A non-invasive assessment of essential trace element utilization at different trophic levels in African wildlife

A.B. Webster[a,*], F.J. Callealta[b], A. Ganswindt[a], N.C. Bennett[a]

[a]Mammal Research Institute, Faculty of Natural and Agricultural Sciences, University of Pretoria, South Africa
[b]Department of Economics, Universidad de Alcalá, Plaza Victoria, 2, Alcalá de Henares, 28802, Spain

*****Corresponding author. andrea.webster@tuks.co.za

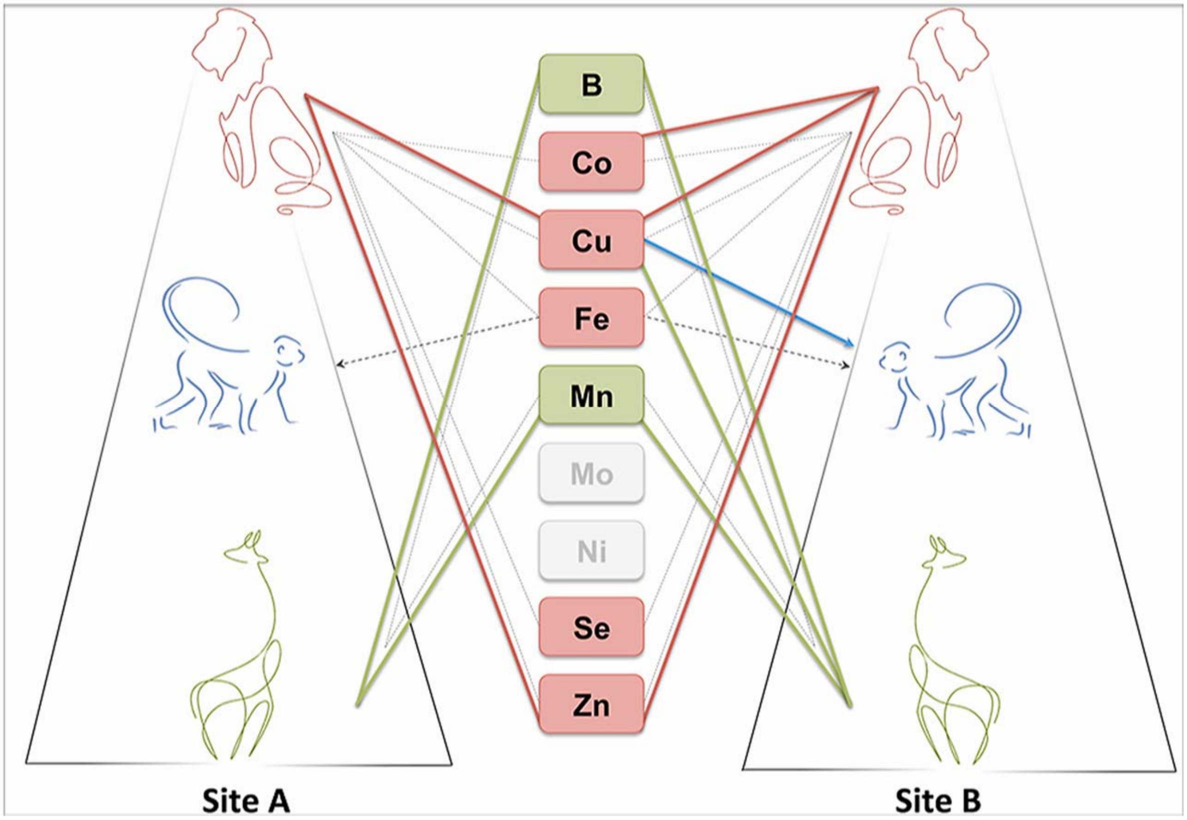
Highlights

- Essential element availability or deficiency difficult to establish in wildlife.
- Trophic level differences investigated non-invasively by faecal sampling.
- Patterns were evident within sites, but absolute values differed between sites.
- Species-specific and essential element interactions need further investigation.
- Broad application of a non-invasive approach for protected area management.

Abstract

The complex relationships that exist between terrestrial mammals and their habitats make African ecosystems highly interactive environments. Anthropogenic activities including climate change have altered geochemical cycles, which influence nutrient availability and deficiency at local, regional and global scales. As synergistic and antagonistic interactions occur between essential elements at both deficiency and excess concentrations, the differences in feeding strategy between trophically distinct groups of terrestrial vertebrates are likely to influence the degree to which overall nutrient needs are met or may be deficient. The overall aim of this study was to investigate and compare quantitative differences of nine essential elements in terrestrial vertebrates occupying different trophic levels within two protected areas; Tswalu Kalahari Reserve (TKR) and Manyeleti Nature Reserve (MNR) South Africa, using faeces as an analytical matrix. Results from linear mixed effects models highlight that concentrations varied widely between individuals. Overall, measured concentrations above their respective means were evident for B and Mn in herbivores, Fe in omnivores and Cu, Co, Fe, Se and Zn in carnivores. Measured concentrations of Mo and Ni did not differ significantly between trophic groups. Although site-specific differences were evident for specific elements, measured mean concentrations of B, Co, Cu, Fe, Mo, Ni, Se and Zn were significantly higher in overall at the MNR study site compared to the TKR site. This is the first study to non-invasively assess essential element concentrations across trophic levels in free ranging African wildlife species within protected areas of the savannah biome. Combined with the assessment of environmental matrices, this approach can be used as an effective diagnostic tool for the assessment of animal welfare and the management of protected areas globally.

Graphical abstract



Keywords: carnivores, environmental health, herbivores, omnivores, South African savannah, terrestrial wildlife management

1 Introduction

The substantial differences in geochemical heterogeneity combined with gradients in precipitation, vegetation and climatic conditions result in nutrient availability or deficiency at different spatial intensities at local, regional and global scales (McNaughton and Georgiadis 1986; Shorrocks and Bates 2015). African soils are typically nutrient poor and soils with higher potential are generally characterized by low pH and relatively low fertility (Steyn and Herselman 2005; Kabata-Pendias 2011). Coupled with anthropogenic activities that have altered geochemical cycles and the biochemical balance of trace elements within the environment (Rzymski et al. 2015), the effects of global climate change, including rising temperatures and CO_2 levels as well as an increase in woody vegetation cover in savannah systems, may significantly alter ecosystem function and capacity (Buitenwerf et al. 2012). In addition, these climate-induced variations in primary production patterns can magnify nutrient disparities (Warne et al. 2010), which ultimately influence how individuals allocate resources to various life history processes, how individuals interact with their environment, and can cause cascading effects across trophic levels (Warne et al. 2012; Warne 2014). Complex intrinsic and extrinsic dynamics including sex, age, life stage and exposure to environmental stressors or infectious pathogens, influence an individual's ability to acquire and assimilate nutrients

(Warne 2014). Periods of nutritional stress, such as drought (van Rooyen and van Rooyen 2017), pregnancy, lactation (Van Der Kolk et al. 2008; Shannon et al. 2008; Keenan et al. 2013) and maintenance of dominance status (van Rooyen 1993; Marshal et al. 2013) also alter individual nutrient requirements and the ability to effectively process nutrients in the body. Additionally, an incomplete understanding of the complex principles that govern element-specific, plant-specific and vertebrate species-specific biological availability, metabolic requirements and interactions, complicate essential element ecosystem assessments (Suttle 2012; Birnie-Gauvin et al. 2017; Garcia-Barrera 2020). Nutrients at deficiency or toxicity levels can have long-term negative effects on free-ranging wildlife, but are not always immediately apparent within a specific environment. Here for the first time, we assess trace element concentrations across free-ranging African vertebrate trophic groups within specific protected areas of South Africa's mesic and arid savannah for comparison.

Essential nutrients are by definition those necessary for survival. Roughly 60 nutrients exist in food and in the body as constituents of bone, proteins, fats, enzymes, co-enzymes, nucleic acids and other components are classified as essential (Baker 2008). Organically bound elements (carbon, hydrogen, oxygen, nitrogen, sulphur and phosphorus) are the components of proteins, carbohydrates and lipids that constitute the bulk of most dietary intake (Schulze-Makuch and Irwin 2005). Macro-mineral cations (calcium, magnesium, sodium and potassium) and anions (phosphorus, chlorine and sulphur) are present as dissolved or precipitated inorganic compounds (Averill and Eldredge 2011) and are vital for a wide range of physiological and biological processes (Warne 2014) that include skeletal formation and maintenance (Weaver and Hill Gallant 2014), neural transmission and energy production (Soetan et al. 2010). In contrast, fat and water soluble vitamins, minerals as well as various trace and ultra-trace elements are required in much smaller quantities, sometimes making it difficult to establish availability in the environment and the degree of utilization by different species (Parker et al. 2009; Chellan and Sadler 2015).

