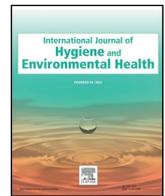




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Biomonitoring of metals and trace elements in urine of central Ethiopian populations

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ABSTRACT

Biomonitoring of chemical concentrations in humans is important for detecting, monitoring, and addressing a wide range of health threats. However, it is virtually absent across many African nations, including Ethiopia. This study aims to determine urinary concentrations for metals and trace elements in populations living in the central Ethiopian Rift Valley. The region is unindustrialized, rural, and characterized by unique geologic rifting and volcanic activities that have produced vast pyroclastic materials, forming its aquifer and fertile agricultural soils. Millions of people in the region rely on wells for drinking water and are engaged in cereal-based subsistence agriculture. We enrolled a total of 386 residents aged 10–50 years old (201 females and 185 males). The levels of 23 elements except F⁻ were quantified in water and urine samples by ICP-MS. Mean concentrations of B, F⁻, Ca, and Mg were measured in mg/L levels, while concentrations of Mo, Zn, Sr, Rb, and Li ranged between 100 and 700 µg/L. Mean concentrations between 5 and 15 µg/L were found for Ni, Cu, and Mn, while Ag, Be, Cd, Co, Pb, Sb, Th, TI, and U were all below 5 µg/L. Arsenic and Al had mean concentrations between 30 and 50 µg/L. Mean urinary concentrations of Ca, Cu, Mg, Pb, Sr, and Zn were significantly higher in males than females, whereas Co and Mn were higher in females. Finally, younger individuals (10–30 years) had significantly higher mean concentrations of B, Cd, Co, Mg, Mo, and Pb than those between 31 and 50 years, whereas only Ca was higher in the older age group. The concentration ranges of B, Mo, Mn, TI, Li, Zn, and in particular F⁻ (0.44–44.6 mg/L) and As (2.2–164 µg/L) in urine were higher than the reference ranges reported in healthy unexposed North American and European populations, while those for the remaining 16 elements were comparable to published reference ranges from such settings. The established concentration ranges are important to monitor future changes in exposure, and risk factors for disease, that might stem from the economic growth and industrialization that is currently underway in the region.

1. Introduction

Biomonitoring of biological matrices has proven to be a useful tool to evaluate exposure to environmental chemicals (Haines et al., 2017; CDC, 2015; Goullé et al., 2005; Woodruff et al., 2011). Biomarkers measured in blood, urine, or other tissues are widely used as indicators of exposure in the general population—both in occupational and environmental settings that more closely reflect total exposure from all possible sources and routes (Angerer et al., 2007; Sexton et al., 2004). While a large number of biomonitoring studies have been conducted in developed countries (e.g., Saravanabhavan et al., 2017; Coelho et al., 2013; CDC, 2012; Alimonti et al., 2005; Wilhelm et al., 2005; Paschal et al., 1998), similar studies are rare in most developing nations and especially in Africa. There are no current large-scale human biomonitoring initiatives on the continent, even at the country level to establish a background level of environmental chemical exposures. Yet, limited studies are available in certain African subpopulations that focus on

specific metals and trace elements in blood (Benin: Yedomon et al., 2017; Egypt: Moawad et al., 2016; Congo: Tuakuila et al., 2015; Tunisia: Khlifi et al., 2014; Morocco: Laamech et al., 2014).

In many countries, where surface water is lacking or polluted, people increasingly depend on groundwater wells as their primary source of drinking water. The Ethiopian Rift Valley (ERV), an arid and semi-arid climate, where fresh surface water resources are scarce or inaccessible to households for most of the year due to the scattered nature of the communities (Paul et al., 2018). The region is extensively covered with volcanic rocks and sediments primarily composed of pyroclastic ashes that release both toxic and essential elements into groundwater and agricultural soils (Chorowicz, 2005; Riemann et al., 2003; Rango et al., 2012, 2014, 2017). There is thus an apparent long-term exposure from naturally-occurring toxic and essential elements in water and food sources. By simultaneously measuring multiple elements in urine using ICPMS (Inductively Coupled Plasma Mass Spectrometer), we can estimate current exposures and determine the

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concentration ranges for these elements in this study population. This allows monitoring of changes in future exposures and related human health risks, as it is routinely done in many countries in North America (Saravanabhavan et al., 2017; CDC, 2012; Ting et al., 1999; Paschal et al., 1998), and Europe (Ganzleben et al., 2017; Pérez-Gómez et al., 2013; Černá et al., 2012; Fréry et al., 2012; Levy et al., 2007; Reis et al., 2004; Schulz et al., 2012). Such biomonitoring studies from different countries are important because reference concentrations reflect a variety of different factors, including geography, ethnicity, background levels of pollution, lifestyle, and diet.

To the best of our knowledge, no biomonitoring data currently exist to establish reference values in Ethiopia. In this study, we measured the concentrations of a range of potentially toxic and essential elements in drinking water and in urine. Measurement of these elements in water is relevant in the region as the communities primarily use well water for drinking and cooking. Measurement in urine, meanwhile, is useful for biomonitoring as many chemicals are excreted via urine. These biomarkers will provide more accurate exposure information and help to generate concentration values of chemicals from non-occupationally-exposed populations in the region. In addition, we compare these concentrations with those established in biomonitoring studies from other countries, where sampling has been conducted in populations that are generally believed to be unexposed to major anthropogenic sources of pollution, although the possibility of low levels of exposure in such studies cannot be excluded entirely. This comparison provides perspective on the sources and levels of trace elements in urine that could pose health risks or benefits among Ethiopians and informs targeting of future health-related studies in the region.

