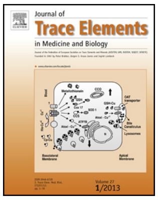
Journal of Trace Elements in Medicine and Biology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

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



Biomonitoring of 29 trace elements in whole blood from inhabitants of Cotonou (Benin) by ICP-MS

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ARTICLE INFO

Article history:
Received 30 August 2016
Received in revised form 25 October 2016
Accepted 4 November 2016

Keywords:
Benin
Biomonitoring
Blood donors
ICP-MS
Trace elements
Whole blood

ABSTRACT

This study aimed to investigate the blood concentration of 29 trace elements, metals or metalloids, in a healthy population of Cotonou not directly exposed to metals in order to propose reference values. Blood samples from 70 blood donors were collected in K2 EDTA tubes for trace elements during September 2015 and a questionnaire was used to assess lifestyle exposure. Blood metal concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS) equipped with a quadrupole-based reaction cell. Among the selected blood donors 51.4% were aged from 18 to 36 years and 49.6% from 37 to 65 years. Among the 29 elements analyzed As, Pb, Mn, Pd, Sb, Co, Se, Sr showed blood concentrations higher than the reference values found in the literature for non-exposed healthy European populations and their geometric means were respectively 5.81; 47.39; 19.71; 1.91; 7.50; 0.66; 163.01; 30.53 µg/L. This study provides the first reference value (5th–95th percentiles) for each element in Cotonou, which enables us to carry out further investigations on environmental and occupational exposure.

1. Introduction

Over the last twenty years, several studies have been conducted in Europe and particularly in developed countries to determine the usual biological concentrations of trace elements in the general population [1–7]. The determination of trace elements in whole blood presents increasing interest, as these values are important indicators of the health and are useful to assess the body burden of pollutants in non-exposed or occupational groups [8]. Among the methods used for the determination of heavy metals, inductively coupled plasma mass spectrometry (ICP-MS) is one of the most powerful tools capable of analyzing several different elements from the same sample with high analytical sensitivity.

Despite the growing number of biomonitoring studies on trace elements in the world, very few data are available concerning African countries. In Africa, urbanization, industrialization and fast population growth induce environmental pollution. Anthropogenic contamination is the main source of heavy metal pollution [9]. In Congo, Tuakuila et al., used ICP-MS spectrometry to show that urinary concentrations of trace elements from an urban area of Kinshasa were mostly higher than those found in rural areas or in the general population in other studies (American, Canadian, French or German) [10]. To our knowledge, apart from studies about blood lead level in semi-rural areas in Benin [11], no published studies have monitored the biological concentration of metals and metalloids in Benin's healthy general population.

The aim of this study was to determine the blood profile of 29 trace elements in a healthy general population of Cotonou (the economic capital of Benin) by ICP-MS to enable us to carry out further investigations.

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http://dx.doi.org/10.1016/j.jtemb.2016.11.004
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2. Materials and methods

2.1. Study population and sample collection

The population studied consisted of 70 healthy male blood donors, aged 18 to 65 years, and randomly selected using an *a priori* procedure with two exclusion criteria. Indeed people with a past or current occupational activity directly exposed to metals and those suffering from systemic diseases were not included. All the participants were of working age and spontaneous volunteers recruited at Cotonou's National Agency for Blood Transfusion. The study took place in September 2015 and authorization from the Ethics Committee of Benin's Institute of Applied Biological Sciences was obtained, as well as the participants' prior consent. A questionnaire was administered to obtain individual socio-anthropometric data about occupational activities (in an office or not), dietary habits (frequency of fish consumption and dietary supplements), traffic exposure, smoking habits and dental amalgams. Whole blood samples were collected in 6 mL plastic K2-EDTA (di-potassium ethylenediaminetetraacetic acid) containing tubes for trace elements (Vacutainer® Becton Dickinson, Le Pont du Clay, France) and carefully homogenized by manual agitation (8–10 inversions to ensure mixing of anticoagulant (EDTA) with blood). Within 2–3 h, blood samples were frozen at – 20 °C until they were flown to Limoges University (in optimal conditions with dry ice). The analyses were performed at Limoges within University Hospital's Department of Pharmacology and Toxicology.

2.2. Instrumentation

All measurements were carried out with a triple quadrupole NexION® 350D spectrometer (Perkin-Elmer, Courtaboeuf, France) equipped with an autosampler "ESI SC4 DX". The instrument features quadrupole-based Universal Cell Technology (UCT$^{\text{TM}}$), allowing the device to be run in three modes (standard, collision or reaction mode). Analyzes were performed in standard mode for 25 elements without any significant interference.

