



Speciated and Total Urinary Arsenic Levels in Belo Horizonte, the Largest Brazilian City within the Mineral-Rich Region “Iron Quadrangle”

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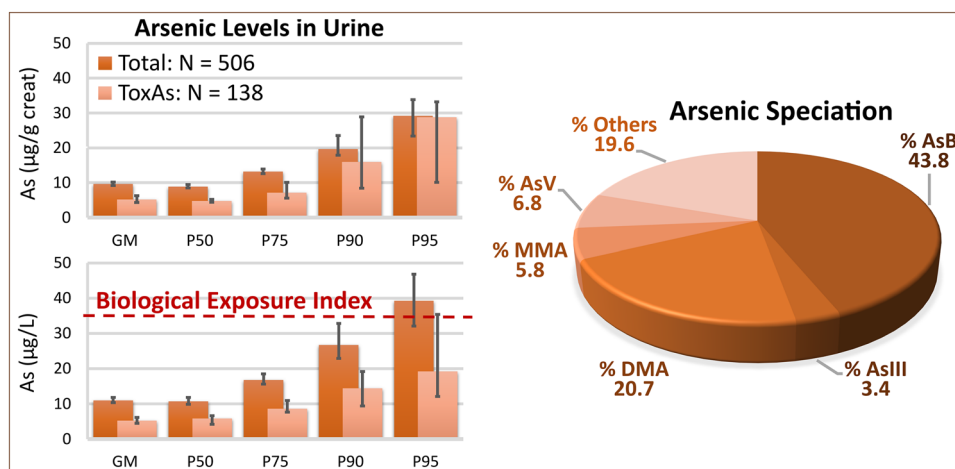
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Abstract

The “Quadrilátero Ferrífero” (Iron Quadrangle) is a mineral-rich province in Brazil, where arsenic anomalies are associated with gold mining and tailings dam failures. Urine samples were randomly collected from residents of Belo Horizonte (BH) city ($N=506$ total/138 speciation) and the metropolitan area ($N=35$ total/10 speciation), with stratifications in age, female-male and geopolitical regions. Creatinine, acid-digested total arsenic (TAs), and five inorganic and organic arsenic species were measured: arsenobetaine (AsB); arsenous (AsIII) and arsenic (AsV) acids and their dissociation products; monomethylarsonic acid (MMA); and dimethylarsinic acid DMA). Overall, the TAs GM ($\mu\text{g/g creat}$) of all stratification groups indicated no statistical difference. The TAs geometric mean (GM) of BH was 9.68 [9.17–10.2] $\mu\text{g/g creat}$, being AsB (43.8%) and DMA (20.7%) the major components. The toxic arsenic (ToxAs) fraction (i.e., AsIII + AsV + MMA + DMA) was 5.23 [4.45–6.15] $\mu\text{g/L}$. These TAs and ToxAs values are lower than international benchmarks and the Brazilian legislated standard of 35 $\mu\text{g/L}$ for ToxAs. The TAs GM ($\mu\text{g/L}$) and creatinine were 36% higher for males than for females ($p < 0.0001$), making TAs GMs comparable, after creatinine correction. Similarly, though the 10–19 age group showed significantly higher ToxAs GM (12.7 [6.49–24.8] $\mu\text{g/L}$) ($p = 0.048$) and creatinine ($p < 0.001$), the ToxAs GM (7.22 [2.92–17.9] $\mu\text{g/g creat}$), was not statistically different than those from the other age groups. The study, the first one in Brazil, identifies the relative contribution of inorganic and organic arsenic and provides a baseline level for clinical reference and bio-monitoring studies, particularly applicable to both occupational and non-occupational populations in a mineral-rich region.

Graphical abstract



Keywords Urine arsenic references · Speciation · Environmental biomonitoring · Non-occupational exposure · Anomalous arsenic

Introduction

Arsenic (As) is ranked number one in the USEPA toxic substance priority list (ATSDR 2019) followed by lead and mercury. It is known that chronic exposure to As can cause cancers, and other non-cancer diseases such as diabetes mellitus, vascular and neurological diseases, hypertension, renal failure, neurological development in children and adverse birth outcomes, and genetic alterations (WHO 2001; IRIS 2003; ATSDR 2007; IARC 2012).

Arsenic is very common in nature in trace amounts, but it can be found in elevated concentrations in mineralized areas. Arsenic-bearing minerals, such as arsenian (As-rich) pyrite (FeS_2), and arsenopyrite (FeAsS) are generally found in gold deposits and associated with sulfides of copper, nickel, antimony, and silver, among others (Smedley and Kinniburgh 2002). Sedimentary iron and manganese ores, as well as phosphate deposits, occasionally contain arsenic levels up to 2900 mg/kg, which can be emitted into the atmosphere from natural and anthropogenic events (WHO 2001).

Population exposure to natural or anthropogenic arsenic anomalies is a matter of concern worldwide. For non-occupational exposure, the sum of inorganic arsenic (iAs) concentrations and its methylated metabolites (MMA and DMA) in urine is usually less than 10 $\mu\text{g/g}$ creat (WHO 2001). Conversion of the result from $\mu\text{g/L}$ to $\mu\text{g/g}$ creat is aimed to reduce the interference of urine dilution, which may cause a change in the actual concentration.

Different reference TAs values have been reported around the world—the European Countries: below 10 $\mu\text{g/L}$ (Buchet et al. 1980); Germany: a mean of 7.17 $\mu\text{g/g}$ creat with a standard deviation of 19.7 (Heinrich-Ramm et al. 2001); Italy: mean of 16.7 $\mu\text{g/L}$, min/max 1/64.5 $\mu\text{g/L}$ (Minoia et al. 1990); United Kingdom: mean of 12.3 $\mu\text{g/L}$ min/max 0.9/1080 $\mu\text{g/L}$ (White and Sabbioni 1998). USA: about 10 $\mu\text{g/L}$ (CDC 2021); USA: Mayo Medical Laboratories in random urine: below 20 $\mu\text{g/g}$ creat or $\mu\text{g/L}$ (Mayo 2021). Mexico: mean for children of 40.28 $\mu\text{g/g}$ creat with a standard deviation of 18.20 to 70.29 $\mu\text{g/g}$ creat (Calderón et al. 2001). Studies carried out in Slovenia and Germany with adults and infants, indicate the value of 15 $\mu\text{g/L}$ as a base reference for the population without previous consumption of seafood for at least 2 days (Tratnik et al. 2019; Schulz et al. 2011). Since 2022, there is no legislation in Brazil for arsenic threshold and reference levels for non-occupational exposure. Before that, the reference level was 10 $\mu\text{g/g}$ creat (Brasil 1994).

The city of Belo Horizonte (BH) is in a mineral-rich province, where gold extraction has occurred for more than two centuries. Arsenic anomalies, associated with long-lasting gold mining, were reported in soil and water, besides high levels in human biological fluids in the neighboring regions (Matschullat et al. 2000). More recent findings of anomalous arsenic concentrations in superficial water were reported, following two disasters (2015 and 2019) in iron ore operations located 150 and 50 km far away from BH (Oliveira and Carvalho 2021).

The above reasons motivated the first investigation of arsenic levels in the BH population with the aim to provide a non-occupational baseline in a mineral-rich region of Brazil. It is important to note there are few studies in the country, which reported only TAs, mostly in blood, or urine, but without As speciation (Freire et al. 2015; Lopes et al. 2019). Total arsenic analysis in all matrices, including urine, serves as a first screening method so that the investigators/regulatory agencies can focus on which samples (e.g., threshold concentration)/areas will require further studies. This is particularly pertinent to areas where specialist speciation laboratories are not readily available. Nevertheless, speciation is the ultimate method when assessing exposure. Levels of urinary creatinine, total acid digest arsenic (TAs), and five arsenic species (arsenobetaine, DMA, MMA, and inorganic AsIII and AsV) were determined in representative sampling in the BH population. The results are compared with the international indexes. The establishment of a specific baseline level of As is important for environmental exposure comparisons and biomonitoring studies in Brazil and perhaps elsewhere. Belo Horizonte is unique considering the features of a large metropolitan area close to the mineralized areas. The differences between males and females, among age groups, regions of the city, and the metropolitan area are also analyzed by considering TAs and ToxAs (defined as the sum of MMA, DMA, and inorganic As). To the best of the authors' knowledge, this is the first epidemiological study of arsenic exposure in a large metropolis in Brazil, with a representative sampling of its population reporting TAs and speciated ToxAs, in the urine.

The Site

Belo Horizonte is a metropolis with the sixth-largest population of about 2.7 million people in Brazil (IBGE 2010a). The city is in the south tropical zone, approximately 450 km from the sea. Due to the elevation (852 m), temperatures are mild throughout the year ranging from 11 to 31 °C, with dry winter and humid summer seasons. The average annual precipitation is about 1200 mm. The BH metropolitan area comprises 34 municipalities and a population of 6 million people. The metropolis has part of its territory within the

boundaries of “Quadrilátero Ferrífero”, a mineral-rich (gold and iron ores) region of approximately 7,000 km² hereafter referred to as Iron Quadrangle (IQ). The IQ region is well-known for its mining activities, mostly represented by gold, which goes back to the eighteenth century, and intensive production of iron ore, especially over the last 80 years. Although there are no mining operations in BH urban area, the city is adjacent to those areas with no uninhabited geographic barrier between them.

