



## Method Article

# Optimised ICP-MS quantification method for using animal faeces as a measure of protected area ecosystem health



Andrea B. Webster<sup>a,\*</sup>, Andre Ganswindt<sup>a</sup>, Charney Small<sup>b</sup>,  
Riana Rossouw<sup>b</sup>

<sup>a</sup> Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Lynwood Road, Hatfield, Pretoria, 0083, South Africa

<sup>b</sup> Central Analytical Facilities, ICP-MS Laboratory, University of Stellenbosch, Cnr Ryneveld and Merriman Street, Stellenbosch, 7600, South Africa

## A B S T R A C T

Pollution is a key threat to biodiversity and ecosystem health within protected areas. Using a non-invasive, multi-matrix approach, sediment, vegetation and faecal material from lion (*Panthera leo*) and giraffe (*Giraffa camelopardalis*) were collected and assessed for the simultaneous quantification of 20 trace elements using an optimised method for Inductively Coupled Plasma–Mass Spectrometry (ICP-MS). Method Linearity was confirmed over an analytical range of 0.1–50 mg/kg for aluminium (Al) and iron (Fe); 0.4–400 µg/kg for vanadium (V), cobalt (Co), molybdenum (Mo), and cadmium (Cd); 0.5–5 µg/kg for mercury (Hg); and 1–1 000 µg/kg for elements arsenic (As), boron (B), barium (Ba), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni), lead (Pb), antimony (Sb), selenium (Se), tin (Sn), strontium (Sr) and zinc (Zn). Coefficient of determination ( $R^2$ ) was above 0.99 for all elements. Accuracy (% recovery) and precision (% RSD) of replicate measurements for certified reference material controls fell within 20% of expected value at lower concentrations and 15% at higher concentrations for all elements except Al. Results for instrument and method limit of detections (LOD), method limit of quantification (LOQ) and expanded uncertainty were satisfactory. Preliminary data indicate As, Cd, Cr, Cu, Hg, Ni, Pb, Se, and Zn were present in all matrices evaluated. This raises concerns regarding the combined action of multiple elements at concentrations that can adversely affect ecosystem and wildlife integrity.

- Sample quantity is reduced due to the power and sensitivity of ICP-MS.
- The optimised method is capable of detecting differences in trace element concentrations over large orders of magnitude in animal faeces containing different amounts of organic content.
- The method can be applied to the quantification of essential and potentially toxic elements in faeces across a wide range of terrestrial species.

DOI of original article: [10.1016/j.jenvman.2021.112820](https://doi.org/10.1016/j.jenvman.2021.112820)

\* Corresponding author.

E-mail address: [andrea.webster@tuks.co.za](mailto:andrea.webster@tuks.co.za) (A.B. Webster).

<https://doi.org/10.1016/j.mex.2021.101441>

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**ARTICLE INFO**

*Method name:* Optimised ICP-MS quantification technique for non-invasive environmental sampling

*Keywords:* Terrestrial mammals, Faeces, Vegetation, Sediment, Ecosystem health, Environmental risk assessment

*Article history:* Received 20 May 2021; Accepted 2 July 2021; Available online 3 July 2021

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**Specifications table**


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|--|---|
| Subject area:                          | Environmental and Biological Sciences   |
| More specific subject area:            | Risk assessment of protected areas/ecotoxicology  |
| Method name:                           | <i>Optimised ICP-MS quantification technique for non-invasive environmental sampling</i>  |
| Name and reference of original method: | United States Environmental Protection Agency (US EPA) (2007). Method 3051a (SW-846): Microwave Assisted Acid Digestion of Sediments, Sludges, Soils and Oils, part of Test Methods for Evaluating Solid Waste, Physical/Chemical Methods. Revision 1. Washington DC.<br>Available from <a href="https://www.epa.gov/esam/us-epa-method-3051a-microwave-assisted-acid-digestion-sediments-sludges-and-oils">https://www.epa.gov/esam/us-epa-method-3051a-microwave-assisted-acid-digestion-sediments-sludges-and-oils</a> .   |
| Resource availability:                 | Certified Reference Materials (CRM) similar in composition to sample matrices under investigation.<br>1) Lake Ontario sediment (WQB-1): National Water Research Institute, Burlington, Ontario, Canada.<br>2) Tomato leaves CRM (NIST 1573a) and Bovine liver (NIST 1577c): National Institute for Science and Technology, Gaithersburg, USA.<br>3) Hg standards: De Bruyn Spectroscopic Solutions, Bryanston, South Africa.<br>4) Milli-Q® water purification and distillation system: Merck, KGaA, Darmstadt, Germany.<br>5) Suprapur (65%) double distilled nitric acid (HNO <sub>3</sub> ) and Suprapur (30%) double distilled hydrochloric acid (HCl): Merck KGaA, Darmstadt, Germany. |

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**Method details**
*Background information*

Trace elements occur naturally within the environment [1]. Contamination results when trace elements bound within soils [2,3] are transferred to vegetation and drinking water where they enter the food chain, bioaccumulate and biomagnify in end consumers [4,5]. Regardless of acceptable levels stipulated in legislation, humans and wildlife are sequentially and simultaneously exposed to ill-defined mixtures of chemical and trace element compounds from historical and contemporary sources on a continuous basis [6–8]. Individual elements and the combined action of multiple elements within the body, even when concentrations are too low to be individually effective, (for example chromium (Cr<sup>6</sup>) with cadmium (Cd) and nickel (Ni) with Cd can result in synergistic effects [9], that cause systemic toxicity and compromise the central nervous and endocrine systems [10].

Pollution therefore poses a substantial threat to ecosystem integrity and the biodiversity that protected areas support [11,12]. Biomonitoring is central to ecosystem management, conservation and restoration. Traditional methods of toxicological risk assessment in wildlife however, are often invasive, involving the collection of matrices (blood, hair or tissue) during routine management procedures or samples (kidney, liver, oesophageal tissue) from carcasses following mass mortality events [13–15]. Further, studies generally focus on a single pollution event or animal species [16–18], a specific matrix [19,20], multiple environmental matrices [21] or various biological matrices from a particular species [22]. Only a few studies have examined trace element concentrations in environmental matrices and animal species occupying specific areas [23,24]. To date, studies investigating trace element concentrations in environmental and biological matrices from species

occupying different trophic positions in protected areas within African savannahs have not been attempted.

Inductively Coupled PlasmaMass Spectrometry or ICP-MS is an extremely powerful and sensitive method capable of detecting and measuring metals at trace and ultra-trace concentrations over large orders of magnitude [25,26]. Sample quantities needed for analysis are therefore greatly reduced [27] and combined with microwave-assisted digestion, ICP-MS is considered the method of choice for isotopic and trace multi-elemental analysis [27].

