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ORIGINAL ARTICLE

Postmortem reference concentrations of 68 elements in blood and urine

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Abstract

Introduction Fatal intoxications, both accidental and intentional, are a global issue. In the Western world, intoxications with pharmaceuticals dominate, but in other parts of the world, other substances are more common. In a forensic setting, elemental intoxications are of great importance when investigating both accidental, suicidal, and homicidal deaths. The current study presents normal postmortem reference concentrations of 68 elements in femoral blood and urine. In addition, possible sources of error such as contamination from sample tubes, preservative potassium fluoride (KF) solution, and storage time are evaluated.

Methods Paired femoral blood and urine samples from 120 cases of death by suicidal hanging in Sweden were collected. Additionally, multiple batches of sample tubes and multiple batches of KF solution were also analyzed. Concentrations of elements were determined by double focusing sector field ICP-MS.

Results Key descriptive statistics for 68 elements are provided in blood and urine. Contamination from sample tubes was minor compared to the overall mean elemental concentrations in both blood and urine. KF solution contained a large assortment of elements, but the overall contribution is relatively minor for most elements given the small amounts of solution added to samples. There were significant differences for 22 elements in blood and 17 elements in urine between samples with short and long storage time.

Conclusion The present study provides an important tool when evaluating postmortem elemental concentrations. It fills a needed gap between large antemortem population studies and postmortem case reports or small case series of elemental intoxications.

Keywords Forensic toxicology · Postmortem toxicology · Elements · Reference concentrations · Blood · Urine

Introduction

Fatal intoxications, both accidental and intentional, are a global problem. In the Western world, intoxications with pharmaceuticals dominate, but in other parts of the world, other substances are more common [1, 2]. Intoxication with elemental compounds is uncommon, for example, heavy metal intoxications only constituting 0.4% of all contacts with American Poison Control Centers in 2019 [3]. However, in a forensic setting, elemental intoxications are of great importance in both accidental, suicidal, and homicidal deaths. For example, arsenic (As) is a classical poison that also has relevance in the modern era [4, 5].

Correctly diagnosing fatal intoxications is difficult. At autopsy there are few and unspecific findings to indicate an intoxication [6]. In the absence of strong circumstantial information, it is often the role of the forensic toxicological investigation to provide the clues needed to correctly diagnose a case. When dealing with postmortem cases, forensic toxicological results face interpretational challenges, such as postmortem redistribution and putrefactive change [6–8], which are not present antemortem. For many elements, it

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is unclear to which extent they are impacted by these factors. One exception is a study by Schier et al. [9], in which postmortem concentrations of cadmium (Cd), lead (Pb), and mercury (Hg) were compared to reference ranges for living persons. The study showed increased concentrations of cadmium (Cd) postmortem compared to living reference values. In addition, it showed concentration variations based on sample site for cadmium (Cd) and mercury (Hg), with higher concentrations in blood sampled from the heart than blood sampled from the femoral vein. As differences between peripheral blood and central blood are indicative of postmortem redistribution [8, 10], it can be assumed that postmortem peripheral blood elemental concentrations better reflect antemortem concentrations.

To correctly interpret postmortem toxicological results, postmortem reference concentrations are needed. Currently there is a wealth of postmortem references describing low, normal, high, and potentially toxic concentrations of narcotics and pharmaceuticals [11–14]. However, there is generally a lack of postmortem reference concentrations describing elements. When evaluating postmortem cases of suspected elemental poisoning, the investigator is often limited to antemortem population studies describing normal conditions [15–19] or postmortem case reports of intoxications. Since it can be suspected that elemental concentrations can change postmortem, a missing piece of the investigative puzzle is postmortem normal elemental concentrations.

The present study aims to provide postmortem normal reference concentrations for 68 elements in both whole blood and urine. Furthermore, as devices used for sampling and storage as well as additives can contaminate the samples [20], a thorough analysis of possible sources of contamination was also done.

Material and methods

Study population and sample selection

In Sweden, the National Board of Forensic Medicine performs around 5500 forensic autopsies divided among the six regional divisions of forensic medicine each year. The regional divisions of forensic medicine are located from Umeå in the north to Lund in the south and are responsible for performing forensic autopsies on cases originating in their regional area. In Sweden, deaths are to be reported to the police whenever there is a suspicion of crime and/or when an external cause of death cannot be dismissed. The police, in turn, refer these cases to the National Board of Forensic Medicine to perform a forensic autopsy. In general, this means that the National Board of Forensic Medicine handles deaths by suspected substance abuse, suicide, transportation and traffic accidents, medical malpractice, unknown identity, and other deaths with unclear circumstances as well as suspected homicide cases.

At autopsy, whole blood (from the femoral vein) and urine samples are collected for forensic toxicological analysis. All samples are then transported from the regional divisions of Forensic Medicine to the Department of Forensic Genetics and Toxicology in Linköping under refrigerated conditions (duration normally 1–2 days) and stored at + 4 °C until analysis. After analysis, the samples are again stored at + 4 °C. Approximately 3–5 months after analysis, the samples are moved to frozen storage (− 20 °C). After 1 year of storage, samples are disposed, unless the forensic pathologist has requested extended storage time.

There is no clear definition as to what constitutes a "normal postmortem population." As the present study aims to provide normal reference concentrations for elements, any included cases must be selected with consideration to rule out death by overexposure to these same elements. This study only includes cases autopsied during 2020–2021 that the forensic pathologist had classified as death by suicidal hanging. As per recommendation of the Clinical Laboratory and Standards Institute (CLSI), 120 cases were selected as a basis for the reference concentrations [21, 22]. To adjust for potential regional differences in elemental exposure, 20 cases were selected from each of the six regional divisions of forensic medicine. Lastly, to adjust for potential differences between refrigerated and frozen samples, the 20 cases from each division consisted of a mix of both. Cases were only included if the deceased was over 18 years old and if both femoral blood and urine were available in a sufficient amount (> 4 mL each).

Selection of sample tubes and additives

As contamination can affect the results of elemental analysis [20], it is important to quantify potential contamination as a source of error. While there exist specialized tubes for trace element analysis, these are not used in routine forensic practice in Sweden. Since requests for elemental analysis are relatively rare, routine use of specialized tubes have been deemed as not feasible in standard casework. In addition, suspicion of elemental poisoning is often raised late in the investigative process when samples have already been collected using regular tubes. As a result, the tested tubes in this study reflect those used in routine casework.

The National Board of Forensic Medicine uses the following tubes for blood and urine samples: Thermo Scientific™ S40304 10-mL transport tube, Thermo Scientific™ Nunc™ 348224 10-mL polystyrene centrifuge tube, Thermo Scientific™ Nunc™ 347856 11-mL polystyrene centrifuge tube, and Thermo Scientific™ Nunc™ 363282 25-mL universal container. Additionally, 75 µL of a 66% solution of

potassium fluoride (KF) is added to each 5 mL of sample to inhibit, for example, degradation of substances [23, 24].

For each type of sample tube, three batches were selected, and three tubes from each batch were selected for analysis (a total of 36 tubes). Additionally, three batches, each containing 10 mL of a 66% solution of KF, were sent for analysis.

Experimental

Instrumentation

All measurements of element concentrations were performed using a double-focusing sector field (SF) inductively coupled plasma mass spectrometry (ICP-MS) ELEMENT XR (Thermo Scientific, Bremen, Germany) instrument equipped with an introduction system consisting of a demountable quartz torch with 1.5 mm i.d. sapphire injector, platinum capacitive de-coupling shield, nickel sampler cone, high sensitivity "X-type" skimmer cone and PFA spray chamber with two gas inlet ports (Cetac Technologies, Omaha, NE, USA), micro-concentric PolyPro nebulizer, FAST SD2 auto-sampler (ESI, Perkin-Elmer, Santa Clara, USA) with a six-port valve, and a 1.5-mL sample loop filled and rinsed by vacuum suction. Methane addition to the plasma was used to decrease formation of oxide-based spectral interferences, to improve sensitivity for elements with high first ionization potentials, and to minimize matrix effects [25]**.** A laboratory MARS5 microwave digestion system (CEM Corporation, Matthews, NC, USA) was used for sample digestions.

