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Reference values of cadmium, arsenic and manganese in blood and factors associated with exposure levels among adult population of Rio Branco, Acre, Brazil

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HIGHLIGHTS

- Data on blood levels of heavy metals in Brazilian general population are lacking.
- Blood Cd levels in adults in Rio Branco were lower to those reported elsewhere.
- Blood levels of As and Mn were higher than in other general populations.

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ABSTRACT

This study aimed to investigate the distribution and factors influencing blood levels of Cadmium (Cd), Arsenic (As), and Manganese (Mn), and to determine their reference values in a sample of blood donors residing in Rio Branco, capital city of Acre State, Brazil. Blood samples were collected from all blood donors attending the Central Hemotherapic Unit in Rio Branco between 2010 and 2011. Among these, 1183 donors (98.9%) answered to a questionnaire on sociodemographic and lifestyle factors. Blood metal concentrations were determined by atomic spectrometry. Association between Cd, As and Mn levels and donors' characteristics was examined by linear regression analysis. Reference values were estimated as the upper limit of the 95% confidence interval of the 95th percentile of metal levels. References values were 0.87 $\mu g\,L^{-1}$ for Cd, 9.87 $\mu g\,L^{-1}$ for As, and 29.32 $\mu g\,L^{-1}$ for Mn. Reference values of Cd and As in smokers were 2.66 and 10.86 $\mu g\,L^{-1}$, respectively. Factors contributing to increase Cd levels were smoking, ethnicity (non-white), and lower education, whereas drinking tea and non-bottled water were associated with lower Cd. Lower levels of As were associated with higher household income, living near industrial facilities, working in a glass factory, a compost plant or in metal mining activities. Risk factors for Mn exposure were not identified. In general, blood Cd concentrations were in the range of exposure levels reported for other people from the general population, whereas levels of As and Mn were higher than in other non-occupationally exposed populations elsewhere.

1. Introduction

Cadmium (Cd), Arsenic (As) and Manganese (Mn) are trace metals naturally occurring in the environment. Among other heavy metals, they are of great significance because they are widely used in the metal industry, contributing to increase environmental levels. Cd and As are ranked among the top ten most toxic substances by the Agency for Toxic Substances and Disease Registry (ATSDR, 2010). Mn is an essential nutrient in human body that is involved in metabolism, bone mineralization, and defense against oxidative

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stress (Aschner and Aschner, 2005; ATSDR, 2008); however, excess accumulation of Mn can cause adverse health effects.

Main anthropogenic sources of Cd in the environment are battery manufacturing, burning fossil fuels, and incineration of municipal waste. In the general population, diet is considered the main source of Cd intake among non-smokers (Skerfving et al., 1999). Inhalation of cigarette smoke is the predominant source of exposure in smokers, whose body burdens of Cd can be approximately twice that of non-smokers. Chronic exposure to environmental levels of Cd has been associated with renal damage and with increased risk for bone fracture and diminished bone mineral density (CDC, 2009). In addition, Cd has been classified as a human carcinogen (Group 1) (IARC, 2004) and has been associated with reproductive outcomes (CDC, 2009).

Human activities causing environmental As contamination include smelting of metals and, to a lesser extent, coal burning. Also, inorganic As compounds can occur in groundwater from natural or anthropogenic sources. In non-occupationally exposed populations, exposure to As occurs mainly through diet and/or drinking water. Smoking tobacco is also a source of inorganic As. Chronic exposure to this metal has been associated with diabetes, hypertension, as it is considered to be a cause of skin, lung, and bladder cancer (NRC, 2001; IARC, 2004). In addition, As has been related to adverse reproductive outcomes and neurodevelopmental disorders in children (McClintock et al., 2012; Rodríguez-Barranco et al., 2013).

Mn compounds are used in the production of dry-cell batteries, in magnetic resonance imaging, glass, ceramics, fungicides, and as antiknock agents in gasoline, among others. For the general population, the majority of Mn is obtained from the diet since it occurs naturally in almost all foodstuffs (Gerber et al., 2002). Excessive Mn exposure can result in adverse effects on lungs, liver, kidney and the central nervous system (ATSDR, 2008), but there is insufficient evidence to indicate that Mn exposure produces cancer in humans (Assem et al., 2011).

Human biomonitoring is an important tool for determining background levels of exposure to environmental chemicals in the population, and for identifying trends in exposure levels and population subgroups with higher exposures (Angerer et al., 2007). The United States, Europe and countries such as Korea have established biomonitoring programs for several environmental pollutants, including heavy metals (Puklová, 2008; CDC, 2009; Lee et al., 2012). In Brazil, information on body concentrations of metals in large groups of the general population is scarce (Nunes et al., 2010; Kuno et al., 2013). The aim of this study was to investigate the distribution and factors influencing blood levels of Cd, As and Mn and to determine reference values of these heavy metals among blood donors from Rio Branco municipality, Acre State, North of Brazil.

2. Material and methods

2.1. Study population

A survey was conducted aiming to determine the concentrations of several environmental chemicals in blood of healthy people residing in Rio Branco, the capital and largest city of the State of Acre. All blood donors attending the Central Hemotherapic Unit (HEMOACRE) of Rio Branco between August 2010 and March 2011 were invited to participate in the present survey providing a blood sample. In this period, 1196 blood donors attended the HEMOACRE, representing 85% of total donations carried out in the state (www.datasus.gov.br). All blood donors followed the inclusion criteria, which were being older than 17 years and to live in the municipality of Rio Branco. After blood sampling, donors completed a questionnaire on information about sociodemographic characteristics and lifestyle factors. Thirteen donors refused to answer the questionnaire, leaving 1183 participants (98.9%) for the present study. The study was approved by the Ethics Committee of the Federal University of Acre (UFAC). A signed informed consent was obtained from all subjects participating in the study.