For arsenic ^{75}As, chromium ^{52}Cr, vanadium ^{51}V and iron ^{56}Fe, the assays were run in reaction mode with high-purity (>99.995%) ammonia as the reaction cell gas (Air Liquide Santé, Nantes, France). A triple nickel cone interface and a quadrupole ion deflector permit a tightly focused ion beam. The spectrometer was monitored with software Syngistix$^{\text{TM}}$ for ICP-MS. The sample introduction system was consisted of a quartz cyclonic spray chamber and a glass Type C nebulizer. Plasma torch argon purity (Linde Healthcare, Saint-Priest, France) was higher than 99.999%. The analytical conditions were optimized daily to obtain the highest signal-to-background ratio for ^{9}Be, ^{115}In, ^{238}U along with the ratios $^{140}\mathrm{Ce}^{16}\mathrm{O}^{+}/^{104}\mathrm{Ce}^{+} < 2.5\%$ and $^{140}\mathrm{Ce}^{2+}/^{140}\mathrm{Ce}^{+} < 3.0\%$. Further ICP-MS operating conditions are summarized in Table 1. Sample preparation and analysis were performed in an ISO class 6 clean room.

2.3. Reagents

Ultrapure de-ionized water was obtained using a Milli-Q® Integral 5 water purification system (Millipore SAS, Molsheim, France). All reagents used were of analytical-reagent grade. Blood samples (100 μL) were diluted 1:51 (v/v) with an aqueous solution containing 0.01% (v/v) Triton® X-100 (Sigma-Aldrich, Saint Quentin Fallavier, France), 0.1 mg/L NH_4OH, 0.1 g/L EDTA and 5 mg/L *n*-butanol (Merck, Lyon, France). The 29 certified standards from SPEX CertiPrep® (Stanmore, UK) as well as four internal standards (^{45}Sc, ^{89}Y, ^{115}In, ^{187}Re) were used to respectively ensure external calibration and correct for possible signal instabilities. To confirm the accuracy of the measurements, internal quality assessment Seronorm$^{\text{TM}}$ Trace Elements Whole Blood controls were obtained from SERO AS (Billingstad, Norway).

Table 1
Operating conditions for ICP-MS NexION® 350D.

Parameters	Settings
RF Plasma power	1600 W
Nebulizer gas flow (Ar)	1.05 L/min
Auxiliary gas flow (Ar)	1.20 L/min
Plasma gas flow (Ar)	15.0 L/min
Reaction gas flow (NH_3)	0.5 mL/min
Cell exit voltage	−3 V
Deflector and Detector voltage	−12 V
Sweeps per reading	20
Reading per replicate	1
Number of replicates	5
Detector mode	Dual
Scanning mode	Peak hopping
Integration time	1000 ms
Wash time	100 s

2.4. Elemental analysis

The screening method used for analysis allowed the simultaneous determination of the 29 following elements in whole blood: ^{7}Li (lithium), ^{9}Be (beryllium), ^{25}Mg (magnesium), ^{27}Al (aluminum), ^{51}V (vanadium), ^{52}Cr (chromium), ^{55}Mn (manganese), ^{56}Fe (iron), ^{59}Co (cobalt), ^{63}Cu (copper), ^{64}Zn (zinc), ^{75}As (arsenic), ^{82}Se (selenium), ^{88}Sr (strontium), ^{98}Mo (molybdenum), ^{103}Rh (rhodium), ^{105}Pd (palladium), ^{111}Cd (cadmium), ^{118}Sn (tin), ^{121}Sb (antimony), ^{138}Ba (barium), ^{140}Ce (cerium), ^{184}W (tungsten), ^{195}Pt (platinum), ^{202}Hg (mercury), ^{205}Tl (thallium), ^{208}Pb (lead), ^{209}Bi (bismuth), ^{238}U (uranium). The use of DRC with ammonia as the reaction gas was only mandatory to remove the polyatomic interferences for arsenic ^{75}As ($^{40}\mathrm{Ar}^{35}\mathrm{Cl}^{+}$), chromium ^{52}Cr ($^{40}\mathrm{Ar}^{12}\mathrm{C}^{+}$), vanadium ^{51}V ($^{35}\mathrm{Cl}^{16}\mathrm{O}^{+}$) and ^{56}Fe ($^{40}\mathrm{Ar}^{16}\mathrm{O}^{+}$). According to the recommendations of the IUPAC (International Union of Pure and Applied Chemistry) and adapted from Boumans' method [12] the LOQs (Limits Of Quantification) were determined by plotting the % RSD (Relative Standard Deviation) of the net signal against the concentration. A linear regression applied to the log–log transformed (% RSDnet) plot enabled the determination of LOQ as equal to 5% of RSDnet [13].