Elements B, Co, Cr, Cu, Fe, I, Mg, Mn, Mo, Ni, S, Se, V and Zn are considered essential or probably essential for biological and physiological functions in many terrestrial vertebrates (Frieden 1974; Mills 1979). Selected elements form multiple components of enzymatic reactions, haemoglobin synthesis (Abbaspour et al. 2014), mineral and hormone metabolism (ATSDR boron: 2010; Białek et al. 2019), maintenance of a healthy immune response (Smith et al. 2018) as well as bone and connective tissue function, steroid hormone production and fertility (ATSDR manganese: 2012). The chemical form in which an element is present in the environment determines its availability for uptake by an organism (Kabata-Pendias 2011). The concentrations at which elements shift between deficient, optimum and toxic are species-specific and specific for each element (Mehri 2020), but the window between these states can be narrow (Tchounwou et al. 2012). Most living organisms have developed various physiological mechanisms to facilitate the uptake of trace elements at concentrations necessary for optimal biological and physiological function (Jaishankar et al. 2014; Koller & Saleh 2018). These same mechanisms however, facilitate the uptake and retention of trace elements above optimal concentrations, which can result in toxic effects (Mann et al. 2011), or secondary deficiency of other trace elements and essential nutrients (Baker 2008). As essential elements do not occur in isolation, numerous synergistic and antagonistic interactions occur at both deficiency and excess concentrations (Garcia-Barrera 2020).

Terrestrial vertebrates must obtain essential nutrients from their environment or through dietary intake at optimal levels to minimize the risk of deficiency or excess (Mehri 2020). Herbivores are classified into broad groups according to the percentage of browse or graze in their diet (McNaughton et al. 1998). Omnivorous mammals with a mixed diet have an advantage over other groups given their ability to utilize a range of available food sources. The relative percentage of various items included in an omnivorous diet is, however, influenced strongly by habitat, rainfall and time of year (Skinner and Chimimba 2005). African carnivores have a large degree of dietary overlap, but different species of carnivore target different prey spectrums (Hayward and Kerley 2008). These differences in feeding strategy between and within groups are likely to influence the degree to which overall nutrient needs are met or deficient. To mitigate periods of nutritional stress (Parker et al. 2009) as well as seasonal fluctuations in nutrient availability and species-specific nutrient requirements (Scogings et al. 2015), terrestrial vertebrates have adopted a number of alternative strategies. Seasonal migration (Lennox et al. 2016) was used by numerous species, but is no longer possible in many areas given the increase in habit fragmentation (Meretsky et al. 2011; Ferguson and Hanks 2012). Dietary flexibility is employed by animals at all trophic levels to meet seasonal changes in nutrient availability (Codron et al. 2007; Hayward and Kerley 2008; Klare et al. 2010; Davidson et al. 2013; Hoffman et al. 2016; Abraham et al. 2019; Straver and Hempson 2020; Beukes et al. 2020). Additionally, coprophagy (Eloff and van Hoven 1980; Reading et al. 2017), osteophagy (Hutson et al. 2013) and geophagy (Engel 2007) are adaptive mechanisms that provide an alternative avenue for accessing nutrients.

Traditional risk assessment of South African protected areas and wildlife species are often invasive and have typically focused on potentially toxic elements that enter the environment through anthropogenic sources (Gummow et al. 1991; Ferreira and Pienaar 2011; Nel et al. 2015; Lewis 2017; du Preez et al. 2018a). In contrast and despite the extensive body of research supporting the fact that essential elements at both deficient and toxic concentrations are capable of causing deleterious health effects in free-ranging wildlife, little attention has been directed towards examining ecosystem integrity and the availability of these nutrients within specific protected areas. Given this gap in savannah ecosystem management knowledge, this study aimed to non-invasively assess concentrations of selected essential trace elements B, Co, Cu, Fe, Mn, Mo, Ni, Se, and Zn in free ranging African herbivore, omnivore and carnivore trophic groups (Table S1) using faeces as an analytical matrix. In addition, we compare overall trace element utilization between trophic groups in two explicitly different protected areas within South African arid and mesic savannah.

2 Materials and methods

2.1 Study sites

The ± 22 497 ha Manyeleti Nature Reserve (MNR), situated at S 24°64'80" and E 31°52'63" in the Mpumalanga Lowveld of South Africa, was established in 1963 from land previously dedicated to pastoral activities by indigenous populations. The MNR shares unfenced borders with the Kruger National Park to the east, the Sabi Sand Game Reserve to the south and the Associated Private Nature Reserves to the northwest (MTPA 2015) and forms part of the Kruger National Park western boundary, which abuts a number of growing rural communities. Manipulation of game movements through provision of artificial water is not used as a management tool on this property and nutrient supplements are not provided, given animals can migrate freely across unfenced boundaries to the north, east and south (Personal communication A Webster – Mark Bourne). Mean temperatures range from 7-22 ºC in the dry winter months and 18-40 ºC in the wet summer months with annual rainfall between 500-700 mm (World weather online Skukuza 2020). The Nwaswitsontso and Mthlowa Rivers drain the northern and central regions, filling the main catchment dams in the centre of the reserve. The Phungwe, Mhluwati and Tswayini Rivers drain the southern regions (Cronje et al. 2005).

Tswalu Kalahari Reserve (TKR) is a fenced reserve situated at S 27°29'61" and E 22°39'43" in the arid Northern Cape Province of South Africa and consists of the Korannaberg (± 101 000 ha) and Lekgaba (± 20 000 ha) sections. Prior to the establishment of the reserve in 1998, land use practices on these owner-managed properties were restricted to domestic livestock farming and hunting. Although natural seasonal pans are present on the property, rotational use of borehole supplied water points are used to manipulate the movement of game to a limited degree. Given the influence of unpredictable rainfall patterns on vegetation biomass in the region, the potential for nutrient deficiency is somewhat mitigated by the provision of supplement salt and mineral blocks (Supplementary material Table S2) throughout the reserve (Personal communication A Webster - Wouter Jordaan). Mean temperatures range from 5-24 ºC in the dry winter months and 22-37 ºC in the wet summer months with a mean annual rainfall of around 325mm (World weather online VanZylsrus 2020). Mountain streams provide seasonal access to water but major rivers do not drain the reserve (van Rooyen and van Rooyen 2017).

2.2 Sample collection

Fresh faecal matter from 12 herbivorous (n = 194), 2 omnivorous (n = 50) and 7 carnivorous (n = 137) terrestrial species (Table S1) within mesic and arid savannah protected areas was collected. Each faecal deposit was homogenised *in situ* before collection of sub-samples from the centre of the faecal pile to avoid contamination by urine, soil or vegetation (Ganswindt et al. 2002). Multiple faecal boli and pellets from monogastric and ruminant herbivores respectively were subsampled from observed defecation events. Although fresh carnivore faecal samples could be collected opportunistically from observed defecation events around kills or dens, in most cases tracks and other sign around overnight faecal deposits left by nocturnal carnivores and porcupine were used to locate and identify faecal samples belonging to various species (Liebenberg 1991a, 1991b). All faecal samples were collected from soil/grass substrate except Steenbok (*Raphicerus campestris*), which had to be uncovered after burial. All faecal samples were collected using gloves, subsequently placed into individual sampling containers (~20 g), labelled and frozen at - 20 °C. 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). The MNR site lies within the “infected zone” for the control of Foot and Mouth Disease (FMD) in South Africa. As a result, faecal subsamples from all samples collected were transported under Red Cross Veterinary Permit to the Agricultural Research Council, Trans-boundary Animal Diseases facility at the Onderstepoort Veterinary Research campus for testing prior to removal from the MNR site. Upon receipt of negative FMD results and termination of fieldwork, frozen faecal samples were transported under Red Cross Veterinary Permit to the University of Pretoria’s Endocrine Research Laboratory for preparation and subsequent diagnostic testing.

2.3 Sample preparation and digestion for ICP-MS analysis

Faecal samples were lyophilized at -50 °C for 5-8 days. Dried faecal samples from all species were subsequently pulverized by hand or using a ceramic grater to homogenise and separate any undigested material and sieved through a plastic-mesh (37 micron) strainer to remove large particles of bone, sinew and vegetation. Individual herbivore and carnivore (± 0.3 g) samples were microwave digested (MARS® -5) in 6.5 ml (65%) HNO_3: 0.5 ml (30%) HCl (US EPA 2007). Post-digestion, de-ionized water was added to make 50 ml final volume of each sample.