Chemicals and reagents

Nitric acid (HNO_3, Sigma-Aldrich Chemie Gmbh, Munich, Germany) and hydrogen fluoride (HF, 48%, Merck, Darmstadt, Germany) used in this work were of Suprapur grade. Water used was de-ionized Milli-Q water (Millipore, Bedford, MA, USA) purified by reverse osmosis followed by ion-exchange cartridges. For dilution of sample digest, water was further purified by sub-boiling distillation in Teflon stills. For quality control (QC)/quality assurance (QA), a set of matrix-matched lyophilized test samples (trace elements in urine and trace elements in whole blood, SERO AS, Billingstad, Norway) were used.

Sample preparation and analysis

Sample preparation was performed in Class 10, 000 clean laboratory areas by personnel wearing clean room attire. General precautions detailed by Rodushkin et al. [26] were taken to minimize contamination. Laboratory materials used during sample preparation were soaked in 0.7 M nitric acid for 24 h at room temperature and rinsed with de-ionized Milli-Q water prior to use. A 0.5 mL sample volume was added into Teflon vials followed by addition of 1 mL HNO_3 and 0.01 mL HF. Vials (up to 40 per batch) were capped and placed on a carousel with numbered slots. The carousel was placed in a microwave digestion system, and a pre-programmed digestion cycle (30 min ramp to 170 °C followed by a 30-min holding time at that temperature) was initiated. Method blanks and test materials for QC were prepared with each batch of samples.

Digests were diluted to 10 mL with water (providing a total digestion factor of 20 v/v), and concentrations of the 68 elements were determined by ICP-SFMS. Matrix effect correction was accomplished by internal standardization (indium added to all measurement solutions at a concentration of 2.5 µg/L), and quantification was done by external calibration with synthetic, concentration-matched, standards. Potential contamination from sampling equipment was evaluated by leaching test with weak acid at room temperature, as discussed in detail by Rodushkin and Ödman [20]. The operation conditions and measured parameters are summarized in supplementary material, Table 1. Further details on the figures of merit of the method can be found elsewhere [27].

Quality control (QC) and quality assurance (QA) procedures

The summary of performance for the analytical method is compiled in Table 1.

The limits of detection (LOD) and the limits of quantification (LOQ) were calculated as three respective ten times the standard deviation for element concentrations detected in preparation blanks ($n > 15$) and varied from below 0.01 µg/L for ultra-trace elements, e.g. rhenium (Re), up to mg/L for major inorganic constituents.

The accuracy of the data was assessed by analyses of test samples (urine and whole blood reference materials from SERO AS) with ICP-SFMS. Results were within 10% RSD range from target, information, or target values for the majority of analytes presented in these samples above respective LODs. Method reproducibility was evaluated from replicate preparation/analysis of test samples and specimens prepared and analyzed in duplicate and as a rule was better than 10% RSD for elements presented in tested matrixes at concentrations above respective LODs.

It should be noted that because of numerous unresolved spectral interferences affecting isotopes of some ultra-trace elements (germanium (Ge), osmium (Os), palladium (Pd), rhodium (Rh), ruthenium (Ru)), accuracy of analytical results (though subjected to mathematical corrections) for these analytes can be affected and should be treated with caution [28].

Table 1 Figures of merit for the analytical method

		LOD[a]/LOQ[b], µg/L	Recovery, urine[c] (SD, $n=4$), %	Recovery, blood[d] (SD, $n=4$), %	Precision, urine[e] %	Precision, blood[e] %
Ag	(Silver)	0.006/0.02	94 (2)	100 (1)	11	10
Al	(Aluminum)	1/3	98 (3)	101 (1)	5	24
As	(Arsenic)	0.04/0.15	96 (3)	101 (3)	3	6
Au	(Gold)	0.006/0.02	97 (1)	NA[f]	16	10
B	(Boron)	4/12	94 (3)	101 (1)	3	2
Ba	(Barium)	0.3/0.9	101 (3)	101 (2)	1	3
Be	(Beryllium)	0.04/0.14	99 (1)	102 (2)	NA[g]	NA[g]
Bi	(Bismuth)	0.005/0.016	105 (2)	100 (2)	13	11
Br	(Bromine)	80/240	98 (20)	111 (2)	4	4
Ca	(Calcium)	60/200	97 (2)	101 (2)	3	2
Cd	(Cadmium)	0.006/0.02	105 (2)	101 (1)	3	1
Ce	(Cerium)	0.02/0.07	95 (3)	99 (9)	7	6
Co	(Cobalt)	0.02/0.05	101 (3)	101 (3)	14	12
Cr	(Chromium)	0.09/0.3	96 (3)	104 (2)	8	3
Cs	(Cesium)	0.01/0.04	90 (1)	103 (3)	2	1
Cu	(Copper)	0.6/2	99 (1)	101 (1)	4	1
Dy	(Dysprosium)	0.003/0.008	96 (5)	118 (7)	NA[g]	NA[g]
Er	(Erbium)	0.005/0.02	94 (2)	118 (14)	22	NA[g]
Eu	(Europium)	0.008/0.03	96 (2)	NA[f]	NA[g]	NA[g]
Fe	(Iron)	14/50	95 (12)	103 (1)	4	2
Ga	(Gallium)	0.01/0.04	92 (2)	81 (7)	63	7
Gd	(Gadolinium)	0.005/0.02	92 (3)	113 (16)	20	11
Ge	(Germanium)	0.2/0.8	103 (6)	NA[f]	21	18
Hf	(Hafnium)	0.009/0.03	98 (2)	NA[f]	3	6
Hg	(Mercury)	0.1/0.4	99 (3)	102 (1)	9	3
Ho	(Holmium)	0.002/0.005	93 (2)	NA[f]	NA[g]	NA[g]
I	(Iodine)	30/100	113 (20)	125 (6)	10	2
Ir	(Iridium)	0.004/0.01	105 (5)	NA[f]	18	19
La	(Lanthanum)	0.01/0.03	93 (1)	110 (6)	22	16
Li	(Lithium)	0.04/0.14	95 (1)	111 (4)	3	3
Lu	(Lutetium)	0.004/0.01	92(2)	NA[f]	NA[g]	NA[g]
Mg	(Magnesium)	14/50	98 (7)	97 (1)	5	1
Mn	(Manganase)	0.15/0.5	96 (5)	103 (1)	3	3
Mo	(Molybdenum)	0.03/0.1	102 (2)	98 (2)	2	4
Nb	(Niobium)	0.006/0.02	100 (4)	86 (8)	9	3
Nd	(Neodymium)	0.01/0.04	95 (1)	105 (7)	10	9
Ni	(Nickel)	0.3/1	99 (3)	103 (1)	6	14
Os	(Osmium)	0.003/0.01	NA[f]	NA[f]	NA[g]	NA[g]

Table 1 (continued)