Internal quality control was carried out during analysis thanks to Seronorm$^{\text{TM}}$ L-3 (reference 1112691) blood samples to ensure that the measured values were within the target ranges. Following every five blood samples, one Seronorm control sample was tested to ensure quality throughout screening. The accuracy of the method was also verified by participation in the QMEQAS external quality assessment scheme (Quebec Multielements External Assessment Scheme). Reference materials produced by Quebec's National Institute of Public Health of (Canada) were analyzed as external quality control. For each result submitted to the QMEQAS committee, a z-score was calculated from unrounded results. Z score values within the +2 to −2 range were satisfactory and indicated analytical accuracy.

2.5. Data analysis

For each element, the distribution was examined and the 5th, 50th and 95th percentiles were determined. The log-transformed values were used to calculate the geometric means. For this determination, individual results below the limit of quantification (LOQ) were replaced by the (LOQ/2) value. In this context, when more than 60% of the samples showed concentrations below LOQs, then the result was given as an estimate rather than a value (the reference values proposed are only indicative, as below the LOQ). The

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Table 2
Whole blood results obtained with QMEQAS-certified reference material (Quebec Multielement External Quality Assessment Scheme) and Seronorm internal control.

QMEQAS 2015–02 External control						Seronorm L-3 Internal control		
Element	Units	Target Value	Measured	Z – Score	Range	Acceptable range (μg/L)	Mean	Standard deviation
^{9}Be	μmol/L	1.41	1.45	0.23	[1.02–1.80]	10.0–15.0	10.37	0.47
^{27}Al			na	na		84.0–126.0	92.35	13.19
^{51}V	nmol/L	20.20	14.90	−1.41	[12.7–27.7]	4.6–6.8	5.63	0.21
^{52}Cr	nmol/L	61.30	39.00	−1.70	[35.1–87.5]	18.5–27.9	22.98	0.80
^{55}Mn	nmol/L	309.00	362.00	1.40	[233.0–385.0]	37.8–56.8	38.61	12.94
^{59}Co	nmol/L	43.20	39.50	−0.73	[33.2–53.2]	10.2–12.6	11.19	0.17
^{63}Cu	μmol/L	54.20	53.50	−0.25	[48.2–60.2]	2220.0–2720.0	2294.51	50.30
^{64}Zn	μmol/L	85.20	71.90	−1.67	[69.3–101.0]	7280.0–10940.0	7459.54	146.99
^{75}As	nmol/L	266.00	247.00	0.56	[191.0–341.0]	23.1–37.7	31.19	0.46
^{82}Se	nmol/L	5.24	4.90	−0.67	[4.2–6.3]	217.0–327.0	234.02	4.95
^{88}Sr	nmol/L	248.00	229.00	−0.72	[196.0–300.0]	na	na	na
^{98}Mo	nmol/L	74.00	67.40	−0.77	[56.8–91.2]	6.0–9.0	6.72	0.24
^{111}Cd	nmol/L	26.60	22.20	−1.61	[21.2–32.0]	10.8–13.4	9.81	0.26
^{118}Sn	nmol/L	76.80	77.00	0.02	[56.3–97.3]	7.8–11.8	10.62	0.25
^{121}Sb	nmol/L	79.90	81.10	0.16	[64.6–95.2]	17.9–26.9	23.23	0.51
^{138}Ba	nmol/L	13.10	10.00	−1.15	[7.8–18.4]	na	na	na
^{195}Pt	nmol/L	9.39	8.82	−0.48	[7.0–11.8]	na	na	na
^{202}Hg	nmol/L	1010.00	946.00	−0.60	[797.0–1220.0]	29.6–44.6	30.55	0.54
^{205}Tl	nmol/L	32.70	30.80	−0.89	[28.5–36.9]	27.2–41.0	88.81	1.98
^{208}Pb	μmol/L	0.86	0.79	−1.16	[0.7–0.9]	401.0–493.0	406.16	10.04
^{209}Bi	nmol/L	50.70	43.00	−1.48	[40.3–61.1]	46.0–69.2	53.09	1.25
^{238}U	nmol/L	1.60	1.89	2.00	[1.3–1.9]	na	na	na

na: not attribuate.

non-parametric Mann-Whitney *U* test was used for comparison of different groups (based on age group, fish consumption frequency, car driving or not, white-collar work or not, dental amalgams or not) and the differences were considered to be significant when $p < 0.05$. Data were analyzed with SPSS.20 software [14].

3. Results

3.1. Population characteristics

According to our questionnaire, the mean age was 37.8 years (±11.4) while the median one was 36.4 years. Among the blood donors, 51.4% were aged 18 to 36 and 49.6% 37 to 65 years. As they were all over 18-years-old they were likely to have an occupation not directly exposed to metals. All the participants were nonsmokers and consumed fish or seafood on average 5.6 times per week (±1.8). Only one reported to be vegetarian (no fish consumption). Three participants (4.3%) mentioned dental amalgams. As regards traffic exposure 15.7% reported being car drivers versus 84.3% who used their own motor bike or a motorcycle taxi. Considering the type of work, 52.9% were classified as white-collar workers (working in an office setting).