Faecal content in herbivores and carnivores (high cellulose and high protein respectively) are the result of differences in diet, digestive specialisation and complex environmental (soil-plant transfer, soil pH, resource availability, rainfall) and biological (sex, age, reproductive stage, metabolism and assimilation of ingested products, physiology) factors that influence element specific absorption at both individual and species levels [28,29]. These differences, as well as the differences between environmental matrices, necessitated the optimisation of an established method, described in the present study for the non-invasive, simultaneous determination of 20 alkali earth elements, barium (Ba) and strontium (Sr), transition metals cadmium (Cd), chromium (Cr), copper (Cu), cobalt (Co), iron (Fe), manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), vanadium (V) and zinc (Zn), basic metals aluminium (Al), lead (Pb), tin (Sn), metalloids antimony (Sb), arsenic (As) and boron (B) and non-metal selenium (Se). Aside from the optimisation of an established quantification technique for the multi-matrix assessment of trace elements, the present study also aimed to: (i) use “test species” to determine effects of different organic content within samples and (ii) using validation parameters, determine whether quantification technique sensitivity in determining concentration differences within and between matrices.

#### Statement of ethics

All samples were collected with the approval of the University of Pretoria Research and Animal Use and Care Committee (Reference EC043–18 and EC043–18-A1) and the South African Department of Agriculture, Forestry and Fisheries (DAFF-18/02/2019).

#### Sample collection and preparation

Environmental and biological samples were collected from two sites in South African savannah; namely Tswalu Kalahari Reserve (TKR), Northern Cape Province (April–June 2019), and Manyeleti Nature Reserve (MNR), Mpumalanga Province, (July to September 2019). Natural water bodies at each site (TKR:  $n = 24$ ; MNR:  $n = 24$ ) were subjectively evaluated for size and proximity to four common plant species widely distributed across each reserve. Single mud-based-sediment samples ( $\pm 50$  g) were collected 3–5 cm below the surface [29] at a central point near the water's edge. The use of metallic sampling equipment was avoided to minimise trace-element transfer to samples and subsequent contamination. At water bodies  $> 20$  m<sup>2</sup>, single sites were sampled for sediment and corresponding vegetation. At water bodies  $> 50$  m<sup>2</sup>, northeast and southwest sediment and vegetation samples were collected, and at water bodies  $> 100$  m<sup>2</sup>, north-south-east-west samples were collected where possible. Leaf vegetation (TKR:  $n = 24$ ; MNR:  $n = 22$ ) within  $\pm 50$  m of sediment collection was randomly stripped from different parts of each plant and pooled. All sediment and vegetation samples were collected using a different pair of gloves to avoid cross-contamination. Samples were placed in separate Ziplock bags, labelled and frozen at  $-20$  °C in a portable freezer until lyophilisation at  $-50$  °C for 4 days. Sediment and vegetation samples were separately milled for 5 min at 90 rpm using a Retsch® agate ball mill to homogenise.

It is unclear whether specific analytes are excreted at the same rate within a faecal deposit. To limit this possible influence on compound concentrations being measured, and to avoid contamination by soil or grass substrate, each faecal deposit was homogenised *in situ* before collection of sub-samples from the centre of the faecal pile [30]. Fresh faecal samples from 21 different terrestrial herbivores, omnivores and carnivores were collected for analysis.

Given the differences in faecal composition between herbivores and carnivores, faecal material from Africa's largest ruminant (giraffe: *Giraffa camelopardalis*) and largest carnivore (lion: *Panthera*

leo), were used as 'test species'. Multiple fresh pellets from giraffe faecal deposits were subsampled ( $n = 20$ ) at observed defecation events, while fresh faecal samples from lion were collected ( $n = 30$ ) at observed defaecation events around kills, or after rest periods. All faecal samples were collected from soil/grass substrate using gloves, placed into individual screw top plastic sampling containers, labelled and frozen in a portable freezer at  $-20\text{ }^{\circ}\text{C}$  until lyophilisation at  $-50\text{ }^{\circ}\text{C}$  for 7–8 days. Dried faecal samples from both species were subsequently pulverized by hand, or using a ceramic grater and sieved through a plastic-mesh ( $37\text{ }\mu\text{m}$ ) strainer to homogenise and remove large particles of bone, sinew and vegetation.

#### *Certified reference materials and reagents*

Decomposition of all samples is required for the extraction of available metals for ICP-MS quantification [31,32]. Following the United States Environmental Protection Agency method 3051a guidelines for total recoverable analytes [33], double distilled 65% nitric acid ( $\text{HNO}_3$ ) was used for microwave digestion of all matrices. Double distilled 30% hydrochloric acid (HCl) was added to improve extraction efficiency and stability of elements Al, Sb, Fe and Hg [33]. Method digestion efficiency was tested using Certified Reference Materials (CRM) similar in composition to the various sample matrices under investigation. Lake Ontario sediment (WQB-1), certified for extractable elements, represents the potential fraction recoverable during microwave acid digestion of sediment samples. Tomato leaves CRM (NIST 1573a) were used to determine the total recoverable elements in vegetation and herbivore faecal samples. Bovine liver (NIST 1577c) was used as a control for total recoverable elements in carnivore faecal samples (which contained a substantial biological component). Ultra-pure de-ionised water ( $18\text{ M}\Omega\text{cm}^{-1}$ ) used for sample dilution was produced using a Milli-Q® Direct water purification and distillation system (Merck, KGaA, Darmstadt, Germany). Suprapur reagents were purchased from Merck, KGaA, Darmstadt, Germany. Certified Reference Materials for sediment, vegetation and herbivore faeces as well as carnivore faeces were purchased from the National Water Research Institute, Burlington, Ontario, Canada, and the National Institute for Science and Technology, Gaithersburg, USA, respectively.

#### *Sample digestion*

All Samples were digested using a Microwave Accelerated Reaction System Microwave Digester (MARS®–5. CEM Corporation) set at 1600 w and 100% power for maximum digestion vessel capacity of 800 psi [32]. Approximately 0.1 g dry mass (dm) of processed sediment was weighed directly into 100 ml Teflon microwave digestion vessels. Subsequently, 6 ml Suprapur 65% double distilled ( $\text{HNO}_3$ ) and 2 mL Suprapur 30% double distilled (HCl) were added to each vessel and the mixture was left to stand for 15–20 min under a fume hood. After pre-digestion, vessels were sealed and placed on a 12-vessel carousel in the microwave digester.