		LOD[a]/LOQ[b], µg/L	Recovery, urine[c] (SD, $n=4$), %	Recovery, blood[d] (SD, $n=4$), %	Precision, urine[e] %	Precision, blood[e] %
Pb	(Lead)	0.06/0.2	103 (2)	103 (1)	6	2
Pd	(Palladium)	0.02/0.05	81 (8)	NA[f]	7	NA[g]
Pr	(Praseodymium)	0.004/0.01	93 (1)	121 (11)	27	18
Pt	(Platinum)	0.008/0.03	100 (1)	78 (14)	14	15
Rb	(Rubium)	0.07/0.2	103 (2)	97 (1)	3	1
Re	(Rhenium)	0.001/0.003	100 (2)	68 (10)	3	23
Rh	(Rhodium)	0.004/0.01	95 (1)	NA[f]	NA[g]	NA[g]
Ru	(Ruthenium)	0.01/0.04	97 (2)	NA[f]	NA[g]	NA[g]
S	(Sulfur)	400/1300	96 (1)	89 (2)	4	1
Sb	(Antimony)	0.008/0.03	98 (3)	97 (2)	1	7
Sc	(Scandium)	0.02/0.07	89 (2)	NA[f]	NA[g]	NA[g]
Se	(Selenium)	0.6/2	101 (4)	97 (1)	12	2
Si	(Silicon)	200/700	NA[f]	NA[f]	3	7
Sm	(Samarium)	0.007/0.02	96 (2)	107 (9)	12	NA[g]
Sn	(Tin)	0.05/0.2	97 (1)	97 (2)	4	8
Sr	(Strontium)	0.1/0.4	90 (1)	102 (1)	18	4
Ta	(Tantalum)	0.003/0.02	124 (7)	NA[f]	52	15
Tb	(Terbium)	0.006/0.02	93 (3)	102 (12)	NA[g]	NA[g]
Te	(Tellurium)	0.03/0.09	107 (9)	NA[f]	12	9
Th	(Thorium)	0.008/0.03	81 (5)	111 (9)	NA[g]	12
Ti	(Titanium)	0.1/0.4	92 (9)	105 (2)	13	13
Tl	(Thallium)	0.001/0.005	100 (2)	102 (1)	5	3
Tm	(Thulium)	0.001/0.004	94 (1)	NA[f]	NA[g]	NA[g]
U	(Uranium)	0.003/0.009	98 (2)	104 (2)	9	12
W	(Tungsten)	0.02/0.08	105 (4)	146 (19)	3	8
V	(Vanadium)	0.02/0.07	94 (1)	104 (2)	15	NA[g]
Y	(Yttrium)	0.03/0.1	95 (2)	142 (20)	5	7
Yb	(Ytterbium)	0.004/0.01	95 (3)	NA[f]	NA[g]	NA[g]
Zn	(Zinc)	1/4	93 (1)	99 (2)	1	1
Zr	(Zirconium)	0.02/0.07	96 (5)	111 (6)	10	17

[a]Limit of detection calculated as three times the standard deviation of 16 preparation blanks from eight digestion batches analyzed in two instrumental sequences

[b]Limit of quantification calculated as ten times the standard deviation of 16 preparation blanks from eight digestion batches analyzed in two instrumental sequences

[c]Calculated as mean ratio of measured concentrations to target/information or spiked concentration of analyte for trace elements in urine test sample from SERO AS (batch 1403081) spiked upon reconstitution at 5 µg/L with Ag, Au, Ce, Dy, Er, Eu, Ga, Gd, Ge, Hf, Ho, Ir, La, Lu, Nb, Nd, Pd, Pr, Pt, Re, Rh, Ru, Sc, Sm, Ta, Tb, Th, Tm, U, W, Y, Yb, and Zr

[d]Calculated as mean ratio of measured concentrations to target or information concentration of analyte for trace elements in whole blood test sample from SERO AS (batch 1702825)

[e]Calculated as the relative standard deviation for concentration obtained in eight specimens prepared and analyzed as duplicates (results below respective LOD are omitted)

[f]Recovery estimate is unavailable because of analyte concentrations below respective LOD or target/information/spike concentrations are absent

[g]Precision estimate is unavailable because of analyte concentrations below respective LOD in all duplicates

Statistical analysis

Data management and descriptive statistics were done using the Python programming language together with the packages NumPy, SciPy and Pandas [29–32].

Concentrations of elements measured in blood and urine are expressed in µg/L. No elemental outlier values were excluded. Concentrations below LOD were replaced with the value (LOD/2). Due to dilution, LOD for the leaching tests were lower (LOD/20) compared to the blood and urine samples. Concentrations below this lower LOD were replaced with half the adjusted LOD value.

For each element, the following descriptive statistics are presented: the frequency of values below LOD, the arithmetic mean (AM), and the geometric mean (GM), median, 75th, 90th, 95th, and 97.5th percentiles, and maximum values. For elements in which the concentration in most cases (> 50%) were below the LOD, only percentiles are presented. All results are presented with at most three significant figures.

Comparisons between refrigerated and frozen samples were tested for significance using the Mann–Whitney *U* test. The confidence level was adjusted for multiple comparisons ($n = 68$, the number of included elements) using the Bonferroni correction.

For the purity tests, the mean and median concentrations for all elements are presented for both tubes and the 66% KF solution. The mean and median value across all analyzed batches were used. However, only 75 µL of KF solution was added to each 5 mL sample. Therefore, an adjusted concentration was calculated to represent the mean additional concentration added to a 5 mL blood or urine sample according to the following formula:

$$\textit{Added mean concentration in } \mu g/L = \left(\textit{Mean KF concentration in } \mu g/L \times \frac{75}{10^6}\right) \times \frac{10^3}{5}$$

The same formula was used to calculate the added median concentration to a 5 mL blood or urine sample (replacing mean concentrations with median concentrations).

Results

Study population

The study population consisted of 120 individuals, of which 85% were male. The demographics of the study population are presented in Table 2. The majority of the samples had been stored in frozen conditions at the time of analysis ($n = 78$, 65%), and the rest had been stored in refrigerated conditions ($n = 42$, 35%).

Elemental blood and urine concentrations

Concentrations of 68 elements in blood and urine are presented in Table 3. Most elements could be detected in both blood and urine. However, for erbium (Er), holmium (Ho), lutetium (Lu), scandium (Sc), samarium (Sm), thorium (Th), thulium (Tm), and ytterbium (Yb), over 90% of the samples have concentrations below the LOD in either blood, urine, or both. In the case of europium (Eu), rhodium (Rh), and terbium (Tb), there were no concentrations above the LOD.

It must also be considered that the arithmetic mean is prone to distortion in the presence of outliers. The average ratio between the arithmetic mean and the median, across all elements where the mean was calculated (e.g., elements for which less than 60 cases were below LOD), was 13.3 in blood and 17.0 in urine. This indicates the presence of high impact outliers skewing the mean. In both blood and urine samples, antimony (Sb) stands out with extremely high ratios of 478 and 440 for blood and urine, respectively. In the case of antimony (Sb), 115 out of 120 concentrations were < 0.75 µg/L. However, the remaining five cases all had concentrations above 150 µg/L. A similar, but not as extreme, pattern exists for other elements, such as the blood and urine concentrations of zirconium (Zr) (ratio 59 and 128, respectively). Excluding antimony (Sb) and zirconium (Zr), the average ratio between the arithmetic mean and the median drops to 1.9 in blood and 4.9 in urine.

Purity tests

The mean and median concentrations of the leaching tests of the sample tubes (aggregated) and the KF solution (aggregated) can be found in supplementary material, Table 2a and 2b, respectively. The tables also show comparisons to the arithmetic mean and median of the blood and urine concentrations for each element.

Most of the sample tubes ($n = 30$) had a majority (> 50%) of the leaching test concentrations below LOD. Only eight elements, aluminum (Al), boron (B), barium (Ba), beryllium (Be), cadmium (Cd), europium (Eu), lithium (Li), and rhodium (Rh), had no concentrations below LOD. In general, the arithmetic mean of the leaching test concentrations was low when compared to both the arithmetic mean

of the blood samples and urine samples for each element. The ratio between the arithmetic mean of the leaching test concentration and the blood sample concentration was > 0.1 for rhodium (Rh), europium (Eu), beryllium (Be), and ruthenium (Ru). Compared with the urine samples, with arithmetic mean ratios > 0.1 for rhodium (Rh), europium (Eu), and beryllium (Be). For most elements, the ratios when compared to the blood and urine sample arithmetic means were very low, and thus, the contribution from sample tubes can be neglected.