2.2. Data collection

Questionnaires were administered by trained staff through face-to-face interviews. Interviewers had no previous knowledge on participants' exposure status. Gathered data included information on age, ethnicity, marital status, education, household income, current (at the time of sampling) cigarette smoking, frequent consumption of selected food items, current vitamin intake, source of drinking water at home, current occupations related with metal exposure, and information on other potential sources of metal exposure. In addition, self-reported information on self-reported weight and height was collected, and blood pressure and microhematocrit measures were taken.

2.3. Sample collection and laboratory analysis

Intravenous blood samples (5 mL) were collected and stored in vacutainer® tubes containing EDTA (BD, trace metals free). Analytical determination of blood metal concentrations was performed through inductively coupled plasma mass spectrometry (ICP-MS, ELAN DRC II, Perkin Elmer) at the School of Pharmaceutical Sciences of Ribeirão Preto (University of São Paulo), Ribeirão Preto, SP, following a methodology previously described (Batista et al., 2009). Briefly, prior to analysis, blood samples (100 μL) were diluted 1:50 in a solution containing 0.01% (v/v) Triton® X-100 (Sigma–Aldrich, USA) and 0.5% (v/v) nitric acid (Sigma–Aldrich, USA). Method detection limits (3 s), based on the analysis of 10 base blood samples, were 0.003 $\mu g\,L^{-1}$, 0.014 $\mu g\,L^{-1}$ and 0.009 $\mu g\,L^{-1}$ for Cd, As and Mn, respectively.

All data were validated based on Internal Quality Control Procedures (ICP) with the analysis of blood Reference Materials produced by the L'Institut National de Santé Publique du Quebec, Canada, before and after the analysis of 10 ordinary samples. Non-significant statistical differences ($p > 0.05$) were observed when comparing observed levels and reference values in paired t test. Within-run and between-run precision for the reference blood samples ranged from 0.6% to 3.1% and from 1.5% to 4.6%, respectively. Moreover, the laboratory participates in External Quality Control Schemes (International Proficiency Testing and Interlaboratory Comparison Studies in Brazil) as an additional guarantee of data accuracy.

2.4. Statistical analysis

Frequency distribution of characteristics of the study population was described according to gender. Metal concentrations that were below the limit of detection were set to the midpoint value between zero and the corresponding detection limit. Geometric means and standard deviations of blood concentrations of Cd, As and Mn were used for descriptive analysis of metal levels according to characteristics of participants, and parametric tests were conducted to examine differences in metal levels.

Linear regression analyses were performed to examine multivariate associations between characteristics of study population and Cd, As and Mn concentrations, respectively, using natural-logarithm transformed (log-transformed) metal blood levels, which fitted normal distributions. Variables associated with metal concentrations at a significance level of $p \leqslant 0.20$ in the bivariate analysis were tested in the multivariate analysis. Following a backward procedure, variables with p-value > 0.10 were sequentially excluded from the model. F test for the change in R^2 in linear regression was used to check the exclusion (or not) of variables step by step. Variables were retained in the final model if they were associated with metal concentrations at a significant level of 0.05. Finally, the estimated regression coefficients (β) were transformed back [exp(β)] on the original scale.

Following the recommendation of the IUPAC and according to the methodology used by the Federal German Environmental Agency (Poulsen et al., 1997; UBA, 2008), reference values of Cd, As and Mn blood concentrations were calculated as the upper limit of the 95% confidence interval (CI) of the 95th percentile (P95). Because Cd, As and Mn concentrations were not normally distributed, percentiles were calculated using log-transformed data and transforming them back to raw values. A reference value of each metal was computed for men, women, subjects aged <30 years, subjects ⩾30 years old, smokers and non-smokers. Age cut-off point was established according to the median age of blood donors in the North Region of Brazil (www.anvisa.gov.br). SPSS version 17.0 (SPSS Inc., Chicago, IL, US) was used for the analyses.

3. Results

Among study participants, 75% were males and 25% were females, they had ages ranging from 18 to 65 years, were mostly non-white (63%), had incomplete or complete secondary education (51%), and a monthly income between US$ 500.00 and US$ 1500.00 (51%) (Table 1). Mean weight was 81 kg for men and 68 kg for women. Characteristics of study population related to lifestyle and occupations have been previously described (Freire et al., 2014) and are shown in Supplemental material (Tables S1 and S2).

Cd was the metal with the greatest number of blood samples below the limit of detection (19%), whereas almost all participants had detected As concentrations (99.5%) and all of them had detected Mn. Geometric means (GM) of Cd, As, and Mn were 0.09 $\mu g\,L^{-1}$ (median = 0.18 $\mu g\,L^{-1}$), 4.19 $\mu g\,L^{-1}$ (median = 3.75 $\mu g\,L^{-1}$), and 12.81 $\mu g\,L^{-1}$ (median = 12.30 $\mu g\,L^{-1}$), respectively. Cd concentrations were positively correlated with As (Spearman correlation coefficient, $r = 0.15$, $p < 0.001$) and Mn ($r = 0.14$, $p < 0.001$). As and Mn were also correlated ($r = 0.37$, $p < 0.001$). Age was positively correlated with Cd ($r = 0.13$, $p < 0.001$) but not with As or Mn.

