The potential of bats as bioindicators for areas currently transformed by opencast mining within the Vhembe Biosphere Reserve, Limpopo Province, South Africa.

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"Anyone who has spent some time in an enclosed space with an excited bat knows what it is to encounter a fundamentally alien form of life" – Thomas Nagel (1974)

Dedications

I dedicate this thesis to my incredibly supportive and encouraging family whom nurtured my passion for nature from a young age and have watched it grow into a healthy obsession; my parents Yvonne and Cedric Cory Toussaint, and my uncle, Denis Cory Toussaint who encouraged my curiosity, to my sister Nancy Barber who tolerated my “weirdness” and grew to be intrigued by it, thank you! To my loving partner Daniel Booyens, thank you for remaining unwavering by my sideand your encourement and belief in me.

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And last but not least, to the incredible bats that have been a great source of fasciation and wonder for many years and hopefully for many more years to come.

How magnificent, magical, marvellous and fascinating the world of bats!
Rhinolophus smithersi (Taylor, Stoffberg, Monadjem, Schoeman, Bayliss and Cotterill 2012)
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Declaration

I, Dawn Cory-Toussaint, hereby declare that the thesis for the degree of Doctor of Philosophy in Mathematical and Natural Sciences at the University of Venda, hereby submitted by me, has not been previously submitted for a degree at this University or any other University, that it is my own work in design and execution and that all referenced material contained therein has been duly acknowledged.

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Publication timeline and disclaimer

Kindly note that chapters 2, 3 and 4 of this dissertation have been compiled as stand-alone papers, thus repetition to a degree was inevitable. The following chapters have been submitted or are in preparation for submission for publication in peer reviewed journals:

Chapter 2: Cory Toussaint, D. and Taylor, P. J. Bats and diamonds: bioindicators of disturbance due to an opencast diamond mine, Northern Limpopo, South Africa.

Chapter 3: Cory Toussaint, D. and Taylor, P. J. Artificial lighting, anthropogenic noise and vegetation cover impacts bat communities on opencast mines in sub-tropical regions: a case study in Africa. (*In Preparation*)

Chapter 4: Cory Toussaint, D, Taylor, P. J., Anderson-Small, C. and Barnhoorn, I. E. J. Non-invasive sampling of bat reflects their potential as ecological indicators of heavy metal and trace metal contamination due to open cast diamond mining.

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Summary

Bats are the second most diverse order of mammals and provide crucial ecosystem services from insect control (including significant crop pest species), to pollination of plants and seed dispersal. Bats are known to be sensitive to alterations in their environment. Globally, bat populations and communities are under pressure due to anthropogenic activities that alter their natural habitats creating unfavourable habitats for some bat species and favourable conditions for others. Anthropogenic activities, impose a multitude of impacts on bats such as artificial lighting throughout nocturnal periods and excessive noise during both diurnal and nocturnal periods. These alterations of habitats affect bat roosting and emergence behaviour, disrupt commuting routes to foraging grounds and alter interspecific competition. Additionally, bats are exposed to and suffer physiological issues relating to contaminants such as heavy metals and trace elements of which their liberation is exacerbated by human activities.

African bat conservation is already under considerable pressure from the development occurring throughout the continent. A looming development of significant concern is the proposed African Mining Vision to develop mining and transport networks throughout sub-Saharan Africa. The fate of bat conservation in Africa is dependent on bat taxonomy (with new species regularly described), an understanding of African bat ecology and the responses of bat species to changes in their environment. Research covering these aspects are greatly needed in order to propose environmental mitigation and remediation strategies. Bats have the potential as bioindicators to provide information concerning ecosystem health pre-, during and post-large scale developments such as mining.

My study focused on how a bat community in northern Limpopo Province was impacted by the de Beers Venetia opencast diamond mining operation. I investigated which bat species have the potential to be bioindicators based on the impact of habitat degradation (Chapter 2). The impact of artificial light and anthropogenic noise was explored (Chapter 3) and the use of bat fur and blood as biomarkers of bat exposure to heavy metals and trace elements (Chapter 4). Bats have the potential to be good bioindicators as they are easy to monitor in areas like mines that are often a challenge to access and or have high safety risks due to heavy machinery movement and human exposure to possible environmental contaminants. By using passive acoustic recording techniques, the composition and activity of the bat community was determined on the Venetia diamond mine and over the natural areas neighbouring the mine. Six bat detectors were deployed for a full summer and winter season to determine what factors (both anthropogenic and natural) influenced total bat activity as well as species richness and diversity. The effect of habitat type (natural habitats Musina Mopane Bushveld and Limpopo Ridge Bushveld vs altered landscapes of the wastewater dam and opencast pit), season, minimum temperature (T_{min}) and moon phase were investigated. Habitat type and season were shown by the best-fit models to be the main factors affecting bat activity, which was lower during winter and consistently lower over the heavily disturbed opencast mining pit. It was evident from the passive acoustic recordings of echolocation calls that the Venetia diamond mine was an important resource for numerous bat species, particularly at the wastewater dam, boasting ~18 out of 22 species/species groups. The wastewater dam provided a sought after resource for foraging and possibly drinking by bats. The infrastructure of the mine was also observed to be used by molossid bats as roosting opportunities. Based on the clear-cut response of three species/species groups to habitat type, *Laephotis capensis/Pipistrellus (Vansonia) rueppellii*, *Afronycteris nana* and *Pipistrellus rusticus/Neoromicia anchietae* were proposed as bioindicators on the Venetia diamond mine. All three species/species groups could be environmental indicators which means that they respond to physical changes in the environment, in this case, changes in preferred habitat. *Laephotis. capensis/P. rueppellii*

generally avoided the mining footprint and were abundant in the natural areas whereas *A. nana* and *P. rusticus/N. anchietae* were prolific over the wastewater dam, particularly during winter. The response of *A. nana* and *P. rusticus/N. anchietae* may be a reflection of changes in insect species composition over the wastewater dam in relation to potential changes in water quality and as such, have also been suggested as ecological indicators as they may be reflecting the change in another taxon or group (invertebrates). Further research in this regard is required. Bat species were characterised as exploiters and adapters to resources provided by the mine such as new artificial roosting opportunities in infrastructure and resource creation, particularly artificial water sources that would otherwise be scarce for bats in the semi-arid environment. Examples of these exploiter and adapter species were *Chaerephon pumilus/Mops condylurus* that exploited resources provided by the mine from roosting in buildings to using the wastewater dam, and *P. hesperidus* as an adapter that used the wastewater dam as an important resource particularly during winter and was only recorded in natural areas during summer but is not known to roost in the infrastructure of the mine.