2. Materials and methods

2.1. Study population

A total of 386 individuals (10–50 years old) were enrolled from 27 rural communities during two sampling periods (June 2015 and May 2016) (Fig. 1). Based on extensive prior work conducted to characterize the range of F^- levels in community water sources in the Ethiopian Rift Valley region, communities covering the range of F^- exposures were selected for the study. Given the high correlation in F^- and other exposures in this location (Rango et al., 2009, 2014), it likely represents most of the range of potential exposures in the Rift population. Individuals in the sample communities are primarily engaged in cereal based agricultural activities. In each sample community, households were selected by counting off every n households (where n was selected based on target sample sizes from each community), and 1 to 2 subjects from each household were enrolled in the study. Study inclusion criteria for selected individuals were: Age between 10 and 50 years that approximately match the length of consumption of well water. In each community, we attempted to select individuals from the randomly sampled households so as to get equal representation across different ages and sexes (e.g., 10–20, 20–30, 30–40, and 40–50 years). Thus, at least 2 subjects from each age group were enrolled in each community, with a nearly equal proportion of males to females. We also collected survey data on anthropometric measures of weight and height for deriving body mass index (BMI), using digital weighing scales to capture individuals' weight, and measuring tapes for their height (Table 1). Included in the questionnaire were questions on smoking habits and use of tobacco products. Field enumerators (graduate students and nurses/medical doctors) were trained on the content of the questionnaire. The study received ethical approval (Protocol No. C0863) from the Institutional Review Board (IRB) at Duke University and by the National Research Ethics Review Committee (NRERC) of Ethiopia. All subjects provided consent, and parents/guardians gave permission for children to participate in addition to children giving their own assent.

Regarding communicating results of our project findings, the communities are generally aware of health issues linked to the drinking

water, though they are not aware of the type and specific concentrations of different contaminants in their water. Given the range of ongoing studies in the region, some are becoming more familiar with the term 'fluoride' and in its role in causing health problems. Typical complaints among populations in the region concern the lack of access to alternative water resources, and pains from teeth and bone disorders. In prior and ongoing work, we have been informing local leaders and participants of the findings from our research on F^- levels in their drinking water, and of the location of alternative low F^- sources or dietary choices that may buffer the toxicity of F^- . Based on our new measurements, we will now include new information about As and other elements in future communications with these populations.

2.2. Water, and urine sampling and analytical methods

Water samples were collected from 27 community wells, each of which served as the primary sources for drinking and cooking purposes used by households in the study communities. The samples were collected from active pumping wells after being allowed to flow for a few minutes prior to sampling. The water samples were then filtered in the field using disposable 60 mL volume luer lock syringes and 0.45 μ m Mixed Cellulose Ester membrane filters directly into 60 mL polyethylene bottles pre-cleaned with trace metal grade \sim 1N HCl and \sim 1N HNO_3 , and then rinsed with deionized water of resistivity $>$ 18M Ω /cm. Twelve-hour urine samples (overnight, 6pm–6am) were collected from 386 individuals. Urine samples were collected in acid pre-cleaned disposable urine collection plastic beakers (each with a capacity of 1 L) that were provided to each individual and immediately transferred to 60 mL acid-washed polyethylene bottles. Participants were instructed to carefully collect urine samples and shown how to best avoid possible contamination. The water and urine samples were acidified with high-purity HNO_3 (Fisher Optima) that was prepared adding it in the bottles before samplings. Water and urine samples were then kept in zero-degree freezer, and properly packed, stored and transported to the laboratory in the United States. The first author has been trained in 'Shipping Biological Materials' and has extensive experience on proper handling of biological samples including sampling, packing, and shipping procedures.

The concentration of metals and trace elements in drinking water, and 12-h urine samples were determined using inductively coupled plasma mass spectrometer (ICP-MS). The F^- concentrations in drinking water and 12-h urine samples were determined electrochemically using the Thermo Scientific Orion Ion-Selective Electrode (ISE). Samples were buffered with an equal volume of total ionic strength adjustment buffer (TISAB II) solution of pH 5–5.5, which allows for optimal analysis of F^- . Calibration standards (1, 10, and 20 mg/L of F^-) were prepared from a 100 mg/L stock solution. The range of electrode calibration slope for a 10-fold change in F^- concentration was -57 to -60 mV, which is within the acceptable range.

Quality control was conducted using freeze-dried urine reference material (Seronorm LOT 0511545; LGC Standards). The accuracy of ICP-MS trace element measurements in urine was within the acceptable range of 82%–115% relative to the LGC standard. The accuracy of ISE's F^- measurements for both urine and water standards ranged from 98% to 102.5% relative to the LGC standard. Repeatability of the trace element measurements in urine was also tested in a second lab at the Research Triangle Institute (RTI) International (Research Triangle Park, United States) for 25 samples, and results were found to be consistent with our measurements. For example, the repeatability of As, Pb, and Mo was 103.8%, 95%, and 102.7%, respectively. The recovery for As, Pb, and Mo in urine samples with respect to the NIST SRM 2668 low standard was 105%, 94.4%, and 98% respectively. The concentrations below detection limits (DLs) were measured (in μ g/L) for silver (0.01), Al (1.7), Be (0.04), Co (0.02), Cd (0.04), Cu (0.57), Mn (0.33), Ni (0.56), Pb (0.06), Sb (0.04), Th (0.05), Tl (0.01), and U (0.02). In this work, we did not collect field blanks and field controls, however, we