High concentrations of arsenic in the soil, groundwater, and surface water have been reported in neighboring municipalities (Borba et al. 2000). Median values (and range) of As concentration in soils of 960(16–13,400), 100(13–467), and 53(16–80) mg/kg were detected in Nova Lima, Santa Barbara e Mariana, respectively (Deschamps et al. 2002). Concentrations as high as 21,000 mg/kg As and 1,700 mg/L As have been found in mining tailings and water samples, respectively (Teixeira et al. 2020). Other studies showed arsenic concentrations between 30 and 255 mg/kg in stream sediments (de Vicq et al. 2015), 78.1 and 85.3 µg/L (Silva et al. 2018), and 57.7 and 414 µg/L (Costa et al. 2015) in surface water samples.

In a study at Nova Lima, urine samples from 126 children living close to a gold mine’s waste disposal areas indicated TAs mean of 25.7 µg/L (min/max: 2.2 to 106 µg/L) (Matschullat et al. 2000). In Nova Lima, Campolina et al. (2007) found that the TAs median of 49 exposed residents to tailing deposits dropped significantly ($p < 0.001$) from 25.30 µg/g creat (min/max 20.22/51.56) in 2003/2004 to 10.74 µg/g creat (min/max 1.89/40.98 µg/g creat) in 2007 after the mitigation actions described by Lima et al. (2006), Alves et al. (2008), and Castro et al. (2011).

In addition to the arsenic directly associated with gold mining, two tailing dam failures caused major environmental impacts and deaths. In 2015, the iron ore tailings released from the impoundment caused devastation along 500 km until reaching the Atlantic Ocean. In 2019, a second disaster involving the collapse of an iron ore tailings dam led to over 270 deaths. Anomalous arsenic concentrations in the water and sediments were reported following the disasters (Oliveira and Carvalho 2021).

The drinking water supplied to the city of BH shows concentrations below 0.7 µg/L (COPASA 2021), considerably lower than the Brazilian maximum permitted limit of 10 µg/L in water for human consumption (Brasil 2021), a threshold aligned with WHO (2011), and therefore, without expected influence on the population’s exposure.

Materials and Methods

Human ethics approval was obtained from the Brazilian Ethics and Research Council of the Minas Gerais State Hospital Foundation (FHEMIG) with the approval certificate number CAAE: 84,417,618.1.0000.5119.

Study Area

Nine regions of Belo Horizonte city—Barreiro, Central-South, East, Northeast, Northwest, North, West, Pampulha, and Venda Nova—were selected for the urine sample collection. The left of Fig. 1 shows the map of Brazil with Belo Horizonte city highlighted in red and Iron Quadrangle province in purple, inside the Minas Gerais state borders. To the right of Fig. 1, the nine sampling regions of the city showing parts of the city are within the Iron Quadrangle province represented in purple with the boundaries in dashed lines.

Study Design

This study is a post-stratification survey divided into three categories: (a) female-male, (b) age (0–9, 10–19, 20–59, 60+), and (c) BH geopolitical regions (Barreiro, Central-South, East, Northeast, Northwest, North, West, Pampulha, Venda Nova and Metropolitan). Urine was randomly collected in a representative sampling from the residents of Belo Horizonte, with volunteer participants from different economic and social characteristics between May 2018 and October 2019. The approach to the participants occurred on the street, in homes, health centers, colleges, and companies, among other places, with no previous information about occupational exposure to arsenic.

There were no previous fish and seafood diet restrictions, although this and other demographical information were collected for further interpretations. The inclusion criteria were residents who had lived in the city of Belo Horizonte for at least six months, and who have understood, agreed, and signed the Informed Consent Form, respecting Resolution 366/2012 of the National Health Council, which guides human research.

Figure 2 shows the representativeness of the sampling for TAs and speciation by comparing the sampling and population proportions according to the three stratification categories, based on the official census (IBGE 2010a, b).

Urine samples were collected in arsenic-free 80 mL plastic containers and transported on ice. The material was aliquoted right after arriving at the survey site into two 10 mL plastic vials and stored at ≤ 4 °C to be collected by a Brazilian commercial laboratory. The total acid-digested arsenic concentration (TAs) in µg/g creat and the creatinine (g/L) of all collected urines was measured using our previously

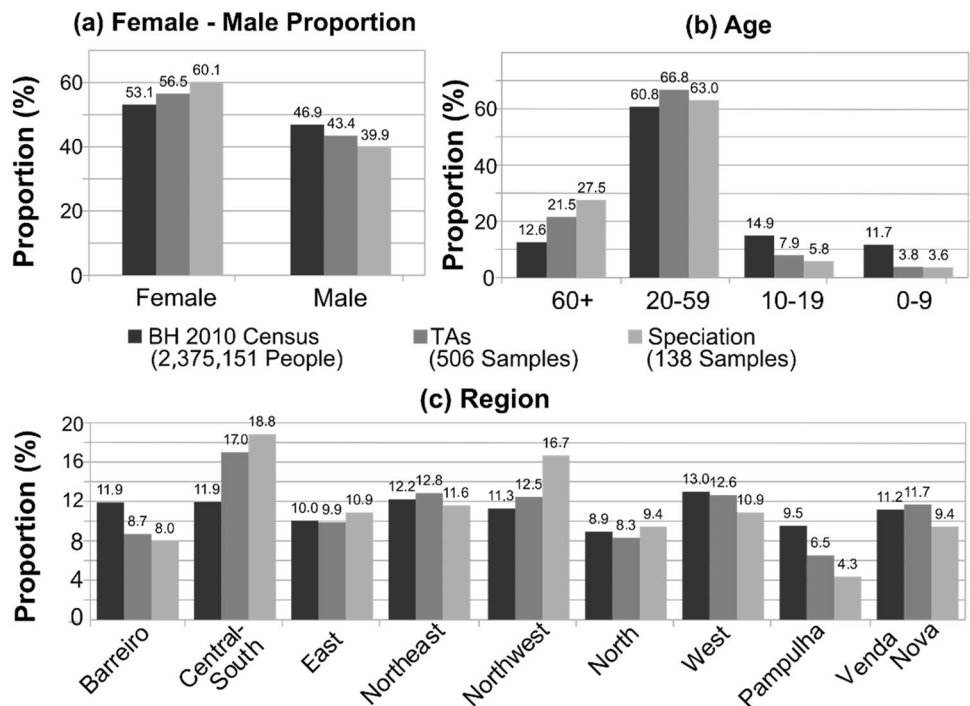
Fig. 1 Map of Brazil showing Belo Horizonte city in the Minas Gerais state (left) with the nine sampling regions (right). The areas within the Iron Quadrangle geological province (in purple) with boundaries in dashed lines are also highlighted



published methods (Tanvir et al. 2021). The concentrations in $\mu\text{g/L}$ were calculated by multiplying those two measurements. Then, three other aliquots were stored frozen at -20°C , one in 2 mL tubes, and two in 10 mL tubes added to 1:100 part of 70% Ultra-Pure nitric acid, Merck®. The frozen urine samples with the total arsenic concentration measured by the local laboratory $\geq 10 \mu\text{g/gCreat}$ or $\geq 10 \mu\text{g/L}$ with a volume of at least 22 mL, were then shipped on dry ice by

an international express courier in 3 batches inside thermo-boxes for urinary arsenic speciation in the laboratories of Queensland Alliance for Environmental Health Sciences (QAEHS), The University of Queensland in Australia. The samples were stored at -80°C until analysis to minimize oxidation of As(III) to As(V). Considering this implication, the speciation results are provided for both As(III) and As(V) separately and combined in ToxAs in this study.

Fig. 2 The sampling representativeness is indicated by comparing urine samples (%) for TAs (506) and arsenic speciation (138) and the population census, according to three stratifications: **a** female-male, **b** age, and **c** region



Data Analysis

Since probability distributions were detected as non-normal, data were compiled as geometric means (GMs) and percentiles (50th, 75th, 90th, and 95th) with 95% confidence intervals of urinary creatinine in g/L, urinary arsenic concentrations in $\mu\text{g/L}$, and creatinine-corrected ($\mu\text{g/g creat}$) for TAs and ToxAs in all the categories, and species of AsB, AsIII, AsV, MMA and DMA for the total population. Arsenic species were also presented as percentages of TAs with 95% confidence intervals. The GMs of the stratification categories were statistically compared. Finally, the levels of BH were also compared to other similar results in the literature.

Laboratory Analysis and Quality Control

In the Brazilian commercial laboratory, TAs was analyzed using ICP-MS while urinary creatinine concentrations were determined using Jaffe's reaction (upper cut-off limit 3 g/L). This laboratory is accredited by the SBPC/ML (Brazilian Society of Clinical Pathology/Laboratory Medicine), CAP (College of American Pathologists), and ISQua (The International Society for Quality in Health Care). The analytical methods follow the national legislation (ANVISA 2005). Due to the recent change in the Brazilian standards and references, the results for urinary arsenic are presented in two concentrations ($\mu\text{g/g creat}$ and $\mu\text{g/L}$), which also facilitates comparisons with the international studies using these two units.

In the QAEHS laboratory, concentrations of TAs and five arsenic species in urine were determined using high-performance liquid chromatography (HPLC) coupled to an inductively coupled plasma mass spectrometry (Agilent 7900 ICP-MS) using helium as the collision gas (HP 1000 HPLC coupled to Agilent 7500 ICP-MS/Octapole Collision Cell Technology). This technology minimizes arsenic chloride and ionic interferences. A Hamilton PRP-X100 ion exchange column (250 mm \times 4.1 mm i.d., 10 μm) was used for the arsenic speciation. The mobile phase consisted of 10 mM $\text{NH}_4\text{H}_2\text{PO}_4$, 3 mM NaNO_3 , and 0.2 mM EDTA- Na_2 (Wu et al. 2011). Urinary creatinine was determined by Jaffe's method using a Beckman Coulter urine calibrator (Lot #10,234) and BIO-RAD Liquicheck urine biochemistry control (Lot #68,540). The five species and limits of detection (LOD) are for arsenobetaine (AsB, $\text{LOD}=0.24 \mu\text{g/L}$), trivalent inorganic As (AsIII, $\text{LOD}=0.08 \mu\text{g/L}$), dimethylarsinic acid (DMA, $\text{LOD}=0.17 \mu\text{g/L}$), monomethylarsonic acid (MMA, $\text{LOD}=0.06 \mu\text{g/L}$), and pentavalent inorganic As (AsV, $\text{LOD}=0.04 \mu\text{g/L}$).