3.2. Quality assurance

Accreditation of the laboratory according to the approved NF ISO 15189: 2012 international standard [15] ensured the technical competence and the existence of a management system of suitable quality. Table 2 shows 22 elements with results for reference materials (internal or external control). The following trace elements (Li, Fe, Mg, Rh, Pd, Ce, and W) are not mentioned in Table 2 as they were not proposed in the external QMEQAS controls and their Seronorm controls acceptable ranges were not certified by the suppliers. As we can see, the QMEQAS external control (Comparison Program from the National Institute of Public Health of Quebec in 2015 – February), was used and the Z score obtained (±2) indicate that our external quality control was satisfactory. For the internal Seronorm controls, the acceptable range limits were set by the control material supplier. The mean values measured for the Seronorm samples and standards deviation are shown in Table 2. The comparison between target values and measured values shows sufficient agreement for internal quality control assurance.

3.3. Sample analysis

The results for trace element levels in whole blood for our study population are summarized in Table 3. Sixteen elements (Mg, Fe, Mn, Co, Cu, Zn, As, Se, Sr, Mo, Rh, Cd, Sb, Hg, Tl, Pb) showed 100% of measured values above the LOQs. For 11 trace elements (Be, V, Cr, Rh, Sn, Ce, W, Pt, Tl, Bi, U), the geometric mean ranged from less than 0.001 to 0.2 μg/L. For six trace elements (V, Cr, Ce, W, Pt, and Bi), very low background levels were found (63 to 89% of their values were below the LOQs). The Reference Values (RV) proposed for these elements are indicative, respectively; (V, RV < 0.1 μg/L), (Cr, RV < 0.24 μg/L), (Ce, RV < 0.010 μg/L), (W, RV < 0.002 μg/L), (Pt, RV < 0.001 μg/L), (Bi, RV < 0.010 μg/L).

For seven other trace elements (Li, Be, Al, Pd, Sn, Ba, U) 4% to 36% of the measured values were below the limit of quantification.

4. Discussion

In Africa, very few studies aiming to determine the concentrations of trace elements in whole blood have been carried out by ICP-MS because of the scarcity of equipment, high cost analyses and pre analytical considerations. The population surveyed in this study is an urban population of a seaport (Cotonou), located on the Atlantic seaside and crossed by a lagoon. This geographical location is a special situation in terms of eating habits.

4.1. Pre-analytical and analytical considerations

A great deal of attention was paid to the pre-analytical and analytical conditions. The use of specific tubes for trace elements (polyethylene terephthalate tubes) allowed to reduce the risks of metallic contamination (by sustained release) found with the glass tubes [16]. However as already noted by some authors, a sporadic contamination by manganese, cobalt or chromium may occur, caused by the stainless steel venepuncture needles [17,18]. The implementation of the cold chain allowed to guarantee the preser-

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Table 3
Whole blood ICP-MS statistical data about 29 elemental concentrations in 70 volunteer blood donors.

					Percentiles		
Elements	LOQ	Mean arythmetic (µg/L)	Geometric mean[a]/(µg/L)	% <LOQ	5%	50%	95%
^{7}Li	0.100	0.474	0.401	4.3	0.120	0.466	0.850
^{9}Be	0.010	0.048	0.020	34.0	<0.010	0.020	0.196
^{25}Mg	1.000	27858.0	27691.0	0.0	23421.0	27638.0	34059.0
^{27}Al	0.410	3.726	1.411	36.0	<0.410	1.932	10.040
^{51}V	0.100	<0.098	<0.098	83.0	<0.100	<0.098	0.160
^{52}Cr	0.240	0.325	<0.240	63.0	<0.240	<0.240	0.990
^{56}Fe	24.500	472457.0	468704.0	0.0	386861.0	476011.4	553716.0
^{55}Mn	0.100	19.936	19.711	0.0	15.700	19.215	25.140
^{59}Co	0.010	0.747	0.673	0.0	0.360	0.653	1.300
^{63}Cu	0.240	874.925	870.000	0.0	720.0	873.4	1027.0
^{64}Zn	2.150	4937.580	4845.446	0.0	3684.0	4862.6	6668.0
^{75}As	0.020	6.126	5.810	0.0	3.520	5.703	10.550
^{82}Se	0.100	165.378	163.013	0.0	123.0	163.4	205.0
^{88}Sr	0.010	31.792	30.531	0.0	21.170	30.691	48.420
^{98}Mo	0.010	1.205	0.912	0.0	0.370	0.814	3.160
^{103}Rh	0.001	0.044	0.044	0.0	0.032	0.044	0.053
^{105}Pd	0.500	1.563	1.027	26.0	<0.500	1.365	3.790
^{111}Cd	0.020	0.356	0.319	0.0	0.150	0.331	0.630
^{118}Sn	0.100	0.257	0.211	9.0	<0.100	0.239	0.480
^{121}Sb	0.100	7.536	7.500	0.0	6.280	7.416	8.940
^{138}Ba	0.240	0.433	0.309	39.0	<0.240	0.326	0.990
^{140}Ce	0.010	<0.010	<0.010	79.0	<0.010	<0.010	0.030
^{184}W	0.002	<0.002	<0.002	63.0	<0.002	<0.002	0.143
^{195}Pt	0.001	<0.001	<0.001	66.0	<0.001	<0.001	0.005
^{202}Hg	0.020	3.762	3.120	0.0	1.110	3.279	7.640
^{205}Tl	0.001	0.142	0.123	0.0	0.050	0.129	0.270
^{208}Pb	0.100	49.487	47.390	0.0	29.370	47.734	74.780
^{209}Bi	0.010	<0.010	<0.010	89.0	<0.010	<0.010	0.015
^{238}U	0.001	0.004	0.002	21.0	<0.001	0.004	0.008