2.4 Trace element analysis

Trace element analyses was performed on an Agilent 7900 quadrupole Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) equipped with a High Matrix Introduction system and Agilent Mass Hunter software (version 4.4) for instrument control and data processing (Agilent Technologies 2009). Detailed parameters for method quantification, adjustments and validation are outlined in

(Webster et al. 2021, under review) and in (Webster et al. 2021a) for analysis of environmental samples. In brief, Argon as the dilution gas was added before sample introduction into the plasmas. Helium collision cell mode using the 4th generation Octopole Reaction System was used for analysis of element isotopes ^{11}B, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{95}Mo. Inter-element corrections were made for possible isobaric interferences of high ^{115}Sn on ^{115}In used as internal standard. Hydrogen reaction gas was used to measure ^{78}Se to improve sensitivity and remove the dual-charged interference from ^{156}Gd observed in some samples during initial investigation. Optimization for sensitivity and low oxide ratios (CeO/Ce < 0.3%) was performed daily.

2.5 Analytical quality control

Respective sets of digestions included dual quality controls of a blank acid mixture and a matrix-suitable Certified Reference Material obtained from the National Institute for Science and Technology, Gaithersburg, USA. NIST 1573a tomato leaf (~ 0.3 g) and NIST 1577c bovine liver (~ 0.2 g) were used as controls for herbivore and carnivore faecal sample digestions respectively. In addition, every third set of carnivore digestions contained (~0.3 g) of NIST 1573a tomato leaf CRM control to ensure consistency throughout the digestion sequence of carnivore faecal samples. 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 (UNODC 2009).

2.6 Data analysis

All statistical analyses were conducted using the R software: v 3.6.1 (R Core Team 2019). In total, 381 faecal samples were collected from 21 different terrestrial mammal species belonging to three groups (herbivore, omnivore and carnivore) at two geographically different sites (MNR and TKR) (Table S1). To balance the comparison between sites and groups, species present at both sites were selected for evaluation. In the event a given species was present at only one site (e.g. Meerkat/Brown hyaena) a similar surrogate species (e.g. Dwarf mongoose/Spotted hyaena) representing the same group and similar trophic position was selected at the other site. Descriptive statistics for all measured essential elements were determined using all samples from each species and evaluated at each site. Consequently, seven outliers that produced extensive compression of scale for a specific element, thus obscuring specific differences between species' boxplots within animal groups, were identified and removed, one by one, before further analyses. To facilitate visual comparison, the scales of measure for each essential element were homogenized (mean = 1), dividing each variable by its mean. Subsequently, plots were created to compare relative concentrations of elements within animal groups and at each site (ggplot2 R package).

Before testing for differences between groups and/or between sites, all possible individual effects were numerically and graphically assessed. To account for the variability of measured element concentrations between species, species (or similar species) were treated as blocks (‘block’ variable in Table S1). Following a 3-way ANOVA randomized block design that considered ‘sites’ and ‘groups’ as respective fixed effect factors and ‘blocks’ as a random effect factor, a linear mixed-effects model assuming heterogeneous variances and including an interaction effect between ‘sites’ and ‘groups’ was built for each essential element and estimated by maximum likelihood (nmle and car R-packages). Using these estimated models (summary of fitted models Table S3), marginal and conditional means and standard errors (SE) were determined for groups and sites using the predicted marginal means method (emmeans R-package). Mean differences between groups or sites were investigated by applying Tukey’s pairwise post-hoc test (multcomp R-package). Basic results are reported as mean ± standard error of the mean (SE) and statistical significance was determined at the $p < 0.05$ (Table 1).

Table 1. Estimates for essential element concentrations between animal groups overall (left) and between sites for any group (right). Note: Overall differences between groups are shown on the left of the table. Comparisons between sites (overall and in any group) and between groups (within each site) are shown on the right. Within row letter subscripts a, b indicate a significant difference between sites (P<0.05) and within column letter superscripts **a, b, c** indicates significant differences between groups (P<0.05).

Element	Group	n	Overall	Gr	n	Manyeleti NR Mean ± SE	S ↔	Gr ↕	n	Tswalu KR Mean ± SE	S ↔	Gr ↕
B	**Overall**	**n**	**Mean ± SE**	↕	**188**	**17763.8 ± 3401.2**	b		**193**	**13106.5 ± 3292.3**	a	
	Herbivore	194	28973.1 ± 3706.3	b	94	34508.7 ± 4307.8	b	b	100	23437.6 ± 3529.2	a	b
	Omnivore	50	15034 ± 7783.6	ab	25	16829.5 ± 7847.8	b	ab	25	13238.5 ± 7811.9	a	ab
	Carnivore	137	2298.4 ± 4896.7	a	69	1953.3 ± 4895.8	a	a	68	2643.5 ± 4906.5	a	a
Co	**Overall**	**n**	**Mean ± SE**	↕	**188**	**2761.2 ± 243.1**	b		**193**	**1224 ± 193.5**	a	
	Herbivore	194	1376.9 ± 181.4	a	94	1838 ± 205.5	b	a	100	915.8 ± 175.2	a	a
	Omnivore	50	2161.9 ± 470	ab	25	2960.6 ± 590.6	b	ab	25	1363.1 ± 488.1	a	a
	Carnivore	137	2438.9 ± 285.9	b	69	3484.8 ± 375.3	b	b	68	1393 ± 260.8	a	a
Cu	**Overall**	**n**	**Mean ± SE**	↕	**188**	**29363 ± 2571.5**	b		**193**	**17785.9 ± 1315.8**	a	
	Herbivore	194	13486.2 ± 1098	a	94	13000.3 ± 1099.1	a	a	100	13972 ± 1167	a	a
	Omnivore	50	19472.8 ± 2944.8	a	25	24485.2 ± 4139.8	b	b	25	14460.4 ± 2498.9	a	a
	Carnivore	137	37764.4 ± 3666.4	b	69	50603.4 ± 6416	b	c	68	24925.4 ± 2824	a	b
Fe	**Overall**	**n**	**Mean ± SE**	↕	**188**	**3958.5 ± 354**	b		**193**	**2979.4 ± 323**	a	
	Herbivore	194	1903 ± 320.1	a	94	2135 ± 338.9	b	a	100	1671 ± 316.5	a	a
	Omnivore	50	3639.7 ± 761.4	ab	25	4070.1 ± 850.2	a	ab	25	3209.3 ± 784.7	a	ab
	Carnivore	137	4864.2 ± 475.7	b	69	5670.4 ± 538.9	b	b	68	4057.9 ± 472.2	a	b
Mn	**Overall**	**n**	**Mean ± SE**	↕	**188**	**196.6 ± 16.4**	a		**193**	**219.7 ± 20.5**	a	
	Herbivore	194	293.5 ± 17.8	b	94	252.2 ± 17.1	a	b	100	334.8 ± 22.7	b	b
	Omnivore	50	197.9 ± 40.4	ab	25	166.4 ± 38.6	a	ab	25	229.3 ± 52.1	a	ab
	Carnivore	137	133 ± 23.1	a	69	171.1 ± 25.3	b	a	68	95 ± 23.7	a	a
Mo	**Overall**	**n**	**Mean ± SE**	↕	**188**	**491.8 ± 57.2**	b		**193**	**340.4 ± 48.5**	a	
	Herbivore	194	304.5 ± 50.1	a	94	356.2 ± 55.7	b	a	100	252.7 ± 49.7	a	a
	Omnivore	50	449.8 ± 113.2	a	25	532.1 ± 125.5	b	a	25	367.5 ± 114.2	a	a
	Carnivore	137	494.1 ± 79.6	a	69	587.1 ± 103.1	b	a	68	401.2 ± 75.2	a	a
Ni	**Overall**	**n**	**Mean ± SE**	↕	**188**	**9272.9 ± 819**	b		**193**	**6182.3 ± 588.8**	a	
	Herbivore	194	7641.1 ± 649.1	a	94	8082.2 ± 749.1	a	a	100	7200 ± 723.3	a	a
	Omnivore	50	8944.8 ± 1480.3	a	25	11547.2 ± 2024.7	b	a	25	6342.5 ± 1283.7	a	a
	Carnivore	137	6596.8 ± 941.7	a	69	8189.3 ± 1172.9	b	a	68	5004.3 ± 974.4	a	a
Se	**Overall**	**n**	**Mean ± SE**	↕	**188**	**526 ± 71**	b		**193**	**362.8 ± 67.3**	a	
	Herbivore	194	202.6 ± 69.8	a	94	246.9 ± 70.6	b	a	100	158.3 ± 69.9	a	a
	Omnivore	50	385.1 ± 158.2	ab	25	506.5 ± 166.3	b	ab	25	263.7 ± 156.3	a	ab
	Carnivore	137	745.5 ± 104.2	b	69	824.5 ± 112.6	b	b	68	666.4 ± 106.9	a	b
Zn	**Overall**	**n**	**Mean ± SE**	↕	**188**	**303474 ± 45940.3**	b		**193**	**190583 ± 38607.5**	a	
	Herbivore	194	61526.4 ± 39202.6	a	94	49162.4 ± 39192.4	a	a	100	73890.3 ± 39278.5	b	a
	Omnivore	50	202354.2 ± 93812	ab	25	307670.7 ± 109329	b	ab	25	97037.6 ± 88830.9	a	a
	Carnivore	137	477204.9 ± 62603.3	b	69	553588.8 ± 74200.5	b	b	68	400821 ± 63096	a	b