Even though the mining activities provided resources, bats were exposed to continuous anthropogenic noise and artificial light during their night-time foraging period. To investigate the impacts of anthropogenic noise and artificial lighting, a transect was installed on the Venetia diamond mine using six SM4BAT acoustic recorders (bat detectors) (Wildlife acoustics, Inc.) to passively record bat species and their associated activity. The transect began in a brightly lit area near some workshops and a conveyor belt system and extended past the well-lit and noisy processing plant, into an open-air laydown storage area and terminated in natural vegetation. Surprisingly, according to mixed-effects models, in general, anthropogenic noise did not have a significant impact on bat activity or species richness. Artificial light only negatively impacted foraging activity and there was little evidence of bats foraging under the floodlights near the workshop and processing plant. Artificial light may possibly impact total bat activity and searching/commuting behaviour. In support of the data concerning the lowest bat activity and species richness over the habitat devoid pit of the mine, the percentage of natural vegetation cover was the most important factor influencing all bat activity and species richness.

With significant bat activity recorded over the mine wastewater dam, there is potential for bats to be exposed to high concentrations of contaminants, particularly heavy metals and trace elements that would otherwise be naturally occurring at lower concentrations in the environment. Therefore, I investigated the potential bioaccumulation of elements in molossid bats from the mining footprint and in a control area adjacent to the mine. There is great interest in using bats as bioindicators of environmental contaminants and with a focus on non-lethal and less invasive methodologies, the fur and blood of several *Tadarida aegyptiaca* and *C. pumilus/M. condylurus* was tested for 23 elements. Fur is a good indicator of exposure to elements over an extended period and blood provides insight into more recent exposure. Fifteen of the 22 elements (barium excluded as it was predominantly below detection limits), were higher in the fur that in the blood samples. Concentrations of boron (B), potassium (K), rubidium (Rb) and cadmium (Cd) in fur and zinc (Zn), and mercury (Hg) in blood were significantly higher in samples from the opencast diamond mine compared with the control site. Manganese (Mn) was higher in the blood of bats from the control site than from the mine. Although weak, the only significant correlations between fur and blood element concentrations were found for Hg and strontium (Sb). Comparative data with other bat species is scant in the literature and there are currently no toxicological thresholds for elements in bats, thus the low concentrations of most elements, except aluminium (Al), iron (Fe) and Zn, may reflect background values. These data are the first presented to compare blood and fur elemental concentrations of South African bats the implications of which are currently unknown.

These data presented in my thesis has great potential to add to the environmental monitoring of the Venetia diamond mine by providing insight into trends in the bat population (including species richness) that utilise the mine associated (drinking and foraging areas over the waste water dams, and roosting opportunities in mine infrastructure). These data can be used as a baseline to monitor the current and future environmental impacts of the mine as production continues and, can be used to monitor and assist rehabilitation efforts soon to be underway.

Chapter 1 - The importance of intact ecosystems and bats in a changing environment

Man's insatiable need and action to develop, produce and consume is altering landscapes and threatening global ecosystems whether it be developments for living (Esch et al. 2017), food production and mining or industry (Kunz et al. 2011). The plethora of unsustainable practices and activities place natural ecosystems under considerable pressure (Lenoir and Svenning 2014, Ancillotto et al. 2016, Anderson et al. 2017, Suarez-Rubio et al. 2018). The results of which include biological community reshaping, threatening biodiversity and disrupting ecosystem services (Bowler et al. 2020). Ultimately, these alterations to the environment and disruption of ecosystems is to our detriment. Healthy ecosystems provide regulatory processes of: water purification, disease mitigation, binding of toxic substances, climate regulation, flood mitigation, pollination, insect population suppression and seed dispersal and supporting processes of: soil formation, nutrient cycling and primary production (Kunz et al. 2011). There is growing interest to re-establish, preserve and sustainably use the environment as the greater public 1) become more aware of their impact on the environment, 2) recognise and 3) appreciate the value of natural spaces from a cultural and aesthetic perspective, and understand the economic value of ecosystem services provided to agriculture and forestry by functional natural ecosystems (Macdonald and King 2015, Anderson et al. 2017, Krauze and Wagner 2019). For example, agroforestry has been shown to be beneficial for soil conservation and enrichment, increased water and air quality, increased carbon sequestration, corridor provision between habitat remnants support biodiversity conservation and maintain integrity of habitat patches, and sustaining local rural livelihoods (Jose 2009, Maas et al. 2013). We are becoming more aware of the detrimental impacts of our actions and the response of organisms concerning habitat encroachment and degradation, terrestrial and aquatic pollution (chemical, noise and light), emergent diseases (including zoonotic diseases with COVID-19 currently at the forefront) and climate change (Bowler et al. 2020). Bats are a great taxon to study in relation to anthropogenic impacts for a variety of reasons.

As with any taxon, a major threat is human-wildlife conflicts that are fuelled by misconceptions, misunderstandings, lack of information and negative social media reports. Bats have been and continue to be persecuted in this regard, particularly from the negative attitude towards to the role of bats in spreading disease. These attitudes have resulted in increased bat evictions from buildings and even governments ordering mass cullings to prevent crop losses and the spread of disease to domestic animals and humans (Frick et al. 2020, Lu et al. 2021). For example, over a third of the population of the endemic flying fox, *Pteropus niger,* in Mauritius was annihilated by an order given by government based on the misconception on the extent of bat damage to litchi crops and associated financial losses (Frick et al. 2020). Disease transmission is not always reduced through culling (legal and illegal) (e.g. rabies transmission by *Desmodus rotundus,* Streicker et al. 2012) and in some instances, results in an increase in the prevalence of a disease (Frick et al. 2020). A pilot study in China conducted by Lu et al. (2021), primarily on students, showed that the attitude towards bats concerning COVID-19 was highly negative. The amount of an individual's bat knowledge, gender and education level were significant in determining the attitude of people towards bats (Lu et al. 2021). However, even people who claimed to have a lot of knowledge about bats had a slightly higher negative attitude towards bats even before the outbreak and that number of people increased after the outbreak (Lu et al. 2021). After the COVID-19 outbreak, the increased negative attitude of people was addressed through educational presentations in order to provide clarity on the environmental importance of bats and their role in COVID-19 (Lu et al. 2021). Unfortunately, the lectures failed to change the misconception that bats transmit COVID-19 directly to humans. A major positive though was that in general, participants highly disagreed with consuming bats as bushmeat, disagreed with ecological and scientific culling of bats and agreed with the conservation of bats (Lu et al. 2021). This highlights the importance of public awareness and bat educational outreach programs.

Why should we give a squeak about bats? The importance of bats in ecosystems
The order Chiroptera is the second most diverse order of mammals containing more than 1400 species, with 77 species listed as endangered on the IUCN Red Data List (Riccucci and Lanza 2014, Frick et al. 2020, Fenton et al. 2020). New bat species continue to be discovered and described such as *Murina rongjiangensis* sp. nov, China (Chen et al. 2017), *Glauconycteris atra* sp. nov, Congo (Hassanin et al. 2018), *Myotis crypticus* sp. nov, Spain (Juste et al. 2018), *M. zenatius* sp. nov, Morocco (Juste et al. 2018), *Pteronotus alitonus* sp. nov, Brazil (Pavan et al. 2018), *Miniopterus nimbae* sp. nov, Liberia (Monadjem et al. 2019), *M. armiensis* sp. nov, Panamá (Carrión-Bonilla and Cook 2020) and recently *M. nimbaensis* from Guinea (Simmons et al. 2021). Bats occur on every continent except Antarctica and are found in most habitats excluding extreme desert and polar habitats (Monadjem et al. 2020). Bats boast a variety of diets and are therefore crucial to ecosystem functioning and benefit humans through ecosystem services they provide such as insect control, pollination and seed dispersal (Blehert et al. 2009, Kunz et al. 2011, Ricci and Lanza 2014, Muylaert et al. 2016, Fenton et al. 2020, Lu et al. 2021).

