Human urinary biomonitoring in Western Kenya for micronutrients and potentially harmful elements

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Highlights

- Survey of Western Kenya for urinary status of potentially harmful elements and micronutrients essential to health
- Reference values (RV95s) generated for urinary biomonitoring in Western Kenya
- Hydration correction for urinary elemental concentrations show significant difference compared to uncorrected data.

Abstract

Spot urinary elemental concentrations are presented for 357 adults from Western Kenya collected between 2016 and 2019 as part of a wider environmental geochemical survey. The aim of this study was to establish population level urinary elemental concentrations in Western Kenya for micronutrients and potentially harmful elements for inference of health status against established thresholds. For elements where thresholds inferring health status were not established in the literature using urine as a biomarker, this study generated reference values with a 95% confidence interval (RV95s) to contextualise urinary elemental data for this population group.

Data are presented with outliers removed based upon creatinine measurements leaving 322 individuals, for sub-categories (e.g. age, gender) and by county public health administrative area. For Western Kenya, reference values with a 95% confidence interval (RV95s) were calculated as follows (µg/L): 717 (I), 89 (Se), 1753 (Zn), 336 (Mo), 24 (Cu), 15.6 (Ni), 22.1 (As), 0.34 (Cd), 0.47 (Sn), 0.46 (Sb), 7.0 (Cs), 13.4 (Ba and 1.9 (Pb).

Urinary concentrations at the 25th / 75th percentiles were as follows (µg/L): 149/368 (I), 15/42 (Se), 281/845 (Zn), 30/128 (Mo), 6/13 (Cu), 1.7/6.1 (Ni), 2.0/8.2 (As), 0.1/0.3 (Cd), 0.05/0.22 (Sn), 0.04/0.18 (Sb), 1.2/3.6 (Cs), 0.8/4.0 (Ba) and 0.2/0.9 (Pb). Urinary concentrations at a population level inferred excess intake of micronutrients I, Se, Zn and Mo in 38, 6, 57 and 14% of individuals, respectively, versus a bioequivalent (BE) upper threshold limit, whilst rates of deficiency were relatively low at 15, 15, 9 and 18%, respectively. Each of the administrative counties showed a broadly similar range of urinary elemental concentrations, with some exceptions for counties bordering Lake Victoria where food consumption habits may differ significantly to other counties e.g. I, Se, Zn.
Corrections for urinary dilution using creatinine, specific gravity and osmolality provided a general reduction in RV95s for I, Mo, Se, As and Sn compared to uncorrected data, with consistency between the three correction methods.

**Keywords:** Urinary biomonitoring, micronutrients, potentially harmful elements, reference values, creatinine, hydration correction, Kenya

**Introduction**

Human biomonitoring is a routine tool for the estimation of chemical exposures and dietary intakes. Urine has increasingly been employed as a non-invasive biomarker in biomonitoring studies to measure both potentially harmful elements (PHEs) and beneficial micronutrients as an integrated quantitative marker of human exposure from multiple pathways. Such information can inform public health, hazard assessments and subsequent mitigation strategies (NRC, 2012) to address excessive or deficient intakes of various environmental and dietary chemicals.

National scale biomonitoring programmes are common in many countries worldwide, particularly in North America (e.g. NHANES, 2021), Europe (HBM4EU, 2021; GHBC, 2021), and South East Asia (Kim & Baek, 2016), with fewer programmes in Africa outside of the occupational setting (Phiri et al. 2020; 2021). More commonly in Africa, single event studies in the range of 100-500 individuals have been reported in the Democratic Republic of Congo-DRC (Tuakila et al. 2015), Ethiopia (Godebo et al. 2019), Malawi, Kenya (Watts 2019b) and Tanzania (Middleton et al. 2018) and on rare occasions on a much larger scale up to 5000 (Farebrother et al. 2018). The complexities of interpreting biomonitoring data were discussed in depth by Saravanabhavan et al. (2017), in which common approaches using descriptive statistics (geometric/arithmetic mean, percentiles) are often compared with reference intervals using appropriate statistical methodologies to account for baseline exposure in a reference population for a health-risk based context (Legrand et al. 2010). A large body of work has accumulated in the scientific literature to establish biomonitoring equivalents (BE) to assist in contextualising biomonitoring data from a reference value based on toxicokinetic data into a biomonitoring concentration (Angerer et al. 2011; Boogaard et al. 2011). For example, Hays et al. (2014; 2016) reported biomonitoring equivalents (BE) for trace elements without established thresholds using external reference doses to relate to urine or blood concentrations. This followed efforts at a Biomonitoring Equivalents Expert Workshop in 2008 to harmonise an approach to interpreting HBM data and to provide guidance in a public health context (Hays & Aylward 2009; 2012) including transparency of discussions of confidence and uncertainty (LaKind et al. 2008).

Increasingly a reference value of background exposure in a population has been reported in the literature using a 95th percentile (RV95s) rather than a geometric mean, providing an upper margin of the current background exposure (i.e. environmental, dietary sources) of a general population to a given substance at a given point in time (Saravanabhavan et al. 2017). The establishment of reference values can be a useful snapshot of population level status, for which a database can be revised and refined with new data. A powerful combination of reference values and BEs can provide a broad comparison to a relatively non-invasive and inexpensive biomonitoring use of urine to reflect human dietary or exposure status to an appropriate range of potentially harmful elements or micronutrients essential for human health. An exceedance of the RV95s may indicate a need to re-test and investigate further, and does not take into account toxicological information to inform clinical
intervention, but are a useful starting point in the absence of population level data for comparison against or in the absence of upper or lower thresholds for exceedance or deficiency of exposure/intake.