3 Results

3.1 Essential element differences between animal groups

Overall, essential element concentrations were variable between individuals and between trophic groups (Figure 1). Measured concentrations of specific elements were >6x higher than their respective means in some individuals. Specifically B and Mn in herbivores, Fe in omnivores and Cu, Fe, Se and Zn in carnivores presented the median concentration in their respective trophic groups above the overall mean. In contrast to measured mean concentrations of B and Mn that were significantly higher in herbivores compared to carnivores, Co, Cu, Fe, Se and Zn concentrations were significantly higher in carnivores (Table 1: left). In omnivores, overall mean concentrations of these seven elements with the exception of Cu were not significantly different from those determined in either herbivores or carnivores. While mean concentrations of Cu in omnivores were similar to those measured in herbivores, they were significantly lower than in carnivores. Measured concentrations of Mo and Ni did not differ significantly between trophic groups.

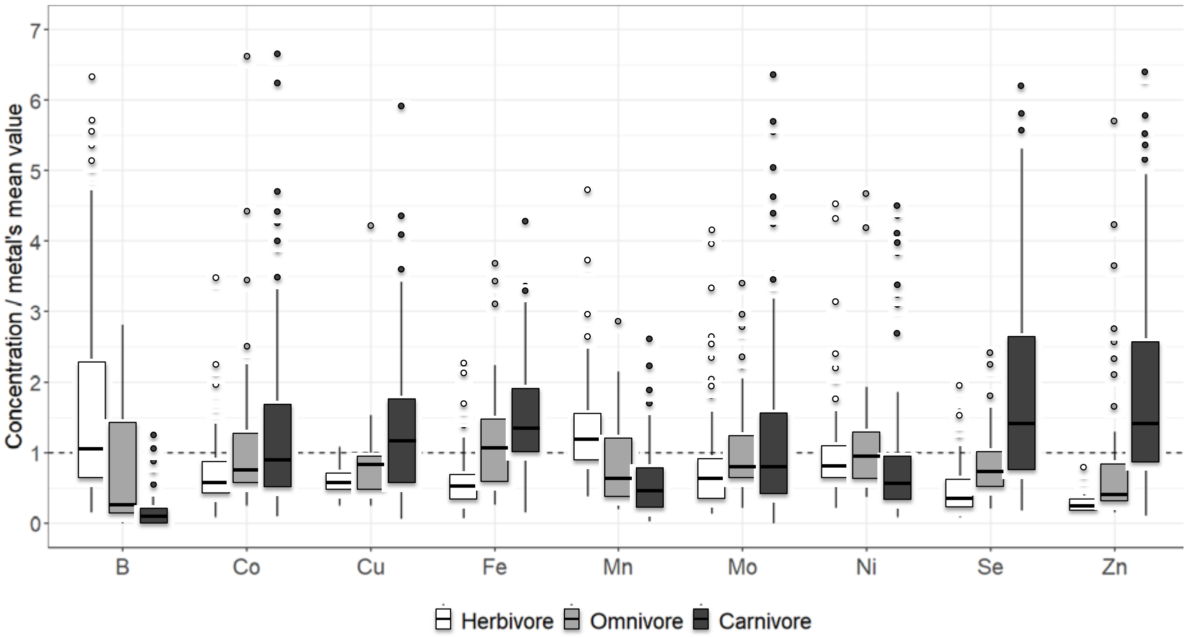


Figure 1. Essential trace element concentrations measured in herbivorous (white), omnivorous (light grey) and carnivorous (dark grey) mammals from relative to the mean for both TKR and MNR sites (dashed line). Box represents the upper (3rd) and lower (1st) quartiles and dark lines within boxes represent median values. Whiskers represent minimum and maximum values excluding outliers. All outliers (excluding seven that obscured differences in animal groups) were included in the analysis and are represented by points.

3.2 Comparison of essential elements in animal groups within and between sites

Despite the provision of mineral blocks throughout the reserve at the TKR site (Table S2), overall measured mean concentrations of B, Co, Cu, Fe, Mo, Ni, Se and Zn were significantly higher across trophic groups at the MNR study site (Figure 2). Statistical differences for each element between sites can be seen in Table 1: right. Although mean Mn concentrations did not differ significantly between sites, Mn and B were significantly higher in herbivores than in carnivores within both sites. In contrast, mean concentrations of Cu, Fe, Se and Zn were significantly higher in carnivores than in herbivores at both sites. Measured Cu and Zn concentrations were also significantly higher in carnivores compared omnivores at the TKR site. At the MNR site however, mean Cu concentrations differed significantly between trophic groups (carnivore > omnivore > herbivore) while mean concentrations of Zn in carnivores and herbivores did not differ significantly to those measured in omnivores. At the MNR site, Co concentrations were significantly higher in carnivores compared to herbivores, but did not differ between trophic groups at the TKR site. Measured concentrations of Mo and Ni did not differ between trophic groups at either site. Although general trends can be established for certain elements within the trophic groups, absolute values for the same elements are different between sites.