Seventy percent of all bat species are insectivorous (Riccucci and Lanza 2014) and thus their assistance with natural arthropod population suppression (Kunz et al. 2011, Suarez-Rubio et al. 2018), particularly of crop pest species, has become an ecosystem service provision of great interest (Riccucci and Lanza 2014, Wanger et al. 2014). In some cases bats provide an annual crop service of billions of dollars in the United States of America (Boyles et al. 2011, Frick et al. 2020). For example, *T. brasiliensis* can provide savings between 2–29% of the US$6 million annual cotton crop in the Winter Garden region (Cleveland et al. 2006). In South Africa, bats have been shown to provide economic savings between 0.53–1.29% of the annual value of macadamia crops (Taylor et al. 2018). The added benefit apart from the reduced cost for pesticides is the reduced risk of pest insect species developing pesticide resistance (Riccucci and Lanza 2014). Biocontrol by bats and birds on the eastern Soutpansberg (Limpopo Province, South Africa) in macadamia orchards against green vegetable bug, litchi moth, macadamia nut borer and two-spotted stink bugs far outweighed the losses due to raiding Vervet monkeys, *Chlorocebus pygerythrus* of approximately ~US$1600 ha^{-1}.yr^{-1} (Linden et al. 2019). Bats and birds could provide farmers with savings of around US$5000 ha^{-1}.yr^{-1} (Linden et al. 2019). In this agricultural system, close proximity of orchards to natural forest patches resulted in the highest provision of ecosystem services by birds and bats (Linden et al. 2019). Crucial habitat is being lost on a yearly basis as ~2000 ha is cleared for additional macadamia orchards resulting in a reduction in ecosystem service value provided by natural fauna (Taylor et al. 2018). The Wrinkled-lipped bat (*T. plicata*) in Thailand contributes to food security for ~26 200 people.yr^{-1} by feeding on white-backed plant hoppers (*Sogatella furcifera*) that cause rice yield losses up to 60% (Wanger et al. 2014). Small colonies of ~300 *T. plicata* living in bat boxes could improve local rice production by protecting ~657kg.yr^{-1} with an approximate saving of US$276 per roost box.year^{-1}. Similarly, bats and birds have been shown to be important pest control agents in Indonesian cacao plantations, which in their absence, resulted in a crop yield decrease of 31% (Maas et al. 2013).

Frugivorous and nectarivorous bat species have been shown in conjunction with birds to be important dispersers of seeds, particularly of pioneer plant species aiding in the early establishment of trees in tropical regions (Duncan and Chapman 1999). Interestingly, bats appear to be more likely to distribute seeds in open areas devoid of trees whereas bird seed dispersal was more evident where trees were already established (Duncan and Chapman 1999). Over 528 species of plants in 67 families and 28 orders have been shown to predominantly rely on bats for pollination (Sritongchuay et al. 2019). Certain plants in Thailand that have a close association with bats place pollen on specific parts of the bat (differential pollen placement) to lower the risk of pollen transfer from other species of plant (Stewart and Dudash 2017). Pollination of the major Mexican cash crop, *Stenocereus queretaroensis* by nectarivorous bat species from the genus *Leptonycteris*, produce higher yields and quality of fruit (pitayas) (Tremlett et al. 2020). When the bats were excluded from pollinating these plants, the yield decreased by 35% and the fruit quality decreased by 46% which has substantial market related value consequences (Tremlett et al. 2020).

A comprehensive review of services provided by bats was compiled by Kunz et al. 2011 detailing not only the above ecosystem service provisions but also:

1. The importance of bats in the redistribution of energy and nutrients in terrestrial (including caves) and aquatic ecosystems through bat guano.

2. The medicinal uses of bats to treat numerous ailments from baldness to paralysis and the actual potential of *Desmodus rotundus* saliva in the treatment of strokes.

3. The cultural and historical value of bats as symbols and heirlooms in ancient Japanese, Egyptian, Mayan and Chinese artefacts and paintings. Museum displays of these artefacts and the sale of associated souvenirs brings in revenue.

4. The use of bats as food and in sorcery, and,

5. The aesthetic value of bats through nocturnal bat tours in National parks, cave visits, and educational nature programs. In South Africa, the Gauteng and Northern Regions Bat Interest Group and the Bat Interest Group of Kwa-Zulu Natal host public bat walks in nature reserves, at local zoos, lead outings in interesting bat areas and host bat workshops to share knowledge about bats (e.g. rehabilitation: Bat Interest Group of Kwa-Zulu Natal).

Bats in an ever changing environment

Bats are known to be sensitive to changes in their environment and due to their low reproductive rates, bat populations tend to take a long time to recover after a decline (Jones et al. 2009). Bat population declines across North America and Europe have been attributed to urbanization, agricultural intensification, changes in resource quantity and quality (available roosts, food and water), climate change, increasing number of wind turbines, disease pressure (white-nose syndrome) and exposure to chemicals through activities linked with historical and current anthropogenic developments (pesticides, waste water treatment plants and organochlorines) (Clark 1981, Naidoo et al. 2013, Bayat et al. 2014, Afonso et al. 2016, Ancillotto et al. 2016, Hernout et al. 2016a, de Souza et al. 2020). The sensitivity of bats to pesticides, such as the organochlorine DDT was identified in the early 1960's resulting in the understanding that bats could face significant fatalities when these pesticides were applied to agriculture and assimilated up the food chain to bats (Clark 1981).

It is well documented in the literature that bats responses to anthropogenic changes in the environment and climate change vary between taxa and species. Species either avoid, utilise or adapt and may even become locally extinct resulting in changes in the composition of animal communities, their associated intraspecific and interspecific interactions, ecosystem functionality and thus conservation strategy (Legakis et al. 2000, Gehrt and Chelsvig 2003, Threlfall et al. 2012, Urban et al. 2012, Urban et al. 2013, Lenoir and Svenning 2014, Nagendra et al. 2014, Ancillotto et al. 2015, Fischer et al. 2015, Treitler et al. 2016). Anthropogenic changes to the environment can be beneficial for some species and detrimental to others. Community structure and functionality can be altered as adaptable species may outcompete more sensitive species or species with similar biology and behaviour, or experience significant range expansions (Hersteinsson and MacDonald 1992, Ancillotto et al. 2016, Urban et al. 2012). Changes in land use and the associated impacts on bats of artificial lighting, noise and exposure to pollutants will be explored further as these impacts are the basis of this PhD thesis.