Study Quality Control

Double-blind assessments were performed at both laboratories to verify the precision of the entire survey process from material handling and transportation to concentration measurement. Two aliquots of a urine sample were assigned to fictitious names and both paired urine samples were sent to the laboratories. To the Brazilian commercial laboratory, 19 pairs of double-blind urine samples were sent resulting in 19 paired results for TAs and creatinine. The paired samples' equality was tested using a ratio-paired t-test with the null hypothesis that the geometric mean of the paired ratios equals one. The null hypothesis was not rejected for the total urinary arsenic ($p=0.619$) and the creatinine ($p=0.358$), meaning that there is no evidence for differences in the double-blind paired measures at a 95% confidence level. The geometric means of the paired ratios and 95% confidence intervals were 1.02 (0.95–1.09) for TAs and 1.02 (0.98–1.05) for creatinine. Another two double-blind paired samples were sent to the QAEHS laboratory resulting in the ratios 1.11 and 0.99 for TAs, and 0.98 and 1.04 for creatinine. Nine specimens of BIO-RAD Liphochek urine metals control Lot #69,190 Level 1 with arsenic-certified mean 63.1 $\mu\text{g/L}$ (50.5–75.7) were analyzed at the QAEHS laboratory. The measured arsenic mean, and 95% CI of 60.0 $\mu\text{g/L}$ (53.62–66.4) were within the acceptable limit of certified reference values. Representative field blanks for each batch of samples were also analyzed and all remained below the detection limit for TAs and arsenic species. With the other in-house assurance/quality control (QA/QC), spiked recovery samples were 104.9% for Certified Reference Material (CRM NIES-18-Japan, TAs and As speciation) (urine), $n=5$, and 100.2% for NWTM 24.3 (LCG 2012) (water, The National Water Research Institute, Canada), $n=2$. The CRM for the measured TAs of $131 \pm 1.4 \mu\text{g/L}$ ($n=2$) agreed with the certified value of $137 \pm 11 \mu\text{g/L}$. The results of CRM for arsenic speciation ($n=9$) and ClinChek-Control I & II for inter-assay variation assessment ($n=14$) are shown in Table S1 and S2 of the Supplementary Material.

Statistical Analysis

Data were processed using the "Survey" package (Lumley 2004) for analysis of complex survey samples in the environment for statistical computing and graphics R (R Core Team 2020). For the survey design, the post-stratification method Generalized Raking was applied to include information on the entire population size and each stratum, generating weightings for estimations and adjustments in the confidence intervals, making them consistent with the population size. For the Metropolitan region, post-stratification was applied only in the female-male stratum and the statistical

parameters were not calculated for them, only for the entire population of the region.

For statistical parameter calculations, concentrations below LOD were replaced by LOD divided by the square root of two, as data fitted the log-normal probability distributions. This substitution is more suitable than LOD/2 for probability distributions close to Log-normal as argued in VerbovSek (2011) and adopted by CDC (2021). The simple exclusion of undetected values biases the estimation of statistical parameters (Helsel 2005).

Urinary arsenic measurements were log-transformed before the geometric means (GM) calculation. Ninety-five percent confidence limits were calculated by the method BetaWald (Korn and Graubard 1998). None of the urinary arsenic species' categories exceeded 50% of concentrations below LOD. If any category exceeded 50% < LOD, a large error would be generated and GM and the 50th percentile would not be reported.

For the double-blind assessment, the ratio paired t-test was used. The ratio t test averages the logarithm of the paired ratios and then tests the null hypothesis that the mean of those logarithms is zero. After the back transformation (exponential), the interpretation of the null hypothesis is that the GM of the ratios equals one. The GM of the ratios and their confidence intervals were also reported.

The categories of GMs were statistically compared inside each stratification following the same transformation principle (log-exponential). Generalized linear models were fit to the log-transformed data from the complex survey design for each stratification. Their transformed means were then compared using Gaussian family functions and back transformed with exponentiation, resulting in comparisons via GM ratios. As the Metropolitan region was not considered part of the BH stratification and had an independent survey design, its GM was compared to the total BH population's GM, using a simple t test, applied to the transformed data. All categories with GM ratios significantly deviated from unity, at a 95% confidence level ($p < 0.05$), and had their p values and proportions reported in the tables.

Results

Table S3 (Supplementary Material) shows the population of BH and the Metropolitan region based on the census (IBGE 2010a, b), and the sample size for each category used in post-stratification weighting corrections. The sample size to the TAs level's estimation of the entire BH population is 506. Among the 506, 138 urine samples were speciated with the criteria defined in the previous section—the total arsenic concentration $\geq 10 \mu\text{g/gCreat}$ or $\geq 10 \mu\text{g/L}$. For the

Metropolitan region, the sample size to the TAs level's estimation is 35, with 10 speciations among them.

All tested urine samples had detectible TAs (i.e., > LOD). Details about LOD and the approach dealing with values below LOD can be found in the previous section. Table S3 shows in the last five columns the number of samples with concentrations values below the LOD (and the percentage concerning each category sample size inside parentheses).

Total Acid Digest Arsenic

Table 1 shows the geometric means and percentiles with 95% confidence intervals of the TAs in $\mu\text{g/g creat}$ and $\mu\text{g/L}$, besides the urinary creatinine in g/L, for all the stratification categories. There was no statistical difference between any GM of TAs in $\mu\text{g/g creat}$. Regarding TAs ($\mu\text{g/L}$), male GM was 35.5% higher than that of females ($p < 0.0001$) and the same difference was observed for creatinine. The TAs ($\mu\text{g/L}$) for ages 10–19 in the Pampulha and Venda Nova regions were also statistically different from the other categories inside their stratification ($p < 0.05$). Details about the statistics of the categories with significant GM ratio deviations are also shown in Table 1.

Toxic Fraction and Arsenic Species

Arsenic speciation was determined in 148 urine samples, 138 from Belo Horizonte and 10 from the metropolitan region. Figure 3 shows the urinary arsenic concentrations of TAs and the ToxAs. The urine samples in Fig. 3 are arranged in ascending order of their TAs concentrations.

Table 2 shows GMs and percentiles with 95% confidence intervals of ToxAs in $\mu\text{g/g creat}$ and $\mu\text{g/L}$ for all stratification categories, together with the details about the statistics of the categories with significant GM ratio deviations. Due to the high percentage of concentrations below the limit of detection (LOD) during As speciation, associated with low sample sizes for some isolated stratum, only the levels of the total population are presented for individual arsenic species. For example, AsIII for the Age 0–9 group has a sample size equal to five and 2 values (40%) < LOD (Table S3). In these situations, determining a statistical parameter for an entire city based only on three detected values would carry a large uncertainty and lack of representativeness.

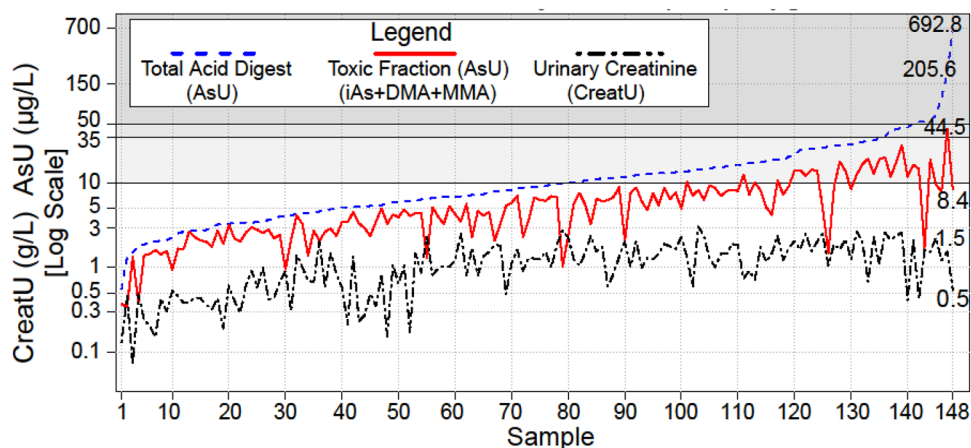
Table 3 shows the geometric mean and percentiles of AsB, AsIII, DMA, MMA, and AsV with 95% confidence intervals for the total population of BH. Table 4 shows the percentages of the urinary arsenic species concerning TAs with 95% confidence intervals, for all sampling stratification categories of BH and the metropolitan region.

Table 1 Geometric means (GM) for total acid digest urinary arsenic levels (µg/g creat and µg/L) and urinary creatinine (g/L) for all the categories, and percentiles (for arsenic) with 95% confidence intervals (C.I.)

