Assessment of trace element concentrations in sediment and vegetation of mesic and arid African savannahs as indicators of ecosystem health

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Highlights

- No established guidelines for allowable levels in South African protected areas
- Matrix-specific differences were evident.
- Potentially toxic element combinations were present at both sites.
- Evidence of geological influence and anthropogenic contamination at both sites
- Risk assessment is essential to maintaining ecosystem integrity.

ABSTRACT

The savannah biome supports unique biodiversity and provides a multitude of ecosystem services. Defining background concentrations for trace elements in the environment is beneficial for the determination of nutrient deficiencies/hotspots and for the management of pollution. Sediment and corresponding vegetation samples were collected around 48 surface water points in two savannah wildlife areas for assessment and comparison of 20 trace elements using ICP-MS. Site-specific and matrix-specific differences were evident for essential *B, Co, Cu, Fe, Mn, Mo, Ni, Se* and *Zn*, potentially toxic *As, Cd, Cr, Hg, Pb* and *V* and additional elements *Al, Ba, Sb, Sn* and *Sr* analysed. Sediment and vegetation from all sampled locations at both sites contained single or multiple potentially toxic elements at various concentrations. Although the presence of all elements can be linked to underlying geology and geochemistry specific to each site, evidence of anthropogenic cause was also

evident at both sites. This paper covers the widest range of trace elements assessed in protected terrestrial wildlife reserves in the South African savannah biome to date and highlights the potential for deleterious consequences of trace element contamination of the environment.

Graphical abstract

Key words: Environmental risk assessment, Ecosystem integrity, Heavy metals, Potentially toxic elements, South African savanna biome, Wildlife management

1. Introduction

The savannah biome covers over 50% of the African Continent (Osborne et al. 2018) and about one third of South Africa. The biome extends from the Eastern Cape through Kwazulu-Natal, the Mpumalanga and Limpopo mesic Lowveld to the North West Province and arid Kalahari region of the Northern Cape (Mucina and Rutherford 2006). A defining characteristic of savannahs is the co-dominance of C_4 shade-intolerant grasses interspersed with a woody

layer of shrubs and trees (Sankaran et al. 2005; Charles-Dominique et al. 2015). The vegetation structure within these ecosystems is substantially influenced by the physical interaction between water, geologic substrate and soils (Knoop and Walker 1985; Vaughn et al. 2015). Additionally, rainfall, herbivory (Coetsee et al. 2019; Owen-Smith et al. 2019) and fire (Higgins et al. 2000; Bond and Keely 2005; Bond 2008;Ribiero et al. 2019) are important drivers of broad and fine-scale spatio-temporal patterns in savannah biomes (Smit et al. 2013). These factors foster productive and diverse ecosystems within the biome that provide a multitude of essential ecosystem services for income generation and development to tens of millions of people (UNEP 2013; World Tourism Organisation 2015; Ryan et al. 2016; Osborne et al. 2018).

Given that an average of $200 per annum is spent per agricultural producer on research and extension in developed countries, soils and crops have been extensively studied (Steyn and Herselman 2005). In contrast, only about $4 per annum is spent in developing countries (Hartemink 2002). As a result, well-established guidelines for permissible limits of trace elements within South Africa are not available (Hartemink 2002; Steyn and Herselman 2005). The absence of enforceable guidelines specific to the geology of different regions within South Africa outside of agricultural practice, coupled with limited environmental legislation and implementation of policies, makes the evaluation of trace element concentrations in specific areas of South Africa a challenge (Herselman et al. 2005). As countless fundamental physiological processes in both plants and animals can be influenced by very low levels, or combinations of organic and trace element pollutants, ecosystems may be vulnerable to their effects (Herselman 2007; Rhind 2009).

The determination and evaluation of trace element content based on parent rock material is complicated (Herselman 2007). Localised impact from environmental and anthropogenic influences specific to that area, substantial geographical differences in underlying geology and geochemistry unique to different regions are factors to consider (McNaughton and Georgiadis 1986; Steyn and Herselman 2005). The specific physical, chemical and biological

properties transferred from parent rock material indicate the functional ability of soils within ecosystems (Kabata-Pendias 2011). Further, these properties determine a soil's ability to perform ecosystem services (Reicosky 2018). Soils are the primary source of trace element transfer into the food chain and bioavailability in soils for absorption by plants is largely dependent on trace element speciation (Kabata-Pendias 2011). Additionally, pH, texture, porosity, organic content, microbe community as well as ion and gaseous exchange capacity are physical characteristics of soils that also influence bioavailability (Beyersmann and Hartwig 2008; Olaniran et al. 2013). Micronutrients, nitrogen, phosphorus, potassium, calcium, magnesium and sulphur are essential to photosynthesis, respiration, cell signalling and many other functions in plants (Epstein and Bloom 2005). In moderate amounts, trace elements *B, Co, Cu, Fe, Mn, Mo, Ni, Se* and *Zn* are also considered essential for normal processes in higher plants (Shahid et al. 2015). Low concentrations of trace elements in soils are expected given their origin, but may exert biological effects disproportionate to their concentrations (Herselman 2007; Rhind 2009). At high concentrations however, trace elements can disrupt optimal plant function and result in toxic effects (Antoniadis et al. 2019). Atmospheric deposition of trace-element-containing particulate matter on leaf surfaces and subsequent adsorption and internalisation through the plant cuticle or trace element diffusion via stomatal openings are important pathways associated with foliar uptake of trace elements (Schreck et al. 2014; Shahid et al. 2017). Plant morphology, physicochemical characteristics of the plant cuticle, habitat and species-specific ion exchange are factors that influence foliar absorption (Beckett et al. 2000). As soil to root transfer and contamination via foliar uptake occurs simultaneously, determining the origin of trace elements in plant tissues is complex (Shahid et al. 2017).

In addition to land transformation, loss of biodiversity (Chapin et al. 2000; Coughenour 2008) and climate change (Osborne et al. 2018), anthropogenic activities associated with trace element pollution are also increasing to meet the demands of a growing human population across the globe (Steyn and Herselman 2005; He et al. 2015; Masindi and Meudi 2018). In this regard, trace element toxicity is a concern of increasing importance for ecological,

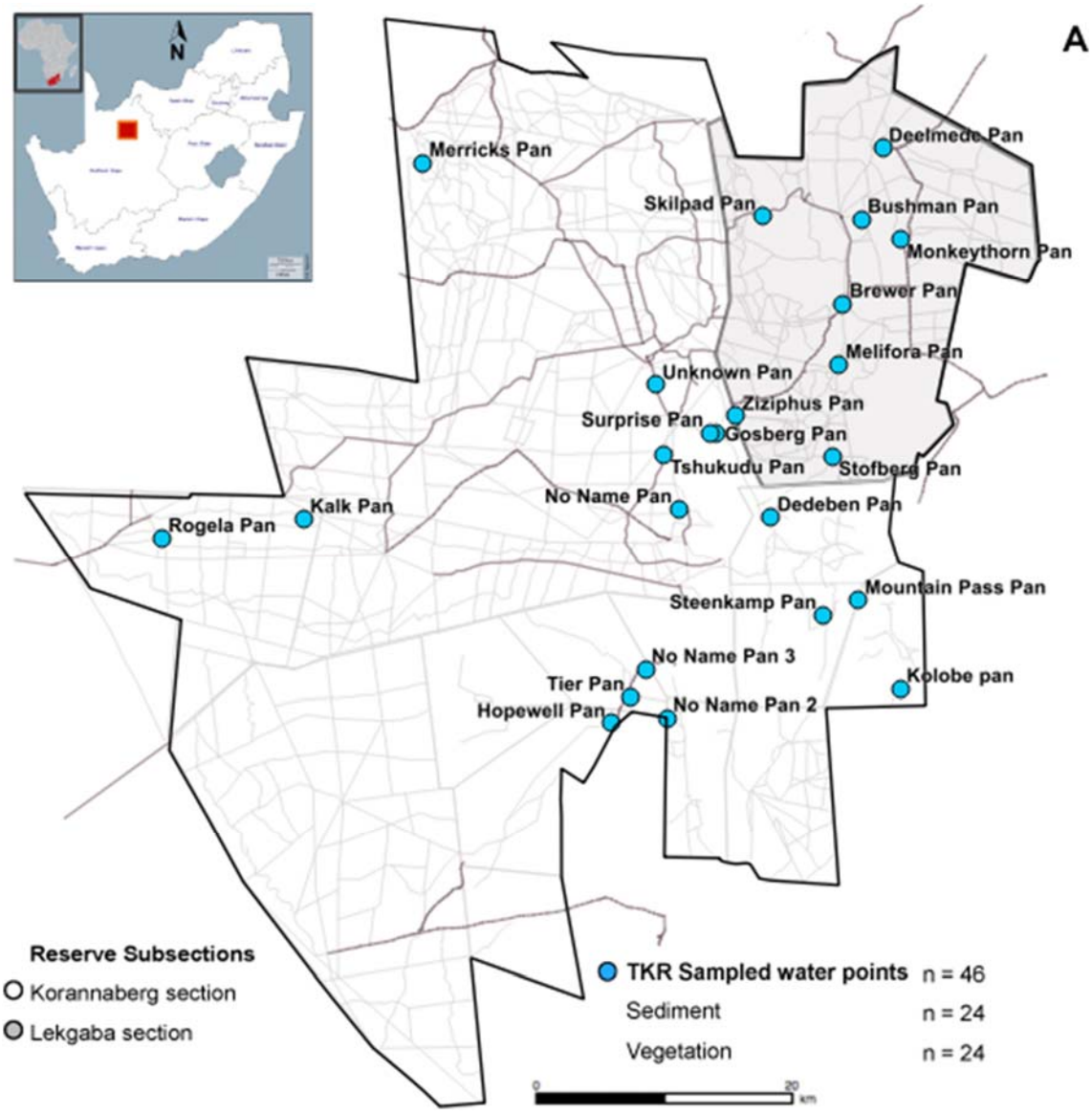
environmental and evolutionary reasons (Nagajyoti et al. 2010). The deleterious effects of mining and associated activities are well documented and have been addressed in detail elsewhere (WHO 1996; McCarthy 2011; Fashola et al. 2016; Shongwe 2018; Olufemi et al. 2018). Atmospheric emissions of trace elements absorbed by dust particles are transported over large geographical distances by wind and water currents. With pollutant levels already above United Nations recommended limits, air quality in South Africa continues to deteriorate (UNEP 2006). Common household items and personal care products that contain trace elements (e.g. selenium) and other pollutants (WHO/UNEP 2012) make their way into water systems where they accumulate and are transported to different regions (ASTDR 2003). Trace elements contained in natural grazing, supplement feed, fertilizers or from nearby industry may contaminate manure (Henja et al. 2017). Subsequent runoff may result in surface and/or ground water contamination (Richards et al. 2014).