Statistically significant differences in mean blood levels of Cd were found according to age, ethnicity, marital status, education, smoking habit, frequent consumption of tea and energy drinks, practicing any activity related with painting, ceramic, pottery, fishing or firearms frequently, and working in galvanoplasty (Tables 2–4). Bivariate associations with As levels were only significant for household income. For its part, levels of Mn were higher in black individuals, those not living near industrial facilities, and not working in a compost plant or in a gasoline station. Regarding hemogram parameters, no significant differences were observed in means of metal concentrations according to elevated pressure (Table S3), whereas subjects with reduced hematocrit had lower Mn levels, particularly males ($p = 0.01$) (data not shown).

Results from multivariate analysis showed ethnicity, education, smoking, drinking tea and origin of water used at home to be independently associated with Cd concentrations (Table 5). The variable that contributed the most to Cd exposure was smoking habit, i.e., Cd levels among smokers were over 3 times higher than levels observed in non-smokers. Levels of Cd were also higher (26%) in non-white than white donors. In contrast, Cd concentrations were lower in donors with superior education (40%), regular

Table 1
Sociodemographic characteristics of blood donors of Rio Branco, Acre, Brazil, 2010–2011.

	All subjects	Men	Women	p-value[a]
N (%)	1183 (100)	890 (75.2)	293 (24.8)	–
Age				
<30 years	582 (49.2)	443 (49.8)	139 (47.4)	0.51
⩾30 years	601 (50.8)	447 (50.2)	154 (52.5)	
Ethnicity				
White	369 (31.2)	272 (30.6)	97 (33.1)	
Black	176 (14.9)	135 (15.2)	41 (14.0)	0.63
Mulatto or indigenous	571 (48.3)	435 (48.8)	136 (46.4)	
Missings	67 (5.7)	48 (5.4)	19 (6.5)	
Marital status				
Single	497 (42.0)	366 (41.1)	131 (44.7)	
Married	629 (53.2)	494 (55.5)	135 (11.9)	<0.001
Widowed or divorced	39 (3.3)	20 (2.2)	19 (6.5)	
Missings	18 (1.5)	10 (1.1)	8 (2.7)	
Education				
Primary incomplete/complete	226 (19.1)	186 (20.9)	40 (13.6)	
Secondary incomplete/complete	605 (51.1)	456 (51.2)	149 (50.9)	0.003
Superior incomplete/complete	325 (27.5)	226 (25.4)	99 (33.8)	
Missings	27 (2.3)	22 (2.5)	5 (1.7)	
Household income (Brazilian reais)				
⩽R$ 500[b]	52 (4.4)	28 (3.1)	24 (8.2)	
R$ 501 – R$ 1000	231 (19.5)	161 (18.1)	70 (23.9)	
R$ 1001 – R$ 1500	269 (22.7)	202 (2.2)	67 (22.9)	<0.001
R$ 1501 – R$ 3000	327 (27.6)	265 (29.8)	62 (21.2)	
>R$ 3000	187 (15.8)	148 (16.6)	39 (13.3)	
Missings	117 (9.9)	86 (9.7)	31 (10.6)	
Smoking habit				
Non smokers	1059 (89.5)	795 (89.3)	264 (90.1)	
Smokers	117 (9.9)	90 (10.1)	27 (9.2)	0.37
Missings	7 (0.6)	5 (0.6)	2 (0.7)	

[a] Chi-square test.
[b] Around US$ 250.

Table 2
Blood concentrations (μg L^{-1}) of Cd, As and Mn according to sociodemographic characteristics of the study population.

	N	Cadmium			Arsenic			Manganese		
		GM	GSD	*p*-value[a]	GM	GSD	*p*-value[a]	GM	GSD	*p*-value[a]
Gender										
Men	890 (75.2)	0.08	8.88	0.63	4.25	1.85	0.27	12.86	1.63	0.64
Women	293 (24.8)	0.09	8.51		4.01	2.27		12.96	1.65	
Age										
<30 years	582 (49.2)	0.07	8.73	0.01	4.17	2.07	0.79	12.61	1.66	0.29
⩾30 years	601 (50.8)	0.10	8.75		4.21	1.84		13.00	1.61	
Ethnicity										
White	369 (31.2)	0.07	9.30		4.26	1.80	0.27	12.29	1.65	
Black	176 (14.9)	0.07	11.98	0.001	4.43	1.67		14.07	1.66	0.01
Mulatto or indigenous	571 (48.3)	0.12	7.17		4.06	2.16		12.71	1.61	
Marital status										
Single	497 (42.0)	0.07	8.83		4.14	2.01	0.89	12.81	1.62	
Married	629 (53.2)	0.10	8.39	0.01	4.22	1.94		12.82	1.64	0.91
Widowed or divorced	39 (3.3)	0.05	11.60		4.15	1.54		12.37	1.55	
Education										
Primary incomplete/complete	226 (19.1)	0.15	7.47		4.08	2.16	0.69	13.22	1.59	
Secondary incomplete/complete	605 (51.1)	0.07	9.20	<0.001	4.17	1.95		12.77	1.65	0.44
Superior incomplete/complete	325 (27.5)	0.08	8.87		4.29	1.85		12.52	1.62	
Household income (reais/month)										
⩽R$ 500	52 (4.4)	0.12	9.33		4.68	1.83	<0.001	14.04	1.65	
R$ 501 – R$ 1000	231 (19.5)	0.10	8.49		3.52	2.60		12.50	1.63	
R$ 1001 – R$ 1500	269 (22.7)	0.10	8.09	0.68	4.32	1.60		12.99	1.65	0.44
R$ 1501 – R$ 3000	327 (27.6)	0.08	8.22		4.44	1.63		12.60	1.60	
>R$ 3000	187 (15.8)	0.09	7.16		4.33	2.00		12.34	1.65	

GM: geometric mean; GSD: geometric standard deviation.
For GM and GSD calculations, values < LD were set to LD/2.
[a] Difference in means in parametric test (T test or ANOVA).

tea drinkers (34%), and those drinking private well water (29%) compared to participants with primary education, non-regular tea consumers and drinking bottled water, respectively. However, these factors only explained 6% of the variability of Cd blood levels.