Categories	Total acid digest urinary arsenic in µg/g creat					Creat. g/L					Total acid digest urinary arsenic in µg/L						
	GM	Percentile (95% confidence interval)				GM	(95% C.I.)	GM	Percentile (95% confidence interval)				GM	Percentile (95% confidence interval)			
		50th	75th	90th	95th				(95% C.I.)	50th	75th	90th		95th			
Total Pop (506)	9.68 (9.17–10.2)	8.90 (8.50–9.50)	13.2 (12.6–13.9)	19.7 (17.9–23.5)	29.2 (23.4–33.8)	1.14 (1.08–1.21)	11.0 (10.3–11.8)	10.7 (9.83–11.8)	16.8 (15.6–18.5)	26.7 (22.9–32.8)	39.3 (32.1–46.8)						
Females (286)	9.70 (9.05–10.4)	8.90 (8.40–9.60)	13.0 (11.7–13.9)	18.8 (16.3–24.2)	29.2 (21.1–34.7)	0.99 (0.91–1.07)	9.52 (8.72–10.4)	8.92 (7.72–10.1)	15.1 (13.0–17.1)	22.0 (19.6–28.9)	34.4 (24.1–40.7)						
Males (220)	9.66 (8.87–10.5)	8.90 (8.10–10.0)	13.2 (12.4–15.8)	19.9 (16.9–24.5)	29.3 (20.0–35.9)	1.34 ^a (1.23–1.45)	12.9 ^d (11.6–14.4)	12.8 (11.1–14.4)	19.3 (16.5–22.7)	31.2 (23.7–40.3)	43.6 (32.2–58.0)						
Age 0–9 (19)	10.9 (8.23–14.5)	8.35 (7.32–16.1)	16.3 (8.16–29.5)	25.1 (12.7–33.8)	33.2 (14.7–33.9)	0.86 (0.70–1.06)	9.39 (7.0–12.6)	8.47 (6.27–15.4)	15.6 (8.30–21.0)	20.4 (12.4–28.7)	22.0 (14.6–30.0)						
10–19 (40)	9.73 (8.29–11.4)	9.30 (6.91–12.7)	14.3 (9.95–17.8)	18.0 (15.0–25.7)	20.0 (16.7–39.7)	1.44 ^b (1.17–1.77)	14.0 ^e (11.1–17.6)	14.6 (9.82–18.5)	22.9 (15.0–36.3)	38.8 (23.0–49.1)	42.2 (27.8–56.7)						
20–59 (338)	9.38 (8.82–9.98)	8.60 (8.20–9.20)	12.7 (11.4–13.6)	19.3 (16.7–23.6)	29.2 (22.0–36.3)	1.14 ^c (1.06–1.21)	10.7 (9.85–11.5)	10.5 (9.34–11.2)	16.3 (14.3–17.7)	24.9 (21.3–32.1)	39.7 (28.8–52.0)						
60+ (109)	10.2 (9.29–11.3)	10.0 (9.30–11.5)	13.2 (12.1–15.0)	18.0 (14.8–24.0)	23.8 (17.3–34.1)	1.07 (0.95–1.20)	10.7 (9.44–12.1)	11.4 (8.88–13.0)	16.1 (14.3–18.9)	23.1 (18.4–31.8)	31.7 (22.2–38.5)						
Barreiro (44)	10.5 (9.14–12.0)	9.86 (8.26–12.7)	13.8 (10.8–17.0)	17.3 (13.6–25.3)	21.1 (15.2–30.4)	1.19 (1.02–1.40)	12.5 (10.5–14.9)	12.8 (9.90–15.9)	17.7 (13.3–21.6)	25.6 (16.7–35.8)	33.3 (17.8–37.5)						
Central-S (86)	10.1 (8.51–11.9)	9.01 (7.42–10.9)	16.0 (11.0–19.6)	25.1 (16.7–37.9)	34.2 (19.7–65.0)	1.19 (1.00–1.41)	11.9 (9.72–14.7)	10.6 (8.54–14.9)	20.7 (15.0–32.4)	40.0 (22.6–56.2)	50.3 (31.7–97.5)						
East (50)	9.90 (8.72–11.2)	9.14 (8.12–9.98)	12.7 (9.57–13.4)	17.5 (12.7–34.3)	26.4 (12.9–39.3)	1.26 (1.09–1.46)	12.5 (10.1–15.5)	11.6 (8.51–16.9)	20.4 (14.1–24.4)	25.2 (21.8–64.0)	52.1 (22.7–80.7)						
Northeast (65)	8.93 (7.72–10.3)	8.62 (7.04–9.60)	12.0 (9.50–16.6)	17.9 (13.7–26.9)	24.7 (16.5–38.7)	1.14 (0.97–1.34)	10.0 (8.29–12.2)	10.3 (7.19–12.3)	14.7 (11.3–22.4)	24.1 (14.9–41.0)	30.8 (16.2–87.1)						
Northwest (63)	11.0 (9.08–13.3)	10.0 (7.98–12.8)	15.9 (11.5–23.8)	27.4 (14.7–37.8)	33.6 (16.6–40.8)	1.16 (0.98–1.38)	12.7 (10.2–15.9)	11.8 (10.5–16.4)	20.0 (15.2–31.7)	39.1 (19.6–48.6)	41.7 (26.4–58.3)						
North (42)	9.31 (7.83–11.1)	9.00 (7.34–11.7)	13.3 (10.7–17.0)	17.5 (13.4–21.5)	18.9 (14.3–24.2)	1.17 (0.97–1.43)	10.9 (8.15–14.5)	11.0 (7.51–16.2)	17.0 (12.7–22.8)	23.3 (16.7–45.2)	32.8 (16.7–53.5)						
West (64)	9.72 (8.33–11.4)	8.68 (7.80–11.1)	13.1 (9.72–19.2)	19.7 (14.1–28.2)	23.6 (14.5–109)	1.09 (0.94–1.27)	10.6 (8.93–12.7)	10.6 (7.26–14.7)	16.2 (13.3–19.2)	20.6 (16.6–31.6)	28.8 (19.6–71.1)						
Pampulha (33)	8.51 (6.95–10.4)	7.80 (6.40–10.1)	11.0 (8.22–13.4)	13.5 (10.1–39.9)	31.3 (11.4–45.2)	1.01 (0.77–1.31)	8.56 ^f (6.52–11.2)	8.88 (5.74–11.7)	12.4 (9.15–15.8)	16.0 (12.1–57.4)	26.7 (12.8–77.0)						
Venda N (59)	9.27 (7.93–10.9)	8.10 (7.30–11.4)	13.1 (9.53–17.5)	18.6 (12.1–28.0)	22.1 (13.5–44.0)	1.05 (0.86–1.29)	9.73 ^g (8.42–11.2)	8.80 (7.34–11.1)	13.1 (10.1–18.4)	19.9 (12.8–25.3)	23.4 (15.4–34.9)						
Metropol (35)	12.4 (9.58–16.0)	11.0 (9.06–13.8)	15.9 (11.9–20.8)	23.6 (16.3–57.0)	36.7 (18.2–412)	1.04 (0.84–1.28)	12.9 (9.77–17.0)	10.6 (9.19–15.3)	16.3 (13.4–22.7)	26.5 (16.3–38.4)	33.1 (19.0–97.9)						

GM with statistical differences inside stratifications: ^a [Male/Female] 135.6% ($p < 0.0001$); ^b [Age 10–19/0–9] 166.7% ($p < 0.0001$); ^c [Age 20–59/0–9] 131.9% ($p = 0.013$); ^d [Male/Female] 135.5% ($p < 0.0001$); ^e [Age 10–19/0–9] 148.8% ($p = 0.04$); ^f [Pamp./Barr.] 68.6% ($p = 0.023$); ^g [Venda N./Barr.] 78% ($p = 0.033$)

Fig. 3 Total arsenic, the toxic arsenic fraction ($\mu\text{g/L}$), and creatinine (g/L) of all speciated urine samples, arranged in ascending order of TAs to facilitate visualization



Discussion

There is no threshold regulatory guideline set for environmental exposure to As in Brazil. Until 2020, the urinary TAs for non-exposed was $10 \mu\text{g/g creat}$, and $50 \mu\text{g/g creat}$ for occupational exposure—Maximum Allowable Biological Indices (IBMP in Brazilian acronym) (Brasil 1994). In the updated Brazilian legislation (Brasil 2020) that came into force in 2022, the Biological Exposure Index (BEI) is $35 \mu\text{g/L}$ for the sum of inorganic As + DMA + MMA (ToxAs). These occupational exposure values agree with the maximum permitted levels for acceptable health risks in various countries (WHO 2001; ACGIH 2017).

The collection of 24-h urine samples has been considered a preferred method but is usually not feasible for large biomonitoring studies (Middleton et al. 2016). Furthermore, since diet and urination time is mere information provided by participants, it is therefore not entirely reliable regarding the intake of arsenic-rich foods or whether the sample collected was indeed the first one in the morning, in addition to several possible internal and external interference factors inherent to 12 or 24-h urine collections. Based on these considerations, the authors claim that a more realistic assessment of the population's exposure to arsenic can be obtained by adopting random collection and spot sampling at the time of the interview, as well as dilution correction.

Hsieh et al. (2019) concluded either urinary creatine or specific gravity correction compared favorably with that of 12 or 24 h urine samples in a review of correction methods for arsenic, cadmium, and mercury. The use of creatinine to correct the effects of urine density is especially relevant in a tropical country, where high temperature and therefore heavier work activity, mainly among men, can influence significantly higher creatinine in this group, as found in this study (Table 1).

The results shown in Table 1 show TAs of five urine samples were higher than $50 \mu\text{g/g creat}$. One of these five urine samples (TAs $205.6 \mu\text{g/L}$) had the ToxAs of $44.5 \mu\text{g/L}$,

greater than the Brazilian threshold for occupational biological exposure for ToxAs ($35 \mu\text{g/L}$). This participant reported he had consumed beverages, seafood, and fish, including fish caught in a mining tailing pond. The individual species concentrations for this participant's urine sample and their contribution to the TAs were AsB $111.1 \mu\text{g/L}$ (54.0%), AsIII $3.18 \mu\text{g/L}$ (1.55%), DMA $28.4 \mu\text{g/L}$ (13.8%), MMA $7.51 \mu\text{g/L}$ (3.65%), and AsV $5.40 \mu\text{g/L}$ (2.63%). These numbers represented increase in AsB (+349.4%), AsIII (+150.0%), DMA (+221.9%) MMA (+218.3%), and AsV (+173.1%) in relation to the 95th percentile (P95th) of the TAs from BH (AsB $31.8 \mu\text{g/L}$ [14.5–97.1], AsIII $2.12 \mu\text{g/L}$ [1.60–3.18], DMA $12.8 \mu\text{g/L}$ [7.48–21.9], MMA $3.44 \mu\text{g/L}$ [2.29–6.37] and AsV $3.12 \mu\text{g/L}$ [2.29–5.23]). Despite the highest increase in the organic and less toxic AsB, the ToxAs for this participant is indeed higher than the P95th of BH ($19.2 \mu\text{g/L}$ [12.1–35.4]), corroborating the information about his exposure to arsenic-contaminated areas reported in the survey form.