According to manufacturer recommendations [32], digestion of sediments was dual-phase; i) ramp from room temperature to  $160\text{ }^{\circ}\text{C}$  over 25 min (held for 10 min) and ii) ramp from 160 to  $210\text{ }^{\circ}\text{C}$  over 15 min (held for 10 min) and cooled for 20 min. Each digestion set included dual quality controls of  $\sim 0.1\text{ g}$  (dm) WQB-1 Lake Ontario sediment CRM and blank acid mixture, respectively. Total microwave digestion time (with cooling) was 80 min.

During separate digestions,  $\sim 0.3\text{ g}$  (dm) of processed vegetation and faecal material were weighed directly into 100 mL Teflon microwave digestion vessels. Subsequently, 6.5 mL Suprapur 65% double distilled ( $\text{HNO}_3$ ) and 0.5 mL Suprapur 30% double distilled (HCl) was added to each vessel and the mixture was left to stand for 15–20 min. After pre-digestion, vessels were sealed and placed in the microwave digester. Digestion of vegetation and faecal material took place over 25 min from room temperature to  $210\text{ }^{\circ}\text{C}$  (held for 12 min), before cooling for 20 min [32]. Again, dual quality controls of  $\sim 0.3\text{ g}$  (dm) NIST 1573a tomato leaves CRM (vegetation and herbivore faecal samples), or  $\sim 0.2\text{ g}$  (dm) of NIST 1577c bovine liver CRM (carnivore faecal samples), and a second blank acid mixture control were included in each digestion set. In addition to the bovine liver CRM and blank acid mixture, every third set of carnivore digestions contained  $\sim 0.3\text{ g}$  (dm) of NIST 1573a tomato leaves CRM control to

ensure consistency throughout the digestion sequence of carnivore faecal samples. Total microwave digestion time (with cooling) was 57 min.

Post-digestion, microwave vessels were de-pressurised and  $\pm 40$  mL of de-ionised water was added to each sample in the digestion vessel. Digestive mixture was decanted into 50 mL Falcon tubes pre-washed in 10%  $\text{HNO}_3$ , and de-ionised water was added to make 50 mL final volume [31–33]. Samples were mixed and left to settle before analysis. Duplicate sediment and vegetation samples from three different water bodies and duplicate faecal samples from each species were digested to verify analytical variability and comparability.

### Instrumentation

Trace element analysis was performed on an Agilent 7900 (Agilent Technologies, Wilmington) quadrupole Inductively Coupled Plasma-Mass Spectrometer (ICP-MS), equipped with a High Matrix Introduction (HMI) system and Agilent Mass Hunter software (version 4.4) for instrument control and data processing [31]. The HMI system reduces high matrix ion effects on analyte measurement by minimizing instrument drift caused by salt deposition on interface parts over time. Additionally, ionization efficiency in the plasma is increased through reduction of easily ionized matrix elements [34].

Individual samples were introduced through  $\sim 0.2$  mL/min concentric nebulizer into a Peltier cooled spray chamber at a carrier gas (argon: Ar) flow rate of 0.83 L/min. Subsequently, Ar dilution gas from the HMI configuration was added at 0.15 L/min before introduction into the high temperature plasma. In addition to known polyatomic interferences, particularly for analytes with isotopic masses between 50 and 80, environmental sample matrices also contribute to oxide interferences from  $^{95}\text{Mo}$  on  $^{111}\text{Cd}$  [35].

All elements, (Isotopes  $^{11}\text{B}$ ,  $^{27}\text{Al}$ ,  $^{51}\text{V}$ ,  $^{52}\text{Cr}$ ,  $^{55}\text{Mn}$ ,  $^{56}\text{Fe}$ ,  $^{59}\text{Co}$ ,  $^{60}\text{Ni}$ ,  $^{63}\text{Cu}$ ,  $^{66}\text{Zn}$ ,  $^{75}\text{As}$ ,  $^{88}\text{Sr}$ ,  $^{95}\text{Mo}$ ,  $^{111}\text{Cd}$ ,  $^{118}\text{Sn}$ ,  $^{121}\text{Sb}$ ,  $^{137}\text{Ba}$ ,  $^{202}\text{Hg}$  and  $^{208}\text{Pb}$ ) with the exception of Se were analysed in helium collision cell mode at 4.8 mL/min flow using the 4th generation Octopole Reaction System (ORS) to mitigate these effects [31]. Inter-element corrections were made for possible isobaric interferences of high  $^{115}\text{Sn}$  on indium ( $^{115}\text{In}$ ) used as internal standard, while Pb was expressed as the sum of isotope masses 206, 207 and 208. Hydrogen ( $\text{H}_2$ ) reaction gas set at 6 mL/min flow was used to measure  $^{78}\text{Se}$  to improve sensitivity and remove the charged interference from (gadolinium)  $^{156}\text{Gd}$  observed in some samples during initial investigation. Optimization for sensitivity and low oxide ratios ( $\text{CeO}/\text{Ce} < 0.3\%$ ) was performed daily.

### Calibration solutions

National Institute for Science and Technology (NIST) traceable multi-element stock solutions purchased from Inorganic Ventures (300 Technology Drive, Christiansburg VA 24,073) were used to prepare instrument calibration standards in 2%  $\text{HNO}_3$ . Suprapur (65%) double distilled nitric acid ( $\text{HNO}_3$ ) and Suprapur (30%) double distilled hydrochloric acid (HCl) purchased from Merck KGaA, Darmstadt, Germany, were used to prepare single element Hg standards purchased from De Bruyn Spectroscopic Solutions, Bryanston, South Africa in 2%  $\text{HNO}_3$  + 2% HCl. Ultra-pure de-ionised water ( $18 \text{ M}\Omega\text{cm}^{-1}$ ) used for stock solution dilution was produced by a Milli-Q® IQ Element (Merck KGaA, Darmstadt, Germany). A separate set of NIST traceable multi-element standards and single element Hg were analysed directly after instrument calibration to verify the accuracy of initial calibration standards. Prior to the introduction of each sample and standard into the ICP-MS instrument, an internal standard element mixture containing scandium ( $^{45}\text{Sc}$ ),  $^{115}\text{In}$ , germanium ( $^{72}\text{Ge}$ ), and rhodium ( $^{103}\text{Rh}$ ) in 2%  $\text{HNO}_3$  was added automatically. The internal standards selected were used to correct for matrix effects and instrument drift over time and cover the entire region of mass.