In general, the 66% KF solution added to the blood and urine samples proved to contain a wide range of elements. Table 2b of supplementary material shows the mean and median concentrations across all elements. The table also contains the added concentration of each element that the KF solution contributes to the overall sample (see calculation in "Statistical analysis" section). The overall ratio of the added concentrations to the overall sample arithmetic means was 1.42 for blood and 1.16 for urine. However, vanadium (V) is an extreme outlier with a ratio of 65 and 50 in blood and urine, respectively. Excluding vanadium (V), the ratios are 0.48 and 0.43 for blood and urine, respectively. Of special note is that for vanadium (V), arsenic (As), tin (Sn), lead (Pd), thorium (Th), barium (Ba), tantalum (Ta), ruthenium (Ru), and chromium (Cr), the ratios were > 1 in blood. In urine, the ratios for vanadium (V), arsenic (As), tin (Sn), thorium (Th), tellurium (Te), and barium (Ba) were > 1. However, for most substances ($n = 45$ and $n = 44$ for blood and urine, respectively), the ratio of means was relatively low (< 0.25).

Comparison between refrigerated and frozen samples

Statistical comparisons between refrigerated and frozen samples for all elements are presented in supplementary materials, Table 3. For most elements, there was no significant difference between refrigerated and frozen samples. However, at a significance level of 0.05, there were significant differences in blood and urine samples for 23 and 17 elements, respectively. After adjustment for multiple comparisons (resulting in an adjusted significance level of 0.00074), eleven elements in blood (gold (Au), bismuth (Bi), cerium (Ce), iron (Fe), iodine (I), magnesium (Mg), platinum (Pt), sulfur (S), silicon (Si), tungsten (W), and yttrium (Y)) and seven elements in urine (barium (Ba), cerium (Ce), lanthanum (La), lithium (Li), magnesium (Mg), strontium (Sr), and yttrium (Y)) retained significance. Of the aforementioned elements, six elements in blood (platinum (Pt), iodine (I), silicon (Si), iron (Fe), magnesium (Mg), and sulfur (S)) showed higher means in the refrigerated samples. For the seven urine elements, all means were higher in the refrigerated samples.

The differences between refrigerated and frozen samples were also compared to the overall arithmetic mean and median for all samples (i.e., all blood samples and all urine samples for a given element). For the blood samples, the largest differences for elements that retained significance after Bonferroni correction were cerium (Ce) and yttrium (Y), in which the ratio between the difference of means in the refrigerated and frozen samples and the whole sample arithmetic mean was 0.72 and 0.5, respectively. For urine samples, the largest ratios were 2.81 and 1.28 for lithium (Li) and barium (Ba), respectively. However, lithium (Li) has extremely high maximum values for both blood and urine (see Table 3) that distorts the arithmetic mean. Disregarding lithium (Li), strontium (Sr) has the highest ratio in urine together with barium (Ba).

Discussion

This work presents postmortem blood and urine concentrations of 68 elements in blood and urine for 120 suicidal hangings aiming to categorize a normal postmortem concentration range. Additionally, the study provides data on possible sources of contamination and variation.

Comparison with antemortem population data

There exist several large studies of antemortem blood and/or urine concentrations of elements, which include participants from France ($n = 1992$) [15], Germany ($n = 130$ and $n = 103$, respectively) [16, 17], and Italy ($n = 5$–959, variable depending on element and matrix) [18]. The studies show that the amounts of naturally occurring elements can differ by region and, therefore, can be found in different populations to different extents. We know of one study by Rodushkin et al. [19] that has presented extensive element concentrations in both blood and urine in a small ($n = 12$) Swedish population. Table 4 shows a comparison for the subset of elements present above LOD/LOQ in all the above studies (and either of the German studies).

It can be noted that the older study by Minoia et al. [18] shows, in general, higher concentrations than the other antemortem studies, which indicates that large variations can be present in different antemortem populations. The degree of elemental exposure in the living population in a region can therefore offer additional insight when evaluating postmortem elemental concentrations.

If we limit the comparison to the Swedish study by Rodushkin et al. [19], and substances with a majority of detections above LOD, it can be noted that several elements in blood (cadmium (Cd), cobalt (Co), germanium (Ge), iridium (Ir), lanthanum (La), antimony (Sb) and ytterium (Y)) show postmortem arithmetic mean concentrations higher than antemortem concentrations by a factor of at least five.

Across all substances present in both the current study and the study by Rodushkin et al., the postmortem arithmetic mean concentrations are on average 80% higher than the antemortem concentrations.

In summary it can be concluded that for many substances, there are large differences between the postmortem samples and the antemortem population data. It must be noted that there are differences in sample collection. The antemortem studies have used special sample tubes for element analysis, while the present study has opted to use a selection of regular sample tubes. While our results indicate relatively minor impact of the sample tubes (see "Purity tests" section), it cannot be ruled out that it has affected some elements [20]. Furthermore, our results indicate that postmortem redistribution plays an important role for elements and must be considered during evaluation.

Comparison with postmortem population data

As noted in the introduction, there is a general lack of larger compilations of elemental concentrations in postmortem populations. However, a recently published study by Issa et al. [33] provides concentration data from a large ($n=400$) autopsy material. Issa et al. analyzed both femoral and cardiac blood and several tissues for eight elements. Interestingly, there are marked differences between cardiac and femoral blood for several elements (such as cadmium (Cd), lead (Pb), and mercury (As)) suggesting postmortem redistribution. Additionally, they have studied the impact of putrefaction noting significant differences for cadmium (Cd) and zinc (Zn).

A comparison between femoral blood concentrations in the current study and the study by Issa et al. is presented in Table 5. It can be noted that while concentrations of several elements are relatively similar, there are also marked differences. Zinc (Zn) shows much higher concentrations in our material, while the concentrations of silver (Ag) are much lower. Regarding zinc (Zn), this might be due to regional factors as our blood concentrations are comparable with European population data [15, 17]. The antemortem population concentrations of zinc (Zn) presented in the study by Issa et al. are lower. Similar differences can be seen for silver (Ag).

Table 2 Demographics of the study population

		n	%
Sex			
	Male	102	85
	Female	18	15
Age (years)			
	18–30	38	32
	30–60	56	47
	60+	26	22
BMI (kg/m^2)			
	< 18.5	7	6
	≥ 18.5–25	71	59
	> 25–30	25	21
	> 30	17	14

Impact of sample tubes, KF solution, and storage conditions

Regarding the influence of the tubes in which blood and urine samples were collected and stored, the impact compared to the overall mean of the blood and urine concentrations seems limited. However, as stated by Rodushkin et al. [20] in their contamination study, the results from leaching tests would be inappropriate to use for correction of the blood or urine concentrations since blood or urine can be expected to affect the tubes differently than water or dilute acid. In addition, the leaching tests do not take storage time into account so the impact of sample tubes could be increased with increased storage time.

Testing showed that the 66% KF solution contained high concentrations of several elements, as can be seen in supplementary materials, Table 2b. When adjusted for the very low amount of solution added to an average blood and urine sample, the impact of this addition still seems substantial for a subset of elements. Ratios above 1 are hard to explain since they signify that the concentrations in the blood and urine samples are less than what should be added by the addition of the KF solution. One possible explanation for these, and other high ratios, is that the KF solution is itself prone to contamination either during preparation or during storage. This would imply that the contribution of the KF solution could be variable over time. Some signs of this have been seen in the different batches analyzed (e.g., one batch had an arsenic (As) concentration of 4950 µg/L, while the two subsequent batches had concentrations close to 1700 µg/L). Other possible explanations could be that there is incomplete mixing of the blood and urine samples with the KF solution or that there exists some imprecision in how much solution is added to samples. While further investigation of this variability has not been possible to evaluate in the present study, it can be concluded that it is of great importance to consider the impact of additives when evaluating postmortem elemental concentrations.

