How does Environmental Geochemistry Affect Elephant Movement?

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Abstract

The aim of this study was to better understand how environmental geochemistry influences mineral provision to African savanna elephants, and thus their home range size and movement choices. Due to increasing human activity and competition for land-use, African savanna elephants globally are constrained into smaller areas. This presents elephants with nutritional challenges, requiring adaptation to their movement patterns to obtain sufficient mineral intake. An *in situ* experiment was enabled by the Palabora Mining Company (PMC) property, and the surrounding national park lands in South Africa, providing a case study of contrasting environments.

The PMC mines copper, phosphates and other minerals and the locale is proposed to act as a micronutrient hotspot for elephants, due to its unique local geochemistry. The increased presence of elephants at the PMC, and incursion to the mine with associated disruption of mining activities has resulted in human-elephant conflict. It was hypothesised that elephants reside within the locality of the PMC property, owing to its potential as a micronutrient hotspot.

To determine the influence of geochemistry on elephant movement, optimum bio-indicators of mineral nutritional or potentially toxic element (PTE) status were required, prior to studying the PMC associated elephant populations, to differentiate between patterns of movement between national park lands and surrounding reserves (background) and mineral processed lands at the PMC (elevated). The validation of optimum bio-indicators (e.g. toenail, tail hair, plasma or faeces) for mineral or PTE status was achieved by collection and elemental analyses of environmental and biological samples from 21 elephants at five UK zoos. A linear mixed model showed that toenail was the best bio-indicator of intake for Mg, P, K, Se and As, with keeper-fed diet being the strongest predictor ($P < 0.05$ for all). Tail hair reflected As and Fe intake ($P<0.001$, 0.029), and Ca intake was reflected in faecal samples ($P= 0.019$).

Validated methods of sampling and analysis were applied to the micronutrient hotspot around the PMC, with sampling and analysis of environmental (soil, water, plants) and biological (faecal and tail hair) samples from elephant populations at the mine and in the surrounding national park and reserves. Results showed that the geochemistry differs at the PMC compared to the surrounding areas, with significant elevations shown in all 15 investigated elements in soil. Significant elevations were seen in Mg, P, Cu, As, Cd, Pb and U in faecal samples at the mine compared to the non-mine samples ($P>0.05$). Tail hair samples from elephants collared at the PMC were significantly higher in Cd and significantly lower in K and Se compared to non-mine counterparts. Correspondingly, the home range of animals collared at the PMC was 59% smaller ($P= 0.001$) than conspecifics from adjacent locations.

This multi-disciplinary study demonstrated the importance of environmental geochemistry on mineral provision, and subsequent influence on elephant population movement, using this case study of contrasting environments and the micronutrient hotspot at the PMC. Application of this information will be valuable to conservation managers, to influence elephant movement and thus reduce associated human-elephant conflicts. Equally, this work benefits zoo elephants, providing comparative baseline data to enable assessment of mineral status in captivity, and in turn this project demonstrates how zoo based research can inform the management of wild counterparts.

Publications arising from PhD programme:

Sach F., Yon, L., Henley M., Bedetti A., Buss P., de Boer WF., Dierenfeld E., Gardner A., Langley-Evans S., Hamilton, E., Lark, RM., Prins HHT., Swemmer AM., Watts M. 2020. Spatial geochemistry influences the home range of elephants**.** 2020. *Science of the Total Environment, 729.* https://doi.org/10.1016/j.scitotenv.2020.139066

Sach F., Dierenfeld E., Hamilton, E., Langley–Evans S., Lark, RM., Yon L., Watts M. 2020. Potential bio-indicators for assessment of mineral status in elephants. *Scientific Reports,* 10, 8032. https://doi.org/10.1038/s41598-020-64780-0

Sach F., Dierenfeld E., Langley–Evans S., Watts M., Yon L. 2019. African savanna elephants (*Loxodonta africana*) as an example of a mega–herbivore making movement choices based on nutritional needs. *Peer J*. DOI: 10.7717/peerj.6260

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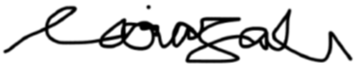
Declaration

Unless otherwise acknowledged the work presented in this thesis is original. No part has been submitted for another degree at The University of Nottingham or elsewhere. Any views expressed in the dissertation are those of the author.

Signed……  ..

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Abbreviations and Acronyms

AfESG	African Elephant Specialist Group
APHA	Animal and Plant Health Agency
APNR	Associated Private Nature Reserves
ASVCP	American Society for Veterinary Clinical Pathology
BIAZA	British and Irish Association of Zoos and Aquariums
CITES	Convention on International Trade in Endangered Species
CRM	Certified Reference Material
DCA	Damage causing animal
DIW	De-ionized water
EA	Elephants Alive
EAZA	European Association of Zoos and Aquaria
EEP	European Ex-situ programme
EPMA	Electron probe microanalysis
FDR	False discovery rate
FERA	Food and Environment Research Agency
HEC	Human-elephant conflict
IATA	International Air Transport Association
ICP-MS	Inductively coupled plasma mass spectrometry
IUCN	International Union for Conservation of Nature
KNP	Kruger National Park
LA-ICP-MS	Laser ablation ICP-MS
LOD	Limit of Detection
PMC	Palabora Mining Company
PPM	Parts per million
PPT	Parts per trillion
PTE	Potentially toxic element
SAEON	South Africa Environmental Observation Network
SANParks	South African National Parks
SEM	Scanning electron microscopy
SEM - EDXA	Scanning electron microscopy - Energy-dispersive X-ray
TAG	Taxon Advisory Group
TOPS	Threatened or Protected Species

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Chapter 1

1.0 Introduction

African savanna elephant incursion into the copper and phosphate mines around Phalaborwa, South Africa has resulted in human-elephant conflict (HEC), elephant and human injury and income loss. The working hypothesis was that elephants were attracted to the mining area due to the micronutrient hotspot created, as a result of the local unique geochemistry of the soil (and plants). Previous evidence suggested that the soil (and plants) in the surrounding national park and reserves, similar to many heavily leached Southern African soils, were low in key minerals such as phosphorus. During the first phase of this project, method validation was conducted using biological samples (plasma, toenails, tail hair, urine and faeces) and samples of environmental inputs (soil, food and water consumed) from elephants at UK zoos, to determine within a relatively controlled environment, the optimum bio-indicator for each mineral or potentially toxic elements (PTEs), in the species. Advanced inductively coupled mass spectrometry (ICP-MS) techniques were used to provide accurate and reliable data.

The second phase of this project applied these validated methods to free-living African savanna elephants around Phalaborwa, determining how environmental geochemistry influenced elephant movement within the area. In addition, assessment was made of PTE levels in biological samples from the elephants (tail hair and faeces) and their environment (soil, plants and water); to investigate if mining activities are causing a risk to wildlife health. This multidisciplinary project combined research on zoo and wild animal health and welfare, environmental geochemistry, drivers for elephant movement and optimum analytical techniques. It is an excellent example of the contribution that captive animals can make to research, to directly benefit their wild counterparts.

1.1 Free Living Elephants

Human activity is threatening one million species with extinction globally; the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services report highlights that biodiversity is declining at unprecedented rates (Brondizio *et al.*, 2019) and The Living Planet Index has reported a 60% decline in wildlife populations since 1970 (Grooten and Almond, 2018). Factors influencing global biodiversity have had a major detrimental impact upon numbers of, and habitats occupied by, elephants. Rising human populations and the global intensification of agriculture are forcing elephants into ever smaller habitats, often with increased fencing restriction and other human intervention (Nyhus, 2016). This presents two issues: first, there is increased pressure exerted upon these restricted areas to meet the resource needs of elephants including those of minerals, and second, it brings elephants into increasing contact and conflict with humans, as human and elephant habitats are forced to overlap. Conflict between people and wildlife is a global conservation issue and is expected to increase over the next 50 years. This is due to the predicted rapid increase in land required for human population growth, and agricultural requirements to feed the expanding population (Woodroffe, Thirgood and Rabinowitz, 2005; Nyhus, 2016).

African savanna elephants (*Loxodonta africana*) are classified as ‘Vulnerable’ and Asian Elephants (*Elephas maximus*) as ‘Endangered’ on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (IUCN, 2019). African savanna elephants are distributed across 37 countries in sub-Saharan Africa, with an estimated population of

415,000 (IUCN, 2019; WWF, 2019) whereas Asian elephants occur in 13 countries across South Asia and South East Asia, with a wild population of *c.* 48,323–51,680 (Menon and Tiwari, 2019). Key threats apply to both species and include habitat fragmentation and shrinkage, habitat alteration due to climate change, poaching driven by the illegal trade of elephant body parts including skin, ivory, and bush meat and human-elephant conflict (HEC; Fischer and Sach, 2019)

When elephant habitat and human activities overlap, conflicts can occur. Approximately 70% of the range of African savanna elephants is outside of protected areas, making HEC conflict almost inevitable (Blanc *et al.*, 2007). Elephants destroy property, the products of agriculture and industry, and cause the loss of human life with associated retaliatory elephant killings (Lee and Graham, 2006; Nyhus, 2016). Elephant populations are challenged by these retaliatory killings, physical collisions of elephants with trains or large vehicles and habitat fragmentation, caused by the use of protection mechanisms such as electric fencing around agricultural land, hindering normal elephant movement and migration (Stephenson, 2004; Nyhus, 2016; Menon and Tiwari, 2019). Conflict mainly occurs in areas adjacent to the natural habitats of elephants (Enukwa, 2017), often outside of protected areas. Conflicts predominantly arise when these animals do not have the resources they need within their habitats, thus they are forced to alter their movements to search for these resources elsewhere, bringing them into conflict with humans (Enukwa, 2017).

Drivers for elephant movement are multifactorial and include availability of food, water and social interaction with conspecifics, access to shade, presence of human activity, and security for the elephant (Stokke and Du Toit, 2002; Osborn, 2004; Grainger, Van Aarde and Whyte, 2005). Several studies highlight examples of elephants moving based upon the need to access key minerals, especially sodium and phosphorus (Jachmann, 1989; Bowell, Warren and Redmond, 1996; Rode *et al.*, 2006; Pretorius *et al.*, 2012; Sach, Dierenfeld, *et al.*, 2019a). Understanding the drivers of elephant movement is essential for land-use planning and management of elephant populations, which is required to understand the causes of HEC, and when attempting to alter elephant movement patterns or behaviours to reduce these conflicts.

Mitigation of HEC is essential for the sustainability of conservation efforts and to improve human-wildlife coexistence. A number of approaches have been developed that aim to achieve this, often with limited success, as they are expensive to construct and maintain and the benefits may only be short-lived, especially when elephants become habituated to the deterrents within their environment (Hoare, 1999; Graham and Ochieng, 2008; Enukwa, 2017). Examples of these methods include lethal control, translocation, exclusion and repellents such as the use of chilli or bees to scare elephants away (Enukwa, 2017). In contrast, habitat manipulation, by improving or providing resource(s) such as food, water, adequate space or improved habitat health, is considered a vital and sustainable method of HEC mitigation (Jackson *et al.*, 2008; Enukwa, 2017). This may be because it deals with the underlying root causes and not just the consequences of the HEC (Hoare, 1999; Barnes, 2002).

Other approaches for HEC mitigation can be divided into lethal and non-lethal control. Lethal control includes reducing the physical number of elephants in an area through culling or hunting. The increased availability of modern weapons has made elephant culling and hunting easier. Culling can either be specifically targeted to damage causing animals (DCA) or can be adopted on a wider population management scale (Enukwa, 2017). Some elephants respond to increased hunting or culling by moving into safer areas, which may in turn cause new additional HEC, further exacerbating the problem (Lee and Graham, 2006). There is limited evidence that wider population management culling reduces elephant numbers; this was discussed by Aarde et al and Own-Smith et al following the Kruger National Park (KNP) cull, 1984-1998 (Aarde, Whyte and Pimm, 1999; Owen-Smith *et al.*, 2006). It was suggested that reproductive rates increased following a period of intense culling to compensate. Culling of targeted DCAs is also reported to be ineffective, as the remaining herd generally returned to the area, or other elephants may move into the area, thus creating new conflicts (Hoare, 2001).

Non-lethal control of elephant populations includes translocation, barriers or exclusionary devices to physically prevent the elephants from entering an area, repellents to deter or scare the elephants away, contraception to limit reproduction and finally habitat manipulation (Nyhus, 2016; Enukwa, 2017). Translocation is "the deliberate and mediated movement of wild individuals or populations from one part of their range to another" (IUCN, 1998) and is a very resource intensive and expensive process. Animals were reported to return to their original locations, if the distance of translocation was insufficient and still within their home range. Additionally, translocated animals had a higher mortality rate than local animals, and displayed increased aggression and extreme behaviours, possibly due to the stress of transport (Noa, 2009; Wong, 2018). Barriers or exclusionary devices included fencing and digging trenches. The most effective fences are electric and must be well constructed and maintained with regular checks conducted, to ensure that there is no damage or breaks in the fence, rendering them ineffective. Installing electric fencing over large areas is very expensive and requires significant labour to install and maintain.

Trenches were generally found to be ineffective as animals can collapse the walls, especially in wetter areas and elephants can cross trenches up to 2.3m wide (Enukwa, 2017).

Methods to scare the elephants away or deter them include repellents such as bee-hive fences, the use of chilli, and spotlights or fire (Enukwa, 2017). Bee-hive fences and the use of chilli were effective on a small scale to deter elephants, as elephants were inherently repelled by them. Chilli was used as a barrier crop, as a paste on fences or burnt, and was found to be effective about 80-89% of the time when tested (Perera, 2009; Enukwa, 2017). However, these deterrent methods required intensive community engagement and participation to assure their success (King *et al.*, 2009). Using spotlights directed into elephants' eyes, to scare them away was effective, but was labour intensive and was only successful if areas were well guarded (Hedges and Gunaryadi, 2010). Bush fires were generally effective to deter elephants, although labour intensive and unless they were well controlled, the fire may extend to destroy crops and vegetation, negating the benefit of reduced HEC (Enukwa, 2017). The use of contraceptives to reduce birth rate and thus slow elephant population growth was ineffective due to the scale required to ensure any reasonable reduction in population growth (Whyte, 2001). In conclusion, habitat manipulation was considered to be a potentially successful mitigation for HEC if conducted with thorough research to identify the drivers for the elephant movement and root cause(s) of the conflict (Woodroffe, Thirgood and Rabinowitz, 2005; Lee and Graham, 2006; Nyhus, 2016; Enukwa, 2017).

1.2 *Ex situ* Elephant Populations

Humans have had a long association with elephants (approximately 4,000-5,000 years); elephants were used in warfare, for transport, in hunting, logging, for religious purposes, in the tourist industry, hunted for meat, skin and ivory and exhibited within zoos (Eltringham, 1990). Elephants became more common in zoos in the 19th century, with the first Asian elephant in the USA arriving in 1796 and African savanna elephant in 1804. Prior to the closing of The Tower of London Menagerie, an elephant was given to the newly founded Zoological Society of London (ZSL), London Zoo which was founded in 1826 (Schulte, 2000). Zoos were consumers of elephants, with poor breeding success and few males were kept in captivity. The first captive births were recorded in the USA in 1880, a still born at ZSL in 1902 and Vienna in 1906. In 1982, 150 European Zoos exhibited approximately 500 elephants, 21 of which were captive born. Elephant breeding increased in the 1980s in both Europe and the USA, as wild import became more difficult (Veasey, 2006; Wiese and Willis, 2006).

Zoo elephants historically experienced compromised survivorship with a lower life expectancy than wild counterparts. Wild elephant life expectancy is about 40-70 years (Nowak, 1991) whereas zoo-born female African savanna elephants had a median life span of 16.9 years and captive-born Asian elephant females had a median life span of 18.9 years (Clubb *et al.*, 2008). A more recent (2019) analysis of long-term data on Asian elephants within the European *ex situ* Programme (EEP) found an average life expectancy of 24 years for males and 32 years for females (analysis includes historic data) with the oldest individuals in the EEP being 59 years (male) and 67 years (female) (Schmidt and Kappelhof, 2019). Reasons for this compromised survivorship are multifactorial and interlinked. They include poor historic keeping practises (e.g. inappropriate indoor substrate) leading to the development of osteoarthritis and foot issues, poor nutrition leading to obesity and reduced reproductive output, high incidence of fatal disease such as elephant endotheliotropic herpesviruses (EEHV) and elephants being kept in inappropriate, unrelated social groups leading to potentially fatal conflicts, increased stress and reduced reproductive success

(Clubb and Mason, 2002; Hermes, Hildebrandt and Göritz, 2004; Hermes *et al.*, 2008; BIAZA, 2019). In 2019, 60% of the European Asian elephant population was captive born compared to 19% in 1999 (Schmidt and Kappelhof, 2019) indicating an increased birth rate and life expectancy in the species. The same trends are beginning to emerge in African savanna elephants in Europe, although improvements are yet to be documented (BIAZA, 2019).

At the time of writing (Jan 2020) there were 26 Asian elephants (5 male and 21 female) in six UK zoos and 24 African savanna elephants (10 male and 14 female) in five UK zoos. Additionally, there were two Asian elephants in the UK not within a zoo. All UK zoo elephants are part of the European population which are managed by the Asian Elephant EEP and the African Elephant EEP. The EEPs are under the auspices of the EAZA Elephant TAG programme which aims to have a self-sustaining, genetically diverse, reproductively and behaviourally competent *ex situ* population (EAZA, 2015).

1.3 Role of *Ex situ* Elephant Populations

Elephants are iconic species in European zoos and have two main purposes within the zoos, first in conservation education and second in research (EAZA, 2015; BIAZA, 2019). Under the governing legislation of 'The Zoo Licencing Act 1981' and 'The EU Zoos Directive' (*Zoo Licensing Act 1981 Guide to the Acts provisions*, 2012; Rodríguez-Guerra *et al.*, 2015), conducting conservation education is compulsory for zoos. Conservation education connects people with nature and inspires them to make positive behaviour changes for conservation benefit (Miller, Luebke and Matiasek, 2018). BIAZA divides the aims of conservation education with elephants into the following broad areas (BIAZA, 2019):

"1. Build knowledge and understanding about elephants and the natural world.

2. Foster positive attitudes and values towards elephants and the natural world.

3. Promote enjoyment, creativity and inspiration about elephants and the natural world.

4. Develop scientific and personal skills.

5. Influence behaviours and activity linked to conservation of elephants and the natural world."

Secondly *ex situ* zoo elephants are invaluable for conservation research. Data obtained from zoo elephants provide information for *in situ* conservation efforts in a variety of areas including general physiology, health, behaviour and nutrition (Bechert *et al.*, 2019), demonstrated in part with this project. Additionally technology such as GPS tracking collars, were developed, trialled and tested with *ex situ* populations (Horback *et al.*, 2012). Finally *ex situ* populations of elephants have a role in capacity building and obtaining conservation funding due to their substantial commercial potential within zoos (Sach, Fitzpatrick, *et al.*, 2019b).

1.4 Elephant Nutrition

Elephants are herbivores and consume a wide variety of plant material including grasses, leaves, twigs, fruits, barks, herbaceous material and soil (Sukumar, 1989; Kabigumila, 1993). A thorough review of elephant diet breakdown, feeding behaviour, seasonal variation and nutrient ranges in natural diets for African savanna elephants is covered in Sach et al. (2019a), in Chapter 3. Both genera of elephants have broadly similar nutritional requirements. The natural diet is characterised by high fibre content (crude fibre 30-50%) and a low to moderate protein content (crude protein 8-12%). Elephants are opportunistic in their food choices and evidence has indicated that they adjust their movement patterns depending upon mineral need (Sach, Dierenfeld, *et al.*, 2019a). Studies have demonstrated that the passage of food through elephants' digestive tract is rapid compared to other monogastric hindgut digesters such as horses; the total gut transit time in elephants is 11-46 hours compared to up to 53 hours in horses (Bax and Sheldrick, 1963; Rees, 1982; Clauss *et al.*, 2003; Van Weyenberg, Sales and Janssens, 2006). Additionally elephants have a correspondingly low digestive efficiency (Clauss *et al.*, 2003; Hatt and Clauss, 2006). In summary, elephants are designed to eat large quantities of nutrient poor fibrous material, which passes quickly through the gastrointestinal tract. Elephants may spend 16 hours a day feeding or foraging and they eliminate 3-5 dung boluses every 1.4 hours or between seven and 29 defecations each day (averaging 12 per day; Sukumar, 1990).

Estimates of daily dry matter intake for an adult elephant are typically around 1-1.5% of body mass (Ullrey, Crissey and Hintz, 1997), however, intakes vary according to the nutritional quality of the feed as well as environmental conditions, activity level, reproductive status and growth stage. These factors also influence energy requirements, and therefore, diet must be adjusted to meet energy needs in a captive setting. Elephants are obligate drinkers, consuming up to 200 litres per day (Olson, 2004; Blanc, 2008). Elephants have a single stomach and a short but voluminous hindgut fermentation chamber (like equids), inhabited by anaerobic bacteria and protozoa like those found in the rumen and reticulum of the ruminant. These microorganisms digest plant fibre that otherwise could not be used, since elephants, like other herbivores, have no fibre-digesting enzymes of their own. Microbial fermentation of plant fibre in the hindgut provides the main energy source for these animals as well as other nutrients including B-vitamins, such as riboflavin, niacin, biotin, folate, B12 and B6 (Hintz, 1975).

1.5 Why is Zoo Nutrition Important?

Zoos have a responsibility to provide optimum nutrition to all animals within their care (*Zoo Licensing Act 1981 Guide to the Acts provisions*, 2012). Failure to do so may result in nutritional-related disease, compromised welfare, decreased reproductive success and a reduced life expectancy. Formulation of an appropriate diet for captive animals requires husbandry skills and applied nutritional science (Dierenfeld, 1997). As feed costs are the second largest day-to-day running costs of a UK captive elephant herd (Sach, Fitzpatrick, *et al.*, 2019b), it is essential that appropriate items of acceptable quality are being fed to zoos elephants. Individual cases of specific mineral deficiencies were documented in zoo elephants (Ullrey, Crissey and Hintz, 1997; Sach, Dierenfeld, *et al.*, 2019a). However, due to elephants' low growth rate and large body size, it is possible that nutritional inadequacies may go unnoticed for relatively long periods of time before clinical signs develop (Ullrey, Crissey and Hintz, 1997), and the requirement for dietary evaluation within a zoo may be overlooked. Work conducted as part of this project to identify the optimum bio-indicators for mineral and PTE status, aimed to improve methodologies to evaluate nutritional status in the species, and thus benefit zoo nutrition and welfare (Sach *et al.*, 2020).

1.6 Legislation and Guidelines for UK Zoo Elephant Nutrition

Due to the limited research on vitamin and mineral requirements in exotic mammals, animal nutritionists frequently use domestic species as a model to make recommendations for captive exotic animals. For elephants, the National Research Council (USA) recommendations for the domestic horse are often used (Hatt and Clauss, 2006; Clauss *et al.*, 2007). Management guidelines for zoo elephant nutrition exist to aid zoos in formulating their elephant diets, with the most up to date guidelines produced by BIAZA in 2019, with extensive information on diet formulation, feed storage and preparation, feed presentation, diet monitoring and evaluation and supplementary feeding of calves and geriatric elephants (BIAZA, 2019). Additional guidelines are in publication, produced by AZA, EAZA and other individual experts within the field; however, these guidelines are superseded by those from BIAZA.

The UK is one of the few countries globally with specific legislation detailing the animal welfare standards required within zoos (Draper, Browne and Harris, 2013), specifically the Zoo Licensing Act, 1981 (Defra, 2012; Draper & Harris, 2012). The Secretary of State's Standards of Modern Zoo Practice (SSSMZP) provide guidance to zoo operators and inspectors on the Act's implementation and represent the minimum standards that zoos are expected to meet (Defra, 2012). In the UK, the SSSMZP were updated in June 2017, with an updated appendix specific to elephants; Appendix 8.8. All UK zoos holding elephants are inspected against the new appendix, by dedicated Department for Environment, Food and Rural Affairs (Defra)-appointed inspectors (Defra, 2017). Within the appendix there are five clauses on nutrition and feeding (8.8.31 – 8.8.35). These focus on food presentation, management of body condition, nutritional content, diet composition, requirements for routine independent feed analysis, good record keeping and water provision. The standards within this appendix set out the minimum standards that UK zoos must adhere to and the BIAZA guidelines (2019) support and exceed these.

1.7 Rationale for the Study

It has been suggested that nutritional factors, most notably mineral availability, are a driver of wild elephant movement (Sach, Dierenfeld, *et al.*, 2019a) and home range size. Elephants will move throughout the landscape to obtain required minerals. Certain areas may act as micronutrient hotspots, due to their local environmental geochemistry, providing elephants with an increased supply of required minerals. When micronutrient hotspots overlap with human activity (such as mining), human-elephant conflict can occur. When elephants are forced into increasingly smaller geographical areas, often restricted by fencing or encroaching anthropogenic activities, they can experience nutritional challenges, as they may not be able to roam over such a large area to obtain their required minerals. Elephants can change their movement patterns and distribution to seek out their required minerals to meet their dietary needs, often making use of any available micronutrient hotspots, potentially causing HEC when these areas overlap with human activity (Sach, Dierenfeld, *et al.*, 2019a).

The rational for the studies described in this thesis was to consider this putative link between minerals and elephant movement. UK zoo animals were used for method validation to determine the optimum bio-indicator(s) for assessing mineral status in the species. This was then applied to free-living elephants that were under study at the Palabora Mining Company (PMC) land in South Africa.

It was suggested that the unique geochemistry surrounding the PMC acts as a micronutrient hotspot and provides the elephants in the area with increased minerals from the local soil, water and plants, and is a factor in attracting the elephants towards the mine. The home range of the collared elephants around the mine is 59% smaller than surrounding conspecifics, indicating that their resource needs are being met within a relatively smaller area. Additionally, a 2015 elephant census showed that the elephant density around the mine is higher than the surrounding area (Lerm and Swemmer, 2015). When elephants spend time around the mining area, as shown in Figure 1.1, it creates HEC, as their movements overlap with mining activities. Human-elephant conflict from elephant encroachment to PMC causes financial loss to the mine, places human and elephant life at risk and disrupts surrounding communities. Three elephants were killed as DCAs since 2015 (M.Henly 2017 pers. com.) and one security guard was killed by an elephant on the neighbouring Foskor mine site (Maphanga, 2019). Therefore, reducing this conflict could have local and wider economic and social benefits, if HEC mitigation strategies were applied here, and to other human-wildlife conflict situations internationally.

Information obtained from this study could be used to determine if minerals are a driver for elephant movement in this landscape. Locations could be proposed within the elephants' home range in which mineral-supplemented forage, or mineral licks, may be placed. This has the potential to reduce the elephants' drive to seek additional sources of minerals, thereby reducing elephant encroachment into the mine and surrounding communities and therefore, mitigating the associated HEC.

Figure 1.1: Elephants on the Palabora Mining Company (PMC) land.

Secondly this project may determine if elephants (or other wildlife) near the mine are exposed to increased or potentially harmful levels of PTEs; if so, this could be highlighted and recommendations could be made to mitigate this, and thereby improve animal health. Copper poisoning was reported in 1989 in impala and buffalo surrounding the study area, and further work suggested that air pollution was the most likely source of contamination. Impala are more area bound and therefore more likely to accumulate copper and develop chronic copper poisoning, compared to buffalo or elephant, which travel over larger distances (Grobler and Swan, 1999). Finally, it may be possible to improve captive elephant health and welfare through establishment of baseline levels for key minerals and PTEs in the species, and establish appropriate bio-indicator(s) to assess elemental status in the species. This will enable better assessment of mineral status in captive and wild elephants. Finally, recommendations could be made using data from this research, to improve zoo elephant diets, to further meet the needs of the species in captivity.

1.8 Validation of Methodologies using Zoo Elephants

Method validation was required to identify the optimum bio-indicator(s) for elemental intake, as a proxy for mineral or PTE status within the elephant. There is no single accepted bio-indicator for analysing animal mineral or PTE status, and none has been established for elephants. A non-invasive method to assess elemental status would be beneficial to assess mineral status of animals in the field and could also be used to determine whether captive dietary mineral provision was sufficient to maintain good health. The principal bio-indicators which could be used for assessing mineral status include plasma, toenail, tail hair, urine and faecal samples.

Blood in the form of plasma samples, are established as the most practical gold standard for assessing an animal's mineral status. For some minerals, liver would be the optimum matrix; however obtaining samples is impossible ante mortem (Mills, 1987; Herdt, Rumbeiha and Braselton, 2000). Collecting a blood sample is an invasive procedure which in captivity requires considerable training, both for the elephant and the keeper (Bourne, 2005), and in the wild requires sedation or anaesthesia of the elephant. Additionally, sample storage in the field to preserve the integrity of the sample, may also be problematic.

Toenail and hair samples generally contain higher concentrations of PTEs than in many other body tissues. Samples are easy to handle in the field and upon return to the laboratory (Asano *et al.*, 2002), although contamination can be an issue, especially in human hair samples where shampoo and hair dyes were used, leading to increased selenium concentrations (Combs, 1987). Human toenails were successfully used to determine selenium status within humans, and correlated to plasma levels (Hays et al., 2014; Sahayestia et al., 2006). Human hair and toenails have also been used to determine mineral status or exposure to PTEs such as arsenic (Bencko, 1995; Button *et al.*, 2009; Middleton, Watts, Hamilton, *et al.*, 2016). Additionally, horse hair samples were found to reflect increased levels of Fe, K and Zn when supplemented in the diet (Armelin, Avila and Piasentin, 2003). Samples can be challenging to collect from a human or animal, although they are less invasive than blood samples. Storage requirements to preserve samples in the field and health, safety, and handling within the laboratory are logistically easier than with blood samples. Sensitivity of analytical techniques has improved greatly, resulting in more accurate data (Middleton, Watts, Hamilton, *et al.*, 2016).

Urine samples can be used to measure excesses of certain minerals that have been absorbed then excreted. Samples can be useful in determining whether a diet is low, adequate or high in minerals such as calcium, selenium and arsenic, provided that established threshold values of deficiency, sufficiency and excess exist (Middleton, Watts, *et al.*, 2016; Phiri *et al.*, 2020). A single sample is of limited value however, due to the homeostatic controls within the mammalian system and variability in hydration status affecting solute concentrations (Caple, Doake and Ellis, 1982; Middleton, Watts, Lark, *et al.*, 2016; Middleton, Watts and Polya, 2019). Sample collection can be challenging, both in captive and free-living elephants, especially as samples need to be collected mid-flow and not from the floor to prevent the risk of contamination. In captivity this requires considerable elephant and keeper training and in the wild, sedation or anaesthesia is needed.

Faecal samples reflect dietary minerals which were not absorbed by the body as well as those excreted into the intestines as excess (Young, Lofgreen and Luick, 1966). However, it is difficult to separate these relative contributions to faecal mineral content. Additionally, when animals consume diets of mixed digestibility, the less digestible components could be over-represented in faecal samples (Sponheimer *et al.*, 2003). However, faecal samples can rapidly reflect dietary changes (Sponheimer *et al.*, 2003), and they are cheap and non-invasive to collect in both captive and free-living elephants, and therefore, could be used to assess mineral status.

Samples from zoo elephants enabled validation of the methodology for this study. Within a zoo setting, it was feasible to sample all of the potential mineral 'inputs' to the elephant. These included all items fed as part of their diet, water, grass and soil from their paddocks. Additionally, due to the routine training carried out with the zoo elephants for health monitoring, it was possible to sample most or all of the 'outputs' or potential bio-indicators, including toenails, tail hair, plasma, faeces and urine.

1.9 Aims and Objectives of the Thesis

1.10 Hypotheses

1. Mineral levels in the plasma, tail hair and toenails of captive African savanna elephants correlate to some extent with dietary intake and act as a proxy for mineral status in the animal.
2. Free-living elephants in the Phalaborwa region, South Africa move towards micronutrient hotspots.
3. Mineral levels in soil, plant material and water surrounding the PMC are higher than typical South African baseline levels

4. Levels of PTEs in soil, plants and water surrounding the PMC are higher than in the surrounding areas (KNP and Associated Private Nature Reserves (APNR)).

1.11 Aim

The aim of this study was to understand the spatial influence of geochemistry on the home range and movement of elephants, using the natural experiment provided by the PMC property and surrounding national park land as a case study of contrasting environments. This may inform on the importance of mineral nutrition in determining population movement and thereby the resulting human-elephant conflict.

The objectives of this work were:

1. Validation of optimum bio-indicators for mineral and PTE status in elephants.
2. Establishment of baseline levels for key minerals and PTEs in African savanna elephant tail hair, plasma and faeces.
3. Determination of whether mineral and PTE levels in soil, plants, and water surrounding the PMC are higher than neighbouring areas within the KNP /APNR.
4. Determination of whether elephants eating and drinking near the PMC have higher mineral and PTE levels in their tissues (tail hairs) and faecal samples than elephants from KNP/APNR.

Further Tasks

1. Measurement of mineral levels in elephant tail hair, plasma, toenails and faecal samples
2. Measurement of PTE levels in elephant tail hair, plasma, toenails and faecal samples
3. Measurement of mineral levels in soil, water and plant material
4. Measurement of PTE levels in soil, water and plant material
5. Establishment of representative sample sites for the PMC, APNR and KNP

1.12 Thesis Structure

Chapter 1: General introduction to the thesis. First, a background to free-living elephant populations, threats and HEC and second, the role and use of *ex situ* elephants within this PhD study for method validation.

Chapter 2: Methods used within the study, especially those adapted for elephant tail hair samples. Appendix to this Chapter contains a list of all analysed elements within the study.

Chapter 3-5: Studies published or under review in peer-reviewed journals. These chapters are presented in 'paper format' with author contributions outlined. Chapter 3 is a review of African savanna elephants making movement choices to meet their nutritional needs. Chapter 4 examines bio-indicators for non-invasive assessment of mineral status in elephants. Chapter 5 examines how environmental geochemistry influences elephant movement around a large copper mine in South Africa. Chapter 5 also contains additional toenail and plasma data that was collected as part of this work but not used within the publication (found in Appendix).

Chapter 6: Conclusion, evaluating how environmental geochemistry affects elephant movement and how this can be applied to understand elephant movement around the PMC and applied to other HEC situations internationally, where mineral provision is suspected as the driver of the movement, resulting in conflict. Additionally, includes limitations of the study and identification of areas for future work.

References are included at the end of each chapter, for published work or chapters which are submitted for publication, references are in the format of the relevant journal.

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Chapter 2

Introduction

This chapter comprises additional methodologies and details not covered in later chapters (Chapters 3, 4 and 5). A literature review looking at how minerals act as drivers for African savanna elephant movement was conducted as detailed in Chapter 3 (Sach *et al.*, 2019), to inform methods for the subsequent experimental chapters within the thesis. Documented examples from other herbivorous species were included where relevant. The review was required to draw together previous work in this field, that was generally on geographically limited populations.

Chapter 4 provides details of a methods validation study to identify optimum biomarkers for elemental status in elephants, using UK zoo elephants. Chapter 5 applies these methods to wild elephants around the Palabora Mining Company (PMC) land, South Africa, which acts as a natural experiment, with areas containing contrasting environmental geochemistry (the mining area and surrounding national parks and reserves). Further information on the methods used within these two studies is detailed in Chapters 4 and 5 respectively.

The analytical techniques used within this study produced data for 58 elements. It was decided to focus on fifteen biologically functional elements throughout the project: calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), selenium (Se), zinc (Zn), arsenic (As), cadmium (Cd), lead (Pb), uranium (U) and vanadium (V). The full list of analysed elements is detailed in Chapter 2, Appendix (Table A0).

2.0 Methods

2.1 Sample Preparation and Analysis

Full details of sample collection and preparation of biological and environmental samples are given in Chapters 4 and 5.

Solid samples (tail hair, toenail, vegetation, faeces and soil) were digested into solution for analysis in batches (groups of at least 31 samples), according to matrix. Liquid samples (urine, plasma and water) were diluted as appropriate for ICP-MS, and analysed in batches according to matrix. Within each batch, ten percent of the total number of samples was run in duplicate, except for tail hair, as duplication was impossible as no two hairs were identical in length. Ten percent of the total number of samples were run blank, to assess for contamination in the sample preparation and to ascertain the practical limit of detection (LOD) for each batch (LOD, 3*STDEV) and ten percent of the total number of samples were run as certified reference materials (CRM), selected according to matrix.

All samples were analysed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS; ICP-QQQ; Agilent 8900x). ICP-MS is the leading technique for multi-elemental analysis as it has high sensitivity, a wide linear dynamic range (low detection limits in the sub parts per trillion range (ppt) and also quantification at the high parts per million level (ppm)), and the possibility to obtain isotopic information, making it the most suitable technique to use for this study (Thomas, 2013). The disadvantage to ICP-MS is the occurrence of spectral interferences (Thomas, 2013; Balcaen *et al.*, 2015). This is overcome by several methods including matrix matched blank subtraction, mathematical correction, trace/matrix separation and the use of the quadrupole based instrument equipped with a collision/reaction cell (Balcaen *et al.*, 2015). All laboratory measurements were carried out at The British Geological Survey laboratories, with required quality assurance, including the use of certified reference materials (CRMs) and duplicate measurements (as detailed in section 2.2), for which data is summarised within each experimental chapter, supplementary information.

2.2 Quality Control

Quality control was measured in the following ways, to produce valid quality data:

1. Use of CRMs selected according to relevant sample matrix. The concentrations of all reference materials were found to be accurate within an acceptable percentage of the certified values for all elements studied here, details are given within Chapters 4 and 5.
2. Duplicate samples - all duplicate sample results were within an acceptable range e.g. ± 5% difference, depending on the matrix and concentration of element in the samples.
3. Blank samples were used to measure potential contamination through the analytical process and to define limits of LODs.
4. Internal standards were used for ICP-MS drift correction: Scandium (Sc), Germanium (Ge), rhodium (Rh), indium (In) and iridium (Ir).

2.3 Ethical Approval

Ethical approval was received from the Ethics Committee at the University of Nottingham, detailed for each study in Chapters 4 and 5.

2.4 Statistical Analysis

Statistical analysis was conducted using ‘R’ version 3.5.0.

Full details of statistical methods used can be found in Chapters 4 and 5.

Statistical method design was guided by Professor R. Murray Lark from the University of Nottingham, School of Biosciences, UK.

2.5 Additional Methods for Validation Study (Chapter 4)

Twenty-one elephants; 10 African savanna elephants *Loxodonta africana* and 11 Asian elephants *Elephas maximus* from 5 UK zoos (Figure 2.1) were selected for the methods validation study. Collections were selected to provide a geographical and geological spread across the UK, an approximately male to female split and approximately equal genus split (within the limitations of the UK *ex situ* population). Only adult animals over 10 years old at the time of sampling were used within the study.

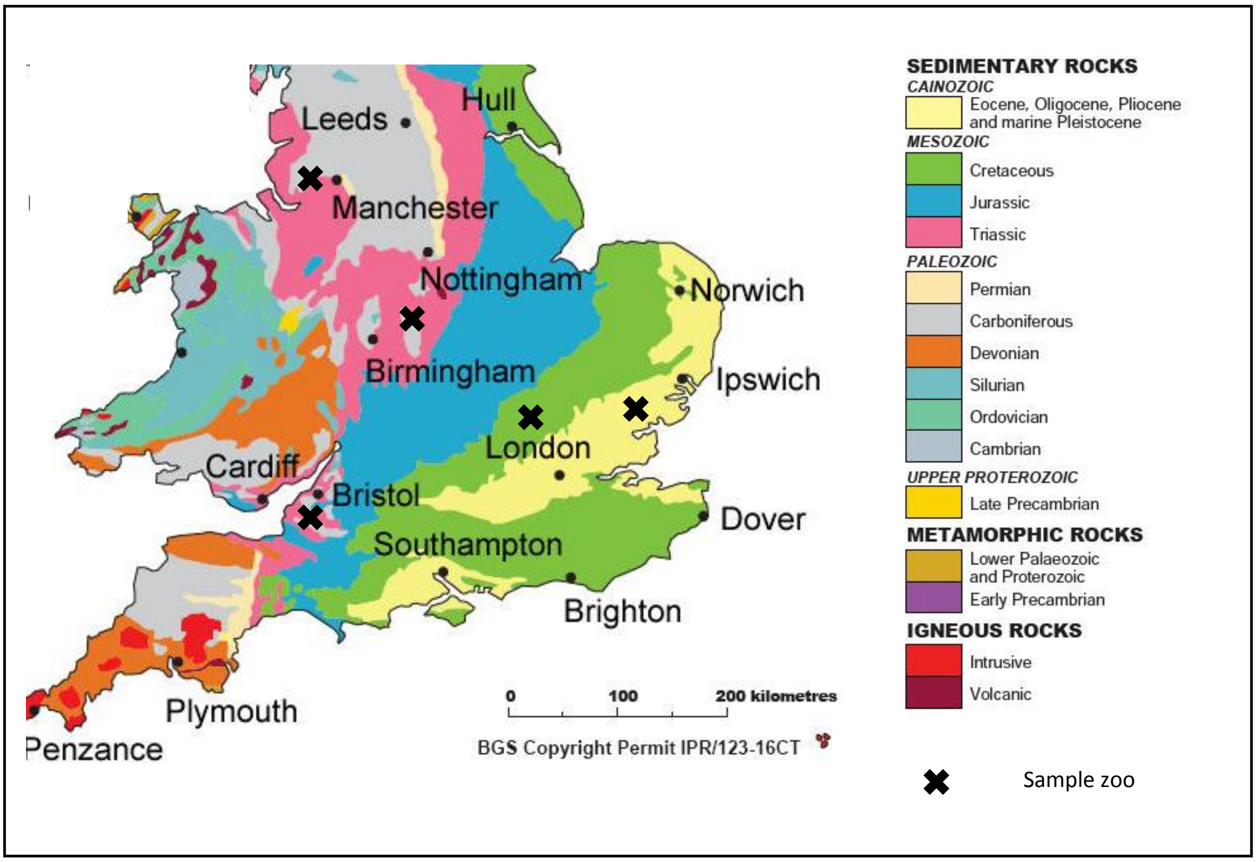


Figure 2.1: Map of UK showing location of sample zoos and the associated regional geology

Within this study, both genera of elephants were used to make use of sampling opportunity within the UK, and to increase sample numbers. Although there are physiological differences between African and Asian elephants, genus was accounted for as a fixed effect in the linear mixed model, indicated as 'species' (Chapter 4, statistical analysis), and thus it was reasonable to use both genera within this study. Both genera have 56 chromosomes (Hungerford *et al.*, 1966), and as large, generalist herbivores they occupy similar niches in their respective environments (BIAZA, 2019). Morphologically, the main differences between the genera include; a more convex back in Asian elephants, twin mounds on the forehead in Asian elephants compared to a single dome in African savanna elephants, a single lip/finger on the trunk of Asian elephants compared to two in African savanna elephants, the absence of tusks in female Asian elephants (sometimes smaller 'tushes' are present), compared to larger tusked male and female African savanna elephants, and five toenails on the hind feet of Asian elephants compared to four in African savanna elephants (Eltringham, 1982; Nowak, 1991; Csuti, Sargent and Bechert, 2001). The body weight ranges of the genera overlap, however, Asian elephants tend to be lighter. The weight range of wild adult Asian elephants is 1,800-5,000 kg compared with a range of 2,700-6,000 kg for adult African savanna elephants. Additionally, Asian elephants are up to one metre shorter in height and length than African counterparts, with males of both genera being larger (Eltringham, 1982; Ullrey, Crissey and Hintz, 1997; Olson, 2004).