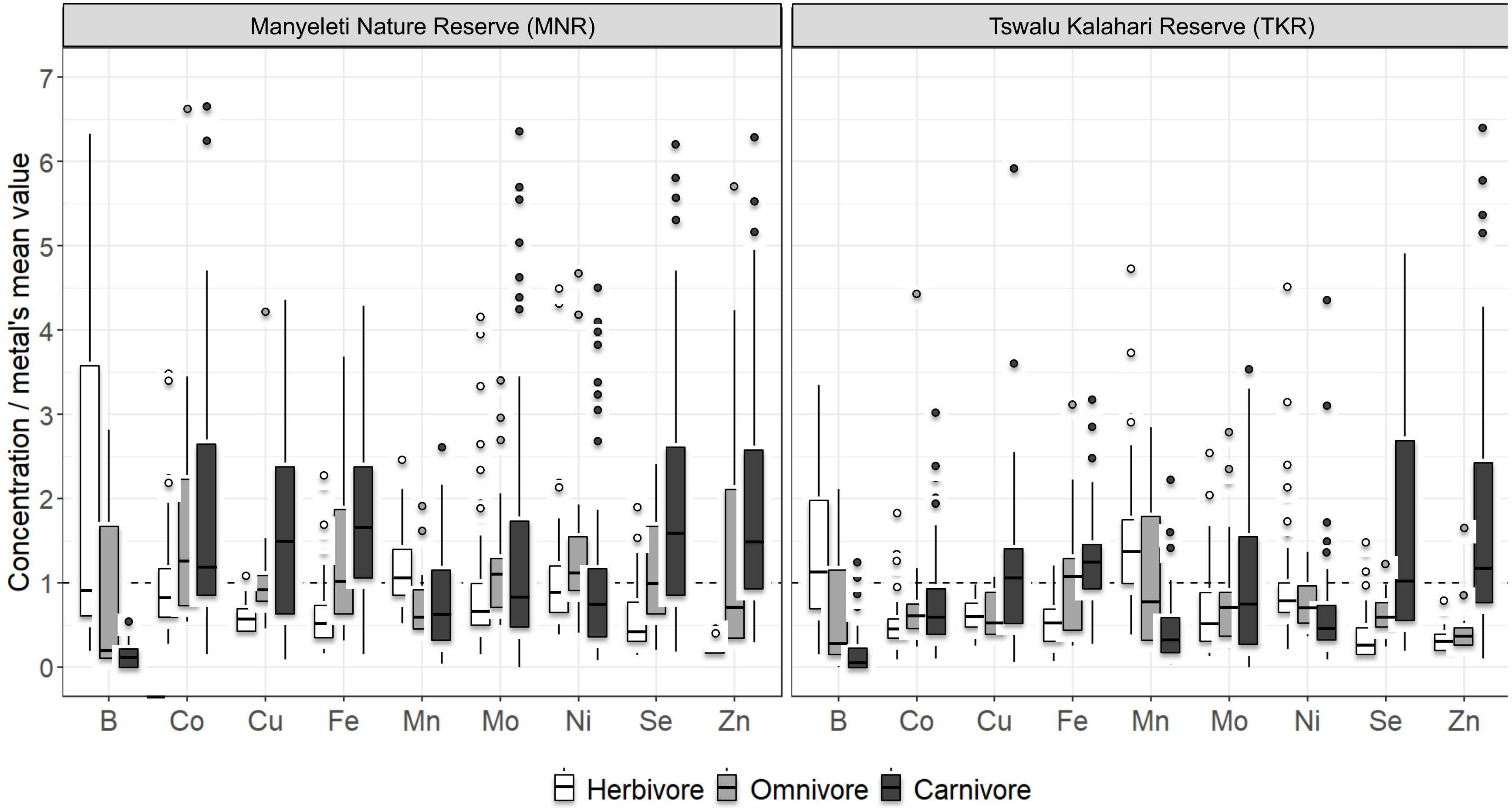


Figure 2. Measured essential element concentrations in sites (MNR: left, TKR: right) in herbivorous (white), omnivorous (light grey) and carnivorous (dark grey) mammals relative to the mean for both sites (dashed line). Box represents the upper (3rd) and lower (1st) quartiles, Dark lines within boxes represent median values. Whiskers represent minimum and maximum values excluding outliers. All outliers (excluding seven that obscured differences in animal groups) were included in the analysis and are represented by points.

4 Discussion

African savannahs support the highest diversity of ungulate (Du Toit and Cummings 1999) and large carnivore (Hayward and Kerley 2008) species compared to any other continent. Despite distinct physiological and morphological differences between animal groups, sub-partitioning of food resources within groups is an important mechanism for the sharing of community resources (McNaughton et al. 1998; Du Toit and Cummings 1999; Owen-Smith et al. 2015). The non-invasive assessment of faecal concentrations has previously been used to assess range quality (Grant et al. 2000), dietary preference (Codron et al. 2007) and the nutritional status (Leslie et al. 2010) of herbivorous wildlife. Here, for the first time, we assess and compare trace element concentrations across free-ranging African vertebrate trophic groups within specific protected areas of South Africa's mesic and arid savannah.

When essential element utilization was compared overall between herbivore, omnivore and carnivore groups at both sites, B and Mn measured concentrations were significantly higher in herbivores than in carnivores. Boron and Mn are both naturally occurring elements in the environment. Although B and Mn can enter the environment through various anthropogenic sources, B is naturally present in numerous silicate minerals found in different soil types (Parks and Edwards 2005), while Mn is naturally abundant in oxide, carbonate and silicate minerals found in soil, water and food (Milatovic et al. 2011). Measured concentrations of B at both sites were higher in sampled vegetation than in sediment (Webster et al. 2021), indicating that herbivores and omnivores to some extent are more likely to accumulate excess levels of this element than carnivores. The reproductive system and developing foetus are most sensitive to B-toxicity (ATSDR 2010). Final consumers exposed to elevated B concentrations may be at the risk of compromised developmental and biochemical processes (Fang et al. 2016), but at what levels this occurs in specific wildlife species is uncertain.

Measured environmental Mn concentrations were higher overall at the MNR site than at the TKR site (Webster et al. 2021), but Mn concentrations in herbivores were higher at both sites compared to carnivores, which may indicate comparable utilization of resources between sites to access specific nutrients. A study on Angolan giant sable antelope (*Hippotragus niger variani*) highlights the selective utilization of termite mounds, rich in several minerals and trace elements as a result of Macrotermes activity, for geophagous activities to supplement sodium and other mineral intake (Baptista et al. 2013). Although the specific use of termite mounds was not observed, geophagous activities were observed on numerous occasions in multiple species at both sites during the study. Between sites, an overall pattern emerges that shows the highest concentrations in herbivores followed by omnivores and carnivores, indicating that concentrations of these elements are either more available for absorption in the herbivore gut or, that through certain behaviours, herbivores are able to gain access to higher Mn concentrations than other groups.

Overall, measured concentrations of Se, Co, Cu, Fe, and Zn were significantly higher in carnivores than in other groups, but on closer inspection site-specific differences in concentrations between elements were more obvious. Measured mean concentrations of Se were significantly higher in carnivores than in herbivores at both sites. Selenium is unevenly distributed within South Africa (van Ryssen 2001) and often occurs at low concentrations in soil and vegetation, frequently at levels below those required for optimum function in terrestrial herbivores (Flueck et al. 2012). The biological interactions between Se and iodine (I) are important for normal thyroid hormone metabolism and deficiency in Se can lead to secondary deficiency of I, which in turn can lead to abortions, stillbirths, increased juvenile mortality and infertility (Shchedrina et al. 2010). Concentrations in plants are dependent on Se chemical speciation and environmental chemistry (Flueck et al. 2012). When present in the soil, Se replaces sulphur (S) and is incorporated into the structure of amino acids. Under these conditions, Se is considered to be in its organic form and is readily absorbed in the digestive tract of foraging animals. Subsequent metabolism in the liver results in the formation of selenocysteine amino acids, which are present in muscle tissue and therefore the source of Se for carnivores (Suttle 2010).

At the MNR site, Co concentrations were significantly higher in carnivores than herbivores, but did not differ between groups at the TKR site. As early as the 1930s, soils of Aeolian origin in Australia were found to be Co-deficient, resulting in naturally occurring diseases in domestic ruminants (Constable et al. 2016). This was the first indication that Co played an important role in ruminant digestion, and that it was necessary for the synthesis of vitamin B_{12}, which serves as a growth factor for many ruminal microorganisms (Suttle 2010). Additionally, Co is a co-factor for the conversion of fermentation by-products to glucose in the liver and kidneys (Waterman et al. 2017). An assessment of environmental sediment and vegetation at the TKR site, the majority of which is situated on soils of Aeolian origin (van Rooyen and van Rooyen 2017), confirms the lower abundance of Co at this site than the MNR site (Webster et al. 2021). Despite the inclusion of Co in mineral blocks at the TKR site, low abundance of this element may be a cause for concern, particularly in herbivores. The higher concentrations of Co found in carnivores at the MNR site may be synonymous with vitamin B_{12} content obtained from a muscle and organ tissue diet. It is worth noting that Co concentrations measured in omnivores were closer to those measured in carnivores than herbivores at each site and this may be representative of the flesh component in their diet during the dry season when seeds, fruit and insects are not as abundantly available as they are in the wet season.

Deficient and excess co-occurrence of between Fe, Cu and Zn can affect absorption and bioavailability of each other. For example excess dietary intake of Fe antagonizes Zn and Cu in the intestinal mucosa, while deficiency causes anaemia (Hooser 2018). Dietary Zn concentrations in excess can negatively impact Fe homeostasis by reducing Cu availability, while Zn deficiency impairs the rate of protein synthesis and transport in the blood (Constable et al. 2016). Copper concentrations above optimal levels impede intestinal absorption of Fe and Zn, while Cu deficiency obstructs the mobilization of iron reserves from the liver, causing anaemia (Suttle 2012). Although environmental deficiencies of Zn and Cu, noted specifically in crop production, are widespread across South Africa (ARC-ISCW 2005), measured concentrations of Cu, Fe and Zn were significantly higher in carnivores compared to herbivorous groups at both sites. A further significant distinction in measured Cu concentrations was evident between carnivores > omnivores > herbivores at the MNR site. Higher concentrations of Zn are expected in carnivores given its functions in skeletal muscle (FOA/WHO 2001) and bone maintenance (Yamaguchi 2009). A recent study of Bearded Vultures (*Gypaetus barbatus*) revealed relatively high content of Fe and Zn in faecal material from an osteophagous diet (Margalida et al. 2020). Myoglobin, the Fe-rich oxygen storage protein, is a key component of muscular tissue (FOA/WHO 2001) while Fe and Cu are major components of blood-rich organs such as the kidney, liver, lungs and heart (Ahmad et al. 2018), which form a substantial part of a carnivorous diet. At the MNR site, measured Cu concentrations in omnivores were also significantly higher compared to herbivores. Omnivores such as Black-backed jackal (*Canis mesomelas*) at the MNR site may have an advantage over those at the TKR site. Higher densities of other carnivores and larger lion prides provide access to kills on a more regular basis and greater opportunities for blood rich meals.