Watercourses that transport chemical and trace element contaminants from upstream (Riddell et al. 2019), wind currents that transport atmospheric pollutants to regions far removed from source (UNEP 2006; SA DEA 2012), and anthropogenic activities occurring within or adjacent to protected areas (Shongwe 2018) contribute to contamination of these biodiversity strongholds. Given the absence of information related to trace element contamination in South African protected areas, the overall aim of this paper was to evaluate and compare gross concentrations of 20 trace elements in sediment and vegetation around surface water points within protected areas of South Africa's mesic and arid savannah biome. Our objectives were more specifically to (1) evaluate and compare overall differences in trace element concentrations in sediment and vegetation between sites. (2) Compare measured concentrations at each site with total investigation levels (TIL) or allowable limits where possible. (3) Determine inter-element associations and spatial variations in sediment and vegetation concentrations within each site. (4) Evaluate whether trace element concentrations in vegetation reflected the same profile as was detected in sediment at each site. (5) Resulting information was then used to assess the influence of site-specific geology and anthropogenic influence on trace element concentrations at each site.

2. Materials and methods

2.1. Study sites

To evaluate trace element concentrations in arid and mesic ecosystems within South Africa's savannah biome, sediment and vegetation samples were collected around water points within Tswalu Kalahari Reserve (TKR), Northern Cape Province, and the Manyeleti Nature Reserve (MNR), Mpumalanga Province, South Africa from April to June and July to September 2019 respectively (Figure 1).



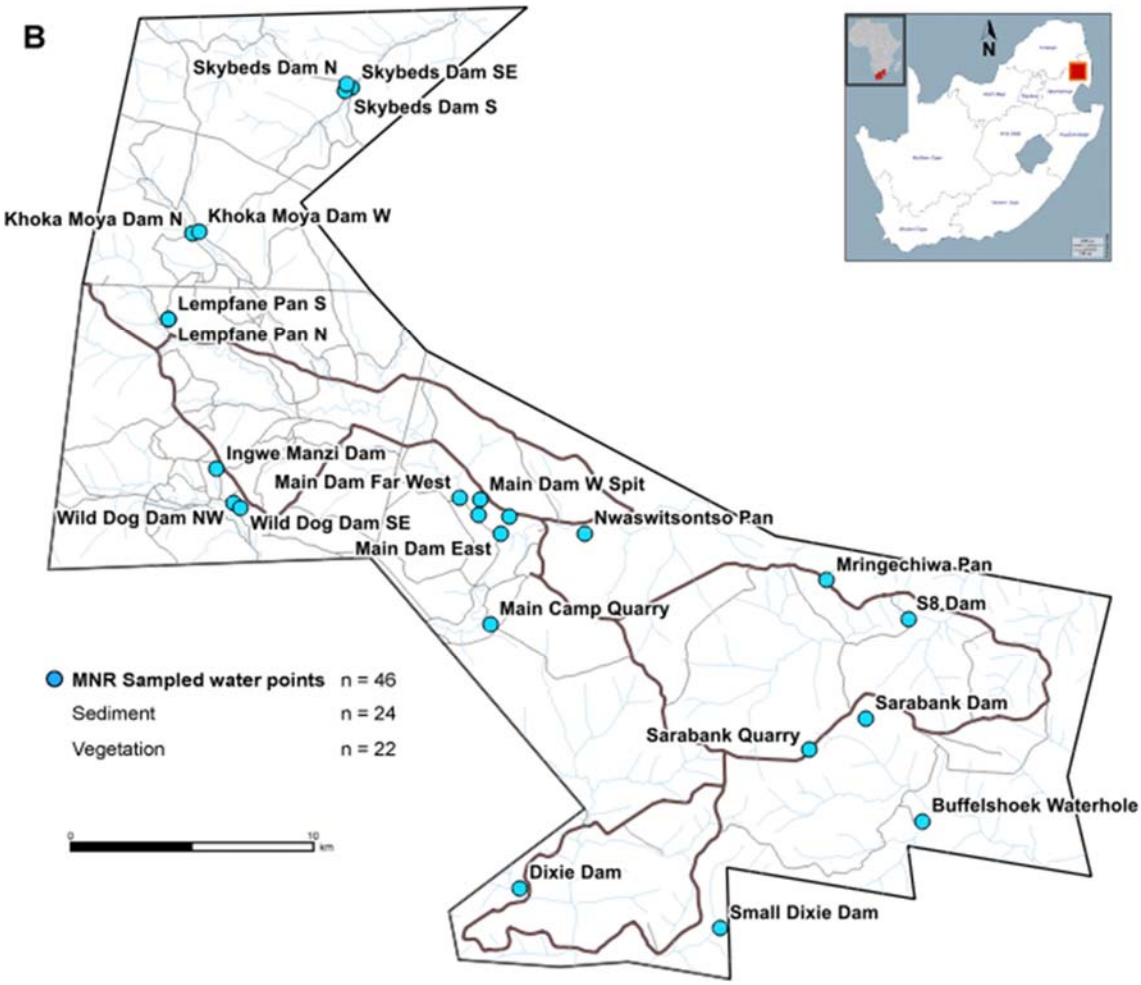


Figure 1. Sampled locations within the arid Tswalu Kalahari Reserve (1A). Sediment and vegetation samples were collected from all marked locations within the Korannaberg section (white: ± 101 000 ha) and the Lekgaba section (grey: ± 20 000 ha). Sampled locations within the mesic Manyeleti Nature Reserve (1B). Sediment was collected from all marked locations and vegetation was collected from all marked locations across the ± 22 497 ha reserve except Main Dam Middle South side and Lempfane Pan N.

2.1.1. Tswalu Kalahari Reserve

Tswalu Kalahari Reserve (TKR) established from rehabilitated livestock farms is situated at S 27°29'61" and E 22°39'43" and is South Africa's largest privately owned game reserve (Figure 1A). The Korannaberg mountain range comprised of subgraywacke, quartzite, slate, dolomite, jasper and conglomerate of the Matsap Formation and Volop Group, runs north to south through the reserve (Visser 1983; Haddon 2005). Aeolian sands of the Gordonia formation are the substrate of sandy plains, dunes and dune valleys (van Rooyen and van Rooyen 2017). Major vegetation types of the TKR include Gordonia Plains Shrubland, Gordonia Duneveld, Kathu Bushveld, Koronna-Langeberg Mountain Bushveld and

Olifantshoek Plains Thornveld (Mucina and Rutherford 2006; van Rooyen and van Rooyen 2017). Anthropogenic activity in surrounding areas is associated with manganese mining within the Kalahari manganese field and power generation at thermal and solar facilities (Taljaardt 1982; Assmang 2016; Wildenboer 2018). Despite the lower resource availability typical of arid savannahs, livestock and game farming are major industries in the region (Sporton and Thomos 2002).

2.1.2. Manyeleti Nature Reserve

The Manyeleti Nature Reserve (MNR) is situated at S 24°64’80” and E 31°52’63” within the Ehlanzeni North District of Bushbuckridge Local Municipality (Figure 1B). The property shares unfenced borders with the Kruger National Park to the east, the Sabi Sand Game Reserve to the south and the Timbavati Private Nature Reserve to the northwest. Granite koppies of Mohlareng and Dixie dominate the north and south respectively. Archean course-grained biotite granite and gneisses underlie the majority of the central, eastern and southern areas. The north western portions overlay part of the extensive Timbavati Gabbro dyke, which have high clay content in derived soils (Breedenkamp1983; Breedenkamp 1985; MTPA 2015). 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. Seasonal watercourses form part of the watershed between the Timbavati River in the north and Sand River in the south (Cronje et al. 2005). The western boundary borders a number of rural communities (MTPA 2015). Anthropogenic activities in these villages include subsistence livestock and crop farming. Entrepreneurial pursuits focus mainly on auto repair, home construction, leather goods, electronic waste salvage, curios and handcrafts. Landfills are used for waste disposal, although burning of all rubbish is frequent. Community boreholes are the main source of water for household use. Natural and artificial water reservoirs are used by community members for subsistence crop irrigation and other general requirements (A. Webster, Pers. obs. and pers. comms. Mnisi Tribal Authority, Hluvukani Village).

2.2. Sample collection

Natural water bodies at each site were subjectively evaluated for size and proximity to four common plant species widely distributed across each reserve. Single mud-based sediment samples (± 50 g) were collected 3 - 5 cm below the surface (Nel et al. 2015) by hand (TKR: n = 24; MNR: n = 24) at a central point near the water's edge. The use of metallic sampling equipment was avoided to minimise contamination. At water bodies > 20 m^2, single sites were sampled for sediment and corresponding vegetation. At water bodies > 50 m^2, northeast and southwest sediment and vegetation samples were collected and at water bodies > 100 m^2, north-south-east-west samples were collected where possible. Leaf vegetation within ± 50 m of sediment collection was stripped randomly from different parts of each plant using gloves to avoid contamination. At the TKR site, leaves from Buffalo thorn (*Ziziphus mucronata)*, Camel thorn (*Vachelia erioloba*), Small-leaved/Common guarri (*Euclea undulata) and Velvet raisin (Grevia flava*) were collected (n = 24). At the MNR site, leaves from Buffalo thorn, Leadwood (*Combretum imberbe),* Blue guarri *(Euclea crispa)* and Red bushwillow *(Combretum apiculatum*) were collected (n = 22). Sediment and vegetation samples were placed in separate Ziplock bags, labelled and immediately frozen at -20 °C.

2.3. Sample preparation and digestion for ICP-MS analysis

Sediment and vegetation samples were lyophilised at -50 °C for 5 days and individually milled for 5 minutes at 90 rpm using a Retsch® agate ball mill to homogenise. Individual sediment (± 0.1 g) and vegetation (± 0.3 g) samples were microwave digested (MARS®-5) in 6 ml (65%) HNO_3: 2 ml (30%) HCl and 6.5 ml (65%) HNO_3: 0.5 ml (30%) HCl respectively (USEPA 2007). Post-digestion, de-ionised 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 quantification, adjustments and validation are outlined in Webster et al. (under review). 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, ^{27}Al, ^{51}V, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{75}As, ^{88}Sr, ^{95}Mo, ^{111}Cd, ^{118}Sn, ^{121}Sb, ^{137}Ba, ^{202}Hg and ^{208}Pb. Inter-element corrections were made for possible isobaric interferences of high ^{115}Sn on ^{115}In used as internal standard. Lead was expressed as the sum of isotope masses 206, 207 and 208. 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

Each set of respective matrix digestions included dual quality controls. A blank acid mixture together with WQB-1 Lake Ontario sediment (~0.1 g) Certified Reference Material (National Water Research Institute, Burlington, Ontario, Canada), and 1573a tomato leaf (~0.3 g) Certified Reference Material (National Institute for Science and Technology, Gaithersburg, USA) were used as controls for sediment and vegetation respectively. 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. Permissible levels for trace elements in South African soils

An enforceable set of guidelines for allowable limits of trace elements in soils and vegetation specific to South Africa has not yet been determined. Criteria set by the Department of National Health and Population Development (Unpublished report 2nd Draft SA DNHPD 1991) were revised in 1997 (SAWRC 1997) as extraction errors produced inaccurate values (Steyn and Herselman 2005). Using USEPA method (3050), 4500 topsoil samples were digested in aqua regia and baseline concentrations for South African soils were subsequently

derived (Herselman et al. 2005). The natural background levels of measured elements were then used as guidelines for investigation and maximum threshold levels in soils that have been used in this study and are summarized in Table 1.