2.6 Additional Methods for Study around the Palabora Mine, South Africa (Chapter 5)

The study areas are shown in Fig. 2.2 (A-F) and consist of the Kruger National Park (KNP), Associated Private Nature Reserves (APNR) and PMC. The PMC borders the ANPR and KNP, with elephants being able to move freely between the three areas (Fig.2.2). The KNP is situated in north-eastern South Africa and shows variation in climate and geological substrate which allows mixed-feeding species, such as elephants to use a variety of vegetation types to meet their dietary needs. From west to east, the geological succession of the national park changes from granitic in the west to basaltic in the east. Granites generally form nutrient poor substrates, whereas basaltic rocks form nutrient rich substrates (Codron *et al.*, 2006). Southern KNP granitic soils are dominated by broad-leaved species such as *Combretum* species, whereas fine-leaved species such as *Acacia* dominate on the basalts. In northern KNP both the granites and basalts are dominated by *Colophospermum mopane* (mopane) shrubveld, a tree species that is completely absent in the south (Venter and Gertembach, 1986). The APNR is located on the western border of the KNP and covers an area of 1800 km^2. Similar to KNP, the south is wetter than the north with about 250mm more rainfall annually. Geologically, the area is mainly made up of gneiss, granite or magmatite (Venter and Gertembach, 1986; Venter, 1990).

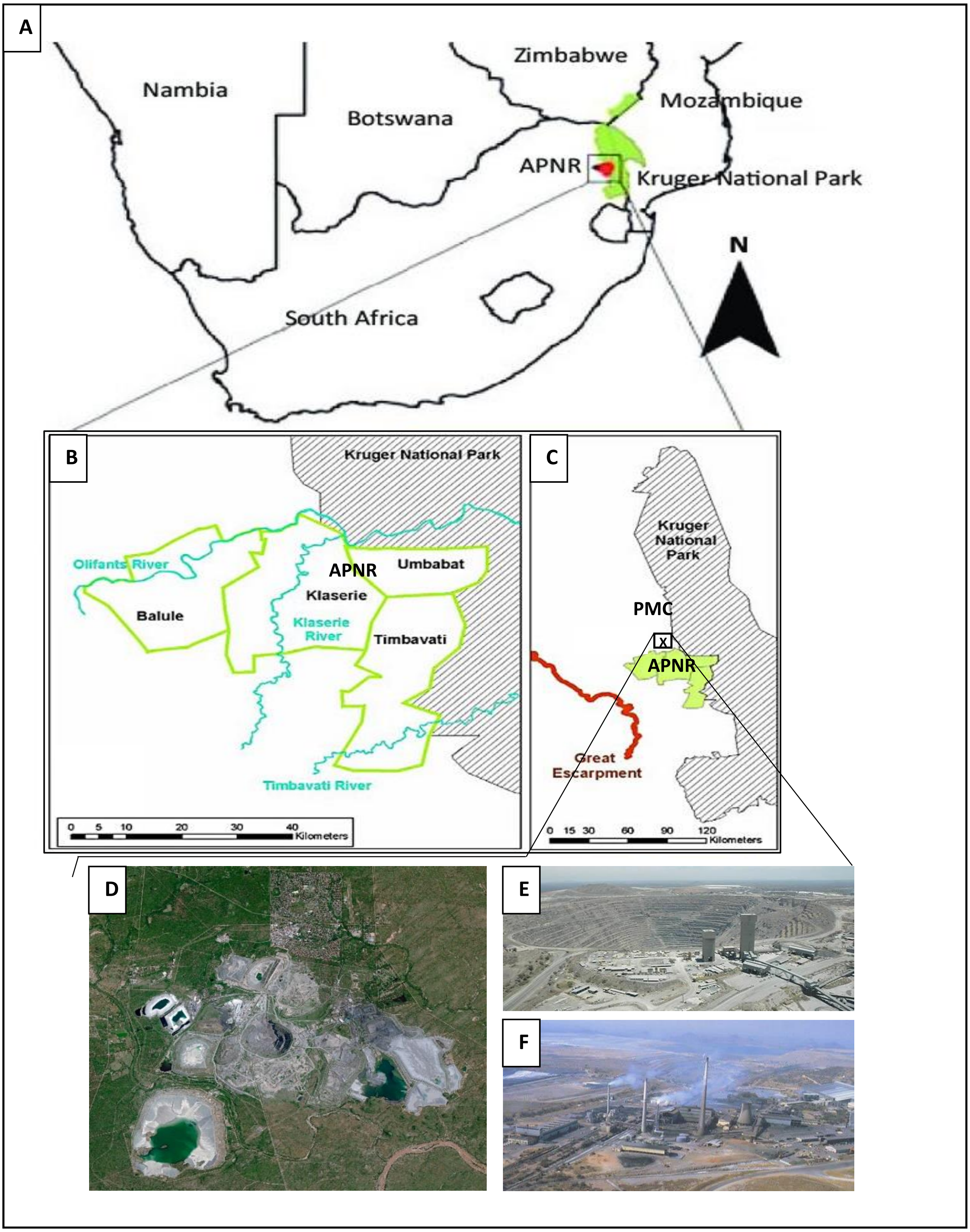


Figure 2.2 (A-F): Study area-Kruger National Park (A, C), Associated Private Nature Reserve (APNR; B, C), Palabora Mining Company (PMC; C, D, E and F)

Prior study in the APNR suggested a localised P deficiency in soil (water and plants) in the area (Fig.2.3). Females in family groups, assumed to have higher mineral requirements, were observed to consume plant parts with higher levels of P compared to larger bodied males (Greyling, 2004). Secondly, within the same study area Pretorius et al observed elephants to consume mopane leaves that were fertilised with P, and concluded that they did so to maximise P intake (Pretorius *et al.*, 2011, 2012).

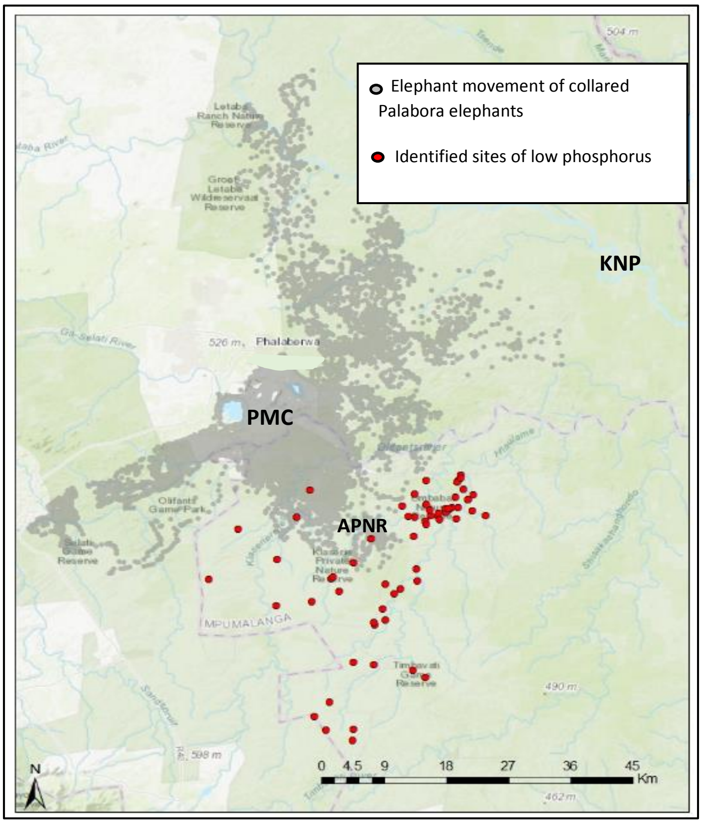


Figure 2.3: Overall elephant movement data from animals collared at the Palabora Mining Company land (PMC), shown as grey dots (2012-2017). Sites of identified low P from previous study in the area (Greyling 2004), shown as red dots. KNP= Kruger National Park, APNR = Associated Private Nature Reserves, PMC = Palabora Mining Company

2.7 Permits and Permissions

UK zoos: Research endorsement for the work within UK zoos was given by the European Association of Zoos and Aquaria (EAZA) Taxon Advisory Group (TAG), Belfast Closed Meeting 2016. Additionally, the British and Irish Association of Zoos and Aquariums (BIAZA) Research Committee gave research support for the work at the five UK zoos. Permission was also obtained from each of the five UK zoos in line with their individual research policies as necessary.

South Africa: Permit numbers for specific samples are detailed within the methods section of Chapter 5.

Permits were required to (1) collect samples in the field (elephant and environmental), (2) export the samples from South Africa, (3) import the samples to the UK and (4) transport the samples within the UK and handle them in UK laboratories.

(1) Sample collection permits:

-SANParks research permit (2017/2018) to collect soil, water, plant and faecal samples from the KNP. Project reference: SACF1444.

-Biological collection permits to collect elephant samples from PMC elephants, used veterinary surgeons with appropriate Threatened or Protected Species (TOPS) permits.

(2) Export permits:

- South African State Veterinary Services sample export permit, to remove samples from Kruger National Park, Skukuza to OR Tambo airport Johannesburg. Soil, water, plants, elephant faecal, elephant tail hair and elephant toenail: DAFF 06/036 322790 and DAFF 06/036 322688. Soil samples were heat treated in accordance with anthrax requirements. Plasma: DAFF 06/036 322782.

- Palabora Mining Company export permit for the transfer of goods from the mine property. Required for faecal samples, soil, water and plants collected within the PMC. Serial number 36196.

- CITES export permit for elephant plasma, toenail and tail hair (South Africa CITES):

  Tail hair (from SANParks) export: 171485.

  Toenail and tail hair (from EA) export: 222457.

  Plasma export: 171485 / 206233.

(3) <u>Sample import permits to bring samples into the UK</u>

- APHA permit number ITMP17.0821B toenail, tail hair, faecal and plasma samples

-BGS FERA licence 2017/2018 52036/198222/3, batch numbers:

  Plant samples DPHLA 17-12 and DPHL-A-18-029.

  Soil samples: DPHLA 17-12, DPHL-A-18-009, DPHL-A-18-26.

  Faecal samples: DPHL-A-18-26.

-CITES import permit for toenail, tail hair and plasma (UK CITES):

  Tail hair import (SANParks and EA): 566134/01 and 568473/01.

  Toenail import: 568473/02.

  Plasma import: 566134/02, seizure notice CET 307/18-19 shipment number 125. 81858556. Restoration: E5115944.

(4) <u>Transport and work with samples in UK</u>

-APHA AB117 U1260259/ ABP/OTHER – transport of biological samples (category 3) in the UK.

-APHA permit ITMP17.0821B for plasma, tail hair, toenail and faeces. All biological samples to be worked under containment level 2 conditions.

- Full Risk Assessment at BGS laboratories.

2.8 Sample Transportation

Plasma samples were transported on dry ice using an international courier. All other environmental and biological samples were transported in airplane hold luggage. All samples were transported in accordance with International Air Transport Association (IATA) transport and packaging requirements. Prior to export, in accordance with the APHA import permit requirements, faecal samples were dried in country and heated to 150 °C for 10 minutes and tail hair and toenail samples were autoclaved at 121 °C for 10 minutes. Plasma, tail hair, toenail and faecal samples were classified under the IATA Dangerous Goods Regulations as ‘Biological Substance Category B UN 3373, Exempt animal specimens’. Samples were transported in line with the requirements set out in 3.6.2.2.3.8 as demonstrated in Fig. 2.4 (Dangerous Goods Regulations, 2020).

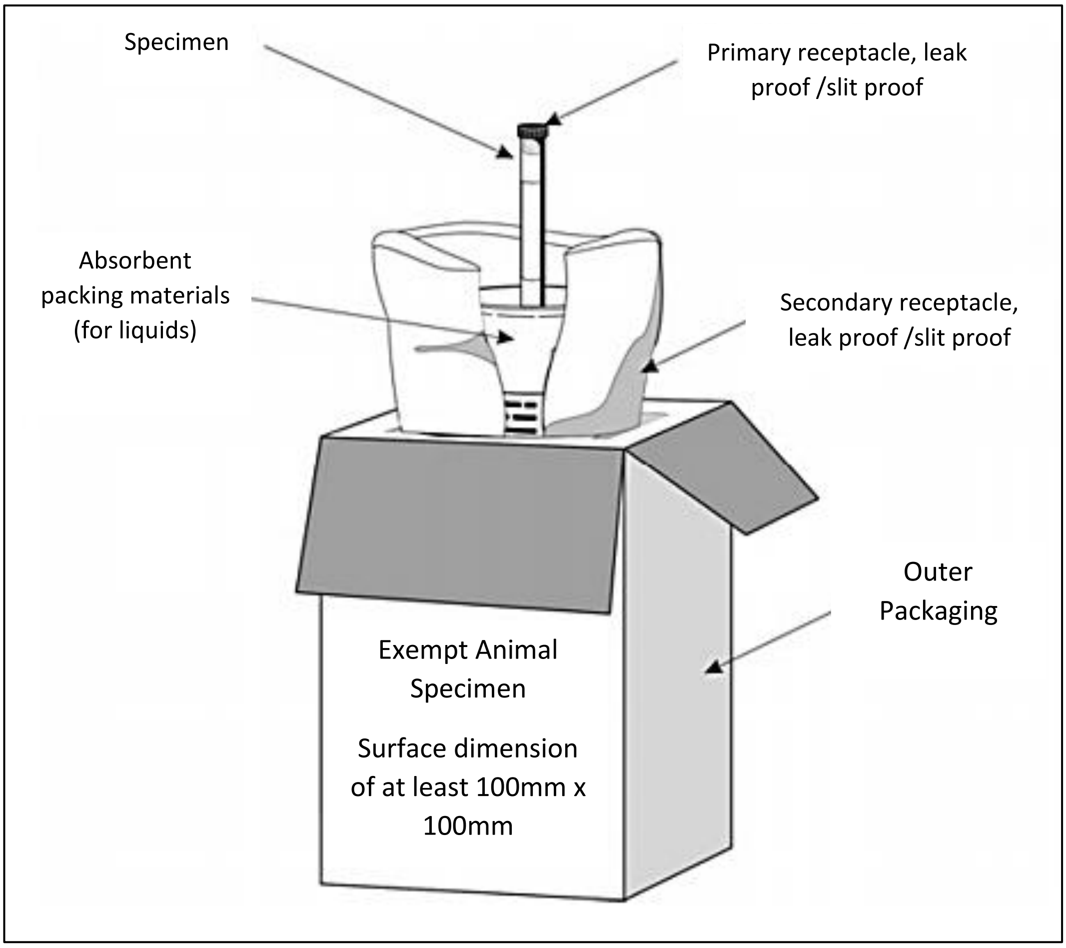


Figure 2.4: Packaging requirements for faecal, toenail, tail hair and plasma samples. Adapted from (Dangerous Goods Regulations, 2020)

2.9 Analysis of Tail Hair

Prior to analysis, tail hair (and toenail) samples were cleaned, based on the method described by Button et al. (2009), as explained in Chapter 4 and 5 and Fig.2.5. The sample was placed in a clean glass beaker (tail hair coiled in a circular shape, depending on length; toenail chopped into approximately 1cm lengths), and sonicated for 5 min using 3 ml of acetone, rinsed first with 2 ml of deionised water and then 2 ml of acetone, sonicated for 10 min in 3 ml of deionised water then twice rinsed with 3 ml of deionised water, ensuring complete submersion of the sample during each step, as shown in Fig. 2.5, steps 1-6. If the tail hair was especially long, double quantities of acetone and water were used to ensure full submersion of the sample at each cleaning step. The acetone used for sample cleaning was HPLC grade (Fisher Scientific, UK).

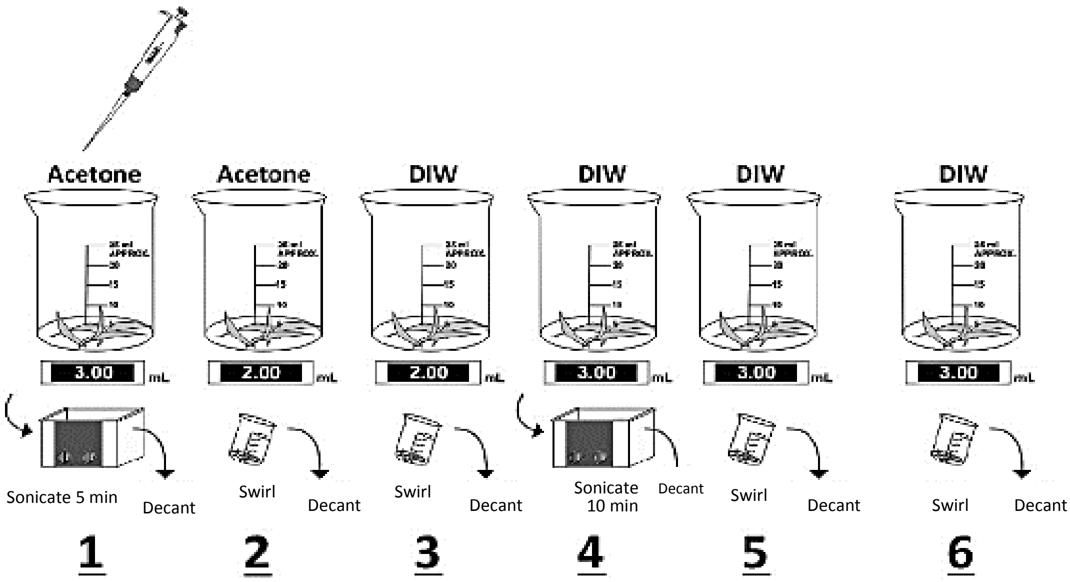


Figure 2.5: Cleaning procedure for elephant tail hair (and toenail) samples, steps 1-6. Based on *Electronic supplementary material (ESI)* (Middleton *et al.*, 2016). DIW = De-ionized water

At the start of the project, the structure of elephant tail hair in relation to elemental distribution across and down the hair was largely unknown. Previous work indicated that the structure was similar to other mammalian species (Hausman, 1920; Yates, Espinoza and Baker, 2010) and that it would be a suitable bio-indicator for elemental status in the elephant. For certain minerals or PTE's, elemental levels in human or horse hair, vary down the length of the hair, reflecting a varying intake over time (Combs, 1987; Bencko, 1995; Armelin, Avila and Piasentin, 2003; Middleton *et al.*, 2016). Chapter 4 demonstrates that tail hair is a reliable indicator of intake and thus proxy for status in Fe and As (Sach *et al.*, 2020). Previous studies indicated an approximate growth rate in wild elephant hair of 0.56 mm/day (± 0.11) in males and 0.81 mm/day (±0.13) in females, based on two studies of 32 and 50 elephant hairs respectively (Cerling *et al.*, 2006; Wittemyer, Cerling and Douglas-Hamilton, 2009) although, acknowledging that this can vary with nutritional plane, weather, health and age of the elephant. Elephant tail hairs from both captive and wild animals are often over 30 cm long, indicating potentially 12-18 months of growth within one hair.

In order to assess the variance in elemental profile down the elephant hair (over time) from the elephants collared at the PMC, additional information was needed. Hairs were sectioned for ICP-MS analysis, to indicate periods of mine/non-mine movement and assess if seasonal changes influence dietary intake. Therefore, information was needed on where to section the hairs for ICP-MS analysis. The minimum mass of sample for ICP-MS is approx. 0.03-5 g to maintain data accuracy. This equated to approximately 3–5 cm of tail hair, depending on hair thickness. The most appropriate technique to quantify mineral and PTE levels down the hair, and provide this information, was laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). This technique has a spatial resolution ranging from 10–100 mm (Becker, Matusch and Wu, 2014). Other techniques such as Scanning Electron Microscopy Energy Dispersive X-ray (SEM-EDX) or Electron Probe Microanalysis (EPMA) were considered (and EPMA was used to produce some mineral maps), but were not suitable alone due to their lower analytical sensitivity. Consequently, information was needed about the overall structure and morphology of the elephant hair, to inform where to direct the laser for LA-ICP MS and the appropriate sampling depth, width and laser cleaning protocol. Scanning electron microscopy (SEM) was identified as the optimum technique for this to examine the structure cross-sectionally as well as the outside surface of the hair. Subsequent ICP-MS analysis using dissolved tail hairs was intended for these samples; therefore, techniques to determine where to section the tail hair had to be non-destructive for ICP-MS (Chapter 5). After the LA-ICP-MS had been conducted, additional examination was undertaken using a

Nikon SNZ 1500 venocular zoo microscope with Nikon NIS elements D camera software with a D5-Fi1 camera.

Tail hair method development, to determine sectioning for ICP-MS was conducted using surplus hairs from both wild and zoo elephants, comprising a variety of ages, thicknesses and lengths of hair, and from both sexes and genera to assess any visual differences in the hair structure. A previous study indicated some difference between the species, with greater pigmentation in Asian elephants and an absence of tubules (vacuities in the field of keratin that are ovoid or compressed) in African savanna elephants compared to uneven scattering in Asian elephants (Yates, Espinoza and Baker, 2010). After method development, scanning electron microscope images were taken of hairs from five animals collared at the PMC, elemental mapping conducted on 2 different hairs (one animal from the PMC), and laser ablation conducted on two hairs of elephants collared at the PMC.

2.10 Scanning Electron Microscopy and X-ray Microanalysis Method

The sub-samples of the elephant hairs, mounted in longitudinal and cross-section, on carbon tape onto a petrographic glass slide were examined in the uncoated state by scanning electron microscopy (SEM) using both secondary electron (SE) imaging and backscattered scanning electron (BSE) imaging. Element distributions in the elephant hairs were studied using digital energy-dispersive X-ray microanalysis (EDXA) elemental mapping and quantitative energy-dispersive electron probe point microanalysis (ED-EPMA). This was carried out simultaneously during backscattered scanning electron microscopy (BSEM). The sub-samples were examined by BSE imaging using the SEM instrument in the low-vacuum environmental mode in order to gain a rapid initial understanding of the reaction products to guide the focus of more detailed observations.

BSEM-EDXA analyses were carried out using an FEI Company QUANTA600 environmental scanning electron microscope (ESEM) fitted with a 2-element (diode-type) backscattered electron detector, and equipped with an Oxford Instruments INCA 450 energy dispersive X-ray microanalysis (EDXA) system with a 50 MM^2 Peltier-cooled (liquid nitrogen free) silicon drift X-ray detector capable of operating at very high input X-ray count rates (up to ~10^6 counts per second). The scanning electron microscope instrument was operated in low vacuum mode (0.95 torr) using a 20 kV electron beam accelerating potential, a beam current of ~0.6 nA, and a working distance of 10 mm. Phase identification was aided by micro chemical information obtained from observation of semi-quantitative EDXA spectra recorded from features of interest.

Digital EDXA X-ray element maps were recorded for key areas at a resolution of 1024 x 1024 pixels, and using a 20 kV electron beam, ~0.6 nA beam currents and at a working distance of 10 mm, to give optimum X-ray count rates of up to 28,000 counts per second. EDXA spectra and digital X-ray elemental maps were processed using the INCA Microanalysis Suite version 5.05 (Oxford Instruments Analytical Limited, 2014) software package. X-ray element maps were produced by summation of data recorded from multiple frame scans to produce maps with sufficient X-ray counts per pixel to enable the key elements, required for the differentiation of the mineral species present, to be detected above background noise (typically between 20-50 frame scans, recorded over 0.5-2 hours). A reference BSE image was recorded for each mapped area. The image brightness in BSE images is related to the average atomic number of the phases observed (Goldstein et al., 1981), thereby enabling the differentiation of the different minerals present. X-ray elemental maps were processed to show relative element concentrations using a ‘rainbow colour scale’ ranging from black (representing zero background) through green, yellow and orange (low to intermediate concentration) to red or white (representing high concentration).

Figures 2.6 and 2.7 show the cross-section elemental distribution for a tail hair. Generally elemental distribution was uniform, with some increased P seen around the edge of the hair in Fig.2.7. Figure 2.8 shows SEM backscatter images of several zoo and the wild elephants sampled at the PMC and the elephants sampled outside of the PMC. Cutting technique affects image quality, and care must be taken to obtain a 'clean cut'. As found by Yates et al. (2010), hair from both species show the presence of tubules, these may be infilled or empty and in African savanna elephants are generally found around the edges of the hair and not in the centre, hence care must be taken to avoid them when sampling for LA-ICP-MS. This is especially visible in Wild African (Int) and PMC elephant Nkozsana (Fig.2.8). Secondly, the surface of the wild elephant hairs appear to be cracked and rough, indicating potential for contamination that is impossible to remove, even with the extensive cleaning protocol (Fig. 2.5), as seen in Wild African (Int) and in the image of the hair surface (Fig. 2.8). Additional elements were examined as part of this method development process using the EDXA, including oxygen (O), sulphur (S), chlorine (CL and carbon (C). These were detected at above background levels in the analysis thus included to give an overall picture.

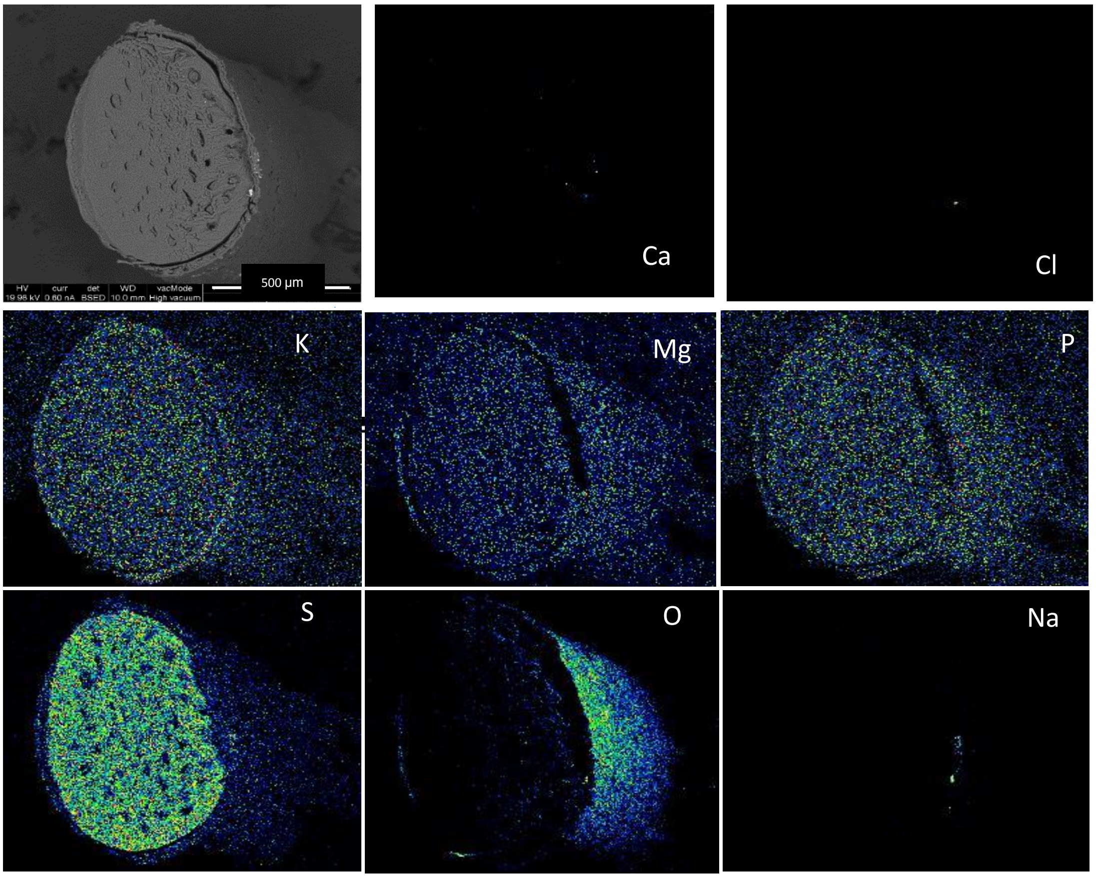


Figure 2.6: Energy-dispersive X-ray microanalysis (EDXA) elemental mapping of a cross section of a female elephant tail hair collared at the PMC (Palabora Mining Company). Black represents zero background through green, yellow and orange representing low to intermediate concentration to red or white, representing high concentration. The image in the top left hand corner shows the overall structure of the hair

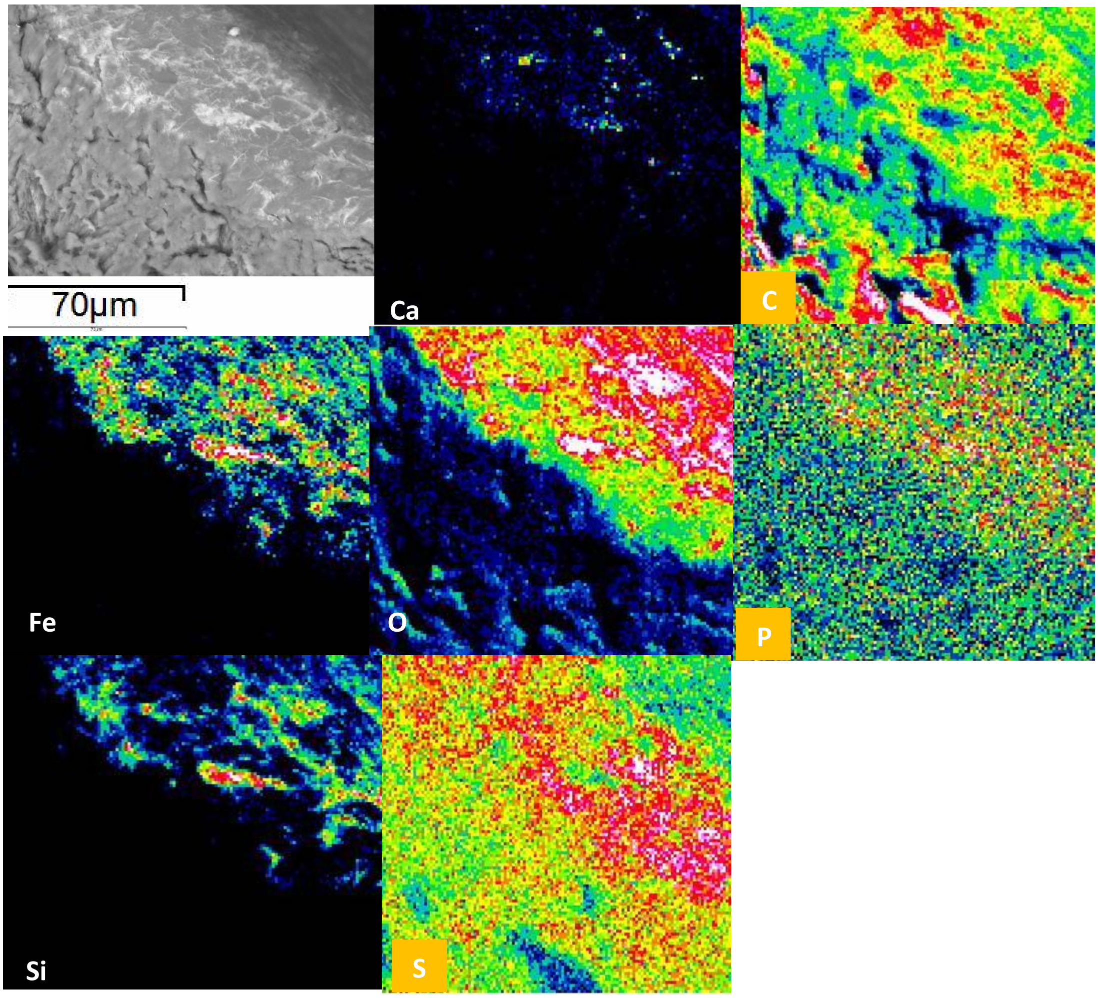


Figure 2.7: Energy-dispersive X-ray microanalysis (EDXA) elemental mapping of a cross section of a tail hair edge from a wild female elephant. Black represents zero background through green, yellow and orange representing low to intermediate concentration to red or white, representing high concentration. The image in the top left hand corner shows the overall structure of the hair.

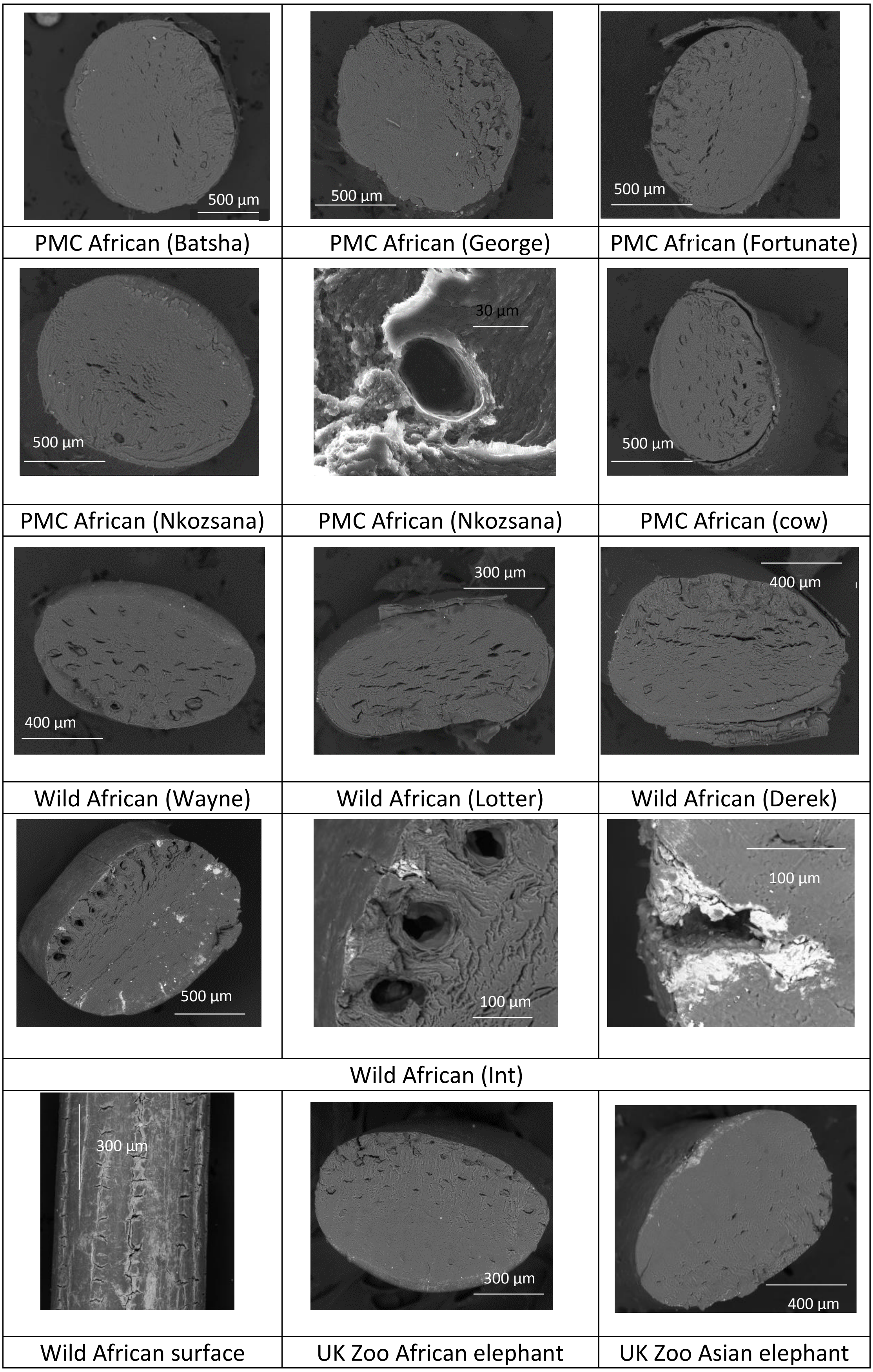


Figure 2.8: Scanning electron microscopy images (cross sectional and surface) of wild and zoo African (*Loxodonta africana*) and Asian (*Elephas maximus*) elephant tail hair. PMC = Palabora Mining Company**,** wild animals collared within the mining area; 'wild' = samples from animals collected within the Kruger National Park or Associated Private Nature Reserves; identification names for the elephants are given in brackets.

2.11 Laser Ablation

Previous work indicated that Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS) was a useful tool for trace element characterisation in a single hair (Sela *et al.*, 2007). Elements of interest were examined both longitudinally down the hair and cross sectionally; Na, Mg, P, K, Ca, Mn, Zn, Pb and U. Longitudinal examination was of principal interest to determine if elemental levels changes down the hair, and therefore, if changes over time could be identified. After method development trials, LA-ICP-MS was conducted on hairs from five animals; three animals collared at the PMC and two from the surrounding area (KNP/ APNR). Cross sectional LA-ICP-MS was also conducted to assess elemental change across the hair to ensure the depth of longitudinal LA-ICP-MS sampling was appropriate.

After cleaning, tail hairs were fixed to the laser plate using double-sided adhesive tape. Hairs were coiled around a glass reference material, SRM 610 (NIST, USA), that was used to ensure appropriate calibration of the laser. The ablation was conducted with a NewWave UP193FX excimer (193nm) laser system, with built in microscope imaging, which was coupled to an Agilent 7500 series ICP-MS, with helium gas, mixed with argon. Laser conditions such as energy and beam size were determined from preliminary ablations on spare hairs; energy was 20 Htz at 100%. The irradiance was typically 1.51 GW/ cm^2 and fluence was 7.54 J/ cm^2. Laser ablation craters were set at 100 µm. In order to remove any surface contamination, and to ensure sample was taken from the mineral crust, two cleaning passes were run over the hair followed by one single data collection pass. The cleaning speed was 200 microns/sec (2 passes) followed by analysis speed of 100 microns/sec (1 pass).

During method development, SEM and microscope images were taken of the lasered hair to assess path of the laser and sample depth as shown in Fig. 2.9.