### Data analysis

To calculate instrument and method validation parameters including limits of detection (LOD) and quantification (LOQ), linearity, working range, accuracy (% recovery), precision (% relative standard

deviation (RSD)) and method uncertainty, respectively standard statistical functions within Microsoft Excel were used for descriptive statistics [36]. Environmental and biological matrices from the different study sites were pooled to enable overall evaluation of method sensitivity and ranges of detection for each element between the different matrices of interest.

To describe concentration differences of the same element between matrices, median and interquartile ranges were calculated. The minimum and maximum concentrations excluding outliers were used to demonstrate the variability of trace elements within each matrix. The R Studio interface, in the programme R [37], was used to calculate measures of central tendency and dispersion as well as for the generation of all graphical presentation of data.

### *Method validation*

Method suitability was evaluated for detection and quantification limits (sensitivity), linearity and working range, accuracy and precision (reproducibility) and expanded method uncertainty, according to Eurachem guidelines [38]. In the absence of specific health guidelines for ICP-MS measurement of trace elements in animal faeces, acceptance criteria outlined in method 6020B (SW-846: Test Methods for Evaluating Solid Waste: Physical/Chemical Methods Compendium) were used [39]. Following matrix-specific optimisation of the method, trace element concentrations from sediment and vegetation, together with faecal material from South Africa's largest carnivore (lion) and largest ruminant (giraffe) as "test species" were analysed.

### *Limits of detection and quantification (sensitivity)*

The limit of detection (LOD) is defined as the minimum distinguishable concentration from background noise with a degree of confidence [38]. The limit of quantification (LOQ) is defined as the minimum concentration of analyte that can be determined with acceptable precision and accuracy [40]. Acceptable signal to noise ratios are 3:1 and 10:1 for LOD and LOQ, respectively [41,42]. The instrument LOD was determined from ten replicate measurements of an unprocessed acid blank solution. Method LOD and LOQ were determined from ten replicate measurements of an un-spiked blank processed in the same manner as samples of different matrices. Limit of detection was calculated as ( $3 \times \text{SD}$ ) of the results, while LOQ was calculated as ( $10 \times \text{SD}$ ) of the results [40].

Overall dilution for vegetation and faecal samples was ~160 (v/m), while for sediment samples dilution was ~480 (v/m). ICP-MS instrument sensitivity allowed for low method limits of detection and quantification (Table 1), allowing variations in element content within different sample matrices to be assessed. Higher method LOD and LOQ values for sediment samples were the result of larger dilution factors from smaller sample quantities being used for more efficient digestion. Adjustment of HCl volume relative to that of  $\text{HNO}_3$  in digestion vessels increased the pressure during sample digestion and, in this regard, optimised digestion and extraction of Al, Sb, Fe and Hg analytes. The physical composition of herbivore and carnivore faecal material differs as a consequence of diet and type of digestive tract, but both herbivore and carnivore faecal material have high organic content. Organic-rich matrices of vegetation and faecal samples, therefore, required smaller volumes of HCl for digestion and recovery of analytes than sediment samples.

To minimise the dissolved salt content of the final solution presented for ICP measurement [26], sediment mass was reduced compared to that used for other matrices of interest. The larger dilution factor for sediment samples possibly limits comparison of low abundance element distribution between the various matrices investigated. However, instrument conditions did not need to be adjusted to accommodate higher plasma loading of sediment samples, and sensitivity was comparable to other sample matrices of interest.

### *Linearity and working range*

Linearity was tested by preparing multi-element calibration standards, (described in the calibration solutions section above) for a working range between 0.1–50 mg/kg for Al and Fe, 0.5–5  $\mu\text{g}/\text{kg}$  for Hg and 1–1 000  $\mu\text{g}/\text{kg}$  for all other elements. A calibration curve coefficient of determination ( $R^2$ ) of 0.95

**Table 1**

Instrument limit of detection, method limit of detection and limit of quantification values for various matrices of interest.

| Trace element | Sediment                               |                                    |                                    | Vegetation and Faeces                  |                                    |                                    |
|---------------|--|------------------------------------|------------------------------------|--|------------------------------------|------------------------------------|
|               | Instrument LOD $\mu\text{g}/\text{kg}$ | Method LOD $\mu\text{g}/\text{kg}$ | Method LOQ $\mu\text{g}/\text{kg}$ | Instrument LOD $\mu\text{g}/\text{kg}$ | Method LOD $\mu\text{g}/\text{kg}$ | Method LOQ $\mu\text{g}/\text{kg}$ |
| Al            | 0.59                                   | 280.0                              | 940.0                              | 0.69                                   | 90.0                               | 290.0                              |
| Fe            | 1.70                                   | 820.0                              | 2720.0                             | 0.30                                   | 40.0                               | 150.0                              |
| Mn            | 0.04                                   | 20.0                               | 70.0                               | 0.04                                   | 10.0                               | 20.0                               |
| As            | 0.013                                  | 6.3                                | 20.9                               | 0.014                                  | 2.7                                | 9.1                                |
| B             | 1.37                                   | 656.0                              | 2186.5                             | 1.26                                   | 476.2                              | 1587.2                             |
| Ba            | 0.014                                  | 6.9                                | 23.0                               | 0.019                                  | 3.3                                | 11.0                               |
| Cd            | 0.011                                  | 1.6                                | 5.5                                | 0.002                                  | 0.6                                | 1.9                                |
| Co            | 0.005                                  | 2.3                                | 7.7                                | 0.005                                  | 0.7                                | 2.2                                |
| Cr            | 0.028                                  | 13.3                               | 44.4                               | 0.051                                  | 8.5                                | 28.3                               |
| Cu            | 0.117                                  | 56.1                               | 187.1                              | 0.152                                  | 21.9                               | 73.0                               |
| Hg            | 0.007                                  | 3.2                                | 10.6                               | 0.005                                  | 1.3                                | 4.2                                |
| Mo            | 0.042                                  | 20.2                               | 67.4                               | 0.042                                  | 12.0                               | 40.1                               |
| Ni            | 0.177                                  | 84.8                               | 450.2                              | 0.044                                  | 7.1                                | 239.7                              |
| Pb            | 0.012                                  | 5.6                                | 18.7                               | 0.006                                  | 1.0                                | 3.2                                |
| Sb            | 0.000                                  | 5.3                                | 17.6                               | 0.002                                  | 1.8                                | 6.1                                |
| Se            | 0.032                                  | 15.2                               | 50.6                               | 0.034                                  | 7.0                                | 23.5                               |
| Sn            | 0.006                                  | 3.1                                | 10.2                               | 0.002                                  | 1.6                                | 5.2                                |
| Sr            | 0.016                                  | 7.7                                | 25.8                               | 0.007                                  | 3.0                                | 10.0                               |
| V             | 0.004                                  | 1.7                                | 5.7                                | 0.005                                  | 0.8                                | 2.7                                |
| Zn            | 0.783                                  | 375.9                              | 1252.9                             | 0.016                                  | 21.1                               | 70.4                               |

or above is considered acceptable for accurate reporting [38]. The working range of the method was determined by method appropriate preparation of all matrix test samples.

