

Intra-household agreement of urinary elemental concentrations in Tanzania and Kenya: potential surrogates in case-control studies

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## **Abstract**

Element deficiencies and excesses play important roles in non-communicable disease etiology. When investigating their roles in epidemiologic studies without prospective designs, reverse-causality limits the utility of transient biomarkers in cases. This study aimed to investigate whether surrogate participants may provide viable proxies by assessing concentration correlations within households. We obtained spot urine samples from 245 Tanzanian and Kenyan adults (including 101 household pairs) to investigate intra-household correlations of urinary elements (As, Ba, Ca, Cd, Co, Cs, Cu, Fe, Li, Mn, Mo, Ni, Pb, Rb, S, Se, Sr, Tl, V, Zn) and concentrations (also available for: Bi, Ce, Sb, Sn and U) relative to external population-levels and health-based values. Moderate-strong correlations were observed for As ( $r=0.65$ ), Cs ( $r=0.67$ ), Li ( $r=0.56$ ), Mo ( $r=0.57$ ), Se ( $r=0.68$ ) and Tl ( $r=0.67$ ). Remaining correlations were  $<0.41$ . Median Se concentrations in Tanzania ( $29 \mu\text{g/L}$ ) and Kenya ( $24 \mu\text{g/L}$ ) were low relative to 5738 Canadians ( $59 \mu\text{g/L}$ ). Exceedances (of reference 95<sup>th</sup> percentiles) were observed for: Co, Mn, Mo, Ni and U. Compared to health-based values, exceedances were present for As, Co, Mo and Se but deficiencies were also present for Mo and Se. For well correlated elements, household members in East African settings provide feasible surrogate cases to investigate element deficiencies/excesses in relation to non-communicable diseases.

**Keywords:** micronutrients, trace elements, urine, cancer epidemiology

## **Introduction**

Chemical elements are integral to human health and nutrition and are typically grouped as essential (e.g. selenium (Se), zinc (Zn), and iron (Fe)) or toxic (e.g. arsenic (As), lead (Pb) and cadmium (Cd)). However, many elements are considered essential but can also be toxic above certain doses, e.g. Se and molybdenum (Mo). Deficiencies of essential elements are associated with numerous non-communicable diseases (NCDs). Essential elements are key components of cellular machinery and many, e.g. Zn and Se, are constituents of proteins involved in DNA damage/repair pathways and antioxidant mechanisms (1, 2). Numerous deleterious health effects are likewise associated with chronic overexposure to certain elements. Notably, inorganic As exposure is associated with cardiovascular disease (3), diabetes mellitus (4) and cancer of the lung, bladder and skin (5); Cd with kidney diseases (6) and Pb with brain development and neurological conditions (7). Arsenic and Cd, as well as hexavalent chromium (Cr VI), beryllium (Be) and some nickel (Ni) compounds are IARC Group 1 (“carcinogenic to humans”) carcinogens (8).

Investigating the role of elemental deficiencies and excesses in NCD etiology, such as cancer, poses many challenges. Epidemiological study designs yielding the strongest evidence utilize biomarkers assessed in biospecimens collected in prospective cohorts (9). In many low and middle income countries, prospective studies are not yet available, thus the case-control study design offers speedy results. However, reverse causality inherent in the case-control design innately invalidates the use of transient biomarkers assessed in biospecimens obtained after disease onset, such as element concentrations in urine or plasma, which are liable to change in response to morbidity. In such instances, the use of longer term biomarkers is also not always feasible: e.g. toenails and hair, which could capture exposures dating from months prior, are often heavily contaminated (10).