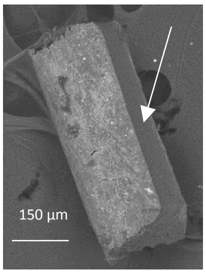
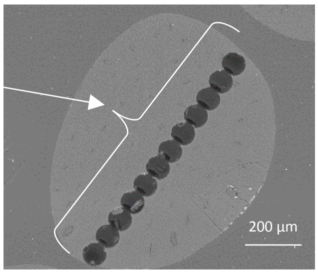
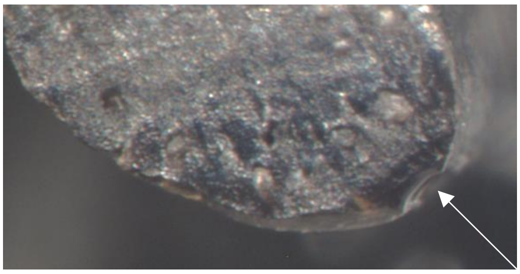
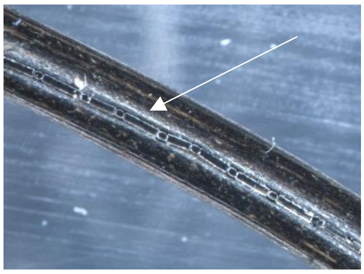
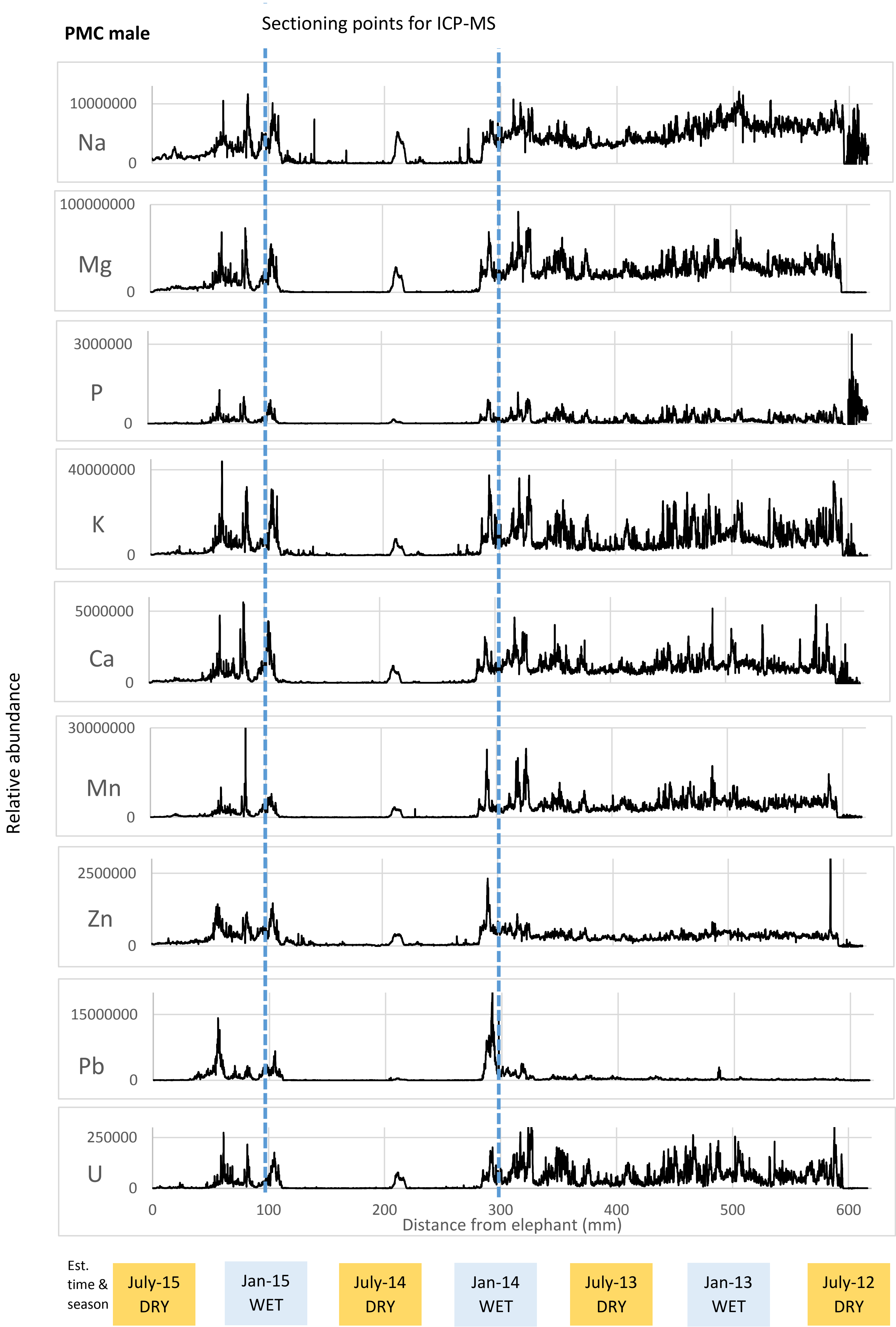
	
SEM image showing laser trench	SEM image of cross sectional hair showing route of laser across hair
	
Microscope image showing depth of laser sampling	Microscope image of hair surface showing longitudinal route of laser

Figure 2.9: Images of tail hair taken after laser ablation was conducted to assess depth and pathway of laser sampling. SEM = Scanning Electron Microscopy



PMC male
Sectioning points for ICP-MS
Relative abundance
10000000
Na
0
100000000
Mg
0
3000000
P
0
40000000
K
0
5000000
Ca
0
30000000
Mn
0
2500000
Zn
0
15000000
Pb
0
250000
U
0
0
100
200
300
400
500
600
Distance from elephant (mm)
Est. time & season
July-15 DRY
Jan-15 WET
July-14 DRY
Jan-14 WET
July-13 DRY
Jan-13 WET
July-12 DRY

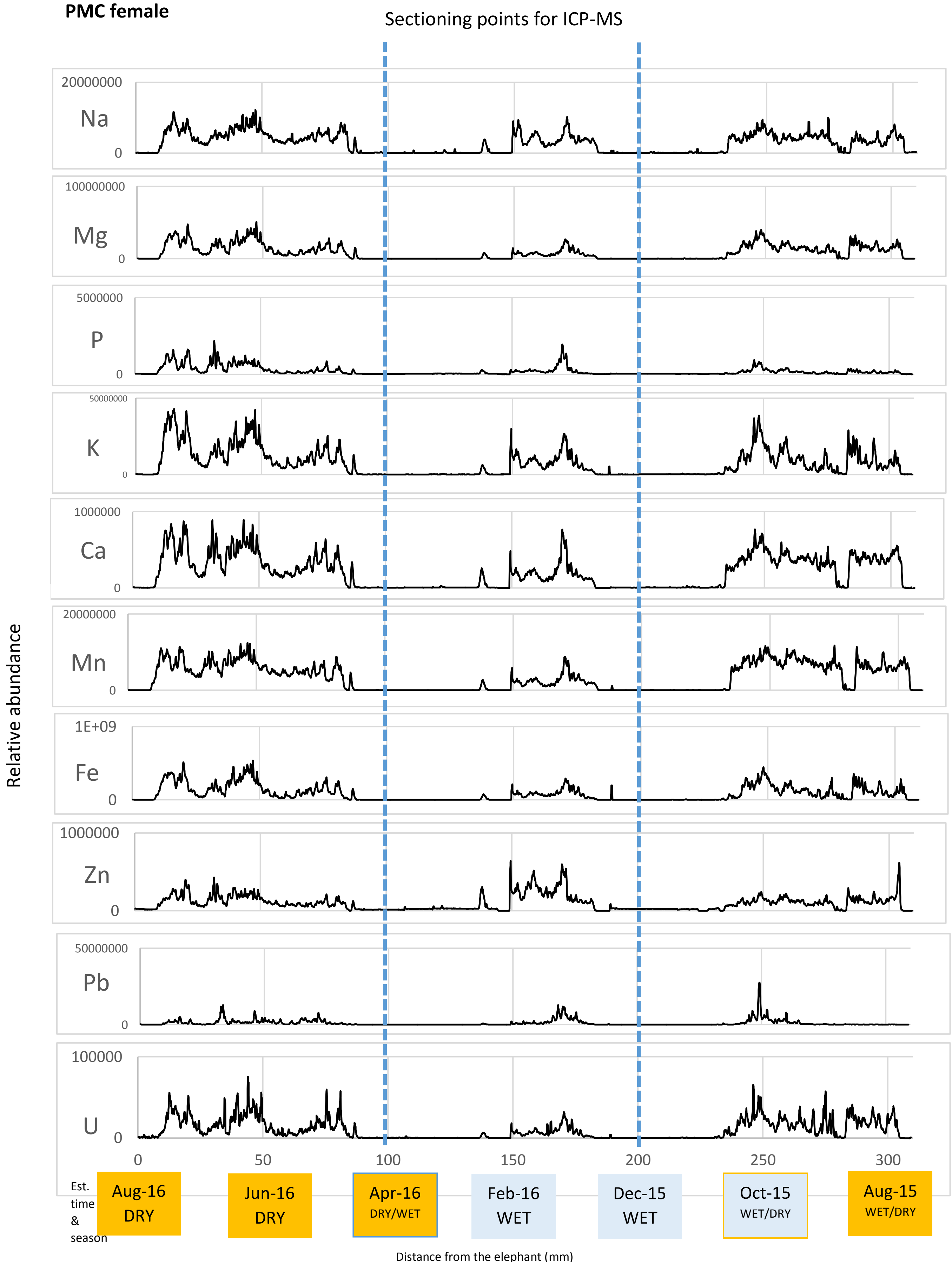

PMC female
Sectioning points for ICP-MS
20000000
Na
0
100000000
Mg
0
5000000
P
0
50000000
K
0
1000000
Ca
0
20000000
Mn
0
1E+09
Fe
0
1000000
Zn
0
50000000
Pb
0
100000
U
0
Relative abundance
0
50
100
150
200
250
300
Est. time & season
Aug-16 DRY
Jun-16 DRY
Apr-16 DRY/WET
Feb-16 WET
Dec-15 WET
Oct-15 WET/DRY
Aug-15 WET/DRY
Distance from the elephant (mm)

Figure 2.10: Laser ablation ICP-MS data (y-axis=relative abundance) for two wild African savanna elephants collared at the Palabora Mining Company (PMC), against estimated time and season data (x-axis). Distance from the elephant, 0=base of hair, attached to elephant tail. These hairs were sectioned for ICP-MS analysis as demonstrated by the blue dashed lines

Figure 2.10 demonstrates how LA-ICP-MS informed where to section the tail hairs depending upon elemental levels, within the minimum mass limitations for subsequent ICP-MS analysis. Results of ICP-MS analysis are detailed in Appendix, Chapter 5. As the date of sampling was known, the approximate growth rate, of the elephant hair (obtained from the literature) was used to estimate the season that the sample was from; growth rate must always be considered as estimation. The sample from the PMC cow showed variation between the wet and dry season with relatively elevated levels of all elements investigated in the 2016 dry season, compared to the 2015/16 wet season, with an indication that this trend was mirrored in the 2015 dry season. Assessing these data in relation to movement data could indicate if animals are spending more/less time within the PMC lands and thus could be consuming increased levels of minerals or PTE's. Unfortunately samples were taken from these animals when GPS tracking collars were fitted, thus movement data for this time period is not available and so the possible relationship between these variables cannot be assessed at this point in time. Figure 2.11 demonstrates the relative change in elements (Mn, Fe and Pb) across a cross section of elephant tail hair. Areas of greatest abundance were found around the edges of the hairs.

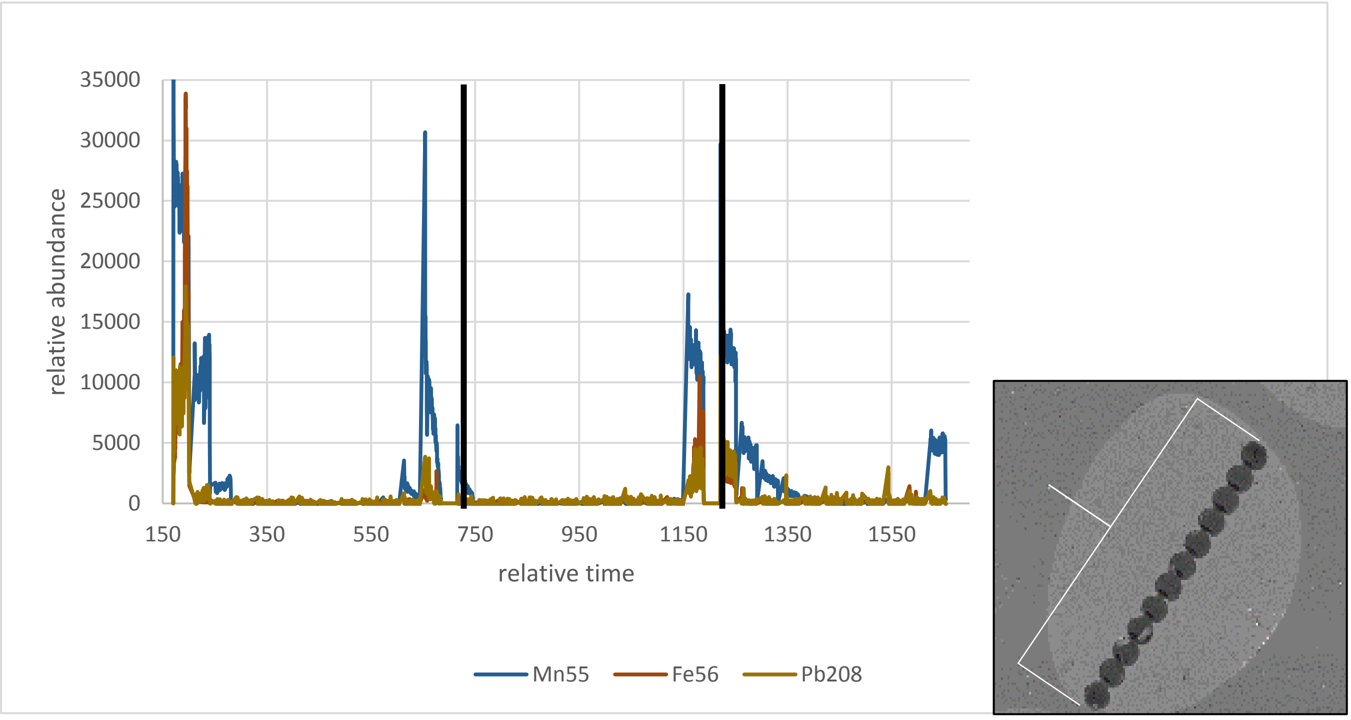


Figure 2.11: Cross sectional laser ablation data across three cross sectional pieces of African savanna elephant tail hair. Elements present in greatest relative abundance are shown: Mn, Fe and Pb. Scanning electron microscopy image demonstrates pathway of the laser cross-sectionally across the hair

2.12 Conclusion

Combining the information in Figures 2.6, 2.7, 2.8, 2.9, 2.10 and 2.11 provided confidence that LA-ICP-MS of the hairs from the elephants collared at the PMC was conducted at an appropriate depth. This was to ensure inclusion of the high mineral crusting around the edge of the hair that was likely to show the greatest elemental variance longitudinally down the hair, whilst avoiding the cracked outside, potentially contaminated surface of the hair. No substantial tubules were seen on SEM images of these hairs from elephants collared at the PMC, giving assurance that the laser was targeted appropriately along the hair avoiding these tubules. It was suspected that elemental levels in tubules would be vastly reduced, as these are essentially air-filled. The experimental study in Chapter 5 compared mineral and

PTE levels in non-mine whole tail hairs with combined median vales for PMC sectioned tail hair. The location of sectioning on these PMC hairs was previously informed by laser ablation, which was informed by SEM imagery.

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Appendix

Table A0: Full list of analysed elements (58), elements included within the study are in bold. Standard scientific abbreviations are used

Ag	**Fe**	**Pb**	**V**
Al	Ga	Pr	W
As	Gd	Rb	Y
B	Hf	S	Y
Ba	Ho	Sb	Yb
Be	**K**	**Se**	**Zn**
Bi	La	Si	Zr
Ca	Li	Sm	
Cd	Lu	Sn	
Ce	**Mg**	Sr	
Co	**Mn**	Ta	
Cr	Mo	Tb	
Cs	**Na**	Th	
Cu	Nb	Ti	
Dy	Nd	Tl	
Er	Ni	Tm	
Eu	**P**	**U**	

Chapter 3

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Supplementary information-*available online*

- Appendix – Papers reviewed

African savanna elephants (*Loxodonta africana*) as an example of a herbivore making movement choices based on nutritional needs

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Abstract

Background

The increasing human population and global intensification of agriculture have had a major impact on the world's natural ecosystems and caused devastating effects on populations of mega-herbivores such as the African savanna elephants, through habitat reduction and fragmentation and increased human-animal conflict. Animals with vast home ranges are forced into increasingly smaller geographical areas, often restricted by fencing or encroaching anthropogenic activities, resulting in huge pressures on these areas to meet the animals' resource needs.

This can present a nutritional challenge and cause animals to adapt their movement patterns to meet their dietary needs for specific minerals, potentially causing human-animal conflict.

The aim of this review is to consolidate understanding of nutritional drivers for animal movement, especially that of African savanna elephants, and focus the direction of future research. Peer reviewed literature available was generally geographically specific and studies conducted on isolated populations of individual species. African savanna elephants have the capacity to extensively alter the landscape and have been more greatly studied than other herbivores, making them a good example species to use for this review. Alongside this, their movement choices, potentially linked with nutritional drivers could be applicable to a range of other species. Relevant case study examples of other herbivores moving based on nutritional needs are discussed.

Methods

Three databases were searched in this review: Scopus, Web of Science, and Google Scholar, using identified search terms. Inclusion and exclusion criteria were determined and applied as required. Additional grey literature was reviewed as appropriate.

Results

Initial searches yielded 1,870 records prior to application of inclusion and exclusion criteria. A less detailed review of grey literature, and additional peer-reviewed literature which did not meet the inclusion criteria but was deemed relevant by the authors was also conducted to ensure thorough coverage of the subject.

Discussion

A review of peer reviewed literature was undertaken to examine nutritional drivers for African savanna elephant movement, exploring documented examples from free-ranging African savanna elephants and, where relevant, other herbivore species. This could help inform prediction or mitigation of human-elephant conflict, potentially when animals move according to nutritional needs, and related drivers for this movement. In addition, appropriate grey literature was included to capture current research.

Introduction

The African savanna elephant (*Loxodonta africana*) is categorised as vulnerable on the IUCN Red List and free-ranging populations have declined rapidly across Africa since 1970, predominantly as a result of increased poaching and competition for resources with an increasing human population (Blanc, 2008). This competition arises due to the intersection of human activities with elephants' home ranges, and much research is devoted to investigating the reasons why the animals move repeatedly through areas which lead them into conflict with humans (Eltringham, 1990; Hoare & du Toit, 1999; Hoare, 2000). The aims of this review are to examine the current knowledge on the mineral requirements of the African savanna elephant, to consolidate the current understanding of nutritional drivers for African savanna elephant movement, to examine how geochemistry may affect herbivore movement and to consider how this knowledge could be applied to predict and mitigate human-elephant conflict in the future. African savanna elephants have the capacity to extensively alter the landscape and have been more extensively studied than other herbivores, making them a good example species to use within this review. Where relevant, examples of other herbivore movement (including other elephant species) based on nutritional needs are included.

Due to their vast food consumption and behaviour, African savanna elephants can cause significant damage to crops and vegetation (Eltringham, 1990; Hoare, 2000) and pose a risk to human life and infrastructure. Continued increase in the global human population, to 9.7 billion by 2050, and the associated intensification of agriculture will have a major impact on the world's natural ecosystems (Nyhus, 2016). This, coupled with a predicted reduction of 200-300 million hectares of wildlife habitat worldwide, will aggravate human-animal conflict. Wide ranging landscape-level herbivores are increasingly threatened globally (Wall et al., 2013). Habitat encroachment and fragmentation poses a substantial threat to elephant populations, forcing them to condense into ever-smaller geographical areas or fenced reserves, whilst putting increased pressure on these areas to meet the animals' resource needs (Nyhus, 2016). This can present a nutritional challenge and might cause animals to adapt their movement patterns to meet their dietary needs, including for specific minerals, presenting wildlife managers with new management issues.

It is the aim of this review to consolidate understanding of nutritional drivers for animal movement especially those of the African savanna elephant, and focus the direction of future research.This will be achieved with the following objectives:

1. Examine current knowledge on mineral requirements in elephants, including the differences between nutritional needs of cows and bulls, activity budget of the species to include time spent feeding,
2. Examine the relationship between the geochemistry and the associated soil of an area, and how this can alter the minerals available in plants to elephants as consumers (herbivores). Use this information to examine how geochemistry may act as a driver for African savanna elephant movement. Only minerals are being considered within this review.
3. Consider how knowledge of mineral distribution in the landscape could be used to predict and mitigate human-elephant conflict in the future.

This review is intended to benefit conservation managers, ecologists, conservation biologists, national park management authorities, and potentially managers of animals under human care both within zoos and fenced reserves.

Methods

The following method was used to ensure comprehensive and unbiased coverage of the literature. Published studies were identified from three databases, using a range of search terms relating to elephant movement choices.

Search terms:

List 1: ‘elephant’, ‘Elephantidae’, ‘Loxodonta’, ‘mega herbivore’

List 2: ‘soil’, ‘mineral’, ’minerals’, ’nutrition’, ‘geochemistry’, ‘movement’

The clause ‘and’ was included between each word in list 1 and list 2. Each search contained 1 word from list 1 and one from list 2. Each word from each list was searched together.

Search terms were selected based on a scan of the literature to give broad covering of subject of interest.

Databases searched: Scopus, Web of Science, and Google Scholar (searched up to 1st April 2018).

Fields searched: titles, keywords, abstracts

Inclusion/exclusion criteria:

Only publications which met the following criteria were included in this review. The publication:

1. Contained at least one of the search terms from each list in the abstract, title or keywords.
2. Was in a published peer-reviewed journal.
3. Was in English.
4. Was relevant to the subject matter (e.g. excluded irrelevant terms such as elephant grass *Pennisetum purpureum*).

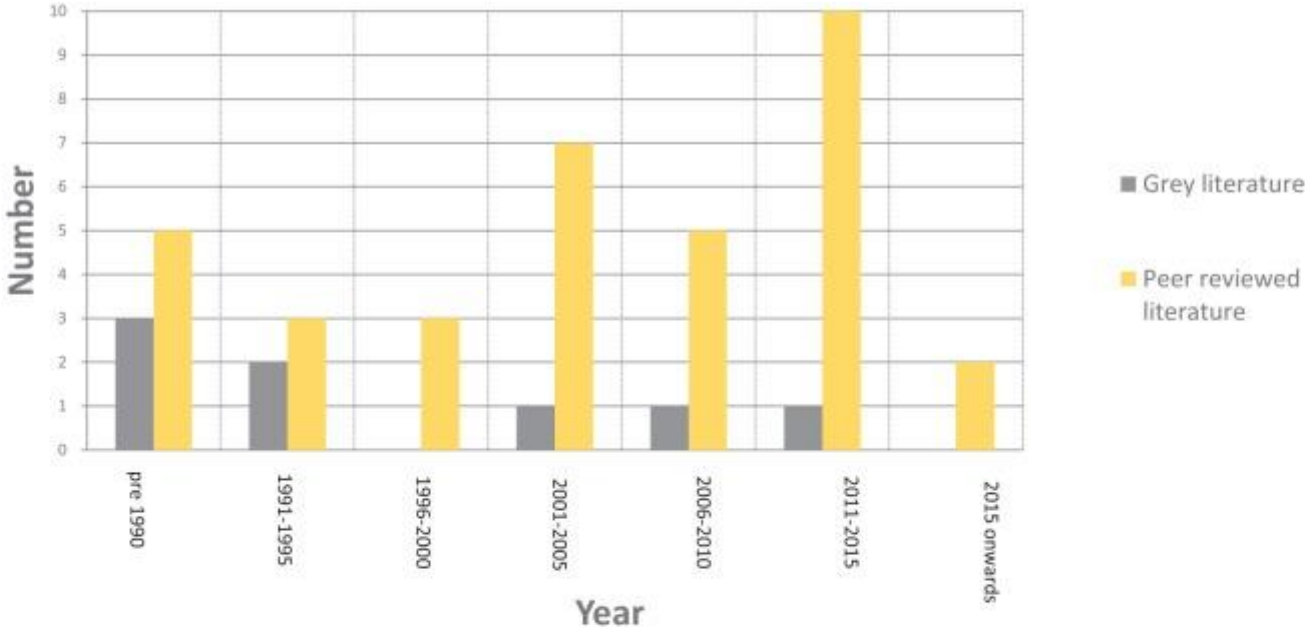

Figure 3.1: Breakdown of the literature by date after the application of the inclusion/exclusion criteria

Grey literature reviewed

Additionally, relevant grey literature which did not meet the inclusion criteria was reviewed. This was identified as follows:

1. Theses books and conference proceedings
2. Using internet searches of key terms and snowballing by searching the reference lists of relevant literature (Sayers, 2007). Keywords were selected based on scan of literature to give a broad coverage of the subject of interest.

Results

Initial searches yielded 1,870 records. After applying the inclusion/exclusion criteria, thirty-five papers were fully reviewed, detailed in Appendix (available online). Current work was generally geographically specific and conducted on isolated populations of individual species with dates ranging from 1969 - 2018. Further details of the breakdown of the literature search can be seen in Figure 3.1. All reviewed papers were on free-ranging African savanna elephants or other herbivore species including wildebeest (*Connochaetes taurinus*), zebra (*Equus burchelli*), roe deer (*Capreolus capreolus*) and black rhino (*Diceros bicornis*). Seventy percent of reviewed papers focused specifically on African savanna elephants, thirty percent of reviewed papers focused more broadly on herbivores.

From the review of grey literature, and additional peer-reviewed literature which did not meet the inclusion criteria, eight further references were identified, which consisted of five books, one thesis and one short report, as detailed in Figure 3.1. Dates of references ranged from 1977-2012, detailed in Appendix (available online).

Elephant Nutritional Needs

Challenges of Estimating Elephant Nutritional Requirements

Due to the lack of knowledge on the digestive physiology of many wild animals, animal nutritionists use domestic species as physiologic models when designing diets for captive exotic animals. For large hindgut fermenters like elephants and rhinos, the horse has been suggested as the appropriate model for most nutrients due to the similarities in gastrointestinal tract anatomy (Clauss, Kienzle & Wiesner, 2003). Therefore, when assessing published nutritional recommendations, the benefits and limitations of using this model must be considered. This approach, using the equid model was validated for white rhinos (*Ceratotherium simum*) and Indian rhinos (*Rhinoceros unicornis*) but not black rhinos (*Diceros bicornis*) or any elephant species (Clauss, Kienzle & Wiesner, 2003). Clauss et al. (2007) demonstrated that black rhinos absorb micronutrients in the same manner as equids, and suggested the same may apply in elephant species. Despite the lack of validation, the horse was extensively used as a model for captive elephant nutrition (Olson, 2004; Clauss et al., 2007; Walter, 2010) and overall, it is considered a suitable model for many aspects of elephant digestion including the mechanisms by which dietary supplements and dietary crude fibre content influence digestibility, calcium absorption, and faecal volatile fatty acid composition. However, elephants have a faster ingesta passage rate than equids, with a total gut transit time of 11-46 hours, compared to an average of 48 hours in equids, and thus digestibility coefficients are lower for all nutrients (Bax & Sheldrick, 1963; Clauss et al., 2003). This must be factored into any comparison with equid recommendations and extrapolation be used with caution.

Reported Mineral Deficiencies in Captive and Free-ranging Elephants

As the evidence for specific mineral needs for elephants (of either species) is very limited, documented values for requirements of both African and Asian elephants (*Elephas maximus*) are included for four key minerals; calcium, iodine, iron and zinc. Because Asian elephants are held in greater numbers in captivity, there has been more research on the mineral needs of this species.

Calcium

It has been suggested that elephants have highest calcium demands when lactating (females) followed by periods of intensive tusk growth (Dierenfeld, 2008). Calcium metabolism in elephants appears to be similar to that of equids, with approximately 60% absorbed from the diet directly in the intestines, independent of total consumption or requirement, with excess excreted in the urine (Ullrey, Crissey & Hintz, 1997). As with other mammals, elephants maintain serum calcium within a narrow range through intestinal absorption, renal excretion and mobilisation of bone (Ullrey, Crissey & Hintz, 1997; Clauss et al., 2003).

Partington (2012), while assessing calcium intake in elephants at 14 UK zoos, determined that a minimum of 0.33-0.77% DM calcium was provided in the offered diets (values represented minimums as calcium provision from grass or browse forages was not included in the calculations). Nonetheless, even the minimum concentrations exceeded the captive adult elephant maintenance recommendation of 0.3% dietary DM (Ullrey, Crissey & Hintz, 1997). Similarly, diets fed to zoo elephants in seven elephant-holding Brazilian zoos contained on average 0.7% Ca DM, showing that minimum recommended levels were being met (Carneiro et al., 2015). Diets of semi-captive Asian elephants in India contained 0.46-0.58% DM calcium (Das et al., 2015) further supporting the conclusion that calcium deficiencies have rarely been documented in healthy adult captive elephants on maintenance diets. There is, however, evidence that incidence of calcium deficiency is higher in cows during partition and lactation, when calcium demand is increased (van der Kolk et al., 2008). Sub-clinical hypocalcaemia was reported in Asian elephants immediately prior to partition at Rotterdam Zoo when calcium demand was not met through dietary provision (van der Kolk et al., 2008).

Metabolic bone disease (rickets) was reported in captive hand-reared Asian elephant calves. This disease results from an imbalance in the calcium to phosphorus ratio or from intestinal malabsorption, and unbalanced milk formulation may have played a role in this report (Ensley et al., 1994).

Iodine

The thyroid mass of an elephant relative to its body mass is double its predicted size, compared to other mammals (Milewski, 2000). This may indicate that the iodine requirements of elephants are proportionally higher than those of other herbivores, and that due to the exclusively herbivorous diet of elephants, they may be susceptible to iodine deficiency (Milewski, 2000). Due to the lack of essentiality of iodine to plant metabolism, land plants have little reason to translocate iodine from soil to foliage, therefore plants consumed by elephants may be low to deficient in iodine (Shetaya et al., 2012; Humphrey et al., 2018). Soil dust deposition has been documented to increase iodine levels of foliage in some situations (Watts et al., 2015). As an alternative iodine source, elephants may seek iodine supplementation from iodine rich water or soil (via geophagy). Humans in Malawi were able to obtain as much as 70% of daily iodine requirements from drinking 2 litres of borehole water per day (Watts et al., 2015). Iodine is required for reproduction, and the high reproductive success of elephants in conservation areas such as Addo Elephant Park, which contained several boreholes, was hypothesised to be linked with an increased supply of iodine (Milewski, 2000; Milewski & Dierenfeld, 2012).

In the Kitum caves, Mount Elgon, Kenya, elephants consume the cave salts correlated with high levels of calcium, sodium, magnesium and phosphorus provided (Bowell, Warren & Redmond, 1996). Iodine was measured in the salt crusts at 1,149 mg/kg, which was >100 times higher than iodine concentrations in the most iodine rich soils in the vicinity. Reproductive outputs of elephant populations consuming these minerals are also high (Bowell, Warren & Redmond, 1996). Given these various lines of inferential evidence, supply or restriction of iodine-rich bore holes could be further investigated as an effective method of population control *in situ*, without affecting reproductive success of smaller herbivores that may have a proportionally lower requirements for iodine, which could be realised by diet, water or geophagy (Milewski, 2000; Milewski & Dierenfeld, 2012).

Iron

Iron deficiency anaemia has rarely been reported in captive or free-ranging elephants, although several cases of anaemia linked with liver fluke infection, retained placenta, tuberculosis, tuberculosis treatment and malabsorption syndrome were documented (Dierenfeld, 2008). Only a single reported iron deficiency anaemia related to low dietary iron intake, affecting three newly imported Asian elephants, was documented. In this case clinical signs resolved upon dietary supplementation (Kuntze & Hunsdorff, 1978). Diets of semi-captive Asian elephants contained 105-126 mg/kg (Das et al., 2015), significantly in excess of the Nutrition Advisory group recommendation of 50 mg/kg iron (Ullrey, Crissey & Hintz, 1997; Das et al., 2015).

Zinc

The dietary recommendation for zinc in captive elephants is 40 mg/kg DM diet, based on determined requirements of equids (Olson, 2004; Ullrey et al. 1997). Partington (2012) reported zinc levels of between 22 and 52 mg/kg DM in zoo elephant diets offered in 14 UK facilities. However, this figure does not account for zinc provision from grass and/or browse forages, which comprise the majority of the diets, hence these data are limited. Nonetheless the lower end values, suggest that some animals may have been consuming inadequate levels of dietary zinc. Semi-captive Asian elephants in India were reported to consume diets containing 38.4 to 45.9 mg/kg zinc (Das et al., 2015); no clinical signs of deficiency were seen and serum concentrations were within the ranges reported for healthy elephants (Ullrey, Crissey & Hintz, 1997; Das et al., 2015). Excess dietary calcium was observed to interfere with zinc bioavailability resulting in skin abnormalities in a zoo elephants (Schmidt, 1989; Dierenfeld, 2008). Schmidt (1989) reported a case of zinc deficiency in a captive Asian elephant, resulting in secondary immune deficiency and skin lesions. Dietary zinc level in that individual was increased from 22 to 54 mg/kg DM and significant clinical improvement was seen within two weeks, with lesions resolved after eight weeks.

Together, these observation confirm that equids may indeed provide a suitable physiologic model for mineral nutrition of elephants.

African Savanna Elephant Feeding Behaviour

African savanna elephants (*Loxodonta africana*) consume a variety of plant material including grasses, leaves, twigs, fruits, barks, herbaceous material and soil (Kabigumila, 1993; Dierenfeld, 2008). Although described as generalist herbivores consuming over 400 species of plants, diet composition may vary regionally and seasonally (Kabigumila, 1993). African savanna elephants are predominantly seasonal grazers and browsers with fruit, barks and soil being consumed as secondary food choices (Kabigumila, 1993). There is debate as to whether savanna elephants are predominantly grazers or browsers, with evidence supporting both feeding strategies: Williamson (1975) reported elephant diets in Hwange National Park, Zimbabwe to consist almost entirely of woody plants whereas Wing and Buss (1970) reported that elephants in Uganda relied primarily on grasses (approximately 90% of bulk) and therefore labelled the species as grazers. Such geographical variations in diet have prompted some authors to classify elephants as browsers (Jachmann & Bell, 1985), whereas others maintain they are primarily grazers (Beekman & Prins, 1989; Tangley, 1997). Therefore it is thought that savanna elephant adopt both feeding strategies, and switch depending on environment and season.

Several studies indicate that savanna elephants spend over half of their daily time budget feeding. Elephants in Tsavo National Park, Kenya were observed to feed for 48-63% of daylight hours (Dougall & Sheldrick, 1964) and elephants in Lake Manyara National Park, Tanzania were observed to spend on average 76% of daylight hours feeding (Beekman & Prins, 1989). Where feeding conditions improved and food availability increased, Guy (1975) observed elephants in Zimbabwe to reduce the total amount of time spent feeding to 50-60% of overall time budget, from a greater proportion of their time budget when food resources were limited. Likewise, savanna elephants in areas of food scarcity in Uganda were reported by Beekman and Prins (1989) to spend as much as 74% of their total time budget feeding. Flexibility in food items consumed and time spent feeding, indicated that elephants respond and adapt their feeding strategy accordingly, with varying availability of food resources.

Savanna elephants were documented to feed throughout the day, with decreased feeding and increased resting during the middle part of the day; 12:00-14:00 hrs (Laws, 1970; Beekman & Prins, 1989; Shannon et al., 2008). This pattern was observed in both sexes. Seasonally, the total amount of time spent feeding per day has not been documented to change, although elephants were observed by Shannon et al. (2008) to adjust the time of day spent feeding in the hotter summer months. Evidence suggests that plant selection and feeding strategy changes depending upon availability. During the wet season elephants were observed by Beekman and Prins (1989) to spend 67% of time grazing with 8% browsing, whilst during the dry season proportions shifted to 23% of time grazing and 60% browsing. During the dry season, the protein content of the grasses decreased. When the protein content of grasses dropped to <2.5%, elephants in Tanzania were seen by Barnes (1982) to increase their browse consumption. Browse typically contains higher levels of secondary compounds such as tannins than grass (Ellis, 1990) and thus, as a by-product of this intensified browse consumption during the dry season, tannin and associated levels of toxin accumulation were seen to increase (Barnes, 1982).

Mineral levels in plants vary seasonally, geographically and between different parts of the plant (Joy et al., 2015; Table 3.1 provides specific examples). Due to the generalist feeding nature of African savanna elephants, it is thought they are able to adapt food selection as required to meet their target levels of (as yet undetermined) mineral requirements (Bax & Sheldrick, 1963). This was demonstrated in elephants within the Kruger National Park (KNP), South Africa, where there is substantial geographical and seasonal variation in plant type consumption by elephants (Codron et al., 2006). Stable carbon isotope analysis of faecal material indicated that during the dry season elephants in northern KNP consumed significantly more grass than their southern counterparts; 40% of their diet was grass in the northern part of the park during the dry season, compared to just 10% in southern KNP (Codron et al., 2006). In contrast, this difference in grass consumption between elephants in the northern and southern parts of this national park was not apparent during the wet season, when elephants throughout the park consumed grass as approximately 50% of their overall diet (Codron et al., 2006). This is in accordance of the observed trend of increased grass consumption during the wet season (Beekman and Prins, 1989). Although elephants consume a vast number of different plant species, they generally receive the bulk of their diet from a few selected species which vary seasonally and geographically (Meissner et al., 1990; Kabigumila, 1993). Bax and Sheldrick (1963) observed elephants in the Tsavo National Park, Kenya, to select specific plant parts, notably bark rich in calcium.

Free-ranging African savannah elephant daily food intake is estimated from either the weight of the stomach contents (post mortem) or from extrapolation of data on feeding rates and time spent feeding. Both methods have produced similar estimates of daily dry matter intake by adults of about 1.0-1.5% of body weight (Meissner et al., 1990; de Villiers et al., 1991; Ullrey, Crissey & Hintz, 1997). Dry matter intake relative to body weight is influenced by a number of factors: dry matter digestibility, environmental stressors, activity levels and life stage of the animal (adult maintenance, growth, pregnancy or lactation; Meissner et al., 1990). Laws (1970) concluded that non-pregnant females and males consumed 1.0-1.2% BW DM (percentage of body weight on a dry matter, dry matter is feed excluding moisture content) whereas pregnant females consumed 1.2-1.5% BW DM. On an as-fed basis (feed including moisture content) elephants consumed about 4% of their body weight per day (Laws 1970).

Evidence shows differences between elephant bulls and reproductively active cows in their nutritional needs and associated diet choices, with cows possibly requiring higher levels of minerals and protein to support growing calves (Dierenfeld, 2008). Greyling (2004) documented that in the Associated Private Nature Reserves (APNR), South Africa, there was a nutritional difference between various parts of the plants consumed by savanna elephants, with leaves containing more calcium and phosphorus than twigs. It is therefore suggested that cows and bulls meet their differing nutritional needs primarily through plant part selection. Family groups with pregnant and lactating females consumed proportionally more leaves and bark in their diet compared to bulls. In the dry season, females consumed 3% leaves and 14% bark, whereas males consumed 1% leaves and 6% bark and additional twigs (Greyling, 2004). This agreed with the previous work of Stokke and DuToit (2002), who found bulls consumed more twigs than cows, and cows engaged in more leaf stripping than bulls.

Greyling (2004) also documented bulls to consume more plant species with higher calcium content than adult cows at maintenance (without calves) throughout the year. Greyling suggested that such mineral selectivity may be due to a higher calcium requirement for tusk growth in males compared to females at maintenance. This observation supports previous work conducted by McCullagh (1969) who suggested a calcium requirement for male elephants of 8-9g per day. Additionally, lactating females were found to have significantly higher calcium needs than adult females at maintenance as summarised in Table 3.2.

During the dry season, Greyling (2004) found bull faeces contained significantly lower phosphorus levels than faeces of cows in family groups. On average, cow faecal samples contained 18% more phosphorus than bulls. Faecal phosphorus levels were used in agriculture to estimate dietary phosphorus in livestock, and they are a more reliable index to diet quality than faecal nitrogen as they are not influenced by tannins (Holechek et al., 1985; Wu, Satter & Sojo, 2000). Lower faecal phosphorus in bulls suggests that less phosphorus was consumed in the diet, which might indicate that the requirement for bulls was lower than that of cows (Grant, Meissner & Schultheiss, 1995; Wrench, Meissner & Grant, 1997). Feeding time budgets of populations of both sexes, studied in three reserves in South Africa, were found to be similar (Shannon et al. 2008). This suggests that cows obtained the required increased dietary energy for pregnancy or lactation, by altering plant selection to preferentially select more energy dense plants, rather than by increasing time spent feeding (Shannon et al., 2008). This finding contradicts that of Guy (1975) who concluded that bulls consumed more 'trunk fulls' of plant material per minute than cows, especially in the dry season, and bulls stayed for longer at feeding sites than family groups do (Stokke & Du Toit, 2002). Stomach fill post mortem of non-pregnant or lactating females and males was smaller than that of pregnant and lactating females, suggesting that females increased overall food consumption to meet their nutritional demands of pregnancy and lactation (Laws, 1975). These pieces of mixed evidence suggest that several feeding strategies may be adopted by elephant cows and bulls to meet their specific individual nutritional needs, depending upon the unique environments in which they live, and seasonal resources available to them.

Table 3.1: Macro-mineral concentrations (%dry matter) in native plants consumed by African savanna elephants (*Loxodonta africana*) in southern and eastern Africa

Location	Season	Plant part	Calcium	Phosphorus	Magnesium	Sodium	Source
Hwange National Park, Zimbabwe	Unknown	Mature leaves	0.02–3.12		0.08–0.64	0.02–0.06	*Holdø, Dudley & McDowell (2002)*
		Young leaves	0.01–1.32		0.1–0.57	0.005–0.05	
		Stems, twigs	0.11–1.85		0.02–0.20	0.001–0.02	
		Bark	0.13–3.93		0.01–0.33	<0.001–0.02	
	End wet season	Browse	0.35–2.47	0.11–0.33			*Williamson (1975)*
		Grass	0.41–0.66	0.09–0.20			
Kasungu National Park, Malawi	Unknown	Tree leaves (12 sp.)				0.10–1.25	*Jachmann & Bell (1985)*
Tsavo National Park, Kenya	Unknown	Grass and browse (59 sp.)	0.37–3.61	0.08–0.36		0.01–1.67	*Dougall & Sheldrick (1964)*
	Wet season	Mixed plant sp.	0.13–0.38				*McCullagh (1969)*
	Dry season		0.38				
	Unknown	Grasses and herb type vegetation	0.36–1.44				
		Shrub	0.53–8.92				

Documented literature on specific mineral needs in elephants is very limited and requirements *per se* have not been experimentally determined (Das et al., 2015). Table 3.2 documents minerals for which estimates have been recorded for African savanna elephants directly. As these values were reached from various different studies, on different populations (captive and free-ranging), parameters of measurement were different e.g. grams required per day compared to mg required per kg dry matter intake or body weight of the animal. This table does not include requirements extrapolated from domestic equids.

Table 3.2: Reported dietary mineral recommendations for African savanna elephants (*Loxodonta africana*)

Mineral	Species	Detail	Daily estimated mineral requirements	Source
Calcium	*L. africana*	Lactating females intensive tusk growth	60 g 8–9 g	*McCullagh (1969)* and *Dierenfeld (2008)*
Sodium	*L. africana*		9 mg Na kg^{-1} BW	*Holdø, Dudley & McDowell (2002)*
Iodine	*L. africana*		0.03 mg I kg^{-1} BW	*Milewski (2000)*

Note:
Estimated mineral requirements for African elephants.