Table 1. Existing and recommended values for trace element levels in South African soils.

Trace element	Maximum permissible value for soil (SADNHPD 1991)	Range in South African top soils	Total Investigation Level (TIL) (Herselman et al. 2005)	Total Maximum threshold level
	mg/kg	*mg/kg*	*mg/kg*	*mg/kg*
Zn	185	12.0 - 115	185	200
Cr	80	5.82 - 353	80	350
Ni	15	3.43 - 159	50	150
Cu	100	2.98 - 117	100	120
Co	20	1.51 - 68.5		-
Pb	56	2.99 - 65.8	56	100
As	2.0	-	2	2
Cd	2.0	0.62 - 2.74	2	3
Hg	0.5	-	0.5	1

2.7. Data analysis

All statistical analyses were conducted using the R software (version 3.6.1; R Core Team 2019). Descriptive statistics were calculated for each trace element in sediment and vegetation respectively for each site. Mean values in sediment were subsequently compared to available total investigation levels (TIL) for South African soils (Herselman et al. 2005) and against Department of National Health and Population Development (1991) value for *Co* (SADNHPD 1991). Mean values for vegetation were compared to available World Health Organisation (1996) guidelines. Differences between trace element concentrations at protected area sites were tested using the Student's t-test for two independent sample sets (where t= test statistic; df= degrees of freedom; n= sample size; p= significance and η= effect size: Pearson's r) if normality could be assumed. The Approximate Two-Sample Fisher-Pitman Permutation Test (where Z= test statistic; p= significance and η= effect size) was used when normality could not be assumed. Normality was tested using the Shapiro-Wilk

test. Homogeneity of variances was checked using the Levene's test and the Fligner-Killeen's test. Statistical significance was determined at the $p < 0.05$ α level, unless otherwise indicated.

To determine the relative abundance of each element within sediment and vegetation respectively at each site, the scales of measure were homogenised (mean = 1) by dividing each variable by its mean. Consequently, the Pearson's Variation coefficient was used to measure the variability in abundance of each element at each sampling site. Cluster analysis using absolute values of the Pearson's correlation coefficient as proximity measure and Adequation analysis were both used to identify and select the groups of more associated metals. To determine inter-element associations between trace elements in sediment and vegetation respectively relative to geographical location, geology and anthropogenic influence, a Principal Component Analysis (PCA) was conducted using the covariance matrix of transformed data to account for different relative variability of trace elements. Following this, a Varimax rotation was carried out to identify reducible groups of metals and to reduce them to factors. Biplots of waterholes in the Rotated Factor Space helped to identify waterholes in which groups of reducible elements were more or less present. Cluster Analysis of reduced groups using the squared Euclidean distance and Ward.D2 link method was applied to obtain groups of waterholes containing similar abundance of trace elements. Descriptive statistics were used to analyse and describe the differences between groups.

2.8. Statement of ethical approval

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).

3. Results

3.1. Overall comparison in sediment and vegetation between sites

The overall profile of trace element concentrations in sediment between the TKR and MNR sites relative to the mean are summarised in Figure 2. When outliers from both sites were excluded, the differences between trace element concentrations in sediments between sites were tested for significance. Mean concentrations were significantly higher at the TKR site for Group A essential elements *B* and *Mo*, while *Co, Cu, Fe, Mn* and *Zn* were significantly higher at the MNR site. Group B potentially toxic elements, *As, Cd* and *Pb* were significantly higher at the TKR site, while *Cr* and *V* were significantly higher at the MNR site. Group C additional element *Sb* was significantly higher at the TKR site, while *Al* and *Sr* were higher at the MNR site. Statistically significant differences between sites were not evident for *Se*, *Hg Ba* and *Sn.* Statistical test scores for this analysis are supplied in supplementary material (Table S1: column sediment).

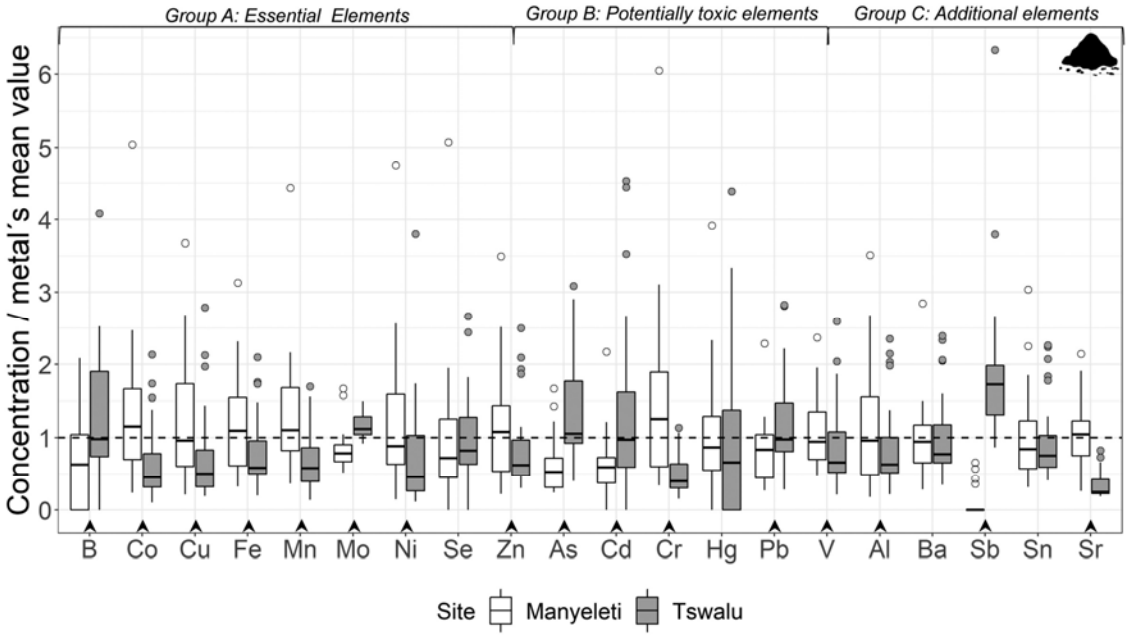
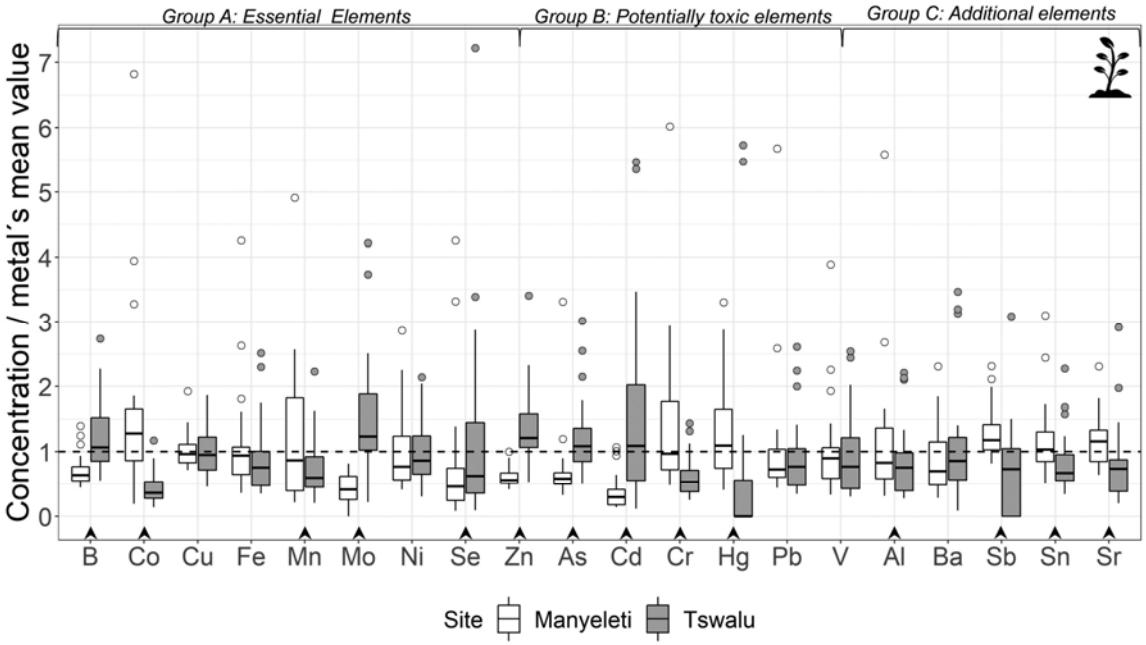


Figure 2. Overall profile of Group A essential, Group B potentially toxic and Group C additional trace elements evaluated in sediment between Tswalu Kalahari Reserve (TKR: grey) and Manyeleti Nature Reserve (MNR: white) relative to the mean for both sites (dashed line). Black arrows above element symbols highlight significant differences in element concentrations between sites. Dark lines within boxes represent median values; edges of the box represent the upper (3rd) and lower (1st) quartiles. Lines extending above and below the box represent minimum and maximum values excluding outliers. Points represent outliers. Transformed data excludes an extreme outlier for *Sr* at Rogela Pan (15.58 outside of scale).

The same overall comparison made for vegetation between the TKR and MNR sites relative to the mean after outliers were excluded are summarised in Figure 3. Mean concentrations were significantly higher at the TKR site for Group A essential elements *B, Mo, Se* and *Zn*, while *Co* and *Mn* were significantly higher at the MNR site. Group B potentially toxic elements *As* and *Cd* were higher at the TKR site, but *Cr* and *Hg* were significantly higher at the MNR site. Group C additional elements *Al*, *Sb*, *Sn* and *Sr* were significantly higher at the MNR site. Statistically significant differences between sites were not evident for *Cu*, *Fe, Pb*, *V* or *Ba*. Statistical test scores for this analysis are supplied in supplementary material (Table S1: column vegetation).