Regarding As, variables that remained statistically significant in the multivariate model were household income, living near an industrial site, working in a glass factory, in a compost plant or in metal mining activities. Determination coefficient was also low for As model ($R^2 = 6\%$) and the explanatory variables were inversely associated with As blood levels (Table 6). Thus, levels of As were 78% lower in subjects working in a glass factory, 30% lower in those working in a compost plant, and 15% lower in metal mine workers compared to those employed in other occupations. In addition, As was 27% and 11% lower, respectively, among donors with a household income between US$ 250.00 and 500.00 compared to <US$ 250.00, and those living near an industrial site. No factor was significantly associated with Mn levels in multivariate analysis.

Table 7 presents distribution and reference values of Cd, As and Mn blood concentrations in all subjects and stratified by gender, age and smoking status. References values were 0.87 μg L^{-1} for Cd, 9.87 μg L^{-1} for As, and 29.32 μg L^{-1} for Mn, and were slightly higher among women. Reference values of Cd and As were higher in subjects >29 years and smokers, whereas Mn reference value was slightly higher in younger subjects and non-smokers, although differences were not statistically significant.

4. Discussion

The main purpose of the present survey was to determine the Cd, As and Mn exposure profile in blood donors of a capital city of the North of Brazil. Carrying out a health risk assessment was not the aim of this study, neither was to investigate exhaustively sources of exposure to the three metals, although the use of a questionnaire allowed us to identify some potential factors related to exposure. Results show that blood levels of Cd in these blood donors are lower than levels observed in other general populations of developed and developing countries, as detailed below. Factors potentially contributing to increase Cd blood levels were smoking, ethnicity (non-white) and lower education; whereas drinking tea and private-well water were associated with lower Cd. Levels of As and Mn were, in general, higher than those described in the literature for non-occupationally exposed individuals. Higher household income, living near an industrial site, working in a glass factory, a compost plant or in metal mining activities was associated with lower levels of As. However, risk factors for Mn exposure were not identified.

4.1. Cadmium

Levels of Cd in nonsmoking blood donors of Rio Branco were similar to those found in a sample of 653 nonsmoking blood donors from São Paulo non-occupationally exposed to this metal (Kuno et al., 2013). In such study, Cd reference value, which also calculated as the upper limit of the 95% CI of the P95, was 0.60 μg L^{-1} (in the present study reference value for non-smokers is 0.65 μg L^{-1}). According to our findings, women in São Paulo had higher Cd levels than men (i.e., 0.80 *vs.* 0.10 μg L^{-1}). However, range of Cd levels found in the current study are higher than those described in a Brazilian study with 1125 subjects from five states (including the State of Pará, in the North), which ranged from 0.09 to 1.10 μg L^{-1} (Nunes et al., 2010).

Levels of blood Cd in general populations of developed countries were in general higher than levels in this study. Mean Cd level revealed in the present study was similar to that found in 25–74 year-old Swedes (arithmetic mean, AM = 0.47 *vs.* 0.30 μg L^{-1} in the current study) (Wennberg et al., 2006), and much lower than that in blood donors 18–58 years old in Czech Republic (GM = 0.60 *vs.* 0.09 μg L^{-1}) (Batáriová et al., 2006) and 50–65 year-old residents in Belgium (GM = 0.42 *vs.* 0.09 μg L^{-1}) (Schroijen et al.,

Table 3
Blood concentrations (μg L^{-1}) of Cd, As and Mn according to lifestyle characteristics of the study population.