Urinary biomonitoring offers a route to supporting public health professionals, with fewer logistical requirements compared to blood or in settings where resources and infrastructure are challenging for sample collection and storage, alongside capacity for sensitive analyses of trace elements with appropriate quality control measures. Therefore, the aim of this paper was to establish urinary biomonitoring reference values for Western Kenya covering both micronutrients and potentially harmful elements by: (1) presentation of a community based urinary biomonitoring dataset; (2) calculation of population level urinary reference values (RV₉₅s) for Western Kenya, and (3) examine the influence of hydration corrections on the calculated RV₉₅s for each element using creatinine, osmolality and specific gravity compared to commonly used uncorrected data.

Methods

Ethical approval

Ethical approval was obtained from the Institutional Research and Ethics Committee of Moi University (000921). Permission and assistance was then requested from the Ministry of Health office for each County before proceeding to the field areas and subsequent engagement with participants via community health workers. Additional research permission granted in Kenya NACOSTI/P/19/43659/29731.

Study setting

Sample collection between October 2016 and November 2019 was part of a wider project as described in Watts et al. (2019a,b), which collected residential samples of soil, crops, drinking water and a urine sample from households. Each sampled household is shown in Figure 1, spanning administrative areas in the ‘n’ Western Kenyan counties of Bomet, Bungoma, Busia, Elgeyo Marakwet, Kericho, Kakamega, Kisumu, Nandi, Siaya, Uasin Gishu, Kisii, Nyamira, Homa Bay and Vihaga. Few sample points are shown in Uasin Gishu and Nandi counties owing to urine samples not being collected in the first field survey when field collections were focussed solely on geochemical samples and when the majority of visits were made for these counties.

Recruitment methods and collection of urine

With the need to collect these samples from the home of each participating house, households were approached through an in-person visit of the study team (often by local community health workers or village leaders) which was travelling by vehicle and stopping at homes in close proximity to a pre-determined sampling location set out for each field team using Maps.me™. These pre-determined sampling locations were selected according to geochemistry, determined by soil parameters and geology of each county, but also considering an appropriate spatial distribution across the county where logistical access was feasible. Upon approaching a house, an adult member (> 18 years) of the household was informed about the study and invited to participate. Consenting participants were confirmed by Kenyan counterparts/community practitioners verbally for ages 18 years and older at each site, where urinary samples alongside other environmental samples were requested following an explanation of the study and its rationale. In general, we attempted to collect from a minimum of 30 different sites that were spread out evenly across each county, representing rural land-use, although the geographic size and accessibility resulted in a slight variation in numbers per county. One sample was generally collected from each
household, a second adult participant provided a sample in <10% of households.
Participants urinated into a 30 mL nalgene LDPE bottle, which was transported in a coolbox
(−4°C) and filtered into an 8 mL nalgene LDPE bottle using a nylon 0.45 µm syringe filter at
the end of each day, followed by storage in a coolbox and freezng at -20°C on return to the
University of Eldoret laboratory in Kenya. Urine samples were transported frozen to the UK
for immediate elemental analyses and urinary dilution measurements for subsequent
corrections.

ICP-MS elemental analyses
Urine samples were analysed for a general suite of trace and major elements. Samples were
diluted x10 with 1% nitric acid / 0.5% hydrochloric acid prior to total element determination
by Inductively Coupled Plasma Mass Spectrometry (ICP-QQQ-MS, Agilent 8900), with Sn in
no gas mode; Zn, Mo, Cu, Ni, Cd, Sn, Sb, Cs, Ba, Pb in He mode; Se in H₂ mode; and As in
O₂ mode and Sc, Ge, Rh, In, Te and Ir elements used as internal standards. Iodine was
measured separately as described in Watts et al. (2019b) with a x20 dilution of urine
samples in 0.5% Tetramethyl ammonium hydroxide (TMAH) solution prior to analyses by
ICP-MS with the reaction/collision cell in no gas mode. Tellurium was used as an internal
standard to correct for minor signal drift. Calibration standards, quality control solutions and
certified reference material were matrix matched with either 1% nitric acid / 0.5%
hydrochloric acid or 0.5% TMAH. Performance characteristics for the limit of detection and
accuracy measured for the Seronorm™ Trace Elements Urine L-1, produced by SERO AS is
presented in full in Supplementary Table 1. Seronorm solutions were analysed on a ratio of
1 for every 15 urine samples. Calibration standards were analysed at the beginning and end
of each analytical batch and analytical run quality was verified using a series of chemical
quality control standards prepared on the day of analysis (Ni, Cu, Zn, As, Se, Mo, Sb, Ba, Pb
at 5 µg/L, Cd, Sn, Cs at 1 µg/L, I at 50 µg/L), produced from an independent source to the
calibration standards (SCP Science, UK and Sigma Aldrich for I). Analytical trends were
monitored via charting in SPC for Microsoft Excel™ version 5 as described in Abellanosa et
al. (2018).

Statistical analyses
Urinary element concentrations that were below their respective limit of detection (LOD)
were assigned a value of LOD/2. Information on age and sex were obtained from household
questionnaires. Element data from participants with urinary creatinine values <0.3 g/L or
>3.0 g/L were excluded from statistical analysis following guidelines from the Human
Biomonitoring Commission (2007) before testing exclusion variables (WHO, 1996; Wilhelm
et al. 2008). Summary statistics were then calculated for each element: arithmetic mean, SD,
median, median absolute deviation, minimum and maximum values, percentiles (P25, P75,
and P90), skewness, and kurtosis. Summary statistics were also calculated based on sex
and age groups. Human biomonitoring reference values (RV₉₅₉) aim to represent current
background exposure; therefore, extreme values were removed, as they may
disproportionately influence the final values. The data were natural log-transformed, and the
normality of the data distributions evaluated using the Kolmogorov-Smirnov test. Extreme
values were identified and removed using Tukey’s approach (Tukey, 1977) if the data were
not skewed, or a modified Tukey’s approach (Hubert and Van der Veeken, 2008) if the data
remained skewed after log-transformation. After extreme value removal, RV₉₅₉ were
estimated statistically as the rounded 95th percentile and its corresponding 95% confidence
interval (95%CI). Statistical analysis was conducted using R version 4.0.3 (R Core Team 2020).