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3.2. Site-specific evaluation of trace element concentrations

3.2.1. Tswalu Kalahari Reserve (TKR)

Trace element concentration mean and SD have been summarised for sediment and vegetation at the TKR sampling site (Table 2). All trace element means in sediment fell below available TIL stipulated by Herselman et al. (2005) or SADNHPD (1991*) for *Co*. In

vegetation, essential elements *Ni* and *Cu* fell below recommended guidelines, while *Zn* concentrations were 42x higher than allowable limits (WHO 1996). Potentially toxic element *Pb* also fell below recommended guidelines, while concentrations for *Cr* were 17% higher and for *Cd* were 3x higher than allowable limits (WHO 1996).

Table 2. Summary of mean and SD trace element concentrations in sediment for comparison against Total Investigation levels (Herselman et al. 2005; SADNHPD 1991*) and in vegetation for comparison against permissible values in plants (WHO 1996) at the TKR sampling site.

Trace element	Sediment (TKR) Mean	Sediment (TKR) SD	Total Investigation Level (Herselman et al. 2005)	Vegetation (TKR) Mean	Vegetation (TKR) SD	Plant Permissible Value (WHO 1996)
	mg/kg	*mg/kg*	*mg/kg*	*mg/kg*	*mg/kg*	*mg/kg*
Group A	**Essential elements for plant physiology and function**					
B	6.44	4.64	**-**	83.25	37.17	**-**
Co	3.78	3.05	**20.00 ***	0.31	0.18	**-**
Cu	11.36	10.04	**100.00**	5.44	2.05	**10.00**
Fe	10568.89	6670.53	**-**	530.42	347.16	**-**
Mn	128.88	79.25	**-**	113.97	70.71	**-**
Mo	0.25	0.03	**-**	0.32	0.20	**-**
Ni	16.64	17.79	**50.00**	4.37	2.28	**10.00**
Se	0.16	0.09	**-**	0.55	0.71	**-**
Zn	17.01	11.97	**185.00**	***25.30***	11.33	**0.6**
Group B	**Potentially Toxic Elements**					
As	1.68	0.94	**2.00**	0.17	0.08	**-**
Cd	0.03	0.03	**2.00**	***0.06***	0.06	**0.02**
Cr	27.42	15.23	**80.00**	***1.53***	0.82	**1.3**
Hg	0.03	0.03	**0.5**	0.02	0.03	**-**
Pb	5.15	2.97	**56.00**	0.29	0.19	**2.00**
V	29.91	19.62	**-**	1.29	0.90	**-**
Group C	**Additional elements**					
Al	16247.93	11349.16	**-**	678.09	470.61	**-**
Ba	94.30	54.28	**-**	120.54	98.98	**-**
Sb	0.10	0.06	**-**	0.01	0.01	**-**
Sn	0.54	0.33	**-**	0.03	0.02	**-**
Sr	30.75	95.71	**-**	116.78	87.70	**-**

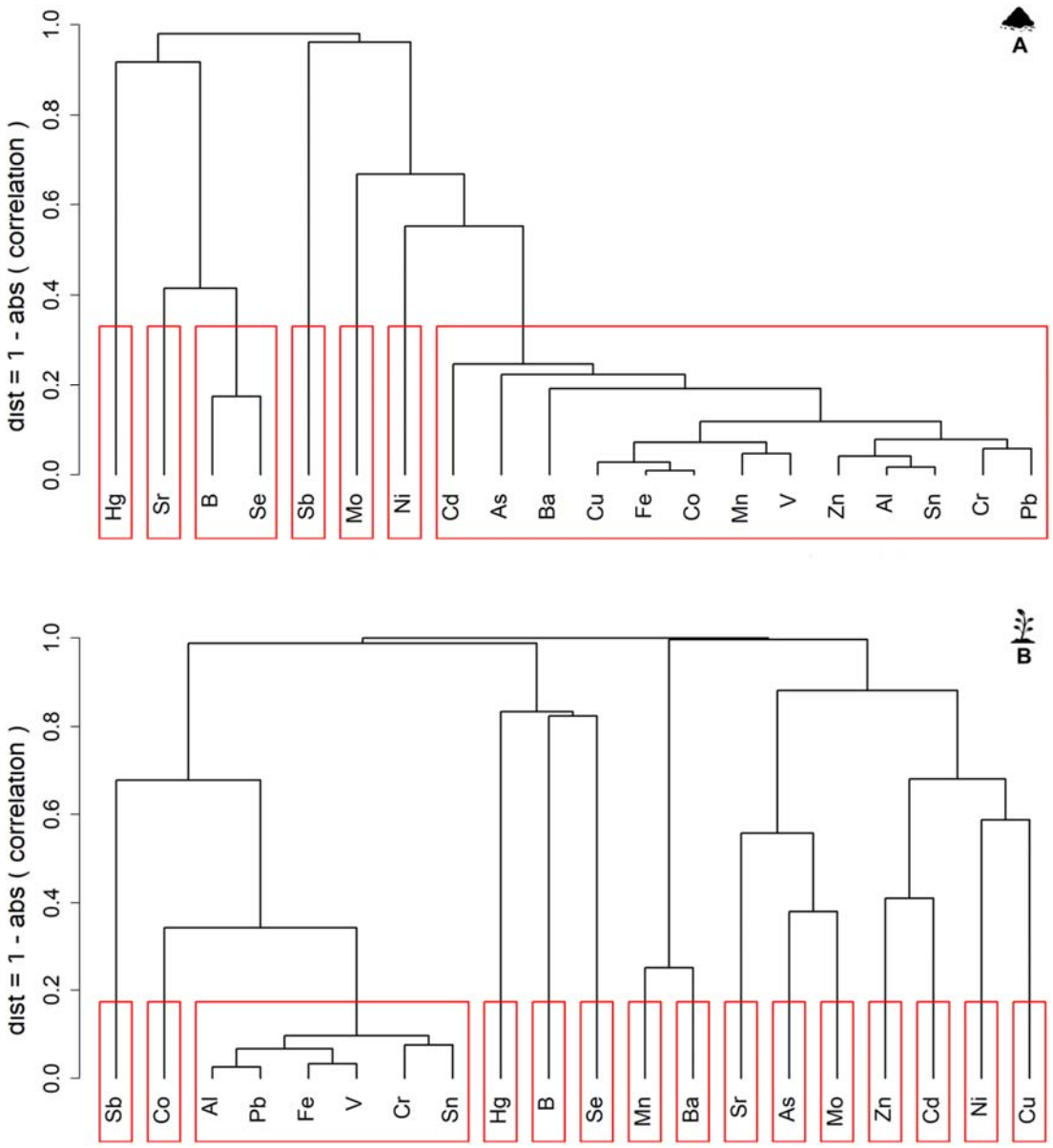

Figure 4. Ward D2 linkage method: Complete cluster-generated dendrograms (minimum 0.75 correlation within groups) highlight different inter-element associations in sediment (4A) and vegetation (4B) at the TKR site.

At the TKR site, inter-element associations differed in sediment and vegetation (Figure 4). In TKR sediment (Figure 4A), close associations in element distribution between sampled locations were evident between essential *Co, Cu, Fe, Mn* and *Zn*, potentially toxic *As, Cd, Cr, Pb* and *V* and additional element *Al.* Highest concentrations of these elements alone or in combination were measured at Surprise Pan, Gosberg Pan, Hopewell Pan and Unknown Pan on the Korannaberg section and at Bushman Pan and Monkeythorn Pan on the Lekgaba section (Figure 5A). In TKR vegetation (Figure 4B), close associations were evident between

essential *Fe*, potentially toxic *Cr, Pb* and *V* and additional elements *Al* and *Sn*. Highest concentrations of these elements alone or in combination were measured at Merricks Pan in the extreme northwest and Tier Pan in the extreme south of the Korannaberg section and at Stofberg Pan and Bushmans Pan on the Lekgaba section. No Name Pan 3 and Hopewell Pan in the south of the Korannaberg section contained disproportionate concentrations of *Cd* when compared to other sites (Figure 5B). Kalk Pan and Rogela Pan in the extreme west had highest concentrations of *As* and *Mo*, comparatively lower levels of *Mn* and the lowest levels of *Ni* compared to other sites on both Korannaberg and Lekgaba sections.

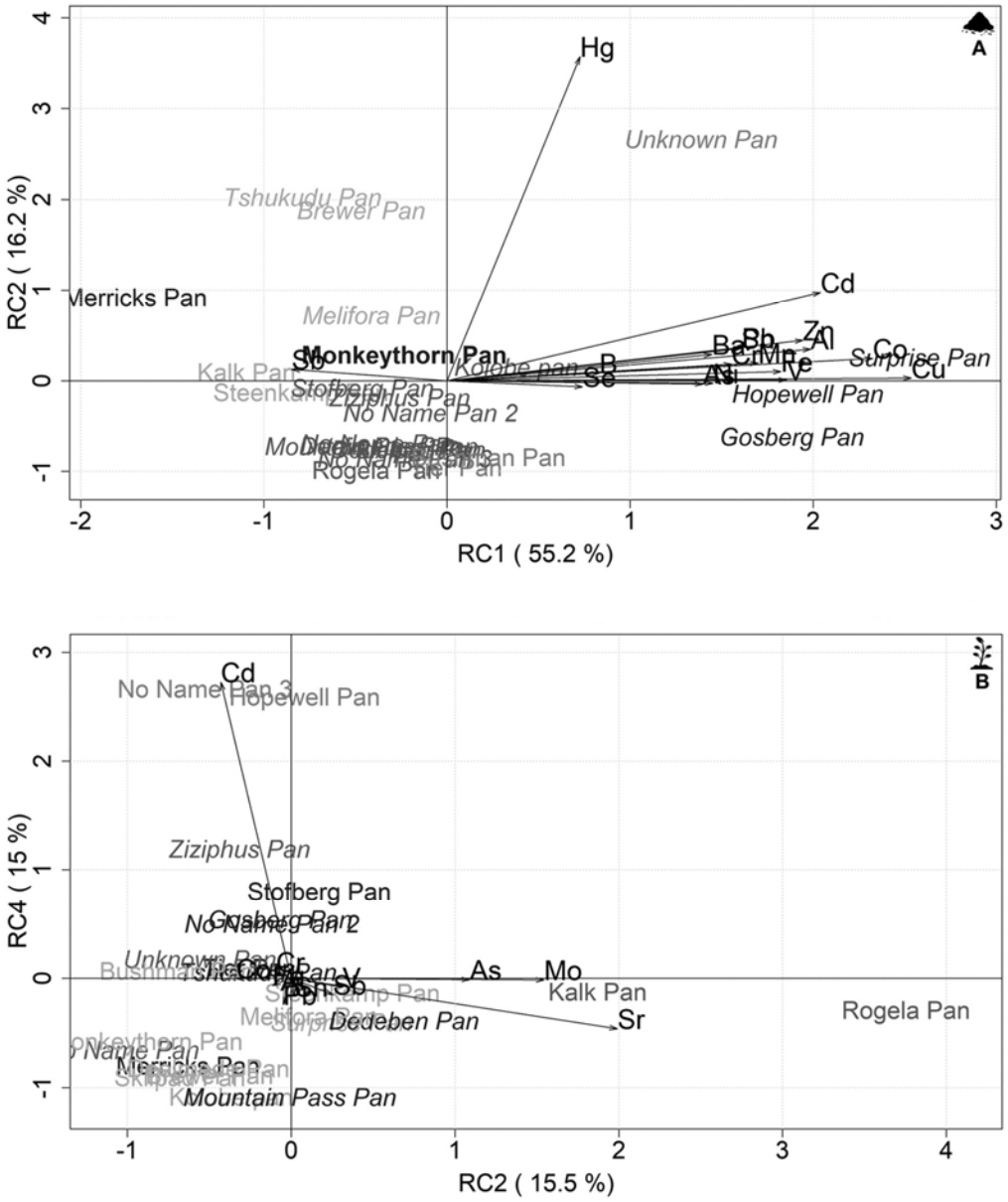

Figure 5. Principal Component Analysis solutions rotated using the Varimax method highlight spatial variation in trace element concentrations in sediment (5A) and vegetation (5B) within TKR sampled sites.