	N	Cadmium			Arsenic			Manganese		
		GM	GSD	*p*-value[a]	GM	GSD	*p*-value[a]	GM	GSD	*p*-value[a]
Smoking habit										
Non smokers	1059 (89.5)	0.08	8.46	<0.001	4.22	1.93	0.30	12.84	1.64	0.42
Smokers	117 (9.9)	0.27	8.75		3.91	2.19		12.42	1.50	
Frequent consumption of fish and seafood										
No	59 (5.0)	0.11	7.31	0.38	4.08	1.58	0.69	12.81	1.58	0.96
Yes	1116 (94.3)	0.08	8.88		4.18	1.97		12.77	1.63	
Frequent consumption of manioc flour (farinha)										
No	138 (11.7)	0.09	7.95	0.74	4.07	2.08	0.61	12.11	1.59	0.15
Yes	1040 (87.9)	0.08	8.89		4.21	1.94		12.87	1.63	
Frequent consumption of Brazil nuts										
No	273 (23.1)	0.10	8.76	0.35	4.22	1.84	0.79	12.88	1.67	0.79
Yes	900 (73.1)	0.08	8.78		4.18	1.99		12.76	1.62	
Frequent consumption of açai fruit juice										
No	93 (7.9)	0.09	9.25	0.85	4.49	1.76	0.23	12.37	1.61	0.50
Yes	1084 (91.6)	0.09	8.72		4.17	1.97		12.81	1.63	
Current vitamin intake										
No	1060 (89.6)	0.09	8.93	0.96	4.17	1.99	0.42	12.85	1.63	0.55
Yes	123 (10.4)	0.08	7.59		4.35	1.66		12.48	1.68	
Frequent consumption of tea										
No	639 (54.0)	0.14	12.74	0.006	4.23	2.02	0.62	12.54	1.64	0.14
Yes	539 (45.6)	0.07	9.43		4.15	1.88		13.07	1.61	
Frequent consumption of energy drinks										
No	882 (74.6)	0.10	8.57	0.004	4.16	1.99	0.53	12.82	1.64	0.83
Yes	294 (24.8)	0.06	9.20		4.28	1.86		12.73	1.60	
Painted or refurnished the house recently										
No	744 (62.9)	0.19	9.09	0.85	4.16	1.97	0.64	12.83	1.65	0.68
Yes	432 (36.5)	0.08	8.28		4.24	1.93		12.68	1.60	
Origin of water used at home										
Bottled	747 (63.1)	0.09	7.99	0.51	4.11	2.04	0.43	12.57	1.62	
Tap water	132 (11.2)	0.09	9.95		4.34	2.15		14.03	1.67	0.13
Private well	242 (20.5)	0.07	10.13		4.41	1.66		12.83	1.64	
Others	52 (4.4)	0.07	12.05		3.96	1.62		12.78	1.57	
Frequent consumption of home garden products										
No	921 (77.9)	0.08	8.71	0.96	4.21	1.91	0.71	12.83	1.63	0.86
Yes	236 (19.9)	0.09	9.50		4.13	2.16		12.75	1.64	
Living near industrial facilities										
No	1019 (86.1)	0.08	8.80	0.41	4.23	1.88	0.19	12.93	1.64	0.01
Yes	139 (11.7)	0.10	8.47		3.81	2.50		11.67	1.54	
Leisure activities: frequent practice of painting, pottery, fishing or firearm shooting										
No	757 (64.0)	0.08	8.81	0.05	4.12	2.05	0.81	12.75	1.66	0.22
Yes	377 (31.9)	0.10	8.27		4.16	1.78		12.30	1.55	

GM: geometric mean; GSD: geometric standard deviation.
For GM and GSD calculations, values < LD were set to LD/2.
[a] Difference in means in parametric test (T test or ANOVA).

2008). Similarly, the 95th percentile of Cd blood levels found in over 4,000 adults surveyed in the 2001–2002 US NHANES (i.e., 1.60 μg L^{-1}) doubled our P95 of Cd (CDC, 2005). Mean level of Cd in 2007–2008 NHANES was still higher than ours (0.55 *vs.* 0.30 μg L^{-1}) (Chen et al., 2013). Cd levels in 1188 adults 18–59 years old participating in the Czech Republic biomonitoring program in 2001–2003 (P95 = 3.0 μg L^{-1}), 2005 (median in non-smokers = 0.50 μg L^{-1}) and 2007 (median in non-smokers = 0.30 μg L^{-1}) were around 2 and 3 times higher compared to this study (Lustigová and Puklová, 2006; Puklová, 2008). In Germany, upper limit of Cd blood levels among non-smoking adults surveyed in 1997–1999 was 1.0 μg L^{-1} (Wilhelm et al., 2004), 1.5-fold higher than upper limit found in blood donors in the current study.

As expected, levels of Cd in blood donors of Rio Branco were also lower than those described for people living near industrial areas. For instance, the P95 of Cd was 2.31 μg L^{-1} in adults living in an industrial area in Tunisia (Khlifi et al., 2014). Mean Cd blood level was 24.10 μg L^{-1} in Chinese people residing in a polluted area (Wang et al., 2011); 9.81 μg L^{-1} in adults living in the vicinity of a cement factory in Pakistan (Afridi et al., 2011a); and 1.70 μg L^{-1} in Korean residents near municipal waste incinerators (Lee et al., 2012).

Levels of Cd in this study were higher in men but gender difference did not remain significant in the multivariate analysis. Reports from Spain and the Czech Republic also found significantly higher Cd blood levels in men (Lustigová and Puklová, 2006; Gil et al., 2011), whereas other studies did not find gender differences (CDC, 2005; Khlifi et al., 2014; Kuno et al., 2013) or revealed higher levels in females than males (Wennberg et al., 2006; Forte et al., 2011; Huang et al., 2013). Because men are more likely to smoke and to be occupationally exposed to metals, these divergent findings may be explained by the different composition of the study populations, which include occupational exposed individuals, people from the general population, smokers and non-smokers, or only non-smokers.

According to previous reports, Cd blood levels were significantly higher in smokers (Gil et al., 2011; Feki-Tounsi et al., 2013b; Khlifi

Table 4
Blood concentrations (μg L^{-1}) of Cd, As and Mn according to current metal-related occupations.