Elephant Movement Patterns, as Related to Geochemistry/Nutritional Factors

The availability of minerals to the plant from the soil underpins the relationship between herbivores and their food supply. The distribution of vegetation was suggested to be strongly associated with the geomorphology of the soil (Lawson, Jenik & Armstrong Mensah, 1968; Bell, 1982). Generally plants will reflect the soil profile and those growing in mineral deficient areas will lack key minerals, thus potentially resulting in deficiencies in the consumer. In contrast, those growing in mineral abundant areas will reflect this, and pass the mineral abundance to the organism consuming them (Hurst et al., 2013; Joy et al., 2015). The ability of an area to supply minerals to an animal does not solely depend on the mineral status of the soil and geochemical parameters (such as organic matter and soil pH), but also on the ability of the plant to incorporate the minerals (Bowell & Ansah, 1994). Additional factors affect the mineral levels within a plant: the pathway of nutrients from the soil to the plant depends upon the amount of element present, the various soil factors that affect the minerals' bioavailability and the plant factors which determine the rate of uptake of the mineral (Maskall & Thornton, 1996).

Soil factors which affect a minerals' soil-to-plant transfer include the composition of the parent material, quantity and composition of organic matter and the soil pH (Hurst et al., 2013). The relationship between mineral status of the soil and parent rock was strongest where there was minimal chemical weathering (Bowell & Ansah, 1994). Organic matter also affects bioavailability, especially that of iodine (Shetaya et al., 2012; Humphrey et al., 2018). Soil pH greatly influences the metal availability (Maskall & Thornton, 1996); in alkali soils, generally the bioavailability of molybdenum and selenium increases, whilst that of copper, cobalt and nickel decreases (Sutton, Maskall & Thornton, 2002). Further, increased availability of phosphorus in alkali soil contributes to its enhanced uptake into the plant (Maskall & Thornton, 1996; Sutton, Maskall & Thornton, 2002).

Plant factors affecting rate of uptake of a mineral include: age of the plant (with levels of trace elements decreasing in older plants), rate of plant growth (with rapidly growing plants displaying reduced levels of trace elements), and plant species (with differences seen between levels of trace elements in different plant species grown in the same soil (Maskall & Thornton, 1996). The greatest differences in mineral content were reported between grasses and browses (Gomide et al., 1969; Ben-Shahar & Coe, 1992). Seasonally, trace element levels were reported to be higher in plants in the wet season: in the grazing pastures in the Kenyan highlands (Howard & Burder, 1962), in grasses by Lake Nakuru in the Rift Valley (Maskall & Thornton, 1991) and in the Mole National Park, Ghana (Bowell & Ansah, 1994). Finally grazing status of the plant was seen to influence plant mineral levels, with increased mineral concentrations of up to 300% in grazed areas, notably sodium, phosphorus and calcium, compared to un-grazed areas supporting low animal densities (McNaughton, 1988).

Forage mineral analysis data is routinely used to assess mineral levels in agriculture, and despite limitations, it is believed to be a reliable index to be used to assess the general ability of forages to meet animals' mineral needs (McNaughton, 1988; Nellemann, Moe & Rutina, 2002). However, the mineral profile of the soil can be depleted by soil, plant, topography and weather factors. In the Sabi Sands Reserve, South Africa, ten species of grass were analysed and grasses from soils of higher mineral levels accumulated lower mineral concentrations, compared to grasses from soils where the minerals were found in lower levels (Ben-Shahar & Coe, 1992). In this case, this was thought to be due to sampled species attributes, and the effect of the local micro-climate on the plants.

Movement Choices of Elephants

Several studies concluded that elephant habitat use is not random, but that elephants have specific preferences for various habitats and move to fulfill their resource needs (Whitehouse & Schoeman, 2003; Osborn, 2004; Douglas-Hamilton, Krink & Vollrath, 2005; Dolmia et al., 2007; Thomas, Holland & Minot, 2008; Leggett, 2015). There are a myriad of factors that contribute towards an elephants' movement choices including availability of food and water, opportunity for social interaction, human presence and associated activities. Hydrology and topography may also influence animal movement (Bowell & Ansah, 1994; Wall, Douglas-Hamilton&Vollrath, 2006). Elephants tend to avoid steep slopes due to the increased energy expenditure required to climb them, even minor hills are considerable energy barriers to an elephant (Wall, Douglas-Hamilton&Vollrath, 2006). De Knegt et al. (2011) suggested that daily movement of elephants related predominantly to food availability, and movements become extended by the distance traversed to water sources. Elephants in that study area of the KNP, South Africa concentrated foraging within areas of high forage availability that were closest to water, whilst still being large enough areas to optimise efficiency of movement and foraging.

The significance of the impact of human activity on the natural movements of elephants is rapidly increasing (Nyhus, 2016). Tucker et al. (2018) concluded that in areas with a high level of human presence, mammal movement decreased by 35-50% across 57 species, compared with areas of low human presence. Over the last 150 years, expansion of human settlement into elephant habitat, and an increase in elephant killing (from poaching and hunting) has significantly altered elephants' home ranges across continental Africa (Eltringham, 1990; Hoare, 2000; Osborn, 2004; Nyhus, 2016). Initially it was thought that there would exist a simple linear relationship between rising human and declining elephant densities at a national or subcontinental scale (Hoare & du Toit, 1999). However, Hoare and du Toit (1999) found that in an area of 15,000 km^2 in northwest Zimbabwe, the relationship turned out to be more complex. Using data from human populations, and observed elephant densities in the region, the authors determined that there was a threshold beyond which elephant and human coexistence could no longer occur, and elephant populations rapidly declined. This threshold was related to agricultural development, and was reached when land was spatially dominated by agricultural use, and the original woodland (that constituted the elephants' habitat) became sub-dominant.

When analysing elephant movement, water availability must be taken into account, elephants are obligate drinkers (Wall et al., 2013). Water availability is considered to affect elephant movement, both on a daily and seasonal basis and may be a greater driver for elephant movement than mineral availability. Three studies conducted in South Africa and Kenya, indicated that elephant movement increased throughout the wet season when water availability was greatest, and then rapidly decreased throughout the dry season, with elephants, especially lactating females, confining themselves to areas within 1-2 days' travel from water to enable them to conserve energy (Western & Lindsay, 1984; Codron et al., 2006; Thomas, Holland & Minot, 2008; Birkett et al., 2012).

Pretorius et al. (2011) concluded that elephants made movement choices based on nutritional provision in a specific area. Fertiliser was applied to mopane trees (*Colophospermum mopane*) in the APNR, South Africa, in various patches, resulting in an increase in the phosphorus and nitrogen levels in mopane leaves. Elephants consumed more mopane leaves per patch in fertilised patches compared to unfertilised patches, regardless of patch size. Furthermore at a 100-m^2 patch size scale, elephants stripped leaves more in fertilised than unfertilised patches, but were more likely to tree kill (through uprooting or breaking main trunks) in unfertilised patches. Therefore, it was suggested that elephants caused more impact to trees of lower value (through tree killing) whilst preserving trees of higher value (fertilised mopane) through coppicing (Pretorius et al. 2011).

Secondly Pretorius et al. (2012) suggested that phosphorus may be a key driver for elephant movement, with elephants moving throughout the year to maximise intake of this mineral. In this study area in the APNR, there was a suspected local deficiency in phosphorus, potentially explaining why the elephants prioritised obtaining this mineral. During the wet season, when food availability was greatest, nitrogen provision was prioritised, possibly to meet the elephants' needs for growth and reproduction. During the dry season, when food was potentially limited, energy was prioritised by the elephants. This could be because energy costs to obtain food and water during the dry season were often higher as elephants had to travel further, due to reduced abundance of forage and availability of water (Pretorius et al., 2012).

Nutritional Factors Affecting Elephant Movement

Minerals can be provided to elephants from multiple sources, including plants, water or soil (through geophagy). Examples of mineral provision from plants include sodium, calcium, magnesium and phosphorus. Forest elephants (*Loxodonta cyclotis*) in the Kibale National Park, Uganda, were reported by Rode et al. (2006) to be crop raiding to meet their sodium need. It was reported in the literature that minerals such as copper and sodium, rather than energy and/or protein, were limited in the elephants' wild food plants, and were found in higher concentrations in crops. Often, wild elephant food plants which are high in sodium are also high in secondary compounds (Rode et al., 2006), which might inhibit the uptake of essential minerals and increase sodium excretion, and thus may further exacerbate low sodium intake (Jachmann, 1989). Crops contained lower levels of secondary compounds compared to wild plants, which allows the elephants to solve the complexities of meeting their sodium need, without interference from secondary compounds. For example, the highest sodium wild plant in this study, *Uvariopsis congensis* also contained high levels of secondary compound, saponin and had a high alkaloid score (Jachmann, 1989). Jachmann (1989) has also reported examples of elephant populations in the Miombo biome, Africa, making plant choices to create diets that contained high sodium and digestible sugar concentrations, and low concentrations of indigestible fibre and secondary compounds. Especially the elephants avoided plants with high phenol and steroidal saponin levels. Additionally in Kibale National Park, seasonal availability of wild food was not correlated to the timing of crop-raiding events (Chiyo et al., 2005). This suggests that elephants may be selecting specific food crops due to their nutritional provision, rather than just being attracted to the presence of food crops and increased overall availability of food (Chiyo et al., 2005).

Finally, savanna elephants within the Mount Elgon region, Kenya, consumed salt deposits within the Kitum caves, which are rich in a variety of minerals including calcium, sodium, magnesium and phosphorus (Bowell, Warren & Redmond, 1996). Cases of uneven tusk wear were noted and presumed to result from the use of tusks to scrape salts from the ceiling and walls (Bowell, Warren & Redmond, 1996). The environment within the cave can be warmer at 13.5^{o}C than surrounding areas where night temperature can drop to 8^{o}C, and although this could be encouraging the elephants to remain in the area overnight, it was suggested that there exists a nutritional drive causing them to seek out and consume the salt deposits on the rocks (Bowell, Warren & Redmond, 1996).

Minerals can also be provided to elephants through drinking water. Sienne, Buckwal and Wittemyer (2014) investigated elephant use of bais (natural forest clearings which often have seasonal or year round sources of water present as surface waters) in the central African rainforest and concluded that mineral provision from water is likely to be attracting elephants to specific bais. Mineral concentrations in water from elephant-evacuated pits were higher than in surface water, and thought to be a causative factor behind bai visitation choice. In particular iodine, sodium, sulphur and zinc were elevated, while calcium, magnesium, manganese, iron and tin concentrations were at least ten times higher in elephant-evacuated water compared to surface waters. Blake (2002) observed that elephants congregated around bais during the dry season, correlating with a seasonal peak in mineral levels in pit water, which may be due to the seasonal ebbing of spring water flow.

Likewise, savanna elephants in the Hwange National Park, Zimbabwe were recorded by Weir (1972) in greater numbers surrounding water sources with higher sodium content. Pans of high sodium water were reported to have three times as many elephants when censured, compared to the lowest sodium areas, indicating elephants might make movement choices based upon sodium need (Weir 1972).

Finally geophagy appears to be a normal behaviour of all elephant species in the majority of habitats and is thought to aid elephants in meeting their nutritional (mineral) needs (Holdø, Dudley and McDowell, 2002). There is some evidence that elephants also conduct geophagy to support detoxification of unpalatable secondary compounds of their diet (Mwangi, Milewski & Wahungu, 2004; Chandrajith et al., 2009). In other ungulate species, clay may decrease the harmful effects of secondary plant compounds and intestinal infections (Klaus & Schmidg, 1998; Ayotte et al., 2006). Soil is never consumed randomly within an elephants' home range, but instead consumed from specific spatially circumscribed sites (Klaus & Schmidg, 1998). It is thought that elephants principally consume soil(s) at specialised licks to supplement sodium intake, although calcium, magnesium and potassium are also often higher in lick soils compared to the surrounding soils (Holdø, Dudley & McDowell, 2002). Additionally elephants are known to consume soil on termite mounds, although it remains unclear as to the driving mineral(s) behind this behaviour. In contrast to the situation at lick sites, sodium levels do not seem to be persistently higher in termite mounds than surrounding soils (Holdø & McDowell, 2004).

A further example of geophagy by elephants was reported by Mwangi, Milewski & Wahungu (2004) in the Aberdares National Park, central Kenya, where elephants rely on browse and unripe fruits to make up the majority of their diet due to limited availability of grasses.

Browse, unripe fruits and seeds generally contain more tannins and alkaloids than grasses, suggesting that the elephants in this national park consume more potentially harmful substances compared to elephants that consume higher levels of grasses. As hindgut fermenters, neutralisation of these harmful substances is not possible in the same way as it is for ruminants (where foregut fermentation is used to neutralise these harmful substances). As the geophagic soils also contained higher levels of sodium and iodine than surrounding soils, it is not possible to identify if minerals or clays are the driving force behind this geophagic behaviour, however it was considered that both factors were important (Mwangi, Milewski & Wahungu, 2004).

In the Kalahari-sand region of Hwange National Park, elephants consumed high-sodium lick soils during the dry season possibly in response to an unmet requirement for sodium (Holdø, Dudley & McDowell, 2002). Lactating and pregnant females consumed more soil per visit to a high sodium lick than males (Holdø, Dudley & McDowell, 2002). The latter might be due to their increased requirement for sodium during pregnancy and lactation (Michell, 1995). This suggests that there is a physiological cause for this geophagy and that in these cases, lick use is driven by a nutritional need. Female elephants will increase geophagy to meet their additional nutritional needs during pregnancy and lactation. Table 3.1 documents sodium levels in browse species during the dry season that are lower than during the wet season, and were suggested by Holdø Dudley & McDowell (2002) to be insufficient. The soil in the mineral lick areas also contained elevated levels of magnesium and calcium, however, these minerals were also available in adequate amounts from other sources such as termite mounds or dietary browse. Interestingly consumptions of termite mounds were not observed. Therefore the authors concluded that these elephants were conducting geophagy based on sodium need (Holdø, Dudley & McDowell, 2002).

As well as the increased clay in the soil in the Aberdares National Park, Mwangi, Milewski and Wahungu (2004) found the soil consumed by the elephants also contained higher sodium and more concentrated levels of iodine than surrounding areas, but was significantly lower in zinc, manganese and iron levels. Additionally, there was 250% more phosphorus and 50% more magnesium in the consumed soil than surrounding control soil (Mwangi, Milewski & Wahungu, 2004). This suggests that elephants of this population chose to consume soil in certain areas based on nutrition provision, and that specific minerals were prioritised.

There is debate as to whether elephants alter their movements to seek out and consume either the soil from termite mounds, or plant material growing on the termite mounds, to meet their mineral needs (Holdø & McDowell, 2004; Muvengwi, Mbiba & Nyenda, 2013; Muvengwi et al., 2014). Soil from termite mounds includes both surface soil and deeper sub soil, raised to the surface by termites. Previous studies generally focused on one geographical area and thus results may be geographically specific depending upon surrounding mineral availability. It appears to be universally acknowledged that soils from termite mounds contain more minerals than surrounding areas as the termites mine deeply into the substrate (Holdø & McDowell, 2004; Muvengwi, Mbiba & Nyenda, 2013; Muvengwi et al., 2014). However, the evidence as to whether elephants move to seek and consume specific soils (and plants) for targeted minerals is variable. Muvengwi, Mbiba & Nyenda (2013) showed that tree diversity did not vary significantly on termite mounds or control plots, in Chewore North, Zimbabwe, net biomass removal by mega-herbivores was up to five times higher on control plots than termite mounds. Specifically when measuring consumption of *Colophospermum mopane*, there was no difference in biomass removal between termite mounds and control plots (Muvengwi et al., 2014).

In contrast, black rhino in Chipinge Safari, Zimbabwe, were observed to browse on foliage growing on termite mounds more than off termite mounds, seen by increased bite intensity on the plants on the termite mounds (Muvengwi et al., 2014). This is suspected to be due to the increased soil and foliar mineral levels. Concentrations of nitrogen, potassium, phosphorus, calcium and sodium were found to be approximately double in the soil and leaves compared to those off the termite mounds (Muvengwi et al., 2014). In the Kalahari Sand Hwange National Park, Zimbabwe elephants consumed soil from the high sodium, sparsely grassed areas on top of the termite mounds if the surrounding soil had a low concentration of sodium, but not if the surrounding soil areas had comparably higher sodium content (Weir, 1969). In western Zimbabwe, 12 paired sample sites were compared. Each site consisted of an area with a termite mound and a corresponding area within woodland, containing no termite mound. Holdø and McDowell (2004) concluded that although the soils within the termite mounds contained more of all tested minerals, the plants on the termite mounds contained less sodium than the plants in woodland plots. Elephants fed more intensively from the plants on the termite mounds than within the woodlands indicating that in this situation, the animals were probably seeking other minerals in addition to sodium from the termite mounds (Holdø & McDowell, 2004).

Finally, termite mounds which are consumed by elephants within the Mimbo ecosystem of the Ugalla Game reserve, Tanzania, contained more minerals than termite mounds which are not used for geophagy (Kalumanga, Mpanduji & Cousins, 2017). The amounts of each mineral correlated to each other, making it impossible to distinguish a single vs multiple specific driver(s) underlying geophagy. However, it is clear that mineral-rich termite mounds are being selected for consumption over less mineral-rich termite mounds (Kalumanga, Mpanduji & Cousins, 2017).

Applications to Ameliorating Human-Elephant Conflict (HEC)

Human-elephant conflict is caused when elephants make forays into human settlement resulting in some form of damage. Humans retaliate to injure, kill or displace the elephant (Hoare, 2000). The African Elephant Specialist Group (AfESG) conducted an inventory of sites across Africa where HEC occurs. It was concluded that the issue is widespread and HEC occurs where interactions happen between the home range of elephants and human activity. Approximately twenty percent of elephant home range is within legally protected areas however, conflict was documented to occur in both protected and non-protected areas (Said et al., 1995). Crop losses attributed to elephants across Africa was low (5-10%), and elephants were considered to be low on the list of agricultural pests (Hoare, 2000; Naughton-Treves, 2008). However, wide spread low level damage from non-dangerous crop pests were better tolerated by communities than rare, localised catastrophic damage caused by elephants (Said et al., 1995; Hoare, 2000; Naughton-Treves, 2008). There is limited evidence to support the relationship between problems caused by elephants and the level of elephant density or nutritional food limitation (Barnes, Asika & Asamoah-Boateng, 1995; Hoare, 1999). The optimum foraging theory has been suggested to explain the unpredictable nature of crop raiding across the savanna (Hoare, 1999). This theory predicts that animals will maximise quality of nutrient intake where possible and thus when crops of higher nutritional value than wild food plants are available, animals will prioritise consumption over their normal food crops (Begon, Harper & Townsend, 1986).

Applications to Other Herbivore Species in Comparable Environments

Consideration of geochemistry is required for maintenance of healthy animal populations, especially within fenced reserves where animal migration is impossible. For example, in Lake Nakuru National Park, Kenya which is a fenced area of 160 km^2, the soil is derived from volcanic ash, pumice and lake sediment, with low levels of extractable cobalt, copper and acetic acid with a high alkaline soil pH (Maskall & Thornton, 1996). In this region of the Rift Valley, mineral deficiencies including copper and cobalt were seen in domestic cattle, as well as in impala (*Aepyceros melampus*) and waterbuck (*Kobus defass*) (Maskall & Thornton, 1996). The increased soil pH caused increased uptake of molybdenum by the plants, which in turn inhibited the utilisation of copper in ruminant animals, further exacerbating the deficiency of copper (Underwood, 1977). A geochemical survey was conducted and results of this related to observed clinical copper deficiencies in animals (Maskall & Thornton, 1996). Following this investigation, recommendations were made to the Kenya Department of Wildlife Conservation and Management that mineral salts containing cobalt, copper and selenium should be made available to wildlife in the park to mitigate these mineral deficiencies (Thornton, 2002). Due to the physiological differences between copper absorption in ruminants and non-ruminants, elephants are not as sensitive to this deficiency as ruminant species and a similar problem has not been documented in elephants (Maskall & Thornton, 1996).

Clinically observed copper deficiencies caused by an increased uptake of molybdenum by the plant and thus interference in the utilisation of copper by the animal were seen in Grant's gazelle (*Gazelle granti*) from another area of the Kenyan Rift valley (Maskall & Thornton, 1996). Additionally this was seen in moose (*Alces gigas*) in Alaska (Kubota, Rieger & Lazar, 1970) and several herbivores at the San Diego Wild Animal Park (USA) where hypocuprosis was diagnosed, caused by feeding alfalfa with a high molybdenum (and sulphur) concentration (Kubota, Rieger & Lazar, 1970; Nelson, 1981; Maskall & Thornton, 1996). In northeast Zimbabwe, it was suggested that high concentrations of iron in the soil and forage inhibited the availability of phosphorus to the plants, and thus to the cattle consuming the plants. The high iron concentration in the soil also reduced the absorption of copper and zinc in cattle (Fordyce, Masara & Appleton, 1996).

Due to the ever-changing environment in which herbivores live, they are forced to make a series of prioritised decisions to ensure survival. These decisions range from spatial to temporal and vary in scale, from smaller scale decisions around which plant part to select for consumption, through to decisions around seasonal movement patterns (Fryxell, 2008). De Knegt et al. (2011) concluded that forage availability, both in terms of quantity and nutritional quality, varies between seasons and years. Consequently those individual herbivores adapt their ranging behaviour to meet their nutritional needs and ensure survival. This is especially important in times of resource scarcity, where poor decision making may result in a reduced reproductive output or death (Shannon et al., 2010). To discriminate between food items of high or low quality will have a selective advantage for long term survival (Fryxell, 2008).

From tracking data on 803 individuals of 57 species, Tucker et al. (2018) concluded that animal movements are on average shorter in resource rich environments. For example red deer *(Cervus elaphus)* in Slovenia were found to have reduced home ranges due to the enhancement of resources, via supplementary feeding (Jerina, 2012), further agreeing with the work conducted by Morellet et al. (2013) and Teitelbaum et al. (2015). Morellet et al. (2013) showed that the home range of roe deer *(Capreolus capreolus)* at higher altitudes, was significantly larger than roe deer at lower altitudes, despite forage availability at higher altitudes being more abundant and of higher quality, although the growing season was shorter than at lower altitudes. This suggested that home range, on an individual basis, is linked to a balance between metabolic requirements and ability to acquire food, accounting for seasonal variation. Teitelbaum et al. (2015) concluded from a review of 94 land migrations of 25 large herbivore species that there was a ten-fold increase in the migration distance between resource high and low areas. These studies indicated that animals living in resource poor areas will have larger home ranges and longer migration distances than those living in resource abundant areas.

African herbivores are not distributed heterogeneously. In the Serengeti National Park (SNP), areas of high herbivore concentration corresponded with areas providing forages of higher mineral content, implying that mineral content in foods was an important determinant of the spatial distribution of herbivores within this park (McNaughton, 1988). For example, magnesium, sodium and phosphorus had a particular influence on herbivore distribution, with high herbivore density areas having 300% more sodium, 50% more phosphorus and 10-23% more magnesium respectively than low herbivore density areas. Secondly, migratory grazing ungulate species in the SNP were reported to make seasonal movements based on grass mineral content (McNaughton, 1990). Grasses, as is common in many tropical soils, were not sufficient in magnesium and phosphorus to meet the mineral requirements for lactating and growing ruminants, and overall were lower in minerals than grasses growing in temperate soils (McDowell, 1985). The nutritional needs of lactating females and growing young were reported to be influential on movement choices (McNaughton, 1990). Animals have evolved with parturition periods being governed by the nutritional requirements of reproducing females and growing young, seasonal rainfall and distance from forage of sufficient quality being prioritised (McNaughton, 1990).

Herbivores have responded to plant evolutionary development through exhibiting seasonal habitat selection and a reported change in movement behaviour. This was shown by Shannon et al. (2010), from examining ranging behaviours and broad scale decision making of wildebeest (*Connochaetes taurinus*), Thomson's gazelle (*Gazella thomsoni thomsoni*), red deer (*Cervus elaphus*), reindeer (*Rangifer tarandus*) and elk (*Cervus canadensis*). Zebra and wildebeest around the Sabi Sands Reserve, South Africa were seen to move seasonally to habitat types characterised by grass communities with a high proportion of nutritious species, and generally increased level of grass diversity, rather than selecting a particularly nutritious species within a broader habitat (Ben-Shahar & Coe, 1992). Home range movement showed that diet composition and habitat use of these animals was influenced by the availability of nitrogen and phosphorus in grasses (Ben-Shahar & Coe, 1992).

Conclusions

Evidence-based values for mineral requirements of elephants remain undetermined. Suspected deficiencies in local key minerals might force animals to make movement choices to obtain these minerals. In African savanna elephants this behaviour has been reported, although there is a need for further research. The latter might reveal correlation patterns which could aid conservation managers in making informed decisions surrounding elephant movement, and the mitigation of human-elephant conflict.

This review collates evidence to suggest that African savanna elephants (and other herbivores) consider nutritional drivers as a factor in their movement choices. The reasons dictating an animals' daily, seasonal and annual movement are considered to be multifactorial, with availability of water, human activity, social behaviour and topography all playing a role alongside nutrient availability, specifically mineral provision. Minerals are available to elephants from plants, water and soil, and all contribute to meeting their, as yet, undetermined mineral needs. There is a relationship between geochemistry and herbivore movement, respectively mineral provision to the consumer, through consumption of plants, water and soil (through geophagy). This relationship needs to be further explored to aid in predicting animal movement.

National Parks and fenced reserves may occupy marginalised land of poorer quality, which has not been assigned to agriculture. The vast increase in land required from 2014 to 2050 for human population growth and agriculture will lead to a further reduction in land available for herbivores such as savanna elephants, and human-elephant conflict is predicted to increase (Nyhus, 2016). Wide ranging, landscape –level movements made by terrestrial herbivores are increasingly threatened globally (Wall et al., 2013). From a practical conservation perspective, there is limited research on the impact mineral provision may have on prediction or mitigation of human-elephant conflict, and how this could be used as a tool for conflict resolution.

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Chapter 4

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Supplementary Information
Supplementary information for this paper will available online upon publication and is detailed as follows:

Table number	Title	Information
1	Sample collection protocol	Sampling procedure and storage methods for UK zoo elephant, food and environmental samples
2	Keeper fed diet analysis	Elemental breakdown of all food items fed to elephants across 5 zoos and CRM data for all feed items including grass
3	Certified Reference Material (CRM) data	Soil, faeces, urine, plasma, toenail and tail hair
4	Keeper fed diet zoo B	
5	Keeper fed diet zoo A	
6	Keeper fed diet zoo C	
7	Keeper fed diet zoo D	
8	Keeper fed diet zoo E	
9	Grass quantification	Estimation of grass consumption
10	Median summary data outputs	Median summary data for outputs, this includes plasma, faeces, urine (normalised for hydration with creatinine), toenail and tail hair from elephants at each of the 5 UK zoos
11	Results of Linear Mixed Model	Includes all P-values produced from sequential linear mixed model

Potential bio-indicators for assessment of mineral status in elephants

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Abstract

The aim of this study was two-fold: (1) identify suitable bio-indicators to assess elemental status in elephants using captive elephant samples, and (2) understand how geochemistry influences mineral intake. Tail hair, toenail, faeces, plasma and urine were collected quarterly from 21 elephants at five UK zoos. All elephant food, soil from enclosure(s), and drinking water were also sampled. Elemental analysis was conducted on all samples, using inductively coupled plasma mass spectrometry, focusing on biologically functional minerals (Ca, Cu, Fe, K, Mg, Mn, Na, P, Se and Zn) and trace metals (As, Cd, Pb, U and V). Linear mixed modelling was used to identify how keeper-fed diet, water and soil were reflected in sample bio-indicators. No sample matrix reflected the status of all assessed elements. Toenail was the best bio-indicator of intake for the most elements reviewed in this study, with keeper-fed diet being the strongest predictor. Calcium status was reflected in faeces, (p 0.019, R^2 between elephant within zoo - 0.608). In this study urine was of no value in determining mineral status here and plasma was of limited value. Results aimed to define the most suitable bio-indicators to assess captive animal health and encourage onward application to wildlife management.

Keywords: *Loxodonta africana, Elephas maximus, geochemistry, mineral status, bio-indicators for health, nutrition.*

Introduction

Formulation of an appropriate zoo diet requires husbandry skills and applied nutritional science[15]. Although there is limited agreement in the literature, the use of appropriate bio-indicators to assess elemental status was suggested by Combs et al. (2013)[13] to support evidence-based zoo diet assessment. Zoos in the United Kingdom have a responsibility to provide appropriate nutrition to all animals within their care[55] to prevent nutritional-related disease, compromised welfare and potential reproductive failure. Limited information exists for estimated mineral requirements of elephants, with cases of specific mineral deficiency documented [38,40, 47]. Due to elephants' low growth rate and large size, clinical signs of nutrient deficiency may go unnoticed for long periods of time[48], making nutritional evaluation challenging.

Jansman and Pas (2015)[24] defined mineral status as the balance between dietary intake of a nutrient and its requirement in the body. Twenty-eight "essential" mineral elements have known metabolic roles in the mammalian system, for which dietary deficiency will lead to clinical deficiency. These include calcium (Ca), phosphorus (P), magnesium (Mg), selenium (Se) and zinc (Zn) [27,46]. Minerals are utilized within the body in various forms or individual compartments, with a central reserve or interchange compartment, usually blood and one or more storage compartments, usually bone or liver. Element and animal species affects the speed of mobilisation of the mineral between compartment(s) [27,46]. Mineral status can also be altered by interactions between dietary components; for example an increase in dietary P causes a decrease in serum Ca[344,], and variations in individuals' metabolism, circadian patterns and pathological state.

Analysing Elemental Status in Elephants

No single sample matrix exists for analysing elemental status in elephants or for other mammalian species. Circulating blood fraction concentrations, and/or liver tissues have provided the standards for assessment criteria, with limited evidence as to the accuracy of reflection of elemental status in an animal[33]. Having a non-invasive method to assess elephant elemental status would enable diet evaluation, regular assessment of mineral status within the animal and the development of more accurate reference ranges for the species. The practically available sample matrices for this include plasma, toenail and tail hair, urine and faeces.

Blood samples have been significantly correlated to nutritional status in livestock for trace minerals such as copper (Cu), cobalt (Co) and Zn, as determined by health status[24,32].

However, mineral concentrations in the body are under homeostatic control, even when intake is insufficient[25]. Fluctuations in dietary intake may affect plasma mineral levels too slowly or too rapidly to demonstrate true nutritional status in the animal[10,22,32]. Blood sample collection requires elephant and keeper training[8] and sample storage outside the laboratory may be problematic. Urine samples indicate excesses of certain minerals that have been absorbed, potentially metabolised, and then excreted. They can be useful in determining Ca, iodine (I) and arsenic (As) status, however, a single sample from an individual is insufficient to reflect elemental status, due to homeostatic controls within the mammalian system and variability in hydration status affecting solute concentrations[10,29,30,50]. Collection from elephants can be problematic as samples must be collected mid-flow without substrate contamination.

Toenail and tail hair samples reflect longer term patterns of dietary intake, over weeks or months[11,43]. Human toenails have been shown to reflect dietary Se levels, and to correlate with plasma levels[39]. Additionally, both sample matrices have been used to identify exposure to toxic trace elements such as As[5,9,31]. Sample collection is less invasive than for blood samples and storage in the field, handling and health and safety is easier. Faecal samples reflect unabsorbed dietary minerals, as well as those re-excreted into the intestines as excess[54]. Separating these relative component contributions in faecal mineral contents is challenging. Elephants consume diets of mixed digestibility, thus less digestible components, including minerals, could be over-represented in samples[43]. However, faecal samples are inexpensive and non-invasive to collect, in both captive and wild elephants.

From existing evidence, there is no recognised sample matrix for assessing mineral status in elephants, other than by using samples of various sample matrices that will differ in suitability for different elements. The approach proposed in this study attempted to model the different sample matrices in terms of measured intake, focussing on minerals essential for health, including Ca, Cu, iron (Fe), potassium (K), Mg, manganese (Mn), sodium (Na), P, Se and Zn and trace metals including As, cadmium (Cd), lead (Pb), uranium (U) and vanadium (V). The overarching aim of this study was to identify the most suitable bio-indictor to reflect elemental intake, and thus elemental status in elephants.

Materials and Methods

Statement of Ethical Approval

i) Ethical approval was obtained from the University of Nottingham School of Veterinary Medicine and Science Clinical Ethical Review Panel (Reference: 1499 150622) prior to commencing the study.

ii) All experiments were performed in accordance with relevant UK guidelines and regulations and appropriate permission obtained from each participating zoo.

Site and Elephant Selection

The study was conducted between March 2016 and July 2017, using 21 elephants at five UK zoos, as detailed in Table 4.1. Each zoo was visited four times over one year throughout the study period to account for potential seasonal variation within the keeper-fed diet and in pasture grazing. Zoos were selected to provide a geographical spread across the UK so as to have regional geochemical variation in soils. Approximately equal numbers of each elephant species were included in the study, with only adult elephants selected (over 10 years old). Animals included in the study represented approximately 40% of the total UK zoo elephant population, sampled from 55% of zoos holding more than one elephant[42].

Table 4.1: Species and sex (male/female) split for UK study elephants, dates for UK zoo visits

	Species	No. animals (male/female)	Visit 1	Visit 2	Visit 3	Visit 4
Zoo A	*L. africana*	0/4	March 2016	June 2016	Sept 2016	Jan 2017
Zoo B	*E. maximus*	0/4	June 2016	Sept 2016	Jan 2017	April 2017
Zoo C	*E. maximus*	1/6	Sept 2016	Feb 2017	May 2017	August 2017
Zoo D	*L. africana*	1/3	Sept 2016	Jan 2017	April 2017	July 2017
Zoo E	*L. africana*	2/0	Sept 2016	Jan 2017	May 2017	July 2017

Sample Collection

Samples of all offered food items, soil and water available to the elephants, elephant tail hair, toenail, plasma, faeces and urine were collected from each study zoo as summarised in Supplementary Information, Table 1.

Sample Preparation

Soil samples were air-dried, crushed and sieved to ≤2mm and further milled to ≤40µm in an agate ball mill (Retsch, Germany). Water samples were filtered with a hydrophilic 25mm Minisart filter in the field and acidified with 1% HNO_3 and 0.5% HCl. Elephant food samples and faecal samples were freeze dried, and passed through a food blender as described by Watts et al. (2019b)[51]. Elephant tail hair and toenail samples were cleaned as described in Middleton et al. (2016c)[31]. Although this method was developed for human hair and toenails, due to the similarities in the sample composition, it was applicable to use with elephant hair and toenails. Blood samples were collected as described in Bourne (2005)[8], using Vacutainer heparin 10 ml collection tubes. Blood samples were mixed by inversion of the sample 10 times immediately after collection; plasma was immediately separated by centrifugation at 1500 g for 10 minutes and transferred into trace element-free tubes (up to 5x1 ml aliquots for each sample) at each individual zoo. Samples were transported on ice and frozen at -20^{0}C upon return to the laboratory.

Sample Digestion for ICP-MS Analysis

Soil samples and elephant faecal samples (0.25 g) were digested in a mixed acid solution (HF: 2.5 ml/HNO_3:2 ml/$HClO_4$:1 ml/H_2O_2:2.5 ml) on a programmable hot block; 0.5 g of elephant food samples were digested in HNO_3:10ml/H_2O_2:1ml mixed solution in a closed vessel microwave heating system (CEM MARS Xpress, USA) as described in Watts et al. (2019b) [51]. In summary, food samples were heated to 100°C over 5 minutes with 10ml HNO_3, held for 1 minute and then heated to 200°C over 5 minutes and held for 15 minutes. The vessels were then cooled and 1ml of H_2O_2 added to the solution, allowed to settle for 30 mins before repeating the heating cycle, but with the latter stage held at 200°C for 25 minutes. Sample digests were then diluted to an appropriate acid matric content for ICP-MS analysis. Elephant tail hair samples and elephant toenail samples (0.1g sample) were digested also using the microwave heating system, but with HNO_3:4 ml/H_2O_2:1 ml and with only one heating cycle, heated to 100°C over 5 minutes, held for 1 minute and then heated to 200°C over 5 minutes and held for 30 minutes as described in Middleton et al. (2016c)[31]. Urine samples were diluted 1-in-10 with 2% HNO_3 prior to analysis as described in Middleton et al. (2016a)[29]. Plasma samples were diluted 1-in-20 with 0.5% HNO_3 prior to analysis as described in, Phiri et al. (2019)[35].

Elemental Analysis

Elemental analysis was conducted on all prepared samples by inductively coupled plasma mass spectrometry (ICP-QQQ; Agilent 8900x, USA) using collision reaction cell mode (reactive gas modes: H_2 for Se at 7.0 ml/min, O_2 for As at 30%, He for all remaining elements at 5.1 ml/min) for a suite of 58 elements using internal standards (Sc, Ge, Rh, In and Ir) for drift correction. ICP-MS operating parameters were: Rf power 1550 W; plasma gas flow 15 L/min; carrier gas flow rate 1.0 ml/min. Fifteen biologically functional elements that are routinely used for health assessment were selected for this study, Ca, Cu, Fe, K, Mg, Mn, Na, P, Se, Zn, As, Cd, Pb, U and V. Internal and external analytical quality controls were used including appropriate certified reference materials, selected based upon the sample matrix. Sample blanks were run to determine practical Limit of Detection (LOD, 3*STDEV).

Additionally, soil pH was measured using 10 g soil and 25 ml $CaCl_2$ and organic matter content was estimated for soil and faecal samples using loss on ignition (LOI) at 450 °C for 1 g of sample, as described in Watts et al. (2019b)[51]. For normalisation across urinary samples, urinary creatinine was determined using the JAFFE method[14].

Analytical Quality Control

The accuracy of the elemental analysis was verified by analysing the following Certified Reference Materials (CRM):

- Human Hair (GBW09101, China)
- Spinach leaves (SRM1570A, NIST, USA)
- Tomato leaves (SRM1573A, NIST, USA)

- Seronorm Trace Elements Urine L-1 (Sero AS, Norway)
- Seronorm Trace Elements Plasma L-1 (Sero AS, Norway)
- Basalt rock (BCR-2 United States Geological Survey, USA)
- Soil (SRM2711a, NIST USA)
- Soil (BGS 102, British Geological Survey, UK)
- In house toenail (BAPS 2014)

The concentrations of all reference materials were found to be accurate within an acceptable percentage of the certified values for all elements studied here (average % recovery = 97% ± 20, see Supplementary Information Table 2 for keeper-fed diet CRM data and Supplementary Information Table 3 for all other CRM data).

Input Sampling and Analysis

Quantities of all food items which were presented to each elephant in their keeper-fed-diets on the day of sample collection were recorded, and water consumption was estimated at 200 litres per elephant per day based on the literature [7,33,45] details of keeper-fed diets are in Supplementary Information Tables 4, 5, 6, 7 and 8. These data were entered into Zootrition software[16] along with elemental analysis data (nutritional breakdown of feed items in Supplementary Information Table 2' keeper-fed diet analysis) to give an estimation of elemental intake in keeper-fed diet for each individual at each sampling point in time.

Elemental intake was estimated from diet, grass and water consumption for each individual elephant to give a combined input value for each element. Grass consumption from pasture grazing for each season was approximately estimated from total dry matter (DM) intake per elephant, based on individual body weight for that season[47], as shown in Supplementary Information Table 9. Soil consumption could not be estimated and thus was not included in the combined input value. Therefore, intake values may be considered a conservative underestimate.

Statistical Analysis

The objective of the analysis was to assess the evidence that particular measures of intake (e.g. soil, water, keeper-fed diet) for each element were predictive of measured status in a particular sample matrix (e.g. toenail, tail hair, plasma, faeces, urine). This was done using a linear mixed model[46] using R software, version 3.5.0[37]; the outcome of interest was the elemental level in elephant sample matrices (as predicted by inputs). In all models, species and sex were included as fixed effects. Zoo, individual and season were treated as nested random effects, accounting for correlations between individuals in the same zoo, and between repeated observations on the same individual. The linear mixed model (lmm) was fitted with the lme procedure from the nlme library for R platform[36].

The evidence that combined input was predictive of element status was assessed by fitting a lmm, with species, sex and combined input as the fixed effects. The models were fitted by residual maximum likelihood. The anova.lme command was applied to test the null hypotheses that the fixed effects on the model were zero. The sequential (default) option was used so that the test on the last-named fixed effect (combined input here) is a test of the null hypothesis given that the other fixed effects were already included in the model.

Next, the separate measures of intake were considered as potential predictors of status by a sequential modelling process. First, and without any reference to data, the measures were ordered from the one regarded as most likely to be predictive to the one least likely. The order of predictors was keeper-fed diet, grass, water and lastly, soil.

The first predictor in this order was then added to sex and species as fixed effects in an alternative model to the null, with sex and species only. The evidence for an effect of the added predictor was assessed by anova.lme command as described above. If the null hypothesis of no effect of the predictor was rejected with $P<0.05$ then the predictor was retained, and a new model was estimated with the second predictor added to the fixed effects as last-named in the sequence. If the null hypothesis for the first predictor was accepted, then it was dropped from the fixed effects, and a new model was fitted with the second predictor added to sex and species as fixed effects. This procedure was iterated until all the predictors had been considered.

The objective of this model fitting was to identify measures of intake that may be predictive of elemental status. The inference in the procedure above from p-values is properly done to test single hypotheses, and the procedure here is multiple hypothesis testing in which our question was `are any of the measures of intake predictive?'. It is well known that multiple hypothesis testing in which each hypothesis is evaluated on the basis of its own *p-valu*e is likely to be anticonservative, in the sense that the final set of selected predictors is likely to contain some, which result from the rejection of null hypotheses, which should have been accepted. Here we used the control of marginal discovery rate to control the error, based on the approach of Benjamini and Hochberg

(1995)[6]. The false discovery rate is the probability that a rejected null hypothesis in some family of tests should have been accepted. We controlled marginal false discovery rate (mFDR) at < 0.05, following the method of alpha-investment proposed by Foster & Stine (2008)[18].