Z-scores from measured elements in TKR sediment on the Korannaberg section identify Merricks Pan *Sb* concentrations at 4σ above the property mean. Rogela Pan in the extreme west had *Sr* concentrations of almost 5σ above the property mean. Surprise Pan, Gosberg Pan, Unknown Pan, Hopewell Pan and Rogela Pan contained disproportionately high levels of *V.* Surprise Pan, Tshukudu Pan and Unknown Pan had highest levels of *Hg* when compared to other sites. On the Lekgaba section, Monkeythorn Pan contained highest levels of *V* and *Ni*, while Mellifera Pan and Bruwer Pan contained the highest levels of *Hg* when compared to other sites. In TKR vegetation, Z-scores from measured elements identify concentrations between 3σ and 4σ above the property mean for *Co* at Tier Pan and *Zn* at No-name Pan 3. Additionally, *Mn* at No-Name Pan 2, *Sn* at Merricks Pan, *Sb* at Surprise Pan and *Se* at Gosberg Pan on the Korannaberg section were high when compared to other sites. Concentrations 3σ and 4σ above the property mean for *Hg* in vegetation at Bruwer Pan and Mellifera Pan on the Lekgaba section mirrored concentrations found in sediment for the same sites. A summary of key results can be found in supplementary material (Table S2).

Potentially toxic elements *As, Cd, Cr, Hg, Pb* and *V* evaluated against the property mean in sediment (Figure 6A) and vegetation (Figure 6B) from sampled locations within the TKR site, were present in various combinations and concentrations at all locations. In sediment, Gosberg Pan, Hopewell Pan, Kolobe Pan, Rogela Pan, Surprise Pan, Tier Pan and Unknown Pan on the Korannaberg section and Bruwer Pan, Bushman Pan and Monkeythorn Pan on the Lekgaba section contained the highest measured concentrations alone or in combination. The vegetation at Merricks Pan, No Name Pan 3 and Tier Pan on the Korannaberg section and Bruwer Pan, Bushman Pan, Mellifera Pan and Stofberg Pan on the Lekgaba sections contained the highest measured concentrations alone or in combination. Essential element and additional element concentrations from sediment and vegetation at sampled locations are available in supplementary material (Figure S1 and Figure S2 respectively).

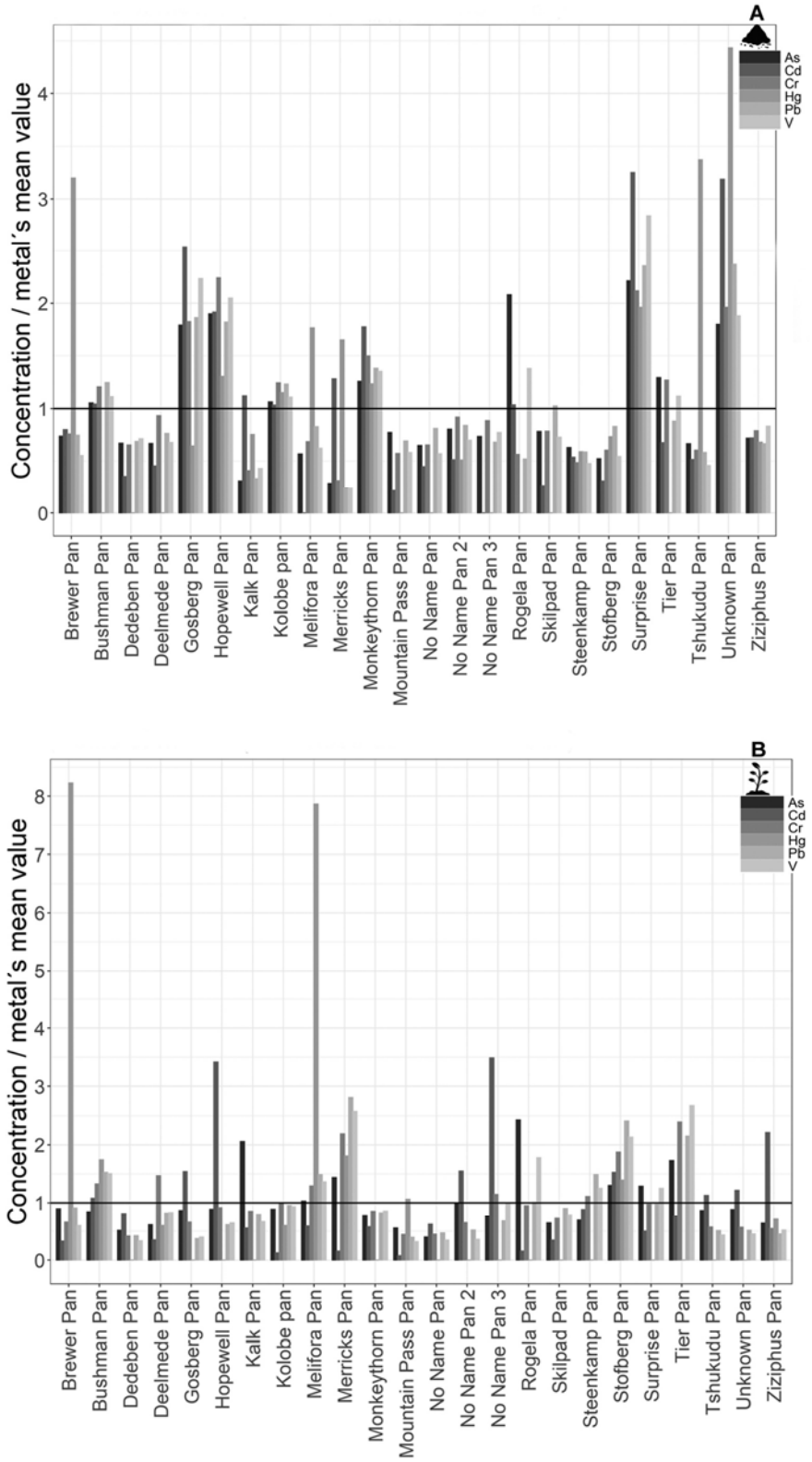

Figure 6. Transformed data for Group B potentially toxic elements against the property mean at sampled water points in sediment (6A) and vegetation (6B) show overall spatial variability within the TKR sampling site.

When mean concentrations of measured elements were evaluated and compared between sediment and vegetation at the TKR site, results were highly variable for most elements. All elements except *B, Ba, Cd, Mo, Se, Sr* and *Zn* were higher in sediment than in vegetation. In contrast, *Mn* concentrations in vegetation were elevated at Merricks Pan, No Name Pan 2, Dedeben Pan and Steenkamp Pan in the Korannaberg section and Mellifera Pan and Stofberg Pan on the Lekgaba section when compared to sediment. Although many elements within sediment and vegetation followed a similar pattern across sampled locations within sites, at specific locations, higher values in sediment or vegetation were not necessarily reflected in the opposing matrix. Significant essential elements *Cu, Mn, Mo* and *Zn a*nd potentially toxic elements *As, Cd, Cr* and *Pb* show variability between sediment and vegetation at the TKR site. These differences can be seen in supplementary material (Figure S3 and Figure S4).

3.2.2. Manyeleti Nature Reserve (MNR)

Trace element concentration mean and SD have been summarised for sediment and vegetation at the MNR sampling site (Table 3). All trace element means for sediments, except *Cr*, fell below available TIL stipulated by Herselman et al. (2005) and SADNHPD 1991*) for *Co*. In vegetation, essential elements *Ni* and *Cu* fell below recommended guidelines, while *Zn* concentrations were almost 20x higher than allowable limits (WHO (1996). Potentially toxic element *Pb* also fell below recommended guidelines at the MNR site, while *Cd* concentrations were equal to the maximum allowable limit. *Cr* concentrations were almost 3x higher than the maximum allowable levels (WHO 1996).

Table 3. Summary of mean and SD trace element concentrations in sediment for comparison against Total Investigation levels (Herselman et al. 2005, SADNHPD 1991) and in vegetation for comparison against permissible values in plants (WHO 1996) at the MNR sampling site.