	N	Cadmium			Arsenic			Manganese		
		GM	GSD	*p*-value[a]	GM	GSD	*p*-value[a]	GM	GSD	*p*-value[a]
Paint factory										
No	1143 (96.6)	0.09	8.76	0.19	4.19	1.91	0.53	12.72	1.63	0.32
Yes	28 (2.4)	0.14	7.52		3.58	3.71		13.94	1.60	
Glass factory										
No	1164 (98.4)	0.09	8.80	0.75	4.19	1.91	0.30	12.74	1.63	0.93
Yes	4 (0.3)	0.10	2.66		0.84	25.11		13.02	1.69	
Compost plant										
No	1084 (91.6)	0.09	8.64	0.64	4.22	1.92	0.09	12.87	1.63	0.05
Yes	87 (7.4)	0.08	9.94		3.59	2.35		11.53	1.64	
Ceramics										
No	1089 (92.1)	0.09	8.67	0.89	4.21	1.92	0.15	12.77	1.63	0.54
Yes	80 (6.8)	0.09	10.29		8.65	2.39		12.32	1.66	
Plastic factory										
No	1141 (96.4)	0.09	8.84	0.19	4.18	1.91	0.83	12.77	1.63	0.67
Yes	24 (2.0)	0.14	5.58		3.92	4.49		13.27	1.55	
Battery repairing										
No	1142 (96.5)	0.09	8.69	0.33	4.19	1.91	0.35	12.75	1.63	0.64
Yes	28 (2.4)	0.05	12.26		3.32	3.67		12.27	1.54	
Galvanoplasty										
No	1151 (97.3)	0.08	8.76	0.04	4.17	1.96	0.42	12.78	1.63	0.84
Yes	15 (1.3)	0.15	11.51		3.93	1.31		12.48	1.65	
Photography developing										
No	1135 (95.9)	0.09	8.70	0.61	4.16	1.97	0.41	12.73	1.63	0.83
Yes	33 (2.8)	0.07	11.92		4.46	1.60		12.98	1.64	
Prosthetics										
No	1149 (97.1)	0.09	8.71	0.32	4.17	1.96	0.67	12.74	1.63	0.65
Yes	22 (1.9)	0.05	11.22		4.36	1.62		13.35	1.60	
Gasoline station										
No	1078 (91.1)	0.09	8.69	0.36	4.17	1.93	0.90	12.83	1.63	0.05
Yes	90 (7.6)	0.07	9.52		4.13	2.28		11.67	1.57	
Metal mining										
No	1129 (95.4)	0.09	8.70	0.64	4.22	1.92	0.08	12.78	1.63	0.42
Yes	40 (3.4)	0.07	10.01		3.10	2.98		12.08	1.54	
Welder										
No	1095 (92.6)	0.09	8.67	0.96	4.18	1.97	0.58	12.78	1.64	0.27
Yes	71 (6.0)	0.09	10.50		4.03	1.65		12.09	1.50	

GM: geometric mean; GSD: geometric standard deviation.
For GM and GSD calculations, values <LD were set to LD/2.
[a] Difference in means in *T* test.

et al., 2014; Puklová, 2008; Schroijen et al., 2008). It is known that cigarette smoking constitutes an important source of exposure to several heavy metals, in particular Cd in ionic form (Joseph et al., 2001; Shih et al., 2003). In the present study, results from multivariate analysis reflect that smoking might be a relevant exposure source to Cd and may in part explain the variability in the blood metal levels. However, tobacco smoking itself cannot explain the Cd exposure burden in the studied population, suggesting that some other unknown exposure sources might be involved.

Additional risk factors for Cd exposure in the present study were age, non-white ethnicity and low education. The observed increase in Cd blood levels with age is in agreement with other studies (Forte et al., 2011; Gil et al., 2011; Bjermo et al., 2013; Huang et al., 2013; Khlifi et al., 2014) and reflects the well-known capacity of metals to accumulate in the body. Thus, blood levels of Cd might be indicative of both recent and past exposure to this metal. The association with ethnicity and education is more difficult to explain and could be related to diet or occupations with Cd exposure. Food is the main source of non-occupational exposure to Cd. In this sense, it is known that food cultured in Cd-rich soil constitutes a major source for this metal (Satarug and Moore, 2004), which can be absorbed by many food crops such as cereal grains, wheat, rice, potatoes, and various seeds. To a lesser extent than food, drinking water is a source for Cd intake. However, we do not have an explanation for the associations between drinking tea and non-bottled water and lower Cd blood levels.

More detailed information on food intake and occupations with potential exposure to Cd (e.g., type of work, years of working), such as pigment production, coatings and plating, contact with electronic batteries, would have provided a further insight on local sources of exposure to Cd in the study population.

4.2. Arsenic

Blood As levels were, in general, higher than those reported for people in different areas of the world, including Brazil (Nunes et al., 2010). Level of As found in the latter study of 1125 Brazilians ranged between 0.1 and 3.2 μg L^{-1}. Our mean As concentration (GM = 4.19 μg L^{-1}) is higher than the mean level found in Italian adolescents (GM = 0.82 μg L^{-1}) (Pino et al., 2012); similar to that reported for control subjects in a bladder cancer case-control study in Tunisia (AM = 3.31 *vs.* 4.91 μg L^{-1} in the present study) (Feki-Tounsi et al., 2013a), and even similar to levels in individuals residing in an industrial area in Tunisia (P95 = 6.79 *vs.* 9.62 μg L^{-1}) (Khlifi et al., 2014) or in steel workers in Pakistan (AM = 4.07 μg L^{-1}) (Afridi et al., 2011b).

Table 5
Multivariate linear regression model for log-transformed blood Cd concentrations (Ln-Cd) and explanatory variables.

	Ln-Cd (R^2 = 6.0)		
	Exp (β)	Standard error	95% CI
Intercept	0.11	0.31	−2.84; −1.64
Ethnicity (ref = white)			
Non white	1.26	0.14	−0.05; 0.00
Education (ref = ⩽ primary school)			
Secondary incomplete/complete	0.62	0.17	−0.82; −0.13
Superior incomplete/complete	0.61	0.20	−0.90; −0.11
Smoking habit = smoker	3.29	0.22	0.76; 1.62
Consumption of tea	0.66	0.13	−0.66; −0.15
Origin of water used at home (ref = bottled)			
Tap water	0.67	0.33	−1.06; 0.25
Private well	0.71	0.17	−0.67; −0.01
Other	0.94	0.21	−0.48; 0.36

Multivariate model include 1081 individuals with complete information on cadmium levels and independent variables.
β: linear regression coefficient; CI: confidence interval.