A possible solution is to revert to a blood or urinary biomarker in a surrogate participant whose levels are expected to be correlated with that of the index subject prior to disease onset. While there is extensive literature on the use of surrogate respondents in epidemiological studies (11, 12), e.g. necessitated when studying conditions like Alzheimer’s disease (13), the use of surrogate biomonitoring results has received less attention. Because urine is a non-invasive specimen, we investigate whether in East African populations, members of the same household have correlated urinary elementary concentrations, thus informing whether household members may serve as viable proxies for investigating disease outcomes in case-control studies. The secondary aim was to report urinary element concentrations in comparison to existing population-based reference values and health based values (where available). Throughout, urinary Se is discussed in the context of oesophageal squamous cell carcinoma (ESCC) - a high incidence cancer in East Africa - as a specific potential application of the findings.

## **Methods**

### *Ethical approval*

Ethical approval was granted at IARC (IEC 14-15), in Tanzania (NIMR/HQ/R.8a) and Moi University in Kenya (IREC 000921). Informed consent was obtained from all participants.

### *Recruitment and data collection*

While conducting community surveys in Tanzania and Kenya into the potential risk factors of ESCC, household paired spot urine samples were collected. Separate cross-sectional studies were conducted in the Kilimanjaro region of Tanzania (Fig.1B) in January-February 2014 and the counties of Kisumu, Bungoma and Trans Nzoia in Kenya (Fig. 1A) in October 2016 (it is noted that paired samples from the same household were always collected on the same date). The study areas were selected to coincide with the broad catchment area of residential locations of ESCC case-control studies being conducted at the Kilimanjaro Christian Medical Centre in Moshi, Tanzania and Moi Teaching and Referral Hospital in Eldoret, Kenya ([esccape.iarc.fr](http://esccape.iarc.fr)). Households were approached at random and two members ( $\geq 18$  years old) from each household were invited to provide a spot urine sample and complete an interview-administered questionnaire in Kiswahili or an appropriate local language to ascertain age and sex and information on other ESCC related factors (e.g. tobacco and alcohol consumption, hot beverage drinking habits and indoor cooking practices). The detailed sampling strategy for the Tanzanian survey has been reported previously (14).

### *Urine sample collection, total element determinations and dilution adjustment*

Spot urine samples were collected in 60 mL wide-mouth LDPE bottles (Nalgene, USA), transported in cool boxes and frozen at  $-80^{\circ}\text{C}$  prior to being shipped to the British Geological Survey in the UK. Urine samples were thawed at room temperature, filtered through  $0.45\ \mu\text{m}$  acrodisc filters and diluted  $\times 10$  prior to analysis (1 mL of urine + 9 mL of deionized water). Total element concentrations (a routine suite of  $\sim 50$  elements) were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) using an Agilent 7500 series instrument and a previously reported methodology (15). Concentrations below the instrumental limit of detection (LOD) were censored with  $0.5 \times \text{LOD}$ . To correct for differences in urinary dilution resulting from varying states of hydration, urinary specific gravity (SG) was estimated using a handheld digital refractometer with built-in temperature correction (Atago PAL-10S). Element concentrations were adjusted for dilution using the Levine-Fahy equation (16):

$$C_{\text{adj}} = C_{\text{vol}} \times ((\text{SG}_{\text{ref}} - 1) / (\text{SG}_{\text{spec}} - 1)), \quad [1]$$

where  $C_{\text{adj}}$  is the SG-adjusted analyte concentration,  $C_{\text{vol}}$  is the unadjusted, volume-based analyte concentration ( $\mu\text{g/L}$ ),  $\text{SG}_{\text{ref}}$  is the SG reference value to which the concentration is normalised (here, the arithmetic mean SG of all samples (both countries combined) was chosen: 1.015) and  $\text{SG}_{\text{spec}}$  is the SG of the individual specimen being adjusted.

### *Element inclusion criteria and quality control*

For the purposes of broader data sharing, all elements that were (i) measured within the acceptable range of Certified Reference Materials (CRM) and (ii) above LOD for both household samples in at least 20 household pairs were reported and included in correlation analysis. Additional elemental concentrations, for which <60% were above LOD (Bi, Ce, Sb, Sn and U) are presented in Table S1 (supplementary material). Kenyan samples were analysed with LGC Ltd (UK) Seronorm Trace Elements Urine L-1 (SERO210605) ( $n=4$ ) and Tanzanian samples with National Institute for Environmental Studies (NIES, Japan) No.18 Human Urine ( $n=8$ ). CRM performance data are reported in Table 1 for elements included in correlation analysis and in Table S2 for the excluded elements.