[a] Concentrations below the limit of quantification (LOQ) are replaced by the LOQ/2 value in order to calculate the geometric mean.

vation of the samples from their sampling in Benin up to their analysis in Limoges University, France.

As mentioned by Hall et al., oxygen (O_2) can be used as a reagent rather than ammonia, for blood arsenic determination [19]. However, our results for arsenic determination using ammonia as a reagent was correct regarding the results of the external QMEQAS quality controls (Table 2). For selenium determination, the most abundant isotope, ^{80}As (49.8% natural abundance) showed major interference with Ar_2 dimer ($m/z = 80$). However, blood selenium can be measured accurately in standard mode at mass 82 and in reaction mode at mass 78. In our method, we determined the ^{82}Se isotope in standard mode [20]. The ^{111}Cd isotope was chosen over the more abundant ^{114}Cd to avoid potential isobaric interference from ^{114}Sn. Judging from the external QMEQAS quality controls (Table 2), the potential polyatomic interference (^{95}Mo^{16}O) does not require using the reaction mode for this element. For palladium we chose to analyze the most abundant (22.23%) free isotope ^{105}Pd. However this element can present polyatomic interferences (^{65}Cu^{40}Ar; ^{89}Y^{16}O; ^{88}Sr^{17}O; ^{88}Sr^{16}O^{1}H) which could increase the signal measured at $m/z = 105$ [21]. Feasible ways are shown to reduce these interferences [22]. As the expected values pose a challenging tasks to the analyst, the assessment of reference values for this metal is problematic. For elements which are present in very low concentrations in biological fluids, such as platinum, it would be worth using Sector Field Inductively Coupled Plasma Mass Spectrometry as it offers better detection power and sensitivity [22].

The participation in inter-laboratories trials and the use of internal Seronorm control before and after every series of 5 samples permitted to guarantee the accuracy of our results (see Table 2).

4.2. General observations

We found a positive association between age, fish consumption and Cu, Se, and Hg blood levels. In the group the over 37-year-olds (79.4%) consume fish more than 4 times per week while only (50%) of the under–37 s usually consume fish. Significantly higher blood levels for copper (Cu, p = 0.014), selenium (Se, p = 0.011), and mercury (Hg, p = 0.002) in the over-37 year-olds could be explained by the cumulative and additional exposure to these elements due to their almost daily fish consumption. Moreover, considering age groups, significantly higher levels were found for cadmium (Cd, p = 0.007), and thallium (Tl, p = 0.02).

The results according to age groups and socio-professional categories of our study sample are representative of Cotonou's 18-to-65 year-old male population [23]. Considering age groups, significantly higher levels were found. Significant difference (p = 0.035) was found for mean cadmium concentration in blood, between white-collar workers (0.3 µg/L ± 0.12) and blue-collar workers (0.4 µg/L ± 0.2). As for traffic exposure, the car drivers (mainly people ≥ 37 years) had a blood mercury level twofold that of motorbike drivers (6.8 ± 4.1 µg/L versus 3.19 ± 1.6 µg/L). As people with dental amalgam were very few (<5%), no further statistical analyses were attempted for this group. For all the other trace elements analyzed, no significant differences were found according to age, socioprofessional job or traffic exposure. Some studies have been conducted on manganese and blood lead in South Africa [24,25] and sub-Saharan Africa [26], as well as among post-partum women in Benin. The Table 4 compares the results of our study with those in the literature (geometric mean, 5th to 95th percentiles).