As this study was conducted in the dry season, nutrient deficiencies would be more pronounced than during times of higher rainfall. The Aeolian sediments of the southern Kalahari are known to be deficient in essential nutrients (Thomas and Shaw 1990; Wang et al. 2007), and despite nutrient supplementation at the TKR site, species within the reserve may be at greater risk of deficiency interactions than those at the MNR site, particularly during times of higher nutrient requirement or nutrient stress. Our findings demonstrate that measured essential element concentrations were highly variable between individuals, a finding consistent with other studies (Wenting et al. 2020) and somewhat expected given the different use of resources within and between trophic groups. Key findings highlight that concentration gradients differed between sites and are specific to a site given the influence of geological signatures, management approaches and other extrinsic factors. Given the lack of guidelines for essential elements within specific regions, it is unclear at this stage whether measured concentrations in various trophic groups equate to deficiency or toxicity within a site. Local and regional geochemical differences, historical land use, prior and current management approaches, anthropogenic influences from surrounding areas, species composition as well as species-specific differences are important factors that influence nutrient availability and aquisition and need to be further investigated.

5 Conclusion

This study provides insights into essential element concentration utilization by terrestrial mammals occupying different trophic levels in African savannahs for the first time. Long-term datasets that account for seasonal variation in trace element availability, fluctuations in trace element concentrations over time and in response to factors associated with climate change such as rising temperatures, an increased woody component and associated changes in species composition within savannahs, pollution and other anthropogenic factors would be extremely valuable in establishing upper and lower range limits for these nutrients as well as a disturbance gradient within sites. Coupled with the assessment of potentially toxic elements and environmental concentrations, this approach could form the foundation for an effective diagnostic tool that can be used to assess and monitor animal welfare as well as the management and maintenance of ecosystem integrity within specific protected areas.

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.

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.

References

Abbaspour N, Hurrell R and Kelishadi R (2014). Review on iron and its importance for human health. Journal of Research in Medical Sciences: the official journal of Isfahan University of Medical Sciences 19(2): 164-174. PMID: 24778671.

Abraham JO, Hempson GP and Straver AC (2019). Drought-response strategies of savannah herbivores. Ecology and Evolution 9: 7047-7056. Doi: https://doi.org/10.1002/ece3.5270

Agency for Toxic Substances and Disease Registry (ATSDR) (1999 revised 2012). Toxicological profile for Manganese. Atlanta, GA, U.S. Department of health and human services, Public health service. https://www.atsdr.cdc.gov/substances/toxsubstance.asp?toxid=23 (Accessed 12 April 2020)

Agency for Toxic Substances and Disease Registry (ATSDR) (2010). Toxicological profile for Boron. Atlanta, GA, U.S. Department of health and human services, Public health service. https://www.atsdr.cdc.gov/substances/toxsubstance.asp?toxid=80 (Accessed 09 April 2020)

Agency for Toxic Substances and Disease Registry (ATSDR) (2004). Toxicological profile for copper. Atlanta, GA, U.S. Department of health and human services, Public health service. https://www.atsdr.cdc.gov/substances/toxsubstance.asp?toxid=37 (Accessed 12 April 2020)

Agilent technologies (2009). Comparing collision/reaction cell modes for the measurement of interfered analytes in complex matrices using the Agilent 7700 series ICP-MS: Technical Overview Available from http://hpst.cz/sites/default/files/attachments/5990-3236en-comparing-collision-reaction-cell-modes-measurement-interfered-analytes-complex-matrices.pdf (Accessed 6 May 2020)

Ahmad RS, Imran A and Hussain MB (2018). Nutritional composition of meat In: Arshad MS (Ed) Meat science and nutrition. Intech Open. Doi: https://doi.org/10.5772/intechopen77045. Available from: https://www.intechopen.com/books/meat-science-and-nutrition/nutritional-composition-of-meat (Accessed 2 November 2020)

Ali H and Khan E (2019). Trophic transfer, bioaccumulation, and biomagnification of non-essential hazardous heavy metals and metalloids in food chains/webs - Concepts and implications for wildlife and human health. Human and Ecological Risk Assessment 25(6): 1353-1376. Doi: https://doi.org/10.1080/10807039.2018.1469398

ARC-ISCW (2005). Overview of the agricultural natural resources of South Africa. ARC-ISCW Report No: GW/A/2004/13. ARC-Institute for Soil, Climate and Water, Pretoria

Averill BA and Eldredge P (2011). General Chemistry: Principals, patterns and applications. Saylor Foundation. Available from https://open.umn.edu/opentextbooks/textbooks/69 (Accessed 16 November 2020)

Baker DH (2008). Animal models in nutrition research. The Journal of Nutrition 138(2): 391-396. Doi: https://doi.org/10.1093/jn/138.2.391

Baptista SL, Pinto PV, Freitas MC, Cruz C and Palmeirim JM (2013). Geophagy by African ungulates: the case of the critically endangered giant sable antelope of Angola *(Hippotragus niger variani*). African Journal of Ecology 51(1): 139-146. Doi: https://doi.org/10.1111/aje.12020

Beukes M, Radloff FGT and Ferreira SM (2020). Spatial and seasonal variation in lion (*Panthera leo*) diet in the southwestern Kgalagadi Transfrontier Park. African Journal of Wildlife Research 50(1): 55-68. Doi: https://doi.org/10.3957/056.050.0055

Białek M, Czauderna M, Krajewska KA and Przybylski W (2019). Selected physiological effects of boron compounds for animals and humans: A review. Journal of Animal Feed Science 28(4): 307-320. Doi: https://doi.org/10.22358/jafs/114546/2019

Birnie-Gauvin K, Peiman KS, Raubenheimer D and Cooke SJ (2017). Nutritional physiology and ecology of wildlife in a changing world. Conservation Physiology 5(1): cox030. Doi: https://doi.org/10.1093/conphys/cox030

Bredin IP, Skinner JD and Mitchell G (2008). Can osteophagia provide giraffes with phosphorus and calcium? Onderstepoort Journal of Veterinary Research 75(1): 1-9. Available from: http://www.scielo.org.za/scielo.php?script=sci_arttext&pid=S0030-24652008000200001&lng=en.

Buitenwerf R, Bond WJ, Stevens N and Trollope WM (2012). Increased tree densities in South African savannas: > 50 years of data suggests CO2 as a driver. Global Change Biology 18(2): 675-684. Doi: https://doi.org/10.1111/j.1365-2486.2011.02561.x

Chellan P and Sadler PJ (2015). The elements of life and medicines. Philosophical Transactions, Series A. Mathematical, physical, and engineering sciences 373(2037): 20140182. Doi: https://doi.org/10.1098/rsta.2014.0182

Codron D, Codron J, Lee-Thorp JA, Sponheimer M, De Ruiter D, Sealy J, Grant R and Fourie N (2007). Diets of savanna ungulates from stable carbon isotope composition of faeces. Journal of Zoology 273(1): 21-29. Doi: https://doi.org/10.1111/j.1469-7998.2007.00292.x

Constable PD, Hinchcliff KW, Done SH and Gruenberg W (Eds) (2016). Diseases of the hemolymphatic and immune systems. In: Veterinary Medicine - E-BOOK: A textbook of the diseases of cattle, horses, sheep, pigs and goats 11 ed. Elsevier Health Sciences pp716-844 (Accessed 28 October 2020).

Cronje HP, Cronje I and Botha AJ (2005). The distribution and seasonal availability of surface water on the Manyeleti Game Reserve, Limpopo Province, South Africa. Koedoe 48(2): 11-21. Doi: https://doi.org/10/4102/koedoe.v48i2.93

Davidson Z, Valeix M, Van Kesteren F, Loveridge AJ, Hunt JE, Murindagomo F and Macdonald DW (2013). Seasonal diet and prey preference of the African lion in a waterhole-driven semi-arid savanna. PLoS ONE 8(2): e55182. Doi: https://doi.org/10.1371/journal.pone.0055182

Du Preez M, Govender D, Kylin H and Bouwman H (2018a). Metallic elements in Nile crocodile eggs from the Kruger National Park, South Africa. Ecotoxicology and Environmental Safety 148: 930-941. Doi: https://doi.org/10.1016/j.ecoenv.2017.11.032

Du Toit J and Cumming D (1999). Functional significance of ungulate diversity in African savannas and the ecological implications of the spread of pastoralism. Biodiversity and Conservation 8: 1643-1661. Doi: https://doi.org/10.1023/A:1008959721342

Eloff AK and van Hoven W (1980). Intestinal protozoa of the African elephant (*Loxodonta Africana)* - Blumenbach. South African Tydskrift Dierkunde 15(2) 83-90. Doi: https://doi.org/10.1080/02541858.1980.11447691

Engel C (2007) Zoopharmacognosy. In: Wynn SG and Fougère BJ (Eds) Veterinary herbal medicine. Pp 7-15. Doi: https://doi.org/10.1016/B978-0-323-02998-8.50006-8

Fang K, Zhang W, Xing Y, Zhang Q, Yang L, Cao Q and Qin L (2016). Boron toxicity causes multiple effects on *Malus domestica* pollen tube growth. Frontiers in Plant Science 7: 208. Doi: https://doi.org/10.3389/fpls.2016.00208