Urinary dilution corrections

Urinary creatinine was determined using a Randox liquid assay kit and a Randox RX Imola chemistry analyser. Osmolality was measured by freezing-point osmometry using an Osmomat 030 (Gonotec, Germany). Specific gravity (SG) was measured with a PAL-10-S digital refractometer (Atago, Japan) prior to filtration. Creatinine, SG and osmolality corrections were performed using Equation 1:

\[
UC_{\text{cor}} = UC_{\text{vol}} \times \frac{D_{\text{ref}}}{D_{\text{meas}}}
\]  

(1)

where \( UC_{\text{cor}} \) is dilution corrected urinary concentration; \( UC_{\text{vol}} \) is the measured, volume-based urinary concentration (in µg/L); \( D_{\text{ref}} \) is the reference value to which UC concentrations are scaled to and \( D_{\text{meas}} \) is that measured in the given specimen (note: \( D_{\text{ref}} \) and \( D_{\text{meas}} \) are used for SG correction). \( D_{\text{ref}} \) was 1 g/L for creatinine – synonymous with the conventional division-based correction and yielding results in µg per g creatinine; and, for both SG and osmolality, the study group medians (n=357) were selected: 1.017 (unitless) and 585 mOsm/kg, respectively (Middleton et al. 2016).

Results & Discussion

Urinary elemental concentrations

Urinary elemental concentrations for 322 adults with outliers removed are summarised in Table 1, along with descriptive summary statistics and the calculated RV95s values for each element. The full dataset is reported in Supplementary Table 2 (including outliers – 357 individuals) and 3 (outliers removed). Descriptive statistics for each county administrative area are presented in Supplementary Table 4 and for age and gender in Supplementary Tables 5 and 6. Supplementary Table 6 includes an individual male/female RV95s gender, to allow for the imbalance in female/male participants (207/112). Comparisons of data will be discussed in detail for each selected micronutrient and PHEs. Elemental concentrations are presented without hydration correction for comparison to the literature, although correction values (creatinine, osmolality, specific gravity) are presented in the supplementary tables for additional information.

Table 1: Descriptive statistics for uncorrected urinary elemental concentrations (µg/L) with calculated RV95s values.

<table>
<thead>
<tr>
<th></th>
<th>I</th>
<th>Se</th>
<th>Zn</th>
<th>Mo</th>
<th>Cu</th>
<th>Ni</th>
<th>As</th>
<th>Cd</th>
<th>Sn</th>
<th>Sb</th>
<th>Cs</th>
<th>Ba</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean</td>
<td>297</td>
<td>36</td>
<td>636</td>
<td>101</td>
<td>11</td>
<td>5.7</td>
<td>7.1</td>
<td>0.20</td>
<td>1.70</td>
<td>0.16</td>
<td>2.9</td>
<td>6.6</td>
<td>0.7</td>
</tr>
<tr>
<td>sd</td>
<td>261</td>
<td>38</td>
<td>473</td>
<td>109</td>
<td>7</td>
<td>9.1</td>
<td>10.4</td>
<td>0.23</td>
<td>6.56</td>
<td>0.33</td>
<td>2.9</td>
<td>19.8</td>
<td>0.9</td>
</tr>
<tr>
<td>median</td>
<td>243</td>
<td>26</td>
<td>529</td>
<td>64</td>
<td>9</td>
<td>3.5</td>
<td>3.9</td>
<td>0.20</td>
<td>0.09</td>
<td>0.08</td>
<td>2.1</td>
<td>1.7</td>
<td>0.50</td>
</tr>
<tr>
<td>P .25</td>
<td>149</td>
<td>15</td>
<td>281</td>
<td>30</td>
<td>6</td>
<td>1.7</td>
<td>2.0</td>
<td>0.10</td>
<td>0.05</td>
<td>0.04</td>
<td>1.2</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>P .75</td>
<td>368</td>
<td>42</td>
<td>845</td>
<td>128</td>
<td>13</td>
<td>6.1</td>
<td>8.2</td>
<td>0.30</td>
<td>0.22</td>
<td>0.18</td>
<td>3.6</td>
<td>4.0</td>
<td>0.9</td>
</tr>
<tr>
<td>P .90</td>
<td>544</td>
<td>68</td>
<td>1261</td>
<td>229</td>
<td>20</td>
<td>10.4</td>
<td>16.8</td>
<td>0.40</td>
<td>4.23</td>
<td>0.38</td>
<td>6.3</td>
<td>12.9</td>
<td>1.4</td>
</tr>
<tr>
<td>RV95s</td>
<td>717</td>
<td>89</td>
<td>1753</td>
<td>336</td>
<td>24</td>
<td>15.6</td>
<td>22.1</td>
<td>0.34</td>
<td>0.47</td>
<td>0.46</td>
<td>7.9</td>
<td>13.4</td>
<td>1.9</td>
</tr>
</tbody>
</table>
The discussion of urinary elemental concentrations will be organised into three sections: micronutrients essential to health with comparative published threshold values (I, Se, Zn, Mo), potentially harmful elements with published threshold values (As, Cd, Sn, Ba) and other elements for which there are no published threshold values (Cu, Ni, Sb, Cs, Pb), but where alternative RV95s values are available in the literature. The range of concentrations for each element are illustrated in Figure 2a-c.