4.3. Essential elements

The essential elements are naturally present in the body. Biomonitoring of these essential elements presents an interest since their deficit or their excess in the body can be responsible for diseases [27]. Concerning manganese, cobalt and palladium, the geometric means found are above the 95th percentiles found in the other studies carried out in unexposed and healthy general popu-

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Table 4
Comparison between Cotonou's population and other studies for essential trace elements in whole blood (expressed in µg/L) from non-occupationally-exposed population.

Reference	Country	n	Parameter	Mn	Se	Cu	Zn	Co	Pd
Our study	Benin	70	GM	19.7 (15.7–25.1)	163 (123–205)	870 (720–1027)	4845 (3684–6668)	0.67 (0.36–1.30)	1.02 (<0.5–3.79)
[36]	Brazil	890 males	GM (5th–95th)	12.8 (6.1–28.4)	–	–	–	–	–
[37]	Congo	100 children	(5th–95th)	(5.7–22.2)	(85–136)	(900–1700)	(3400–6900)	–	–
[6]	Germany	130	GM (5th–95th)	8.6 (5.7–14.6)	132 (105–164)	1020 (804–1620)	–	<0.14 (0.05–0.41)	0.01 (<0.02–0.029)
[3]	France	100	(5th–95th)	(5–12.8)	(89–154)	–	–	(0.04–0.64)	(0.01–0.71)
[35]	Italy	104 males	GM (5th–95th)	8.0 (4.6–14.2)	141.00 (111–185)	957 (769–1200)	6804 (5056–8956)	–	–
[34]	USA		Reference	(4–15)	–	–	–	–	–

n: numbers of subjects in the study; GM: geometric mean.

lations. Thus, for these three elements, the reference values in the Beninese population are above the usual values known in the literature (See Table 4). For selenium, copper and zinc, the results found are within the literature's reference limits, but selenium concentrations are close to the 95th percentile found in other studies, when copper and zinc levels are close to the 5th percentiles (see Table 4).

Se (Selenium): It is an essential nutrient necessary for good health with the particularity of having a relatively narrow range of consumption. Inorganic selenium consumed through drinking water could be toxic. Epidemiological studies reassessing the safe upper limit in drinking water suggest reduction to 1 µg/L in order to adequately protect human health [28]. According to the reference values of selenium in drinking water proposed by the European Union (below 10 µg/L) and as recommended by recent epidemiologic studies, the selenium concentration in tap water in Cotonou is less than 0.25 µg/L [29]. A correlation was found between blood selenium concentration ($p = 0.0004$) and fish consumption frequency. Indeed, as mentioned by Fox et al., fish represents one of the main dietary selenium sources due to the bioavailability of this element in the flesh of fish [30]. Our results are similar to those obtained in a group of electronic waste recycling workers in Agbogbloshie (Ghana) (mean = 164 ± 49 µg/L in Ghana versus 165 ± 28 µg/L) [31], suggesting that higher blood selenium levels could be specific to the West African coastal towns. In addition, the Maize paste (owo) which is one of the daily most common grain preparation could be another source of dietary selenium in urban adults in Benin, as mentioned in a study in Malawi [32,33].

Mn (Manganese): Concerning blood manganese, a single sample in our study was within the reference limits proposed by the Agency for Toxic Substances (4–15 µg/L) [34]. Our results (Geometric mean = 19.71 µg/L) were higher than those obtained in an Italian study (8.01 µg/L) [35] or in a German study (8.6 µg/L) [6], but comparable to those obtained in a recent study in Brazil (see Table 4). The authors of the latter study concluded that the blackskinned and non-smoking people of this country might have blood manganese above 17.88 µg/L [36]. In Congo, concentrations from 5.7 to 22.2 µg/L (5th–95th percentiles) were found for children younger than 6 months-old [37]. In Nigeria (located east of Benin), high manganese concentrations were also found (28 µg/L) in healthy adults and could be due to high manganese contents in the soil of this region [38]. In Benin, the analysis of atmospheric fine particles in Cotonou in 2010 showed values superior to those set by the World Health Organization in the ambient air of Cotonou. The concentrations in manganese in the ambient air were on average 68 ng/m^3 [39,40] and superior to those found in Canada (7–19 ng/m^3) [41]. Environmental pollution, air erosion of dusts or soil pollution contributes to human manganese exposure via inhalation and are factors associated to a high rate of blood manganese [36,42]. Except these environmental risks, a sporadic contamination due to the stainless steel venepuncture needles cannot be excluded [17].

Zn (Zinc): Our results concerning zinc (geometric mean: 4845 µg/L) were low compared to those found in Bocca's Italian study [35], but still within the usual values (5th to the 95th percentiles) suggested in Goullé's French study [3]. The low values observed in our study could be explained by a low-zinc food supply, just as it has been shown that sub-Saharan Africans [43] and children in a rural area of Northern Benin may present this type of deficiency because of a low zinc supply compared to physiological needs [44].