Several studies around the world, especially in Europe but also in other countries, have justified the use of reference values at the 95th percentile as the highest exposure threshold to As. Examples are human biomonitoring surveys carried out in Germany (Schulz et al. 2011, 2012), South Korea (Lee et al. 2012), England (Bevan et al. 2013), Belgium (Hoet et al. 2013), Slovenia (Tratnik et al. 2019), United States of America (CDC 2021), Canada (CHMS 2021), Malaysia (Anual et al. 2021), Japan, among others. The necessary care with the evaluation of sample selection, sample size, collection period, exclusion criteria, pairing criteria, analytical quality, as well as other aspects such as age groups, sex, eating, and smoking habits, in addition to aspects of possible environmental and occupational exposures were discussed (Saravanabhavan et al. 2017; Vogel et al. 2019). Though the maximum exposure in a studied region is defined through the P95th, this value does not necessarily represent normality (Ewers et al. 1999). Therefore, each region must have its reference updated, as proposed

Table 2 Toxic fraction (AsIII + AsV + MMA + DMA) urinary arsenic levels ($\mu\text{g/g}$ creat and $\mu\text{g/L}$): Geometric means (GM) and percentiles with 95% confidence intervals

Categories	Toxic fraction urinary arsenic in $\mu\text{g/g}$ creat					Toxic fraction urinary arsenic in $\mu\text{g/L}$				
	Percentile (95% confidence interval)					Percentile (95% confidence interval)				
	GM (95% C.I.)	50th	75th	90th	95th	GM (95% C.I.)	50th	75th	90th	95th
Total Pop	5.21 (4.32–6.28)	4.78 (4.38–5.25)	7.15 (5.60–10.1)	16.0 (8.40–28.9)	28.8 (10.1–33.2)	5.23 (4.45–6.15)	5.82 (4.21–6.64)	8.63 (7.59–10.9)	14.4 (9.40–19.2)	19.2 (12.1–35.4)
Females	4.77 (3.79–5.99)	4.69 (4.28–5.60)	7.12 (5.24–9.56)	10.1 (7.05–23.2)	16.3 (8.19–28.1)	4.56 (3.80–5.48)	5.50 (2.92–7.06)	8.38 (6.41–10.2)	11.3 (8.57–15.4)	14.1 (10.9–16.8)
Males	5.74 (4.32–7.63)	4.78 (4.16–6.40)	7.11 (5.18–20.5)	23.4 (6.86–33.3)	28.9 (6.87–35.2)	6.08 (4.61–8.04)	5.80 (4.12–8.24)	8.72 (6.45–17.6)	19.2 (8.52–37.1)	21.7 (9.79–43.4)
Age 0–9	5.71 (3.73–8.73)	4.62 (0.82–11.4)	7.21 (1.23–12.2)	10.1 (0.92–12.2)	11.1 (0.42–12.2)	5.61 (3.75–8.38)	5.68 (0.61–8.77)	7.31 (1.02–8.94)	8.14 (0.93–8.94)	8.54 (0.40–8.94)
Age 10–19	7.22 (2.92–17.9)	6.86 (0.96–22.1)	10.8 (4.15–27.7)	19.7 (4.62–28.8)	24.2 (2.64–28.8)	12.7 ^b (6.49–24.8)	14.1 (2.21–23.1)	19.2 (7.29–27.3)	21.3 (10.9–28.2)	24.8 (5.03–28.2)
Age 20–59	5.14 (4.11–6.43)	4.76 (4.25–5.25)	7.04 (5.20–11.5)	20.1 (7.77–29.1)	28.9 (9.50–33.9)	5.19 (4.29–6.28)	5.84 (4.11–7.49)	8.64 (7.22–11.5)	14.5 (9.00–19.1)	18.5 (9.68–38.4)
Age 60+	5.11 (4.11–6.35)	4.86 (4.28–6.08)	6.70 (5.47–10.0)	10.2 (6.28–15.0)	11.5 (7.09–17.0)	4.46 (3.56–5.59)	4.91 (3.30–6.09)	6.94 (5.12–9.52)	10.2 (6.85–13.6)	12.4 (7.22–17.2)
Barreiro	5.44 (4.52–6.55)	4.85 (4.09–6.79)	6.23 (4.60–9.36)	6.99 (4.71–10.5)	8.60 (4.70–10.5)	4.89 (3.34–7.16)	4.95 (2.02–8.39)	6.64 (3.23–13.5)	11.9 (3.75–14.2)	13.2 (4.28–14.2)
Central-South	4.62 (3.15–6.77)	5.29 (3.03–6.93)	7.29 (5.32–15.3)	13.6 (6.53–25.7)	18.6 (6.88–28.6)	4.88 (3.18–7.51)	5.50 (2.57–9.84)	11.2 (5.68–18.0)	18.0 (9.36–25.0)	19.1 (10.7–28.0)
East	5.20 (3.42–7.89)	4.78 (4.01–7.06)	5.94 (4.62–21.6)	10.4 (4.89–28.5)	20.3 (4.78–29.1)	6.99 (5.43–8.99)	8.34 (5.36–9.05)	8.80 (6.48–11.0)	9.76 (8.43–11.9)	10.9 (8.33–11.9)
Northeast	5.96 (2.61–13.6)	5.06 (2.29–15.3)	8.86 (2.77–33.3)	22.9 (2.27–35.2)	29.1 (0.37–35.2)	5.34 (3.22–8.83)	4.43 (2.62–14.5)	10.2 (3.98–14.8)	14.6 (2.57–14.8)	14.7 (0.96–14.8)
Northwest	5.24 (3.82–7.18)	6.26 (3.59–7.22)	7.45 (6.43–12.0)	11.7 (6.96–17.2)	15.8 (6.93–17.5)	3.94 (2.73–5.67)	3.34 (2.18–7.15)	7.23 (3.39–14.1)	14.1 (5.97–18.3)	14.3 (6.16–19.2)
North	4.45 (3.67–5.40)	4.04 (3.03–6.40)	5.48 (3.62–7.64)	7.31 (3.85–7.88)	7.59 (4.27–7.88)	5.36 (3.72–7.73)	5.19 (2.27–8.20)	7.47 (4.15–16.5)	11.8 (4.93–20.3)	15.6 (5.48–20.4)
West	6.96 (3.21–15.1)	4.80 (2.57–24.1)	14.8 (4.13–28.6)	25.9 (4.30–28.9)	27.4 (1.74–28.9)	6.98 (3.38–14.4)	7.85 (2.46–17.7)	8.42 (3.62–39.9)	27.0 (3.28–44.5)	35.7 (2.03–44.5)
Pampulha	5.75 (3.49–9.47)	4.36 (1.45–12.0)	8.90 (1.91–12.6)	11.4 (1.69–12.6)	12.0 (0.62–12.6)	3.04 (1.32–7.01)	1.86 (0.66–15.1)	2.28 (0.92–19.2)	11.6 (0.77–19.3)	15.4 (0.40–19.3)
Venda N	3.64 ^a (2.95–4.49)	3.51 (2.24–4.8)	4.18 (2.78–11.6)	5.67 (3.66–14.3)	7.71 (3.87–14.6)	4.44 (3.62–5.44)	4.03 (3.17–6.53)	6.12 (3.60–11.4)	6.53 (3.49–16.8)	6.82 (4.07–16.8)
Metropolit	5.70 (3.49–9.31)	5.12 (2.02–10.4)	8.64 (2.78–20.1)	13.4 (6.66–21.6)	17.4 (7.34–22.0)	3.34 (2.56–4.36)	2.60 (1.78–7.10)	4.35 (2.63–8.21)	8.09 (2.79–9.42)	8.72 (2.87–9.49)

GM with statistical differences inside stratifications: ^a [VendaN/Barr.] 66.9% ($p < 0.01$); ^b [Age 10–19/0–9] 226.4% ($p = 0.048$);

Table 3 Urinary arsenic species in $\mu\text{g/g}$ creat and $\mu\text{g/L}$ to the total Belo Horizonte population. Geometric means (GM) and percentiles provided with 95% confidence intervals (C.I.)