### *Statistical analysis*

All analyses and results, with the exception of comparisons to reference values and health-based guidance values, concern SG-adjusted urinary element concentrations. These concentrations were positively skewed but approached normality following natural log (ln)-transformation. Intra-household Pearson's correlation coefficients ( $r$ ) and 95% confidence intervals (95% CI) were calculated for ln-transformed concentrations. A sensitivity analysis was performed to compare the effect of omitting censored (i.e. <LOD) concentrations from correlation calculations, urinary SG adjustment and gender matching. To test the significance of the difference in strength between two correlations, Williams's and Steiger tests were used for independent (gender matched versus unmatched) and dependent (unadjusted and SG-adjusted) correlations, respectively. Age and gender determinants of urinary element concentrations were investigated using linear regression of ln-transformed values, accounting for within-household correlations; beta coefficients (differences in means on ln scale) were exponentiated to give the corresponding ratio of geometric means and only the latter were reported. Based on the results of these models, for certain elements with high correlations, we simulated elemental data for 10,000 pairs, with a random assignment of gender, and used this simulated dataset to examine whether matching on gender would improve the within-household correlation. All statistical analysis and plotting was conducted using R (version 3.3.1) (17) and the RStudio GUI (18).

### *Comparisons of urinary element concentrations*

Urinary element concentrations were compared to published, population-based reference values where available. These values were obtained from the US National Health and Nutrition Examination Survey (NHANES) (2013-2014) (19), the Canadian Health Measures Survey (CHMS) (2009-2011) (20), a study of 134 non-occupationally exposed individuals from the UK (21) and 1001 Belgian adults (22). To assess deficiencies of essential elements relative to these external populations, comparisons were

made between median concentrations. To indicate relative exceedances, the number of samples exceeding the highest 95th percentile of the four reference populations was employed. Neither of the above approaches is health-based.

For a limited number of elements, results were compared to health-based biomonitoring equivalent (BE) and BE point-of-departure (BE<sub>POD</sub>) values (As, Cd, Mo and Se, including nutritional BEs for Se and Mo) (23-26) and Biological Exposure Indices (BEI) (Co and U) (27). BEs are risk-assessment tools, i.e. estimated biomarker concentrations at which there are likely to be no adverse health effects and above which such risk assessment investigation is considered medium priority (28). BE<sub>POD</sub> values are less conservative and exceedances are considered a high priority for investigation. Biological exposure indices were developed for occupational settings by the American Conference of Governmental Industrial Hygienists to represent levels below which injury/illness is unlikely to occur (27). The health-based values selected for this study were as follows: for As (hyperpigmentation and vascular complications): BE: 6.4 µg/L; BE<sub>POD</sub> 19.3 µg/L; Cd (renal cortex concentration): BE: 1.5 µg/L; BE<sub>POD</sub>: 4.6 µg/L; Co: BEI: 15 µg/L; Mo (nutritional BE - average estimated requirement): 21.7 µg/L; Mo (USEPA reference dose): BE: 206; BE<sub>POD</sub>: 2063; Se (nutritional BE - average estimated requirement): 10 µg/L; Se (toxicity): BE: 90 µg/L; BE<sub>POD</sub>: 280; U: BEI: 200 µg/L.

## **Results**

### *Study group characteristics*

Socio-demographic characteristics of the study sample are shown in Table 2. Participants were residents of rural, subsistence farming communities and most pairs were male-female.