Changes in land use

Bats can be broadly classified into three categories based on their response to anthropogenic developments. Bat species that make use of foraging and roosting opportunities created by humans are known as "urban exploiters" e.g. Molossids (free-tail bats) (Jung and Kalko 2011, Schoeman 2015). Urban adapters are species that make use of favourable conditions created by humans e.g. *Afronycteris nana, L. capensis, Pipistrellus hesperidus/N. anchietae* (Naidoo et al. 2015, Schoeman 2015). Bat species that avoid anthropogenic developments based on their extreme sensitivities to changes in the landscape such as Rhinolophids and Nycterids, are called urban avoiders (Threlfall et al. 2012, Schoeman 2015).

Pipistrellus kuhlii (Kuhl's pipistrelle) in temperate regions has been shown to benefit from developing urbanisation by producing more pups than conspecifics in rural areas (Ancillotto et al. 2015). Additionally, climate change was recognised as a major driving force behind the significant 394% range expansion of *P. kuhlii* (Ancillotto et al. 2016). Urbanisation in Calgary (Alberta, Canada) negatively affected species richness of prairie bat but positively increased the abundance of the bat species with *M. lucifugus* dominating the activity (Coleman and Barclay 2012). Similarly, in South Africa, the abundance of bats was significantly higher over rural villages than agricultural areas and rangelands with different bat species dominant in different villages (Foord et al. 2018). The observed increase in bat abundance may have been supported by an increase in insect activity and roosting opportunities in the villages (Foord et al. 2018). Diverse bat assemblages, including threatened species, are supported in urban areas in Sydney, Australia, but conversely to Coleman and Barclay (2012), bat activity in the urban Sydney landscape was lower compared to agricultural and forested areas, thus the urban landscape had a strong negative effect on bats (Threlfall et al. 2012). Treitler et al. (2016) showed that in Germany, the interaction between bats and insects was weakened by a combination of local land-use intensification and the loss of forest remnants. As land-use intensity increased insect abundance also increased but the size and diversity of insects decreased and with it, a decrease in bat feeding activity (Treitler et al. 2016). Nine species of Microchiroptera (Vespertilioniformes) and one species of Megachiroptera (Pteropodiformes) have become extinct due extensive land transformation of native forest in Singapore (Lane et al. 2006). Many more bat taxa are expected to become extinct as the heavy deforestation in Southeast Asia continues (Lane et al. 2006). Sometimes bats seem to benefit from changes in land-use, but if this is associated with poor quality resources, for example, foraging over polluted water/land resources, or be more susceptible to predation, it may affect productivity.

Artificial lighting

With urbanisation and anthropogenic developments comes a suite of pollutants that impact on organisms in numerous ways. Ecological light pollution (waste light from anthropogenic developments and activities) or artificial light at night (ALAN) has been largely underemphasised concerning anthropogenic developments, conservation strategies and environmental management (including biodiversity management) (McNaughton et al. 2021). Street lighting is responsible for a large percentage of ecological light pollution (Riley et al. 2013, Lewanzik and Voigt 2014). With a global annual increase in artificial lighting of approximately 6% (Stone et al. 2015, Lacoeuilhe et al. 2014), the impact of artificial light has become a significant global concern (Davies et al. 2012, Gaston and Bennie 2014). As underlined by many studies, ecological light pollution disrupts time measurement by interfering with circadian, seasonal and lunar cycles, alters primary productivity, impacts physiological function recovery and repair (cellular level), alters the recognition of resources and predators, affects temporal niche portioning (diurnal animal activity extending into the night), impairs navigation and causes disorientation (Rowse et al. 2016, Geipel et al. 2019). These impacts affect a variety of taxa including, plants, invertebrates, fishes, crustacean zooplankton, birds, amphibians, reptiles and many mammalian species that could lead to detrimental impacts on biodiversity and ecosystem functioning over time (Moore et al. 2000, Perry et al. 2008, Kempenaers et al. 2010, Davies et al. 2012, Riley et al. 2013, Gaston and Bennie 2014, Minnaar et al. 2014, Stone et al. 2015, Wakefield et al. 2017, Cabrera-Cruz et al. 2018, Owens et al. 2019, Singhal et al. 2019).

Particularly in urban developments, based on the wavelengths of light energy emitted, some types of lighting have been shown to be more harmful than others, particularly to insects (Spoelstra et al. 2017, Longcore et al. 2018, Firebaugh and Haynes 2019). Low-pressure sodium lamps (LPS) and high-pressure sodium lamps (HPS) have highest luminous efficiency, do not emit ultraviolet (UV) light and are predominantly used for street lighting (Riley et al. 2013, Stone et al. 2015, Lewanzik 2017). Low pressure sodium lights produce a monochrome orange light that peaks at 589.3nm and hardly allows the human eye to identify colour. High pressure sodium lights produce a range of wavelengths, allowing for a degree of colour identification and are used more often than LPS lights for street lighting (Stone et al. 2015, Lewanzik 2017). High-pressure mercury (MV) lights and metal-halide (MH) lights emit a multitude of wavelengths that allows for good colour perception, but also emit UV and a high amount of waste light into the environment (Falchi et al. 2011, Lewanzik 2017). There is a movement in cities to replace old streetlight technology with more advanced energy efficient lighting technologies such as broad spectrum light emitting diodes (LEDs) (Gaston et al. 2012, Jin et al. 2015, Lewanzik and Voigt 2017, McNaughton et al. 2021). The benefits of using LED lighting, apart from their reduced energy consumption, is that they can be dimmed and easily managed from a central management system (Kim and Schubert 2008, Gaston et al. 2012). Research on the effects of LED lighting is greatly needed (Stone et al. 2012). The responses of organisms to changes in street lighting from HPS to LED appears to be species specific and location specific with few direct negative impacts (that can be mitigated) on organisms in urban settings (McNaughton et al. 2021).

Research has shown that all types of conventional lighting can impact bats over large and fine scales in well-lit environments (Stone et al. 2009, Minnaar et al. 2014, Lewanzik and Voigt 2014, Rowse et al. 2016). Light sensitivity is generally species-specific (Eklöf 2003, Rowse et al. 2016). It is accepted that as light intensity increases towards daylight, bats light-sensitive eyes decrease in visual sensitivity (Fure 2006, Liu et al. 2015). The flow of information to organisms can be disrupted by artificial light and provides misleading clues resulting in a reduction of individuals emerging from roosts, delays bat emergence from roosts, affects navigation and commuting behaviour, disturbs foraging behaviour, and may even decrease the growth rate of young bats if adults have to forage further afield from maternity roosts and incur higher energetic losses and decreased foraging time (Boldogh et al. 2007, Stone et al. 2009, Gaston et al. 2013, Minnaar et al. 2014, Lewanzik and Voigt 2017).

