)	Chinese (Beijing) [14]	Korean [13, 23]	French [3]	Germany [17]	Italian [10]	Czech [11]	Brazilian [7]	Beninian [24]
Fe (mg/L)	488.98	–	–	–	–	–	–	–	468.70
Ca (mg/L)	56.66	–	–	–	–	–	–	–	–
Mg (mg/L)	39.44	–	–	–	–	–	–	–	27.69
Zn (μg/L)	5850.00	4665.00	–	5805.00	–	6418.00	–	–	4845.00
Cu (μg/L)	783.76	802.40	979.80	–	1020.00	1036.00	–	999.00	870.00
Pb (μg/L)	17.84	42.55	15.97	18.80	19.00	–	33.00	23.90	47.39
Mn (μg/L)	12.40	11.42	11.06	7.71	8.60	8.91	–	12.50	19.71
As (μg/L)	2.25	–	7.19	1.67	0.71	–	–	3.60	5.81
Hg (μg/L)	1.90	–	3.41	1.38	0.90	–	0.82	1.40	3.12
Cd (μg/L)	0.70	0.68	0.78	0.39	0.38	–	0.60	0.13	0.32
Cr (μg/L)	0.36	–	–	0.42		–	–	–	< 0.24
Tl (μg/L)	< 0.05	–	–	0.02	0.02	–	–	–	0.12

Mg, Fe–Zn, and As–Hg (Table 6). Details regarding the analytical quality and observations will be discussed below.

Analytical Quality Assurance

ICP-MS is widely used for trace element determination in human blood, tissue, and body fluid for clinical nutritional or toxicity testing and monitoring [21]. We established a ICP-MS-based method to simultaneously determine 12 essential and toxic metals and metalloids in human blood in the current study. The characteristics including limit of detection and quantification (LoD and LoQ), trueness, and repeatability were evaluated for the in-house method validation (Tables 2 and 3), which showed a good performance and could meet our clinical testing needs. Reference blood controls from SERO (Seronorm™ trace element blood) and NCCL (NCCL certified trace element material) were used. Considering the possible contamination from blood-collection tube and external environment, we using the metal-free tubes (BD Vacutainer Trace Element tubes) for blood collection and all sample preparations were processed in the Clean Bench.

Area Comparisons

Compared with other areas of the world, our results from populations living in Wuhan of central China seem to be mostly consistent (Table 7). Blood levels of Cu (783.8 μg/L), Zn (5850.0 μg/L), Mn (12.4 μg/L), and Cd (0.7 μg/L) in population of Wuhan were almost the same with those of Beijing (Cu 783.8 μg/L, Zn 4665.0 μg/L, Mn 11.4 μg/L, Cd 0.7 μg/L) in China [14], but the blood Pb levels (17.8 μg/L) were obviously lower than those in Beijing (42.6 μg/L), which implied a possible chronic lead exposure in that area [22]. Blood levels of Cu (783.8 μg/L), As (2.3 μg/L), and Hg (1.9 μg/L) were lower in Wuhan population when compared with those in Korean (Cu 979.8 μg/L, As 7.2 μg/L, Hg 3.4 μg/L) [23]. And when compared with European including French [3], Germany [17], Italian [10], and Czech Republic [11], blood levels of Mn, As, and Hg were much higher while Cu was lower in our study. There was a certain similarity for blood metal levels in our study with that in Brazilian of South America [7] and in Beninian of Africa [24], in which blood levels of Mn, As, and Hg were also higher than those of the European. In Brazilian and Beninian, blood Pb levels were much higher than those in our study.

Gender Specificity

Our results showed a total of eight metals and metalloids with a significant gender specificity, in which Cu and Ca levels (geometry averages) were higher while Zn, Fe, Mg, As, Pb, and Hg levels were lower in women ($p < 0.05$) (Table 4). Our observations for blood Cu (4.3% higher in women) and Zn (8.3% lower in women) levels were much consistent with the Chinese national survey (men 767 μg/L; women 822 μg/L) [25] and also consistent with some previous reports [10, 14]. It has been hypothesized that estrogen-induced ceruloplasmin synthesis in the liver may lead to an increased Cu in blood for female [26]. Blood levels of Pb were much higher (31.0%) in men, which has been reported by many previous studies [3, 7, 11, 13, 14]. Blood Hg level that was higher (30.0%) in men in our study was consistent with reports from that in Korean [23], while blood As level that slightly increased in men in our study showed almost no significant gender specificity in other previous reports. These results indicated that men showed more susceptibility of heavy metal exposure than women.

Age Influence

It is well known and frequently reported that trace metals accumulated and loss in the human body with age [20]. We found a total of nine metals and metalloids including Cu, Zn, Ca, Mg, Pb, Mn, As, Cd, and Hg showed significant age-dependent variation in our study ($p < 0.05$). Blood levels of Cu, Ca, Mg, and Mn all showed a high level at age less than 20 years and declined to a low level at age of 20–39, then rose again after 40 years old. While for Zn and heavy metals like Pb, As, Cd, and Hg, blood levels all showed an upward trend with age growing. Our findings that heavy metals increased along with aging have been reported by previous studies, in which Pb, As, Cd, and Hg basically shown a relative high level of blood in the elderly compared with those in the young people [7, 13, 23], but essential elements like Cu and Zn were reported of no significant difference with age.

Metal Correlations

The variation of certain trace elements may be affected by elevated exposure to other elements, either essential or not. Accordingly, highly statistically significant correlations ($p < 0.001$) between Fe–Mg ($r = 0.57$), Fe–Zn ($r = 0.42$), As–Hg ($r = 0.46$), Ca–Cu ($r = 0.34$), Pb–Hg ($r = 0.36$), Pb–Cd ($r = 0.31$), Pb–As ($r = 0.25$), and Ca–Fe ($r = -0.23$) were found in the present study (Table 6). The positive correlations of Fe–Zn, Fe–Mg, As–Hg, and Pb–Cd in blood have been reported previously [23, 27–29]. Fe and Zn have identical outer electron shell configurations and similar chemical nature [28], and Fe appears not to have a negative effect on serum zinc concentrations [30]. These may lead to the mutual dependence and the high positive correlations of the two metals. The negative correlation of Ca–Fe ($r = -0.23$) that found in our study was consistent with previous studies [31, 32], which may due to the significant inhibitory effect of Ca on Fe absorption [29].

Conclusions

This study provided the information regarding blood levels of 12 essential and/or toxic metals and metalloids in residents of Wuhan in central China. Blood levels of several elements such as Cu, Ca, Zn, Mg, As, Pb, and Hg showed a gender- and age-related differences. Inter-element correlations were found for Fe–Zn, Fe–Mg, As–Hg, and Pb–Cd. Thus, special care could be taken when analyzing these elements during biomonitoring studies. Our data will help establish the reference values for blood levels of these elements for the population in central China and shall be useful for future monitoring of occupational and environmental exposure.

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Compliance with Ethical Standards

Approval was received from Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology Review Board in Wuhan, China. All the procedures involving human samples conformed to the principles outlined in the Declaration of Helsinki.

Competing Interests The authors declare that they have no conflict of interest.

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