As Species (S. Size)	Urinary arsenic species: total population, Belo Horizonte				
	GM	Percentile (95% confidence interval)			
	(95% C.I.)	50th	75th	90th	95th
Concentrations in $\mu\text{g/g}$ creat					
AsB (138)	2.70 (1.95–3.74)	2.55 (1.93–3.68)	6.89 (4.27–9.48)	13.49 (8.29–71.0)	65.0 (9.31–76.7)
AsIII (138)	0.31 (0.23–0.43)	0.46 (0.24–0.65)	0.90 (0.72–1.13)	1.42 (1.03–2.80)	2.12 (1.14–4.91)
DMA (138)	2.51 (2.05–3.08)	2.28 (1.94–2.69)	4.01 (2.70–5.26)	8.38 (4.70–18.5)	16.5 (5.19–19.1)
MMA (138)	0.53 (0.37–0.74)	0.76 (0.70–0.87)	1.22 (1.02–1.69)	2.63 (1.35–5.10)	4.75 (1.63–7.4)
AsV (138)	0.89 (0.70–1.13)	1.10 (0.85–1.31)	1.86 (1.47–2.29)	3.51 (2.03–5.38)	5.26 (2.96–7.53)
Concentrations in $\mu\text{g/L}$					
AsB (138)	2.71 (1.99–3.69)	2.46 (1.83–3.31)	7.58 (4.64–14.6)	18.9 (12.2–36.5)	31.8 (14.5–97.1)
AsIII (138)	0.32 (0.24–0.42)	0.38 (0.24–0.49)	0.97 (0.50–1.28)	1.80 (1.05–2.41)	2.12 (1.60–3.18)
DMA (138)	2.52 (2.07–3.08)	2.70 (2.00–4.04)	4.98 (4.19–6.87)	8.05 (6.04–13.2)	12.8 (7.48–21.9)
MMA (138)	0.53 (0.39–0.72)	0.78 (0.57–0.89)	1.44 (1.09–2.04)	2.83 (1.81–3.47)	3.44 (2.29–6.37)
AsV (138)	0.90 (0.71–1.12)	1.20 (0.92–1.37)	1.99 (1.51–2.24)	2.59 (2.10–3.20)	3.12 (2.29–5.23)

Table 4 Urinary arsenic species (%) in the total acid digested As, with 95% confidence intervals (C.I.) for all stratification categories

Categories (S. Size)	Urinary Arsenic Species: % of the total acid digested as				
	AsB	AsIII	DMA	MMA	AsV
	(95% C.I.)	(95% C.I.)	(95% C.I.)	(95% C.I.)	(95% C.I.)
Total Pop. (138)	43.8 (36.2–51.3)	3.38 (2.11–4.65)	20.7 (16.6–24.7)	5.75 (4.45–7.05)	6.77 (4.36–9.18)
Females (83)	41.8 (30.0–53.5)	3.77 (2.20–5.33)	22.7 (17.3–28.1)	5.84 (4.42–7.25)	7.61 (4.72–10.5)
Males (55)	45.1 (37.9–52.3)	3.13 (1.72–4.55)	19.4 (15.2–23.5)	5.70 (4.14–7.26)	6.23 (3.58–8.87)
Age 0–9 (5)	32.4 (10.9–53.9)	2.41 (0.00–4.82)	36.2 (24.9–47.5)	6.86 (2.53–11.2)	13.5 (8.03–18.9)
Age 10–19 (8)	28.8 (22.8–34.7)	8.81 (3.82–13.8)	30.5 (24.0–36.9)	8.70 (6.50–10.9)	9.59 (7.65–11.5)
Age 20–59 (87)	45.4 (38.4–52.4)	2.79 (1.80–3.78)	19.4 (15.8–23.1)	5.31 (4.19–6.44)	6.18 (4.04–8.32)
Age 60+ (38)	36.0 (29.0–42.9)	7.04 (4.15–9.93)	26.5 (21.8–31.3)	8.77 (6.93–10.6)	10.8 (7.33–14.2)
Barreiro (11)	40.3 (33.5–47.0)	5.21 (3.37–7.06)	27.1 (20.5–33.8)	8.55 (6.21–10.9)	11.5 (7.32–15.8)
Central-South (26)	39.0 (25.0–53.0)	4.76 (1.41–8.10)	23.8 (14.7–33.0)	6.36 (3.55–9.17)	6.37 (3.54–9.19)
East (15)	49.8 (36.9–62.7)	3.39 (1.17–5.60)	18.6 (13.2–24.0)	5.93 (3.84–8.01)	6.26 (2.62–9.90)
Northeast (16)	50.9 (39.8–62.0)	2.48 (0.00–5.76)	17.9 (12.2–23.5)	6.48 (4.89–8.07)	8.31 (4.86–11.8)
Northwest (23)	33.6 (23.7–43.4)	5.44 (2.37–8.51)	22.6 (13.6–31.7)	7.19 (3.75–10.6)	11.7 (6.55–16.8)
North (13)	36.1 (25.9–46.3)	4.34 (2.01–6.68)	28.9 (24.0–33.8)	8.77 (6.77–10.8)	9.45 (6.97–11.9)
West (15)	48.3 (38.9–57.7)	2.09 (1.06–3.12)	17.2 (11.5–22.8)	4.47 (3.02–5.93)	4.34 (1.43–7.24)
Pampulha (6)	26.1 (23.5–28.6)	3.58 (1.17–5.99)	24.2 (22.5–25.9)	4.95 (2.48–7.43)	6.92 (1.44–12.4)
Venda N. (13)	25.6 (13.7–37.5)	5.29 (1.77–8.80)	33.5 (25.7–41.3)	5.15 (2.30–7.99)	10.3 (3.72–16.9)
Metropolit. (10)	67.2 (59.4–75.0)	1.02 (0.00–2.97)	3.82 (0.00–10.8)	1.06 (0.00–3.01)	1.38 (0.00–3.76)

here, due to possible variations in the exposure factors (Kristiansen et al. 1997; Apostoli et al. 1999).

The GM of TAs (9.68 $\mu\text{g/g}$ creat [9.17–10.2]) and ToxAs (5.23 $\mu\text{g/L}$ [4.45–6.15]) in BH are both lower than those of Brazilian legislation and close to the international reference values discussed above. Around 50% of the samples collected from the volunteer participants are close to or less than the reference value, as the 50th percentile for TAs in BH

was 10.7 $\mu\text{g/L}$ [9.83–11.8] and 8.90 $\mu\text{g/g}$ creat [8.50–9.50]. The species AsB (43.8%) and DMA (20.7%) were the major species in the TAs composition. The participants reported a daily intake of rice and beans in large amounts, which could contribute to the increase in arsenic concentration (Ciminelli et al. 2017; Borges et al. 2020). It was observed that the TAs increase is not necessarily followed by an increase in ToxAs,

and the ToxAs only approximates TAs up to the concentration of 10 µg/L (Fig. 3).

The GM of creatinine among men was 35.6% higher than in women ($p < 0.0001$), as well as GM of TAs in µg/L, was 35.5% higher in males ($p < 0.0001$), therefore, the values of females and males TAs GM in the unit µg/g creat were quite close to each other (9.70 [9.05–10.4] µg/g creat and 9.66 [8.87–10.5] µg/g creat), reinforcing that both units should be considered in male–female comparisons. Specific gravity is a better method of hydration correcting the concentration of urinary arsenic because creatinine is influenced by the body mass, diet, and renal health status, as shown by Islam et al. (2022). However, as Brazilian legislation and the international references utilized here for comparisons inform only the hydration correction by creatinine, we also reported the unit µg/g creat. No other stratification of TAs in µg/g creat indicated GMs with statistical differences between them.

Various studies have shown the importance of performing arsenic speciation in human samples due to the lesser toxicity of some organic arsenic compounds than the inorganic and methylated species (Kales et al. 2006; deCastro et al. 2014; Saoudi et al. 2012). Health problems have been related to exposure to metals and metalloids in naturally contaminated or industrialized areas, especially in children who are more susceptible to these exposures. Previous similar studies measuring As in human samples in Brazil did not include As speciation (Freire et al. 2015; Lopes et al. 2019).

Regarding BH ToxAs (µg/L), only age 10–19 had its GM (12.7 µg/L [6.49–24.8]) significantly increased ($p = 0.048$), but also its creatinine ($p < 0.001$), resulting in ToxAs GM of 7.22 µg/g creat [2.92–17.9] and no statistical difference between categories in age stratification after creatinine-correction. Even with this rise in ToxAs GM of age 10–19 in µg/L, the upper limit of its 95% CI (24.84 µg/L) remained below the BEI of 35 µg/L for ToxAs (Brasil 2020). The concern about children is confirmed here as ages 10–19 had the highest percentage of DMA (36.2% [24.9–47.5%]) and AsV (13.45% [8.03–18.9%]), and the age 10–19 category had the highest percentage of AsIII (8.81% [3.82–13.8%]), in relation to TAs, despite the low ToxAs levels in both age-categories (age 0–9 GM was 5.71 [3.73–8.73] µg/g creat and age 0–9 GM was 7.22 [2.92–17.9] µg/g creat). A study in Bangladesh, with 142 school-aged children (aged 6–16) in industrialized areas of Eastern South Asian Megacity Dhaka, showed significantly higher ($p < 0.001$) urinary TAs median (77.0 µg/L) than the reference control area (TAs median 51.0 µg/L) (Tanvir et al. 2021). Both levels are higher than the BH levels for the 0–9 and 10–19 age groups, with TAs median values of 8.47 µg/L [6.27–15.4] and 14.6 µg/L [9.82–18.5], respectively.

When considering ToxAs with creatinine correction, only Venda Nova had a statistical deviation (decrease) between regions ($p < 0.01$), with GM of 3.64 µg/g creat [2.95–4.49%].