Table 6
Multivariate linear regression model for log-transformed blood As concentrations (Ln-As) and explanatory variables.

	Ln-As (R^2 = 6.0)		
	Exp (β)	Standard Error	95% CI
Intercept	1.61	0.09	1.43; 1.79
Household income (ref = ⩽ R$ 500)			
R$ 501 – R$ 1000	0.73	0.10	−0.51; −0.12
R$ 1001 – R$ 1500	0.89	0.10	−0.32; 0.07
R$ 1501 – R$ 3000	0.90	0.10	−0.29; 0.09
>R$ 3000	0.90	0.10	−0.30; 0.10
Industry near home	0.89	0.06	−0.23; 0.00
Glass factory	0.22	0.32	−2.17; −0.90
Compost plant	0.70	0.12	−0.59; −0.13
Metal mining	0.85	0.08	−0.31; −0.01

Multivariate model include 1034 individuals with complete information on cadmium levels and independent variables.

As exposure among blood donors of Rio Branco was higher in males, older individuals and smokers, which is in agreement with the literature (Feki-Tounsi et al., 2013a; Khlifi et al., 2014; Lee et al., 2012; Pino et al., 2012). However, differences were not statistically significant.

Since single doses of As are rapidly and extensively cleared from the blood via the kidney, blood concentrations have been considered to reflect only recent exposure (Pomroy et al., 1980). However, with chronic and continuing exposure, steady-state concentrations in blood are achieved and these have the potential to serve as biomarkers of past exposure (Hall et al., 2006). According to this, lack of association between blood As and water origin may indicate that Rio Branco is not an area with groundwater As contamination. It is known that the lower the concentration of As in water, the greater is the role of smoking and occupational exposures (ATSDR, 2010). Regarding occupations, two studies observed that As blood levels increased in workers exposed to pesticides (Khlifi et al., 2014) and construction workers (Feki-Tounsi et al., 2013a). As concentration in our study was inversely associated with some occupations related to heavy metal exposure. In this regard, working in a glass factory, compost plant or in metal mining activities does not necessarily mean that the person is exposed to As. In addition, finding of higher As levels in individuals with little household income together with that of inverse association with those occupations could be reflecting a higher exposure in low-paying jobs specifically related to As, such as farming or workers exposed to wood fumes, that were not considered here.

Fish and seafood consumption is another potential source of As exposure (Lee et al., 2012), although most of the As in fish is an organic form that is rapidly excreted in urine (ATSDR, 2007). Thus, blood levels of As might not be a good biomarker of fish/seafood consumption since no information was gathered on intake in the last days or even weekly frequency of fish consumption. In the studied population, regular fish/seafood consumers had higher As concentrations, but difference was not statistically significant.

4.3. Manganese

In the present study, 37% of the subjects had blood concentrations of Mn above the range of normal blood Mn in U.S. population that is 4–14 μg L^{-1} (ATSDR, 2009). Mn levels were also higher compared to other studies. The mean level of Mn in blood donors of Rio Branco (GM = 12.81 μg L^{-1}) is higher than the mean level found in adults of the general population of Korea (GM = 10.8 μg L^{-1}) (Lee et al., 2012), Canada (GM = 10.8 μg L^{-1}) (Clark et al., 2007), Denmark (AM = 9.06 μg L^{-1} *vs.* 14.54 μg L^{-1}) (Kristiansen et al., 1997) and people from five Brazilian states (range = 6.9–18.4 μg L^{-1}) (Nunes et al., 2010). It is noteworthy to mention that Mn concentrations in the state of Pará, North of Brazil, were significantly higher than in people from other states (Nunes et al., 2010).

Surprisingly, Mn levels in this study are greater than those described for Spanish workers exposed to metals (GM = 7.05 μg L^{-1}) (Gil et al., 2011), and also higher than levels found in children living in the vicinity of a ferromanganese alloy plant in the State of Bahia, North-East Brazil (AM = 8.2 μg L^{-1}) (Menezes-Filho et al., 2011). In Quebec, Canada, blood Mn concentrations higher than 7.5 μg L^{-1} were found to be associated with neuromotor impairment in adults living in the vicinity of a ferromanganese refinery (Beuter et al., 1999). About 89% of the subjects in the present study had Mn concentrations ⩾7.5 μg L^{-1}.

Attempting to gain further insight into the occurrence of these high concentrations of Mn in blood, characteristics of blood donors with Mn in the upper range (>75th percentile = 17.88 μg L^{-1}) were compared to those of donors with Mn levels <75th percentile. Women, non-white individuals and non-smokers were more likely to have Mn levels >17.88 μg L^{-1} than lower levels (Table S4). As levels were about 2-fold higher in blood donors with Mn in the upper range compared to Mn < 17.88 μg L^{-1}. No statistically significant differences in the distribution of frequencies were observed for the remaining characteristics of participants (data not shown).