Trace element	Sediment (MNR) Mean	Sediment (MNR) (SD)	Total Investigation Level (TIL) (Herselman et al. 2005)	Vegetation (MNR) Mean	Vegetation (MNR) (SD)	Plant Permissible Value (WHO 1996)
	mg/kg	*mg/kg*	*mg/kg*	*mg/kg*	*mg/kg*	*mg/kg*
Group A			**Essential elements for plant physiology and function**			
B	3.45	3.27	**-**	47.52	16.56	**-**
Co	7.71	5.92	**20.00 ***	1.18	1.04	**-**
Cu	18.41	13.34	**100.00**	5.69	1.54	**10.00**
Fe	15916.82	9131.98	**-**	668.66	521.20	**-**
Mn	239.49	156.68	**-**	182.85	161.74	**-**
Mo	0.18	0.06	**-**	0.10	0.04	**-**
Ni	27.91	22.63	**50.00**	4.44	2.83	**10.00**
Se	0.14	0.15	**-**	0.37	0.48	**-**
Zn	22.28	15.58	**185.00**	***11.04***	2.77	**0.6**
Group B			**Potentially Toxic Elements**			
As	0.73	0.46	**2.00**	0.10	0.08	**-**
Cd	0.01	0.01	**2.00**	***0.02***	0.01	**0.02**
Cr	***80.90***	69.52	**80.00**	***3.66***	3.14	**1.3**
Hg	0.03	0.03	**0.5**	0.02	0.01	**-**
Pb	3.50	1.91	**56.00**	0.34	0.35	**2.00**
V	35.57	17.17	**-**	1.43	1.08	**-**
Group C			**Additional elements**			
Al	20224.95	14763.02	**-**	934.43	902.71	**-**
Ba	87.21	49.90	**-**	96.68	57.90	**-**
Sb	0.00	0.01	**-**	0.01	0.00	**-**
Sn	0.56	0.37	**-**	0.05	0.02	**-**
Sr	33.54	15.43	**-**	169.48	60.12	**-**

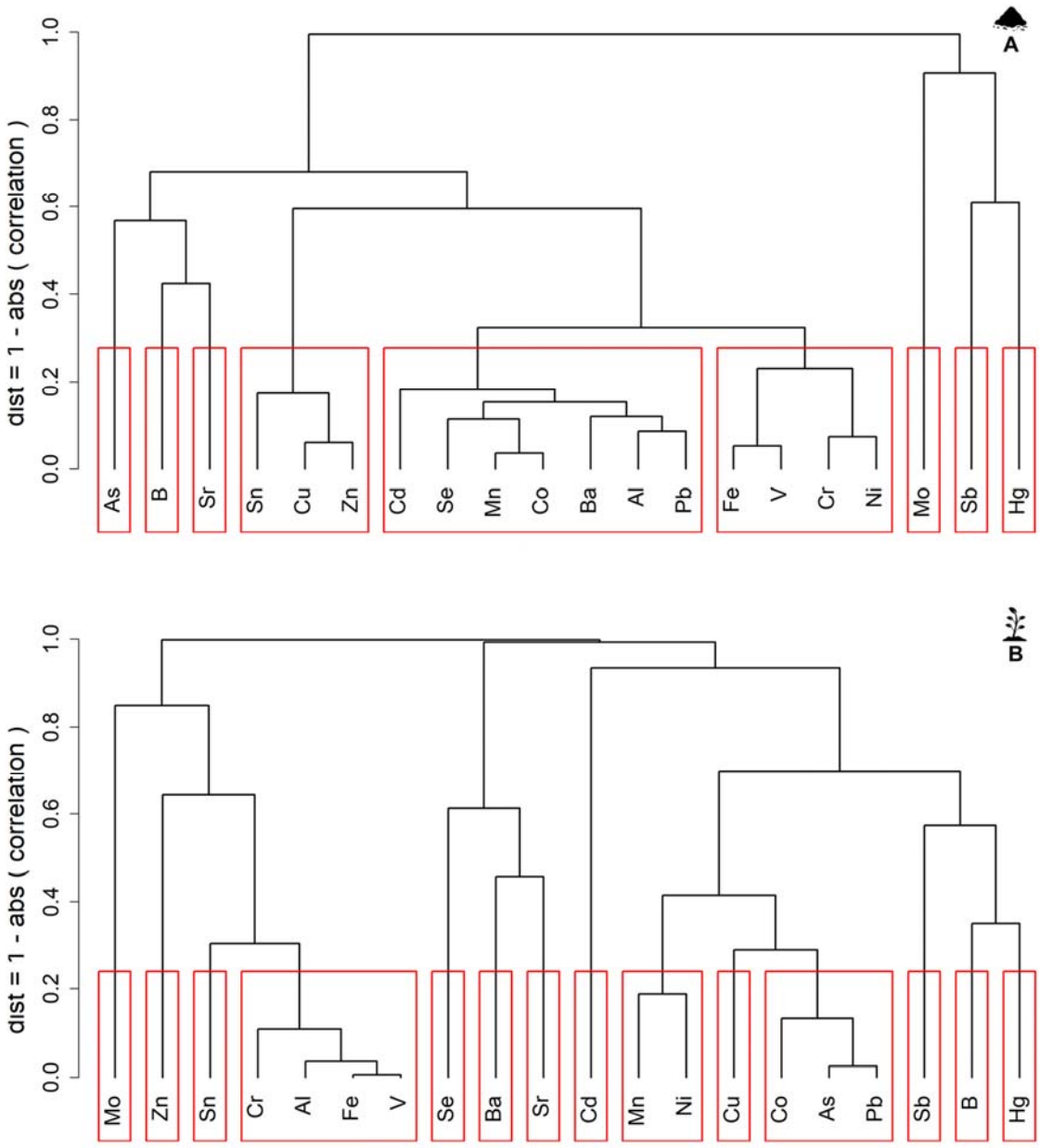

Figure 7. Ward D2 linkage method: Complete cluster-generated dendrograms (minimum 0.75 correlation within groups) highlight different inter-element associations in sediment (7A) and vegetation (7B) at the MNR site.

At the MNR site, inter-element associations differed for sediment and vegetation. In MNR sediment (Figure 7A) close associations between sampled locations were evident for essential *Mn, Co, Fe,* and *Ni* and to some extent *Cu* and *Zn*, potentially toxic *Cd, Cr, Pb* and *V* and additional element *Al*. In MNR vegetation (Figure 7B), a prominent association was evident between essential *Fe*, potentially toxic *Cr* and *V* and additional elements *Al* and *Sn.* A limited association was also evident between essential elements *Cu, Mn* and *Ni* as well as between *Co* and potentially toxic *As* and *Pb*.

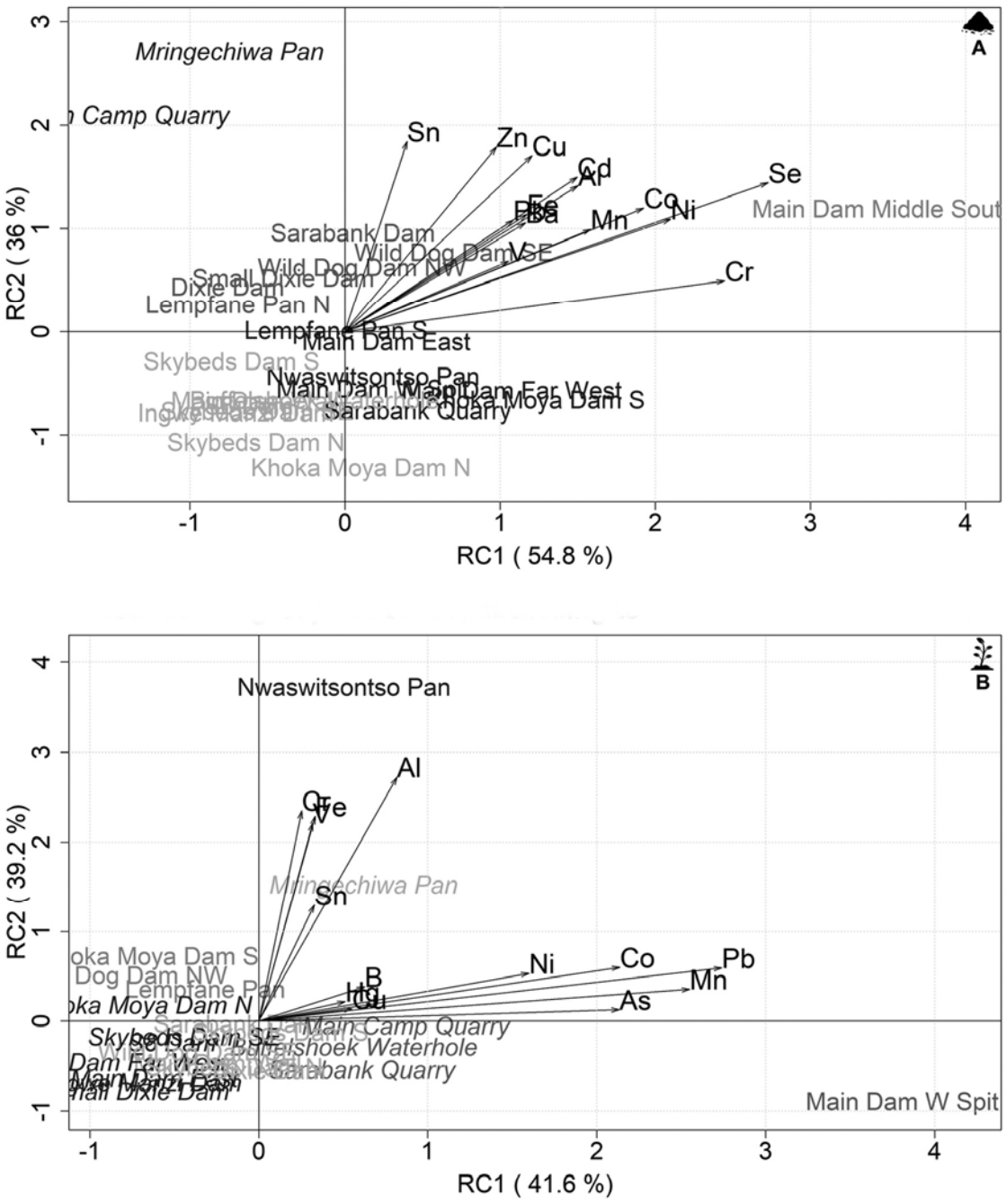

Figure 8. Principal Component Analysis solutions rotated using the Varimax method highlight spatial variation in trace element concentrations in sediment (8A) and vegetation (8B) within MNR sampled sites.

An examination of PC plots from measured elements in sediment at the MNR site (Figure 8A) revealed some spatial distinction in sediment chemistry at specific sites. *Cr* was identified as the most significant element. Concentrations above available TIL were measured for *Cr* at sites around the middle of the reserve, from Wild Dog Dam in the northwest, through the Main Dam and Nwaswitsontso Pan areas to Mringechiwa Pan in the

southeast. Main Camp Quarry had *Ni* concentrations equivalent to TIL, while Main Dam Middle South side exceeded TIL for *Ni.* Mringechiwa Pan contained elevated levels of *Al, Fe* and *Sn* in addition to *Cr* when compared to other sites. Z-scores at Main Dam Middle South side were 3σ and 4σ above the property mean for essential *Co, Mn, Ni* and *Se,* potentially toxic *Cd, Cr* and *Pb* and additional element *Ba.* Values for essential *Ni* and *Co* and potentially toxic *Cr* and *Pb* at this site were greater than any recommended guidelines locally. Although Dixie Dam, Sarabank Dam and S8 Dam contained elevated *Hg* concentrations, Main Dam Western Spit contained the highest *Hg* values in sediment when compared to all other sites.

Z-scores and PC plots for measured elements in vegetation at the MNR site (Figure 8B) highlight Main Dam Western Spit access as having the highest concentrations of essential *Co, Cu, Mn* and *Ni* and potentially toxic *As*, *Cd, Hg,* and *Pb* relative to other sampled sites. *Cu* and *Ni* concentrations were 7% and 25% higher respectively in vegetation than maximum allowable limits (WHO 1996). *Pb* concentrations were just below, while Cd concentrations were 2x higher than maximum allowable limits (WHO 1996). Vegetation at Nwaswitsontso Pan had the highest measured concentrations of essential *Fe*, potentially toxic *Cr, V* and additional element *Al* when compared to other sites. Dixie Dam in the extreme south had highest measured concentrations for *Se*. A summary of key results can be found in supplementary material (Table S2).