Ferguson K and Hanks J (2012). The effects of protected area and veterinary fencing on wildlife conservation in Southern Africa. Parks 18(1): 49-60. https://www.iucn.org/sites/dev/files/import/downloads/parks_ferguson.pdf (Accessed 27 July 2020)

Ferreira SM and Pienaar D (2011). Degradation of the crocodile population in the Olifants River Gorge of Kruger National Park, South Africa. Aquatic Conservation: Marine and Freshwater Ecosystems 22: 155-164. Doi: https://doi.org/10.1002/acq.1175

Flueck WT, Smith-Flueck JM, Mionczynski J and Mincher BJ (2012). The implications of selenium deficiency for wild herbivore conservation: a review. European Journal of Wildlife Research 58(5): 1-20. Doi: https://doi.org/10.1007/s10344-012-0645-z

FOA/WHO (2001). Report of a joint FOA/WHO expert consultation on human vitamin and mineral requirements. Bangkok, Thailand. Pp 1-303

Frieden E (1974). The evolution of metals as essential elements [with special reference to iron and copper. In: Friedman M (Ed) Protein-metal interactions (volume 48) of Advances in Experimental Medicine and Biology. Springer, New York, NY, USA. Pp. 1-31.

Ganswindt A, Heistermann M, Borragan S and Hodges JK (2002). Assessment of testicular endocrine function in captive African elephants by measurement of urinary and fecal androgens. Zoo Biology 21: 27-36. Doi: https://doi.org/10.1002/zoo.10034

García-Barrera T, Gómez-Ariza JL González-Fernández M, Moreno F, Carcia-Sevillano MA and Gómez-Jacinto V (2012). Biological responses related to agonistic, antagonistic and synergistic interactions of chemical species. Analytical and Bio-analytical Chemistry 403(8): 2237-2253. https://doi.org/10.1007/s00216-012-5776-2

Grant CC, Peel MJS and van Ryssen JBJ (2000). Nitrogen and phosphorus concentration in faeces: an indicator of range quality as a practical adjunct to existing range evaluation methods. African Journal of Range & Forage Science 17(1-3): 81-92. Doi: https://doi.org/10.2989/10220110009485743

Gummow B, Botha CJ, Basson AT and Bastianello SS (1991). Copper toxicity in ruminants: Air pollution as a possible cause. Onderstepoort Journal of Veterinary Research 58: 33-39. PMID: 2052318

Hayward MW and Kerley GIH (2008). Prey preferences and dietary overlap amongst Africa's large predators. South African Journal of Wildlife Research 38(2): 93-108. Doi: https://doi.org/10.2957/0379-4369-38.2.93

Hoffman T, Beamish E, Kaplan B, Lewis M, O'Riain MJ, Sithaldeen R and Stone O. (2016). A conservation assessment of *Papio ursinus*. In: Child MF, Roxburgh L, Do Linh San E, Raimondo D, Davies-Mostert HT (Eds). The Red List of mammals of South Africa, Swaziland and Lesotho. South African National Biodiversity Institute and Endangered Wildlife Trust, South Africa

Hooser SB (2018). Iron In: Gupta RC (Ed) Veterinary Toxicology Basic and Clinical Principles 3rd ed. Academic Press. Pp 433-437.

Hutson JM, Burke CC and Haynes G (2013). Osteophagia and bone modifications by giraffe and other large ungulates. Journal of Archaeological Science 40(12): 4139-4149. Doi: https://doi.org/10.1016/j.jas.2013.06.004

International Union for Conservation of Nature (IUCN) Red list of threatened species. Available from https://www.iucnredlist.org (Accessed 12 December 2017, August 2020)

Jaishankar M, Tseten T, Anbalagan N, Mathew BB and Beeregowda KN (2014). Toxicity, mechanism and health effects of some heavy metals. Interdisciplinary Toxicology 7(2): 60-72. Doi: https://doi.org/10.2478/intox-2014-0009

Kabata-Pendias A (2011). Trace elements in soils and plants. 4th edn. Boca-Raton, FL: CRC press.

Keenan K, Bartlett TQ, Nijland M, Rodriguez JS, Nathanielsz PW and Zürcher NR (2013). Poor nutrition during pregnancy and lactation negatively affects neuro-development of the offspring: evidence from a translational primate model. American Journal of Clinical Nutrition 98(2): 396-402. Doi: https://doi.org/10.3945/ajcn.112.040352

Klare U, Kamler JF, Stenkewitz U and MacDonald DW (2010). Diet, prey selection, and predation impact of Black-backed jackals in South Africa. Journal of Wildlife Management 74(5): 1030-1042. Doi: https://doi.org/10.2193/2009-211

Koller M and Saleh HM (2018). Introducing heavy metals. In: Hosam El-Din S and Aglan RF (Eds) Heavy Metals. IntechOpen Books on Demand. Doi: https://doi.org/10.5772/intechopen.74783. Available from https://www.intechopen.com/books/heavy-metals/introductory-chapter-introducing-heavy-metals (Accessed 13 March 2020)

Lennox RJ, Chapman JM, Souliere CM, Tudorache C, Wikelski M, Metcalfe JD and Cooke SJ (2016). Conservation physiology of animal migration. Conservation Physiology. 29: 4(1): cov072. Doi: http://doi.org/10.1093/conphys/cov072

Leslie DM, Bowyer RT and Jenks JA (2010). Facts from feces: Nitrogen still measures up as a nutritional index for mammalian herbivores. Journal of Wildlife Management 72(6): 1420-1433. Doi: https://doi.org/10.2193/2007-404

Lewis AE (2017). Examining heavy metal concentrations in hair of South African Vervet monkeys *(Chlorocebus pygerythrus)* to assess anthropogenic impacts. MSc Dissertation, East Carolina University, US.

Liebenberg L (1990a). The art of tracking: the origin of science. David Philip Publishers and Creda Press. Cape Town, South AFrica

Liebenberg L (1990b). The field guide to the animal tracks of Southern Africa. David Philip Publishers Cape Town and Johannesburg, South Africa.

Mann RM, Vijver MG and Peijnenburg WJGM (2011). Metals and metalloids in terrestrial systems: Bioaccumulation, biomagnification and subsequent adverse effects. In: Sánchez-Bayo F, Van Den Brink J and Mann RM (Eds) Ecological impacts of toxic chemicals, Bentham, UK. Pp 43-62. Available from https://edepot.wur.nl/192580 (Accessed 23 March 2020)

Margalida A, Schulze-Hagen K, Wetterauer B, Domhan C, Oliva-Vidal P and Wink M (2020). What do minerals in faeces of Bearded Vultures reveal about their dietary habits? Science of the Total Environment 728:138836. Doi: https://doi.org/10.1016/j.scitotenv.2020.138836

Marshal JP, Grange S and Marneweck DG (2012). Seasonal variation in body condition of impala at Manyeleti Game Reserve, South Africa. South African Journal of Wildlife Research 42(2): 128-137. Doi: https://doi.org/10.3957/056.042.0212

McNaughton SJ, Ruess RW and Seagle SW (1988). Large mammals and process dynamics in African ecosystema. Bioscience 38(11): 794-900. https://about.jstor.org/terms (Accesses 2 Nov 2020)

McNaughton SJ and Georgiadis NJ (1986). Ecology of African grazing and browsing mammals. Annual Review of Ecology and Systematics 17: 39-65. Doi: https://doi.org/10.1145/annurev.es.17.110186.000351

Mehri A (2020). Trace elements in human nutrition (II) - An update. International Journal of Preventative Medicine 11(2): 1-87. Doi: https://doi.org/10.4103/ijpvm.IJPVM_48_19

Meretsky VJ, Atwell JW and Hyman JB (2011). Migration and conservation: Frameworks, gaps and synergies in science, Law and Management. Environmental law (Northwestern School of Law) 41(2): 447-534. PMID: 29332970

Milatovic D, Gupta RC, Yin Z, Zaja-Milatovic S and Aschner M (2011). Manganese In: Gupta RC (Ed) Reproductive and Developmental Toxicology. Elsevier Academic Press. Pp 439-450

Mills CF (1979). Trace elements in animals. Philosophical Transactions of the Royal Society of London Series B: Biological Sciences 288(1026): 5163. JSTOR, www.jstor.org/stable/2398725 (Accessed 3 Nov. 2020).

Mpumalanga Tourism and Parks Agency (MTPA) (2015). Integrated Management Plan: Manyeleti Nature Reserve, South Africa.