Under Foster and Stine's (2008)[18] procedure, the p-values for a set of tests conducted in some order are compared against a set of threshold values. The threshold value for the i^{th} test is not fixed in advance but depends on a quantity called the alpha-wealth, which is depleted (by acceptance of null hypotheses at positions 1 to (i-1) in the sequence) or augmented by rejection of these hypotheses. Foster and Stine (2008)[18] present rules for the development of the alpha wealth, which ensure that the false discovery rate is controlled below the specified value. These mean that judicious ordering of the hypotheses, so that the least plausible nulls are tested early, (i.e. the predictors most likely to be informative) will increase the power of the procedure to detect real effects (although this is not valid if the ordering is based on prior examination of the data). More detail of the theory of this procedure is presented by Foster and Stine (2008)[18] and Lark (2017)[26] gives an example of its application. It has been used in various studies to improve the efficiency with which large data bases are interrogated to identify effects of interest while controlling false discovery rate[1].

The p-values from the successive tests of null hypotheses for each element and sample matrix were evaluated against threshold values determined by the method of Foster and Stine (2008)[18,] specifying that the false discovery rate be kept below 0.05.

The process above results in a predictive model for a mineral in a particular sample matrix, with selected predictors related to possible sources of intake, or no predictors are selected. In the former case we compute a measure of the extent to which the predictive model succeeds in accounting for variation in the observed concentrations in the sample matrix of interest. This is called the approximate adjusted R^2 and it is computed for each random effect in the model: the between-zoo, between-elephant within zoo and between observations within elephant random effects (the latter including measurement error). If the estimated between-zoo variance component for the null model, with sex and species the only fixed effects, is $s^2_{Z,0}$, and the corresponding variance component for the model with additional selected predictors is $s^2_{Z,1}$, then the approximate adjusted R^2 at between-zoo level may be computed as $R^2_Z = 1 - s^2_{Z,1} / s^2_{Z,0.}$ [1]

We may think of this quantity as the approximate proportion of variance (at the between-zoo level) accounted for by adding the extra predictors to sex and species. It should be noted that, unlike the R^2 values customarily computed with statistical software, this one is based on likelihood-based estimates of variance components rather than those obtained by partition of a sum of squared residuals. It is therefore possible that an estimated variance component could be negative, due to estimation error[23], and so the value computed in Equation [1] might not be bounded by [0,1]. Negative values simply imply that the effect of adding the predictor, at this level, is smaller than the estimation error, and a value of 1 implies that the variance component in the model with predictors is very small and has been estimated as zero as (or less than zero). A value of $R^2_Z < 0$ should therefore be interpreted as evidence that the predictor has negligible effect in terms of accounting for variation in the observations at the between-zoo level, and a value of 1 that it accounts for most of the variation at this level.

Results

Overall results

Tables 4.2 and 4.3 summarise results of all intakes sampled (keeper-fed diet, grass, soil and water) at the five study zoos. All individual data points are shown in Supplementary Information Tables 2 and 4 to 8.

Table 4.2: Median summary data for inputs (this includes keeper-fed diet and pasture grass) to all elephants at each of the five UK zoos (A, D, E = *Loxodonta africana*, B, C= *Elephas maximus*) at the four collection time points. CRM data for all diet items can be found in Supplementary Information Table 2. Keeper-fed diets n= number of diets (consisting of multiple feed items) at zoo, grass n= number grass samples collected

			Keeper-fed diet - as fed					Grass - estimated input, as fed					
		Zoo	A	D	E	B	C	units	A	D	E	B	C
		Species	*L.africana*			*E.maximus*			*L.africana*			*E.maximus*	
		Number elephants (m/f)	4 (0/4)	4 (1/3)	2 (2/0)	4 (0/4)	7 (1/6)		4 (0/4)	4 (1/3)	2 (2/0)	4 (0/4)	7 (1/6)
		Units											
Ca	median	g	312	529	340	147	327	g	33	72	12	261	37
	IQR		156	190	59	66	40		34	56	7	127	38
	n		16	16	8	14	28		16	16	8	6	26
Mg	median	g	73	157	76	53	118	g	17	17	3	141	10
	IQR		15	28	13	19	7		14	14	5	16	12
	n		16	16	8	14	28		16	16	8	6	26
Na	median	g	49	107	65	41	207	g	4	4	1	6	4
	IQR		24	64	49	27	68		4	3	1	1	3
	n		16	16	8	14	28		16	16	8	6	26
P	median	g	121	215	143	86	128	g	19	35	5	68	17
	IQR		8	111	9	27	68		21	20	7	97	20
	n		16	16	8	14	28		16	16	8	6	26
K	median	g	736	1488	929	579	460	g	126	203	40	524	129
	IQR		36	622	163	54	48		125	98	41	755	171
	n		16	16	8	14	28		16	16	8	6	26
Cu	median	g	0.9	1.3	0.8	0.6	0.8	mg	373	636	30	1063	255
	IQR		0.8	0.6	1.1	0.6	1.0		825	620	61	655	514
	n		16	16	8	14	28		16	16	8	6	26
Fe	median	g	8	18	9	6	11	g	35	13	6	215	8
	IQR		3	15	2	3	12		56	18	13	304	16
	n		16	16	8	14	28		16	16	8	6	26
Mn	median	g	7.6	8.0	3.8	1.6	12	g	17	2	0	6	1
	IQR		5.1	6.8	2.4	0.7	6.3		4	3	2	4	1

	n		16	16	8	14	28		16	16	12	6	26
Se	median	mg	2.4	13.0	2.9	1.5	3.2	mg	1.2	0.9	0.2	3.0	0.5
	IQR		0.7	7.1	0.3	1.1	0.8		1.4	.06	0.2	1.7	1.0
	n		16	16	8	14	28		16	16	8	6	26
Zn	median	g	3.2	5.0	3.1	1.0	2.2	mg	386	482	85	1529	229
	IQR		1.8	1.6	0.6	0.4	0.3		422	501	193	476	257
	n		16	16	8	14	28		16	16	8	6	26
As	median	mg	4	13	8	2	4	mg	18	6	4	78	3
	IQR		0.9	7.6	3.5	1.0	4.9		30	9	9	96	6
	n		16	16	8	14	28		16	16	8	6	26
Cd	median	mg	39	11	10	3	8	mg	5.0	0.6	0.6	5.0	0.4
	IQR		32	10	40	3	4		7.2	0.9	1.0	2.0	0.5
	n		16	16	8	14	28		16	16	8	6	26
Pb	median	mg	24	56	29	9	22	mg	121	20	13	323	12
	IQR		27	30	33	4	35		181	30	29	399	22
	n		16	16	8	14	28		16	16	8	6	26
U	median	mg	4	8	6	3	8	mg	1	0	0	8	0
	IQR		1	5	1	10	1		2.1	0.6	0.5	11.4	0.4
	n		16	16	8	14	28		16	16	8	6	26
V	median	mg	13	48	20	11	24	mg	68	24	10	397	18
	IQR		5	45	3	12	23		112	41	24	552	36
	n		16	16	8	14	28		16	16	8	6	26

Table 4.3: Summary data for inputs (water and soil) to all elephants at each of the five UK zoos (A, D, E = *Loxodonta africana*, B, C = *Elephas maximus*), samples were taken at the first visit. CRM data can be found in Supplementary Information Table 3

	Water - based on estimated input of 200 litres per elephant per day					Soil - Dry Matter					
Zoo		A	D	E	B	C	units	A	D	E	B	C
Species	units	*L.africana*			*E.maximus*			*L.africana*			*E.maximus*	
Element	Number elephants (m/f)	4 (0/4)	4 (1/3)	2 (2/0)	4 (0/4)	7 (1/6)		4 (0/4)	4 (1/3)	2 (2/0)	4 (0/4)	7 (1/6)
Ca	g	4	22	18	11	22	mg/kg	3369	1742	8045	10044	3354
Mg	g	0.5	3.4	2.3	2.1	0.8		4194	1352	5272	14985	2398
Na	g	2.8	10.9	3.5	3.9	3.5		3206	2054	2171	3437	3992
P	mg	322	356	366	4200	4		1086	298	790	493	817
K	g	0.2	1.7	0.5	2.6	0.7		13323	9397	27705	24989	10771
Cu	mg	0.1	12	3	1.6	0.6		76	11	19	16	19
Fe	mg	3	0	15	20	11		23600	15565	33053	26721	27092
Mn	mg	0.0	0.1	2.2	13.0	0.8		595	152	1883	462	955
Se	mg	0.0	0.6	0.1	0.1	0.1		0.63	0.17	0.54	0.25	0.45
Zn	mg	1	2	144	6	109		101	36	191	91	81
As	mg	22	44	342	56	36		11	7	28	9	123
Cd	mg	2	2	32	6	2		2.0	0.1	1.4	0.2	0.4
Pb	mg	0	14	252	12	32		74	12	87	39	49
U	mg	2	138	81	88	48		1.7	0.9	2.5	1.6	1.8
V	mg	20	20	140	40	40		61	36	70	62	74

Elemental Data for Intakes (diet, grass, water and soil)

Based on observations of the elephants, and keeper feedback, on all four visits to each of the five zoos, it was clear that the elephants consumed all the food presented to them in their keeper-fed diets, over a 24-hour period. At all five zoos, hay made up the largest contribution to the diet by weight, on average 52 kg per elephant per day (+/- 15 kg) out of an average keeper-fed diet of 81 kg per elephant per day (over 60% of intake), other than possible grazing for which accurate quantification was not possible. Seasonally, there was little variation in mineral provision from the hay and amounts fed were consistent throughout the year in all but one zoo (Zoo C), as shown in Supplementary Information Tables 2, 3, 4,5,6,7 and 8.

Commercial pellets were the main source of dietary minerals. Across the five zoos, 10 different commercial pellets were fed with four out of the five zoos feeding a pellet that was manufactured specifically for the species. One pellet had a Zn supplement milled into it (fed at Zoo C) and was fed to specific individuals that were suspected to be Zn deficient from previous in house assessment. All but one zoo fed wheat bran to their elephants in minimal quantities as a medicant carrier. Five additional nutraceutical or vitamin/mineral powdered supplements were added to the keeper- fed diets across three zoos (Zoos A, C and D): Bladder-rite (Gold Label, UK), Newmarket Joint Supplement (Newmarket, UK), multivitamin supplement (Farm and Stables Supplies, UK), multivitamin with additional vitamin E and Se supplement (Farm and Stables Supplies, UK), and calcium carbonate ($CaCO_3$) (Farm and Stables Supplies, UK). Two zoos provided no additional supplements (Zoos B and E).

Grass from pasture grazing was not available to all elephants throughout the year, as access depended on the weather conditions; however, all zoos offered grazing to all animals for at least part of the year (generally in spring, summer or autumn). Mineral content in grass from pasture grazing, and from browse consumption, varied considerably across seasons and between zoos. Generally, less browse was presented to elephants during winter months due to the challenges of sourcing palatable material in the UK climate. Fruit and vegetables comprised a very small proportion of all elephant diets (by weight) despite the wide variety of items fed (18 varieties of vegetables and 9 varieties of fruit over the five zoos), thus contributed minimally to the mineral provision in the diet due to the very small quantities fed per day and their high water content.

For certain minerals, the provision from water, contributed substantially to the overall mineral intake for the elephants. There was considerable variation between zoos noted, as shown in Figure 4.1. Specific elements of interest provided through drinking water included Zn where provision in zoos C and E were noteworthy, Ca in all the study zoos, and As and U in which provision from water contributed to intake more than keeper-fed diets. Additionally, at Zoo E, levels of Cd, Pb and V in the water contributed substantially to overall intake of these elements at this zoo. All water samples fell within the safe water limits issued for humans by the World Health Organization (WHO 2018)[53].

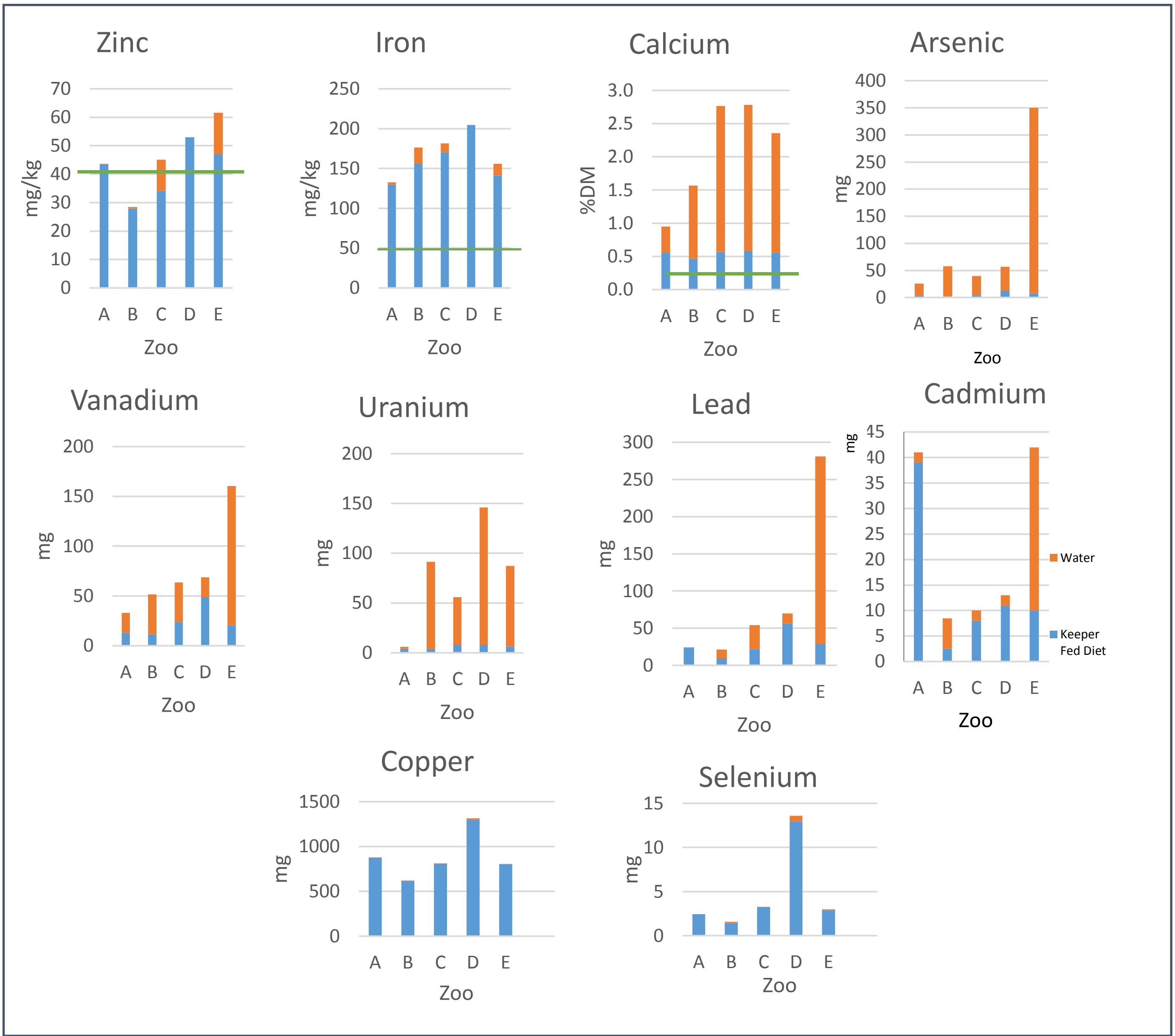

Figure 4.1: Median dietary element intake per zoo from keeper-fed diets and water (estimated at 200 litres per animal per day), in line with dietary recommendations[47], as shown by the green line

It was not possible to quantify elemental provision from soil, although anecdotally, keepers reported that elephants were seen to consume soil in very small quantities on occasion. Table 4.3 indicates that there is considerable variation in elemental provision from soil at the various zoos. This is likely to be due to geographical location of each zoo and geological makeup of the associated soil[19].

Dietary Provision compared to Published Recommendations

Calcium, Fe and Zn all have published recommended dietary intakes for elephants[38]. Figure 4.1 shows a comparison of mineral levels in keeper-fed diets from each zoo to captive recommendations produced by Ullrey et al. (1997)[47]. In all zoos, Ca and Fe concentrations in provisioned diets were well in excess compared with intake recommendations, whereas Zn was below the recommended levels in keeper-fed diets in two of the five zoos. However, when Zn provision from water was also considered (based on assumed consumption of 200 litres per elephant per day[7,33,45]), the Zn level in Zoo C rose to within the recommended levels and only intake in Zoo B remained below the recommended level of 40 mg/kg (DM basis).

Elemental Reflection in Elephant Sample Matrices

Tables 4.4, 4.5 and Supplementary Information Table 10, summarise the results of all outputs from elephants at the five study zoos (toenail, tail hair, faeces, urine and plasma), divided into fluids and solids. Urine elemental analysis was corrected for hydration status using creatinine normalization[14]. Elephant tail hair is estimated to grow at approximately 2–3.5 cm per month, with two studies of 50 and 32 hairs, respectively, documenting this growth rate and reported that tail hair of males grew up to 1.5 cm more slowly per month than those of females[11,52].

These data reported for zoo elephants are therefore assumed to represent the median elemental analysis for approximately the most recent 6 months of growth: 3x3cm sections for males and 5x3cm sections for females, given that growth rate of males is documented to be slower than females.

Table 4.4: Median summary elemental data for sample matrices (fluid outputs) from elephants at all UK zoos; this includes plasma and urine (corrected for hydration status using a ratio with creatinine), from all sampled elephants (21 individuals) at each of the five UK zoos (A, D, E = *Loxodonta africana*, B, C = *Elephas maximus*), at the four collection time points. All data reported on a wet basis, see Supplementary Information Table 10 for further data points

	Median plasma						Median Urine: creatinine		
Zoo	units	A	D	E	B	C	Ratio	D	C
Species		*L.africana*			*E.maximus*			*L.africana*	*E.maximus*
Number elephants (m/f)		4 (0/4)	4 (1/3)	2 (2/0)	4 (0/4)	7 (1/6)		3 (1/2)	6 (0/6)
Number samples		9	16	4	5	28		11	21
Ca	mg/l	107	105	104	86	108	Ca:Cre	2.3	5
Mg	mg/l	28	25	26	22	28	Mg:Cre	0.6	1.7
Na	mg/l	2955	3038	3109	2603	3218	Na:Cre	0.1	2.1
P	mg/l	91	81	95	67	78	P:Cre	0.01	<0.001
K	mg/l	284	205	192	178	182	K:Cre	5.6	19
Cu	µg/l	919	988	1001	736	1096	Cu:Cre	<0.001	<0.001
Fe	µg/l	1.6	3.2	0.9	1.5	1.1	Fe:Cre	<0.001	<0.001
Mn	µg/l	2.1	1.6	3.8	2.3	1.5	Mn:Cre	<0.001	<0.001
Se	µg/l	125	162	118	106	187	Se:Cre	<0.001	<0.001
Zn	µg/l	1399	1056	1129	614	737	Zn:Cre	<0.001	<0.001
As	µg/l	0.5	0.8	1.0	0.3	0.5	As:Cre	<0.001	<0.001
Cd	µg/l	0.2	0.06	0.08	0.1	0.03	Cd:Cre	<0.001	<0.001
Pb	µg/l	0.1	0.48	0.81	0.2	0.18	Pb:Cre	<0.001	<0.001
U	µg/l	0.02	0	0.00	0.02	0.00	U:Cre	<0.001	<0.001
V	µg/l	0.23	0.22	0.22	0.22	0.28	V:Cre	<0.001	<0.001

Table 4.5: Median summary elemental data for sample matrices (solid outputs); this included faeces, toenails and tail hair from all sampled elephants (21 individuals) at each of the five UK zoos (A, D, E = *Loxodonta africana*, B, C = *Elephas maximus*), at the four collection time points. All data reported on a dry matter basis. Further data points are reported in Supplementary Information Table 10

			Faeces dry matter					Toenail					Tail hair				
Zoo		units	A	D	E	B	C	A	D	E	B	C	A	D	E	B	C
Species			*L.africana*			*E.maximus*		*L.africana*			*E.maximus*		*L.africana*			*E.maximus*	
Number animals (m/f)			4 (0/4)	4 (1/3)	2 (2/0)	4 (0/4)	7 (1/6)	4 (0/4)	4 (1/3)	2 (2/0)	4 (0/4)	7 (1/6)	4 (0/4)	4 (1/3)	2 (2/0)	4 (0/4)	7 (1/6)
Ca	median	mg/kg	4229	3887	7758	2764	3615	2355	3807	3716	4496	3296	2958	3155	1658	2046	3118
	n		16	16	8	10	27	16	16	8	15	28	20	13	6	5	29
Mg	median	mg/kg	1013	1330	1442	1230	1620	224	587	304	456	224	450	422	198	230	175
	n		16	16	8	10	27	16	16	8	15	28	20	13	6	5	29
Na	median	mg/kg	1522	2766	2446	2384	4415	37	108	180	88	57	351	132	390	218	99
	n		16	16	8	10	27	16	16	8	15	28	20	13	6	5	29
P	median	mg/kg	2709	2946	4337	2895	3680	57	106	93	57	76	65	159	63	102	79
	n		16	16	8	10	27	16	16	8	15	28	20	13	6	5	29
K	median	mg/kg	13408	11625	14654	11965	10738	252	874	350	825	225	1643	301	202	1073	126
	n		16	16	8	10	27	8	16	8	11	28	20	13	6	5	29
Cu	median	mg/kg	18	30	29	28	30	1.8	2.4	2.2	1.1	2	17	15	14	10	11
	n		16	16	8	10	27	16	16	8	15	28	20	13	6	5	29
Fe	median	mg/kg	735	1428	1651	645	562	247	730	463	356	351	58	0	17	314	93
	n		16	16	8	10	27	16	16	8	15	28	15	13	6	5	29
Mn	median	mg/kg	197	143	174	80	295	14	18	25	11	21	29	13	36	13	37
	n		16	16	8	10	27	16	16	8	15	28	20	13	6	5	29
Se	median	mg/kg	0.05	0.16	0.1	0.03	0.1	0.3	0.6	0.2	0.4	0.4	1.0	1.5	0.8	1.0	0.2
	n		16	16	8	10	27	16	16	8	15	28	20	13	6	5	29
Zn	median	mg/kg	76	68	89	53	61	51	55	39	39	30	125	94	108	94	101
	n		16	16	8	10	27	16	16	8	15	28	20	13	6	5	29
As	median	mg/kg	0.3	0.6	1.2	0.3	0.3	0.2	0.8	0.8	0.3	0.4	0.2	0.2	0.2	0.3	0.1
	n		16	16	8	10	27	16	16	8	15	28	20	13	6	5	29
Cd	median	mg/kg	1.90	0.12	0.35	0.20	0.15	0.05	0.02	0.07	0.05	0.02	0.12	0.02	0.05	0.09	0.05
	n		16	16	8	10	27	16	16	8	15	28	20	13	6	5	29
Pb	median	mg/kg	2.7	1.85	3.78	1.2	1.2	0.48	0.66	1.87	1.03	0.49	0.45	0.48	0.45	1.02	0.25

	n		16	16	8	10	27	16	16	8	15	28	20	13	6	5	29
U	median	mg/kg	0.20	0.19	0.38	0.10	0.34	0.01	0.03	0.02	0.03	0.02	0.002	0.012	0.003	0.010	0.004
	n		16	16	8	10	27	16	16	8	15	28	20	13	6	5	29
V	median	mg/kg	1.65	3.85	3.37	1.2	1.59	0.37	1.42	0.84	0.41	0.87	0.10	0.3	0.00	0.50	0.10
	n		16	16	8	10	27	16	16	8	15	28	20	13	6	5	29

Identifying Best Sample Matrices for Each Element

Table 4.6 summarises results from the linear mixed model, and the significance of correlation with combined inputs, including the keeper-fed diet, estimated grass consumption and estimated water consumption. Toenail was found to best reflect Na and K intakes. Iron intake was best reflected in both tail hair and toenail samples. Phosphorus and Ca levels in faeces correlated with combined inputs and Se intake correlated with toenail and faecal levels. Finally, Cd and U intake were reflected in plasma samples. Elemental levels in urine were found to have no significant correlation with any combined inputs.

Table 4.6: Linear mixed model p-values of combined input values compared to sample matrices. Combined inputs include keeper-fed diet, estimated elemental provision from water and grass consumption based on estimated intakes. Urine is not included as there was inadequate balanced replication to estimate model. R^2 E = between-elephant within zoo and R^2 O = between observations within elephant random effects (the latter including measurement error)

Element		Tail hair	Toenail	Plasma	Faeces
Ca	p-value	0.513	0.159	0.992	0.019
	R^2E				0.608
	R^2O				0.092
Mg	p-value	0.450	0.599	0.357	0.924
Na	p-value	0.076	0.039	0.309	0.136
	R^2E		<0.000		
	R^2O		0.061		
P	p-value	0.318	0.165	0.812	0.012
	R^2E				<0.000
	R^2O				0.142
K	p-value	0.791	0.031	0.869	0.411
	R^2E		1.000		
	R^2O		<0.000		
Cu	p-value	0.467	0.445	0.475	0.671
Fe	p-value	0.029	0.012	0.390	0.582
	R^2E	0.406	<0.000		
	R^2O	0.256	0.149		
Mn	p-value	0.630	0.707	0.650	0.498
Se	p-value		0.031	0.887	0.000
	R^2E		0.186		0.521
	R^2O		<0.000		0.077
Zn	p-value	0.824	0.502	0.068	0.691
As	p-value	0.175	0.237	0.644	0.511
Cd	p-value	0.088	0.975	<.0001	0.907
	R^2E			<0.000	
	R^2O			0.287	
Pb	p-value	0.224	0.397	0.485	0.394
U	p-value	0.356	0.124	0.004	0.552
	R^2E			<0.000	
	R^2O			0.150	
V	p-value	0.090	0.412	0.288	0.481

Table 4.7 shows summary results from within the linear mixed model to identify the best sample matrices for reflecting bio-indicators of intake and therefore a good proxy of elemental status. The R^2 value (in Table 4.6 and 4.7) considers other factors which may

influence the significant predictor; R^2 E = between elephant within zoo and R^2 O = between observations within elephant random effects (the latter including measurement of error). This value increases confidence towards the predictor. Supplementary Information Table 11 details all p-values resulting from this model. Inputs were considered sequentially, with diet as the first predictor followed by grass, water and finally soil. Toenail was found to reflect the greatest number of elements, followed by faeces and plasma. Tail hair and urine reflected the least number of elements. Diet was the most significant predictor for the greatest number of elements (8) followed by grass, soil and water. Due to the reduced sample size for urine, there is inadequate balanced replication to estimate the full model, so sex and species effects were removed as random effects. No significant predictors were found for Cd, Na, Pb and V.

Table 4.7: Linear mixed model results to identify bio-indicators of elemental status depending on multi-layered model using diet, grass, water and soil as predictors. R^2 E = between-elephant within zoo and R^2 O = between observations within elephant random effects (the latter including measurement error)

Element		Tail hair	Toenail	Plasma	Urine	Faeces
Ca	predictor	no significant predictors				diet
	R^2E					0.919
	R^2O					0.085
Mg	predictor	no significant predictors	diet	no significant predictors		
	R^2E		<0.000			
	R^2O		0.212			
	predictor		soil			
	R^2E		0.175			
	R^2O		0.227			
P	predictor	no significant predictors	soil	no significant predictors		
	R^2E		0.320			
	R^2O		<0.000			
K	predictor	no significant predictors	diet	no significant predictors		
	R^2E		1.000			
	R^2O		0.146			
	predictor		grass			
	R^2E		<0.000			
	R^2O		0.586			
Cu	predictor	no significant predictors				diet
	R^2E					<0.000
	R^2O					0.063
Fe	predictor	no significant predictors		soil	no significant predictors	
	R^2E			0.588		
	R^2O			0.030		
Mn	predictor	no significant predictors		soil	no significant predictors	

	R^2E			<0.000		
	R^2O			<0.000		
Se	predictor	no significant predictors	diet	no significant predictors		diet
	R^2E		0.140			0.663
	R^2O		<0.000			0.103
Zn	predictor	no significant predictors	no significant predictors	diet	no significant predictors	no significant predictors
	R^2E			<0.000		
	R^2O			0.056		
As	predictor	grass	diet	no significant predictors		diet
	R^2E	0.488	<0.000			<0.000
	R^2O	0.093	0.159			0.115
U	predictor	no significant predictors		diet	no significant predictors	
	R^2E			0.315		
	R^2O			0.120		
	predictor			grass		
	R^2E			<0.000		
	R^2O			0.490		

Discussion

The aim of this study was to identify appropriate sample matrices to use as bio-indicators of elemental status in elephants. The variability in dietary provision by zoos presented a challenge, and the complexity of estimating the 'inputs', i.e. keeper-fed diet including browse, grass provision from grazing, and intake of water and soil, demonstrates the need to be able to simply obtain non-invasive samples for assessing elemental status in the species. A strong bio-indicator must respond well to variation in intake, so we examined relationships between elemental composition of sampled sample matrices and known inputs as shown in Tables 4.6 and 4.7.

Tables 6 and 7 demonstrate that non-invasive sampling of tail hairs and toenails can potentially provide useful indications of As, Fe, K, Mg, Na, P, Se, and Zn status in elephants. Results suggest that the current clinical practice of elemental measurement using plasma is of limited value, as such measures are rarely responsive to dietary variation. There were no useful biomarkers of intake for Cd, Na, Pb and V when considered sequentially and for As, Cu, Mg, Mn, Pb, V and Zn when considered on a combined input basis. Additionally, faeces reflected As, Ca, Cu and Se intake on a sequential basis and Ca, Se and P on an estimated combined input basis. Manganese and U intakes were reflected in blood sequentially. Where there is no elephant-derived sample that can indicate intake of an element, zoos may find it useful to be aware of alternative measures (e.g. haemoglobin status for Fe) and consider the elemental composition of their water sources.

Toenails were found to best reflect elemental intake for the largest number of elements in elephants: As, Fe, K, Mg, Na, P and Se as shown in Tables 4.6 and 4.7. Unfortunately, it was not possible to estimate the growth rate of the toenails or the time during which the material analysed was laid down by the elephant. A number of factors including substrate, exercise, weather, foot care, nutritional and health status of the animal, as well as behaviour will affect toenail growth rates[20]. Tail hair proved to be a bio-indicator of total intake of key minerals, including As and Fe. Tail hair growth rate, as with other mammals, is also likely to be affected by multiple factors including overall nutritional plane, weather, health of the elephant and age of the elephant[41]. Therefore, estimated growth rate was used to calculate the analysed length[11,52], which is an estimation of the previous 6 months of growth, although in practice this may not always be accurate due to variability in growth rate. Faecal samples were indicative of estimated elemental intake in the elephant for As, Ca, Cu, Se and P, as shown in Tables 4.6 and 4.7. Due to historic concern around insufficient vitamin E and other antioxidants in zoo elephant diets, Se is likely being fed in excess as an additive within the manufactured elephant pellets[17]. This could indicate why high concentrations were excreted and therefore detected within faecal samples.

Macro-mineral concentrations within plasma were not found to be reflective of intake. This is unsurprising, as mammals homeostatically control levels of these minerals within the blood and store excess as needed[10,22,32]. Urine was not found to indicate estimated intake of any element. It was only possible to obtain 32 samples from 9 animals at two zoos. Therefore, there was inadequate balanced replication to estimate the full model, thus sex and species effects were removed.

Generally, UK zoo elephants are not mineral deficient, and often for several minerals, keeper-fed diets contain excess provision. For specific elements such as Pb, inputs were very low (in keeper-fed diet, water, soil or grass) and thus, Pb was not present in sufficient quantities in any inputs, to be reflected in any elephant samples. A linear mixed model with the application of alpha wealth was appropriate to identify significant relationships between inputs and sample matrices (outputs) both on a combined and sequential method. The use of alpha wealth to reduce the false discovery rate within the linear mixed model provided greater confidence in the findings; nine previously significant relationships were eliminated through this correction. The application of R^2 allows for consideration to be given to the weighting of factors other than the one being investigated that may have affected relationships, including between-elephant within zoo and between observations within elephant random effects (the latter including measurement error). Elemental combined input based on keeper-fed diet, estimated water consumption (200 litres/day) and estimated grass intake from grazing (based on %DM consumption), resulted in less significant relationships with sample matrices than when considered sequentially. This is likely because estimations were made of inputs, especially of grass intake, to form the combined input figure.

Keeper-fed diet was the most likely predictor of elemental values measured in any sample matrix and was the major source of minerals, as shown in Table 4.7. From this source, the pellet ration within the diet contributed most to elemental provision, even though the weight of pellet fed in the overall keeper-fed diet was comparatively low, on average 9% of the diet as fed (an average 7±9 kg per elephant per day). However, only Zoo B and C fed less than the recommended 3 kg of pellet per day[2]. Variation in elemental provision from hay throughout the year was minimal, mineral or trace metal degradation would not be expected with storage. Hay and browse used within each zoo were produced within close proximity (within approx. 20 km) of each zoo. The variation in elemental analysis in hay and browse between zoos, is likely to reflect the differing geology of the five areas surrounding the zoos.

Water mineral concentrations varied widely both among zoos and seasonally, often depending on source / availability, e.g. borehole, rainwater harvesting or mains water supply. Elemental provision from water is rarely considered as significant when evaluating human or animal diets, except when investigating exposure to trace metals. However, Figure 4.1 shows that water contributed substantially to the intake of specific minerals (As, Ca, Cd, Pb, U, V and Zn, Cd) and requires further study. At zoo C, Zn provision from keeper-fed diet was below the recommended levels. With the addition of Zn from the water provided (assuming average levels of water intake from the literature, 200 litres /day), apparent levels of Zn became sufficient. Beal (2017)[4] demonstrated the significance of Ca provision in human drinking water, where Ca from water contributed up to 11% of national Ca supplied, based on consumption of 1.7 litres per adult per day. Within this study, Ca in water was found to contribute up to 8% of Ca supply per day, based on an estimated consumption of 200 litres of water per elephant per day.

Zoos were selected to provide a geographical and geological spread with variance within the soil make up, therefore the variation seen in the elemental analysis of soils sampled was not unexpected. The linear mixed model determined that soil minerals were a significant predictor of Mg and P levels in toenail and Fe and Mn in plasma. For these elements, provision from soil may be important to consider when looking at elemental reflection in these sample matrices.

There are limited published recommendations for elephant dietary mineral provision. Generally the domestic horse is considered to be an acceptable physiological model for elephants[12,38]. Additionally, the BIAZA (British and Irish Association of Zoos and Aquaria) Elephant Management Guidelines (2019)[38] provides recommendations on dietary management, which must be followed by BIAZA member zoos as part of the BIAZA Elephant Management Policy. All zoos within this study fed browse daily throughout the year and provided some grazing access to all animals, in line with these BIAZA recommendations.

Mineral provision from the keeper-fed diet within this study was found to be similar to previous work. Partington (2012)[34] found all UK elephant diets to be excessive in Ca, as was the case in this study, and also detected some possible Zn deficiencies. The study conducted by Partington used intake data from all UK elephant holding zoos at the time of writing but used published elemental data for each food item and did not include elemental provision from browse as was included in the current study. The lowest Zn dietary intake identified by Partington (2012)[34] was 22mg/kg DM, whereas in this study, the lowest Zn dietary intake was 28 mg/kg DM (Zoo B) as shown in Figure 4.1, but still below the recommended 40 mg/kg DM.

Published reference ranges for elephant plasma, urine and faecal mineral levels are very limited. The largest database of this information resides with Species 360, USA[42]. All member zoos internationally are encouraged to submit data as available. Sample sizes of these datasets are often small and animals may be health-compromised at the time of sampling. Results for elemental levels in plasma, urine and faeces in this study (Table 4.4, 4.5 and Supplementary Information Table 10) were within reported reference ranges, Species 360, 2017[42]. Published data on elemental analysis in elephant tail hair or toenails is absent from the literature.

Feed costs are the second largest day-to-day running costs of a captive elephant herd[38,39]. It is therefore essential that zoos are feeding appropriate items of acceptable quality to their animals. The BIAZA Elephant Management Guidelines recommends the use of fruits and vegetables in very limited quantities, less than 1 kg per elephant per day[40]. All zoos in the current study were feeding greatly in excess of this recommendation as shown in Supplementary Information Tables 4,5,6,7 and 8. Given the high incidence of obesity in UK zoo elephants, with estimations of up to 75% of the population being recorded as 'overweight'[21], a seasonal reduction in hay is recommended to offset increased grass from pasture and browse availability during summer months, this is currently only practiced in Zoo C.

Finally, the elephants in this study are under human care, and could arguably be variably stressed or compromised[28], which may alter mineral metabolism. For example, plasma Zn levels can be artificially increased when an animal is stressed or suffering from an inflammatory condition[25]. Caution must be used when comparing these values to wild elephants, or as target values for elephants. Likewise, as seen within this current study, UK zoo elephants are unlikely to be experiencing nutritional compromise or substantial mineral deficiency. Diets fed in these five UK zoos, in general, were appropriate to meet species' documented mineral needs.

Conclusion

The results from the current study indicate that no single sample matrix from elephants are sufficient to reflect elemental intake within the animal, and thus be a good proxy for elemental status, a variety of sample matrices are needed. Of the five sample matrices investigated in this study, toenail reflected inputs for the largest number of elements assessed, and is likely to be the best reflection of status for these elements. Faeces and tail hair were also found to significantly correlate to inputs into the elephant. Plasma was of limited value with a small number of elements being responsive to dietary variation. Urine did not correlate with any inputs for any element and thus was not a useful bio-indicator. Predicting how elemental status is reflected in various sample matrices presents a challenge, as the sample matrix concentrations may not be indicative unless levels are below an excess threshold. Sample availability may also influence sample matrix choice when investigating mineral status. Finally, mineral provision from water should never be overlooked when assessing zoo animal diets, especially for species that consume such large volumes as elephants.

Future work should investigate how the methods described in this study could be applied to free-living populations of elephants, especially those within smaller fenced reserves, to identify individuals with mineral deficiencies, or elephants exposed to uncharacteristically high levels of trace metal intake. Opportunities exist to address the United Nations Sustainable Development Goals (SDGs) 3 (Good Health and Well-Being), 15 (Life on Land) and 17 (Partnerships for the Goals) from this work. Advancing zoo animal health and welfare will increase opportunities to (re) connect people with nature and promote well-being through the visiting of zoos (SDG 3), with further potential for education and creation of livelihoods (SDG 4, 8). Secondly, application of this work provides the opportunity to protect ecosystems through benefitting wildlife management (SDG 15). Finally, global partnerships can be developed between North and South with the opportunity for studies on captive animals in a controlled environment to inform research and welfare of wild counterparts (SDG 17).

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Declaration of Interests

The authors declare the following financial interests/personal relationships, which may be considered as potential competing interests:

-Ellen Dierenfeld is employed by Ellen S. Dierenfeld, LLC.

-Fiona Sach is employed by the Zoological Society of London.

-Simon Langley-Evans, Michael Watts, R.Murray Lark, Elliott Hamilton and Lisa Yon have no competing interests

Chapter 5

Sach F., Yon, L., Henley M., Bedetti A., Buss P., de Boer WF., Dierenfeld E., Gardner A., Langley-Evans S., Hamilton, E., Lark, RM., Prins HHT., Swemmer AM., Watts M. 2020. Spatial geochemistry influences the home range of elephants**.** 2020. *Science of the Total Environment, 729.* https://doi.org/10.1016/j.scitotenv.2020.139066

Author contributions

FS, MW, LY, SLE, ED, MH and AS conceptualization and methodology; FS and AB data curation; FS, MH, AG, EH and PB investigation; MW, MH, AS and PB resources; FS and RML formal analysis; FS writing original draft. All authors contributed critically to drafts and final approval for publication.

Supplementary information for this paper will be available online upon publication:

1	GPS coordinates of sample sites
2	Certified Reference Material (CRM) data
3	Soil data
4	Plant data
5	Water data
6	Tail hair data
7	Faecal data
8	Original P-values and adjusted P-values for biological and environmental samples
9	Home range analysis

As part of this work, evidence of further analysis work can be found in the Appendix.

Analysis of 293 wild South African plasma samples taken at random from the South African National Parks Biobank and 25 toenail samples from animals collared by Elephants Alive.

These data are made available for other future publication or research studies.

Spatial geochemistry influences the home range of elephants.

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Abstract

The unique geochemistry surrounding the Palabora Mining Company (PMC) land may act as a micronutrient hotspot, attracting elephants to the area. The PMC produces refined copper and extracts phosphates and other minerals. Understanding the spatial influence of geochemistry on the home range size of African savanna elephants is important for elephant population management and conservation.

The home ranges of collared elephants surrounding the PMC were significantly smaller (P=0.001) than conspecifics in surrounding reserves, suggesting that their resource needs were met within these smaller areas. Environmental samples (soil, water and plants) were analysed from the mine area and along six transects radiating from the mine centre. Tail hair and faecal samples from elephants at the PMC, and conspecifics within the surrounding area were analysed. All samples were analysed for minerals essential to health and potentially toxic elements (PTEs; As, Ca, Cd, Cu, Fe, K, Mg, Mn, Na, P, Pb, Se, U, V and Zn). Results show that the geochemistry at the PMC is different compared to surrounding areas, with significant elevations seen in all analysed minerals and PTEs in soil closer to the mine, thereby drawing the elephants to the area. Additionally significant elevations were seen in elements analysed in water and vegetation samples. Elephant tail hair from elephants at the mine was significantly greater in Cd, whilst Mg, P, Cu, As, Cd, Pb and U concentrations were significantly greater in elephant faecal samples at the mine compared.