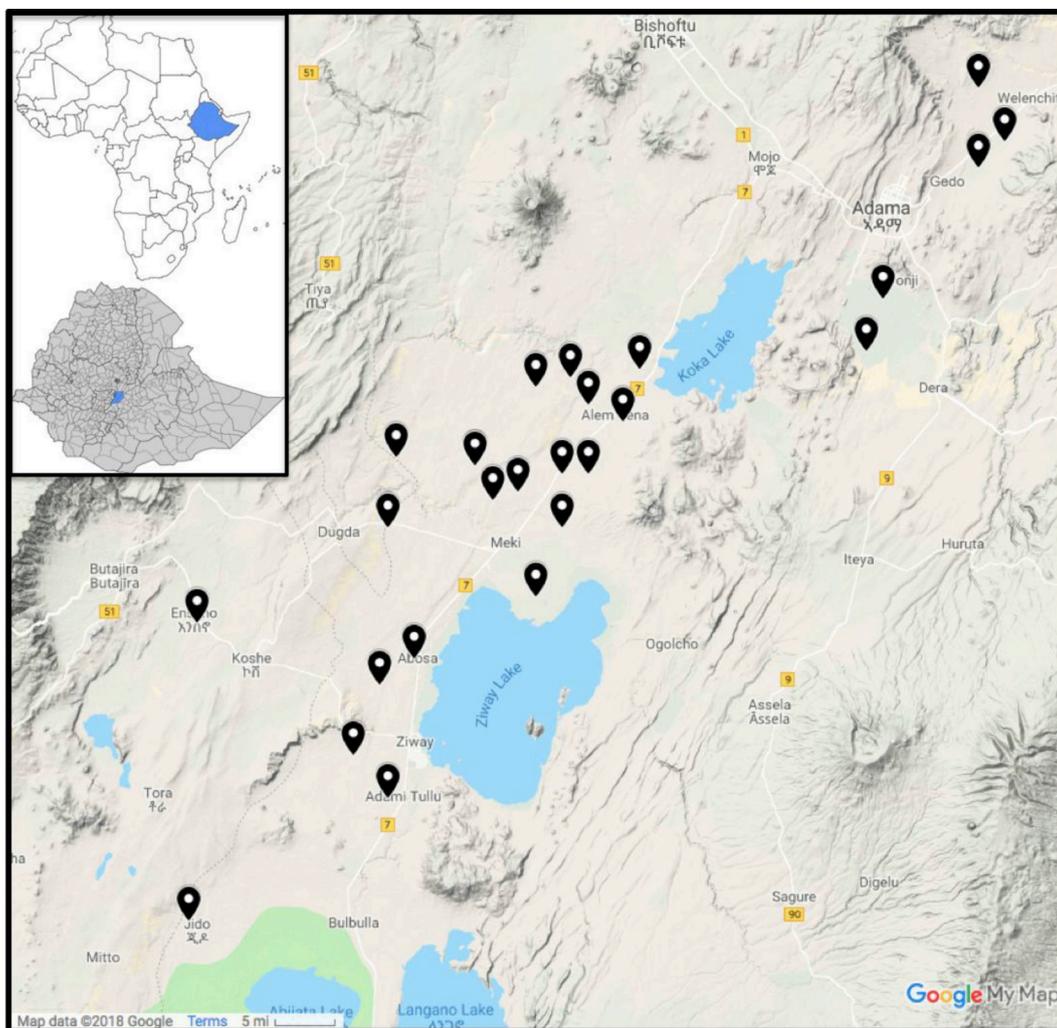


Fig. 1. Groundwater and 12-h urine sampling sites in the central Ethiopian Rift Valley.

have taken the necessary precaution to avoid any contamination in water and urine samples including proper collection, storage and transportation of sampling materials such as pre-cleaned polyethylene bottles and beakers, and syringes/filters.

2.3. Statistical analysis

Descriptive analyses were carried out using percentiles, means, and standard deviations for continuous variables. The data were not normally distributed and thus required log-transformation prior to statistical analysis. The relationships between trace elements concentrations in water, and urine were evaluated using Pearson's correlation coefficient. Comparison of urinary metals and trace element concentrations in the subjects based on sex, age, and BMI was performed using t-tests. For the biomarker data falling below the detection limit, we recorded

concentrations as DLs/2, prior to log-transformation. If the proportion of biomonitoring results below the DL was greater than 40%, geometric means (GM) were not calculated. Biomonitoring percentiles that are less than the DL are reported as bd. The statistical significance was set at $p < 0.05$.

3. Results and discussion

3.1. Characteristics of the study population

The average age of study individuals was 24.6 ± 11 years old. Females represented 52% ($n = 201$) of the respondents (Table 1). The majority of the population in the study region is engaged in cereal-based subsistence farming, and most respondents have low education and socio-economic status (e.g., Paul et al., 2018). The mean BMI of the

Table 1 Statistical descriptions of the characteristics of study population.

	N	Mean \pm SD	Min	Percentiles					Max
				5th	25th	50th	75th	95th	
Age (years)	386	24.6 \pm 11.1	10.0	11.0	15.0	22.0	34.0	45.0	50.0
Weight (kg)	382	50.4 \pm 11.8	21.0	27.7	43.8	52.0	58.5	67.1	82.9
Height (m)	382	1.59 \pm 0.11	1.24	1.36	1.54	1.60	1.67	1.77	1.86
BMI (kg/m ²)	382	19.6 \pm 3.17	8.7	14.7	17.7	19.4	21.3	25.4	30.7

Table 2
Summary of EC, TDS, and major and trace elements concentrations in 27 community water samples considered in this study.