Micronutrients with threshold values (I, Se, Zn, Mo)

Median urinary iodine was 243 µg/L - slightly lower than the 261 µg/L reported by Watts et al. (2019b), which comprised of a smaller component of this same dataset and without outliers removed. Urinary iodine concentrations were between 9 and 3,146 µg/L, P25 and P75 were 149 and 368 µg/L, respectively. Approximately 15% were considered to represent a status that was moderately deficient (<100 µg/L), whilst 38% of samples were considered to represent an excess of iodine intake (>300 µg/L) (WHO/UNICEF/ICCIDD, 2007). In comparison, the calculated RV95s of 740 and 663 µg/L (female/male) are both considerably higher than the threshold associated with excess iodine intake, and is high when comparing to biomonitoring equivalent (BE) values calculated by Hayes et al. (2018) using Estimated Average Requirement (EAR), Recommended Dietary Allowance (RDA) and for toxicity, Tolerable Upper Intake Level (UL) and Minimal Risk Level (MRL). For example, the BE values derived for adults by Hays et al. (2018) were; 60,100, 730 and 450 µg/L, respectively.

These urinary iodine data are comparable to other African studies reporting the prevalence of excess iodine intake. For example, Farebrother et al. (2018) reported uncorrected median urinary iodine in women across central Kenya of 289 µg/L (IQR 173, 458 µg/L). Median uncorrected urinary iodine reported in Malawi (Watts et al. 2015) were 221 µg/L (141-344 µg/L); in Port Sudan 464 and 561 µg/L, Medani et al. (2012) and Hussein et al. (2012), respectively. In Sadami, Ethiopia, women of reproductive age (WRA) and school age children (SAC) presented median urinary iodine of 143 and 187 µg/L, respectively.
months after a salt iodisation campaign commenced, although inconsistent iodine content of
salt was found in this study (Tafere & Stoecker, 2020). In Somalia, WRA provided a median
urinary iodine of 329 µg/L (Kassim et al. 2014) and in Lesotho, a median urinary iodine of
280 µg/L (Sebotsa et al. 2005). Tanzanian SAC in Kindoni presented a very high median
urinary iodine at 400 µg/L, with one third >500 µg/L (Venance et al. 2020). The Iodine Global
Scorecard (IGN, 2021) summarised median uncorrected urinary iodine in Kenyan SAC as
208 µg/L, although this summary of global progress used data from a 2011 national survey
(Kenya Ministry of Health, 2011) – underlining the need for timely and relevant data. Food
supply calculations previously suggested a 100% risk of iodine deficiency for this same study
area in Western Kenya from dietary source sampling reported in Watts et al (2019a),
although did not include iodised salt reported to be present in 98% of Kenyan households
(Joy et al. 2014). Figure 3a illustrates the range of urinary iodine within each County
administrative area with little variation in the median values, although some examples of
excess values as outliers at concentrations >1000 µg/L are clearly illustrated in Figure 1 for
Homa Bay, Kisumu and Saiya Counties bordering Lake Victoria, possibly representing
elevated fish consumption (Watts et al. 2015) as the main source of protein or possible salt-
preserved fish (personal observation).

The median urinary selenium concentration was 26 µg/L, with P25 and P75 of 15 and 42
µg/L, respectively. These values are comparable to Middleton et al. (2018) reported median
urinary selenium of 24 µg/L for 45 individuals in Kenya and 29 µg/L for 200 individuals in
Tanzania. Fourteen percent of individuals were below the lower threshold of 10 µg/L using a
BE estimation (Hays et al. 2014), below which could infer a status of deficiency. As Figure
2a shows, just 15% indicated deficiency using a 10 µg/L (BE) low threshold, but with still 6%
of samples above an upper/excess threshold of 90-100 µg/L (BE). The calculated RV95s for
females of 78 µg/L was slightly below the upper threshold, whereas the male RV95s was
significantly different from females and above the threshold at 103 µg/L. The urinary
selenium concentrations contrasts with the calculated risk of deficiency for male and females
of 100 and 93%, respectively for dietary supply data and measured food items from the
same households in Western Kenya as the urine collections. Median urinary selenium for
male (n = 111) and female (n = 204) were 30 µg/L and 25 µg/L, respectively. Phiri et al
(2020) reported a greater potential deficiency in Malawi indicated by urinary selenium in
~35% of the study (n = 1618) and similar ~5% exhibiting an excess status. The median
urinary selenium from this national survey was relatively lower at 16.2 and 15.0 µg/L in
women of reproductive age and school age children, respectively. In contrast, Joy et al.
(2015) calculated a risk of deficiency for Se intake of 74% from dietary supply calculations in
Malawi. Figure 3a shows a similar range of urinary selenium across each of the County
administrative areas, with the exception of outlier values in Homa Bay and Kakamega
counties and in particular, Vihaga county where the median urinary selenium of 42 µg/L is
represented by a very small number of samples. Phiri et al. (2019) also demonstrated
geographical variation for urinary selenium in Malawi.