### *Urinary element concentrations and their demographic determinants*

Detection limits and detailed descriptive statistics of urinary element concentrations (including elements omitted from correlation analysis) are shown in Table S1 (supplementary material) for Kenya and Tanzania separately in comparison to published population-based reference values. Elements for which >40% of samples were below LOD were Bi, Ce, Sn for Kenya and Bi, Ce, Mn, Sb, Sn, Tl, U, V for Tanzania.

Of the recognised essential elements for which population-based reference values were available, median Se concentrations were lower than the Canadian population (59 µg/L) for both Kenya (24 µg/L) and Tanzania (29 µg/L), but comparable to the Belgian median (25 µg/L) and higher than that reported for the UK (13 µg/L). Median Cu concentrations were comparable to all four reference values in Kenya (10 µg/L) and Tanzania (8.9 µg/L). Median Zn concentrations were elevated in both Kenya (479 µg/L) and Tanzania (427 µg/L) relative to Canadian (350 µg/L), UK (180 µg/L) and Belgian (256 µg/L) medians.

Of those elements often investigated in relation to their toxicity, relative exceedances were present for Co (Kenya: 45%; Tanzania: 16%), Mn (Kenya: 45%; Tanzania: 46%), Mo (Kenya: 25%; Tanzania: 18%), Ni (Kenya: 11%; Tanzania: 5%) and U (Kenya: 24%; Tanzania: 8%). No exceedances were observed for the established toxic elements As, Cd and Pb. Elemental exceedances for which the health implications are less clear were present for Ba (Kenya: 16%; Tanzania: 14%), Ce (Kenya and Tanzania: 100%), Rb (Kenya: 45%; Tanzania: 71%), Sr (Kenya: 15%; Tanzania: 12%), Tl (Kenya: 82%) and Zn (Kenya: 11%; Tanzania: 8%).

In relation to health-based values, 3 (5%) samples in Kenya and 1 (0.5%) sample in Tanzania were below the nutritional BE for Se (10 µg/L) and 6 (11%) samples in Kenya and 16 (8%) samples in Tanzania were below the nutritional BE for Mo (21.7 µg/L). No exceedances were found in either Kenya or Tanzania for Cd (BE: 1.5 µg/L; BE<sub>POD</sub>: 4.6 µg/L) or U (BEI: 200 µg/L). For As, 14 (25%) and 31 (16%) of samples exceeded the BE (6.4 µg/L) in Kenya and Tanzania, respectively. The less conservative As BE<sub>POD</sub> (19.3 µg/L) was exceeded by 1 (2%) Kenyan and 6 (3%) Tanzanian samples. It is noted that the As health-based values refer to inorganic As and its metabolites and not total arsenic as measured in the present study (see discussion). The BEI for Co (15 µg/L) was exceeded by a single sample from Tanzania. Three (5%) Kenyan and 17 (9%) Tanzanian samples exceeded the Mo BE (206 µg/L), but none exceeded the Mo BE<sub>POD</sub> (2063 µg/L). The Se BE (90 µg/L) was exceeded by 4 (2%) samples in Tanzania only and no exceedances of the Se BE<sub>POD</sub> (280 µg/L) were found. It is noted that for some elements, such as Se and Mo, both deficiencies and exceedances are possible.

Regression analyses indicated that, for some elements, age and gender were significant determinants of urinary element concentrations (Table 3).