Some bat species appear to benefit from artificial lighting through increasing their foraging efficiency by identifying and exploiting insects swarming around lights and are referred to as light-tolerant (Lewanzik and Voigt 2014, Rowse et al. 2016,). The swarming behaviour of insects around artificial lights is due to insects being attracted to all types of conventional lighting, although they are attracted to certain types of lighting more so than others (Gaston et al. 2013, Rowse et al. 2016). Open-air and clutter-edge forager bat species have echolocation calls adapted for open and semi-open habitats and are often observed foraging around artificial light sources in open habitats apparent around artificial lighting, and are referred to as light-tolerant (Rowse et al. 2016). At artificial light sources, interspecific competition for food resources within the same lit area can arise caused by the extension of diurnal species foraging activity into the night-time period (e.g. birds) (Longcore and Rich 2004, Lewanzik and Voigt 2014). There is evidence that some clutter-edge forager species do make use of the foraging opportunities created by artificial lighting, namely *Eptesicus nilssonii, E. serotinus, Vespertilio murinus, P. kuhlii, P. pipistrellus, Hypsugo savii* and *Nyctalus leisleiri* (Rydell 1992, Azam et al. 2016, Azam et al. 2018, Russo et al. 2019). Whereas other species, such as *M. daubentonii*, which is considered as one of the most sensitive species to lighting, foraging activity decreases under lit conditions, but was not affected by artificial light when the bats were commuting (Spoelstra et al. 2018, Russo et al. 2019). In a study undertaken in 1952 by W. E. Curtis (reported by Eklöf 2003), *E. fuscus* was shown to exhibit brightness discrimination (being able to search for and find food under illuminated conditions) at a light intensity of 10 lux (the light level at dawn and dusk) and remained very good in conditions as low as 0.001 lux (Eklöf 2003).

Many bat species appear to be intolerant of light and avoid lit areas, particularly species of the genera Rhinolophus and Nycteris, which are slow flying highly manoeuvrable species, adapted for foraging in cluttered environments, which tend to emerge from their roosts in complete darkness (Schoeman 2015, Lewanzik and Voigt 2014). Due to the physical sensitivity of their eyes to light (and ultra violet), bats may experience potential vision impairment under lit conditions (Jones et al. 2009, Lewanzik 2017). Artificial lighting can create “barriers” that may limit the effective dispersal of species, isolating habitat patches and populations from immigration and reduce the connectivity of habitats in the landscape e.g. *Rhinolophus hipposideros* (Stone et al. 2009, Gaston and Bennie 2014). The disruption of commuting routes and fragmentation of habitats known to be caused by traditional streetlight lighting remains an issue concerning LED lighting for light sensitive species *Rhinolophus hipposideros* and *Myotis* spp. where low lux levels of 3.6 were avoided (Stone et al. 2012).

Bats may also perceive a higher predation risk (Minnaar et al. 2014, Spoelstra et al. 2017). Species, particularly clutter foragers, may avoid lit areas since lighting is usually placed in open or semi-open habitats that are generally not favoured by clutter forager bat species as their echolocation call structure is not well suited for foraging in open and semi-open habitats created around artificial lighting (Rowse et al. 2016). Artificial lighting can also disrupt ecosystem services provided by frugivorous bat species that avoid lit areas e.g. *Carollia sowelli* (Sowell’s short-tailed bats) avoided foraging in artificially lit areas resulting in fewer fruits of pepper plants (Piperacea) harvested and consumed, reducing the probability of seed dispersal of these plants (Lewanzik and Voigt 2014).

Natural and anthropogenic noise

Anthropogenic noise is an emerging pollutant that is gaining more attention in terms of species sensitivity to noise, resulting physiological and ecological impacts and implications for biodiversity conservation (Bunkley and Barber 2015). Natural abiotic (weather, vegetation, moving water etc.) and biotic noise (conspecifics, heterospecifics, potential predators and prey) are important auditory cues for animals (Gallego-Abenza et al. 2019). Emerging *Micronycteris microtus* and *Molossus molossus*, rely on the sound of rain, together with increased humidity, decreased barometric pressure and odour changes, as an important auditory cue concerning the costs and benefits in delayed roost emergence (Geipel et al. 2019). In naturally noisy environments, some animals are flexible in their signal production and response (Gallego-Abenza et al. 2019). Native Australian frogs, *Limnodynastes convexiusculus* and *Litoria rothii*, had two different responses when exposed to the call of the invasive cane toad, *Rhinella marina*, which breed in the same ponds as the native Australian frogs. *Limnodynastes convexiusculus* adjusted their calling rate and the interval between calls in order to exploit the gaps in the noise from the cane toads whereas, *L. rothii* significantly reduced their calling rate in order to possibly conserve energy during the noisy periods (Bleach et al. 2015).

The impact of anthropogenic noise has been studied in a variety of terrestrial (e.g. birds, insects, bats and other small mammals) and aquatic organisms (e.g. marine mammals, crabs, mussels, frogs) (Melcón et al. 2012, Hotchkin and Parks 2013, Kruger and Du Preez 2013, Shannon et al. 2016, Gallego-Abenza et al. 2019, Tidau and Briffa 2019, Nagelkerken et al. 2019, Wale et al. 2019, Eastcott et al. 2020, Halfwerk and van Oers 2020, Sathyan and Couldridge 2020). A caution is highlighted that predicting responses of species to anthropogenic noise will be difficult as commonalities in responses to noise may not exists even between closely related species (Francis et al 2011).

Severe fitness consequences can result from exposure to anthropogenic noises that disrupts information transmission between individuals and erroneous interpretation of auditory cues. The results of which can lead to stress (e.g. European sea bass), unnecessarily aggressive behaviour, selection of poorer quality mates, missed foraging opportunities, perceived predation threats and reduced attention that can lead to increased predation (Brown et al. 2012, Hage and Metzner 2013, Hotchkin and Parks 2013, Luo et al. 2015, Gomes et al. 2016, Manson et al. 2016, Spiga et al. 2017, Gallego-Abenza et al. 2019, Geipel et al. 2019, Eastcott et al. 2020, Halfwerk and van Oers 2020).

Bats are auditory specialists with the highest sensitivity to sounds generally within their species-specific call spectral range (Bohn et al. 2006). Potential conflicts in the perception of auditory cues can hinder bat foraging success in anthropogenically noisy environments if anthropogenic sounds overlap with the spectral ranges of bats echolocation calls or the sounds produced by prey (acoustic masking) that bats use to locate and capture prey (Simmons et al. 1978, Schaub et al. 2008, Luo et al. 2015, Gomes et al. 2016). In response to noise stimulus, *M. daubentonii* did not experience acoustic masking nor reduced attention but rather avoided the noise stimulus that resulted in reduced foraging success (Luo et al. 2015). Sensitivity to noise studied in the laboratory was shown to differ even between conspecifics of *M. daubentonii* with a single individual completely unaffected by the noise trials (Luo et al. 2015). *Antrozous pallidus* has been shown to increase their searching time under anthropogenically noisy conditions that could result in decreased fitness or survival in the wild as energy budgets might not be met during the night time foraging period (Bunkley and Barber 2015). Free-living bat communities in England are negatively impacted by traffic noise (playback) with a significant reduction in feeding behaviour particularly in *P. pipistrellus* and *P. pygmaeus* (Finch et al. 2020). In some instances, bats habituate to noise. Resting (torpid) *M. myotis* habituated quickly to anthropogenic noise (Luo et al. 2014). However, during their active period, *M. myotis* actively avoided foraging areas that were heavily degraded by noise or experienced a significant reduction in foraging efficiency when exposed to traffic noise (Schaub et al. 2008, Siemers and Schaub 2011). Similar adverse effects of anthropogenic noise and avoidance behaviour is expected by bat species that relay on passive listening for prey capture namely: *M. blythii, M. evotis, M. septentrionalis, Euderma maculatum,* and species from the genera *Plecotus* and *Corynorhinus* (Schaub et al. 2008, Bunkley and Barber 2015). It is evident that bats responses to anthropogenic noise is species-, individual- and behavioural context specific (Schaub et al. 2008, Luo et al. 2014, Luo et al. 2015).