According to our finding, previous studies have shown higher levels of Mn in women (Clark et al., 2007; Nunes et al., 2010; Lee et al., 2012). Ingestion is the main Mn exposure route in non-occupationally population and, in general, intake is higher for vegetarians and those consuming more foods and beverages high in Mn, such as nuts, tea, cereals, and some fruit juices and drinks (WHO, 2004). A recent report found that *acai* fruit, a typical Amazon region berry, has high levels of essential minerals, especially Mn (Da Silva Santos, 2014). Other Amazonian fruits may also have high levels of Mn, representing potential sources of human exposure to Mn in this region, although data confirming this hypothesis have not been published yet. In the present survey, frequent consumption of *açai* fruit was not related to Mn blood levels, which could be explained by the high variability and short half-life of Mn in blood. Blood Mn may reflect exposure over a recent period and, in general, studies have found no or weak relations between blood

Table 7
Distribution and reference values of blood concentrations (μg L^{-1}) of Cd, As and Mn in blood donors of Rio Branco, Acre, Brazil, 2010–2011.

	N	AM	GM	GSD	Min	Max	P5	P25	P50	P75	P95	95%CI[a]	
												LL	UL
Cadmium													
ALL SUBJECTS	1183	0.30	0.09	8.78	<LD	9.83	<LD	0.05	0.18	0.39	0.82	0.77	0.87
Men	890	0.29	0.08	8.88	<LD	9.83	<LD	0.05	0.18	0.38	0.83	0.77	0.89
Women	293	0.32	0.09	8.51	<LD	8.54	<LD	0.04	0.19	0.44	0.76	0.63	0.90
<30 years	582	0.25	0.07	8.73	<LD	8.54	<LD	0.03	0.17	0.35	0.66	0.60	0.72
⩾30 years	601	0.35	0.10	8.75	<LD	9.83	<LD	0.06	0.21	0.45	0.99	0.90	1.08
Non smokers	1059	0.25	0.08	8.46	<LD	9.03	<LD	0.04	0.17	0.35	0.61	0.57	0.65
Smokers	117	0.81	0.27	8.75	<LD	9.83	<LD	0.22	0.52	0.98	2.30	1.94	2.66
Arsenic													
ALL SUBJECTS	1183	4.91	4.19	1.96	<LD	30.75	2.31	3.07	3.75	6.27	9.62	9.37	9.87
Men	890	4.93	4.25	1.85	<LD	30.75	2.28	3.10	3.77	6.39	9.61	9.32	9.90
Women	293	4.84	4.01	2.27	<LD	26.28	2.37	2.93	3.68	6.11	9.77	9.28	10.26
<30 years	582	4.94	4.17	2.07	<LD	30.75	2.24	3.09	3.83	6.55	9.61	9.25	9.97
⩾30 years	601	4.87	4.21	1.84	<LD	29.51	2.34	3.02	3.69	6.21	9.71	9.36	10.06
Non smokers	1059	4.92	4.22	1.93	<LD	30.75	2.31	3.08	3.78	6.39	9.61	9.35	9.87
Smokers	117	4.75	3.91	2.19	<LD	29.51	2.02	3.00	3.65	5.64	9.94	9.02	10.86
Manganese													
ALL SUBJECTS	1183	14.54	12.81	1.63	1.96	119.12	6.00	9.38	12.30	17.88	28.58	27.83	29.32
Men	890	14.48	12.76	1.63	1.96	119.12	6.13	9.40	12.27	17.28	28.42	27.54	29.30
Women	293	14.73	12.96	1.65	2.94	49.90	5.88	9.31	12.50	18.71	29.58	28.20	30.96
<30 years	582	14.39	12.62	1.66	1.96	89.95	5.45	9.24	12.12	17.51	28.78	27.72	29.85
⩾30 years	601	14.68	13.00	1.61	3.60	119.12	6.22	9.49	12.36	18.40	28.45	27.40	29.50
Non smokers	1059	14.61	12.84	1.64	1.96	119.12	5.98	9.35	12.33	18.15	28.60	27.80	29.40
Smokers	117	13.55	12.42	1.50	3.92	40.69	6.57	9.49	12.00	15.54	27.52	25.81	29.23

AM: arithmetic mean; GM: geometric mean; GSD: geometric standard deviation; *P*: percentile.
For means and SD calculation, values < LD were set to LD/2.
Numbers in the gray-shaded column are the reference values of blood metal levels, which were established as the upper limit of the 95% confidence interval of the 95th percentile.
[a]95%CI of the 95th percentile; LL: lower limit; UL: upper limit.

Mn and exposure (Laohaudomchok et al., 2011). Overall, donors in this study with higher blood Mn levels seem to be healthier (i.e., less likely to be smokers and to have low hematocrit), which could be in part explained by a "healthier" diet with a higher intake of Mn enriched foods. However, interpretation of our findings with respect to such status is limited by the lack of detailed information on diet or recent food intake. Anyhow, consumption of *açai* and other fruits with high levels of Mn could be responsible for the high blood Mn levels found in the studied population.

Overall, the lack of more detailed data on dietary patterns, occupations and environmental exposure prevents us from identifying more specific exposure pathways to Cd, As and Mn in blood donors from Rio Branco. Nonetheless, this study represents the first human biomonitoring of Cd, As and Mn exposure in the North of Brazil, and adds some information on potential local sources of exposure in the population living in the largest city of the State of Acre. Additional strengths of this study include the large sample size; the use of whole blood, which is the most widely used and accepted matrices for biomonitoring heavy metal exposure in environmental toxicology (Wilhelm et al., 2004; Wang et al., 2011); and the use of blood donors, who have a good predisposition to collaborate and are likely to be healthy individuals.

5. Conclusions

In this exposure assessment study, profile of Cd, As and Mn blood levels was determined in adult blood donors from the North of Brazil, providing important reference data that can be useful for the surveillance of exposure of Brazilians to heavy metals. Ongoing efforts should be made to assess the body burden of environmental pollutants among the general population in the country and to evaluate the contribution of various exposure sources and pathways.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2014.12.083.

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