#### *Intra-household urinary element correlations*

Intra-household urinary element correlations of the 20 elements included are presented in Table 4. Correlations were initially estimated with the inclusion of all samples, including censored concentrations below LOD. Using this approach, variation among censored concentrations results solely from the application of SG adjustment. The following elements exhibited moderate to strong (i.e.  $r > 0.50$ ) correlations: As ( $r = 0.65$ ; 95% CI: 0.52, 0.75); Cs ( $r = 0.67$ ; 95% CI: 0.54, 0.76); Li ( $r = 0.56$ ; 95% CI: 0.41, 0.68); Mo ( $r = 0.57$ ; 95% CI: 0.42, 0.68); Se ( $r = 0.68$ ; 95% CI: 0.56, 0.77); Tl ( $r = 0.67$ ; 95% CI: 0.54, 0.76). A sensitivity analyses was then conducted by omitting censored concentrations. Elements for which a moderate to strong correlation was found following the omission of censored concentrations are plotted in Figure 2. Of the 6 aforementioned elements, only Tl concentrations were substantially reduced in number following the omission of censored data and their correlation strengthened ( $r = 0.85$ ; 95% CI: 0.66, 0.94) after omission. Additionally, moderate-strong correlations were found for Ni ( $r = 0.63$ ; 95% CI: 0.44, 0.77) and V ( $r = 0.63$ ; 95% CI: 0.37, 0.80). Urinary SG adjusted concentrations yielded stronger correlations to unadjusted ones e.g. for Se, the correlation prior to adjustment was 0.43,

significantly weaker ( $p=0.01$ ). Of the elements with a significant age and gender determinant (Table 3), only Se had a moderate household correlation of 0.68 and a significant gender difference. A simulation revealed that this gender-unmatched correlation of 0.68 improved by gender-matching to 0.71, and was higher than the correlation among gender-discordant pairs (0.65).

## Discussion

This study was the first to report on such a wide range of urinary trace element concentrations in Kenyan and Tanzanian populations and one of few studies worldwide to report their intra-household correlations. In relation to population-based reference values, of the essential elements investigated, only Se showed indications of being low (based on comparisons of Tanzanian (29  $\mu\text{g/L}$ ) and Kenyan (24  $\mu\text{g/L}$ ) medians that of the CHMS (59  $\mu\text{g/L}$ )) whilst, of those elements broadly considered toxic, Co, Mn, Mo, Ni and U were relatively elevated in some samples. Compared to the limited number of health-based values available, exceedances were observed for As, Co, Mo and Se and deficiencies for both Mo and Se. These exceedances/deficiencies should be interpreted with due caution, but warrant further consideration for research. Moderate-strong intra-household correlations were observed for As ( $r=0.65$ ), Cs ( $r=0.67$ ), Li ( $r=0.56$ ), Mo ( $r=0.57$ ), Se ( $r=0.68$ ) and Tl ( $r=0.67$ ), with the remainder of elements having correlations below 0.41. These correlations were all a marked improvement on those calculated with urinary concentrations that were not adjusted for urinary SG – reiterating the necessity of hydration adjustments in urinary biomonitoring to improve interpretability (29). Correlations were only marginally improved by matching pairs on gender.

Expectedly, the intra-household correlations reported varied greatly by element. For many elements, this may be due to the limitations already discussed. Urinary Cu and Zn both had weak intra-household correlations of 0.01 and 0.35, respectively. Neither element is routinely measured in urine and are both primarily excreted via the faeces (30). The relative strengths of common exposure sources of many elements, and their differences between individuals from the same households, are responsible for variations in strength of correlations. Manganese concentrations were weakly correlated (0.17) and higher in females, whereas the contrary was found for Se, i.e.  $r=0.68$  and higher concentrations in males. Diet is a dominant source of both Mn and Se and higher concentrations are found in specific food items. A dominant source of Se in the Kenyan and Tanzanian diet is likely meat and dairy products, based on food composition studies (31) and findings from neighbouring Burundi (32), whereas Mn is present at highest concentrations in cereals and vegetables (31). Dietary heterogeneity and how it differs between household members may therefore drive the strength of some elemental correlations relative to others. In a previous study (15) an intra-household correlation of 0.66 was measured for urinary As, the dominant source of which was the household drinking water supply – shared by both members. Furthermore, correlative strength is also driven by between household variation which, given the



localised source of the majority of samples (Figure 1B), may have weakened correlations in the present study.