Potentially toxic elements *As, Cd, Cr, Hg, Pb* and *V* evaluated against the property mean in sediment (Figure 9A) and vegetation (Figure 9B) from sampled locations within the MNR site, were present in various combinations and concentrations at all locations. Of all sediment at sampled locations in the MNR, all locations except Ingwe Manzi Dam, Main Dam Wall and Skybeds Dam contained one or a combination of toxic elements above the property mean. The same comparison for vegetation highlights Lempfane Pan, Main Dam W Spit, Mringechiwa Pan, Nwaswitsontso Pan and Wild Dog Dam NW with highest concentrations of multiple toxic elements above the property mean. Vegetation at remaining water points

contained all toxic elements with one or more being above the property mean. Essential element and additional element concentrations from sediment and vegetation at sampled locations is available in supplementary material (Figure S5 and Figure S6 respectively).

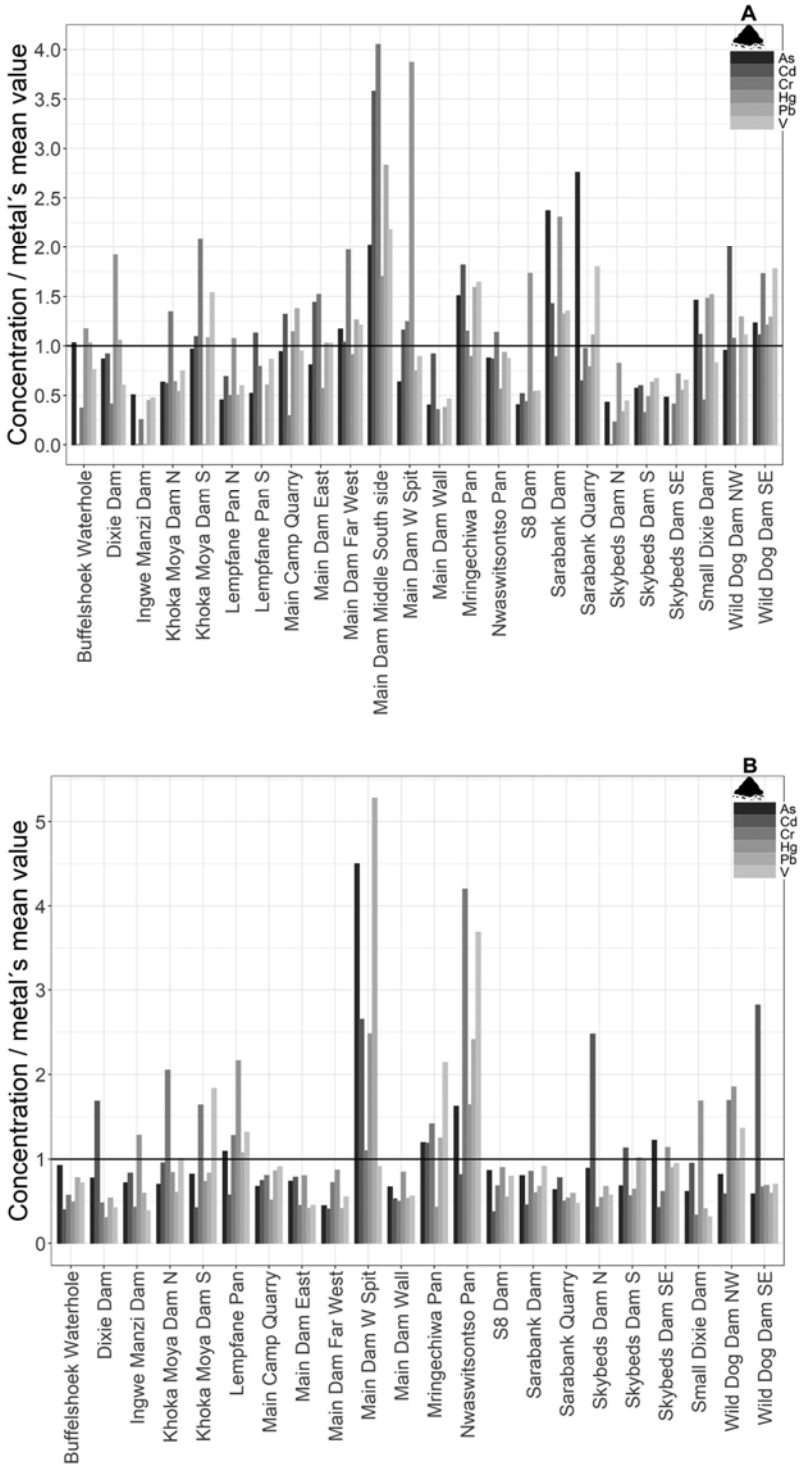

Figure 9. Transformed data for Group B; potentially toxic elements against the property mean at sampled water points in sediment (9A) and vegetation (9B) show overall spatial variability within the MNR sampling site. Absence of vegetation at the Main Dam Middle South Side site prevented sample collection. The proximity of Lempfane Pan N and S forced collection of a combined vegetation sample for both sites.

When mean concentrations of measured elements were evaluated and compared between sediment and vegetation at the MNR site, results were highly variable for most elements. All elements except *B, Ba, Cd, Sb* and *Sr* were higher in sediment than in vegetation. In contrast, *Mn* concentrations at Buffelshoek Waterhole, Sarabank Quarry, Nwaswitsontso Pan and Main Dam East were higher in vegetation than in sediment and *Zn* concentrations were higher in vegetation at Skybeds Dam SE and Skybeds Dam N. As was evident at the TKR site, although many elements within sediment and vegetation followed a similar pattern across sampled locations within sites, at specific locations, higher values in sediment or vegetation were not necessarily reflected in the opposing matrix. At the MNR site, significant essential elements *Co, Cu, Mn* and *Zn* and potentially toxic elements *Cr, Ni, Pb* and *V* demonstrate these differences and can be seen in supplementary material (Figure S7 and Figure S8).

4. DISCUSSION

Defining background concentrations for trace elements in soils and plants is necessary for the determination of nutrient deficiencies/hotspots and for pollution management. However, differences in geological and geochemical specifics at local, regional and global scales complicate matters, particularly when established guidelines are absent (Herselman et al. 2005). Given the absence of established guidelines, “baseline concentrations” reflecting the natural range in which trace elements could be expected in a South African context prior to contamination are used as a reference in this study. Geochemical peculiarities specific to South Africa indicate that although these elements have low bioavailability, concentrations of *Cr* and *Ni* are expected to be high while *Zn, Co* and *Cu* are expected to show deficiencies in concentrations at different spatial intensities (Herselman et al. 2005). In this context, sediment and vegetation trace element concentrations in protected areas within mesic and arid African savannahs were evaluated and compared for the first time.

4.1. Overall differences between sites

Given the geographical and environmental differences between sampled sites, some variation in trace element concentrations was expected. The Hotazel iron ore formation within the Kalahari Manganese field to the north east of the TKR site is extensively mined for *Mn* and *Fe and r*elatively high levels of *Mo, Cr, Ni, Cd, Pb, As, Sb, Se* and *Zn* are associated with the banded iron ores of this region (Varentsova et al. 2019). These associations were reflected to some extent for *Mo, As, Cd, Pb* and *Sb* but not for *Cr, Ni, Se* or *Zn* in sediment at the TKR site. The higher measured concentrations of *Mn* and *Fe* at the MNR site were unexpected given the comparatively lower concentrations of these elements within parent rock at the MNR site. As the majority of TKR *Mn* and *Fe* ore deposits are below the surface, sufficient weathering of the parent material may not have occurred to significantly impact the surrounding area and are therefore not reflected in the top layer of sediment above ground (Tsikos et al. 2003). At the MNR site, Archaean gneiss underlies much of the reserve and prominent intrusions of Timbavati gabbroic rocks are evident in the west (Walraven 1986). Alternating layers of olivine and quartz gabbro in the sections of gabbro closest to the MNR are associated with relatively high levels of *Ba, Cr, Co, Fe* and *Mn, Ni, V* and *Zn* (Walraven 1986). These associations were reflected to some extent in the higher levels of *Cr, Co* and *Ni* present in the measured sediment signature for this site.

With the exception of *B, Ba, Cd, Se* and *Sr*, all other elements at both sites were higher in sediment than in vegetation. Concentrations of essential, potentially toxic and additional elements were highly variable within and between sites and overall, a clear pattern or linear relationship for bioaccumulation of specific elements did not appear to be evident. This could be linked to the types of minerals present in parent rock, the susceptibility of specific minerals to weathering and subsequent degree to which these elements become available in soils (Thornton and Webb 1980). In addition to the physical characteristics of soils, physico-chemical properties specific to each trace element, the bioavailability of each element within the soil and the species-specific abilities of plants to extract these elements from soil solution, further complicate trace element transfer between matrices (Mann et al. 2011; Arif

et al. 2015; Nyika et al. 2019). An anticipated outcome of this study was to establish if trace element and mineral profiles within soils at each study site would generally be reflected in the sampled vegetation and for which elements this would apply (Joy et al. 2015). Results from this study however, indicate that the relationship of most elements in sediment and vegetation was highly variable across sampled locations and between sites.

4.2. Sampling site-specific differences

4.2.1. Tswalu Kalahari Reserve

Upon closer examination of the TKR site, concentrations of specific elements or mixtures of elements were highlighted. The highest concentrations of associated elements *Al, As, Cd, Co, Cu, Cr, Pb, Mn, V* and *Zn* were identified in sediment at Unknown Pan, Surprise Pan, Gosberg Pan and Hopewell Pan in the central and southern parts of the Korannaberg section and at Bushman Pan and Monkeythorn Pan in the north eastern section of Lekgaba. The brown quartzite and subgraywacke ridges in close proximity to these pans is likely to influence the geochemistry of these sediments compared to other pans in the reserve that are more heavily influenced by the abundant Aeolian sand fraction (van Rooyen and van Rooyen 2017). In TKR vegetation, highest concentrations of associated elements *Al, Cr, Fe, Pb, Sn* and *V* were measured at Merricks Pan in the extreme northwest and at Tier Pan in the extreme south of the Korannaberg section as well as Bushmans Pan in the extreme north and Stofberg Pan in the extreme south of the Lekgaba section. There is little similarity in topography between these sites and water supplies are from different sources. The spatial variation may indicate similarities of previous land use, but we were not able to establish an explanation for why these sites, so far removed from each other, seemed to exhibit the highest concentrations of associated elements. Rogela Pan had the highest measured concentration of *Sr* in sediment. A likely explanation lies in the fact that this is the only water point on the property supplied by the Kalahari East Pipeline (A. Webster, pers. comms. W. Jordaan, Conservation Manager TKR). This mega-water project, initiated almost 60 years ago, was recently upgraded to facilitate future water supply to Botswana and meet the increased demands of Kalahari mining operations, solar parks and communities and

livestock farms in the Hotazel, Olifantshoek and Khatu regions bordering the TKR (Energy and Mining News, South Africa 2016). Although stable forms of *Sr* are not considered particularly toxic to plants, combination effects may compromise growth (Seregin and Kozhevnikova 2004) and influence calcium uptake ability (Burger and Lichtscheidl 2019). The highest measured concentrations of *Mo* in vegetation at Rogela Pan and Kalk Pan in the extreme west were not reflected in sediment at these sites. Anthropogenic contamination from coal combustion or current and historic use of phosphate fertilizers may be possible sources.