Nel L, Strydom NA and Bouwman H (2015). Preliminary assessment of contaminants in the sediment and organisms of the Swartkops Estuary, South Africa. Marine Pollution Bulletin 101: 878-885. Doi: 10.1016/jmarpolbul.2015.11.015

Owen-Smith N, Martin J and Yoganand K (2015). Spatially nested niche partitioning between syntopic grazers at foraging arena scale within overlapping home ranges. Ecosphere (69): 152. Doi: https://doi.org/10.1890/ES14-00487.1

Parker KL, Barboz PS and Gillingham MP (2009). Nutrition integrates environmental responses of ungulates. Functional Ecology 23: 57-69. Doi: https://doi.org/10.1111/j.1365-2435.2008.01528.x

Parks JL and Edwards M (2005). Boron in the environment. Critical Reviews in Environmental Science and Technology 35(2): 81-114. Doi: https://doi.org/10.1080/10643380590900200

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reading RP, Tshimologo B and Maude G (2017). Coprophagy of African Wild dog faeces by Hooded vultures in Botswana. Vulture News 72: 34-37. Doi: https://doi.org/10.4314/vulnew.v72i1.5

Rzymski P, Tomczyk K, Rzymski P, Poniedziałek B, Opala T and Wilczak M (2015). Impact of heavy metals on the female reproductive system. Annals of Agricultural and Environmental Medicine 22(2): 259-264. Doi: https://doi.org/10.5604/12321966.1152077

Schulze-Makuch D and Irwin LN (2005). Building blocks of life. In: Life in the universe. Advances in astrobiology and biogeophysics vol 3. Springer, Berlin, Heidelberg. Doi: https://doi.org/10.1007/10825622_5

Scogings PF, Hattas D, Skarpe C, Hjältén J, Dziba L, Zobolo A and Rooke T (2015). Seasonal variations in nutrients and secondary metabolites in semi-arid savannas depend on year and species. Journal of Arid Environments 114: 54e61. Doi: http://dx.doi.org/10.1016/j.jaridenv.2014.11.003

Shannon G, Page BR, Mackay RL, Duffy KJ and Slotow R (2008). Activity budgets and sexual segregation in African elephants (*Loxodonta africana*). Journal of Mammalogy 89(2): 467-476. Doi: https://doi.org/10.1644/07-MAMM-A-132R.1.

Shchedrina VA, Zhang Y, Labunskyy VM, Hatfield DL and Gladyshev VN (2010). Structure-function relations, physiological roles and evolution of mammalian ER-resident selenoproteins. Antioxidants & Redox Signalling 12(7): 839-849. Doi: http://doi.org/10.1089/ars.2009.2865

Shorrocks B and Bates W (2015). The biology of African savannahs. Biology of habitats. Illustrated and revised edn. Oxford University Press.

Skinner JD and Chimimba CT(2005). The Mammals of the Southern African Subregion 3rd edn. Cambridge University Press

Smith AD, Panickar KS, Urban JF Jr and Dawson HD (2018). Impact of micro-nutrients on the immune response of animals. Annual Review of Animal Biosciences 6(1): 227-254. Doi: https://doi.org/10.1146/annurev-animal-022516-022914

Soetan KO, Olaiya CO and Oyewole OE (2010). The importance of mineral elements for humans, domestic animals and plants: A review. African Journal of Food Science 4(5): 200-222. Available from http://www.academicjournals.org/ajfs (Accessed 3 October 2020)

Steyn CE and Herselman JE (2005). Trace elements in developing countries using South Africa as a case study. Communications in Soil Science and Plant Analysis 36(1-3):155-168. Doi: https://doi.org/10.1081/CSS-200043017

Staver AC and Hempson GP (2020). Seasonal dietary changes increase the abundances of savanna herbivore species. Science Advances 6: eabd2848. Doi: https://doi.org/10.1126/sciadv.abd2848

Suttle NF (2012). Copper Imbalances in ruminants and humans: Unexpected common ground. Advances in Nutrition 3(5): 666-674. Doi: https://doi.org/10.3945/an.112.002220

Suttle NF (2010). Mineral nutrition of livestock, 4th ed. CAB International, Oxfordshire UK. Pp 223-426.

Tchounwou PB, Yedjou CG, Patlolla AK and Sutton DJ (2012). Heavy metals toxicity and the environment. National Institute of Health, Public Access 101: 133-164. Doi: https://doi.org/10.1007/978-3-7643-8340-4_6

Thomas DSG and Shaw PA (1991). The Kalahari environment. Cambridge University Press.

United Nations Office on Drugs and Crime (UNODC) (2009). Guidance for the validation of analytical methodology and calibration of equipment used for testing of illicit drugs in seized materials and biological specimens. Laboratory and Scientific Section UNODC, Vienna, United Nations, New York

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. Available from https://www.epa.gov/esam/us-epa-method-3051a-microwave-assisted-acid-digestion-sediments-sludges-and-oils (Accessed 24 April 2020)

Van Der Kolk JH, Van Leeuwen JPTM, Van Den Belt AJM, Van Schaik RHN and Schaftenaar W (2008). Subclinical hypocalcaemia in captive elephants (*Elephas maximus*). Veterinary Record 162(15): 475-479. Doi: https://doi.ort/10.1136/vr.162.15.475

van Rooyen N and van Rooyen G (2017). Ecological evaluation of Tswalu Kalahari Reserve. An Ecological Report compiled by Ecotrust CC

van Rooyen AF (1993). Variation in body condition of impala and nyala in relation to social status and reproduction. African Journal of Wildlife Research 23: 36-38.

Van Ryssen (2001). Geographical distribution of the selenium status of herbivores in South Africa. South African Journal of Animal Science 31(1): 1-9. Doi: https://doi.org/10.4314/sajas.v31i1.3840

Wang L, D'Odorico P, Ringrose s, Coetzee SH and Macko SA (2007). Biochemistry of Kalahari sands. Journal of Arid Environments 71(3): 259-279. Doi: https://doi.org/10.1016/j.jaridenv.2007.03.016

Warne RW (2014). The micro and macro of nutrients across biological scales. Integrative and Comparative Biology 54(5): 864-872. Doi: https://doi.org/10.1093/icb/icu071

Warne RW, Gilman CA, Garcia DA and Wolf BO (2012). Capital breeding and allocation to life-history demands are highly plastic in lizards. American Naturalist 180(1): 130-41. Doi: https://doi.org/10.1086/665995.

Warne RW, Pershall AD and Wolf BO (2010). Linking precipitation and C_3-C_4 plant production to resource dynamics in higher-trophic-level consumers. Ecology 91(6): 1628-38. Doi: https://doi.org/10.1890/08-1471.1

Waterman RC, Kelly WL, Larson CK and Petersen MK (2017). Comparison of supplemental cobalt form on fibre digestion and cobalamin concentrations in cattle. The Journal of Agricultural Science 155 (5): 832-838. Doi: https://doi.org/10.1017/S0021859617000107

Weaver CM and Hill Gallant KM (2014). Nutrition In: Burr DB and Allen MR (Eds) Basic and Applied Bone Biology 2nd edn. Academic Press Pp 283-297.

Webster AB, Rossouw R, Callealta FJ, Bennett NC and Ganswindt A (2021). Assessment of trace element concentrations in sediment and vegetation of mesic and arid African savannahs as indicators of ecosystem health. Science of the Total Environment 760: 143358. Doi: https://doi.org/10.1016/j.scitotenv.2020.143358

Webster AB, Rossouw R, Small C, Callealta FJ, Bennett NC and Ganswindt A (2021a). Assessment of trace elements in African savannah ecosystems using a non-invasive approach and ICP-MS quantification. Method X (Under review)

Wenting E, Siepel H and Jansen PA (2020). Stoichiometric variation with and between a terrestrial herbivorous and a semi-aquatic carnivorous mammal. Journal of Trace elements in Medicine and Biology 62: 126622. Doi: https://doi.org/10.1016/j.jtemb.2020.126622

World Weather Online Skukuza, Kruger National Park, Mpumalanga Province https://www.worldweatheronline.com/lang/en-in/skukuza-weather-averages/mpumalanga/za.aspx (Accessed 30 July 2020)

World Weather Online Van Zylsrus, Northern Cape Province https://www.worldweatheronline.com/van-zylsrus-weather-averages/northern-cape/za.aspx (Accessed 30 July 2020)

Yamaguchi M (2010). Role of nutritional zinc in the prevention of osteoporosis. Molecular and Cellular Biochemistry 338(1-2): 241-54. Doi: https://doi.org/10.1007/s11010-009-0358-0

Zhu Z, McKendry R and Chavez CL (2000). Signalling in copper ion homeostasis In: Storey KB and Storey J (Eds) Environmental Stressors and Gene Responses. Elsevier Science BV. Pp 293