Linearity was established over the specified working range from different concentrations of reference standards within the calibration curve. The coefficient of determination ( $R^2$ ) for all elements across the working range was consistently greater than 0.99. For elements Al and Fe, the working range was 0.1–50 mg/kg. For elements V, Co, Mo, Cd and Sn the working range was 0.4–400  $\mu\text{g}/\text{kg}$ . The working range for Hg was 0.5–5  $\mu\text{g}/\text{kg}$  and 1.25–1 000  $\mu\text{g}/\text{kg}$  for all other elements (As, B, Ba, Cr, Cu, Mn, Ni, Pb, Sb, Se, Sr and Zn).

Method sensitivity was optimised for lowest quantification limits and simultaneous reduction of matrix effects resulting from high salt content of samples [43]. Trace element content of CRMs, each representing a matrix similar to that of the samples, was used to estimate sample dilution requirements during digestion to ensure measured concentrations fell within the anticipated working range of the instrument [44]. Given the nature of the samples ( $> 0.5\%$  total dissolved solids, in a matrix of  $> 5\%$  (v/v) acid content), a more robust set-up of the ICP-MS instrument was required to minimise matrix effects and instrument drift [26]. In this regard, instrument sensitivity is inadvertently reduced resulting in higher limits of quantification for all elements of interest [44]. The quantification parameters of instrument LOD, method LOQ, and linear working range are interdependent, thus an attempt to optimize one parameter may result in another parameter being compromised [34]. The final determination of measurement conditions set for this method facilitated simultaneous determination of all elements of interest in the different matrices evaluated.

#### Accuracy and precision (reproducibility)

Accuracy is defined as proximity of the measured values to that of reference values and is expressed as % recovery [45,46,41]. Three different Certified Reference Materials; WQB-1 for sediment, NIST 1573a for vegetation and herbivore faeces, and NIST 1577c for carnivore faeces were digested in the same manner as samples. Subsequent analysis was conducted to evaluate the efficiency of the digestion method, ability to amass extractable trace element content from different sample matrices and whether the CRM could be accurately and repeatedly measured by ICP-MS. Accuracy of the method for each matrix, respectively was calculated from the average measurement of all associated

CRM values. Results are deemed acceptable according to general analytical methods when recovery falls between 80 and 120% ( $\pm 20\%$  of the expected value at lower concentrations and within  $\pm 15\%$  at higher concentrations) [41].

Precision is defined as the proximity between replicate measured values and can be used to evaluate repeatability of a method [46]. The relative standard deviation (% RSD) of all repeated CRM measurements throughout the analytical process was used to calculate method precision and should not exceed 20% at lower concentrations and 15% at higher concentrations [41].

Accuracy (% recovery) and precision (% RSD) results differed between matrices, but fell within acceptable limits for all elements (Table 2). An exception to acceptable limits was NIST 1573a, representative of vegetation and herbivore faecal samples, which demonstrated low recovery (75%) for total Al and high% RSD for Hg present at low concentrations. Precision values for all elements were less than 10% RSD for sediment CRM (WQB-1). For tomato leaves CRM (NIST 1573a), used in analysis of vegetation and herbivore faecal samples, precision was below 15% RSD for all elements. In bovine liver CRM (NIST 1577c) used in analysis of carnivore faecal samples, precision was below 20% RSD for all elements.

### Uncertainty

Measurement uncertainty is an estimate attached to a test result that defines an interval within which the true value of measurement is believed to lie [41,47]. All errors associated with the various stages of analysis over several days including sample homogenization, weighing, microwave extraction, instrument measurement and calibration, are taken into account for the determination of combined standard uncertainty of the method [47]. Random samples within each matrix are selected for duplicate measurement. The subsequent variance from pooled matrix-specific duplicate sets was then used to calculate the combined standard uncertainty. When a confidence interval is defined within which a result is expected to fall, a coverage factor is applied to the combined standard uncertainty in order to define an expanded uncertainty [48]. At the 95% level of confidence, this factor has a value of 2 and is considered acceptable when uncertainty does not exceed  $\pm 15\%$  [41,47].

The 'true' value of results of each analyte is believed to lie within the interval  $x \pm U95\%$  with 95% confidence (where  $x$  is the reported result in  $\mu\text{g}/\text{kg}$ ). The expanded method uncertainty, expressed as  $U95\%$ , (Table 3) falls within the accepted range at 95% level of confidence for all elements in sediment except Hg, Cd, B and Se that were present at low concentrations and Ni, Mo and Sb which showed elevated uncertainty in duplicate samples. In vegetation, all elements except Hg and Sb, which were present in low concentrations in this matrix, fell within the acceptable range at 95% level of confidence. Hg and Sb in herbivore faeces and Sb in carnivore faeces approach the limit of quantification, but all elements in the biological matrices fell within the accepted range at 95% level of confidence.

Accuracy, precision and uncertainty parameters are influenced by two prominent steps in analysis; sample preparation and quantification [49,26]. Validation results for accuracy indicate that selected sample digestion methods were suitable for the extraction of total recoverable analytes from all three certified reference materials representative of the sample types of interest in this study. Reference materials are typically well homogenized, and repeated extractions generally result in replicates with good precision [50]. Validation results reflect acceptable precision for all elements except Al (75%) in tomato leaf CRM. In some cases, aluminium bound to silicate minerals within plant material cannot be efficiently digested by  $\text{HNO}_3$  /  $\text{HCl}$  acid digestion, and in this regard the addition of hydrofluoric acid (HF) may facilitate a more complete extraction in future analyses [51,21]. The level of confidence in method extraction and quantification applied to the different matrices of interest is confirmed by CRM analytical results.

### Matrix specific trace element variation

Digestion methods and ICP-MS quantification enabled successful detection of all trace elements of interest within the environmental (Fig. 1) and biological matrices (Fig. 2) evaluated.