When micronutrient hotspots overlap with human activity (such as mining), this can lead to poor human-elephant coexistence and thus conflict. When managing elephant populations, the influence of mineral provision on elephant movement must be considered. Such detailed resource information can inform conservation efforts for coordinated programmes (UN SDGs 15 and 17) and underpin sustainable economic activity (UN SDG 8, 11 and 12).

Keywords: *Loxodonta africana*, minerals, mining, potentially toxic elements, elephant movement

Introduction

The increase in human population and global intensification of agriculture have significantly reduced African savanna elephant (*Loxodonta africana*) populations, through habitat reduction and fragmentation, causing the overlap of human and elephant habitation, leading to increased human-elephant conflict (HEC; Blanc, 2008). Elephants are forced into increasingly smaller areas, often restricted by fencing or encroaching anthropogenic activities, resulting in increased pressures on these areas to meet the elephants' resource needs. This can present nutritional challenges, resulting in altered elephant movement patterns and distribution in efforts to seek out required minerals. Elephants move to meet their mineral needs, and use available micronutrient hotspots, causing HEC, when these overlap with human activities (Sach *et al.*, 2019). Minerals are required by elephants for a variety of biological processes including energy metabolism, organ and immune function, reproduction and cellular growth (Ishiguro, Haskey and Campbell, 2018).

Geochemistry influences mineral availability in soils, and thereby in plants and water to elephants (Prins and Langevelde, 2008). Understanding how the geochemistry of an area, and presence of micronutrient hotspots, influences mineral provision to the animal, informs how geochemistry influences home range size, especially when anthropogenic activities constrain long-distance movements. Largely, plants reflect the soil mineral profile, plants growing in deficient areas lack key minerals, which can result in deficiencies in the consumer (elephant). In contrast, plants growing in mineral rich areas pass on the mineral abundance to the consumer (Joy *et al.*, 2015). Geochemical properties (including organic matter and soil pH), and the ability of plants to extract minerals from the soil will influence the availability of these minerals to elephants (Bowell and Ansah, 1994; Maskall and Thornton, 1996).

African savanna elephants move and adapt their food selection, to meet their target levels of (as yet undetermined) minerals (Bax & Sheldrick, 1963). It is suspected that in volcanic areas such as the Palabora Mining Company (PMC), levels of micronutrients will be elevated, acting as a micronutritent hotspot, with a reduction in elephant home ranges size (Greyling, 2004). This may be beneficial or detrimental to elephants. In areas where the soil is generally deficient in minerals, it may allow elephants to meet their mineral needs within a small area. However, as with other mammals, dietary excess of minerals or potentially toxic elements (PTEs) can occur from overconsumption, causing toxic effects; data is limited as to these threshold levels for elephants (Sach *et al.*, 2019). Elephants are large, slow-growing and can accommodate extended periods of nutrient deficiency due to their nutrient stores (Prins and Langevelde, 2008). Excess consumption of minerals or PTEs to harmful levels is likely to take several years (Ullrey, Crissey and Hintz, 1997).

As well as micronutrients, drivers for elephant movement include availability of food and water, social interaction, human activities, safety and access to shade (Wall, Douglas-Hamilton and Vollrath, 2006). The distance travelled by elephants to meet their resource needs, will be reflected in their home range size (de Knegt *et al.*, 2011). Mineral provision influences elephant food selections; for example, the Associated Private Nature Reserves (APNR), South Africa are suspected to have a localised phosphorus (P) deficiency, elephants increased their consumption of leaves from trees that had been fertilised with P (Pretorius *et al.*, 2011, 2012). Secondly, females in family units maximised P intake by ingesting leaves with higher P content, to meet their increased requirements, compared to larger bodied males who consumed other lower P plant parts (Greyling, 2004). Phosphorus plays a role in reproduction and lactation (Groenewald and Boyazoglu, 1980). It is predicted that if an area such as the PMC is a micronutrient hotspot, elephants will remain within the locality, to meet their resource needs for minerals as demonstrated by Tucker et al. (2018), especially if the surrounding soils are poor in several essential micronutrients such as P, as suggested by Greyling (2004) and Pretorius (2011, 2012).

The aim of this study was to understand the spatial influence of geochemistry on the home range size of elephants, using the Palabora Mining Company (PMC) property and surrounding national park land as a case study of contrasting environments. The following objectives were used to achieve the aim: (1) Determine if mineral levels in soil, forage and water near the mine are greater than the nearby Kruger National Park (KNP)/APNR and hence may influence a reduced elephant home range size; (2) Establish baseline levels for key minerals and PTEs in African savanna elephant tail hair and faeces as potential biomarkers, and (3) Determine if the elephant tissues (tail hair and faecal samples) collected near the mine contain greater concentrations of essential minerals and PTEs, compared to elephants in surrounding reserves, away from the mine.

Materials and methods

Statement of Ethical Approval

iii) Ethical approval from the University of Nottingham (Reference: 1499 150622) and South African National Parks (SANParks), project number SACF1444.

iv) The Palabora Copper (Pty) Limited, a subsidiary of Palabora Mining Company Ltd, is a copper mine that also operates a smelter and refinery complex. Its majority shareholder is the Hebei Iron & Steel Group. The study design, methodology or results were not influenced by the management or staff of these commercial entities. The manuscript was not vetted or otherwise influenced.

Permits

i) 'Department for Environment, Food and Rural Affairs (DEFRA) authorisation for the importation from third countries of research and diagnostic samples', ITMP17.0821B - tail hair and faecal samples.

ii) Convention on international trade in endangered species (CITES) of wild fauna and flora: Export 171485/Export 222457, Import 566134/01/Import 568473/01

iii) Plant and soil samples-British Geological Survey FERA Licence 2017/2018.

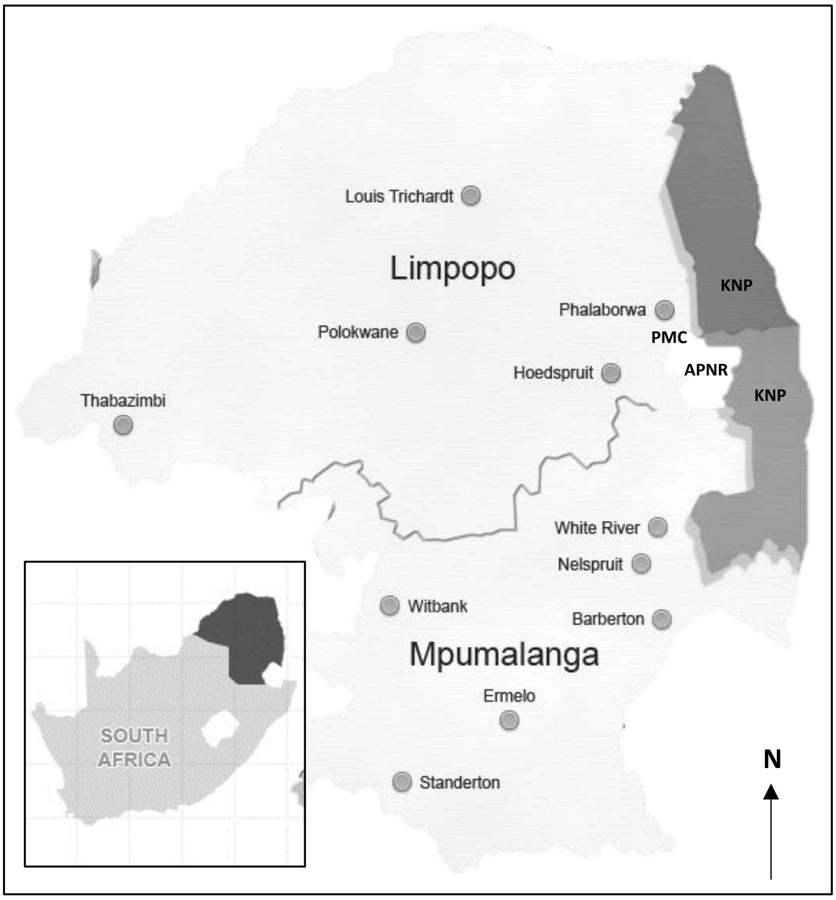


Figure 5.1: Study area showing the Kruger National Park (KNP), Associated Private Nature Reserves (APNR) and Palabora Mining Company (PMC)

The study was conducted on the Palabora Mining Company (PMC) land near Phalaborwa town, South Africa and adjacent areas within the KNP and the APNR (Fig. 5.1). From west to east, the geological succession of the KNP changes from granitic to basaltic. Granites generally form nutrient poor substrates whereas basaltic rocks form nutrient rich substrates (Venter and Gertembach, 1986). The APNR is located on the western border of the KNP, and is made up of gneiss, granite or magmatite (Venter and Gertembach, 1986). Elephants can move freely amongst the KNP, APNR and PMC lands. Elephant incursion into the PMC can cause financial losses and risk to elephant and human life. Elephants can damage infrastructure, inhibit mining operations and cause elephant, vehicle and train collisions.

In this generally micronutrient poor environment, the Palabora Igneous Complex has a unique mineral rich rock formation. Commercial mining began in 1954, with open-cast mining of foskorite and pyroxenite, thereafter the PMC began mining the same ores for copper and magnetite, developing into the country's main producer of refined copper, operating over 1950 ha (Roux *et al.*, 1989). The NGO Elephants Alive (EA) have collared elephants throughout the APNR, and seven elephants utilising the mine area (movements in Fig. 5.2). The home range of these mine collared elephants was calculated using a-LoCoH 90% (Getz and Wilmers, 2004), and was smaller than that of neighbouring elephants within the APNR (Table 5.1), animals of the same sex, age category and wearing collars for the same time period were compared. Elephant census data showed that elephant density within the operational PMC (1.4 per km^2) was larger than that within the surrounding KNP (0.8 per km^2; Lerm and Swemmer, 2015).

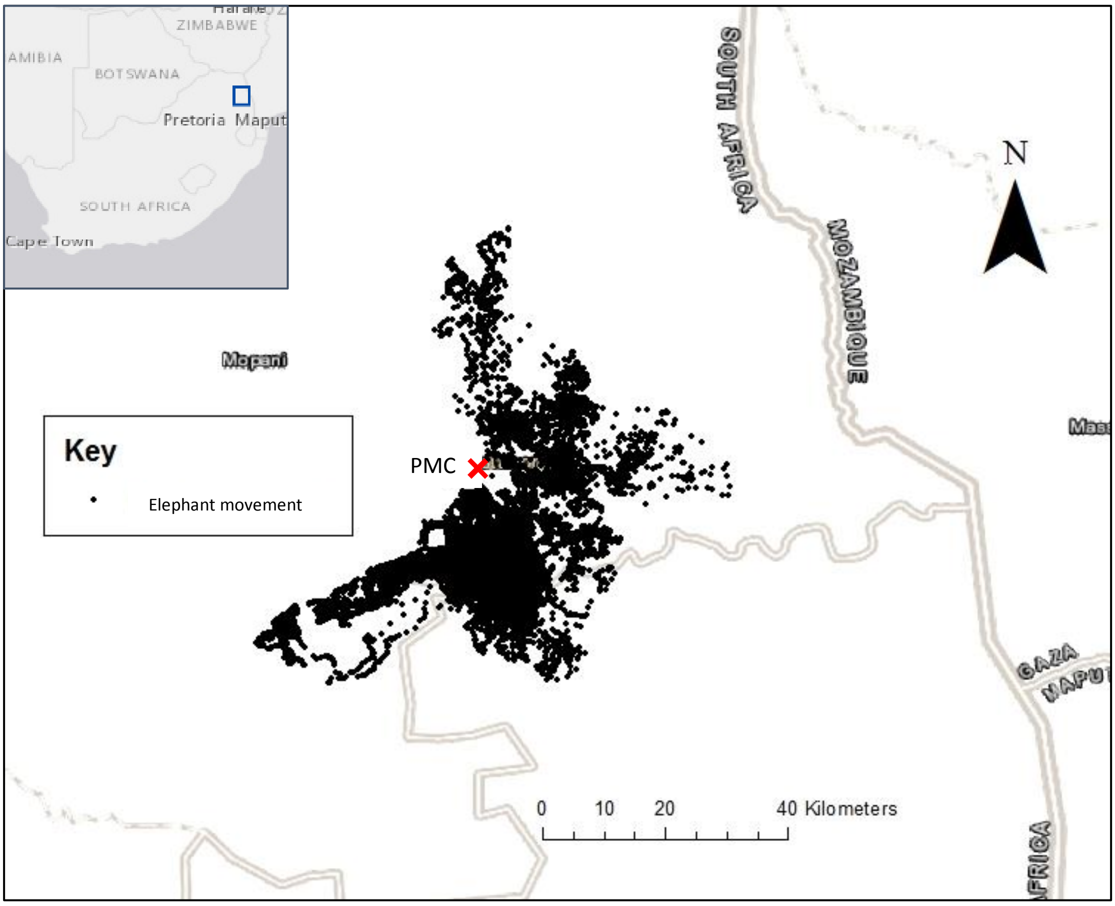


Figure 5.2: Fixes of collared elephants surrounding the Palabora Mining Company (PMC) site between 15.6.2012 and 23.7.2017

Table 5.1: Home ranges of elephants within the Palabora Mining Company (PMC) land and neighbouring reserves. Full data given in Supplementary Information Table 9

	Average Home Range calculated using LoCoH 90% (km^2)	Standard error of mean	Min/max (km^2)	Number of elephants
PMC	529	78	200/728	7
Neighbouring reserves	1305	265	498/2244	7

Sample Site Selection

Fifty-three sampling sites were selected on six transects radiating out from the PMC, to include points within and outside of the area occupied by the collared elephants at the mine (Fig. 5.3). Transects were used to observe if an elemental gradient from the PMC was present. Additionally, 43 sampling sites were identified within the PMC (Fig. 5.3). Sample sites were not selected to the north west of the PMC area; this is a fenced urban area with minimal elephant movement.

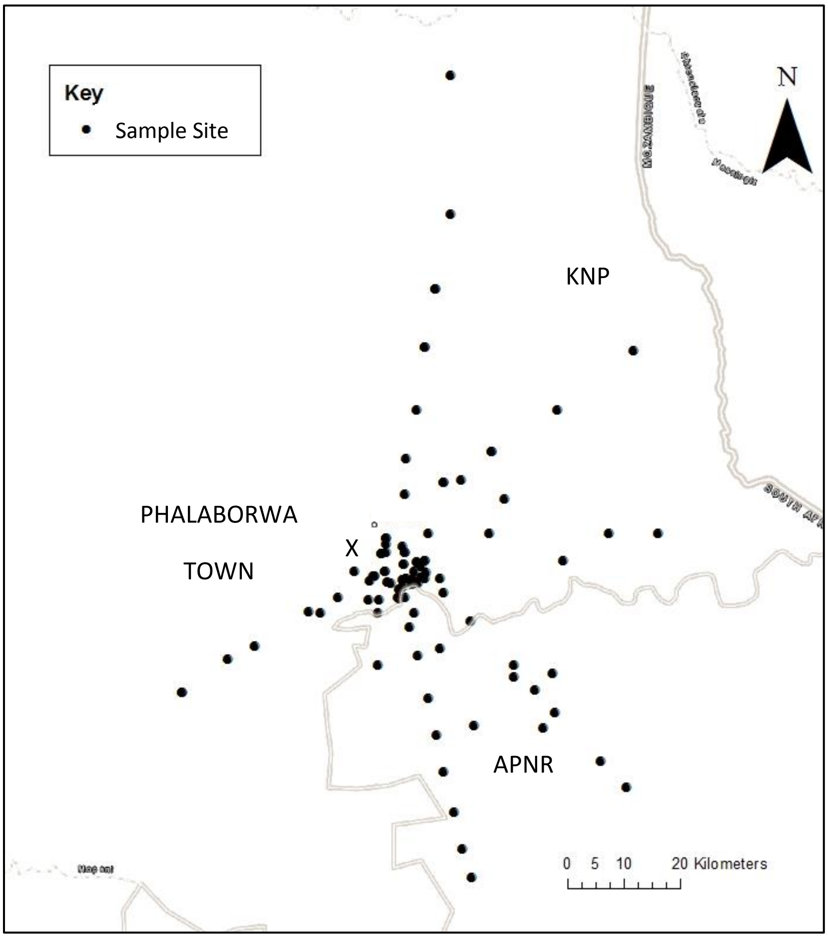


Figure 5.3: Sampling sites for environmental and faecal samples. KNP=Kruger National Park; APNR=Associated Private Nature Reserves. The PMC (Palabora Mining Company) is located where transects cross, south of Phalaborwa town

Sample Collection

Environmental and faecal sampling was conducted during September 2017 and September 2018, within a 50 m radius around each sampling point. Tail hairs were sampled between March 2002 and July 2018. Trace element free paper bags were used for plant, soil, faecal and tail hair samples. All samples were transported to the lab within 8 h of collection; plant and water samples in a cooler, tail hair, faecal and soil samples at ambient temperature.

Environmental sampling

Plant parts (approx. 500g per sample, n=100) were sampled from seven species commonly consumed by elephants (Table 5.2; Smallei and O'Connor, 2000; Holdø, Dudley and McDowell, 2002; Codron *et al.*, 2006; Pretorius *et al.*, 2011, M Henly pers. comm 2017). Not every species or part was found at the sampling site. Samples were taken randomly from the plant, mixed sized leaves were sampled, branches/ roots of approx. 5cm in length were cut using secateurs and bark was scraped off the trunk using a chisel.

Table 5.2: Plant species and plant parts sampled within this study

Species	Common name	Part sampled
Colophospermum mopane	Mopane	Leaves
Grewia monticola	Silver Raisin	Bark
		Leaves
Senegalia nigrescens	Knob Thorn	Bark
Combretum apiculatum	Red Bushwillow	Roots
Lannea schweinfurthii	False Marula	Leaves
		Inner bark
Dichrostachys cinerea	Sicklebush	Branches
		Leaves
Maerua parvifolia	Dwarf Bush-cherry	Branches
		Bark
		Leaves

Soil samples (n=97, approx. 500g per sample) were collected at each site, from surface soil using a trowel, to a depth of 15cm, from five separate points within a 1-m^2 grid. Water was sampled opportunistically at sample sites, key rivers or identified elephant drinking points (n=36); two 30 ml samples were collected in Nalgene HDPE bottles, filtered (0.45 µm).

Biological Sampling

Elephant faecal samples (n=94, approx. 500g per sample) were taken from the centre of fresh, intact boluses with circumference of >40cm, to indicate adult size (Jachmann and Bell, 1985), as calf samples on a pre-weaned diet could bias results (Cook *et al.*, 1994). On return to the laboratory, samples were oven dried at 50 ^{o}C for 24 h.

Tail hair samples were plucked from the tail (1-3 hairs per animal) between March 2002 and July 2018 during routine collaring operations or management activities throughout the KNP, APNR and PMC, as part of the South African National Parks Bio-bank (SANParks), or by EA. Tail hair samples were taken up to 170km from the PMC (n=200 from non-mine collected by SANParks and EA, n=7 from mine area collected by EA). All sedations were carried out using the SANParks SOPs for Capture Transport (*Standard Operating Procedures for Capture Transport and maintenance in Holding Facilities of Wildlife*, 2017).

Sample Preparation

Soil samples were air-dried, crushed and sieved to ≤2mm particle size and further milled to ≤40µm in an agate ball mill. Water samples were filtered with a hydrophilic 25mm Minisart filter and acidified to 1% HNO_3 and 0.5% HCl. Plant and faecal samples were oven dried at 50 ^{o}C for 24 h, and passed through a food blender as described by Watts et al. (2019). Elephant tail hair samples were cleaned as described in Middleton et al. (2016) and autoclaved in line with DEFRA requirements.

Sample Digestion for ICP-MS Analysis

Soil samples (0.25 g) were digested in a mixed acid solution (HF: 2.5 ml/HNO_3:2 ml/$HClO_4$:1 ml/H_2O_2:2.5 ml) on a programmable hot block; 0.5 g of plant samples or faecal samples were digested in HNO_3:10 ml/H_2O_2:1 ml mixed solution in a closed vessel microwave heating system (MARS Xpress) as described in Watts et al. (2019). Elephant tail hair samples (variable weight) were digested in HNO_3:4 ml/H_2O_2:1 ml mixed solution in a closed vessel microwave heating system (MARS Xpress) as described in Middleton et al. (2016). Tail hairs from the non-mine elephants were digested and analysed whole, and those from the collared elephants at the PMC were cut into 3–5 cm sections, down the length of the hair, for future profiling, prior to digestion and subsequent analysis. Soil, plant, faecal material and tail hair data is presented as dry weight.

Elemental Analysis

Elemental analysis was conducted on all prepared samples by inductively coupled plasma mass spectrometry (ICP-QQQ; Agilent 8900x) using collision cell mode (gas modes: H_2 for Se, O_2 for As, He for all remaining elements). Fifteen biologically functional elements were selected for this study; Ca, copper (Cu), iron (Fe), potassium (K), Mg, manganese (Mn), Na, P, selenium (Se), zinc (Zn), arsenic (As), cadmium (Cd), lead (Pb), uranium (U) and vanadium (V). Sample blanks were run to determine the practical limit of detection (LOD, 3*STDEV).

Analytical Quality Control

The accuracy of the elemental analysis was verified by analysing the following certified reference materials (CRM)s:

- Human Hair (GBW07601, China)
- Spinach leaves (SRM1570a, NIST, USA)
- Tomato leaves (SRM1573a, NIST, USA)
- Basalt rock (BCR-2 United States Geological Survey, USA)
- Soil (SRM2711a, NIST, USA)

- Soil (BGS 102, British Geological Survey, UK)
- In house human toenail (BAPS 2014) reference material

The concentrations of all elements of interest in the reference materials had an acceptable accuracy to the target values, of 97% ± 39%, data detailed in Supplementary Information Table 3.

Statistical Analysis

The evidence for differences between mine and non-mine elephant home range size was assessed by a Wilcoxon-test of the null hypothesis that the median home range size value was the same for the collared mine and non-mine elephants.

The evidence for differences between mine and non-mine elephant tail hair and faecal samples with respect to analytes was assessed by a Student's t-test of the null hypothesis that the mean value was the same for samples from the mine and non-mine. Boundaries to define the mine and non-mine were based on physical land ownership. The t-test was performed assuming that the variances within the two groups were not necessarily the same, and computing effective degrees of freedom for the resulting t-statistic according to the Satterthwaite-Welch equation (Welch, 1947). This is a conservative approach when, as here, the sample sizes are unequal.

Each family of tests (t-tests on one matrix for the set of minerals, or tests of trend models for some environmental matrix on the set of minerals) can be regarded as a multiple hypothesis testing exercise, because each mineral was not considered in turn, but rather examined for evidence that specific minerals display behaviour of interest. For that reason we undertook false discovery rate control (FDR) following Benjamini and Hochberg (1995).

The FDR is the expected proportion of rejected null hypotheses that should have been accepted. Here we controlled the FDR at 0.05, computing adjusted P-values for each family of tests using the p.adjust command in the base statistical library of the R package (R Core team, 2017).

The environmental data, on soil, water and plants, were examined for evidence that there is a dependency of the measured concentration on distance from the mine. This was done using a polynomial function of distance. For plants, leaves only were used to demonstrate spatial variation, a full dataset and comparison for the plant data (all plants versus leaves) is in Supplementary Information Table 4. The data on soil, water and plants were collected from transect points radiating from the mine, sampling at more or less regular intervals. Because the samples are not collected from sites selected independently and at random, it is not possible to make sound inferences based on standard ordinary least squares methods (Lark and Cullis, 2004). Rather, it is necessary to fit a linear mixed model (LMM) to the data, with the fixed effects comprising polynomial terms in distance to the mine, and the random effect comprising both an independent and identically distributed error term and a spatially correlated random effect. The models were fitted using the lme and update functions from the nlme library for the R platform (R Core Team, 2017; Pinheiro *et al.*, 2018).

A quartic polynomial (first, second, third and fourth order terms) in distance was initially fitted to the data by ordinary least squares, and summary statistics and the histogram of the residuals were examined to decide whether to analyse the data on their original units or after transformation to natural logarithms. The full model was then fitted as a LMM using residual maximum likelihood (REML), and models with spherical and exponential correlation functions for the spatially-dependent random effect were compared on their likelihood. The selected spatial correlation function was then retained for all further models for this variable on the matrix being considered. The full quartic model was then re-estimated using ordinary maximum likelihood to allow comparisons with alternative models with different fixed effects. A cubic model was then fitted (i.e., dropping the quartic term), and the quartic and cubic models were compared on the log-likelihood ratio statistic to test the null hypothesis that the coefficient for the quartic term was zero. If this null hypothesis was rejected then the full model was retained and compared with a null model in which the only fixed effect was a constant mean. This latter test was recorded as the strength of evidence for a trend with distance to the mine. If, on the other hand, the null hypothesis was accepted, then the quartic term was dropped and the cubic model compared with a quadratic, and so on.

As with the comparisons between the mine and non-mine areas by the t-test, each set of spatial models over all elements on a particular matrix was treated as a family of multiple hypotheses to be tested with FDR control. The same method was used to do this as described above for the t-tests.

One data point furthest from the mine was removed, because of the considerable leverage that this could have on a trend model. It was also necessary to "jitter" some of the spatial coordinates, moving them 1 metre in a random direction. This is because, although none of the environmental samples on any matrix were actually from the same location, the GPS coordinates were duplicated as GPS readings are only precise within 6 m. It was one observation out of any such pair that was "jittered" in this way using the jitterDupCoords function from the geoR package in R (Ribeiro and Diggle, 2018).

Results

Home Range Size

The null hypothesis that the mean home range size was the same for the mine and non-mine areas could be rejected. The Wilcoxon test showed a significant difference between mine and paired conspecifics outside of the mine (P= 0.001; Table 5.1; Supplementary Information Table 9).

Environmental Samples

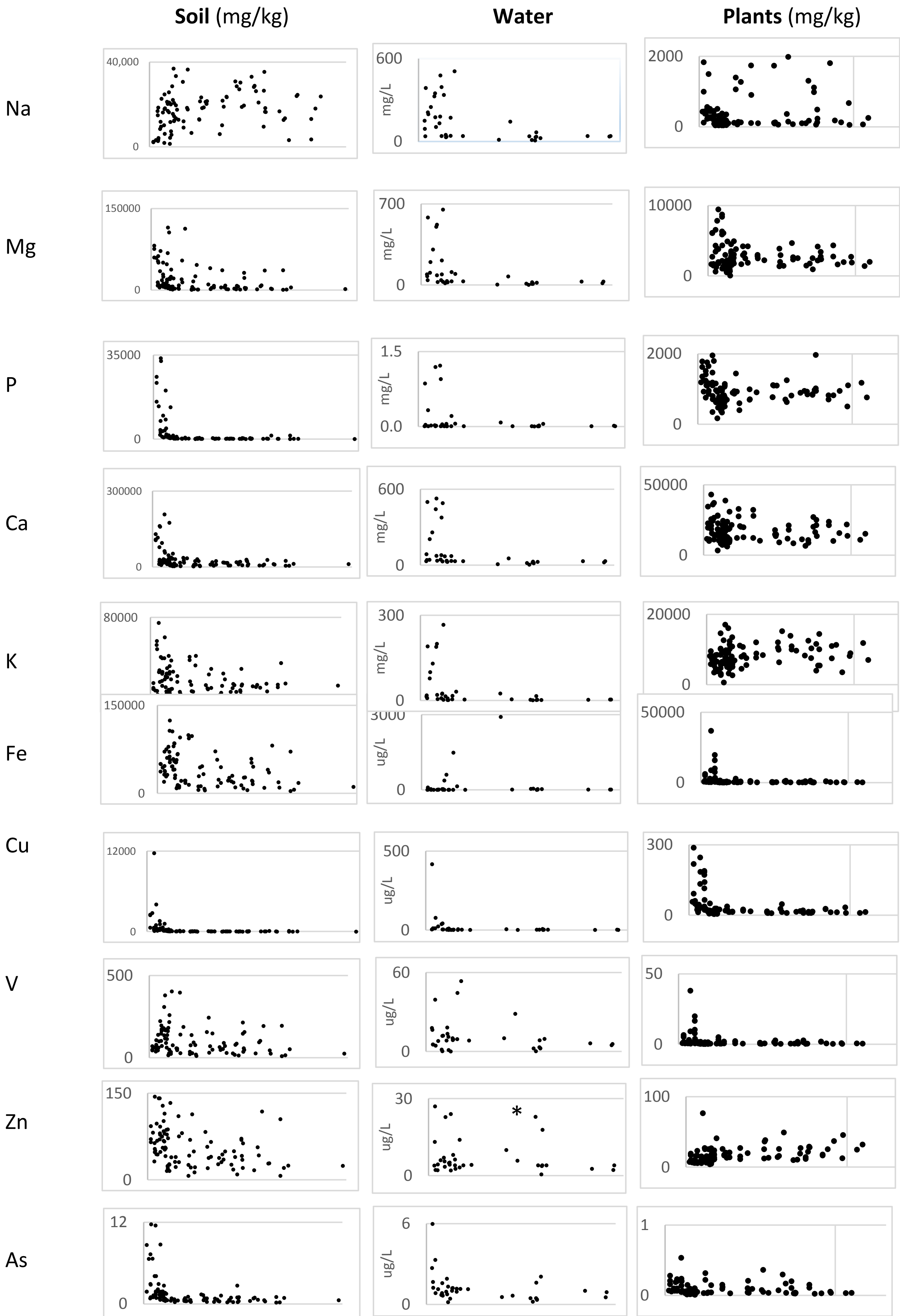
With FDR control at 0.05, the null hypothesis of no spatial trend in concentration in the soil with distance from the mine could be rejected for all investigated elements. Inspection of the trend model shows that in all cases concentrations decline with distance from the mine (Fig. 5.4; Table 5.3; Supplementary Information Table 3 and 8). With FDR control at 0.05, the null hypothesis of no spatial trend in concentration in the water with distance from the mine could be rejected for four investigated elements (Ca, K, Fe and Cu), with concentrations declining with distance (Fig. 5.4; Table 5.3; Supplementary Information Table 5 and 8). With FDR control at 0.05, the null hypothesis of no spatial trend in concentration in plants (leaf samples) with distance from the mine could be rejected for nine investigated elements (P, Mg, Mn, Fe, Cu, Zn, Se, Cd, and U), with concentrations declining with distance (Fig. 5.4; Table 5.3; Supplementary Information Table 4 and 8).

Elephant Biomarkers

With FDR control at 0.05, the null hypothesis of no difference between the mine and non-mine faecal samples could be rejected for Mg, P, Cu, As, Cd, Pb and U (larger concentrations in the elephants near the mine) and Na, Mn, Zn and Se (smaller concentrations in the elephants near the mine; P<0.05; Figure 5.5; Supplementary Information Table 7; 8).

With FDR control at 0.05, the null hypothesis of no difference between the mine and non-mine tail hair samples could be rejected for Cd (larger concentrations in the elephants near the mine) and K and Se (smaller concentrations in the elephants near the mine; $P<0.05$; Figure 5.5; Supplementary Information Table 6; 8).

Environmental Data



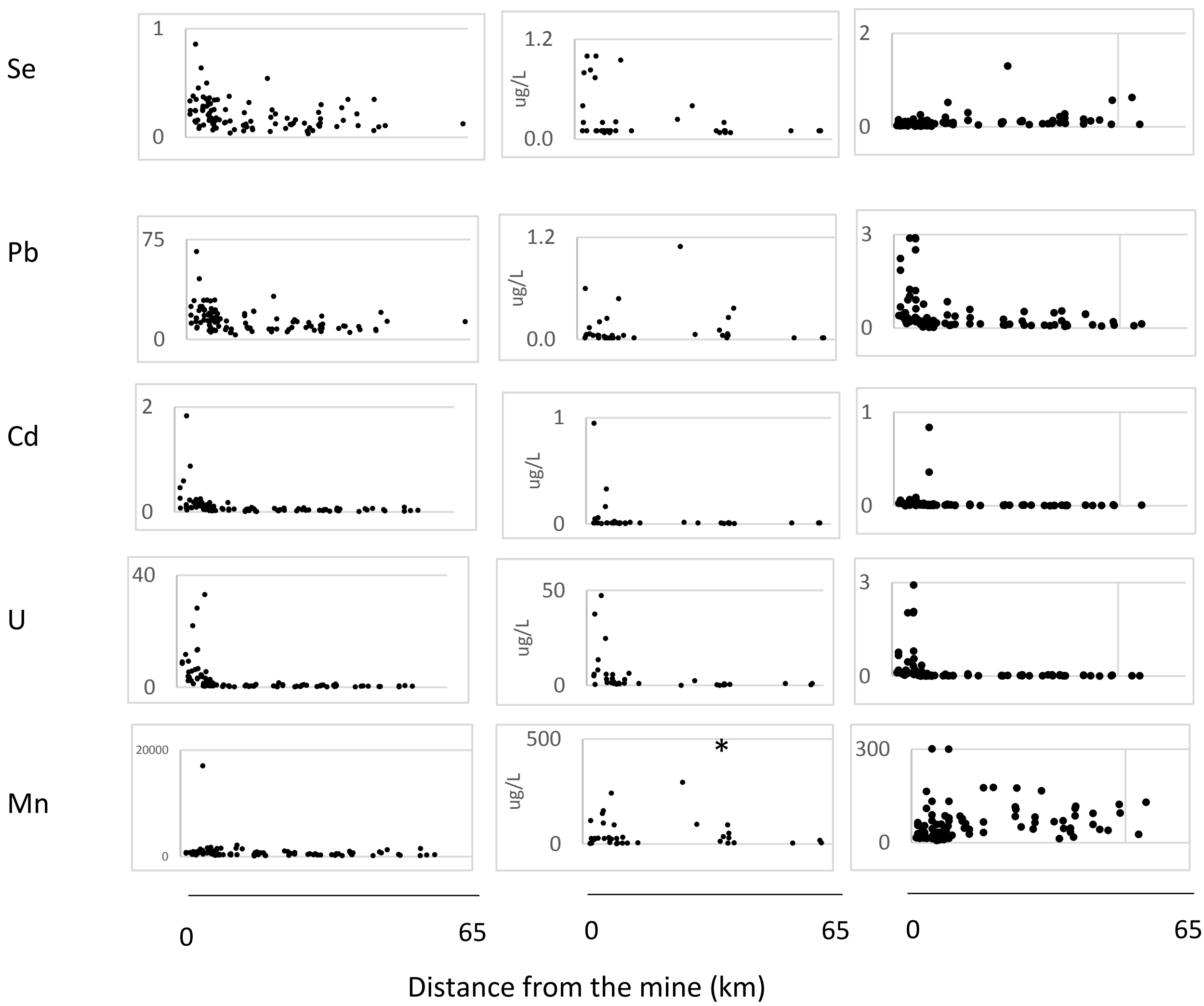


Figure 5.4: Overview of elemental analysis of environmental samples (y-axis), against distance from the mine (x-axis). Plant data=median of all samples collected (leaves, twigs and branches).

Table 5.3: Results of linear mixed model to show significant differences in soil, water and plant (leaf) concentrations as distance from the mine increased. P-value (<0.05) and adjusted P-values to control the false discovery rate (FDR) reported.

	Soil		Water		Plants/ leaves	
Element	P-value	Adjusted P-value	P-value	Adjusted P-value	P-value	Adjusted P-value
Ca	**0.05**	**0.043**	0.42	**0.038**	0.336	0.360
l ratio	5.84		0.65		0.926	
number	2		1		1	
P	**<.0001**	**0.000**	**0.03**	0.060	**<.0001**	**0.005**
l ratio	40.27		4.82		19.52	
number	4		1		4	
Mg	**<.0001**	**0.000**	**0.02**	0.075	**<.0001**	**0.005**
l ratio	31.88		5.42		68.08	
number	4		1		2	
Na	**0.04**	**0.050**	**0.01**	0.525	0.5317	0.532
l ratio	4.44		6.05		1.26	
number	1		1		2	
K	**0.04**	**0.043**	**0.01**	**0.038**	0.33	0.360
l ratio	4.44		7.88		0.93	
number	1		1		1	
V	**0.002**	**0.003**	0.31	0.423	0.10	0.136
l ratio	9.18		1.02		4.60	
number	1		1		2	
Mn	**0.001**	**0.002**	0.60	0.692	**<0.0001**	**0.020**
l ratio	10.47		1.86		6.9662	
number	1		3		1	
Fe	**0.0003**	**0.001**	**<.0001**	**0.000**	**0.03**	**0.050**
l ratio	12.94		42.50		6.77	
number	1		1		2	
Cu	**0.009**	**0.012**	**0.01**	**0.038**	**0.01**	**0.021**
l ratio	9.42		8.78		8.68	
number	2		2		2	
Zn	**0.02**	**0.025**	0.96	0.960	**0.003**	**0.009**
l ratio	7.96		0.00		8.78	
number	1		2		1	
As	**<.0001**	**0.000**	0.16	0.267	0.30	0.360
l ratio	47.96		1.95		3.66	
number	2		1		3	
Se	**0.00**	**0.008**	0.11	0.206	**0.02**	**0.038**
l ratio	9.60		2.51		5.65	
number	1		1		1	
Cd	**<.0001**	**0.000**	0.08	0.171	**<0.0001**	**<0.0001**
l ratio	51.20		0.08		25.54455	
number	2		1		1	
Pb	**<.0001**	**0.000**	0.77	0.825	**0.04**	0.060
l ratio	114.46		0.08		6.24	
number	1		1		2	
U	**0.002**	**0.003**	0.26	0.390	**0.0027**	**0.009**
l ratio	14.44		1.28		11.84	
number	3		1		2	

Biological Data

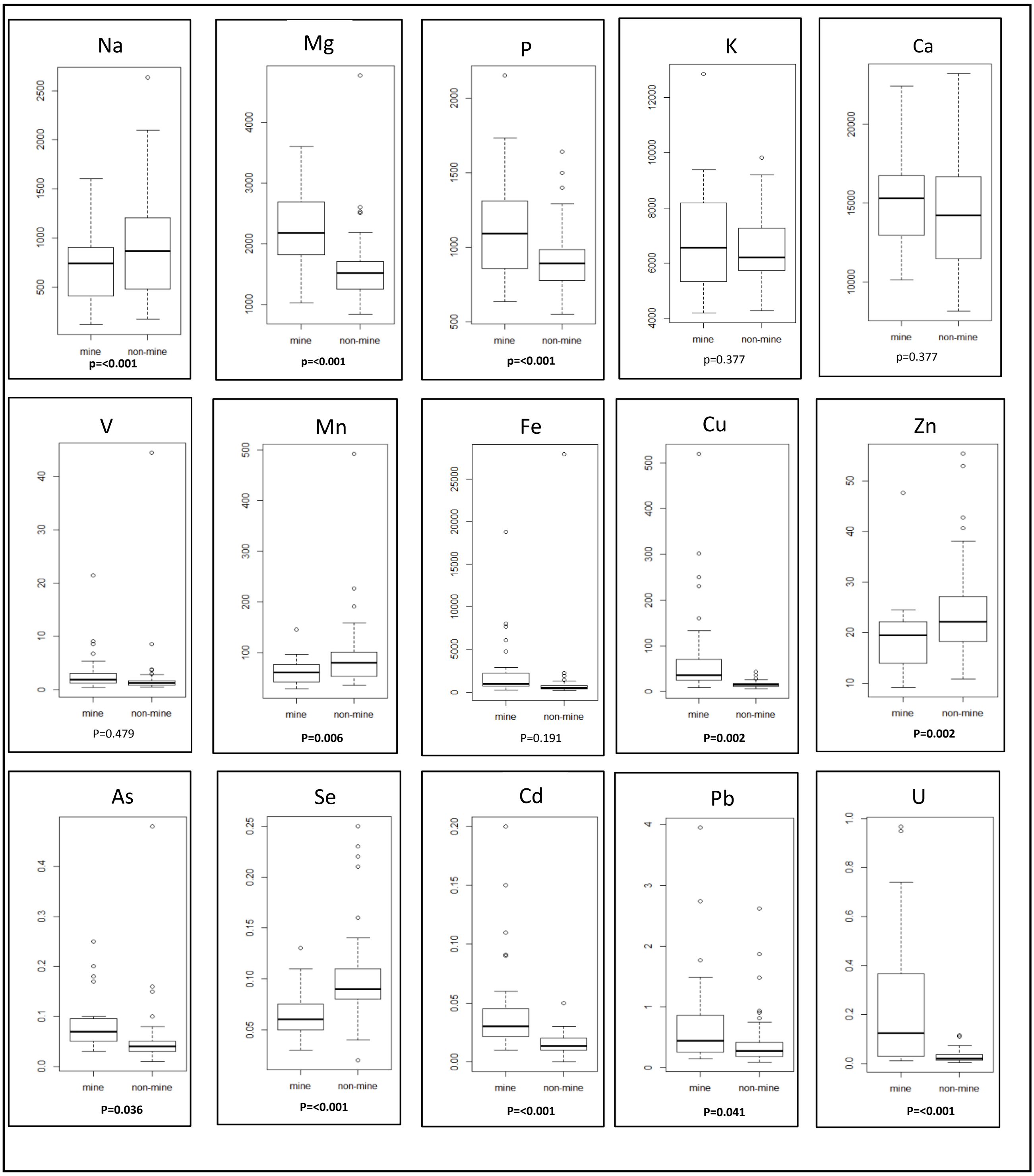


Figure 5.5: Elemental analysis data (y-axis, mg/kg) for faecal samples. Box plots show median, Q2, Q3, max and min. Outliers are defined as 1.5*IQR. Adjusted P-values are reported to control for false discovery ($p<0.05$). For mine samples n =37, non-mine n=57

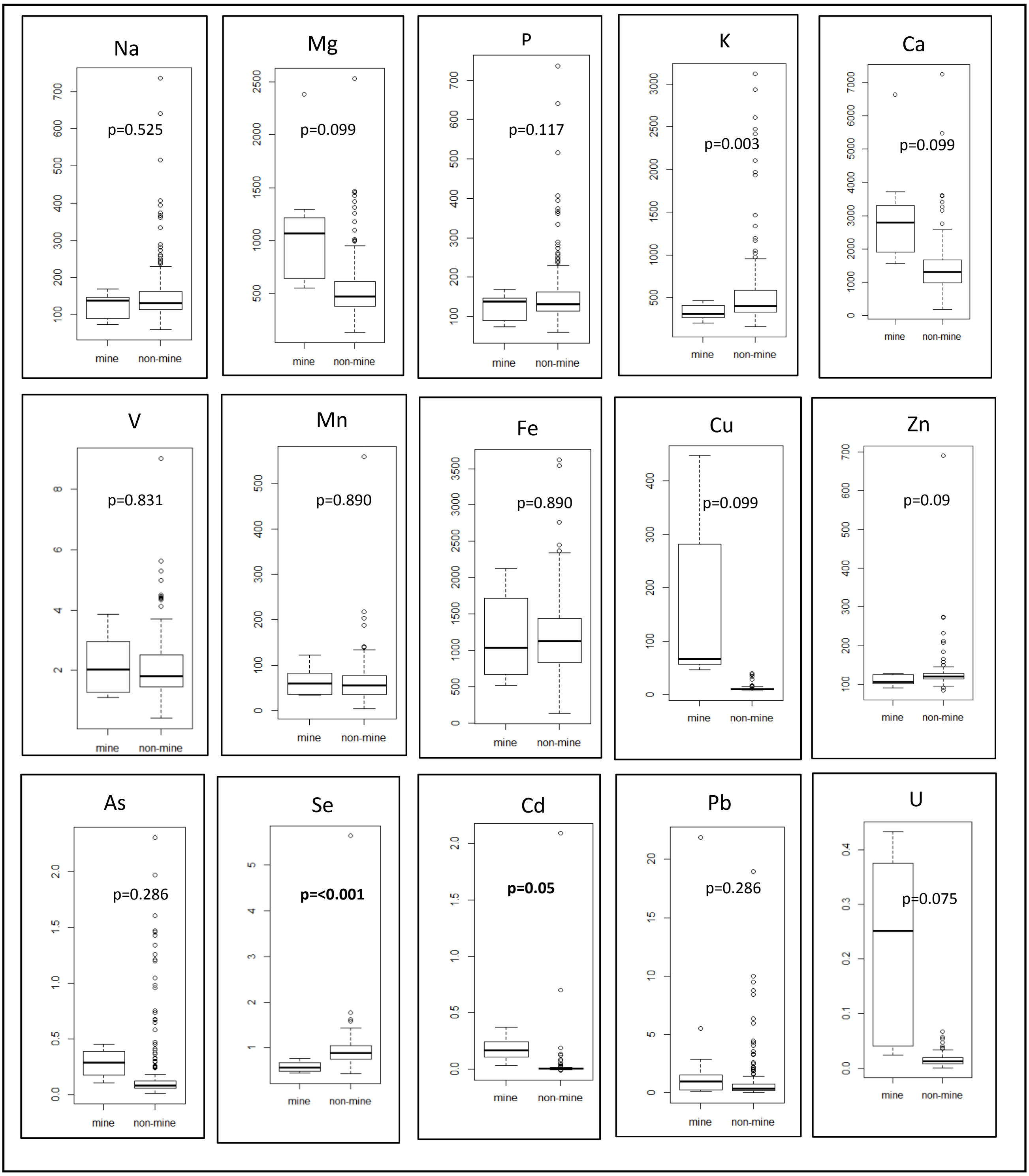


Figure 5.6: Elemental analysis data (y-axis mg/kg) from tail hair samples from mine and non-mine elephants, y axis=element concentration (mg/kg). Box plots show median, Q2, Q3, max and min. Outliers are defined as 1.5*IQR. Adjusted P-values are reported to control for false discovery (p<0.05). For mine samples n=7, for non-mine samples n=200

Discussion

Mineral provision at the PMC was greater than the surrounding areas (Table 5.3; Fig.5. 4). Home ranges of the mine elephants were significantly smaller (59% P=0.001) than those in the surrounding areas (Fig. 5.2; Table 5.1), suggesting that their resource needs, including minerals, were met within this smaller area, close to the PMC (Tucker *et al.*, 2018). A trade-off is likely whereby elephants consume soil and water (or plants) near the PMC to obtain increased levels of Ca, Mg, P, Cu, Zn and Se but also consume PTEs (Pb, U and V). Selenium and Zn are fertility augmenters, benefiting elephants in early life (Hidiroglou and Knipfel, 1984; Mistry *et al.*, 2012), whereas the effects on fertility from consuming PTE's to toxic levels may take decades to realise, having a lesser effect on total reproductive output (Kincaid, 1999). An evolutionary advantage may be gained in consuming increased micronutrients at the PMC, at the cost of the increased consumption of PTEs. High consumption of macro-minerals (seen in plants) are under homeostatic control within the elephant, and thus the elephant can buffer increased intake (Kincaid, 1999).