For a subset of both urine and blood samples, there were significant differences based on storage conditions. For urine samples, even at a significance level of 0.05, all but three of the significant elements (thallium (Tl), iron (Fe), and zirconium (Zr)) showed higher mean concentrations in the refrigerated samples. However, there was no general trend for blood samples as refrigerated samples showed both increased and decreased means compared to frozen samples for different elements. In addition, for most elements, both urine and blood samples with significant differences showed the same direction of change (i.e., blood and urine samples

Table 3 Concentrations of 68 elements in postmortem femoral blood and urine (μg/L)

Element	Source[a]	$n <$ LOD	AM[b]	GM[c]	Median	75th percentile	90th percentile	95th percentile	97.5th percentile	Max
Ag	Blood	1	0.08	0.048	0.047	0.103	0.181	0.255	0.322	0.534
(Silver)	Urine	54	0.026	0.009	0.008	0.016	0.036	0.114	0.230	0.408
Al	Blood	10	10.6	4.18	3.86	7.97	16.9	40.7	60.2	273
(Aluminum)	Urine	52	8.25	1.71	1.39	3.84	10.8	28.3	47.4	354
As[d]	Blood	0	4.25	2.34	2.25	4.98	9.36	12.5	16.3	50.4
(Arsenic)	Urine	0	11.1	3.61	3.28	8.48	17.6	69.9	98.3	135
Au	Blood	3	0.042	0.013	0.011	0.014	0.021	0.044	0.187	1.89
(Gold)	Urine	34	0.01	0.007	0.008	0.01	0.014	0.022	0.049	0.198
B	Blood	0	80.9	45.3	36.4	49.5	98.3	274	715	1100
(Boron)	Urine	0	484	325	345	601	915	1350	1670	4590
Ba	Blood	2	1.65	1.05	0.916	1.56	3.02	6.19	9.02	11.8
(Barium)	Urine	65	-	-	0.141	0.633	2.28	3.14	6.44	85.7
Be	Blood	119	-	-	0.02	0.02	0.02	0.02	0.02	0.246
(Beryllium)	Urine	117	-	-	0.02	0.02	0.02	0.02	0.02	0.061
Bi	Blood	17	0.016	0.01	0.009	0.013	0.024	0.073	0.14	0.156
(Bismuth)	Urine	45	0.01	0.006	0.006	0.008	0.018	0.044	0.068	0.1
Br	Blood	0	1260	1190	1130	1460	1820	2120	2370	3580
(Bromine)	Urine	0	1700	1540	1580	2030	2690	2950	3310	5020
Ca	Blood	0	29800	26700	28300	38700	48200	54500	59100	71300
(Calcium)	Urine	7	19900	1920	1280	12800	59600	99400	139000	416000
Cd	Blood	0	21.2	6.14	5.92	16.7	38.3	68.5	81.1	655
(Cadmium)	Urine	0	2.49	0.795	0.727	1.94	4.62	9.02	14.1	79.9
Ce	Blood	18	0.19	0.056	0.044	0.105	0.259	0.54	1.33	4.66
(Cerium)	Urine	82	-	-	0.011	0.024	0.041	0.064	0.092	0.293
Co	Blood	0	0.636	0.343	0.277	0.505	0.93	1.59	3.79	16.0
(Cobalt)	Urine	0	0.566	0.309	0.312	0.717	1.25	2.04	3.03	4.22
Cr	Blood	0	2.45	0.633	0.473	1.25	2.75	5.71	7.25	86.4
(Chromium)	Urine	0	0.634	0.374	0.294	0.508	1.25	1.76	2.16	14.0
Cs	Blood	0	9.37	8.30	8.01	10.5	13.6	14.4	19.8	83.4
(Cesium)	Urine	0	8.84	7.54	7.77	10.2	13.3	13.8	15.0	91.0
Cu	Blood	0	684	673	665	733	810	954	1070	1190
(Copper)	Urine	0	75.5	45.0	39.0	101	182	232	273	571
Dy	Blood	104	-	-	0.001	0.001	0.003	0.006	0.018	0.047
(Dysprosium)	Urine	102	-	-	0.001	0.001	0.004	0.005	0.011	0.038
Er	Blood	113	-	-	0.003	0.003	0.003	0.015	0.233	0.478
(Erbium)	Urine	110	-	-	0.003	0.003	0.003	0.023	0.083	0.287
Eu	Blood	120	-	-	0.004	0.004	0.004	0.004	0.004	0.004

Table 3 (continued)

Element	Source[a]	$n < LOD$	AM[b]	GM[c]	Median	75th percentile	90th percentile	95th percentile	97.5th percentile	Max
(Europium)	Urine	120	-	-	0.004	0.004	0.004	0.004	0.004	0.004
Fe	Blood	0	756000	748000	751000	817000	884000	930000	950000	1030000
(Iron)	Urine	1	819	279	290	623	1440	3310	5410	15500
Ga	Blood	5	0.055	0.047	0.052	0.070	0.086	0.101	0.110	0.264
(Gallium)	Urine	73	-	-	0.006	0.021	0.050	0.078	0.117	0.390
Gd	Blood	92	-	-	0.003	0.003	0.04	0.336	0.623	1.71
(Gadolinium)	Urine	92	-	-	0.003	0.003	0.04	0.119	0.911	34.2
Ge[e]	Blood	0	1.51	1.48	1.50	1.68	1.83	1.93	2.18	3.13
(Germanium)	Urine	7	0.687	0.607	0.693	0.868	1.10	1.15	1.23	1.40
Hf	Blood	95	-	-	0.004	0.004	0.017	0.025	3.97	8.34
(Hafnium)	Urine	101	-	-	0.004	0.004	0.071	0.516	2.58	11.3
Hg	Blood	0	1.26	0.825	0.813	1.30	2.34	3.74	5.03	14.2
(Mercury)	Urine	28	0.272	0.181	0.181	0.271	0.515	0.881	1.29	2.19
Ho	Blood	113	-	-	0.001	0.001	0.001	0.002	0.004	0.015
(Holmium)	Urine	115	-	-	0.001	0.001	0.001	0.001	0.004	0.011
I	Blood	8	53.5	44.9	42.2	51.7	77.7	95.8	178	434
(Iodine)	Urine	1	94.3	82.5	81.9	108	161	184	205	555
Ir	Blood	48	0.004	0.004	0.004	0.005	0.006	0.007	0.008	0.019
(Iridium)	Urine	51	0.004	0.003	0.004	0.005	0.007	0.007	0.008	0.010
La	Blood	0	0.159	0.057	0.04	0.093	0.207	0.586	1.22	3.10
(Lanthanum)	Urine	61	-	-	0.005	0.022	0.054	0.074	0.138	0.27
Li	Blood	82	-	-	0.217	0.598	1.40	2.27	2.70	2110
(Lithium)	Urine	9	526.1	5.87	7.53	9.66	13.0	16.2	19.0	62200
Lu	Blood	110	-	-	0.002	0.002	0.002	0.006	0.021	0.106
(Lutetium)	Urine	115	-	-	0.002	0.002	0.002	0.002	0.011	0.026
Mg	Blood	0	59800	59000	57800	63900	72800	76900	79100	113000
(Magnesium)	Urine	1	15000	1210	798	11300	46400	58200	81200	390000
Mn	Blood	0	23.4	17.9	16.1	23.9	47.2	65.5	76.9	164
(Manganase)	Urine	16	10.6	1.15	1.02	3.11	9.92	16.3	27.0	702
Mo	Blood	0	3.62	1.83	1.38	3.57	8.80	13.9	19.8	50.0
(Molybdenum)	Urine	0	17.5	10.9	11.9	22.5	38.1	52.3	62.7	107
Nb	Blood	0	0.067	0.058	0.053	0.069	0.087	0.115	0.140	0.685
(Niobium)	Urine	3	0.021	0.017	0.015	0.02	0.038	0.056	0.071	0.146
Nd	Blood	78	-	-	0.006	0.018	0.043	0.123	0.19	0.617
(Neodymium)	Urine	100	-	-	0.006	0.006	0.022	0.027	0.070	0.275
Ni	Blood	34	2.15	0.638	0.584	1.22	2.53	4.13	5.95	92.4