	Min	Max	Percentiles					Mean	Water quality standards			Samples Exceeding lowest standard (% of villages)
			5	25	50	75	95		WHO	U.S EPA	EU	
Major elements (mg/L)												
F ⁻	0.55	15	1.41	2.53	5.59	9.88	13.7	6.48	1.5	4.0	1.5	25 (96%)
Ca ²⁺	0.36	40.7	0.61	5.47	15.2	23.5	38.77	15.8	–	–	–	–
Mg ²⁺	bd	13.8	0.02	1.06	3.66	5.11	11.63	3.84	–	–	–	–
Trace elements (µg/L)												
As	0.16	54.9	0.59	1.98	4.9	17.7	54.9	10.2	10	10	10	7 (26%)
Cd	bd	0.08	bd	bd	0.01	0.02	0.08	0.02	3	5	5	0 (0%)
Pb	bd	1.59	bd	bd	0.04	0.34	0.73	0.2	10	15	10	0 (0%)
U	bd	25.5	0.5	1.23	2.96	6.25	25.5	4.67	15	30	–	1 (4%)
Ag	bd	0.04	bd	bd	bd	bd	bd	bd	–	100 ^a	–	0 (0%)
Al	2.3	310	2.74	5.9	18.2	52.2	200	43.6	–	200 ^a	–	2 (7%)
B	9.0	638	18.9	54.7	111	289	556	185	2400	–	1000	0 (0%)
Be	bd	0.17	bd	bd	bd	0.07	0.15	0.04	–	4	–	0 (0%)
Co	bd	0.44	bd	0.01	0.02	0.07	0.12	0.05	2000	13000	2000	0 (0%)
Cu	0.03	4.91	0.03	0.11	0.36	0.61	3.89	0.9	2000 ^a	1300	–	0 (0%)
Li	3.6	156	5	12.0	32.6	57.9	143	41.8	–	700	–	0 (0%)
Mn	bd	422	bd	0.86	3.41	28.3	211	37.8	400	50 ^a	–	3 (11%)
Mo	0.34	73.7	0.93	4.24	7.16	12.5	73.7	13.4	–	–	–	–
Ni	bd	0.99	bd	bd	0.05	0.45	0.72	0.22	70	–	20	0 (0%)
Rb	0.84	52.2	1.31	4.44	8.59	15.5	35.7	11.5	–	–	–	–
Sb	bd	0.74	bd	bd	0.01	0.13	0.74	0.11	20	6	5	0 (0%)
Sr	3.9	227	4.16	32.9	86.8	136	223	91.4	–	4000	–	0 (0%)
Th	bd	0.16	bd	0.01	0.01	0.02	0.06	0.02	–	–	–	–
Tl	bd	0.01	bd	bd	bd	bd	bd	bd	–	2	–	0 (0%)
Zn	1.24	143.4	1.85	7.8	16.1	41.3	88.3	28.5	5000 ^a	–	–	0 (0%)

Notes: bd = below detection; ^a Secondary standard.

Regulatory limits for inorganic chemicals in drinking water established by the WHO (2011), the U.S. EPA (2009), and the EU Council (1998).

participants was 19.6 ± 3.2 kg/m² (range: 8.7–30.7 kg/m²). Based on the WHO classification, most participants were categorized as normal weight (56%; n = 215) or underweight (39%; n = 148). Five percent (5%; n = 19) of respondents were overweight.

3.2. Distribution of trace elements in drinking water

The distribution of metals and trace elements measured in the 27 sampled community wells is presented in Table 2. The highest concentrations (relative to levels normally considered safe) were for F⁻ and As, and these exceeded the WHO recommended limits of 1.5 mg/L and 10 µg/L, respectively. Arsenic in the drinking water wells ranged from 0.16 to 54.9 µg/L; 26% of samples contained As concentrations above 10 µg/L. Fluoride concentration ranged from 0.6 to 15 mg/L, with 93% of samples exceeding the WHO recommended limit. In addition, the concentration of Mn was elevated in three of the wells (163, 211, and 422 µg/L), exceeding the 50 µg/L USEPA secondary standard. Only one sample (25.5 µg/L) had a concentration of U that exceeded the 15 µg/L WHO drinking water standard. Overall, the concentrations of other elements (such as B, Cd, Li, Pb, Sb, Sr, Ni, Co, Cu, Be, Zn, and Tl) was below the lowest of these three agencies drinking water standards (Table 2). Due to Rift aquifer geochemistry, the water samples contained very low levels of Ca (mean: 15.8 mg/L) and Mg (mean: 3.8 mg/L), compared to levels found in groundwater in the Ethiopian highlands (Rango et al., 2009). The alkaline pH of the Rift groundwater allows the release of F⁻ and other oxyanion forming elements such as As, B, and Mo to occur at elevated levels. Yet, this high pH limits the occurrence of metals such as Mn, Al, Cu, Pb, Zn, and Ni in the Rift groundwater (Rango et al., 2014).

The health effects of F⁻ in the region have been studied (Rango et al., 2012, 2017); however, the health concerns from low-to moderate-concentrations of As in the region require further investigation. Many epidemiological studies suggest risk of cancer, cardiovascular disease, respiratory illness, and diabetes from As exposures in water

below the EPA maximum contamination level of 10 µg/L (Moon et al., 2013; García-Esquinas et al., 2013; Leonardi et al., 2012; Navas-Acien et al., 2008). This suggests there may be no safe threshold for As exposure (Schmidt, 2014). It is therefore reasonable to consider that the level of As exposure found in our study may be associated with adverse health effects. Overall, the very low levels of metals measured in drinking water suggest no immediate health risks for conditions such as kidney disease (e.g., from U and Cd), or neurodevelopment disorders (e.g., Pb) in this population.

3.3. Distribution of metals and trace elements in urine

Table 3 illustrates the statistical distribution (percentile, min, max, and means) of metals and trace elements in urine samples of the study population, as well as the reference ranges and 95th percentiles reported in other studies from developed countries. We found that mean concentrations of B, F⁻, Ca, and Mg were measured in mg/L levels, whereas concentrations for Mo, Zn, Sr, Rb, and Li fell between 100 and 700 µg/L. Mean concentrations between 5 and 15 µg/L were found for Ni, Cu, and Mn, and below 5 µg/L were observed for Ag, Be, Cd, Co, Pb, Sb, Th, Tl, and U. Arsenic and Al had mean concentrations between 30 and 50 µg/L. Since no biomonitoring studies in urine have been carried out in Ethiopia or many other African countries, we mainly depended on established reference concentration ranges from North American and European populations for comparison. While these comparisons relate to populations with very different characteristics and environmental exposures, they do provide a benchmark for consideration of the observed elemental levels. The concentrations of Ag, Al, Be, Ca, Cu, Pb, Rb, Sb, Th, and U were found to fall within the reference ranges. Cadmium, Mg, Co, Sr, and Zn were slightly higher, but even these were within the 95th percentile of the ranges found in studies of healthy unexposed populations (Table 3). One of the elements with exceptionally high concentration ranges was F⁻. Levels of As, B, Mo, Mn, Tl, Li and Zn were also found to exceed the comparison ranges. All of

Table 3
Summary of metals and trace elements concentrations in urine samples of individuals in the study area.