The median urinary zinc concentration was 529 µg/L, with P25 and P75 of 281 and 845 µg/L
and median urinary zinc of 700 and 448 µg/L for male and females, in comparison to a BE
for nutritional requirement reported by Poddalgoda et al. (2019) of 206 and 159 µg/L for
male and female, respectively. In general, urinary zinc suggests a status of deficiency in 9%
of volunteers (Figure 2a) in contrast to 57% of urinary zinc exhibiting an excess status when
using the lower exposure guideline value for North America of 439 µg/L, yet no exceedances
when using the more conservative European value of 3,489 µg/L (Poddalgoda et al. 2019).
The RV95s for this study was 2,030 µg/L for males and 1551 µg/L for females, both between
the two proposed upper BE thresholds. In general, Figure 3a shows that each of the County
administrative areas were similarly above minimum nutritional requirements for Zn. This
contrasts with food supply calculations in the same area by Watts et al. (2019a) that
suggested a risk of deficiency for Zn status of 85 to 100% for males and females and
equivalent published data of 100% risk, suggesting greater food diversity for this region of
Kenya or perhaps greater consumption of dairy, meat and fish deriving a higher Zn status
than national survey data would suggest (FAOSTAT, 2019).

Urinary zinc data for this study were generally much higher than other African studies. For
example, a national survey in Malawi (Phiri et al. 2021) presented a median urinary zinc of
322 µg/L for women of reproductive age (n=741) and 346 µg/L in school age children (n =
645). Similarly, Godeboa et al. (2019) presented urinary zinc for the Ethiopian Rift valley with
a median of 287 µg/L (P25 167; P75 502) (n = 386). However, Middleton et al. (2018)
broadly similar median urinary zinc of 479 and 427 µg/L for Kenya and Tanzania,
respectively.

The median urinary molybdenum concentration was 64 µg/L, with P25 and P75 of 30 and
128 µg/L, respectively. Median urinary molybdenum were broadly similar for male and
females at 68 and 64 µg/L, respectively. These values were relatively high in comparison to
a BE for nutritional requirement reported by Hays et al. (2016) of 21.7 µg/L using an
estimated average requirement (EAR) assumption or recommended daily allowance (RDA)
of 28.4 µg/L. Upper thresholds ranged from 206 µg/L for North American and 7,516 µg/L for
OECD values. Using the lower EAR threshold, 18% were considered deficient using urinary
molybdenum BE, whilst 14% exceeded the upper BE threshold (Figure 2a). The calculated
RV₉₅s of 379 µg/L for males and 314 µg/L for females both exceeded the lower of the
published BE upper threshold values of 206 µg/L. Middleton et al. (2018) reported a slightly
higher exceedance rate of 25 and 18% for Kenya and Tanzania, respectively. In general,
each of the county administrative areas demonstrated a level of Mo sufficiency, with notable
exceptions illustrated in Figure 3a for Homa Bay and Kisumu bordering Lake Victoria,
although not for Saiya which also borders the lake. The general Mo sufficiency derived from
urinary molybdenum agrees with the predicted risk of deficiency close to zero calculated for
this area by Watts et al. (2019a) in which vegetable groups, seeds and pulses were reported
to be a significant contributor of Mo to the daily dietary intake. Godeboa et al. (2019)
reported significantly higher urinary molybdenum values for the Ethiopian Rift valley, with a
median of 367 µg/L and P25 and P75 of 197 and 614 µg/L (n = 386).

Potentially harmful elements (PHEs) with published threshold values (As, Cd, Sn, Ba)
Median urinary arsenic concentrations were 3.9 µg/L, with P25 and P75 of 2.0 and 8.2 µg/L,
with median UAsC for male and females showing no contrast at 3.9 and 4.2 µg/L,
respectively. The median values were below the biomonitoring equivalent upper threshold
representing toxicity of 6.4 µg/L for inorganic As published by Hays et al. (2010). It should
be noted that urinary arsenic values in this study (Figure 2b) represent total As, which
incorporates both inorganic and organic As, the latter most likely derived from dietary
sources. Therefore, care should be taken in interpreting that 34% of participants had total
urinary inorganic arsenic values that exceeded 6.4 µg/L. The calculated RV₉₅s of 23.6 µg/L
for males and 22.1 µg/L for females in this study fall midway between studies for adults in
Germany, Belgium and South Korea with RV₉₅s reported as 15, 49 and 106 µg/L (Wilhelm et
al. 2004; Hoet et al. 2013; Lee et al. 2012). Further investigation is required to understand
the higher measurement of urinary arsenic, using arsenic speciation to derive inorganic and
organic As species to provide additional interpretation and to support differentiation between
occupational, environmental or dietary sources (Hays et al. 2010; Middleton et al. 2016). In
general, the majority of administrative county areas as illustrated in Figure 3b were below
Hays et al. (2010) upper threshold for urinary arsenic, or at least with few outliers, with the
exception of Homa Bay, and Saiya in particular and to a lesser extent Kisumu county, all
bordering Lake Victoria. In these instances, greater fish consumption may contribute to
elevated As dietary intake, represented as organic-arsenic (Middleton et al. 2016). Godeboa
et al. (2019) reported higher urinary arsenic in the Ethiopian Rift Valley, with a median of
18.9 µg/L and 25/75th percentiles of 11.4 and 38.9 µg/L (n = 386). Tuakila et al. (2015) also
reported significantly higher urinary arsenic in the Democratic Republic of Congo (DRC) with
a median of 171 µg/L (n=60) in the age group of 6-14 years old, although these volunteers
represented occupational exposure from artisanal mining. Middleton et al. (2018) reported
exceedances of 25 and 16% in Kenya and Tanzania, respectively, when considering
inorganic-As.