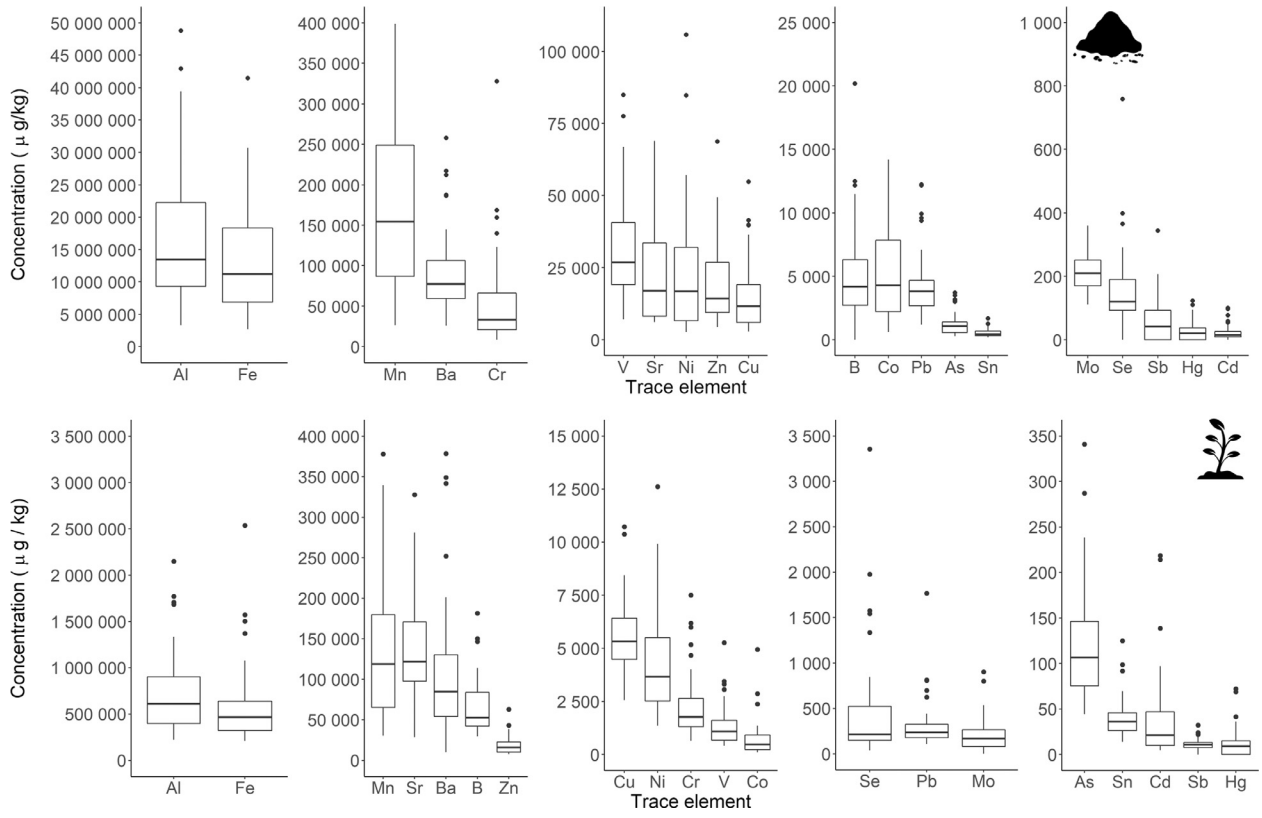
**Table 2**

Accuracy (%) and precision (%RSD) for digestion and analysis of Certified Reference Materials as applicable to matrices and analytes of interest.