Exposure to contaminants: Heavy metals and trace elements

Heavy metals have been characterised by having an atomic density that is usually equal to or greater than five times the density of water or alternatively, have a density ≥ $3 g.cm^{-3}$ and can be toxic even at low concentrations (Nagajyoti et al. 2010, Bánfalvi 2011, Tchounwou et al. 2012, Gall et al. 2015). Although debatable concerning the definition based on relative weights, the term "heavy metal" is closely associated with elements that have the potential to be toxic and or result in harmful biological and ecological effects in animals and plants for example: arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), mercury (Hg), manganese (Mn), molybdenum (Mo), nickel (Ni), vanadium (V) and zinc (Zn) are considered heavy metals (Bánfalvi 2011, Singh et al. 2011, Nagajyoti et al. 2010, Zocche et al. 2010). Elements that are present in minute quantities are known as trace elements (Bánfalvi 2011). Some trace elements are heavy metals such as Cu, Fe and Zn, and there are those that are not, such as boron (B), aluminium (Al) and magnesium (Mg) (Bánfalvi 2011). Heavy metals and trace elements can be broadly classified into two groups: 1) essential elements vital for physiological and biochemical processes within plants and animals but become toxic at certain concentrations or at concentrations higher than what is physiologically required by an organism (Aggett 1985, Nagajyoti et al. 2010, Bánfalvi 2011, Jakimska et al. 2011, Tchounwou et al. 2012, Flache et al. 2018) and 2) non-essential elements that have no physiological role or function (Reis et al. 2010, Ferrante et al. 2018). From this point forward, heavy metals and trace elements will be referred to collectively as elements.

Concentrations of elements released from natural sources depends on the release process. Elements released by the formation of soil depends on parent rock material, environmental conditions and the weathering process are usually present in low concentrations whereas high concentrations of elements are rapidly released during volcanic eruptions (Nagajyoti et al. 2010). Sea sprays and associated oceanic aerosols have been shown to transport heavy metals inland (Nagajyoti et al. 2010, Sen and Peuker-Ehtenbrink 2012). The natural release of elements is exacerbated by anthropogenic activities that result in the accumulation of high concentrations of these elements in the soil often near the source of emission (Sen and Peuker-Ehtenbrink 2012, Flache et al. 2015, Nagajyoti et al. 2010). Some sources of anthropogenic element release are in the form of mining activities and associated transport and processing of ore (dust fallout, smelting, amalgamation processes), burning of fossil fuels, fertilizers (organic and inorganic), pesticides, continued irrigation of agricultural crops, construction activities, paper and plastic processing, wood preservation, waste water treatment plants and urban storm water runoff (Sen and Peuker-Ehtenbrink 2012, Naidoo et al. 2016, Nagajyoti et al. 2010). But, even remote areas do not escape metal contamination as some metals emitted through anthropogenic activities and volcanic eruptions like mercury, are able to be transported over vast distances in the atmosphere and contaminate areas far from the emission point (Flache et al. 2018, Nagajyoti et al. 2010). Terrestrial and aquatic (marine and freshwater) ecosystems are heavily impacted by certain elements that persist in the environment due to long half-lives or do not degrade, resulting in their bioaccumulation in living tissue and assimilation up food chains causing toxicity at high trophic positions (Jakimska et al. 2011, Gall et al. 2015, Bat et al. 2020).

Various element concentrations have been studied in a variety of species ranging from the study of negative behavioural, biological and reproductive effects of Fe, Cu, Zn and Cd in fruit flies (*Drosophila melanogaster)* (Bahadorani and Hilliker 2009) to Hg levels in polar bears (Basu et al. 2009). Studies have shown that effects of elements are intra- and interspecific concerning exposure, bioaccumulation, tolerance, excretion and how elements move through trophic levels (Sánchez-Chardi and Nadal 2007, Sánchez-Chardi et al. 2007, Gall et al. 2015). The effects of chronic or acute exposure to high or low concentrations of certain elements may include tissue damage, severe organ damage and dysfunction (particularly kidneys and liver as they are responsible for detoxification), hepatotoxicity due to increased free radicals, cancers and tumours, increased mutagenic activity of other toxic substances, loss of motor function, disorientation, interspecific communication disruption, reproductive interference (testicular reduction, embryo development disruption), DNA damage and repair inhibition, impairment of cellular respiration, disruption of metabolic processes, negative effects on the central nervous system (reduced concentration and learning abilities, neurodegenerative diseases), neuronal lesions, tremors and convulsions, blindness, respiratory arrest, pain, nervous depression, cellular apoptosis and necrosis, induction of anaemia, and death in animals and humans (Smith and Rongstad 1982, Hardin et al. 1998, Basu et al. 2005, Sánchez-Chardi and Nadal 2007, Zocche et al. 2010, Reis et al. 2010, Jakimska et al. 2011, Zwolak and Zaporowska 2012, Tchounwou et al. 2012, Naidoo et al. 2013 Valli et al. 2015, Lovett and McBee 2015, Morcillo et al. 2017, Bjørklund et al. 2017, Flache et al. 2018, Kern et al. 2020).

Bats can be exposed to environmental contaminants through direct exposure by drinking from contaminated water sources, and through consuming prey species that breed in contaminated bodies of water (Zocche et al. 2010). Lead, Fe, Cu, Mn, Cd, Ni and Al are toxic at low concentrations, causing gene mutations, DNA damage and cancers (Beyersmann and Hartwig 2008) but the concentration at which these elements are toxic and or fatal to bats is unknown. Heavy metal and trace element pollution can alter bat ecological, physiological and behavioural parameters such as diversity, relative abundance, population structure, flight activity, DNA damage in blood cells, changes in blood parameters, plasma glucocorticoids, activity of ligands, neurochemical alterations and the possibly suppress the immune system and result in death (Naidoo et al. 2013, Zukal et al. 2015). Zocche et al. (2010) reported preliminary results that the exposure to and resulting uptake of elements associated with coal mining such as Al, silicon (Si), Mn, Fe, Cr, Cd, Zn, Pb, Cu and Ni, resulted in DNA damage in *Eptesicus diminutus* and toxic heavy metal accumulation in *Molossus molossus* and *Tadarida brasiliensis.* They suggested that the above bat species occurring in the Carboniferous Basin of Santa Catarina could be used as bioindicators species for ecosystems that are directly and indirectly affected by coal mines to detect bioaccumulation of heavy metals (Zocche et al. 2010).