Table 3 (continued)

Element	Source[a]	$n < LOD$	AM[b]	GM[c]	Median	75th percentile	90th percentile	95th percentile	97.5th percentile	Max
(Nickel)	Urine	18	2.97	1.36	1.48	3.42	7.36	10.5	17.8	24.3
Os[e]	Blood	110	-	-	0.002	0.002	0.002	0.003	0.004	0.007
(Osmium)	Urine	102	-	-	0.002	0.002	0.004	0.004	0.005	0.005
Pb	Blood	0	20.0	16.6	15.8	25.2	34.6	44.1	56.8	88.6
(Lead)	Urine	1	0.33	0.248	0.228	0.414	0.572	0.846	1.21	2.64
Pd[e]	Blood	120	-	-	0.008	0.008	0.008	0.008	0.008	0.008
(Palladium)	Urine	106	-	-	0.008	0.008	0.017	0.027	0.038	0.13
Pr	Blood	82	-	-	0.002	0.005	0.011	0.02	0.056	0.263
(Praseodymium)	Urine	92	-	-	0.002	0.002	0.006	0.008	0.008	0.031
Pt	Blood	80	-	-	0.004	0.009	0.011	0.013	0.016	0.022
(Platinum)	Urine	99	-	-	0.004	0.004	0.01	0.011	0.012	0.024
Rb	Blood	0	4200	4120	4150	46200	5440	5790	5990	7210
(Rubium)	Urine	0	2492	2300	2370	3190	3829	4090	4240	4750
Re	Blood	63	-	-	0	0.002	0.002	0.002	0.003	0.011
(Rhenium)	Urine	3	0.008	0.005	0.005	0.01	0.015	0.021	0.024	0.063
Rh[e]	Blood	120	-	-	0.006	0.006	0.006	0.006	0.006	0.006
(Rhodium)	Urine	120	-	-	0.006	0.006	0.006	0.006	0.006	0.006
Ru[e]	Blood	88	-	-	0.006	0.011	0.014	0.018	0.020	0.028
(Ruthenium)	Urine	3	0.023	0.022	0.023	0.027	0.03	0.034	0.037	0.047
S	Blood	0	1940000	1930000	1940000	2040000	2140000	2240000	2280000	2430000
(Sulfur)	Urine	0	448000	385000	406000	610000	773000	867000	946000	1000000
Sb	Blood	0	14.8	0.052	0.031	0.049	0.12	0.662	333	503
(Antimony)	Urine	0	29.5	0.154	0.067	0.119	154	236	340	530
Sc	Blood	113	-	-	0.01	0.01	0.01	0.02	0.18	0.423
(Scandium)	Urine	109	-	-	0.01	0.01	0.01	0.036	0.13	0.616
Se	Blood	0	123	120	121	135	158	169	186	274
(Selenium)	Urine	0	17.2	13.2	14.4	22.4	35.0	45.6	47.5	55.8
Si	Blood	0	1050	854	834	1050	1450	1700	4710	10800
(Silicon)	Urine	0	3160	2210	1950	3630	6970	10800	13800	18500
Sm	Blood	115	-	-	0.004	0.004	0.004	0.004	0.011	0.023
(Samarium)	Urine	117	-	-	0.004	0.004	0.004	0.004	0.004	0.018
Sn	Blood	0	0.535	0.287	0.249	0.400	0.569	0.944	1.44	22.4
(Tin)	Urine	0	0.660	0.479	0.435	0.762	1.16	1.71	2.14	7.04
Sr	Blood	0	9.18	7.73	7.93	11.1	16.0	20.3	24.1	36.2
(Strontium)	Urine	7	15.4	1.86	1.26	9.87	37.2	69.9	97.5	431
Ta	Blood	21	0.005	0.004	0.004	0.005	0.008	0.012	0.015	0.032

Table 3 (continued)

Element	Source[a]	$n < LOD$	AM[b]	GM[c]	Median	75th percentile	90th percentile	95th percentile	97.5th percentile	Max
(Tantalum)	Urine	14	0.014	0.005	0.004	0.006	0.014	0.045	0.075	0.669
Tb	Blood	120	-	-	0.003	0.003	0.003	0.003	0.003	0.003
(Terbium)	Urine	120	-	-	0.003	0.003	0.003	0.003	0.003	0.003
Te	Blood	31	0.029	0.027	0.03	0.035	0.04	0.042	0.046	0.066
(Tellurium)	Urine	105	-	-	0.014	0.014	0.029	0.03	0.036	0.05
Th	Blood	107	-	-	0.004	0.004	0.009	0.013	0.034	0.123
(Thorium)	Urine	112	-	-	0.004	0.004	0.004	0.009	0.021	0.059
Ti	Blood	0	1.05	0.616	0.545	0.766	1.27	5.48	7.35	12.6
(Titanium)	Urine	11	1.45	0.420	0.342	0.574	1.50	5.34	7.09	49.2
Tl	Blood	0	0.135	0.123	0.124	0.162	0.198	0.255	0.271	0.538
(Thallium)	Urine	0	0.103	0.077	0.087	0.138	0.21	0.227	0.31	0.336
Tm	Blood	115	-	-	0.001	0.001	0.001	0.001	0.005	0.011
(Thulium)	Urine	116	-	-	0.001	0.001	0.001	0.001	0.002	0.007
U	Blood	43	0.03	0.005	0.003	0.008	0.071	0.17	0.197	0.801
(Uranium)	Urine	51	0.069	0.007	0.004	0.025	0.072	0.118	0.209	4.47
W	Blood	9	0.064	0.045	0.043	0.057	0.088	0.115	0.24	1.13
(Tungsten)	Urine	12	0.074	0.056	0.058	0.094	0.133	0.174	0.199	0.568
V	Blood	70	-	-	0.011	0.045	0.115	0.216	0.328	0.827
(Vanadium)	Urine	51	0.07	0.032	0.027	0.076	0.16	0.26	0.409	0.662
Y	Blood	6	0.141	0.119	0.136	0.159	0.178	0.231	0.411	0.666
(Yttrium)	Urine	1	0.094	0.084	0.079	0.117	0.158	0.182	0.192	0.407
Yb	Blood	110	-	-	0.002	0.002	0.002	0.006	0.03	1.27
(Ytterbium)	Urine	110	-	-	0.002	0.002	0.002	0.009	0.015	0.113
Zn	Blood	0	9750	96000	9570	10900	12100	12500	12800	13900
(Zinc)	Urine	0	3220	12000	1250	2670	7680	11100	21000	53800
Zr	Blood	0	9.05	0.237	0.154	0.230	0.672	1.40	143	357
(Zirconium)	Urine	0	9.87	0.143	0.077	0.150	1.47	18.1	93.4	427

[a]*Blood*, femoral vein blood

[b]*AM*, arithmetic mean

[c]*GM*, geometric mean

[d]Total arsenic (i.e., combining all arsenic species)

[e]Concentrations should be treated with caution, see "Quality control (QC) and quality assurance (QA) procedures" section

Table 4 Comparison of arithmetic mean of blood and urine element concentrations between the current postmortem study and different antemortem studies