While numerous studies have reported biomarker concentrations from multiple individuals within the same household (15, 33), to our knowledge, this is one of the first studies to explore them in the context of surrogate participants in case-control studies. In the example of investigating the potential role of Se deficiency and ESCC risk, deficiencies of Se and other essential elements have been linked to an increased risk of ESCC (34-39). This may be pertinent in Kenya and Tanzania, which are part of an ESCC high incidence corridor (40, 41). Reliance on subsistence farming and low estimates of geochemically mediated (42) micronutrient supplies have been reported (43). However, there are no suitable cohorts to address the hypothesis, and cases were often severely underweight with solid and liquid dysphagia at the time of diagnosis. Because we found a relatively strong intra-household correlation of urinary Se ( $r=0.68$ ), surrogate household members could be considered in a case-control study. The implications of measurement errors introduced through the use of surrogates on statistical models appropriate for the analysis and on sample size calculations would need to be considered. We crudely quantified the effect of a surrogate study design using a sample size calculation. A correlation of 0.68 translates into a doubling of study participants to detect the effect of a 5  $\mu\text{g/L}$  difference in urinary Se between cases and controls (up to 528 from 244 case-control pairs if surrogate household members for Se biomarkers were employed as opposed to index cases and controls). The cost of such a study design, both statistically and logistically (i.e. the required compensatory increase in participant numbers) would need to be taken into account.

The findings of this study are subject to several caveats. Element concentrations were reported if they passed analytical QC criteria, which alone does not validate their robustness, individually, as human biomarkers - whose applicability depend on numerous physiological and pharmacokinetic factors (44). For many elements, adequacy of nutritional status is assessed via blood, e.g. ferric Fe and Se in serum. Blood is also the preferred matrix for many toxic elements, e.g. Pb, which is subject to less variation in blood than in urine and reflects recent intake and stored concentrations (45). Conversely, urinary concentrations of some elements are more robust in urine, such as As, a rapidly excreted element (46), which is cleared from the bloodstream too quickly (47). In addition, urinary Se concentrations have previously been demonstrated to agree with those measured in blood plasma (48). The efficacy of spot urine samples, opposed to longer term sampling protocols, has often been questioned and, as mentioned, adjustment for hydration-driven dilution variation is necessary. However, we note that such adjustment cannot correct for other forms of variation in spot results, such as the timing of sampling relative to intake. The interpretive values of urinary concentrations are also limited by the paucity of population-based reference values and health-based criteria and the robustness of those already derived, all of which can only serve as a guide. Notwithstanding the aforementioned limitations, it was opted to report all concentrations, should they be of future relevance in light of new interpretive tools and toxicological

understanding. Readers are advised to review these factors on an element-specific basis before interpreting the results. Additionally, only total element concentrations are reported. This is particularly relevant to As, with likely contributions of non-toxic, organic As species having been extensively documented (15). The determinants of urinary trace element concentrations, particularly those that showed indications of deficiency or exceedance, in the study population warrant further investigation. Sampling designs will need to account for geospatial, environmental, socio-demographic and environmental variables.

## **Conclusions**

Intra-household correlations of urinary trace element concentrations measured in these rural Tanzanian and Kenya populations suggest that, for some elements, the use of biomarkers from household members as proxies for the index subjects may provide a feasible means to investigate nutrient deficiencies and excesses in relation to occurrences of non-communicable diseases.

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## **Conflict of Interest**

All authors declare no competing interests.

Supplementary information is available at <https://www.nature.com/jes/>

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## Tables

**Table 1** Certified Reference Material performance data for the 20 elements reported. Nominal certified values and acceptable ranges or reference values (marked with asterisks) are listed with mean measured

values. Accuracy and precision are shown with mean percentage recoveries and relative standard deviations (RSD), respectively.