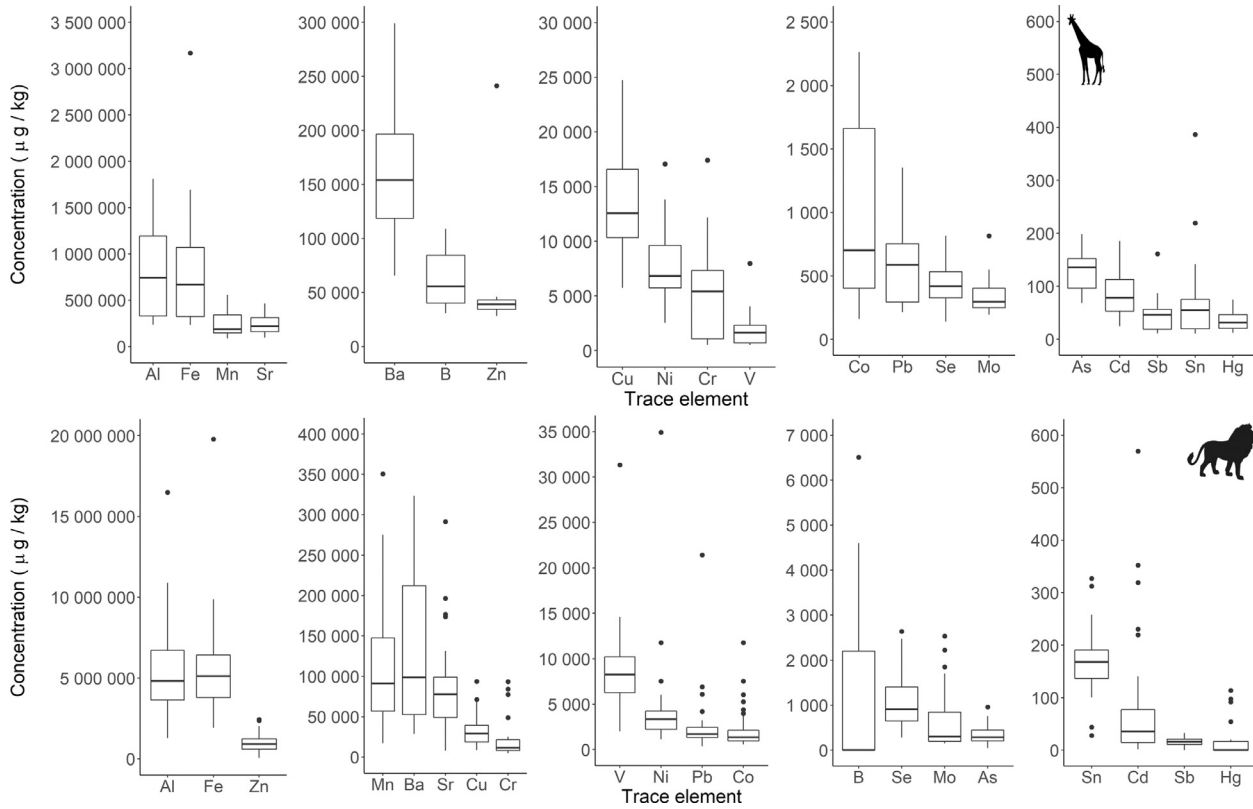
| Trace element | Sediment samples CRM (WQB-1) sediment |                     |                      | Vegetation & herbivore faeces CRM (NIST-1573a) Tomato leaves |                 |                   | Carnivore faeces CRM (NIST-1577) Bovine liver |                     |                   |
|---------------|---------------------------------------|---------------------|----------------------|--|-----------------|-------------------|---|---------------------|-------------------|
|               | Certified (mg/kg)                     | Accuracy (%)        | Precision (% RSD)    | Certified (mg/kg)  | Accuracy (%)    | Precision (% RSD) | Certified (mg/kg)                             | Accuracy (%)        | Precision (% RSD) |
| <b>Al</b>     | <b>58,414</b>                         | 115                 | 4                    | <b>598.4</b>   | 75              | 5                 | –   | –                   | –                 |
| <b>Mn</b>     | <b>2289</b>                           | 95                  | 3                    | <b>246.3</b>   | 96              | 4                 | <b>10.46</b>                                  | 98                  | 3                 |
| <b>Fe</b>     | <b>47,856</b>                         | 91                  | 3                    | <b>367.5</b>   | 96              | 4                 | –   | –                   | –                 |
|               | <b>Certified</b>                      | <b>Accuracy (%)</b> | <b>Precision (%)</b> | <b>Certified</b>   | <b>Accuracy</b> | <b>Precision</b>  | <b>Certified</b>                              | <b>Accuracy (%)</b> | <b>Precision</b>  |
|               | ( $\mu\text{g}/\text{kg}$ )           |                     | (% RSD)              | ( $\mu\text{g}/\text{kg}$ )                                  | (%)             | (% RSD)           | ( $\mu\text{g}/\text{kg}$ )                   |                     | (% RSD)           |
| <b>As</b>     | <b>23,100</b>                         | 97                  | 3                    | <b>112.6</b>   | 105             | 13                | <b>19.6</b>                                   | 90                  | 9                 |
| <b>B</b>      | <b>77,000</b>                         | 117                 | 1                    | <b>33,130</b>  | 102             | 8                 | –   | –                   | –                 |
| <b>Ba</b>     | <b>413,000</b>                        | 113                 | 2                    | <b>63,000*</b>   | 100             | 6                 | –   | –                   | –                 |
| <b>Cd</b>     | <b>1790</b>                           | 93                  | 2                    | <b>1517</b>  | 98              | 4                 | <b>97</b>                                     | 100                 | 5                 |
| <b>Co</b>     | <b>18,100</b>                         | 96                  | 5                    | <b>577.3</b>   | 93              | 4                 | <b>300</b>                                    | 101                 | 3                 |
| <b>Cr</b>     | <b>77,200</b>                         | 104                 | 3                    | <b>1988</b>  | 93              | 5                 | <b>53</b>                                     | 83                  | 19                |
| <b>Cu</b>     | <b>78,400</b>                         | 90                  | 4                    | <b>4700</b>  | 93              | 5                 | <b>275,200</b>                                | 95                  | 2                 |
| <b>Hg</b>     | <b>1090</b>                           | 101                 | 3                    | <b>34.1</b>  | 110             | 66**              | –   | –                   | –                 |
| <b>Mo</b>     | <b>1200</b>                           | 116                 | 6                    | <b>460*</b>  | 92              | 9                 | <b>3300</b>                                   | 103                 | 2                 |
| <b>Ni</b>     | <b>63,100</b>                         | 98                  | 8                    | <b>1582</b>  | 99              | 8                 | <b>44.5</b>                                   | < LOQ               | –                 |
| <b>Pb</b>     | <b>85,000</b>                         | 97                  | 2                    | <b>700*</b>  | 92              | 12                | <b>62.8</b>                                   | 119                 | 13                |
| <b>Sb</b>     | <b>710</b>                            | 99                  | 4                    | <b>61.9</b>  | 101             | 13                | <b>3.13</b>                                   | < LOQ               | –                 |
| <b>Se</b>     | <b>1530</b>                           | 95                  | 7                    | <b>54.3</b>  | 102             | 13                | <b>2031</b>                                   | 91                  | 7                 |
| <b>Sn</b>     | <b>5920</b>                           | 110                 | 4                    | –  | –               | –                 | –   | –                   | –                 |
| <b>Sr</b>     | <b>67,000</b>                         | 111                 | 3                    | <b>85,000*</b>   | 100             | 4                 | <b>95.3</b>                                   | 98                  | 12                |
| <b>V</b>      | <b>107,000</b>                        | 105                 | 3                    | <b>835</b>   | 92              | 7                 | <b>8.17</b>                                   | 116                 | 18                |
| <b>Zn</b>     | <b>279,000</b>                        | 93                  | 5                    | <b>30,940</b>  | 92              | 3                 | <b>181,100</b>                                | 93                  | 2                 |

\* Values are not certified.

\*\* Some replicate values &lt;LOQ.



**Fig. 1.** Trace element concentrations in environmental matrices: sediment (above) and vegetation (below). Dark lines represent median value; box represents upper (3rd) and lower (1st) quartiles. Whiskers extending above and below the box represent minimum and maximum values excluding outliers and points represent outliers. Take note of different concentration values for each group of elements.



**Fig 2.** Trace element concentrations in biological matrices: cellulose rich giraffe (*Giraffa camelopardalis*) faeces (above) and protein rich lion (*Panthera leo*) faeces (below). Dark lines represent the median value; box represents upper (3rd) and lower (1st) quartiles. Whiskers extending above and below the box represent minimum and maximum values excluding outliers, while points represent outliers. Take note of concentration differences between element groups.