Mo (Molybdenum): With variations from one author to another, the reference range (5–95th percentiles) proposed for Mo is (1.02–6.03 µg/L) in the Italian population [1], and (0.78–1.13 µg/L) for the (25th–75th percentiles) in the Swedish population [7]. These values are close to the values measured in our study (5–95th percentiles) (0.37–3.1 µg/L). Molybdenum concentrations in air are higher in urban areas than in rural areas. In Cotonou, molybdenum levels in drinking water are within acceptable ranges (<0.7 µg/L) [29], and the concentration of molybdenum in sea water is approximately 0.01 mg/L [45]. These observations suggest that the molybdenum concentration in blood could be related to the geographical location of Cotonou and the exposure to ambient air, soil, or legumes.

Co (Cobalt) and Pd (Palladium): These two essential elements show respective geometric mean concentrations of 0.67 and 1.02 µg/L which are above the usual values found in the European studies (see Table 4). For palladium, the high level found in our study could come from the polyatomic interferences, as mentioned in the sub chapter Pre-analytical and analytical considerations. For cobalt, no particular exposure seems to explain the observed difference.

4.4. *Toxic trace elements*

Metal pollution in Benin represents a particular environmental problem and numerous works have been led about anthropogenic pollution. Atmospheric pollution, spraying-waters, and ground pollutants can reach sufficient levels to contaminate the species consumed by people (fish and vegetables) [9,46,47]. In our study the average blood concentrations in lead, arsenic, strontium, mercury were within the normal range, but tended towards the upper limits (95th percentiles) found in the literature (Table 5). Blood cadmium alone showed values very similar to those found in the literature for the non-smokers.

Pb (Lead): The average blood lead level in our study (49.5 µg/L) was comparable to those obtained among 225 Beninese mothers (mean = 51.4 µg/L) [11], but slightly higher than those found in the study of Heitland [6] and Goullé [3] in German and French populations respectively (see Table 5). Air pollution in Cotonou comes from transport, waste and industries combined. In addition to this baseline exposure we noted that – the ban on leaded gasoline in

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Table 5
Comparison between Cotonou's population and other studies for toxic trace elements in whole blood (expressed in µg/L) from non-occupationally-exposed population.

Reference	Country	n	Parameter	As	Hg	Pb	Cd	Sr	Sb
Our study	Benin	70	GM (95th) percentiles	5.81 (10.55)	3.12 (7.64)	47.39 (74.78)	0.32 (0.63)	30.53 (48.42)	7.50 (8.94)
[11]	Benin	225 Mothers	(5th–95th) percentiles	–	–	(36.50–60.10)	–	–	–
[36]	Brazil	890 Males	GM (95th) percentiles	4.25 (9.61)	–	–	0.08 (0.83)	–	–
[49]	Tunisia	350	(5th–95th) percentiles	0.01–6.79	–	–	0.01–2.31	–	–
[6]	Germany	130	(5th–95th) percentiles	0.16–2.3	0.2–3.3	8–47	0.12–1.9	11–39	<0.013–0.04
[3]	France	100	(5th–95th) percentiles	2.6–17.8	0.94–8.13	11.4–62.8	0.15–2.04	9–41	0.05–0.13
[1]	Italy	110	(5th–95th) percentiles	–	1.97–14.50	12.80–79.50	0.25–1.97	–	–
[59]	Canada	2576 Males	GM (95th) percentiles	–	0.68 (5.60)	15.0 (42.0)	0.30 (3.40)	–	–

n: number of subjects in the study; GM: geometric mean.

France and Germany before the 2000s, contributed to lower the lead induced atmospheric pollution, but in Benin this ban occurred only in 2005; – the Convention on the prohibition of white lead in paint adopted in Geneva on October 1921, was not ratified by Benin until December 1960, while in France this rule has been implemented since 1948. Then the relatively high concentration found in Cotonou's population could be related to environmental pollution and to the consumption of lead-contaminated food [46–48].

As (Arsenic): The result for blood arsenic in our study (5.70 µg/L) was higher than the average found in a study in Germany [6]. However it should be highlighted that this geometric mean in the Beninese population remains within the acceptable limits in comparison to the French [3], Tunisian [49] and Brazilian [36] studies (see Table 5).

Sb (Antimony): The mean blood concentration in our study (7.5 µg/L) was much higher than that observed in Heitland's study (Table 5) [6]. Due to the density of the traffic in Cotonou city, environmental pollution coming from the fallout particles emitted into the atmosphere by planes, car and motorbike admittedly reaches vegetation and soils [50,51]. As antimony is mostly emitted as oxides from traffic and could easily concentrate in diverse vegetables before being ingested [52], the higher levels found in our study could be related to environmental pollution. However, the few studies conducted on antimony in the environment in Benin do not allow us to identify exposure risks with greater certainty.