<b>Element</b>	<b>Certified/reference value* (acceptable range) (µg/L unless specified)</b>	<b>Mean measured value (µg/L unless specified)</b>	<b>Mean recovery±RSD</b>
<b>Kenya (SERO210605, n=4)</b>			
Arsenic (As)	79 (47-111)	71	90±1%
Barium (Ba)	28*	26	92±1%
Calcium (Ca), mg/L	71*	73	103±1%
Cadmium (Cd)	0.2 (0.13-0.27)	0.24	118±17%
Cobalt (Co)	0.72 (0.43-1.01)	0.72	100±10%
Caesium (Cs)	5.8*	5.7	98±1%
Copper (Cu)	31*	28.5	92±3%
Iron (Fe)	13.7*	12	89±3%
Lithium (Li)	7*	8	114±17%
Manganese (Mn)	0.73 (0.44-1.02)	0.62	85±15%
Molybdenum (Mo)	37*	40	108±1%
Nickel (Ni)	1.51 (0.91-2.11)	1.7	110±12%
Lead (Pb)	0.66 (0.39-0.93)	0.54	82±1%
Rubidium (Rb)	990*	974	98±1%
Selenium (Se)	13.9 (8.3-19.5)	14	101±3%
Strontium (Sr)	89*	87	98±2%
Sulphur (S), mg/L	521*	545	105±3%
Thallium (Tl)	0.16 (0.13-0.19)	0.16	102±15%
Zinc (Zn)	334 (200-468)	321	96±2%
<b>Tanzania (NIES No.18, n=8)</b>			
Arsenic (As)	137 (126-148)	130	95±3%
Copper (Cu)	10*	9	90±1%
Lead (Pb)	1.1*	0.88	80±2%
Selenium (Se)	59 (54-64)	67	114±5%
Vanadium (V)	0.66 (0.5-0.9)	0.68	103±4%
Zinc (Zn)	620 (570-670)	634	102±4%

**Table 2** Socio-demographic characteristics of the study sample.

	<b>Tanzania, <i>n</i> (%)</b>	<b>Kenya, <i>n</i> (%)</b>
<b>Total</b>	190	55
<b>Gender<sup>a</sup></b>		
Male	83 (44)	16 (29)
Female	105 (55)	38 (69)
<b>Age group<sup>b</sup></b>		
18-29	21 (11)	1 (2)
30-39	24 (13)	15 (27)
40-49	30 (16)	15 (27)
50-59	46 (24)	7 (13)
60-69	28 (15)	9 (16)
≥70	39 (20)	6 (11)
<b>Household pairs</b>	<b>Both countries: 101 pairs (204 samples)</b>	
Male-female pairs	79 (78%)	
Female-female pairs	15 (15%)	
Male-male pairs	4 (4%)	

<sup>a</sup> Gender information missing for 2 and 1 participants from Tanzania and Kenya, respectively.

<sup>b</sup> Age information missing for 2 participants from both Tanzania and Kenya.

**Table 3** Ratios of geometric mean urinary element concentrations (SG-adjusted and log-transformed) as a function of gender (male to female ratio) and 10 year increase in age (mutually adjusted). \*, \*\* and \*\*\* denote significance to  $p < 0.05$ , 0.01 and 0.001, respectively.

<b>Element</b>	<b>Male to female ratio</b>	<b>Ratio with 10-year increase in age</b>
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As	0.95 (0.76, 1.20)	0.95 (0.89, 1.02)
Ba	0.64** (0.47, 0.88)	1.04 (0.95, 1.14)
Ca	0.94 (0.71, 1.24)	0.99 (0.91, 1.07)
Cd	1.06 (0.89, 1.27)	0.94* (0.89, 0.99)
Co	0.62*** (0.47, 0.81)	0.79*** (0.73, 0.85)
Cs	1.13 (0.97, 1.32)	0.94** (0.89, 0.98)
Cu	0.98 (0.84, 1.13)	1.00 (0.96, 1.05)
Fe	0.85 (0.65, 1.13)	0.98 (0.90, 1.06)
Li	1.07 (0.83, 1.38)	0.96 (0.89, 1.03)
Mn	0.43*** (0.29, 0.63)	1.03 (0.92, 1.16)
Mo	1.08 (0.88, 1.33)	0.85*** (0.80, 0.90)
Ni	0.93 (0.72, 1.20)	0.83*** (0.77, 0.90)
Pb	1.11 (0.90, 1.36)	0.92** (0.86, 0.97)
Rb	1.04 (0.89, 1.22)	1.02 (0.98, 1.07)
S	1.08 (0.97, 1.19)	0.95*** (0.92, 0.98)
Se	1.21** (1.05, 1.39)	0.93*** (0.89, 0.97)
Sr	0.99 (0.80, 1.23)	0.96 (0.90, 1.02)
Tl	0.95 (0.67, 1.35)	0.94 (0.84, 1.04)
V	1.04 (0.86, 1.26)	0.99 (0.93, 1.05)
Zn	1.35** (1.13, 1.62)	1.06* (1.00, 1.12)