Internal organs are good biomarkers of element concentrations have been widely used due to the above reasons (e.g. livers of fish; Dragun et al. 2019, kidneys and or liver of bats; Zocche et al. 2010 and Hernout et al. 2016a, kidneys of mink and otters; Harding et al. 1998). Brain, blood, liver, kidney and whole animal sampling for the determination of biomarkers and elemental contamination in bats are highly-invasive (e.g. liver, kidney biopsies) or destructive sampling methodologies (e.g. Smith and Rongstad 1982, Naidoo et al. 2013, Flache et al. 2018). Fur is gaining attention as a good biomarker since elements are incorporated into the fur during growth as the roots are constantly in contact with bloodstream (Hernout et al. 2016a) and additionally fur also stores external airborne particles, thus external exposure and ingestion of elements could be investigated (de Souza et al. 2020). Long-term exposure to elements and element concentration at the time of tissue formation can be indicated by fur (Fraser et al. 2013, Hernout et al. 2016b). There is a current desire to use less-invasive and non-destructive methodologies to determine and monitor element concentrations in wildlife using fur (Hernout et al. 2016b, Powolny et al. 2019). Karouna-Renier et al. (2014) used blood, fur and wing punches to determine mtDNA damage associated with Hg level in these tissues in *M. lucifugus* collected near a river contaminated with Hg in north-western Virginia (South River). Although higher Hg concentrations were found in the blood and fur of the bats in Virginia than those samples from a control area in Moscow, only a weak correlation existed between Hg levels and mtDNA damage from the wing punches. An interesting observation was that even though high concentrations of Hg were present at one site (Grottoes) that presented high Hg concentrations in fur and blood samples, the level of mtDNA damage was not the highest. Samples collected from another site with a lower Hg concentration (Mt. Sydney) presented significantly higher mtDNA damage than at Grottoes. Genotypic differences, in addition to environmental and ecological factors were suggested to be responsible for the observed difference between samples from Grottoes and Mt Sydney (Karouna-Renier et al. 2014). Flache et al. (2015) used bat fur to monitor bat's exposure to potentially toxic metals in their foraging habitats in Germany and reported trace metal concentrations of Cd, Cu, Mn, Pb and Zn in fur samples collected from *Myotis bechsteinii, M. daubentonii, M. myotis* and *Pipistrellus pipistrellus.* Other studies investigating the bioaccumulation of heavy metals in bats report varying concentrations of elements in fur and other tissues and organs of bats from a variety of contaminated and uncontaminated sites (see Hickey et al. 2001).

In South Africa, data on the impact of heavy metals on bats is scant and limited to *A. nana* foraging over wastewater treatment works (WWTW) (Naidoo et al. 2013, 2015 and 2016, Hill et al. 2016). *Afronycteris nana* active over contaminated water sources of WWTW exhibited kidney, liver and DNA damage when non-essential heavy metals Ni, Cr and Cd were present in internal tissues (Naidoo et al. 2013, 2015 and 2016), changes in fatty acid profiles when exposed to a combination of pollutants which could affect energy use, mitochondrial functioning and torpor use (Hill et al. 2016) and elevated levels of arsenic in brain samples that may affect protection mechanisms and cellular processes (Hill et al. 2017). Hill et al. (2017) compared metal levels in the fur with the brains of bats as a potential, non-invasive marker.

There is still a major gap in our knowledge of how elements interact within organisms, how they are metabolised and what concentrations are "normal" or toxic with associated health impacts (disease, organ damage and failure, neurological issues etc.). Thus, the need for destructive methodologies in order to identify and understand the impact of elements on the health and function of animals still exists. The specific effects of element toxicity in wild animals is limited and it is challenging to provide a concentration range of heavy metal and trace elements that reflects "normal" conditions (Sánchez-Chardi et al. 2007). Bats is one such group where studies of bioaccumulation of elements is lacking, there is very little to no baseline data or "normal" levels to compare element concentrations to from polluted areas and there are even fewer comparative studies available concerning element concentrations in blood and fur.

Additional threats to bats and bat's potential role as bioindicators

Additional threats to bats that are just as disconcerting as the aforementioned are mortality rates in relation to White-nose syndrome (WNS) caused by *Pseudogymnoascus destructans* that was introduced to America from Europe that persists as a major issue with the initial die-off in 2006 and continued decline of some bat populations by 90% (Blehert et al. 2009, Frick et al. 2010, Frick et al. 2020, Fenton et al. 2020). Unfortunately, regardless of strong selection pressures, bats continue to return to habitats (hibernacula) with high WNS severity and lowest survival rates that exacerbate continued population declines (Hopkins et al. 2021). These declines are compounded by mortalities associated with wind energy facilities that are also responsible for large numbers of bat fatalities (mostly migratory species) due to direct collisions with turbine blades (Kunz et al. 2007, Frick et al. 2020, Lawson et al. 2020). The loss of bats from ecosystems will result in a loss of the essential ecosystem services that they provide and will result in both known (increased crop losses of certain crops and affect food security of local people) and unknown negative consequences for the ecosystems in which bats have evolved (Frick et al. 2010, Kunz et al. 2011, Wanger et al. 2014).

Monitoring of natural, transformed and areas under use or in the process of rehabilitation can be achieved using specific organisms that have close associations with either directly or indirectly with the quality of the environment for example dragonflies (Chovanec and Raab

1997) and frogs (Sisman et al. 2021). These organisms are known as bioindicators. Three categories of bioindicators are recognised based on Jones et al. (2009): 1) biodiversity indicators are species that capture the responses of a variety of species and represent species diversity and richness, 2) ecological indicators are species or assemblages that are sensitive and respond to environmental stressors that mirror the response of other taxa in the environment and 3) environmental indicators are species that respond to changes in the environment in a predictable way (Jones et al. 2009).

Bats are ideal bioindicators due to their taxonomic stability, long life expectancies, low reproductive outputs, ease of monitoring short- and long-term effects on populations, crucial ecological roles, occupancy of high trophic levels (particularly insectivorous bats) with changes in activity possibly reflecting changes of their prey groups, their sensitivity to environmental changes, co-habitation with humans thus increased exposure to chemical pollutants and disease (e.g. WNS), and global distribution allowing for the effects of environmental change to be comparable (Jones et al. 2009, Jones et al. 2013, Russo and Jones 2015, Zukal et al. 2015, Frick et al. 2016, Smith et al. 2016). In response to changes in the environment, bats are categorised into three groups based on their responses: 1) urban exploiters that make use of foraging and roosting opportunities created by humans (*C. pumilus, M. condylurus, T. aegyptiaca, Otomops martiensseni* and *Scotophilus dinganii*), 2) urban adapters that make use of favourable conditions created by humans (*L. capensis, A. nana, P. hesperidus/N. anchietae*), and 3) avoiders which are species that avoided the lit areas and were typically clutter foragers (*Nycteris thebaica* and *R. simulator*) (Schoeman 2015, Jung and Threlfall 2018).