Median urinary cadmium concentrations were 0.2 µg/L, with P25 and P75 of 0.1 and 0.3
µg/L in comparison to a BE upper threshold of 2.5 µg/L using underlying kidney and urinary
Cd concentration data (Hays et al. 2008). No individuals were above this threshold as shown
in Figure 2b, whilst the calculated RV95s was 0.22 µg/L for males and 0.35 µg/L for females.
Figure 3b shows a similarly low status using UcdC for all county administrative areas. The
urinary cadmium were low in comparison to Godeboa et al. (2019) study in Ethiopia with a
median of 0.61 µg/L, P25 and P75 of 0.27 and 1.05 µg/L (n = 386). Al-Saleh et al. (2020)
reported a similarly low median urinary cadmium of 0.4 µg/L for non-occupationally exposed
women in Saudi Arabia, but with an RV95s of 1.2 µg/L, which was significantly higher than
this study. Wilhelm et al. (2004) reported a median urinary cadmium value of 0.2 µg/L and
an RV95s of 0.8 µg/L. For an African comparison, Tuakila et al. (2015) reported a much
higher median urinary cadmium in the DRC of 1.7 µg/L than this study for children
occupationally exposed to mining activities, albeit below the BE threshold. Similarly,
Middleton et al. (2018) reported no exceedances for urinary cadmium in Kenya or Tanzania
for non-occupationally exposed individuals.

Median urinary tin concentrations were 0.09 µg/L, with P25 and P75 of 0.05 and 0.22 µg/L,
respectively, were very low in comparison to a BE of 20 µg/L for inorganic tin (Poddalgoda et
al. 2016). Just 2% of volunteers exceeded this threshold and can be seen as outliers in
Figures 2b and 3b. The majority of administrative counties exhibited low urinary tin ranges
with the notable exception of Bomet and Vihaga counties and to a lesser extent Bungoma,
albeit with the majority of their urinary tin below the upper threshold, confirmed with the
RV95s calculated as 1.3 µg/L for males and much lower at 0.4 µg/L for females. Few non-
occupationally derived studies exist for urinary tin, particularly for Africa.

Median urinary barium concentrations were 1.7 µg/L, with P25 and P75 of 0.8 and 4.0 µg/L,
were generally low with just 1% of individuals exceeding the BE upper threshold of 190 µg/L
(Poddalgoda et al. 2017) largely representing outliers in this study as illustrated in Figures 2b
and 3b. This contrasts with relative exceedances reported by Middleton et al. (2018) of 16
and 14% for Kenya and Tanzania. The RV95s calculated for this study was 8.2 µg/L for
males and a much higher value of 17.2 µg/L for females. Median urinary barium for male
and females were 1.9 and 1.4 µg/L, respectively. All counties exhibited a similarly low
urinary barium, with a slight difference in Saiya, albeit well below the upper threshold. There
is a paucity of data for UBaC in the literature, particularly for Africa. Therefore, the NHANES
survey for North America from which the BE value was derived is an exceptional resource
for a range of biomonitoring matrices and elements, particularly for elements such as barium
for which the health consequences are less certain (Poddalgoda et al. 2017).
Elements with no published thresholds, but comparative RV$_{95s}$ values (Cu, Ni, Sb, Cs, Pb)

Median urinary copper concentrations were 9 µg/L, with P25 and P75 of 6 and 13 µg/L (Figure 2c). Male and female median urinary copper were 9.6 and 8.8 µg/L, respectively. There are currently no thresholds or BE values reported in the literature for copper, although the calculated RV$_{95s}$ value of 29 and 21 µg/L for male and females, respectively for this study can be compared to RV$_{95s}$ values for population groups reported in other countries for some context, although should not be interpreted in depth. For example, Hoet et al. (2013) reported that non-occupationally exposed individuals in Belgium had an RV$_{95s}$ value of 19.6 µg/L (n = 1001), and Saravanabhavan et al. (2017) 25 µg/L for a Canadian study (n = 1513), both of a similar magnitude to this study. Godeboa et al. (2019) reported a broader range of urinary copper data, with a median urinary copper of 5.6 µg/L, with P25 and P75 of 2.2 and 9.1 µg/L in the Ethiopian Rift valley. Middleton et al. (2018) reported similar median urinary copper of 10 and 8.9 µg/L for Kenya and Tanzania, respectively. There were no marked differences in the range of urinary copper across the County administrative areas as illustrated in Figure 3c.

Median urinary nickel concentrations were 3.5 µg/L, with P25 and P75 of 1.7 and 6.1 µg/L (Figure 2c), with male and female median values of 2.7 and 3.6 µg/L, respectively. There are no thresholds or BE values reported in the literature for Ni, although similarly to Cu, the calculated RV$_{95s}$ value of 15.4 µg/L for this study can be compared to RV$_{95s}$ values for population groups reported in other countries for some context, although should not be interpreted in depth. For example, Hoet et al. (2013) reported that non-occupationally exposed individuals in Belgium had an RV$_{95s}$ value of 4.7 µg/L (n = 1001), Wilhelm et al. (2004) reported an RV$_{95s}$ for adults in a German study of 3.0 and Saravanabhavan et al. (2017) 4.4 µg/L for a Canadian study (n = 5602), each of them of a similar magnitude to this study. Very few studies have reported urinary nickel, with Godeboa et al. (2019) also reporting Ni in a broad elemental suite with a broader range of urinary nickel data, with a median of 7.4 µg/L, with 25/75th percentiles of 3.5 and 11.6 µg/L in the Ethiopian Rift valley. There were no marked differences in the range of urinary nickel across the County administrative areas as illustrated in Figure 3c.

Median urinary antimony concentrations were 0.08 µg/L, with P25 and P75 of 0.04 and 0.18 µg/L (Figure 2c). There are no thresholds or BE values reported in the literature for Sb, although similarly to Ni and Cu, the calculated RV$_{95s}$ value of 0.43 and 0.48 µg/L for male and females, respectively, in this study can be compared to RV$_{95s}$ values for population groups reported in other countries for some context, although should not be interpreted in depth. For example, Hoet et al. (2013) for adults in Belgium reported a much lower RV$_{95s}$ of 0.24 µg/L in Germany, whilst a Canadian study reported 0.17 µg/L (Saravanabhavan et al. 2017). No African comparison is available, as urinary antimony measurements in the Ethiopian Rift valley were below the limit of detection (Godeboa et al. 2019). The urinary antimony were generally similar across county administrative areas (Figure 3c), with the exception of Busia and Nyamira.