Element	Source	Current study (µg/L)	Nisse et al. (2017) [15] (µg/L)	Heitland and Köster (2006) [16] (µg/L)	Heitland and Köster (2021) [17] (µg/L)	Minoia et al. (1990) [18] (µg/L)	Rodushkin et al. (2001) [19] (µg/L)[a]
As	Blood	4.25	2.62	0.93	0.87	7.9	2.35
(Arsenic)	Urine	11.1	37.6	-	10.3	16.7	8.5
Cd	Blood	21.2	0.56	0.57	0.55	0.6	0.32
(Cadmium)	Urine	2.49	0.53	-	0.23	0.86	0.23
Co	Blood	0.64	0.33	0.19	0.16	0.39	0.12
(Cobalt)	Urine	0.57	0.82	-	0.34	0.57	0.43
Cr	Blood	2.45	0.6	-	0.045	0.23	0.33
(Chromium)	Urine	0.634	0.66	-	0.17	0.61	0.17
Hg	Blood	1.26	2.03	1.4	0.65	5.3	3.98
(Mercury)	Urine	0.27	2.02	-	0.25	3.5	2.17
Mn	Blood	23.4	8.09	9	8.9	8.8	5.85
(Magnesium)	Urine	10.6	0.45	-	0.06	1.02	0.15
Ni	Blood	2.15	1.47	0.11	0.4	2.3	0.54
(Nickel)	Urine	2.97	2.72	-	1.35	0.9	1.47
Pb	Blood	20.0	22.8	22	13	157.7	25.5
(Lead)	Urine	0.33	1.5	-	0.46	17	1.51
Tl	Blood	0.14	0.05	0.02	<LOQ	0.39	0.02
(Thallium)	Urine	0.10	0.26	-	0.34	0.42	0.30

[a]Study presents min and max concentrations only. Concentrations in this column reflect the average of the two extremes, with concentrations below LOD set as equal to LOD

both had either higher or lower concentrations in the same storage condition). Surprisingly, for gold (Au) and cerium (Ce), blood and urine samples seem to have behaved differently under storage with blood and urine concentrations changing in reverse directions to each other. The reason for this discrepancy is not clear. Contamination from sample tubes or absorption of elements into a tube during storage should behave similarly in both urine and blood samples.

The changes found regarding storage could be related to the sample tubes, even though the leaching test concentrations were, in general, low. The increased contact time could reasonably impact the concentrations. Further studies with repeat elemental sampling over a sample's entire storage time must be conducted to provide further details. However, at least for a subset of elements, storage time and conditions can be an important factor in interpretation. The relevance of these differences varies depending on the circumstances of each specific case and must be evaluated together with information and findings from the cause of death investigation.

Confounding factors

Apart from the factors discussed in "Impact of sample tubes, KF solution, and storage conditions" section, there are other confounding factors that need to be considered when evaluating elemental concentrations.

It is known that factors such as sex, smoking, dietary habits, and alcohol intake can influence element concentrations [34, 35]. A well-known example is arsenic (As), in which dietary

Table 5 Comparison of arithmetic mean of femoral blood and urine element concentrations between the current study and the study by Issa et al. [33]

Element	Source	Current study (µg/L)	Issa et al. (2022) [33] (µg/L)
Ag	Blood	0.08	1.52
(Silver)	Urine	0.026	2.78
As	Blood	4.25	8.92
(Arsenic)	Urine	11.1	3.00
Cd	Blood	21.2	12.98
(Cadmium)	Urine	2.49	1.56
Hg	Blood	1.26	2.21
(Mercury)	Urine	0.271	3.56
Pb	Blood	20.0	14.1
(Lead)	Urine	0.33	17.8
Sb	Blood	14.8	1.75
(Antimony)	Urine	29.5	2.94
Se	Blood	123	245.7
(Selenium)	Urine	17.2	169.3
Zn	Blood	9750	396.5
(Zinc)	Urine	3220	157

factors can have a large impact on the measured concentration [36, 37]. Furthermore, cadmium (Cd) is present in tobacco smoke and appears in increased concentrations in smokers [38].

Some elements, such as gallium (Ga), are used in positron emission tomography (PET) [39]. Other elements, such as lithium (Li) and magnesium (Mg), can be found in pharmaceuticals and dietary supplements. Still other elements are present in occupational exposure settings, such as for welders [40].

Regarding urine samples, the elemental concentrations can be influenced by differences in diuresis. However, the results can be normalized by taking the urine creatinine concentration into account, as has been done with antemortem concentrations of cannabis and other drugs of abuse [41]. However, in the postmortem setting, to our knowledge, creatinine is seldom analyzed in urine. While studies indicate that it is stable in postmortem serum [42, 43], other studies indicate that concentrations of creatinine change postmortem [44, 45]. Thus, the present study has not adjusted the urine concentrations of elements for possible differences in diuresis, which need to be considered in evaluation.

In the postmortem forensic setting, confounding factors are often unknown due to lack of circumstantial information. However, taking known confounding factors into account is important when evaluating an individual case. The reference concentrations in the present study have not been stratified according to different confounding factors and represent an all-cause overview of a postmortem normal population. Thus, the interpretation must be moderated considering known confounding factors.

Strengths and limitations

This is, to our knowledge, the most extensive study on postmortem elemental concentrations to date. The large number of included cases allow for the establishment of reference concentrations. Additional information regarding the impact of sample tubes and storage conditions aid in interpretation. Together with case circumstantial information, we believe the current study can serve as an important tool in postmortem forensic casework.

Our work is limited in the extent to which storage conditions are reviewed. The current approach does not allow for a detailed stability study and does not provide time-concentration curves. Thus, further studies are needed to provide a more detailed and extensive picture of the behavior of elemental concentrations during both short- and long-term storage as well as sources of contamination. In addition, there seem to be a presence of high concentration outliers for many elements (especially antimony (Sb) and zirconium (Zr)). Further studies are needed to elucidate possible reasons for the high concentrations in these cases.

The present study does not take several known confounding factors into account. This represents a weakness in comparison with many antemortem studies and must also be considered in the evaluation of postmortem cases. Further studies, in which the presence or absence of confounders is known, are needed to investigate their impact in the postmortem setting.

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Data availability Not applicable.

Declarations

Ethical approval The study has been approved by the Swedish Ethical Review Authority (No: 2020–07053).

Research involving human participants and/or animals Not applicable.

Informed consent Not applicable.

Conflict of interest The authors declare no competing interests.

References

1. Ajdacic-Gross V (2008) Methods of suicide: international suicide patters derived from the WHO mortality database. Bull World Health Organ 86(9):726–732. https://doi.org/10.2471/BLT.07.043489
2. Eddleston M (2000) Patterns and problems of deliberate self-poisoning in the developing world. QJM 93(11):715–731. https://doi.org/10.1093/qjmed/93.11.715
3. Gummin DD et al (2020) 2019 annual report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 37th Annual Report. Clin Toxicol 58(12):1360–1541. https://doi.org/10.1080/15563650.2020.1834219
4. Hughes MF, Beck BD, Chen Y, Lewis AS, Thomas DJ (2011) Arsenic exposure and toxicology: a historical perspective. Toxicol Sci 123(2):305–332. https://doi.org/10.1093/toxsci/kfr184
5. Duncan A, Taylor A, Leese E, Allen S, Morton J, Mcadam J (2015) Homicidal arsenic poisoning. Ann Clin Biochem 52(Pt 4):510–515. https://doi.org/10.1177/0004563214559222
6. Skopp G (2010) Postmortem toxicology. Forensic Sci Med Pathol 6(4):314–325. https://doi.org/10.1007/s12024-010-9150-4
7. Richardson T (2000) Pitfalls in forensic toxicology. Ann Clin Biochem 37(1):20–44. https://doi.org/10.1258/0004563001901498
8. Pélissier-Alicot A-L, Gaulier J-M, Champsaur P, Marquet P (2003) Mechanisms underlying postmortem redistribution of drugs: a review. J Anal Toxicol 27(8):533–544. https://doi.org/10.1093/jat/27.8.533
9. Schier JG et al (2010) Postmortem blood cadmium, lead, and mercury concentrations: comparisons with regard to sampling location and reference ranges for living persons. J Anal Toxicol 34(3):129–134. https://doi.org/10.1093/JAT/34.3.129
10. Zilg B, Thelander G, Giebe B, Druid H (2017) Postmortem blood sampling—Comparison of drug concentrations at different sample