**Table 4** Pearson's correlation coefficients (ln-transformed and SG-adjusted variables) and 95% confidence intervals for paired urinary element concentrations (moderate-strong correlations are emboldened). Correlations are presented separately for all paired samples and following the omission of pairs where at least one of the household concentrations was censored for being below the limit of detection.

Element	Pearson's correlation (r, 95% CI) (censored concentrations included, n=101 pairs)	Pearson's correlation (r, 95% CI) (censored concentrations omitted)	n pairs (after omission of censored concentrations)
As	<b>0.65 (0.52, 0.75)</b>	<b>0.69 (0.57, 0.78)</b>	98
Ba	0.18 (-0.02, 0.36)	0.20 (-0.01, 0.40)	86
Ca	0.23 (0.04, 0.41)	0.18 (-0.02, 0.37)	98
Cd	0.24 (0.04, 0.41)	0.18 (-0.09, 0.43)	55



Co	0.26 (0.07, 0.43)	0.23 (0.02, 0.42)	89
<b>Cs</b>	<b>0.67 (0.54, 0.76)</b>	<b>0.67 (0.54, 0.76)</b>	101
Cu	0.01 (-0.18, 0.21)	-0.06 (-0.26, 0.15)	89
Fe	0.26 (0.06, 0.43)	0.41 (0.17, 0.60)	59
<b>Li</b>	<b>0.56 (0.41, 0.68)</b>	<b>0.66 (0.45, 0.80)</b>	45
Mn	0.17 (-0.02, 0.36)	0.07 (-0.31, 0.44)	27
<b>Mo</b>	<b>0.57 (0.42, 0.68)</b>	<b>0.57 (0.41, 0.69)</b>	100
<b>Ni</b>	0.41 (0.23, 0.56)	<b>0.63 (0.44, 0.77)</b>	53
Pb	0.16 (-0.04, 0.34)	0.14 (-0.10, 0.37)	67
Rb	0.35 (0.16, 0.51)	0.35 (0.16, 0.51)	101
S	0.31 (0.12, 0.48)	0.31 (0.12, 0.48)	101
<b>Se</b>	<b>0.68 (0.56, 0.77)</b>	<b>0.68 (0.56, 0.77)</b>	101
Sr	0.21 (0.01, 0.39)	0.21 (0.01, 0.39)	101
<b>Tl</b>	<b>0.67 (0.54, 0.76)</b>	<b>0.85 (0.66, 0.94)</b>	21
<b>V</b>	0.45 (0.27, 0.59)	<b>0.63 (0.37, 0.80)</b>	34
Zn	0.35 (0.16, 0.51)	0.30 (0.11, 0.47)	100

### Figure legends

**Figure 1** Sampling locations in (A) Bungoma, Kisumu and Trans Nzoia counties, Kenya and (B) the Kilimanjaro region of Tanzania.

**Figure 2** Moderate to strong correlations for intra-household urinary concentrations of As, Cs, Li, Mo, Ni, Se, Tl and V. Pearson's correlation coefficients were calculated for ln-transformed variables following omission of censored (<LOD) concentrations and adjustment to a SG of 1.015.