**Table 3**

Summary of 2-tailed Expanded Method Uncertainty values for all elements in environmental and biological matrices of interest at 95% level of confidence.

| Trace element | SedimentU <sub>95%</sub> | VegetationU <sub>95%</sub> | HerbivoreU <sub>95%</sub> | CarnivoreU <sub>95%</sub> |
|---------------|--------------------------|----------------------------|---------------------------|---------------------------|
| Al            | ± 3.3                    | ± 1.9                      | ± 5.1                     | ± 3.4                     |
| Fe            | ± 3.9                    | ± 1.9                      | ± 5.0                     | ± 1.9                     |
| Mn            | ± 6.3                    | ± 3.2                      | ± 4.1                     | ± 2.7                     |
| As            | ± 11.0                   | ± 6.2                      | ± 5.4                     | ± 7.2                     |
| B             | ± 16.9                   | ± 2.4                      | ± 4.3                     | ± 5.6                     |
| Ba            | ± 4.8                    | ± 1.4                      | ± 4.2                     | ± 4.4                     |
| Cd            | ± 20.0                   | ± 9.3                      | ± 6.1                     | ± 9.3                     |
| Co            | ± 7.1                    | ± 8.7                      | ± 4.8                     | ± 4.6                     |
| Cr            | ± 5.6                    | ± 6.8                      | ± 4.5                     | ± 4.8                     |
| Cu            | ± 12.9                   | ± 4.7                      | ± 3.6                     | ± 2.7                     |
| Hg            | ± 30.0                   | ± 15.3                     | ± 13.5                    | ± 7.1                     |
| Mo            | ± 23.3                   | ± 1.2                      | ± 3.6                     | ± 4.9                     |
| Ni            | ± 25.2                   | ± 8.8                      | ± 4.6                     | ± 5.0                     |
| Pb            | ± 3.4                    | ± 9.0                      | ± 5.7                     | ± 5.9                     |
| Sb            | ± 32.5                   | ± 20.4                     | ± 12.8                    | ± 14.5                    |
| Se            | ± 22                     | ± 8.3                      | ± 9.5                     | ± 4.5                     |
| Sn            | ± 7.1                    | ± 8.8                      | ± 7.9                     | ± 6.7                     |
| Sr            | ± 6.8                    | ± 1.3                      | ± 2.7                     | ± 5.2                     |
| V             | ± 4.5                    | ± 2.5                      | ± 3.4                     | ± 3.8                     |
| Zn            | ± 6.6                    | ± 2.3                      | ± 5.1                     | ± 4.1                     |

**Table 4**

Summary of differences in trace element concentrations between matrices.

| Trace Element                 | Environmental matrices | Biological matrices |
|-------------------------------|------------------------|---------------------|
| Al, Fe                        | Sediment > Vegetation  | Lion > Giraffe      |
| Mn, Ni                        | Sediment > Vegetation  | Giraffe > Lion      |
| B, Ba, Sr                     | Vegetation > Sediment  | Giraffe > Lion      |
| Se, Zn                        | Vegetation > Sediment  | Lion > Giraffe      |
| As, Co, Cr, Cu, Mn, Pb, Sn, V | Sediment > Vegetation  | Lion > Giraffe      |
| Cd                            | Vegetation > Sediment  | Giraffe > Lion      |
| Sb, Hg                        | Sediment > Vegetation  | Giraffe > Lion      |

In environmental matrices, with the exception of B, Ba, Cd, Zn, Se and Sr, trace element concentrations were higher in sediment compared to vegetation. In biological matrices, Fe, Al, As, Cr, Cu, Pb, Se, Zn, Co, Mo, V and Sn concentrations were higher in lion faeces compared to giraffe faeces while Mn, Cd, Hg, Ni, B, Ba, Sb and Sr concentrations were higher in giraffe faeces compared to lion faeces (Table 4).

The complex nature of sediments assimilates soluble and insoluble components with variations in chemical abundance due to lithological and anthropogenic factors, affect its composition making quantifying trace element concentrations in sediments relatively complicated [52]. To confirm that each sample portion was representative of the whole, and that element variation within a specific sample matrix was not the result of inconsistent sample processing, several duplicate samples of each matrix were digested and analysed [43]. Repeat analysis demonstrated acceptable levels of uncertainty (< 15%) for elements with relatively high concentration [41]. Given the lower accuracy and precision of measurement for analytes such as Hg, Cd, B and Se in sediments and Hg and Sb in vegetation that occur in lower concentrations, greater uncertainty can be expected. Increased uncertainty associated with Ni, Mo and Sb in sediments may result from heterogeneous distribution of minerals containing these elements within samples. Lack of homogeneity in the sample may be caused by differences in sample composition and particle size [53]. This could be minimised in future studies by additional milling and homogenisation of samples. Alternatively, minerals within samples may be present at

low concentrations, and this would require sample analysis in duplicate throughout the quantification process as increasing sample mass for digestion is not optimal given the resulting increased matrix effects during ICP-MS measurement [25].

Considering that each sample analysed represents a specific set of conditions with a number of unique variables, variation in trace element concentrations within and between matrices was to be expected [54]. In sediment for example, considerable variation of element concentrations in different sediment samples, may result from minerals within the sample being resistant to acid digestion [55]. Elemental composition in vegetation is influenced by various environmental factors, one of which is soil chemistry [56]. Measured concentrations in vegetation may vary according to plant-specific selectivity for element absorption from soil, water and atmospheric conditions [57]. Herbivores that feed on cellulose-rich plant matter digest their food through fermentation in the microbial-rich environment of the rumen or hindgut, while carnivores have a short digestive tract and protein-rich matter is digested in the stomach by digestive enzymes [28]. Faecal samples are therefore the result of a complex mixture of the above variables, and additionally may be influenced by biological and physiological processes that influence element-specific absorption at both the individual and species level [28,29].

## Conclusion

All trace elements were detectable over a wide range across matrices. Potentially toxic elements, typically associated with adverse health effects at low concentrations were detected within all four matrices. This highlights the need for further investigation of the complex relationships between trace elements in abiotic and biotic components within terrestrial ecosystems. The value of this study lies in its non-invasive approach to sample collection and the application of an optimised method allowing for the simultaneous detection of multiple trace elements in a variety of environmental matrices [58] Webster et al. 2021 and [59] Webster et al. 2021b for terrestrial ecosystem monitoring. The use of a single, sufficiently sensitive technique to quantify and evaluate variability of trace element concentrations (within and between) matrices of different organic content is clearly advantageous and facilitates the risk assessment of multiple components involved in ecosystem health.

## Acknowledgements

Tswalu Kalahari Reserve and Mpumalanga Tourism and Parks Agency, Manyeleti Game Reserve are thanked for facilitating this research. Central Analytical Facilities, ICP-MS Laboratory, University of Stellenbosch is thanked for sample analysis. The University of Pretoria Endocrine Research laboratory and CSIR Stellenbosch are thanked for assistance with sample preparation. Professor Nigel Bennett is thanked and acknowledged for comments and edits to the manuscript, Javier Callealta is thanked for assistance with graphics

**Funding:** This research was supported by the Department of Science and Technology and National Research foundation SARChI chair of Mammalian Behavioural Ecology and Physiology, South Africa (GUN number 64756), The University of Pretoria Post-graduate Scholarship Programme and The Tswalu Foundation, South Africa. The National Institute for Science and Technology, Gaithersburg, United States of America is acknowledged for donation of domestic sludge and tomato leaf Certified Reference Materials. Funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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