Median urinary caesium concentrations were 2.1 µg/L, with P25 and P75 of 1.2 and 3.6 µg/L (Figure 2c). The calculated RV$_{95s}$ was 7.4 and 8.2 µg/L were for male and females, respectively, in comparison to a Canadian RV$_{95s}$ of 12 µg/L (Saravanabhavan et al. 2017). No other studies exist for non-occupationally exposed populations, particularly in Africa.
Across county administrative areas, urinary caesium were generally within a similar range, with the exception of Busia, Kakamega and Vihaga (Figure 3c).

Median urinary lead concentrations were 0.5 µg/L, with P25 and P75 of 0.2 and 0.9 µg/L (Figure 2c). The calculated RV95s was 2.2 and 1.8 µg/L for male and females, respectively, which compared closely to a Canadian study also 1.9 µg/L (Saravanabhavan et al. 2017) and a Belgian study at 2.8 µg/L (Hoet et al. 2013). Godeboa et al. (2019) reported lower median 0.14 µg/L and P25 to be less than the lower limit of detection and P75 at 0.36 µg/L in Ethiopia, yet Al Saleh et al. (2020) reported a much a higher median of 14 µg/L in non-occupationally exposed women, which was comparable to a group of occupationally exposed miners in DRC at 19.3 µg/L (Tuakila et al. 2015). In general, county administrative areas presented a similar range of urinary lead (Figure 3c), although Bomet and Vihaga exhibited a broader and higher range of concentrations in comparison to other counties.

Influence of urinary hydration corrections

Data in this study was presented without hydration corrections for comparison with literature values, where such corrections are inconsistently presented or where they are present, presented with and without correction. This study employed hydration corrections using creatinine, osmolality and specific gravity (SG). Only occasionally, published data includes both corrected and uncorrected data. For example, Tuakila et al. (2015) usefully presented differing RV95s values with and without creatinine corrections, ranging from a -31% reduction following correction for urinary arsenic, -113% for urinary cadmium and +20% for urinary lead. The employment of the appropriate correction factor to each urinary elemental concentration should be considered, with significant differences in individual values possible with and without correction or even between correction methods. For example, urinary iodine (Watts et al. 2015; 2019b) exhibited greater uncertainty when deploying creatinine compared to osmolality and SG, whilst Middleton et al. (2016) observed a similar pattern for urinary arsenic. Phiri et al. (2020) presented a comparison of hydration factors for urinary selenium in women of reproductive age (WRA) and school age children (SAC) groups and subsequently employed SG corrections for urinary zinc (Phiri et al. 2021) in a national Malawian survey. For urinary selenium, mean corrected concentrations were similar, yet lower than uncorrected values, explained as a consequence of protein energy malnutrition, particularly for SAC.

For this study, urinary elemental concentrations and associated RV95s values are summarised as uncorrected or with one of the three hydration correction methods in Table 2. The urinary iodine, urinary selenium, urinary molybdenum, urinary arsenic, urinary tin RV95s values show a significant difference between uncorrected and corrected data, but with general agreement for the three correction methods. It is likely that the removal of outliers for creatinine as suggested by Saravanabhavan et al. (2017) has improved the comparison with SG and Osmolality against previously reported UIC in Watts et al. (2019b).

Table 2: Influence of hydration adjustment methods on urinary elemental concentrations (µg/L).