Inorganic elements	Percentiles					Mean		Geometric Mean		Reference range in healthy and unexposed population in studies in other countries				
	Min	Max	5	25	50	75	95	Mean	Geometric Mean	*not reported for those > 40% bd	^{a,b,c,d} Range	^e Range	95% Percentile	Geometric Mean
Ag	bd	0.20	bd	bd	bd	0.01	0.04	0.01	*		0.04–0.88(c)	< 0.01–0.02	0.03(i)	
Al	bd	1851	4.0	15.0	23.8	37.4	94	45.8	25.0		2.4–19.5(c); 2.3–110(d)	0.6–5.1		9.27(j)
As	2.15	164	5.54	11.4	18.9	38.9	102	30.7	20.9		< 0.5–48.2(a); 15(b)	5.3–11.7	27(f)	48.8(j)
B	227	8216	591	1152	1723	2579	4737	2019	1677		490–3290(c)	380–3600		
Be	bd	0.77	bd	bd	bd	0.04	0.31	0.05	*		0.04–0.76(c)	< 0.01–0.018	0.79(h)	< 0.007(j)
Ca	1.73	382	3.3	10.6	26.1	55.4	155	43.5	24.3		Mean: ~120 mg/L(d)	–		
Cd	bd	3.35	bd	0.27	0.61	1.05	2.28	0.76	0.61		0.05–1.64(a); 0.8(b)	0.005–0.46	1.3(f); 0.42(i)	1.06(j)
Co	bd	9.44	bd	0.52	1.13	1.93	3.93	1.44	1.05		< 0.12–2.05(a)	0.04–0.81	8.3(h); 1.53(i)	1.003(j)
Cu	bd	47.8	bd	2.21	5.59	9.07	17.4	6.5	5.21		4.6–40.4(a)	1.9–15.9	25(f); 13(i)	19.6(j)
F ⁻	0.44	44.6	2.2	4.6	8.5	13.4	23.9	10.1	7.76		0.3–1(d)	–	1.4(f)	
Li	5.0	589	24.6	66	110	164	279	126	100		Mean: ~0.5(d)	5.2–23	115(i)	75(j)
Mg	0.57	611	28.4	67	105	164	275	125	97.1		~90 mg/L(d)	–		
Mn	bd	298	bd	bd	0.51	2.95	31	8.4	*		< 0.09–7.8(a)	< 0.05–0.24	3.33(h); 0.21(i)	0.355(j)
Mo	11.7	2884	64	197	367	614	386	484	335		2.8–288(a)	12–108	170(f); 168(h); 94(i)	116(j)
Ni	bd	61.0	bd	3.49	7.4	11.6	21.5	8.63	6.95		< 0.3–59(a)	0.24–2.7	4.4(f); 2.5(i)	4.73(j)
Pb	bd	3.00	bd	bd	0.14	0.36	0.88	0.26	0.21		0.3–30(a)	0.12–2.9	1.9(f); 6.4(h); 2.1(i)	2.81(j)
Rb	83	4186	143	321	502	777	1671	637	494		284–4096(c)	500–5500	2424(i)	0.277(k)
Sb	bd	2.0	bd	bd	bd	bd	0.05	0.02	*		0.19–1.1(c)	0.022–0.104	0.17(f); 4.17(h); 0.18(i)	0.236(j)
Sr	6.5	788	17	38.4	84.1	162	303	117	79.4		Mean: ~220(d)	18–260	444(i)	0.043(k)
Th	bd	3.3	bd	bd	bd	bd	0.3	0.07	*		0.01–0.28(d)	0.0005–0.005	0.61(f); 0.0031(g)	299(k)
Tl	bd	11.9	0.15	0.65	1.47	2.72	6.25	2.06	1.44		0.07–0.7(c)	0.05–0.54	1.18(h); 0.43(i)	0.5(j)
U	bd	4.3	bd	bd	bd	bd	0.05	0.03	*		0.01–0.35(d)	0.0007–0.019	0.0345(g); 0.01(f)	0.03(j)
Zn	26.7	2983	66	167.3	287	502	1066	386	283		266–846(c); 27–850(d)	40–430	1100(f); 692(i)	1048(j)

Notes: bd = below detection. All concentrations are in µg/L, except Ca, Mg, and F⁻ in mg/L. For each element, the range 95th percentiles and geometric mean of reference values reported for healthy populations in different geographical regions is also presented.

^a White and Sabbionia, 1998 – UK population; ^b Wilhelm et al., 2004 – German population; ^c Minoia et al., 1990 – Italian population; ^d Caroli et al., 1994, and ^e Rodushkin et al., 2000 – Sweden population; ^f Saravanabhavan et al., 2017 – Canada population; ^g Ting et al., 1999; ^h Paschal, et al., 1998 – United States population; ⁱ Heitland and Köster, 2006 – German population; ^j Hoet et al., 2013 – Belgium population; ^k CDC, 2018 – United States population; * GMs were not calculated if > 40% of samples contain bd.

these elements were significantly positively correlated with the respective elements in the drinking water, except for Mn and Zn. Here we discuss these and other elements in additional detail.