Biological Samples

Tail hair reflects up to 18 months residence, whereas faecal material reflects short-term dietary intake (Bencko, 1995; Wittemyer, Cerling and Douglas-Hamilton, 2009). The differences in tissue biomarkers indicated that short-term environmental differences in availability of minerals consumed by the elephants, appeared to be reflected directly by faecal samples. Whereas, the tail hair data suggested that the elephants moved to obtain required minerals over time, thereby not showing significant differences in as many elements, between mine and non-mine samples (11 of 15 elements in faecal material versus 3 of 15 elements in tail hairs). Such temporal variability must be considered in evaluating the use of biomarkers for assessing nutrient status/habitat quality.

This study covers the widest range of minerals and PTE analysis in elephant faeces to date (n=97; Fig. 5.5; Supplementary Information Table 7). In Hwange National Park, Zimbabwe, Mg, Na and K data were similar to concentrations found in this study (Holdø, Dudley and McDowell, 2002). However, in this study, faecal Ca concentrations, both from mine and non-mine samples were substantially larger than reported by Holdø et al. (2002) with the minimum and maximum level in this study being 8,100 and 23,100 mg/kg DM, respectively, versus 920 and 12,000 mg/kg DM. Additionally, in the APNR, Greyling (2004) reported similar P levels in faecal samples (median 1100 versus 990 mg/kg DM in this study). Faecal samples reflect Ca intake (Sach et al., 2020), therefore increased Ca levels found in in this study could be attributed to increased intake.

Faecal samples may not represent a specific location or plant consumed; elephants have a total gut transit time of 11–46 h (Clauss *et al.*, 2003) and walk over 22 km daily (Thomas, Holland and Minot, 2012). Faecal samples were a reliable indicator of Ca, P, Se, Cu and As intake (Sach et al., 2020) and thus a proxy for elemental status. Significantly greater levels of faecal P, Cu and As were seen in mine samples compared to non-mine samples, indicating that intake of these elements were greater in mine versus non mine. This is also seen in soil, in Cu in water and P and Cu in plants (leaves), supporting this increased intake. Additionally, elephants are frequently documented to participate in geophagy (Holdø, Dudley and McDowell, 2002), and although not specifically reported at PMC, could be in part obtaining these increased elemental levels via geophagy.

This study provides the largest multi-element dataset on mineral and PTE analysis data in elephant tail hair (Fig. 5.6; Supplementary Information Table 6). Hair analysis is routinely used in humans and livestock to assess Se and As levels (Bencko, 1995; Middleton *et al.*, 2016). Duer, Tomasi and Abramson, (2016) analysed an elephant tail hair from a deceased healthy individual from Tsavo National Park, Kenya and reported 11 elements for which concentrations were comparable to the non-mine elephants within this study. However, levels of Mg, Ca, Mn, Cu and Pb in the mine elephant tail hairs were considerably greater than those reported by Duer, Tomasi and Abramson (2016).

Environmental Samples

This study agrees with work reported by Ramahlo (2013) within the Phalaborwa region, regarding the impact of mining on soil at surrounding farms, where P, As and Pb levels in soil decreased with increasing distance from the mine. African soils contain high levels of Fe (Siyame *et al.*, 2013), and thus a significant difference between mine/ non-mine faecal or tail hair samples may not be seen (Fig. 5.5 and 5.6), as all animals may be consuming to excess. Studies demonstrated elephants selectively drank water with elevated mineral levels; notably Na, iodine (I), sulphur (S), Zn, Ca, Mg, Mn and Fe (Weir, 1972; Sienne, Buchwald and Wittemyer, 2014). Additionally, elephants may spend more time at the PMC site during the dry season, either due to mineral deficiencies in natural forage being heightened in the dry season, or simply for increased water availability within the PMC area (Purdon and van Aarde, 2017).

Elemental analysis of plant samples do not always reflect soil due to a variety of factors including soil pH, organic matter and differences in the capacity of individual plant species to accumulate certain elements (Bowell and Ansah, 1994; Maskall and Thornton, 1996). In the Sabi Sands Reserve, South Africa, grasses were analysed from soils of higher mineral levels, yet they accumulated less minerals compared to grasses from soils where the minerals were lower (Ben-Shahar & Coe, 1992), due to differences in soil-to-plant transfer between plant species and the effect of the local micro-climate. Similarly, this variation in soil-to-plant transfer was reflected in this study for Ca, Na, K, V, As and Pb (Fig. 5.4; Table 5.3). These elements decreased significantly in soil with distance from the mine, although plants did not follow the same trend. The igneous Phalaborwa apatite would be expected to have low reactivity (i.e. low solubility), hence the elements in the soil may be less available for uptake by plants (Appleton, 2002). Finally high soil Fe, typical of African soils, could also reduce the availability of P to animals via plants, thus the increase in soil P may not be reflected within mine tail hair samples (Fordyce, Masara and Appleton, 1996).

Conclusion

The home ranges of the collared PMC elephants are considerably smaller (59% smaller) than elephants in surrounding areas, implying that their resource needs are being met within this relatively compact area. Drivers for elephant movement are multifactorial, yet evidence suggests that these key differences in the geochemistry of the mine compared to the surrounding areas, could act as a driver for elephant movement, resulting in reduced home range size compared to other elephants within this geographical region. Mineral provision to the elephants at the PMC is significantly greater than in surrounding areas, seen most significantly in the soil where all investigated mineral and PTE levels decreased significantly with increasing distance from the mine. These differences suggest that elephants are attracted to this micronutrient hotspot at the PMC, to obtain required minerals.

The increased mineral provision and trade off of increased PTE levels were reflected in biological samples of elephant tail hair and faeces. Baseline levels of key minerals and PTEs in African savanna elephant tail hair and faeces were established from this work. The methods described within this natural experiment to investigate how environmental geochemistry influences elephant home range size and potentially movement, facilitates the consideration of intervention to reduce associated HECs at the PMC. This approach could be applied to similar situations, with wider benefits to a variety of stakeholders, informing broader conservation efforts.

CRediT Author Statement

FS, MW, LY, SLE, ED, MH and AS conceptualization and methodology; FS and AB data curation; FS, MH, AG, EH and PB investigation; MW, MH, AS and PB resources; FS and RML formal analysis; FS writing original draft. All authors contributed critically to drafts and final approval for publication.

Declaration of Interest

Ellen Dierenfeld is employed by Ellen Dierenfeld Consulting LLC

No other declarations of interest

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- Funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

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Appendix 5

Additional data produced from this research not included within the manuscript:

- Toenail data from Elephants Alive (EA) from 25 elephants sampled within the Associated Private Nature Reserves (APNR), Table A1

- Plasma data from South African National Parks (SANParks) BioBank from 293 elephants sampled within the Kruger National Park (KNP), sampling locations detailed in Figure A1, data presented in Table A2.

Table A1: Elemental analysis of toenail samples from 25 elephants, collected by Elephants Alive within the Associated Private Nature Reserves (APNR)

Sample Name	**Na**	**Mg**	**P**	**K**	**Ca**	**V**	**Mn**	**Fe**	**Cu**	**Zn**	**Se**	**Pb**	**U**	**As**	**Cd**
14313 0001-0025 (lab ID number)	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Khumo ave	247	529	80	602	1448	3.61	33.6	1628	3.76	43.1	0.221	1.675	0.039	0.104	0.003
Nthaba	142	265	66	281	484	1.25	13.4	669	1.79	47.4	0.238	4.346	0.020	0.063	0.002
General	390	1591	76	459	1600	2.18	38.9	828	5.35	75.5	0.294	0.739	0.011	0.067	0.104
Lotter	268	208	69	376	814	1.15	9.0	470	1.92	57.2	0.220	5.366	0.005	0.092	0.003
Draco	327	390	94	394	1	2.43	26.0	1070	3.32	65.7	0.342	0.421	0.020	0.077	0.002
2010-3033	143	564	58	427	639	0.77	6.6	334	1.44	50.3	0.261	0.203	0.006	0.036	0.002
Crystal	149	243	100	340	1027	1.16	17.0	540	2.08	65.9	0.395	0.165	0.006	0.036	0.002
Lekotla	271	973	105	960	2172	4.60	74.4	2335	4.90	35.3	0.231	0.593	0.040	0.186	0.020
Intwandamela	137	193	64	158	694	0.71	13.1	450	1.3	61.4	0.39	0.171	0.005	0.06	0.002
Wessa	143	649	125	718	1432	4.49	55.4	2125	12.74	52.6	0.372	0.928	0.072	0.083	0.008
Classic ave	598	1747	40	890	5346	1.06	28.8	352	1.36	33.6	0.270	0.601	0.009	0.037	0.002
2010-3035 ave	175	330	64	514	1062	1.47	15.9	691	2.19	47.0	0.164	0.232	0.013	0.037	0.004
RiffRaff	116	774	115	1317	2053	7.50	61.1	2775	5.68	59.7	0.195	0.519	0.045	0.245	0.013
2010-3037	228	193	194	241	905	0.37	4.8	176	1.79	82.4	0.236	0.378	0.002	0.017	0.002
Charlie	265	278	59	348	967	1.49	11.3	610	2.32	42.2	0.234	0.709	0.005	0.040	0.002
Bambuti	181	281	54	243	1033	0.50	5.8	296	1.93	74.6	0.373	4.883	0.004	0.014	0.000
Bell	181	445	82	552	883	4.25	36.9	1886	4.25	51.6	0.202	0.543	0.039	0.117	0.009
Bacha	271	318	88	441	1048	1.79	20.1	910	2.57	39.4	0.204	0.405	0.017	0.054	0.002
Induna ave	208	286	53	419	741	1.75	17.6	729	2.46	29.3	0.332	1.291	0.014	0.082	0.002
Derek	113	178	64	302	682	0.70	6.7	314	1.37	44.4	0.206	1.970	0.003	0.080	0.002
Lapajuma	507	1376	34	1805	2963	1.14	16.9	482	2.85	27.4	0.186	2.474	0.009	0.094	0.004
Elon	137	313	69	378	884	1.47	14.1	610	2.53	54.5	0.266	1.319	0.012	0.064	0.009
Dex	122	186	65	241	621	0.74	7.0	373	1.78	65.7	0.175	1.031	0.007	0.021	0.001
Wayne	167	192	102	285	996	0.66	5.6	261	1.69	65.1	0.196	1.545	0.004	0.051	0.001
George	141	467	74	198	1021	1.77	18.9	861	5.39	68.4	0.246	0.178	0.018	0.147	0.008

	Na	Mg	P	K	Ca	V	Mn	Fe	Cu	Zn	Se	Pb	U	As	Cd
DL this data set	**<17**	**<2**	**<8**	**<18**	**<324**	**<0.04**	**<0.3**	**<3**	**<0.07**	**<0.6**	**<0.009**	**<0.009**	**<0.001**	**<0.007**	**<0.003**
mean	225	519	80	516	1261	1.96	22.4	871	3.15	53.6	0.258	1.307	0.017	0.076	0.008
median	247	529	80	602	1448	3.61	33.6	1628	3.76	43.1	0.221	1.675	0.039	0.104	0.003
stdev	122	446	33	378	1044	1.69	18.5	711	2.42	14.8	0.070	1.476	0.017	0.053	0.021
min	113	178	34	158	1	0.37	4.8	176	1.34	27.4	0.164	0.165	0.002	0.014	-0.002
max	598	1747	194	1805	5346	7.50	74.4	2775	12.74	82.4	0.395	5.366	0.072	0.245	0.104
q1	142	243	64	285	741	0.77	9.0	373	1.79	43.1	0.204	0.405	0.005	0.037	0.001
q3	268	564	94	552	1432	2.18	28.8	910	3.76	65.7	0.294	1.545	0.020	0.092	0.008
IQR	126	321	30	267	691	1	20	538	2	23	0	1	0	0	0
difference between mean and med	-22	-11	-1	-87	-187	-2	-11	-758	-1	10	0	0	0	0	0
% diff	-10	-2	-1	-17	-15	-84	-50	-87	-19	19	14	-28	-130	-37	59

CRMs

GBW07601	164	355	117	15	2989	0.19	6.1	39	10.07	190.9	0.534	8.851	0.056	0.215	0.111
GBW07601	169	365	146	10	3056	0.21	6.0	40	10.26	197.9	0.555	8.975	0.059	0.231	0.123
average	166	360	131	13	3022	0.20	6.1	39	10.16	194.4	0.545	8.913	0.057	0.223	0.117
SD	**98**	**207**	**74**	**10**	**1752**	**111.90**	**3.3**	**23**	**5.25**	**108.5**	**6.596**	**10.011**	**0.111**	**18.843**	**17.385**
RSD%	**169**	**174**	**177**	**132**	**173**	**0.18**	**183.5**	**172**	**193.58**	**179.1**	**8.260**	**89.032**	**51.565**	**1.184**	**0.673**
Expected	152	360	170		2900		6.3	54	10.60	190.0	0.600	8.800		0.280	0.110
%Bias	**9**	**0**	**-23**		**4**		**-4**	**-28**	**-4**	**2**	**-9**	**1**		**-20**	**6**
BAPS2014	562	152	291	938	391	0.01	0.3	34	4.34	129.8	0.676	0.189	0.002	0.163	0.003
Expected	502	137	237	1015	778	0.03	0.4	37	4.40	119.5		0.219	0.007	0.093	0.006
% diff	**12**	**11**	**23**	**-8**	**-50**	**-76**	**-20**	**-8**	**-1**	**9**		**-13**	**-69**	**75**	**-45**

Values below the detection limit (DL) are reported as half the DL

Table A2: Elemental analysis data of plasma from the South African National Parks Biobank, from elephants in Kruger National Park

		Na	Mg	P	K	Ca	V	Mn	Fe	Cu	Zn	As	Se	Pb	U	Cd
UKAS ID	*Customer ID*	*mg/l*	*mg/l*	*mg/l*	*mg/l*	*mg/l*	µ/l	µ/l	*mg/l*	µ/l	µ/l	µ/l	µ/l	µ/l	µ/l	µ/l
14296-0001	149	2460	27.8	59.6	165	86.7	0.2	2	0.7	872	901	0.2	163.3	1.4	0.1	0.1
14296-0002	147	2409	26.9	91.0	226	109.4	0.4	13	0.9	796	582	0.6	129.5	1.3	0.4	0.4
14296-0003	148	1533	18.1	47.8	149	70.6	0.0	5	0.5	623	580	0.5	80.7	1.3	0.1	0.1
14296-0004	150	1552	17.7	46.7	146	68.5	0.9	18	0.6	475	317	1.2	90.7	2.2	0.3	0.4
14296-0006	81	2454	27.5	63.3	187	99.7	0.4	8	0.8	848	1077	0.8	123.5	1.1	0.2	0.3
14296-0007	80	3012	37.2	72.3	182	116.4	0.1	1	0.8	857	1231	0.2	134.4	0.7	0.0	0.1
14296-0008	82	1892	17.8	50.2	127	70.5	0.5	12	0.7	533	701	0.7	74.1	1.4	0.3	0.3
14296-0009	83	1167	14.2	32.5	85	49.5	-0.1	4	0.3	434	484	0.5	43.4	2.0	0.2	0.3
14296-0010	17	3336	42.8	107.5	187	119.4	0.2	1	1.0	977	1290	0.2	165.3	0.5	0.0	0.0
14296-0011	15	3001	36.7	93.1	203	112.7	0.1	2	1.2	973	1239	0.3	131.9	0.7	0.0	0.1
14296-0012	128	2104	20.7	61.3	124	83.5	0.4	8	1.9	715	834	0.1	87.4	1.2	0.0	0.1
14296-0013	51	3114	26.3	68.2	190	88.1	0.1	1	0.7	1097	865	1.6	80.5	0.6	0.1	0.1
14296-0014	129	1710	22.0	56.0	114	84.8	0.2	4	0.4	612	589	0.3	42.5	2.4	0.1	0.1
14296-0015	130	1214	12.8	39.0	75	56.0	0.5	20	0.6	387	397	1.6	26.1	1.7	0.5	0.5
14296-0017	52	3068	30.8	99.5	180	101.7	0.0	1	1.0	919	857	0.3	113.8	0.4	0.0	0.0
14296-0018	8	2864	36.7	83.5	171	93.5	0.2	1	1.0	901	956	0.5	53.9	0.6	0.0	0.1
14296-0019	84	1689	18.7	45.2	111	68.4	0.3	1	0.7	608	712	0.4	69.7	1.2	0.1	0.1
14296-0020	9	2738	28.1	65.9	161	95.6	-0.1	0	0.6	917	886	0.5	91.0	0.8	0.0	0.1
14296-0021	10	2481	26.6	80.1	135	104.4	0.2	0	0.6	1076	771	0.7	87.0	0.9	0.0	0.0
14296-0022	48	2711	30.3	52.4	182	96.9	-0.1	0	0.9	836	841	0.4	75.2	0.8	0.0	0.0
14296-0023	73	2447	21.7	66.8	135	71.7	0.2	0	0.8	790	639	0.2	46.9	0.3	0.0	0.0
14296-0025	90	1810	19.4	40.0	98	65.8	0.2	0	0.3	546	739	0.2	36.3	0.9	0.0	0.1
14296-0026	94	1956	18.8	54.5	140	56.6	0.1	0	0.4	584	734	0.1	82.9	0.9	0.0	0.1
14296-0029	96	2427	28.1	57.5	213	88.5	0.2	2	0.3	740	875	0.0	88.9	1.6	0.0	0.0
14296-0030	79	2500	33.8	59.5	163	96.5	0.1	3	0.9	766	1040	0.1	92.3	0.8	0.1	0.0
14296-0031	85	2784	31.8	64.6	144	105.9	0.2	7	0.7	955	812	0.9	94.1	1.0	0.0	0.1

14296-0032	87	2747	32.5	76.3	161	106.4	0.2	5	0.6	848	978	0.1	59.4	1.4	0.0	0.1
14296-0033	86	2483	25.3	61.4	144	92.5	-0.1	2	0.9	892	694	0.7	65.4	1.1	0.0	0.1
14296-0034	1	2600	17.3	91.5	129	71.2	0.0	5	0.5	978	587	0.3	96.6	0.6	0.0	0.1
14296-0035	6	2523	27.5	163.6	458	73.7	0.9	46	3.3	1011	1979	0.2	120.6	0.7	0.0	2.3
14296-0036	7	3049	31.3	93.9	410	111.9	0.0	1	1.1	974	920	0.1	55.8	0.4	0.0	0.0
14296-0037	11	2742	26.8	70.5	144	93.1	0.0	3	0.8	1106	1266	0.3	50.6	0.8	0.0	0.1
14296-0038	12	2861	28.5	79.2	144	97.9	0.0	4	0.8	1035	824	-0.1	68.6	0.4	0.0	0.1
14296-0039	13	2503	25.7	58.1	135	77.9	0.1	4	0.7	887	765	-0.1	50.6	0.4	0.0	0.0
14296-0040	14	1707	16.4	42.3	90	51.5	0.1	3	0.2	707	571	0.1	23.9	0.5	0.0	0.0
14296-0041	32	2985	15.7	111.8	200	45.2	0.2	3	0.8	931	371	0.1	76.0	0.4	0.1	0.1
14296-0042	40	2543	28.4	77.7	129	94.9	0.1	5	0.6	955	957	0.4	63.0	0.5	0.1	0.1
14296-0043	41	2941	36.8	55.0	197	75.8	-0.2	4	1.3	859	674	0.0	131.1	1.1	0.0	0.0
14296-0044	43	2128	24.4	67.6	118	74.6	0.0	2	1.3	749	612	0.0	86.8	0.5	0.0	0.0
14296-0045	60	2764	34.0	74.6	147	96.3	-0.1	2	1.3	673	720	0.0	74.7	0.6	0.1	0.0
14296-0046	107	2735	30.6	111.8	185	85.5	0.3	19	1.3	711	1563	0.0	113.0	0.5	0.0	0.1
14296-0047	110	2637	25.7	72.0	171	99.1	0.2	1	0.7	1148	939	0.1	125.2	1.0	0.0	0.0
14296-0048	123	2586	25.8	62.3	152	91.4	0.2	0	0.5	882	898	0.3	108.3	0.8	0.0	0.0
14296-0049	132	2644	29.6	81.0	207	119.3	-0.1	2	1.0	1132	1239	0.4	126.4	0.9	0.0	0.2
14296-0050	135	3005	35.6	85.9	163	116.1	0.1	1	1.2	949	1614	0.2	179.9	0.2	0.0	0.1
14296-0051	138	3083	31.0	71.5	175	85.8	0.0	1	1.2	1024	1220	0.1	104.9	0.5	0.0	0.0
14296-0052	142	2864	24.9	78.4	180	84.1	0.1	2	0.7	687	818	0.5	42.5	1.1	0.0	0.1
14296-0053	145	3033	44.0	88.3	178	117.0	0.3	3	1.7	1095	1476	0.0	181.1	0.3	0.0	0.0
14296-0054	152	3036	40.7	91.1	187	131.1	0.8	103	1.6	935	1717	6.1	160.3	4.1	0.2	0.6
14296-0055	177	1624	19.2	49.7	104	62.2	-0.1	5	0.6	598	805	0.3	38.3	0.7	0.0	0.1
14296-0056	179	2218	22.8	61.7	126	89.0	0.4	7	1.8	888	1108	0.8	95.0	1.6	0.0	0.1
14296-0057	180	2866	26.5	77.4	177	95.0	0.9	15	1.5	896	1219	3.9	107.3	2.2	0.2	0.2
14296-0059	189	2797	32.5	83.3	160	96.5	0.0	3	0.8	891	1005	0.1	79.2	0.6	0.0	0.1
14296-0060	196	2372	28.1	66.7	140	78.6	0.0	6	0.9	787	1927	0.9	87.4	1.2	0.0	0.3
14296-0061	197	1924	20.9	56.3	122	66.2	0.4	5	1.2	660	1503	0.8	56.3	2.0	0.0	0.0

14296-0062	198	2442	24.1	65.5	162	79.2	0.3	6	1.5	805	1580	1.1	81.9	0.9	0.0	0.2
14296-0064	200	2211	24.0	52.3	145	87.0	0.4	10	1.5	351	1539	0.6	70.0	2.2	0.0	0.1
14296-0065	201	2295	24.9	68.7	114	86.0	0.4	9	1.6	490	1701	0.7	60.5	1.4	0.0	0.1
14296-0066	202	2302	22.2	47.8	143	67.9	0.5	3	0.8	694	1456	0.0	78.9	0.6	0.0	0.1
14296-0067	203	2793	29.7	71.0	157	99.1	0.3	6	1.1	700	2079	0.2	58.0	0.9	0.0	0.0
14296-0068	204	3118	29.9	85.4	167	109.8	0.5	4	0.8	945	2068	0.5	80.6	0.7	0.0	0.0
14296-0069	205	2516	27.5	72.6	131	94.7	0.1	26	1.1	775	1695	2.6	50.0	2.0	0.2	0.4
14296-0070	207	2970	32.4	91.5	164	103.8	0.5	5	1.3	1117	1250	1.1	151.3	1.3	0.0	0.0
14296-0071	208	3049	35.2	121.3	214	119.7	0.1	5	0.8	1061	1235	0.9	146.9	0.7	0.0	0.0
14296-0072	209	2274	29.9	67.8	140	141.7	-0.1	21	0.7	678	1387	0.8	103.9	5.3	0.1	0.4
14296-0073	210	1539	16.4	36.1	98	57.4	-0.8	9	0.7	546	1128	2.2	72.8	6.0	0.1	0.2
14296-0074	211	3486	43.5	138.4	237	136.1	0.1	3	1.4	1150	1766	0.1	197.0	1.0	0.0	0.1
14296-0075	212	2870	27.1	101.8	206	97.4	0.0	3	1.4	839	7059	1.1	87.5	2.4	0.0	0.1
14296-0076	213	3010	21.6	64.0	151	93.3	0.0	4	0.9	890	1555	0.2	67.9	1.4	0.0	0.2
14296-0077	214	2900	27.0	93.2	187	46.4	0.4	8	1.8	675	2196	2.6	37.4	2.5	0.1	0.2
14296-0078	215	2459	26.7	66.7	146	90.0	-0.4	10	1.2	656	1858	2.0	44.6	2.5	0.0	0.4
14296-0079	216	2487	24.1	60.7	158	81.7	0.7	3	1.1	963	1710	0.3	42.3	1.0	0.0	0.1
14296-0080	217	2971	27.1	69.4	170	88.8	0.5	5	1.0	872	2227	0.9	54.7	0.5	0.0	0.0
14296-0081	218	2788	27.0	69.7	162	93.8	0.9	4	1.0	719	2147	0.6	50.5	0.6	0.0	0.0
14296-0082	219	2791	26.9	62.7	154	76.8	0.2	3	0.5	942	1838	0.0	122.6	1.0	0.0	0.0
14296-0083	220	1756	19.9	42.4	118	64.7	0.6	6	1.6	589	1363	0.1	92.1	0.9	0.0	0.0
14296-0084	221	2449	25.6	66.5	148	72.3	-0.1	3	0.9	919	1901	0.5	112.3	2.8	0.0	0.1
14296-0085	222	2607	27.6	57.0	148	82.2	0.0	3	0.6	812	2321	0.0	116.8	0.3	0.0	0.1
14296-0086	223	2327	21.6	54.7	134	48.4	0.4	6	1.5	678	1568	1.4	97.5	2.1	0.0	0.1
14296-0087	224	1995	42.8	95.8	132	69.6	0.4	9	0.7	502	1381	0.4	92.3	0.9	0.1	0.1
14296-0088	225	2000	16.0	40.0	129	56.8	0.6	8	1.7	602	972	0.3	60.5	1.4	0.0	0.1
14296-0089	226	2099	21.8	53.7	126	68.3	0.1	10	2.3	696	930	1.0	55.9	1.3	0.1	0.2
14296-0090	227	1757	24.7	76.7	110	49.3	0.1	5	0.9	558	625	1.0	33.2	0.9	0.0	0.1
14296-0091	228	2808	26.2	58.9	161	64.9	0.0	1	0.4	828	1058	0.1	123.9	0.3	0.0	0.0

14296-0092	229	2977	36.7	88.9	189	86.3	1.2	13	0.8	763	1300	0.2	120.2	0.9	0.0	0.1
14296-0093	230	3008	20.3	111.1	169	86.2	0.5	2	1.8	556	901	0.2	117.2	0.9	0.0	0.1
14296-0094	231	3043	30.3	75.6	193	89.2	0.3	3	0.7	963	1466	0.1	148.2	0.9	0.0	0.1
14296-0095	232	2943	31.3	90.6	185	94.9	0.6	4	1.2	1020	1882	0.3	97.1	0.9	0.0	0.1
14296-0096	233	3276	30.7	128.9	181	93.2	0.9	13	1.1	1111	1694	0.7	80.5	1.3	0.1	0.2
14296-0097	234	3111	27.3	114.1	190	57.9	0.8	3	1.6	852	1404	0.6	88.3	0.9	0.0	0.1
14296-0098	235	3191	32.5	94.7	191	96.2	0.3	3	1.2	1281	1695	0.3	103.2	1.9	0.0	0.1
14296-0099	236	2928	24.8	89.3	169	30.3	0.4	1	0.8	782	1333	0.5	79.0	0.9	0.0	0.1
14296-0100	237	3232	28.5	95.7	178	63.3	0.4	3	1.2	1156	1678	0.4	101.1	0.9	0.0	0.1
14296-0101	238	2848	25.4	76.1	158	62.5	0.8	3	0.9	995	1436	0.4	82.4	1.1	0.0	0.1
14296-0102	239	2974	26.2	75.8	171	76.2	0.3	3	0.8	828	1319	0.2	75.0	1.5	0.0	0.1
14296-0103	240	3028	27.3	80.2	169	76.6	0.5	3	1.1	1014	1892	0.3	102.3	1.5	0.0	0.1
14296-0104	241	3143	25.5	100.4	189	54.2	0.9	3	2.3	914	1601	1.1	81.1	1.1	0.0	0.1
14296-0105	242	2934	24.1	74.7	162	120.8	0.5	6	0.7	877	1546	0.4	89.4	2.2	0.0	0.1
14296-0106	243	3223	35.1	96.3	190	102.6	0.3	2	0.9	1260	1411	0.6	117.4	0.9	0.0	0.1
14296-0107	244	2980	29.7	82.2	175	85.7	0.1	1	0.7	989	1024	0.5	85.7	0.8	0.0	0.1
14296-0108	245	3033	36.6	87.4	148	95.8	0.1	2	0.8	1092	1416	0.4	68.0	1.0	0.0	0.1
14296-0109	246	2626	33.8	72.5	148	94.3	0.3	2	1.1	1073	1280	0.5	59.4	1.0	0.0	0.1
14296-0110	247	2941	31.8	77.9	173	85.2	0.2	4	0.9	902	896	0.1	69.9	1.0	0.0	0.1
14296-0111	248	3271	29.5	74.8	173	67.8	0.3	2	0.8	926	1017	0.2	69.8	1.1	0.0	0.5
14296-0112	249	2935	25.6	59.5	176	62.8	0.0	23	0.6	702	1016	0.1	83.7	1.2	0.0	0.1
14296-0113	250	2513	15.9	41.4	161	40.4	0.1	1	0.4	549	688	0.2	65.8	0.9	0.0	0.0
14296-0114	251	2665	18.6	80.2	135	21.7	0.1	2	0.8	764	895	0.2	92.0	0.1	0.0	0.1
14296-0115	252	2573	23.1	62.5	124	68.9	0.1	1	1.0	687	925	0.1	88.1	0.2	0.0	0.0
14296-0116	253	2719	26.9	78.2	156	75.6	0.1	4	1.2	831	1281	0.2	116.8	0.3	0.0	0.1
14296-0117	254	2617	31.1	92.9	192	85.5	0.4	3	2.2	1011	1469	0.1	122.4	0.2	0.0	0.0
14296-0118	255	2602	28.3	87.0	197	80.8	0.3	1	0.9	828	1214	0.1	124.7	0.4	0.0	0.0
14296-0119	256	2908	46.8	109.0	160	127.7	0.2	2	0.9	1122	1650	0.2	163.5	0.6	0.0	0.0
14296-0120	257	2283	30.0	83.3	148	85.3	0.4	1	0.9	826	990	0.1	94.7	0.8	0.0	0.0

14296-0121	258	2701	30.0	96.4	160	75.2	0.2	1	1.1	879	1228	0.1	120.2	0.1	0.0	0.0
14296-0122	259	2590	28.9	74.2	143	82.7	0.0	0	0.7	784	1140	0.0	108.3	0.0	0.0	0.0
14296-0123	260	2691	31.6	85.1	145	87.1	0.2	0	0.8	984	1197	0.0	135.1	0.2	0.0	0.0
14296-0124	261	2758	36.2	85.7	150	92.4	-0.1	1	0.7	822	1054	0.1	121.5	0.1	0.0	0.0
14296-0125	262	2578	28.5	77.8	144	77.7	0.1	1	0.4	838	881	0.1	92.3	0.3	0.0	0.0
14296-0126	263	2633	29.8	70.7	155	72.9	0.1	1	0.5	797	973	0.1	93.8	0.1	0.0	0.0
14296-0127	264	2718	33.2	84.3	148	79.4	0.0	1	0.7	938	1026	0.1	113.9	0.2	0.0	0.0
14296-0128	265	2949	24.9	98.5	135	68.0	0.8	2	0.9	1091	941	0.2	91.5	0.4	0.0	0.0
14296-0129	266	2878	22.2	98.6	141	70.0	1.9	1	1.4	1092	826	0.0	110.2	0.3	0.0	0.0
14296-0130	267	2848	32.1	77.1	148	76.8	0.6	29	0.9	875	933	1.0	107.1	0.6	0.4	0.3
14296-0131	268	2653	36.4	70.9	136	81.1	0.2	8	0.6	800	917	0.2	114.4	0.4	0.1	0.2
14296-0132	269	2619	33.2	92.6	141	73.2	0.1	5	0.6	740	883	0.6	91.5	0.4	0.0	0.1
14296-0133	270	2733	26.7	61.7	142	82.7	-0.1	1	0.8	766	1175	0.2	68.7	0.7	0.0	0.0
14296-0134	271	2614	18.1	55.4	127	64.1	0.3	11	0.5	842	861	1.0	62.6	0.6	0.1	0.2
14296-0135	272	2847	24.2	106.6	173	70.0	0.9	24	1.9	821	1037	1.3	53.9	0.9	0.3	0.3
14296-0136	273	2639	23.0	75.4	146	56.8	0.0	8	0.9	780	974	1.2	83.2	0.5	0.1	0.1
14296-0137	274	2720	23.3	73.2	150	75.2	0.2	3	0.9	836	1054	0.9	92.5	0.6	0.0	0.0
14296-0138	275	2521	18.9	51.6	137	64.7	0.3	3	1.4	637	939	0.8	61.0	0.6	0.0	0.0
14296-0139	276	2868	26.3	79.0	152	83.3	0.2	4	0.6	980	1165	1.1	86.0	0.6	0.0	0.1
14296-0140	277	2979	21.9	68.5	153	80.6	0.2	3	0.9	912	1299	1.3	92.0	0.6	0.0	0.1
14296-0141	278	2888	19.8	62.8	153	58.5	0.1	5	0.9	899	853	0.8	86.6	0.5	0.0	0.1
14296-0142	279	2673	25.3	38.6	143	74.8	0.5	23	1.5	938	564	0.3	47.5	0.5	0.1	0.1
14296-0143	280	2500	24.8	48.8	153	65.3	0.2	10	1.3	623	731	0.1	138.1	0.9	0.1	0.1
14296-0144	281	2540	26.1	51.4	143	78.5	0.1	10	1.1	894	948	0.1	150.4	1.0	0.1	0.1
14296-0145	282	2561	28.8	52.1	140	69.0	0.3	10	0.5	842	976	0.2	107.6	0.6	0.1	0.0
14296-0146	283	2298	26.8	69.9	114	62.0	0.0	27	0.5	662	983	0.1	98.3	1.1	0.0	0.1
14296-0147	284	2448	21.6	48.4	124	44.0	0.0	12	0.4	645	713	0.1	75.9	9.4	0.0	0.0
14296-0148	285	3066	28.4	87.0	165	76.3	0.1	2	1.2	946	1208	0.0	121.0	0.8	0.0	0.1
14296-0149	286	2910	25.2	69.7	185	52.3	-0.1	9	0.6	884	918	0.0	104.3	0.6	0.0	0.0

14296-0150	287	2420	20.4	63.3	131	56.1	0.2	7	0.5	750	979	0.3	101.9	2.0	0.0	0.0
14296-0151	288	2435	18.4	50.4	140	40.6	0.1	13	1.5	668	932	0.3	98.7	1.2	0.1	0.4
14296-0152	289	2582	24.9	62.7	142	77.1	0.2	8	0.5	742	797	0.2	97.1	0.9	0.0	0.0
14296-0153	290	2536	24.1	56.9	129	60.1	0.3	14	0.7	870	877	0.2	97.2	1.0	0.1	0.0
14296-0154	291	2477	24.3	69.6	148	83.5	0.1	2	0.8	880	980	0.2	138.9	1.3	0.0	0.0
14296-0155	292	2843	29.7	93.8	180	136.9	0.2	3	0.6	1004	1061	0.1	123.9	1.3	0.0	0.1
14296-0156	293	2220	20.3	37.5	131	65.9	0.3	7	0.4	715	794	0.1	127.4	1.2	0.0	0.1
14296-0157	294	2514	23.1	82.6	163	73.4	3.6	11	1.0	911	865	0.0	104.6	1.3	0.0	0.1
14296-0158	295	2591	27.4	74.1	139	101.2	-0.1	8	0.7	801	795	0.2	113.9	1.1	0.0	0.1
14296-0159	296	2429	20.8	57.7	144	37.1	0.4	7	1.2	721	707	0.2	105.8	1.6	0.0	0.2
14296-0160	297	2709	25.2	54.6	156	135.7	0.2	3	0.5	854	874	0.1	115.5	1.0	0.0	0.1
14296-0161	298	3239	37.0	97.1	195	61.2	0.0	4	1.3	1391	1506	0.3	180.7	0.9	0.0	0.1
14296-0162	299	2435	22.2	37.4	126	66.7	0.1	3	0.4	588	628	0.1	103.2	0.7	0.0	0.0
14296-0163	300	2485	28.9	73.4	131	84.8	-0.1	1	0.8	807	801	0.2	87.5	0.7	0.0	0.0
14296-0164	112	2334	22.9	69.3	171	75.5	0.0	0	0.6	735	683	0.1	117.3	0.6	0.0	0.0
14296-0165	113	3152	34.4	86.8	249	100.3	0.0	1	1.2	1142	800	0.2	198.5	1.0	0.0	0.1
14296-0166	111	3183	35.8	85.8	230	96.3	0.4	1	1.1	1025	803	0.2	167.4	1.0	0.0	0.1
14296-0167	24	2862	30.7	69.9	163	84.6	0.0	0	0.6	778	1031	0.0	114.3	0.5	0.1	0.1
14296-0168	154	2800	31.3	82.5	172	98.8	0.0	1	1.2	944	817	0.0	150.8	0.6	0.0	0.0
14296-0169	155	2631	30.7	70.7	167	95.2	0.1	1	0.6	1057	730	0.1	151.1	0.6	0.0	0.0
14296-0170	153	2774	27.0	71.3	178	94.8	0.1	5	0.7	746	918	0.0	185.2	0.4	0.0	0.1
14296-0171	186	3093	34.2	82.2	190	99.3	0.1	16	1.0	1230	1178	0.4	222.6	0.5	0.1	0.2
14296-0172	157	2753	22.8	69.0	196	112.3	0.2	3	1.7	1185	1257	0.2	155.3	1.0	0.0	0.0
14296-0173	156	2072	19.0	50.9	138	109.6	0.4	7	0.5	740	1350	0.7	102.8	1.7	0.0	0.2
14296-0174	93	2922	34.2	61.9	251	90.8	0.2	2	1.1	932	1084	0.3	136.4	1.1	0.1	0.1
14296-0175	108	3069	29.0	81.8	211	93.6	0.2	2	0.9	899	1338	0.2	143.3	1.1	0.0	0.1
14296-0176	109	3022	27.3	82.1	191	87.6	0.0	2	0.6	903	1088	0.1	108.5	1.3	0.0	0.1
14296-0177	74	2961	34.6	102.5	204	109.3	0.0	2	1.0	952	845	0.3	198.2	0.5	0.0	0.0
14296-0178	75	2885	31.2	75.4	207	114.7	0.0	2	1.4	892	930	0.1	195.3	0.7	0.0	0.0