The Metropolitan region was the category with the highest percentage of AsB (67.2% [59.4–75.0%]) relative to the TAs, and Barreiro had the highest percentage of MMA (8.55% [6.21–10.9%]). Among regions, Barreiro, North, and Venda Nova had the highest DMA value at their P95th. The regions closest to industries are West and Barreiro (placed in the southwest). The As species DMA had the highest levels among the ToxAs in BH, with GM of 2.51 µg/g creat [2.05–3.08] and 2.52 µg/L [2.07–3.08], and the 95th percentile of 16.46 µg/g creat [5.19–19.1] and 12.80 µg/L [7.48–21.9]. These results are consistent with previous studies showing relatively high DMA concentrations in rice available in Brazilian supermarkets (Ciminelli et al. 2017; Borges et al. 2020). One should be aware that urinary DMA is a metabolite of arsenolipids which are abundantly present in seafood, particularly in oily fish. Thus, for high urinary arsenobetaine concentration (i.e., seafood consumers), abundant DMA is expected not only from inorganic As metabolite but also from arsenolipids metabolism, and this may lead to an overestimation of "ToxAs" of inorganic origins. Some rice varieties are known to contain a significant concentration of DMA in the rice grains. Therefore, rice can be also a major contributor to DMA and this is relevant in regions such as in the present study, where rice is a major staple, not fish. The total arsenic intake from rice ingestion in Southeast Brazil was shown to vary from 50 to 79%, whereas fish lay within 0.7–4.3% (Ciminelli et al. 2017). Also, contrary to Asian countries, where inorganic As often predominates in rice, DMA accounts for approximately 39–50% in Brazil (Borges et al. 2020; Batista et al. 2011). Finally, the legislation that came into force in 2022 (Brasil 2020) adopted 35 µg/L for ToxAs, which was defined as the sum of DMA, MMA, and inorganic AsIII and As V—the definition applied in the present work.

Comparing the TAs levels of BH to previous Brazilian studies in Nova Lima (a bordering city), Matschullat et al. (2000) reported TAs mean as 25.7 µg/L (min/max: 2.2 to 106 µg/L) in urine samples from 126 children living close to gold mine waste areas, collected in 1997/98. This level is close to the BH P95th of TAs (22.0 µg/L [14.6–30.0]) from the age 0–9 group ($N = 19$) and more than twice the GM of TAs from the same BH group (9.39 µg/L [7.0–12.6]). Campolina et al. (2007) reported the arsenic levels in a longitudinal study undertaken during and after mitigation actions in Nova Lima. The participants with TAs > 20 µg/g creat from exposed areas ($N = 49$) had a TAs median that dropped significantly ($p < 0.001$) from 25.30 µg/g creat (min/max 20.22/51.56) in 2003/2004 to 10.74 µg/g creat (min/max 1.89/40.98 µg/g creat) in 2007, after undertaking the mitigation actions. This TAs median after 2 years from the end of the mitigation actions in Nova Lima is slightly higher than the TAs median in BH (8.90 µg/g creat [8.50–9.50]) as shown in Table 1.

In another Brazilian locality called Vale do Ribeira, around 960 km from BH between São Paulo (SP) and Parana (PR) states, de Figueiredo et al. (2007) compared the urinary As levels of a group living near a contaminated river to a control group living 158 km distant upstream. The study reported the means of urinary TAs for children and adults from the exposed group as 8.94 $\mu\text{g/L}$ ($N=89$) and 8.54 $\mu\text{g/L}$ ($N=86$), and for children and adults from the control areas

as 3.60 $\mu\text{g/L}$ ($N=73$) and 3.87 $\mu\text{g/L}$ ($N=83$). The levels of the exposed group are close to the GM of TAs from BH for the age 0–9 group (9.39 $\mu\text{g/L}$ [7.0–12.6]) and age 20–59 group (10.7 $\mu\text{g/L}$ [9.85–11.5]) despite these levels are well below, for example, the TAs threshold of 50 $\mu\text{g/L}$ for typical arsenic levels in the United States (ATSDR 2007). The levels found in BH were also compared to other studies worldwide with representative population sampling, with TAs and

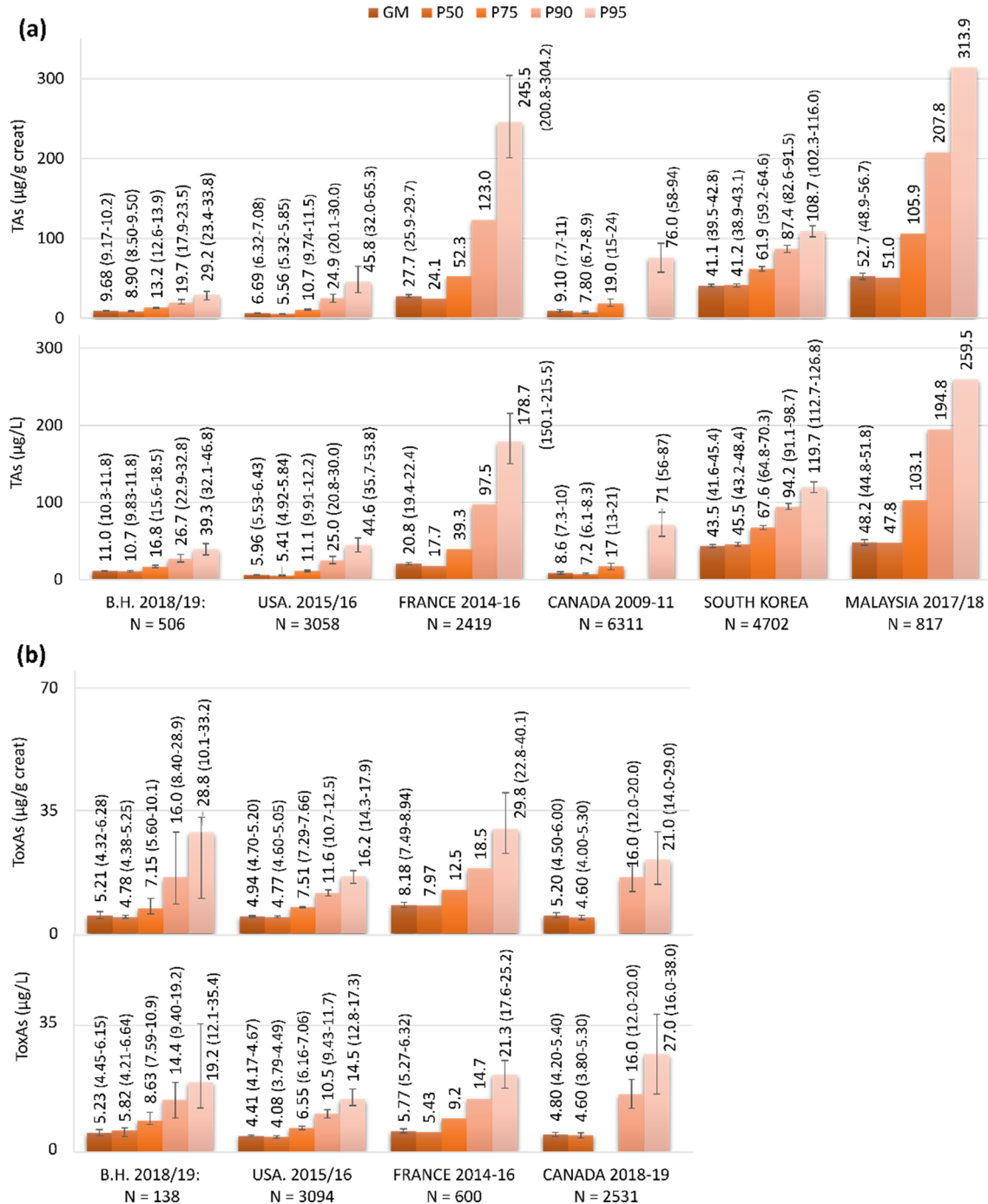


Fig. 4 Total acid digested urinary arsenic and toxic fraction levels $\mu\text{g/g creat}$ **a** and $\mu\text{g/L}$ **b** in Belo Horizonte compared to other studies

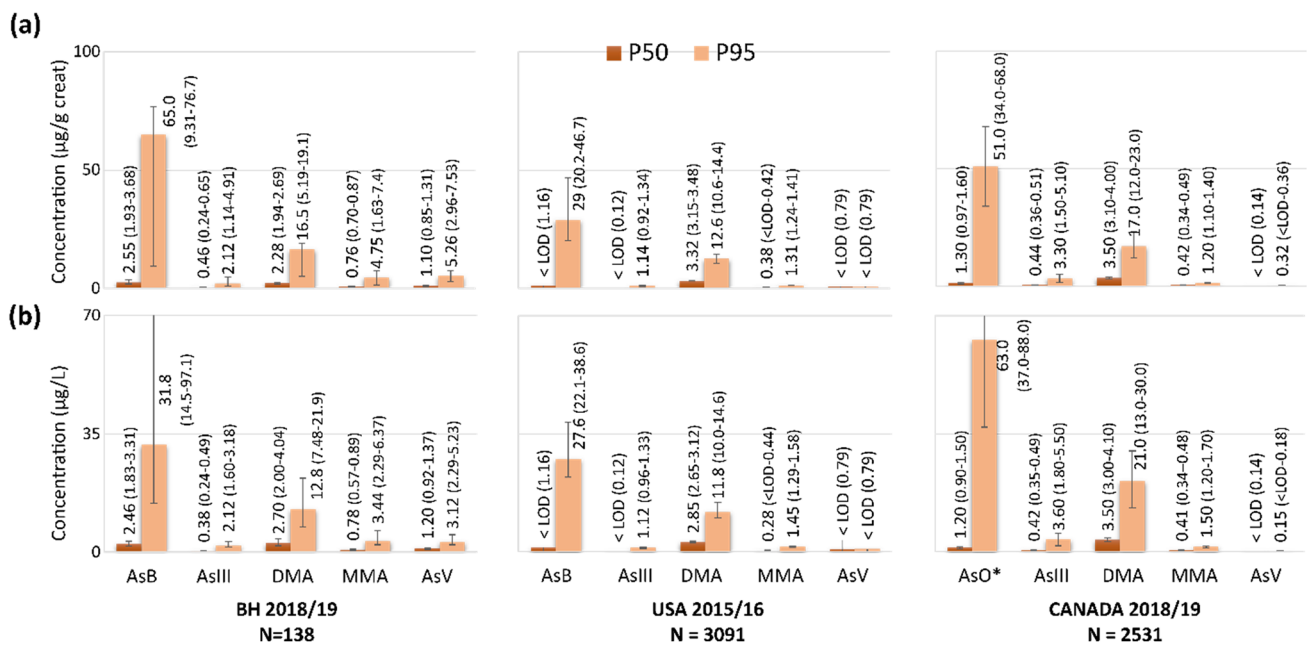


Fig. 5 Urinary arsenic species $\mu\text{g/g creat}$ **a** and in $\mu\text{g/L}$ **b** to the total population of Belo Horizonte compared to other studies. <LOD means less than the limit of detection in $\mu\text{g/L}$. AsO* = arsenobetaine (AsB) + arsenocholine