Arsenic concentrations in urine of studied individuals were found to range from 2.2 to 164 µg/L (mean: 30.7 ± 29.7 µg/L). The majority (82%; n = 317) of individuals urine samples had As concentrations below 50 µg/L and 19.4% (n = 75) of individuals had As below 10 µg/L. Only 5% of individuals had As between 100 and 164 µg/L. The range of As levels was higher than those in other populations—United Kingdom (< 0.5–48.2 µg/L), Sweden (5.3–11.7 µg/L), Belgium (95th percentile; 48.8 µg/L), and Canada (95th percentile; 27 µg/L). More than 60% of individuals had As level exceeding the reference value of 15 µg/L reported in German studies (Wilhelm et al., 2004, 2005). The 30.7 µg/L observed in our study was also higher than the mean As concentrations of 16.7 µg/L in the urine of individuals from uncontaminated areas of Lombardy, Italy (Reimann and de Caritat, 1998). The strong correlation of As in drinking water and urine in our study population indicates the As in urine mainly comes from drinking water or non-fish dietary elements, as sea food consumption is uncommon in the region. One of the major factors determining the toxicity of As is its metabolism, since As is excreted in urine as organic arsenical species (monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA)), as well as unmetabolized inorganic As (Aposhian and Aposhian, 2006). The inorganic As species, arsenate (As-V) and arsenite (As-III), are more toxic and carcinogenic than MMA and DMA, and As-III is more toxic than As-V (Heinrich-Ramm et al., 2002; Aposhian and Aposhian, 2006). A study of As speciation in water, food, and urine is needed to more fully characterize the inorganic and organic components of As.

Fluoride concentrations fell between 0.44 and 44.6 mg/L (mean: 10.1 ± 7.1 mg/L). More than 96% of the urine samples contained F⁻ at levels higher than the Biological Exposure Index (BEI) value of 2 mg/L established by industrial hygienists as a guideline for occupational exposure (ACGIH, 2012). The maximum of 44.6 mg/L is much higher than reported levels seen in other studies including from endemic areas (e.g., Susheela et al., 2002). We also compared our data with Biomonitoring Equivalents (BEs), which refer to the concentration or range of concentrations of chemicals in a biological medium that is consistent with an existing health-based exposure guideline. The F⁻ range we have obtained in our study is far beyond the established range for BEs (1.1–2.1 mg/L) (Aylward et al., 2015), indicating high potential health risks to the Rift population.

Boron concentrations were found to range between 227 and 8216 µg/L (mean: 2018 ± 1287 µg/L), with a 95th percentile of 4737 µg/L. These values are higher than reported reference values of 490–3290 µg/L and 380–3600 µg/L in other studies (Minoia et al., 1990; Rodushkin et al., 2001). Optimal intake of B has been shown to be essential for healthy bones, joints and cartilage, interact with Ca, Mg, and vitamin D and to play a vital role in the metabolism of bone (Khaliq et al., 2018; Nielsen and Stoecker, 2009; Newnham, 1994; Meacham, 1994).

The highest Mo value encountered was 2884 µg/L. The Mo ranges were much higher than the reference ranges in other studies (White and Sabbionia, 1998: 2.8–288 mg/L; Rodushkin et al., 2001: 12–108 µg/L) and the 95th percentile (170 µg/L) value reported by Saravanabhavan et al. (2017). The 95th percentile (386 µg/L) value was higher than the 116 µg/L reported by Hoet et al. (2013) and 94 µg/L reported by Heitland and Köster (2006). The Mo concentrations in drinking water (0.34–74 µg/L) were well below the levels in urine, indicating that diet is the main source of Mo exposure. The urine Mo concentration obtained in our study had a range of 11.7–2884 µg/L, which overlaps with the established BEs for Mo (200–7500 µg/L; Hays et al., 2016), indicating increased health risks to the Rift population.

Manganese concentrations varied from below the detection limit to 298 µg/L, with a 95th percentile of 31 µg/L, which significantly exceeds the 95th percentile of 0.37 µg/L (CDC, 2018) and ranges (< 0.09–7.8 µg/L and < 0.05–0.24 µg/L; White and Sabbionia, 1998;

Rodushkin et al., 2001) observed in reference populations. About 38% of individuals had Mn exceeding the maximum value of 7.8 µg/L reported for reference populations (White and Sabbionia, 1998). Only twelve of the individuals had Mn between 90 µg/L and the maximum value 286 µg/L measured in our study. The concentration range of 1–8 µg/L is considered normal (ATSDR, 2012), which correlates with what was observed in the reference populations in Table 3. While Mn is essential at low levels, it is neurotoxic and carcinogenic at higher concentrations (Bouchard et al., 2007).

Thallium concentrations varied from below the detection limit to 11.9 µg/L, which is higher than the reported ranges of 0.07–0.7 µg/L (Caroli et al., 1994) and 0.05–0.54 µg/L (Rodushkin et al., 2001) in reference populations. The 95th percentile (6.25 µg/L) value was also higher than the 95th percentiles of 0.42, 0.5, and 1.18 µg/L reported in several other studies (CDC, 2018; Hoet et al., 2013; Paschal et al., 1998). The German human biomonitoring (HBM) Commission established HBM I concentration values of 5 µg/L for TI, for the concentration at and below which there is no risk of adverse health effects for the general population (Apel et al., 2017). In our study subjects, 92% (n = 355) had TI concentration below 5 µg/L. Current knowledge on the health effects of TI is limited, but it may be associated with headaches, anorexia, and pain in the arms, thighs, and abdomen (ATSDR, 2013; Peter and Viraraghavan, 2005).

Lithium concentrations were found between 5 and 589 µg/L, which is higher than the reported ranges of 5.2–23 µg/L (Caroli et al., 1994) and 0.8–40.5 µg/L (Abou-Shakara et al., 1989). The 95th percentile (179 µg/L) value was higher than the 75 µg/L reported by Hoet et al. (2013) and 115 µg/L reported by Heitland and Köster (2006). Chronic high-dose Li medication is well known to cause health effects such as gastrointestinal pain, diarrhea, weight gain, edema, hypothyroidism, and renal tubular damage (Aral et al., 2008). On the other hand, studies have showed Li in drinking water to be associated with lower suicide rates in Texas (Schrauzer and Shrestha, 1990), Japan (Ohgami et al., 2009), and Austria (Kapusta et al., 2011). The Li level (3.6–156 µg/L) found in drinking water in our study matches the Li ranges in the Texas study (< 1–160 µg/L).