14296-0179	35	3119	34.8	84.5	183	117.4	0.3	8	2.0	1050	1541	0.2	169.7	0.6	0.1	0.1
14296-0180	167	2635	26.0	91.0	243	89.3	0.1	1	3.6	957	951	0.1	144.4	0.5	0.1	0.0
14296-0181	2	3074	43.1	110.2	169	129.2	0.2	2	1.3	1228	1423	0.3	173.3	0.4	0.0	0.0
14296-0182	100	3238	33.1	82.0	225	101.8	0.1	2	1.1	1083	1916	0.6	153.4	1.2	0.0	0.2
14296-0183	101	2753	30.4	78.3	189	97.6	0.2	3	1.0	880	1365	0.2	111.7	0.3	0.0	0.1
14296-0184	25	2931	36.7	88.2	187	114.7	0.3	2	0.7	1036	1621	0.1	145.2	0.6	0.1	0.1
14296-0185	34	2831	31.4	75.8	175	119.4	0.1	2	1.0	1168	1546	0.1	151.8	0.5	0.0	0.0
14296-0186	91	2726	30.7	76.0	156	110.6	0.3	9	0.9	843	1242	0.3	148.0	0.8	0.1	0.1
14296-0187	137	2786	24.8	62.4	158	86.5	0.4	12	0.7	749	1314	2.9	110.7	2.4	0.1	0.1
14296-0188	30	2857	35.0	81.0	172	125.9	0.3	2	1.3	1002	1516	0.2	166.1	0.3	0.1	0.1
14296-0189	103	2735	27.0	73.8	193	102.6	0.3	7	0.9	1031	1853	0.8	155.8	0.6	0.1	0.1
14296-0190	102	2507	32.6	73.7	179	103.8	0.2	24	1.1	793	1651	1.8	130.6	1.1	0.3	0.3
14296-0191	99	2603	27.3	68.1	194	89.8	0.3	21	0.9	833	1299	0.7	118.8	0.6	0.3	0.2
14296-0192	98	2806	28.4	90.1	213	110.9	0.2	18	1.5	1022	1450	0.6	136.2	0.5	0.2	0.2
14296-0193	97	3035	28.9	92.7	202	99.3	0.2	2	0.8	771	1589	0.1	132.0	1.5	0.0	0.1
14296-0194	77	2725	30.2	70.6	161	100.0	0.1	4	1.0	1000	1094	0.4	92.1	0.4	0.0	0.0
14296-0195	44	2515	27.4	65.9	157	94.3	0.0	2	0.7	706	824	0.1	118.9	0.6	0.0	0.0
14296-0196	76	3330	21.3	125.8	140	103.5	0.2	3	0.6	1115	734	2.9	68.4	0.5	0.0	0.0
14296-0197	143	2836	30.1	81.1	176	110.5	0.6	23	0.9	1047	1519	7.4	119.6	6.0	0.2	0.3
14296-0198	16	2852	27.9	67.9	172	96.2	0.3	2	0.5	1449	883	2.5	35.7	1.1	0.0	0.1
14296-0199	36	2960	31.4	103.9	160	108.2	0.1	1	1.6	540	1605	0.2	129.4	0.5	0.0	0.0
14296-0200	164	2648	26.6	78.4	148	81.3	0.1	1	0.6	815	722	0.0	139.6	0.7	0.0	0.0
14296-0201	165	2915	28.2	80.9	144	95.8	0.3	2	1.5	894	812	0.1	144.1	0.5	0.0	0.1
14296-0202	169	2801	27.3	78.8	173	101.8	0.6	24	1.8	1090	1182	0.7	121.0	0.6	0.3	0.3
14296-0203	168	2758	30.3	85.7	180	97.4	0.2	14	1.0	773	1015	0.5	116.8	0.6	0.2	0.2
14296-0204	162	2891	29.6	79.2	178	99.5	0.2	3	1.6	1204	1175	0.1	172.1	1.3	0.0	0.1
14296-0205	39	2855	32.8	75.4	173	102.5	0.1	1	0.9	1058	1065	0.2	117.6	0.4	0.0	0.1
14296-0206	38	2987	33.5	78.5	182	120.4	0.0	6	1.0	1119	1233	0.3	119.2	0.5	0.1	0.1
14296-0207	37	2776	32.0	65.8	155	103.1	0.1	3	1.5	1135	1532	0.2	129.8	0.5	0.0	0.0

14296-0208	166	2832	34.0	88.6	170	115.0	0.1	2	1.2	1019	1085	0.1	166.6	1.0	0.0	0.1
14296-0209	114	2795	31.1	78.9	141	100.3	-0.1	116	0.7	967	1089	0.4	63.0	0.5	0.0	0.0
14296-0210	161	2725	30.6	79.2	168	97.1	0.0	2	0.9	1004	1133	0.1	133.4	0.7	0.0	0.1
14296-0211	191	2937	30.6	66.3	165	98.6	0.1	3	1.1	1208	1250	0.1	126.4	1.0	0.0	0.2
14296-0212	47	2694	30.1	63.2	141	99.8	0.1	3	0.5	840	734	0.2	116.2	1.7	0.0	0.1
14296-0213	170	2771	28.3	65.3	176	98.4	0.1	3	0.8	921	1298	0.0	129.7	0.6	0.0	0.0
14296-0214	171	2736	28.2	60.7	158	99.3	0.2	2	1.5	788	1186	0.1	116.3	1.1	0.0	0.0
14296-0215	192	2806	32.7	67.0	174	99.3	0.4	6	2.1	950	1214	0.2	149.1	1.8	0.0	0.1
14296-0216	46	3048	28.6	72.2	165	112.5	-0.1	3	0.9	990	943	0.2	96.5	1.0	0.0	0.0
14296-0217	190	2950	35.6	95.9	183	125.5	0.0	2	1.3	735	1102	0.1	155.9	1.1	0.0	0.1
14296-0218	193	2746	32.1	82.3	174	115.1	0.0	2	1.1	380	839	0.1	136.9	1.4	0.0	0.1
14296-0219	19	2817	29.9	75.9	193	102.8	0.0	2	0.8	931	763	0.2	124.3	0.6	0.0	0.1
14296-0220	50	2783	33.6	79.9	181	109.9	0.0	3	1.2	976	1110	0.3	141.7	0.5	0.0	0.0
14296-0221	65	2901	26.4	74.6	176	97.3	-0.1	2	0.6	876	1032	0.1	100.2	0.6	0.0	0.3
14296-0222	174	2792	27.3	66.4	171	98.9	0.2	6	1.9	766	993	0.1	125.9	0.8	0.0	0.1
14296-0223	172	2678	27.4	57.3	168	98.7	0.3	2	0.7	970	971	0.1	106.8	0.7	0.0	0.1
14296-0224	173	2803	29.1	55.1	165	68.8	0.1	2	1.0	1048	905	0.1	112.1	0.4	0.0	0.1
14296-0225	195	3252	40.6	94.3	178	130.5	0.2	3	0.7	1069	1241	0.1	128.5	0.8	0.0	0.1
14296-0226	163	2819	33.4	87.9	163	106.7	0.3	3	1.7	1156	949	0.5	137.0	1.0	0.0	0.1
14296-0227	160	2813	28.5	79.6	178	99.0	0.1	2	0.9	950	1176	1.3	87.9	0.9	0.0	0.0
14296-0228	62	2876	26.1	78.8	156	100.9	0.1	2	0.9	950	1009	0.4	78.4	0.5	0.0	0.0
14296-0229	178	2879	28.4	80.7	199	94.4	-0.1	3	1.8	947	1402	1.0	88.3	0.7	0.0	0.0
14296-0230	61	2864	26.2	72.1	177	98.5	0.1	1	0.6	855	900	0.1	86.0	0.4	0.0	0.0
14296-0231	57	2922	28.9	72.3	152	97.3	0.3	3	1.6	893	623	0.5	88.9	0.7	0.0	0.0
14296-0232	23	2938	39.7	90.7	185	121.8	0.2	2	3.5	931	990	0.2	103.9	0.6	0.0	0.0
14296-0233	124	2904	31.0	79.6	183	96.3	0.2	2	0.7	942	1065	0.4	86.9	1.4	0.0	0.2
14296-0234	125	3083	34.7	99.9	168	127.9	-0.1	2	1.0	1115	1386	0.4	93.2	0.3	0.0	0.0
14296-0235	56	2962	28.8	79.1	165	99.7	-0.1	1	0.9	986	852	0.2	111.2	0.6	0.0	0.0
14296-0236	144	2839	33.8	84.7	166	103.5	0.4	4	1.0	846	1218	1.0	92.4	1.3	0.0	0.1

14296-0237	78	2617	32.1	68.3	164	96.1	0.2	3	1.1	896	487	0.4	98.7	1.2	0.1	0.1
14296-0238	49	2707	32.2	108.5	182	105.6	0.1	0	1.0	964	1119	0.6	116.1	1.0	0.0	0.1
14296-0239	118	2405	25.5	65.8	144	82.1	0.2	0	0.4	666	545	0.1	54.7	0.9	0.0	0.0
14296-0240	184	2620	24.7	67.1	150	79.6	0.2	4	0.4	818	821	1.0	102.4	0.8	0.0	0.0
14296-0241	120	2568	26.9	72.0	138	92.7	0.1	2	0.6	820	942	0.0	58.3	0.9	0.0	0.1
14296-0242	119	2879	31.3	77.3	191	120.9	0.1	1	1.4	952	1146	0.3	85.8	1.0	0.0	0.1
14296-0243	136	2008	18.6	55.2	109	79.3	0.0	1	0.7	637	1334	1.5	51.6	0.4	0.1	0.0
14296-0244	141	2805	32.2	84.2	182	113.9	0.2	2	0.7	1023	1236	0.7	102.7	2.3	0.1	0.2
14296-0245	33	2101	24.0	57.9	120	94.8	0.5	47	1.2	822	1062	0.9	62.1	1.6	0.4	0.2
14296-0246	121	3136	32.3	78.2	154	112.4	0.0	37	1.2	1038	1099	2.7	78.5	0.5	0.4	0.2
14296-0247	158	2248	26.2	48.3	127	83.1	0.3	1	0.8	791	649	0.4	59.3	1.2	0.0	0.1
14296-0248	159	2826	27.5	93.2	160	101.9	0.1	2	0.7	968	594	0.8	77.3	1.0	0.0	0.0
14296-0249	133	1293	13.5	35.5	75	57.9	0.2	8	0.3	504	635	0.4	39.1	1.8	0.0	0.1
14296-0250	117	2804	32.3	90.3	152	102.6	0.3	21	1.0	879	1007	0.7	65.2	1.9	0.2	0.3
14296-0251	88	1892	20.9	60.6	111	77.9	0.0	3	0.7	705	891	0.2	57.5	1.5	0.1	0.1
14296-0252	139	2757	30.3	77.1	177	102.4	0.3	10	1.2	899	1008	1.2	74.6	1.3	0.2	0.2
14296-0253	104	2664	32.0	66.2	181	116.4	0.0	6	1.3	881	1201	0.6	88.7	1.6	0.1	0.1
14296-0254	175	2805	34.0	73.2	196	99.1	0.2	6	0.9	1159	1145	0.6	92.5	1.4	0.1	0.2
14296-0255	31	2893	26.6	73.6	188	94.9	0.1	1	0.3	1154	539	0.4	49.1	1.2	0.1	0.1
14296-0256	127	1756	20.6	49.7	90	69.2	0.0	2	0.4	517	707	0.3	39.9	1.6	0.0	0.1
14296-0257	115	2655	28.7	83.7	153	97.5	0.2	2	0.8	949	924	0.1	59.2	0.4	0.1	0.0
14296-0258	45	2586	28.8	96.7	129	78.9	0.3	12	0.8	836	745	0.3	104.3	0.6	0.1	0.1
14296-0259	126	2897	34.3	81.5	156	107.0	0.2	2	0.8	1104	1013	0.4	77.5	0.5	0.0	0.1
14296-0260	140	2858	35.6	84.6	152	101.8	0.2	0	0.6	797	915	0.0	69.7	0.4	0.0	0.0
14296-0261	116	2700	29.7	72.7	147	103.0	0.2	1	0.9	913	689	0.0	55.6	0.4	0.0	0.0
14296-0262	5	2451	18.3	56.1	135	80.6	0.0	0	0.2	954	453	0.0	61.2	0.8	0.0	0.0
14296-0263	3	2699	30.9	75.8	137	85.5	-0.2	10	0.4	861	642	0.9	61.5	0.6	0.1	0.1
14296-0264	63	2567	25.5	67.5	148	84.9	0.1	2	1.4	797	598	0.0	78.6	0.5	0.0	0.0
14296-0265	59	2955	28.2	78.4	160	94.6	0.0	2	1.3	852	867	0.2	78.6	0.6	0.0	0.0

14296-0266	68	2907	28.9	90.4	184	100.8	0.0	3	1.3	1019	977	0.2	64.6	0.7	0.0	0.1
14296-0267	42	2797	25.3	68.8	154	100.3	0.0	1	0.7	891	1104	0.3	101.1	0.6	0.0	0.1
14296-0268	26	2751	32.2	87.4	152	95.0	0.3	4	0.7	785	839	0.2	37.6	1.0	0.0	0.0
14296-0269	185	2670	28.0	69.4	174	88.0	0.0	17	2.2	985	1165	1.4	131.7	0.6	0.0	0.0
14296-0270	188	446	6.4	15.2	25	71.2	0.2	4	0.1	200	455	0.1	9.9	1.6	0.0	0.2
14296-0271	54	2885	25.5	87.3	183	89.2	0.3	5	0.8	831	795	0.2	84.4	1.0	0.1	0.1
14296-0272	55	2956	28.8	88.0	168	109.5	0.3	6	2.3	924	1131	0.1	94.6	1.1	0.0	0.0
14296-0273	58	3108	27.2	86.3	180	112.0	0.2	3	2.0	779	1065	0.2	77.2	0.7	0.0	0.0
14296-0274	53	2755	25.6	77.8	158	90.3	-0.1	3	0.9	946	685	0.1	74.3	1.0	0.1	0.1
14296-0275	22	2785	30.3	77.2	148	99.1	0.1	12	1.3	1032	909	0.4	76.3	0.7	0.1	0.1
14296-0276	69	2927	34.9	93.8	166	100.5	0.1	3	1.2	1155	819	0.6	67.2	0.7	0.0	0.0
14296-0277	18	2989	32.8	99.5	186	113.8	0.4	45	2.3	1075	1063	1.3	84.4	1.6	0.5	0.5
14296-0278	66	2906	24.1	71.2	144	85.9	0.3	7	0.8	1032	925	0.5	78.0	1.2	0.1	0.0
14296-0279	194	2876	26.0	84.6	179	87.1	0.3	2	0.8	1269	744	0.3	84.6	0.8	0.0	0.0
14296-0280	4	2915	34.1	66.7	160	109.7	0.2	3	1.6	1149	1031	0.3	166.5	1.8	0.0	0.0
14296-0281	183	2576	36.7	73.6	144	88.7	0.6	3	0.9	1039	1079	0.1	72.9	0.5	0.0	0.0
14296-0282	70	2994	30.1	80.0	174	110.3	0.1	3	1.5	841	989	0.2	96.2	1.0	0.1	0.0
14296-0283	72	2921	39.8	95.9	178	109.9	0.0	4	2.2	1038	1089	0.2	69.9	0.8	0.0	0.0
14296-0284	20	2808	28.1	89.8	146	89.1	0.3	7	1.1	951	1017	0.1	71.3	0.8	0.1	0.0
14296-0285	182	2843	30.6	88.9	154	97.0	0.0	2	1.3	882	1176	0.0	68.9	0.7	0.0	0.1
14296-0286	151	2676	39.4	96.4	145	103.8	0.2	5	0.9	839	1121	0.2	83.2	0.7	0.0	0.0
14296-0287	67	3086	28.7	96.6	179	107.6	0.2	4	1.8	1161	979	0.2	72.0	0.8	0.1	0.0
14296-0288	176	2833	32.1	76.0	151	97.3	0.3	8	1.2	1064	1177	0.3	69.2	0.8	0.1	0.1
14296-0289	64	2855	27.5	82.4	141	84.9	0.1	3	1.0	1042	798	0.2	73.4	0.7	0.1	0.1
14296-0290	105	3034	33.2	97.5	213	116.2	0.0	2	2.3	1002	1095	0.3	103.4	0.6	0.0	0.0
14296-0291	21	3077	36.7	94.5	178	99.7	0.1	0	0.6	1125	669	0.1	79.4	0.6	0.0	0.0
14296-0292	206	2640	29.2	65.0	167	76.2	0.4	5	1.5	889	695	0.0	53.8	1.0	0.0	0.0
14296-0293	71	2994	33.7	83.1	156	97.8	0.2	5	1.1	973	1071	0.5	57.5	1.0	0.1	0.0
14296-0294	106	2969	34.6	92.8	177	123.8	0.0	2	5.7	1097	1936	0.3	138.3	0.5	0.0	0.0

14296-0295	181	2650	30.9	72.9	156	97.6	0.1	3	0.9	904	961	0.0	99.3	0.8	0.0	0.0
14296-0296	134	3362	35.5	82.5	189	218.4	0.0	11	0.6	859	1622	0.7	63.3	1.8	0.0	0.1
14296-0297	29	2819	29.4	74.0	138	93.0	0.2	0	0.7	857	897	0.5	67.5	0.4	0.0	0.0
14296-0298	28	2967	33.8	91.7	159	118.8	0.1	0	1.1	1077	1100	0.9	80.6	0.7	0.1	0.0
14296-0299	27	2964	38.9	90.7	166	127.7	0.1	3	1.3	1035	894	1.8	67.7	1.1	0.1	0.0
14296-0300	122	2907	30.7	80.6	210	111.7	0.3	3	0.6	1196	1639	0.1	128.2	0.4	0.1	0.0
n=293	mean	2693	28.3	75.7	163	90.6	0.2	6	1.0	885	1114	0.5	98.8	1.0	0.1	0.1
	median	2786	28.4	75.8	161	93.6	0.2	3	0.9	892	1024	0.2	93.2	0.8	0.0	0.1
	stdev	396	5.9	18.3	37	21.7	0.3	11	0.6	184	507	0.8	36.7	0.9	0.1	0.2
	min	446	6.4	15.2	25	21.7	-0.8	0	0.1	200	317	-0.1	9.9	0.0	0.0	0.0
	max	3486	46.8	163.6	458	218.4	3.6	116	5.7	1449	7059	7.4	222.6	9.4	0.5	2.3
	Q1	2567	25.3	65.5	144	76.8	0.0	2	0.7	782	845	0.1	72.8	0.6	0.0	0.0
	Q3	2934	32.1	86.8	179	102.5	0.3	6	1.2	1004	1290	0.6	121.0	1.2	0.1	0.1
	IQR	367	6.8	21.3	36	25.8	0.2	5	0.5	222	445	0.5	48.3	0.6	0	0
	mean-med	-93	-0.1	-0.1	2	-3.0	0.0	3	0.1	-7	91	0.2	5.6	0.2	0	0
	%diff	-3	-0.3	-0.1	1	-3.3	22.9	52	12.4	-1	8	49.1	5.7	16.6	48	34

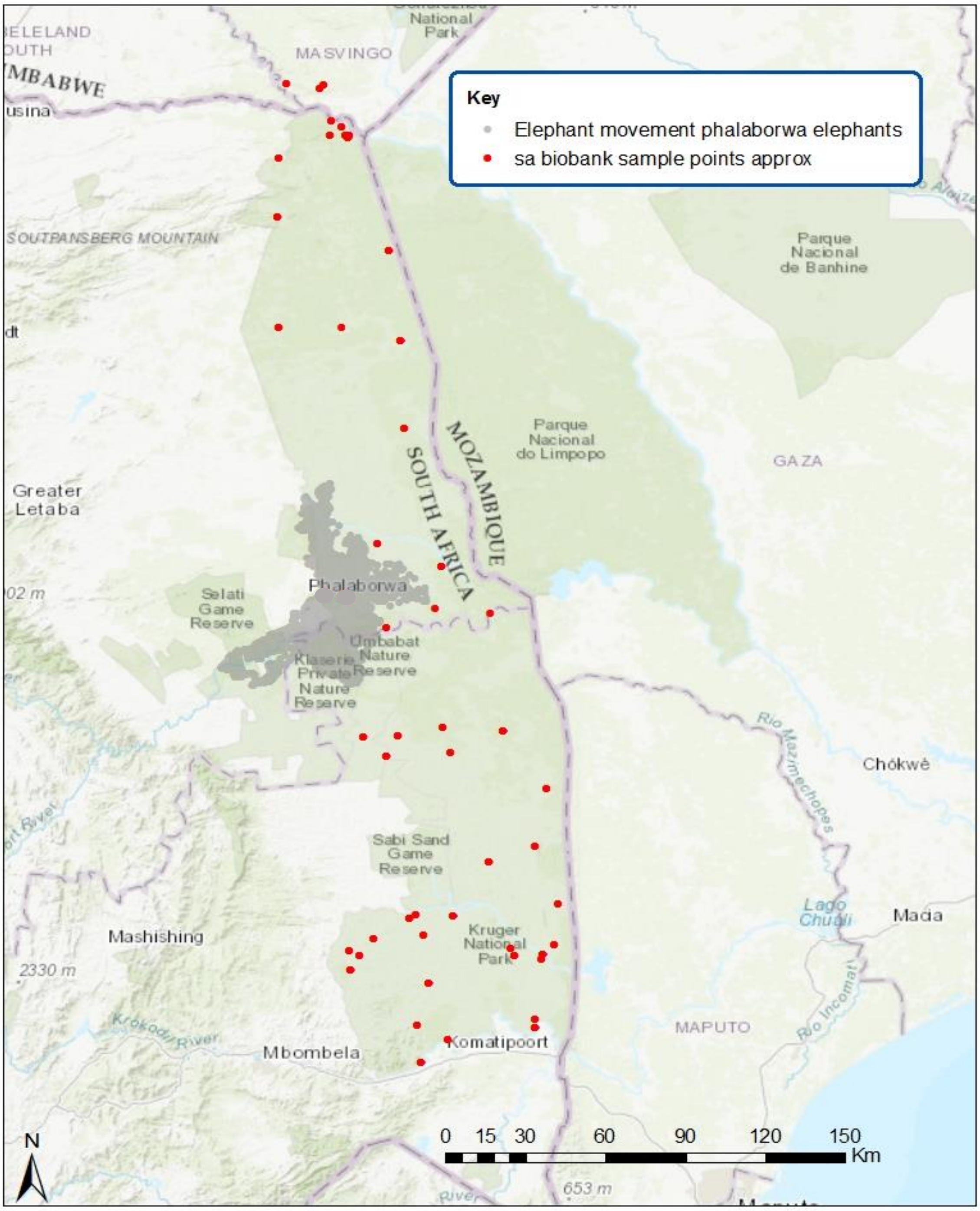

Figure A1: Locations of plasma sampling for the South African National Parks Biobank samples. GPS coordinates were not available for all samples, multiple samples were sometimes taken from the same GPS location if multiple animals available

Chapter 6

Conclusion

The aim of this research was to understand the spatial influence of geochemistry on the home range and movements of elephants, using the natural experiment enabled by the differences in environmental geochemistry in the region of the Palabora Mining Company (PMC) and surrounding national park lands, as a case study of contrasting geochemical environments. With a continually rising human population and an increase in land required for agriculture, historic elephant movements will be ever more limited with elephants forced into increasingly smaller, fenced reserves, and coming under increased human management for protection and survival (Nyhus, 2016). Therefore, it is essential that conservation managers consider the influence of mineral provision, affected by local geochemistry, on elephant movement and home range size. Within these restricted areas, it will also be necessary to consider the potential for increased accumulation of potentially toxic elements (PTEs), if they are present in the environment. Because elephants may be foraging within a relatively restricted home range, with less opportunity to move over longer distances to obtain food resources, they could potentially be consuming higher levels of PTEs, if present in environment.

The investigation addressed the project aims through the following objectives:

1. Validation of optimum bio-indicators for mineral and PTE status in elephants.
2. Establishment of baseline levels for key minerals and PTEs in African savanna elephant tail hair, plasma and faecal samples.
3. Determination of whether mineral and PTE levels in soil, plants, and water surrounding the PMC are higher than neighbouring areas within the KNP /APNR.

4. Determination of whether elephants eating and drinking near the PMC have higher mineral and PTE levels in their tissues (tail hairs) and faecal samples than elephants from KNP/APNR.

Chapter 3 summarized the literature concerning movement choices of African savanna elephants based on nutritional needs. While the mineral requirements of elephants remain undetermined, estimations of daily intake needs were made for Ca, Na, Zn and Fe (Sach, Dierenfeld, *et al.*, 2019) based on captive and wild studies. The link between environmental geochemistry and mineral availability to the consumer must be considered when investigating elephant movement. Plants will often reflect soil mineral profile(s), with those growing in mineral deficient areas passing this onto the animal consuming them. Likewise, those plants growing in mineral abundant areas will also pass on these higher levels to the consumer (Hurst *et al.*, 2013; Joy *et al.*, 2015). Numerous factors affect the soil-to-plant transfer of nutrients, including geochemical parameters such as organic matter content and soil pH, and the ability of the plant to incorporate specific minerals as affected by plant age, growth rate, and species (Maskall and Thornton, 1996).

Chapter 3 highlighted the fact that captive elephant nutrition is understudied, and evidence for specific micronutrient needs of either genus of elephants is limited. Animal nutritionists therefore use domestic species as physiological models when designing diets for elephants. The domestic horse was suggested as the most appropriate model for determining mineral needs in elephants, as it is a large hindgut fermenter, with a similar gastrointestinal tract anatomy (Clauss *et al.*, 2003). However, elephants have a faster ingesta passage rate than horses, and thus digestibility coefficients are lower in elephants for all nutrients. Therefore, when using dietary recommendations from the domestic horse for zoo elephants, caution must be used. Evaluation of the suitability of a zoo elephant's diet linked with overall health is conducted through routine assessment of weight and body condition, skin and foot status, overall vitality and measurement of blood biochemical parameters. Having validated and expanded bio-monitoring techniques and tissues as indicators of mineral or PTE status in elephants, such as toenail, tail hair or faeces (in addition to plasma provides new indices that can be used to monitor these aspects of elephant health, in captive and wild populations, as explored in Chapter 4 and 5.

Results provided in Chapter 4 informed methodologies for wild study (as covered in Chapter 5), and fulfilled the first objective of the thesis, which was to validate optimum bio-indicators for mineral and PTE status in elephants. Elephants from UK zoos were used for the validation elements of this research. The zoo environment provided an opportunity to quantify intakes of the elephants, within a relatively closed system, from keeper-fed diet, water and soil. Outputs, in the form of biological samples of toenail, tail hair, faeces, urine and plasma were analyses to identify the optimum bio-indicator for intake, as a proxy for status of each mineral or PTE. Toenail proved to be the best bio-indicator matrix of intake and thus a proxy for status of As, Fe, K, Mg, Na, P and Se within the elephant. Keeper-fed diet had the greatest influence over total mineral or PTE intake. Tail hair reflected intake of Fe and As, as a proxy for status of these elements. Faecal samples reflected Ca, Cu, Se, As whilst urine was of no value in determining mineral or PTE status for any analysed element. Plasma, previously considered the optimum bio-indicator, was found to be of limited value for most minerals, but reflected Fe, Mn, Zn and U intake.

Results provided in Chapter 4 also highlighted the substantial contribution that water can make to mineral nutrition, especially zinc intake in zoo elephants. Two studied zoos provided diets that contained low zinc concentrations relative to dietary recommendations, although there were no clinical signs of deficiency in their animals. When the zinc provision from water was included, the total zinc intake at one of the zoos increased considerably, to above the minimum recommendation. Potential mineral provision from drinking water should be considered for other large herbivore nutrition assessments, especially when such large volumes of water are consumed. Water as a significant factor to nutrient balance has also been demonstrated in human nutrition, where Ca from water contributed up to 11% of the Ca supplied, based on consumption of 1.7 litres per adult human per day (Beal *et al.*, 2017). Furthermore, results from the current study also indicated that UK zoo elephants are not mineral deficient, nor at risk of consuming detrimentally high levels of PTEs. Generally, all studied animals were fed in line with dietary recommendations, and legal requirements for the species (*Zoo Licensing Act 1981*; BIAZA, 2019) with browse plants fed daily, and grazing provided to all animals at some point throughout the year. Obesity poses the highest nutritional risk to UK zoo elephants, with most animals fed in excess of the energy intake requirements per animal per day.

Feed costs represent the second greatest day-to-day expenses for UK elephant herds (Sach, Fitzpatrick, *et al.*, 2019); therefore, appropriate nutrition is necessary and important not only from an animal health and welfare perspective, but also for a fiscal responsibility. Inadequate nutrition can lead to compromised welfare, reduced reproductive output and life expectancy (Dierenfeld, 1997; Hermes, Hildebrandt and Göritz, 2004). Due to the low growth rate and large body size of elephants, it is possible that nutritional inadequacies and appropriate dietary evaluation may be overlooked within a zoo setting, for relatively long periods of time, before clinical signs develop (Ullrey *et al.*, 1991). Therefore, having improved bio-monitoring techniques to assess mineral and PTE status in elephants, through the use of minimally invasive bio-indicator tissues such as toenail, tail hair or faecal samples, could improve zoo elephant health, with potential application to other species.

Ex situ elephants in UK zoos have an invaluable role to play in research; (1) to benefit wild counterparts; (2) to benefit, share and improve husbandry practices for elephants in range-state countries under human care and; (3) to benefit husbandry, welfare and care of other *ex situ* elephants in western zoos. Method development, such as within this project, and detailed by Bechert et al (2019) would not be possible without *ex situ* zoo elephants. In turn, zoo nutritionists, veterinary and curatorial staff can use data from this research, to better assess mineral status in their elephants, using novel matrices such as toenail, faeces and tail hair, which may also be easier to routinely sample. Additionally, more detailed evaluation of individual keeper-fed diets, incorporating soil and water components, with comparison to available recommendations and data from this study, could be conducted. Further critical review, as to the suitability of the domestic equid model for elephant mineral requirements in captivity should be considered in light of the improved tools and understanding of optimum best bio-indicators of mineral or PTE status.

When collecting biological samples in South Africa, in the field, there were practical challenges. Samples from both Elephants Alive (EA) and the South African National Parks (SANParks) BioBank were collected over a number of years, prior to the commencement of this project. Toenail samples were not routinely collected as part of collaring or management activities. Therefore the number of available toenail samples was significantly smaller than plasma or tail hair samples. Additionally, plasma was not collected from the animals collared at the PMC, so these two matrices could not be used for comparison between the animals collared at the PMC and those from the surrounding areas. However the results from the zoo validation indicated that toenail was a preferential bio-indicator matrix for mineral status in elephants. Based on the results of this project, it is recommended that in the future, toenail should be routinely sampled when wild elephants are anesthetized for collaring or management purposes, to build a dataset of baseline mineral and PTE levels from assumed healthy animals. In-country laboratories could be used for analysis of all samples (biological and environmental). Method development and validation, especially in regard to sectioning of tail hair for ICP-MS analysis, as described in Chapter 2, was made possible from the UK zoo validation study, as discussed in Chapter 4. Suitable sample cleaning, preparation, digestion and analysis methods including quality control were identified from the validation study in Chapter 4 using zoo samples, meaning in the future this sample preparation and analysis could be conducted in laboratories in country, so as to enable more readily accessible, less expensive mineral and PTE analysis, if required by conservation managers or the National Parks Authorities.

This research has produced the largest datasets to date of mineral and PTEs present in wild African savanna elephant tail hair and faecal samples (detailed in Chapter 5), as well as that in toenail and plasma samples (details in Appendix of Chapter 5). The methods for analyzing these biological samples from elephants were adapted using state of the art analysis technology, from those used in prior studies conducted on elephants, horses, antelopes and humans (Wiedner *et al.*, 1638; Wrench, Meissner and Grant, 1997; Armelin, Avila and Piasentin, 2003; Middleton *et al.*, 2016; Hu, Fernandez and Cerling, 2018).

Chapter 5 draws upon method development given in Chapter 4, supported by information gained from the literature as detailed in Chapter 3. This chapter addressed the second, third and fourth objectives of the thesis, which were: (2) Establishment of baseline levels for key minerals and PTEs in free-ranging African savanna elephant tail hair, plasma and faecal samples, (3) Determination of whether mineral and PTE levels in soil, plants, and water surrounding the PMC are higher than neighbouring areas within the KNP /APNR and (4) Determination of whether elephants eating and drinking near the PMC have higher mineral and PTE levels in their tissues (tail hairs) and faecal samples than elephants from KNP/APNR.

Baseline levels for key minerals and PTEs in elephant tail hair, plasma and faecal samples were established as part of this study. Tail hairs were analysed as whole samples from 200 elephants within the KNP and APNR, collected during routine collaring or management activities within these areas. Faecal samples were collected from 58 locations within the KNP and APNR as part of the environmental sampling strategy, and represented a broad covering of these areas. Twenty five toenail samples were analysed from animals within the APNR as part of routine collaring operations conducted by Elephants Alive (EA). Finally 293 elephant plasma samples were analysed from elephants from across the KNP as part of the work of the SANParks BioBank. Establishment of these baseline levels was required for comparison with samples from animals at the PMC. Previous data reported to date represented very small, geographically specific populations and therefore, unsuitable for comparison to the animals collared at the PMC. Comparison of these established baseline levels to samples from elephants at the mine fulfilled objective 4.

Mineral and PTE levels in soil were significantly higher at the mine compared to surrounding areas, decreasing in concentration as distance from the mine increased. Concentrations of Ca, K, Fe and Cu in water were considerably higher at the mine, also decreasing as distance from the mine increased. Concentrations of P, Mg, Mn, Fe, Cu, Zn, Se, Cd and U in leaves from plants were considerably higher at the mine, decreasing as distance from the mine increased. This data indicates that the mine offered the elephants a unique environment and that the geochemistry in the locality of the mine was significantly different to the surrounding national park and reserve lands. This information could be used to influence and manipulate elephant movement through the use of supplementary mineral provision, which could draw them towards areas, where human-elephant conflict (HEC) would be minimised, away from the working areas of the mine. A selected number of plant species were sampled based upon information from the literature on local plants consumed by elephants, which may not always represent direct elephant consumption. Further research and monitoring of specific plant species and parts consumed, to obtain a more detailed representative diet sample, could be considered in the future. Physical water provision as well as mineral provision from water influenced elephant movement, especially during the dry season, when plants are likely to be at their most mineral depleted and water availability scarcer within surrounding areas. To reduce elephant incursion into the PMC, consideration could be given to blocking off water sources in the PMC that are accessible to the elephants.

An evolutionary advantage for elephants may be gained in consuming increased micronutrients at the PMC, but potentially at the cost of the increased consumption of PTEs. Fertility augmenters in the form of Se and Zn benefit elephants in early life (Hidiroglou and Knipfel, 1984; Mistry *et al.*, 2012), whereas the effects on fertility from consuming PTE's at toxic levels may take decades to be realised, having a lesser effect on total reproductive output on the individual or the population (Kincaid, 1999). Data produced from this project on soil, water and plants within the KNP, APNR and PMC will substantially augment the small body of literature to date on mineral and PTE levels in soil, water and plants in this geographical region (Venter and Gertembach, 1986; Greyling, 2004; Codron *et al.*, 2006).

Limitations to the study

Sample size was a limitation of this study, particularly the biological sample size(s) for the elephants collared at the PMC. Identifying appropriate animals to collar at the PMC, availability of funds, obtaining permits and addressing the considerable logistics for such a study are all challenges to overcome when sampling in the field; however, increasing the sample size in this area would be beneficial. Investigating other similar locations in which elephant movement may be driven by unique differing environmental geochemistry would increase evidence for this factor impacting elephant movement, and increase the validity of the conclusion that elephants move based in part on mineral provision and the interactions with local environmental geochemistry. Finally, although when calculating the baseline mineral and PTE levels in African savanna elephant tail hair, plasma and faecal samples, the samples sizes were very large, (and those for plasma were above the required numbers recommended by the American Society for Veterinary Clinical Pathology (ASVCP)), a larger sample size covering a broader geographical area, would increase confidence in the values.

Future Work

Further biomonitoring work could be conducted to compare mineral and PTE levels in plasma and toenail samples from animals collared at the PMC with African savanna elephants outside of the mine area, in the surrounding national parks and reserves. If collared elephants were re-collared or sedated for other management purposes, it may be possible to apply the techniques on the sectioned tail hairs, detailed in Chapter 2, to assess how movement correlates to changes in mineral or PTE as tail hair grows.

The plasma data from 293 wild African savanna elephants is the largest data set of its kind and it is intended that reference ranges will be produced for the 15 investigated minerals and PTEs, following the ASVCP reference range framework. This will be of use to zoo and wild elephant managers and veterinary staff. In the future it could be helpful to widen the study to more sites, to increase the number of elephants within the study and consider the effect of seasonality as all environmental sampling was all conducted solely at the end of the dry season.

This research strengthens zoo-wild interactions, relationships and collaborative research, demonstrating how zoo elephants can benefit their wild counterparts. In turn, zoos can benefit from the nutrient data produced from environmental and biological samples, enabling comparison with *in situ* populations. Furthermore, the PMC and surrounding mines can benefit from data produced, informing drivers for HEC within their locality, and provide information which could be used to inform mitigation strategies for this conflict.

The information derived from the studies conducted in Chapter 4 and 5 both contribute towards achieving the United Nations Sustainable Development Goals (SDGs). Specifically, zoos contribute towards SDG 3 (Good health and well-being), 15 (Life on land) and 17 (Partnerships for Goals). Advancing zoo animal health and welfare will increase opportunities to (re)connect people with nature and promote well-being though the visiting of zoos (SDG 3). Linking zoos with wild counterparts, encouraging two-way knowledge exchange and the development of global partnerships contributes towards SDG 17. The PMC produces refined Cu and minerals such as P, and acts as an opportunistic micronutrient hotspot to the elephants in the area, due to its unique local geochemistry. Where elephant movements overlap with mining activities, elephant incursion into the mine occurs, and thus results in HEC. In order to reduce this HEC, conservation managers could consider using habitat manipulation. Provision of alternative sources of minerals could be placed, away from the mining area and the PMC land, encouraging elephants to meet their resource needs elsewhere and thereby reducing HEC in the area. Principles of environmental geochemistry help us better understand how mineral provision drives elephant movement. This should be applied by elephant population managers internationally both *in situ* and *ex situ*, to influence elephant movement for conservation gain, and to optimise elephant health and welfare; in a world where elephants are experiencing greater human initiated movement restriction and threat.

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Additional Papers produced:

- Fischer M. & **Sach F**. 2020: Editorial: Conservation of Elephants. *International Zoo Yearbook* 53: 9–16.
- **Sach F**., Fitzpatrick M., Masters N., Field D. 2019. Financial planning required to keep elephants in zoos in the United Kingdom in accordance with the Secretary of State ’ s Standards of Modern Zoo Practice for the next 30 years. 24:1–11. DOI: 10.1111/izy.12213.
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Conference Presentations:

- Agilent User meeting, BGS lunchtime lecture (20 minutes), December 2019

- 34th International Conference of the Society Environmental Geochemistry and Health- Geochemistry for Sustainable Development 2018, Livingstone, Zambia. (Oral presentation)

- 13th AZA NAG conference on Zoo and Wildlife Nutrition, St Louis 2019 (Oral Presentation – Roy McClements Best Presentation Award)

- 16th International Elephant Foundation Conservation and Research Symposium 2019, Bela, South Africa presentation

- European Association of Zoo and Aquaria (EAZA) 2019, Valencia Poster presentation

- European Association of Zoo and Aquaria (EAZA) 2017, Emmen Poster presentation

- European Association of Zoo and Aquaria (EAZA) 2016, Emmen presentation to Taxon Advisory Group (TAG) for research support and TAG endorsement

- Savannah Network meeting 2018, Kruger National Park, poster presentation

In Addition:

- Two oral presentations (2017, 2019), one poster presentation (2018) at postgraduate research events at the University of Nottingham (Postgraduate Symposium) – One first place prize

- Two poster presentations (2017, 2018) at BGS postgraduate science festivals (BUFI) - One first place prize

- Soapbox Science, Manchester Blue Dot Festival 2018

- Radio interviews – following soapbox science on local radio station and Zambia local radio station at SEGH conference