<table>
<thead>
<tr>
<th>Element</th>
<th>Correction Method</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>RV95s (95% CI)</th>
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<tr>
<td>I</td>
<td>Uncorrected</td>
<td>297</td>
<td>361</td>
<td>243</td>
<td>717 (643-811)</td>
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<tr>
<td>Element</td>
<td>Uncorrected</td>
<td>Corrected</td>
<td>Value</td>
<td>Reference Range</td>
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<td>-------------</td>
<td>-----------</td>
<td>-------</td>
<td>-----------------</td>
<td></td>
</tr>
<tr>
<td>Osmolality</td>
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<td>539</td>
<td>(502-585)</td>
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<tr>
<td>Creatinine</td>
<td>255</td>
<td>530</td>
<td>(482-588)</td>
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</tr>
<tr>
<td>Specific gravity</td>
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<td>Se</td>
<td>36</td>
<td>89</td>
<td>(79-103)</td>
<td></td>
<td></td>
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<td>70</td>
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<td>Creatinine</td>
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<td>34</td>
<td>63</td>
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<tr>
<td>Zn</td>
<td>636</td>
<td>1753</td>
<td>(1549-2016)</td>
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<td>678</td>
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<td>530</td>
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<td>Mo</td>
<td>101</td>
<td>336</td>
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<td>98</td>
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<td>80</td>
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<td>11</td>
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<td>11</td>
<td>20</td>
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<td>Creatinine</td>
<td>9</td>
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<tr>
<td>Specific gravity</td>
<td>11</td>
<td>18</td>
<td>(17-19)</td>
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<tr>
<td>Ni</td>
<td>5.7</td>
<td>15.6</td>
<td>(13.4-18.6)</td>
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<td>5.5</td>
<td>14.4</td>
<td>(12.6-16.8)</td>
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<td>11.9</td>
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<td>14.4</td>
<td>(12.6-16.8)</td>
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<tr>
<td>As</td>
<td>7.1</td>
<td>22.1</td>
<td>(18.8-26.6)</td>
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<td>6.7</td>
<td>18.1</td>
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<tr>
<td>Creatinine</td>
<td>5.6</td>
<td>16.7</td>
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<tr>
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<td>18.1</td>
<td>(15.8-21.1)</td>
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<tr>
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<td>(0.31-0.38)</td>
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<td>0.55</td>
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<td>0.46</td>
<td>(0.40-0.54)</td>
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<td>Sn</td>
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<td>0.47</td>
<td>(0.37-0.58)</td>
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<td>0.76</td>
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<td>0.81</td>
<td>(0.65-0.98)</td>
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<tr>
<td>Sb</td>
<td>0.16</td>
<td>0.46</td>
<td>(0.39-0.55)</td>
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<tr>
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<td>0.17</td>
<td>0.50</td>
<td>(0.43-0.60)</td>
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<tr>
<td>Creatinine</td>
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<td>0.42</td>
<td>(0.36-0.50)</td>
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<td>0.46</td>
<td>(0.40-0.55)</td>
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<tr>
<td>Cs</td>
<td>2.9</td>
<td>7.9</td>
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<tr>
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<td>(6.2-7.8)</td>
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<tr>
<td>Creatinine</td>
<td>2.4</td>
<td>5.8</td>
<td>(5.1-6.6)</td>
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<td>(6.6-8.3)</td>
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<tr>
<td>Ba</td>
<td>6.6</td>
<td>13.4</td>
<td>(11.0-16.9)</td>
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<td>Osmolality</td>
<td>Creatinine</td>
<td>Specific gravity</td>
<td>Pb Uncorrected</td>
<td>Osmolality</td>
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<tr>
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<td>6.4</td>
<td>0.7</td>
<td>0.9</td>
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<td>16.3</td>
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<td>1.8</td>
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<tr>
<td>95% Confidence Interval (CI)</td>
<td>14.1 (11.7-17.6)</td>
<td>13.4 (11.0-17.0)</td>
<td>14.8 (12.1-18.5)</td>
<td>1.9 (1.6-2.2)</td>
<td>1.6 (1.5-1.9)</td>
</tr>
</tbody>
</table>

### Application of urinary biomonitoring data

In general, most biomonitoring studies are reported with descriptive statistics for aggregated data, with comparison to a reference or population value to establish a public health context. As Morrens et al. (2021) pointed out, there is no universal consensus to communicate individual results to study participants, where most surveillance studies, including this study, are designed to communicate aggregated results. For this study, aggregated data has been communicated with public health administrators for onward public health contextual interpretation with community workers, alongside individual data for follow-up on exceedances or where specifically requested by the participants. The latter often involved individuals sensitised and with an interest in health conditions in their local areas (e.g. iodine deficiency-goitre; fluorosis). The majority of participants wanted to contribute to research, with a small proportion curious about their own results. The RV\textsubscript{95}s values provide some context and aid to explaining results, particularly for elements where there are no established bioequivalent thresholds that can infer health status, but can be used as a reference point against the rest of the study participants. However, care should be used where health-based/bioequivalent guidance values are not available and the RV\textsubscript{95}s values used as a comparison with other population groups to generate a body of evidence/requirement for investment in bioequivalent calculations.

### Conclusion

Health studies will benefit from a dual approach to deconvolute and design mitigation strategies to deficiencies of micronutrients essential to health and reduce exposure to potentially harmful elements using food consumption surveys alongside human biomonitoring. Biomonitoring through the use of urinary elemental concentrations does provide a cost effective approach compared to accurate food dietary survey/analyses for population background exposure albeit with a snapshot in time. The increasing literature providing comparative reference values (RV\textsubscript{95}s) for study organisers and participants where health-based guidelines are lacking may assist in building evidence and targeting of resources towards the development of bioequivalent calculations to better infer health outcomes and subsequent design of mitigation strategies. For biomonitoring data to be used to inform health interventions, it must have sufficient quality assurance controls and levels of reliability, including consideration of hydration correction factors to derive reference values for a range of elements appropriate to urinary measurements. Further studies should consider targeted hydration corrections for each of the urinary elemental concentration versus uncorrected data for transparent comparison with published studies and building of confidence in the appropriate correction strategy. This will have significance in challenging
environments where lower cost measurements such as SG may be more appropriate or in a 
low-income nation setting where low protein intake may render creatinine a poor method.

**Figure legends**

Figure 1: Location map for collection points and county administrative areas in Western 
Kenya.

Figure 2: Distribution of urinary elemental concentrations (uncorrected) in groups of (a) 
micronutrients - above red line denotes excess biomonitoring equivalent from NHANES most 
conservative value, blue line below which deficiency based on the estimated average 
requirement-EAR bioequivalent from NHANES most conservative value, (b) potentially 
harmful elements - (above red line denotes excess biomonitoring equivalent from NHANES 
most conservative value, and (c) elements with comparable published reference values 
(RV95s) - red line denotes calculated RV95s value.

Figure 3: Distribution of urinary elemental concentrations (uncorrected) by county 
administrative areas in groups of (a) micronutrients - above red line denotes excess 
bioequivalent from NHANES most conservative value, blue line below which 
deficiency based on the estimated average requirement-EAR bioequivalent from NHANES 
most conservative value, (b) potentially harmful elements - above red line denotes excess 
bioequivalent from NHANES most conservative value, and (c) elements with 
comparable published reference values (RV95s) - red line denotes calculated RV95s value.

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permission of the Executive Director of the British Geological Survey.

**Declarations**

The authors declare that there is no financial/personal interest or belief that could affect their 
objectivity.

**Data statement**

All data is included in Supplementary information, but without compromising the anonymity 
of study participants.

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