The Zinc range (26.7–2983 µg/L) was higher than the reference intervals reported by (Rodushkin et al., 2001: 40–430 µg/L; Croli et al., 1994: 27–850 µg/L). The 95th percentile (1066 µg/L) value was comparable to the 1100 and 1048 µg/L reported by Saravanabhavan et al., (2017) and Hoet et al. (2013).

The concentrations of other elements such as Pb, Cd, U, Ni, Co, Cu, and Ag were generally low and within the reference ranges found in healthy populations (Table 3). The 95th percentiles were (in µg/L) for Pb (0.88), Cd (2.28), U (0.05), Ni (21.5), Co (3.9), Cu (17.4), Ag (0.04), Al (94), Be (0.31), Rb (1671), Sb (0.05), Sr (303), Th (0.3), and TI (0.05); and (in mg/L) for Ca (155) and Mg (275). Lead, Cd and U are well known nephrotoxic contaminants (Skerfving et al., 1998; Nordberg et al., 2007; Åkesson et al., 2005; Kurttio et al., 2002). Cadmium concentrations ranged between below DL and 3.4 µg/L (mean: 0.76 ± 0.69 µg/L). Most individuals (73.4%; n = 285) had urinary Cd below 1 µg/L. The Cd concentration in urine is influenced by smoking habits and age. As a few male subjects (n = 10) were smokers, there may not be a major influence of smoking on Cd levels in this population. A study in Germany established a reference value of 0.8 µg/L for non-smoking adults. Yet this value is lower than the 95th percentile of Cd (2.3 µg/L) in our study. The German HBM Commission established HBM I and HBM II values for urinary Cd as guidelines for determining health risks for children and adults (Apel et al., 2017). In our study, 38.3% (n = 59) of the children (10–18 years old) had urinary values below 0.5 µg/L (the HBM I level) with no risk of adverse health effects, whereas 8.4% (n = 13) of them had values exceeding the HBM level II value of 2 µg/L, above which adverse health effects are possible. For adults (18–50 years old), 76.3% (n = 177) had value below the HBMI level of 1 µg/L, and none had values above the HBM II level of 4 µg/L. The maximum urinary Cd concentration obtained in our study was

Table 4
Difference in log urine concentrations by Age, Sex, and BMI.

	a) T-Test by Age (Age < 30 – Age > 30)		b) T-Test by Sex (Female – Male)		c) T-Test by BMI status (normal BMI – underweight BMI)	
Ag	0.11	(1.28)	-0.08	(-1.05)	0.03	(0.41)
Al	0.14	(1.21)	0.12	(1.13)	-0.06	(-0.52)
As	0.06	(0.64)	-0.05	(-0.60)	-0.07	(-0.71)
B	0.14*	(1.99)	-0.06	(-0.91)	-0.06	(-0.92)
Be	0.14	(1.36)	0.03	(0.30)	0.00	(0.01)
Ca	-0.30*	(-2.37)	-0.37**	(-3.19)	0.40***	(3.35)
Cd	0.38*	(2.17)	0.00	(0.01)	-0.15	(-0.90)
Co	0.80***	(4.9)	0.34*	(2.21)	-0.26	(-1.64)
Cu	0.25	(1.75)	-0.29*	(-2.25)	0.05	(0.40)
F ⁻	-0.05	(-0.57)	-0.02	(-0.27)	0.13	(1.64)
Li	0.07	(0.91)	-0.01	(-0.10)	0.10	(1.30)
Mg	0.28**	(3.02)	-0.30***	(-3.56)	-0.06	(-0.70)
Mn	0.34	(1.63)	0.47**	(2.64)	-0.60**	(-3.13)
Mo	0.24*	(2.32)	-0.18	(-1.88)	-0.13	(-1.37)
Ni	0.09	(0.69)	0.00	(-0.00)	-0.07	(-0.55)
Pb	0.42**	(3.17)	-0.30*	(-2.42)	-0.17	(-1.30)
Rb	-0.03	(-0.43)	0.12	(1.64)	0.08	(1.09)
Sb	0.05	(0.80)	-0.02	(-0.30)	-0.04	(-0.79)
Sr	0.10	(0.95)	-0.24*	(-2.50)	0.07	(0.75)
Th	0.02	(0.18)	0.12	(1.33)	-0.01	(-0.09)
Tl	0.14	(0.89)	0.15	(1.03)	-0.10	(-0.68)
U	0.03	(0.42)	0.11	(1.48)	-0.01	(-0.19)
Zn	0.04	(0.46)	-0.48***	(-5.98)	-0.11	(-1.30)

t statistics in parentheses: *p < 0.05; **p < 0.01; ***p < 0.001.

3.4 µg/L, well above the BE range for Cd (1.1–1.5 µg/L), indicating potentially heightened health risks.

Calcium and Mg concentrations ranged between 1.7 and 382 mg/L, and 0.57 and 611 mg/L, respectively. The mean values for Ca (43.5 mg/L) and Mg (124.4 mg/L) were lower and higher than what is measured for Ca—145.7 mg/L and Mg—78 mg/L in other research (e.g., Dlugaszek et al., 2011). Caroli et al. (1994) found a mean Ca concentration of 90 mg/L, which is also higher than the mean Ca level measured in our study. The low level of Ca measured among our study individuals demands further investigation, particularly to identify the possible effect chronic F⁻ exposure on Ca status and its urinary excretion.