ToxAs data shown in Fig. 4, and arsenic species shown in Fig. 5. Complementary information to Figs. 4 and 5 are in Tables S4 and S5 in Supplementary Material. It can be seen in Fig. 4 that the distribution of TAs for BH is flatter than in the USA (CDC 2021) and Canada (CHMS 2013) since the GM and P50th in BH are higher than both, and the P95th is lower, for both concentrations $\mu\text{g/g creat}$ and $\mu\text{g/L}$. The same pattern is not observed for ToxAs, since the BH levels are slightly higher than USA (all 95% CIs overlap) and quite close to Canada in concentrations $\mu\text{g/g creat}$. The GM of TAs in BH is different from France (Fillol et al. 2021), South Korea (Lee et al. 2012), and Malaysia (Anual et al. 2021), as both concentrations $\mu\text{g/g creat}$ and $\mu\text{g/L}$ and the P95th of BH are far smaller. The levels of ToxAs from BH are also slightly lower than in France (Fillol et al. 2021). Therefore, there is no clear indication of a significant influence of geogenic arsenic anomalies nearby.

It can be seen in Fig. 5 that the DMA P50th from the USA (CDC 2021) and Canada (CHMS 2021) were higher than BH (3.32 and 3.50 $\mu\text{g/g creat}$ against 2.28 $\mu\text{g/g creat}$) with a statistical significance (no overlap of their 95% CI), but relative to the P95th, BH (16.5 $\mu\text{g/g creat}$) and Canada (17.0 $\mu\text{g/g creat}$) are close and both slightly higher than USA (12.6 $\mu\text{g/g creat}$). BH had a higher P95th of MMA than USA and Canada (4.75 $\mu\text{g/g creat}$ against 1.31 and 1.20 $\mu\text{g/g creat}$) with no 95% CI overlap, and a higher P95th of AsB (65.0 $\mu\text{g/g creat}$) than USA (29.0 $\mu\text{g/g creat}$) and the sum of AsB and arsenocholine of Canada (1.30 $\mu\text{g/g creat}$). A study in Japan with a population not occupationally exposed, but

with a high intake of seafood has also shown the predominance of DMA among toxic species, as well as the elevation of DMA when AsB is high (Hata et al. 2007).

The percentages of DMA, AsIII, and MMA relative to TAs in BH (20.7% [16.6–24.7%], 3.38% [2.11–4.65%], and 5.75% [4.45–7.05%]) are quite different from the reported in France (Fillol et al. 2021), respectively as 84.2%, 12%, and 3.7%, being more consistent with the percentages of USA with AsB 43.4% and DMA 29.8% (Caldwell et al. 2009), Korean with AsB 56.7% and ToxAs 43.3% (Lee et al. 2022), and Japan with AsB 40% and DMA 30% (Hata et al. 2007).

The European Union is advocating using the 95th percentile to establish a population background of any urinary As concentration. The P95th of ToxAs for BH was 19.2 $\mu\text{g/L}$ [12.1–35.4], slightly lower than that reported in France (Fillol et al. 2021) as 21.3 $\mu\text{g/L}$ [17.6–25.2], higher than in the US (CDC 2021) 14.5 $\mu\text{g/L}$ [12.8–17.3], but lower than in Canada (CHMS 2021) 27.0 $\mu\text{g/L}$ [16.0–38.0]. Taking the upper limit of the P95th, the maximum exposure to ToxAs for BH is 35.4 $\mu\text{g/L}$, showing consistency with the Brazilian occupational legislation BEI 35 $\mu\text{g/L}$ (Brasil 2020). This occupational comparison was due to the lack of Brazilian non-occupational legislation.

The various exposure pathways to arsenic and their health risk apportionment to the residents of Paracatu, a gold mining town, 500 km away from BH were reported (Ng et al. 2019). The gold mine is the largest gold producer in the country and stands out by the proximity (e.g., 2 km) of the open pit mine to residential areas. Food was the main

contributor to the daily total intake of arsenic (81.5%) with rice (37.5%) and beans, a staple food in Brazil, being the most significant ones (Ciminelli et al. 2017). The contribution of water intake (1.34 $\mu\text{g/L}$ As) to total intake was 16.5% and of geogenic material (intake plus inhalation) was 2%. Despite very high concentrations of arsenic in soil samples, the bioaccessibility was low, due to the entrapment of arsenic in iron oxyhydroxides (Antonio et al. 2021; Morais et al. 2019; Ciminelli et al. 2018). As rice and most food items surveyed were produced elsewhere, an exposure pattern dominated by food is expected in other urban areas in Brazil. An investigation of As exposure to the residents for BH has not been undertaken. But food is likely the main exposure pathway whereas water ingestion (0.7 $\mu\text{g/L}$ As) is less relevant to total intake if compared to Paracatu. The abundance of iron oxide deposits in the Iron Quadrangle offers a possibility for As remediation. In the present investigation, no clear influence of the nearby arsenic anomalies is evident.

The current study results can serve as a baseline of population levels for clinical reference, bio-monitoring, and future studies in cities with similar characteristics. The study showed the importance of speciation in assessing arsenic exposure, since the non-toxic species, AsB, in BH showed the largest contribution 43.8% [36.2–51.3%] in the TAs. The results highlight the danger of misinterpreting risks when considering only TAs in clinical evaluation. The determination of reference values should focus on the ToxAs with concentrations above 10 $\mu\text{g/L}$ or 10 $\mu\text{g/g}$ creat. If speciation is not performed, the dietary restrictions for seafood for 3 days before sample collection should be mandatory.

Conclusion

The present study provided, for the first time, the baseline urinary levels of total arsenic (TAs) and arsenic species concentrations in a representative population sampling in Belo Horizonte (BH), with stratifications in age, female-male and geopolitical regions. The levels of TAs geometric mean 9.68 [9.17–10.2] $\mu\text{g/g}$ creat in BH were close to those reported in the USA and Canada, and below the values in France, South Korea, and Malaysia. The fractions referred to as toxic arsenic (i.e., ToxAs: AsIII + AsV + MMA + DMA) geometrical mean (GM: 5.23 [4.45–6.15] $\mu\text{g/L}$) are significantly below the international BEI (35 $\mu\text{g/L}$) The P95th (19.2 $\mu\text{g/L}$) is still 55% lower than the BEI value, whereas the upper limit is close to this index. We argue that the P95th threshold is suitable to represent the non-occupational index for BH, in agreement with many other authors who advocate this parameter as the exposure threshold for populations worldwide. The TAs GM of males was 36% higher than those of

females when using the $\mu\text{g/L}$ unit but equal in $\mu\text{g/g}$ creat, as urinary creatinine was also about 36% higher in males. The findings reinforce the authors understanding that both units should be considered, especially in male–female comparisons. In summary, no stratification group showed statistically different total arsenic concentrations (geometric mean) TAs GM ($\mu\text{g/g}$ creat). Now regarding ToxAs ($\mu\text{g/L}$), the age 10–19 group showed GM (12.7 [6.49–24.8] $\mu\text{g/L}$) and creatinine statistically higher than the other age groups, thus resulting in no statistical difference in age stratification after creatinine-correction. In one of the nine regions (Venda Nova), the ToxAs with creatinine-correction (GM of 3.64 $\mu\text{g/g}$ creat [2.95–4.49%]) were statistically inferior ($p < 0.01$) than the values from the other regions Venda Nova is distant from the central and industrialized areas. This result suggests that further in-depth biomonitoring studies in areas closer to industries, soil anomalies, or mining sites—beyond the focus of the present investigation—are justifiable.

The main arsenic species found in BH was the non-toxic fraction AsB (43.8%), thus supporting the importance of speciation in exposure analysis. The overall findings from this work show the relative contribution of inorganic and organic arsenic in a large metropolis and offer a baseline population level for clinical reference and bio-monitoring studies.

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Author Contributions DC: Conceptualization, supervision of sample collection and measurement, analysis of results, and the draft manuscript preparation. VSTC: data analyses, writing, and editing. JCN: supervision on speciation analysis, writing, and editing. TAMC: statistical analysis, writing, and editing. CLC: data analyses, writing, and editing. XGA, MNI: measurements, writing, and editing. All authors commented on all versions of the manuscript and approved the final manuscript.

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Data Availability The data supporting the results of this study are available from the corresponding authors upon reasonable request.

Declarations

Competing interests Délio Campolina and Thiago A.M. Campolina have received consultant honoraria and research funding from Kinross Brasil Mineração. Ximena Gutiérrez Aviña, Md Nazrul Islam declare no competing financial interests. Virginia S. T Ciminelli, Jack C. Ng, and Cláudia L. Caldeira have received research funding from the Kinross Brasil Mineração in other projects.

Ethical Approval The present study was approved by the Brazilian ethics and research council of the Minas Gerais State Hospital Foundation (FHEMIG) with the approval certificate number CAAE: 84417618.1.0000.5119.

Consent to Participate All study participants provided written informed consent; Children's written informed consent was obtained from their parents or legal guardian.

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