Sr (Strontium): The geometric mean for blood strontium in our study was 30.53 µg/L, which is almost twofold that found in the German population (19 µg/L) [6]. However, if the (5th to 95th percentiles, 21–48 µg/L) values are considered as reference values, our result is consistent with the limits suggested by Heitland's study in Germany [6], Alimonti's in Italy [1] and Goullé's in France [3]. Strontium is widely distributed in the earth's crust and oceans and is considered as having a low toxicity. Small amounts of strontium are ubiquitous in the environment and humans can be exposed through the inhalation of aerosols and the ingestion of food (vegetables or fish) and drinking water [53].

Hg (Mercury): The regular consumption of fish and other sea products is responsible for an increase in the blood content in mercury as shown in Wilhem's study [5]. In our study, people who consume fish more than 4 times per week ($N = 45$) show higher blood mercury than people who consume it less than 4 times (mean = 4.07 µg/L versus 3.16 µg/L). For all participants, the geometric mean for blood mercury (3.12 µg/L) was lower than the 95th percentile found in German, French, Italian and Canadian studies (see Table 5). Regarding road traffic exposure, we noticed that the mercury level in the blood of those who reportedly rode by car (6.8 µg/L ± 4.1) was significantly different ($p = 0.0012$) from that of those who did not travel by car (3.2 µg/L ± 1.6). After analysis it was found that age played an important role in this result because the oldest had higher rates of blood mercury and were also mostly car-owners.

Cd (Cadmium): We found a significant higher level in the elderly participants (Cd, $p = 0.007$). As our study participants were non-smokers, this situation could be related to the cumulative nature of cadmium [54] and exposure via food consumption. As noted by Koumolou et al., there is a potential risk of exposition to this toxic through regular consumption of vegetables in Benin [46]. However the geometric mean in our study (0.32 µg/L), was within the reference values proposed in a review study by Herber et al. (below 0.8 µg/L) for white-collar workers [55] and similar to the geometric mean found for non-smokers, in studies by Becker et al. (0.28 µg/L) and Heitland et al. (0.29 µg/L) [6,56].

Be (Berylium): The geometric mean found in our study was (GM = 0.02 µg/L) versus (GM < 0.03 µg/L) in a French study [57] and (GM < 0.008 µg/L) in a German study [6]. The general population is exposed to trace amounts of beryllium through inhalation of air, cigarette smoke and ingestion of drinking water and food. One of the major anthropogenic emission sources for the environment is the combustion of coal which releases particulates and fly ash that contain beryllium into the atmosphere [58]. As the participants were no smokers and beryllium in drinking water in Cotonou was within the low range (<0.125 µg/L), this study suggests the possible involvement of other sources of beryllium described in the literature like the use of charcoal for cooking or food contamination. There are no data on exposure to this metal in the general population in Benin.

4.5. *Perspectives and limitations of the study*

This study will serve as a reference to identify specific at-risk groups for occupational and environmental exposure in the general population. These reference data (5th–95th percentiles), however, remain limited to the 18–65 year-old male population in Cotonou, representing a first stage in the determination of reference values. The limited number of participants did not allow to highlight the cumulative effect of certain toxic metals such as lead. From this study in Cotonou, it will from now on be possible to detect occupational exposures earlier by comparing certain toxic trace element concentrations with the 95th percentile values of the present study. Furthermore, among the essential trace elements presented in this study, it will now be possible to identify the deficiencies in essential trace elements poorly known (when the values found are lower than the values of the 5th percentiles).

5. Conclusion

Among the 29 elements analyzed, three essential trace elements (manganese, cobalt and palladium) had blood concentrations higher than the reference values found in the literature for non-exposed healthy populations. For toxic trace elements, the mean blood levels for arsenic, mercury, lead, and strontium were within the normal range proposed by other studies, but tended towards the upper limits (95th percentile) found in the literature. Some differences in trace elements reference blood level could be due to environmental pollution and eating habits. This study provides the first reference values for trace elements in Cotonou, which enables

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us to carry out further investigations on environmental and occupational exposure. For the next biomonitoring studies on Cotonou's male population, the measures equal to or above the 95th percentile should be considered as indicators of exposure.

Acknowledgements

This investigation was funded within the financial support of the Région du Limousin, the Limoges University and the Faculty of Health Sciences of Cotonou. The authors are grateful to all the team of ANTS in Cotonou (National Agency for Blood Transfusion). The authors would like to thank Sylvie Gautier for proofreading this paper.

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Please cite this article in press as: B. Yedomon, et al., Biomonitoring of 29 trace elements in whole blood from inhabitants of Cotonou (Benin) by ICP-MS, J Trace Elem Med Biol (2016), http://dx.doi.org/10.1016/j.jtemb.2016.11.004