sites. Forensic Sci Int 278:296–303. https://doi.org/10.1016/j.forsciint.2017.07.006
11. Ketola RA, Ojanperä I (2019) Summary statistics for drug concentrations in post-mortem femoral blood representing all causes of death. Drug Test Anal 11(9):1326–1337. https://doi.org/10.1002/dta.2655
12. Druid H, Holmgren P (1997) A compilation of fatal and control concentrations of drugs in postmortem femoral blood. J Forensic Sci 42(1):14071J. https://doi.org/10.1520/jfs14071j
13. Nedahl M, Johansen SS, Linnet K (2019) Postmortem brain–blood ratios of amphetamine, cocaine, ephedrine, MDMA and methylphenidate. J Anal Toxicol 43(5):378–384. https://doi.org/10.1093/jat/bky110
14. Jönsson AK et al (2014) Sedative and hypnotic drugs-fatal and non-fatal reference blood concentrations. Forensic Sci Int 236:138. https://doi.org/10.1016/j.forsciint.2014.01.005
15 Nisse C et al (2017) Blood and urinary levels of metals and metalloids in the general adult population of Northern France: the IMEPOGE study, 2008–2010. Int J Hyg Environ Health 220(2 Pt B):341–363. https://doi.org/10.1016/j.ijheh.2016.09.020
16. Heitland P, Köster HD (2006) Biomonitoring of 37 trace elements in blood samples from inhabitants of northern Germany by ICP-MS. J Trace Elem Med Biol. https://doi.org/10.1016/j.jtemb.2006.08.001
17. P Heitland, HD Köster (2021) "Human biomonitoring of 73 elements in blood, serum, erythrocytes and urine," J Trace Elem Med Biol 64 https://doi.org/10.1016/J.JTEMB.2020.126706
18. Minoia C et al (1990) Trace element reference values in tissues from inhabitants of the European community I. A study of 46 elements in urine, blood and serum of Italian subjects. Sci Total Environ 95:89–105. https://doi.org/10.1016/0048-9697(90)90055-Y
19. Rodushkin I, Ödman F, Olofsson R, Burman E, Axelsson MD (2001) Multi-element analysis of body fluids by double-focusing ICP-MS. Recent Res Dev Pure Appl Chem 5:51–66
20. Rodushkin I, Ödman F (2001) Assessment of the contamination from devices used for sampling and storage of whole blood and serum for element analysis. J Trace Elem Med Biol 15(1):40–45. https://doi.org/10.1016/S0946-672X(01)80025-0
21. M et al (2010) Gary L. Horowitz, MD, Sousan Altaie, PhD, James C. Boyd, "EP28-A3c: defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline—Third Edition," in Clinical and Laboratory Standards Institute 2010. 56238–682–4.
22. Horowitz GL (2008) Reference intervals: practical aspects. EJIFCC 19(2):95–105
23. M Robertson, O Drummer (1998) "Postmortem drug metabolism by bacteria," J Forensic Sci 40 3 https://doi.org/10.1520/jfs13791j
24. Robertson MD, Drummer OH (1998) Stability of nitrobenzodiazepines in postmortem blood. J Forensic Sci 43(1):5–8
25. Rodushkin I, Nordlund P, Engström E, Baxter DC (2005) Improved multi-elemental analyses by inductively coupled plasma-sector field mass spectrometry through methane addition to the plasma. J Anal At Spectrom 20(11):1250–1255. https://doi.org/10.1039/B507886E
26. Rodushkin I, Engström E, Baxter DC (2010) Sources of contamination and remedial strategies in the multi-elemental trace analysis laboratory. Anal Bioanal Chem 396(1):365–377. https://doi.org/10.1007/S00216-009-3087-Z
27. Rodushkin I, Odman OF, Olofsson R, Axelsson MD (2000) Determination of 60 elements in whole blood by sector field inductively coupled plasma mass spectrometry. J Anal At Spectrom 15(8):937–944. https://doi.org/10.1039/B003561K
28. Rodushkin I et al (2018) Application of double-focusing sector field ICP-MS for determination of ultratrace constituents in samples characterized by complex composition of the matrix. Sci Total Environ 622–623:203–213. https://doi.org/10.1016/J.SCITOTENV.2017.11.288
29. "Python programming language." [Online]. Available: www.python.org
30. W McKinney (2010) "Data Structures for Statistical Computing in Python," 56–61 https://doi.org/10.25080/Majora-92bf1922-00a
31. Harris CR et al (2020) Array programming with NumPy. Nature 585(7825):357–362. https://doi.org/10.1038/S41586-020-2649-2
32. Virtanen P et al (2020) SciPy 1.0: fundamental algorithms for scientific computing in Python. Nat Methods 17(3):261–272. https://doi.org/10.1038/S41592-019-0686-2
33. SY Issa, NM Zaghloul, MK al Mazroua (2022) "Estimation of blood and urine levels of eight metals and essential trace elements collected from living Subjects compared to urine, cardiac and femoral postmortem blood, and other postmortem samples: a forensic toxicology study" J Forensic Leg Med 92 https://doi.org/10.1016/J.JFLM.2022.102435
34. Kristiansen J, Christensen JM, Iversen BS, Sabbioni E (1997) Toxic trace element reference levels in blood and urine: influence of gender and lifestyle factors. Sci Total Environ 204(2):147–160. https://doi.org/10.1016/S0048-9697(97)00155-1
35. Saravanabhavan G, Werry K, Walker M, Haines D, Malowany M, Khoury C (2017) Human biomonitoring reference values for metals and trace elements in blood and urine derived from the Canadian Health Measures Survey 2007–2013. Int J Hyg Environ Health 220(2Pt A):189–200. https://doi.org/10.1016/J.IJHEH.2016.10.006
36 Vahter M (1994) What are the chemical forms of arsenic in urine, and what can they tell us about exposure? Clin Chem 40(5):679–80
37 Vahter M, Lind B (1986) Concentrations of arsenic in urine of the general population in Sweden. Sci Total Environ 54(C):1–12. https://doi.org/10.1016/0048-9697(86)90252-4
38. P Richter, O Faroon, RS Pappas (2017) "Cadmium and cadmium/zinc ratios and tobacco-related morbidities," Int J Environ Res Public Health 14(10) https://doi.org/10.3390/IJERPH14101154
39. Ramogida CF, Orvig C (2013) Tumour targeting with radiometals for diagnosis and therapy. Chem Commun (Camb) 49(42):4720–4739. https://doi.org/10.1039/c3cc41554f
40. AL Insley et al (2019) "Occupational survey of airborne metal exposures to welders, metalworkers, and bystanders in small fabrication shops," 16 6 410–421 https://doi.org/10.1080/15459624.2019.1603389
41. Lafolie P et al (1991) Importance of creatinine analyses of urine when screening for abused drugs. Clin Chem 37(11):1927–1931
42. Palmiere C, Mangin P (2012) Postmortem chemistry update part I. Int J Legal Med 126(2):187–198. https://doi.org/10.1007/S00414-011-0625-Y
43. Coe JI (1993) Postmortem chemistry update. Emphasis on forensic application. Am J Forensic Med Pathol 14(2):91–117. https://doi.org/10.1097/00000433-199306000-00001
44. Palmiere C, Mangin P (2015) Urea nitrogen, creatinine, and uric acid levels in postmortem serum, vitreous humor, and pericardial fluid. Int J Legal Med 129(2):301–305. https://doi.org/10.1007/S00414-014-1076-Z
45. Takasu S, Matsumoto S, Kodama S, Sakamoto K, Shimmura S, Iwadate K (2022) Accuracy of urea nitrogen and creatinine measurements in postmortem serum and pericardial fluid compared with antemortem data. Am J Forensic Med Pathol 43(1):33–39. https://doi.org/10.1097/PAF.0000000000000710