We observed age, sex and BMI differences in the levels of the elements. Individuals in younger age groups (10–30 years) had significantly higher mean concentrations of B, Cd, Co, Mg, Mo, and Pb than older ones between 31 and 50 years, whereas only Ca was significantly higher in the older age group (Table 4a). Mean urinary concentrations of Ca, Cu, Mg, Pb, Sr, and Zn were significantly higher in males than females, whereas only Co and Mn were higher in females. In total, mean values of 14 of the 23 elements (Ag, As, B, Ca, Cd, Cu, F, Li, Mg, Mo, Pb, Sb, Sr, and Zn) were higher in males than females (Table 4b). Individuals with normal or overweight BMI (> 18.5 kg/m²) had significantly higher urinary Ca and lower Mn concentrations than underweight (< 18.5 kg/m²) individuals (Table 4c). While several studies (e.g., Aprea et al., 2018; Heitland and Köster, 2006; Hoet et al., 2013) have documented age and sex dependence for biomonitoring levels of metals and trace elements, we are unsure at this time of the physiological reasons for such dependence; this issue therefore warrants further investigation.

We observed significant positive correlation between As, B, Cd, F⁻, Li, Mo, Rb, and U in drinking water and urine, indicating the important role of drinking water as the source of exposure to these elements (Table 5). Conversely, we found significant negative correlation between Ag, Ca, Co, Mg, Mn, Sr, Tl, and Zn in drinking water and urine. No correlation was observed between the rest of the elements in drinking water and urine.

Limitations of the study: Since biomonitoring data in other regions

Table 5
Pairwise correlation between log urine and water concentrations.

	Pearson's correlation coefficient	Significance level
Ag	-0.12	0.0218*
Al	-0.09	0.0787
As	0.55	0.0000**
B	0.26	0.0000**
Be	-0.01	0.8914
Ca	-0.11	0.0243*
Cd	0.15	0.0025**
Co	-0.33	0.0000**
Cu	0.09	0.0881
F	0.70	0.0000**
Li	0.44	0.0000**
Mg	-0.18	0.0004**
Mn	-0.25	0.0000**
Mo	0.11	0.0245*
Ni	-0.05	0.3563
Pb	0.02	0.7515
Rb	0.14	0.0071**
Sb	-0.07	0.2016
Sr	-0.11	0.0333*
Th	-0.01	0.7855
Tl	-0.11	0.0316*
U	0.15	0.0029**
Zn	-0.19	0.0002**

* = p < 0.05; ** = p < 0.01.

of Ethiopia is absent, it was not possible to compare levels in the Rift population relative to individuals in other regions of the country. Due to the unique geologic setting of the Rift, the concentration values primarily serve as baseline concentration ranges for these sample populations. We also did not measure creatinine or the specific gravity of urine, which would have allowed normalization for dilution. However, many similar biomonitoring studies report concentration values as we do, allowing comparison with our findings. In addition, our study collected 12-h urine, which captures a more representative variation of elemental concentrations, unlike spot samples that are likely to vary over time reflecting short-term changes in diet, lifestyle, or activities (e.g., Smolders et al., 2014). We also anticipate that the relatively uniform diet, water sourcing, and lifestyle of individuals in the Rift would yield relatively stable correlations between individuals' spot and 12-h urine. Nonetheless, it would be worthwhile to study variation in the urinary excretion of elements in both spot and longer collection times.

4. Conclusion

The established concentration ranges are relevant in providing baseline levels of exposure in this study population, which resides in a central Ethiopian Rift Valley known for its unique geology and geography. This unique location features vast volcanic pyroclastic materials that play a major role in the chemical composition of water, soil, and therefore food sources and biomarkers. We found urinary concentrations of As, F⁻, B, Mo, Mn, Li, and Zn that exceeded reference values from healthy unexposed populations in other regions, whereas other elements were within or close to the ranges of reference populations. While it is important to note that this comparison provides a picture of concentrations, it is not necessarily indicative of deficient, optimal, or toxicity thresholds above which pathological states would be expected in our populations. Several factors (including lifestyle, nutritional status, ethnicity, sex, age, and interaction of the elements) besides chemical exposures determine health and disease. In fact, the health effect of excess F⁻ has already been well-recognized in the region, but the health risk of low-to-moderate levels of As in Rift water and urine remains unknown. Meanwhile, the established baseline values in the Rift population may reflect those in other countries in the

Great African Rift Valley with similar geologic settings. Overall, there is a need to initiate comprehensive biomonitoring studies in Ethiopia and other African countries to establish reference ranges that allow better monitoring of environmental and other changes that might result from ongoing economic development and industrialization. We stress that the presented concentration ranges can be integrated into future studies of the toxicity or deficiency of the measured elements. This will help to define normal or pathological ranges and will inform establishment of public health policies.

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Abbreviations

- Ag: Silver
 Al: Aluminum
 As: Arsenic
 B: Boron
 Be: Beryllium
 Ca: Calcium
 Cd: Cadmium
 Co: Cobalt
 Cu: Copper
 F⁻: Fluoride
 Li: Lithium
 Mg: Magnesium
 Mn: Manganese
 Mo: Molybdenum
 Ni: Nickel
 Pb: Lead
 Rb: Rubidium
 Sb: Antimony
 Sr: Strontium
 Th: Thorium
 Tl: Thallium
 Zn: Zinc
 U: Uranium
 BMI: body mass index
 CDC: Centers for Disease Control and Prevention
 DL: Detection limit
 EU directives: European Union Directives
 ICP-MS: Inductively Coupled Plasma Mass Spectrometry
 IRB: Institutional Review Board
 ISE: Ion Selective Electrode
 LGC: LoGiCal[®] reference materials
 mg/L: milligram per liter
 µg/L: microgram per liter
 TISAB: Total Ionic Strength Adjuster Buffer
 U.S. EPA: U.S. Environmental Protection Agency
 WHO: World Health Organization