Due to its latent toxicity and carcinogenicity, antimony has been included as a priority pollutant by the United States Environmental Protection Agency (Coralles et al. 2014; Zhou et al. 2018). When *Sb* is present in soil solution, it is easily absorbed by some plant species (Shtangeeva et al. 2012) and at high concentrations may compromise photosynthesis, synthesis of various metabolites and growth (Feng et al. 2013). The higher concentrations of this element measured at Merricks Pan are therefore a concern and may be related to atmospheric pollution. Further investigation into the source of these concentrations would be prudent. The high concentrations of *Hg* in sediment at Unknown Pan, Surprise Pan and Tshukudu Pan on the Korannaberg Section and in sediment and vegetation at Mellifera Pan and Bruwer Pan on the Lekgaba section are of concern given the potential effects on plants and final consumers. Affected ground water may be a possible source, but a more logical explanation for the localized influence may be related to the landing strip north of Dedeben.

Boron, essential for normal functioning of vascular plants (Warington 1923; Butterwick et al. 1989; Epstein and Bloom 2005; Matthes et al. 2020) primarily exists as boric acid ($B(OH)_3$) in soil solution (Camacho-Cristóbal et al. 2008). In contrast to high rainfall conditions where ($B(OH)_3$) is easily leached, in more arid climates leaching does not occur which leads to accumulation of ($B(OH)_3$) levels in soil (Shorrocks 1997; Yan et al. 2006). Extremely high mean concentrations for *B* in sediment (21x higher) and vegetation (270x higher) than US recommended limits were present at locations in the extreme west of the property (ATSDR

2010). Although many of the mechanisms involved in *B*-toxicity are still unknown, concentrations of this magnitude may impact several developmental and biochemical processes in some plant species and possibly final consumers (Fang et al. 2016). Although the chemical similarity between essential *Zn* and toxic *Cd* is well known, the mechanisms resulting from toxic effects of both elements are not yet clearly understood (Paunov et al. 2018). Elevated concentrations of both elements at the TKR site could be associated with the historical addition of fertilizers or atmospheric contamination (Wuana and Okieman 2011), but without further investigation the source cannot be confirmed. Although *Zn* is essential for normal physiological and biological function in plants (Kabata-Pendias 2011), early assimilation at high concentrations (42x allowable WHO (1996) limits at some TKR locations) can induce phytotoxic effects (Chaney 1993; Rout and Das 2003). Even at low concentrations *Cd* is extremely toxic to plants (Clemens and Ma 2016), but at concentrations 3x above WHO (1996) limits in combination with elevated *Zn* levels, can result in oxidative stress functions (Tsonev and Lidon 2012), compromised photosynthesis, growth and ultimate survival (Szopiński et al. 2019).

Overall, the TKR site exhibited higher concentrations of single elements and mixtures of elements in both sediment and vegetation in the central part of the Korannaberg and Lekgaba sections, with hot spots located to the south and extreme northwest sections. It is clear that high concentrations of single or multiple elements measured at various locations in the reserve are not the result of a single contributing source. The surrounding mining activities may have some influence on the presence of high concentrations of certain elements. However, other sources including possible contamination from ground water, atmospheric pollution, exhaust fumes from vehicles driving along the municipal road separating the Greater Korannaberg and Lekgaba sections, as well as historical legacy of prior land use may be evident.

4.2.2. Manyeleti Nature Reserve

Overall associated elements in sediment and vegetation differed at the MNR site. Similar to the TKR site, mean concentrations of *Zn*, 20x above the allowable WHO (1996) limit, were observed particularly in vegetation around the Skybeds area in the far north. These levels may be associated with the geochemistry of Archean gneiss underlying the area to some degree (Walraven 1986). Concentrations 3x higher than TIL for *Cr* are indicative of the geochemical signature of this area. Elevated *Cr* and *Ni* concentrations in Wild Dog Dam, Ingwe Manzi Dam, Main Dam, Nwaswitsontso Pan and Mringechiwa Pan could be related to runoff from gabbros located in higher lying regions to the north and west of these sites (Walraven 1986). Further, *Cr* and *Ni* may be channelled into these catchment areas by the Nwaswitsontso and Mthlowa Rivers, which drain the northern and central regions outside of the reserve (Cronje et al. 2005). Atmospheric pollution and improper treatment of domestic wastewater coming in from surrounding areas adjacent to the reserve may also contribute to high *Cr* and other toxic element levels (ATSDR 2012). As plants lack a specific transport system for *Cr*, sulphate or *Fe* essential ion carriers facilitate *Cr* uptake in vegetation, which ultimately compromises plant growth and final production (Shanker et al. 2005). Depending on which form of *Cr* is present (Cr^{+6} or Cr^{+3}) concentrations of this magnitude may result in high bioavailability for uptake and subsequent deleterious effects in plants and final consumers (Oliviera 2012; ATSDR 2012). Concentrations of *Ni* above essential levels measured around Main Camp Quarry and at other scattered locations may impact metabolic reactions in plants causing oxidative stress in addition to inhibition of the root-meristem, reduced water content, seed germination and final production (Bhalerae et al. 2015; Hassan et al. 2019). Phytotoxic effects resulting from the consumption of high *Ni* concentrations by end consumers may compromise hypothalamic pituitary gonadal axis activity resulting in negative reproductive effects (Rehman et al. 2017).

In addition to *Zn, Cr* and *Ni*, sediment on the southern banks of Main Dam had high concentrations of *Ba, Cd, Co, Cu, Mn, Pb* and *Se.* Vegetation at the western access to the catchment area contained the highest levels of *As, Cd*, *Co, Cu, Hg, Mn, Ni* and *Pb*. Runoff

and water flow from the Nwaswitsontso and Mthlowa Rivers access the main dam catchment area at this site, which appears to be a hotspot for accumulation. It seems probable that transfer of *Cd, Co, Cu* and *Mn* from sediment to vegetation occurs at this site. (Hutchinson 1979; USEPA 2007: OSWER Directive 9285.7-68). The concentrations of *As*, *Hg* and *Pb* are worrying and do not appear to be related to any geological association, but are considered to be a major threat to soil systems (Masindi and Muedi 2018). *As* is extremely toxic even at relatively low concentrations because it can cause morphological, physiological and biochemical changes in plants (Abbas et al. 2018). At trace concentrations, *Hg* is hazardous to microorganisms (Boening 2000). While some bacterial and fungal organisms are able to modify available *Hg* within the environment through methylation (Azevedo and Rodriguez 2012), organic forms of *Hg* are hazardous to aquatic organisms and birds (Boening 2000). Atmospheric *Hg* is a primary source of contamination, particularly from the burning of fossil fuels and production of coal and cement (ATSDR 1999). The presence of *Hg* together with elements *Cd, Se, Pb* and *Ba* may be influenced by diesel exhaust emissions and brake and tyre wear, resulting from high levels of tourist related game drive vehicle traffic in this area of the reserve (UNEP 2006; SADEA 2012; Grigoratos and Martini 2014; Adamiec et al. 2016). In addition to the above, mercury-containing fertilizers, anti-parasitic veterinary drugs used outside the reserve and direct runoff from contaminated livestock manure into surface water may be contributing factors (Richards et al. 2014). Phytotoxic effects of *Pb* are dependent on the concentration and duration of exposure. In addition the disturbance of water and nutrient balance cause oxidative damage (Zulfiqar et al. 2019) and compromised enzyme reactions, carbon metabolism and membrane structure (Sharma and Dubey 2005).

Coal mining activities in the Mpumalanga Highveld region to the southwest of the MNR site (Shongwe 2018) may contribute to the atmospheric deposition of contaminated dust particles containing elements *Sb, As, Ba, Cd, Cr, Co, Pb, Mn, Hg, Ni* and *Se* (Aneja et al. 2012; Shongwe 2018). Copper mining activities approximately 140 km to the north of the MNR site produces *Ni*-sulphate and a host of other minerals (Southwood and Cairncross 2017). A recent study related to the spatial geochemistry around the Phalaborwa mining site indicates

that elements associated with the mine decrease as distance from the mine increases (Sach et al. 2020). Without further investigation, it is unclear whether atmospheric processes might play a role in the transport of *Cu*-associated mining pollutants to the MNR site. Sarabank Quarry, Sarabank Dam, Dixie Dam and Buffelshoek Waterhole were identified as hotspots that showed high levels of localised contamination. Given the variation in spatial distribution and difference in element concentrations however, it is impossible to say with any certainty whether external or internal source(s) are responsible. Most elements were measured at high concentrations within the central portions of the MNR around the main catchment area, particularly in sediments, but also for vegetation specific regions within this area.

This preliminary assessment highlights that although underlying geology and mineral profiles contribute significantly to trace element concentrations in sediment and vegetation, specific geochemical interactions and spatial variability within sites are not homogenous. Despite inter-element associations being evident in sediment and vegetation, concentrations of these elements were highly variable between matrices across sites and between sites. Anthropogenic contributions from multiple sources at both sites were apparent, but the absolute cause of high concentrations of specific elements or groups of elements cannot be determined with certainty without further investigation. The study draws attention to the vulnerability of protected areas, which are at risk of pollution from sources within and outside of protected area boundaries. All measured elements that could be compared to allowable levels and those above the property mean have the potential to cause deleterious effects in end consumers.

5. Conclusion

Trace element concentrations within sediment and vegetation were influenced at both sites by the underlying geology to some extent. However, numerous anthropogenic factors were also identified and considered that may contribute to the high concentrations of some elements at each site. It is clear that investigation of multiple matrices is required to form a more complete picture of trace element concentrations within an area and that variability of

trace elements may differ according to local and regional heterogeneity. Valuable information would be gained by future studies that include monitoring of external sites, and that focus on distinguishing trace element speciation, seasonal variation, endemic woody and grass species-specific transfer and comparison of concentration differences in plant parts.

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