Bats as bioindicators on the Venetia diamond mine

Situated in the semi-arid, subtropical Limpopo River Valley, the Venetia diamond mine (VDM: -22.427708°, 29.324158°) is an opencast mine that has been in operation since 1992. The climate of the areas is characterised by very dry winters and hot summers with mean annual precipitation between 300-400mm (Mucina and Rutherford, 2011). The mining footprint is situated in the Limpopo Ridge Bushveld (LRB). The mine is nestled predominantly in the Musina Mopane Bushveld (MMB) that flank the western, northern and southern borders with the LRB adjoining the eastern border (Figure 1). The vegetation structure is open woodland to savanna on poorly developed soils, as well as moderately closed shrubveld (Figure 2). The MMB (considered to be the most diverse mopaneveld type in South Africa) together with the LRB, is dominated by Mopane (*Colophospermum mopane*), Red Bushwillow (*Combretum apiculatum*) and Purple-pod Cluster-leaf (*Terminalia prunoides*) with a scattering of other iconic tree species such as Baobab (*Adansonia digitata*), Marula (*Sclerocarya birrea*) and Knobthorn (*Senegalia nigrescens*) (Mucina and Rutherford, 2011).

The mine footprint itself is situated in the Limpopo Ridge Bushveld. The natural vegetation and geological features on the operational mining footprint were removed resulting in the elimination of natural foraging areas and potential roosting sites in trees and rocky outcrops. The natural landscape has been replaced with a large open-pit, a large tailings dam to the west of the pit, numerous waste water dams and mining infrastructure e.g. offices, sorting and storage areas and mine processing plant (Figure 1). The mine is operational 24 hours a day. As a result, there is continuous noise and the active mining footprint is artificially lit with stationary and mobile floodlights for operational and security reasons during the entire night-time period.

Figure 1. The location (black dot on map insert) and Google image of the Venetia diamond mine in the northern Limpopo Valley, South Africa. The extent of the study area is bordered in red. The open circle indicates the Musina Mopane Bushveld and the open square indicates the Limpopo Ridge Bushveld

Figure 2. The typical Mopane dominated open woodland of the Limpopo Ridge Bushveld (left) and Musina Mopane Bushveld habitat types with the Venetia diamond mine in the background (right).

Research on the availability of heavy metals and the degree of environmental contamination by these elements during opencast diamond mining is scarce in scientific literature and few publications are available. Liberation of heavy metals and trace elements is a possibility during the extraction and processing of the diamond containing kimberlite rock. Although not as polluting as coal and gold mining, which are often responsible for acid mine draining (Zocche et al. 2010), the kimberlite rocks of the Venetia diamond mine belong to the Group I Kimberly kimberlites that have high yet variable trace elements within the rock (Le Roex et al. 2003, Allsop et al. 1995). These elements have the potential to be liberated as the rock is crushed to remove the diamonds. The water used during the “washing” process of the crushed rock contains incredibly fine kimberlite dust and rock fragments that is then thickened and water is recovered and recovered. The resulting sludge is stored in large open slimes/tailings dams (Morkel 2007). Within these Group I kimberlites abundances of heavy metals and trace elements have been reported by Le Roex et al. (2003) and Allsop et al. (1995): Cr: ~600–2800 ppm, Co: undetectable limits–123 ppm, V: 45–127 ppm, Sr: 0–2260 ppm, Rb: 0–158 ppm, Ni: 556–1590 ppm, Zn: 25–94ppm and Pb: 0.7–86 ppm. Open pit mines can fill with water becoming lakes with potentially toxic levels of heavy metals that animals can drink directly or assimilate through contaminated food (Zocche et al. 2010) additionally, active pits need to be dewatered into wastewater containment dams which can also expose animals to contaminated drinking water.

My PhD study addresses the impacts of opencast diamond mining on a bat community in the northern Limpopo Province, South Africa. The effects of habitat degradation, artificial light, anthropogenic noise and ecotoxicology are not mutually exclusive of each other. These effects should be considered not only the during operation phase but pre- and post- (rehabilitation efforts) mining operations as these impacts will affect bat communities in terms of species richness, abundance and individual health.

Objectives and hypotheses

The second chapter of my thesis addressed habitat degradation associated with the opencast Venetia diamond mine. Since mines are not always easily accessible and the environment on the mining footprint may not be safe to perform surveys on aquatic bioindicators (e.g. frogs and dragonflies), bats could be beneficial for monitoring the environmental impacts over time due to the ease of monitoring bats. The hypothesis was that species composition and bat activity would be negatively impacted by the degradation of the habitat on the mining footprint in comparison to the natural areas (control sites) surrounding the mine. Alternatively, bat species richness and activity may not be affected by the degraded habitat on the mining footprint. The objective was to identify species of bat that could be considered as bioindicators for monitoring ecosystem health on and around the VDM. Additionally, species were identified that readily exploit changes and associated resources in the environment as a result of the mining activities irrespective of the quality of the resources. Passive acoustic sampling using SM4BAT FS recorders (Wildlife Acoustics, Inc.) with SMM-U1 ultrasonic microphones mounted approximately 5m above the ground were used to determine bat assemblages and abundances of species in different habitat types: MMB, LRB, VDM-wastewater (tailings dam) and VDM-pit.

The third chapter focused on the potential impacts of artificial light, mining related anthropogenic noise and vegetation cover on bat activity and behaviour on the VDM. The novelty of this study was that the effects of light and noise was conducted in the field taking into consideration vegetation cover, moon phase, minimum temperature (T_{min})and season where most studies have investigated the impact of light and noise on bats separately. We investigated the impacts of artificial lighting, anthropogenic noise and vegetation cover along a gradient using six SM4BAT FS recorders (Wildlife Acoustics, Inc.) with SMM-U1 ultrasonic microphones mounted approximately 6m above the ground. The expectations were that over areas affected by artificial lighting, high noise levels and reduced vegetation cover, that firstly, bat species richness and secondly, bat activity (total activity and foraging guild activity) will be significantly lower than the dark/dimly lit, quiet, vegetated areas. Alternatively, there may be no difference in bat species richness and activity in areas affected by ecological light and noise pollution, and reduced vegetation cover. Lastly,we hypothesized that foraging activity will be higher under lit conditions due to the expected increase in insect activity around light sources compared to unlit conditions.

In the fourth chapter the heavy metal and trace element concentrations in blood and fur of two species of open-air foragers: *Mops condylurus* (Angolan free-tailed bat (A. Smith 1833)) and *Tadarida aegyptiaca* (Egyptian free-tail bat (É. Geoffroy Saint-Hilaire 1818)) collected during summer were investigated. The tissue samples were analysed by the Central Analytical Facility (CAF), University of Stellenbosch, Western Cape using an Agilent 7900 quadrupole inductively coupled plasma mass spectrometer (ICP-MS). Using non-lethal methods our aim was to determine if bats would be suitable bioindicators for the monitoring of ecosystem health over and near opencast diamond mining operations. The expectation was that bioaccumulation of elements in the fur and blood samples would be significantly higher over the mining areas compared to the adjacent control area. Alternatively we may find no difference in heavy metal concentration in the fur and blood of bats active over the opencast mine and adjacent control area. We also investigated if there were any correlations between concentrations of elements between fur and blood to determine if fur could be a reliable indicator of internal element concentrations.

The fifth chapter summarises and places into context the impacts of opencast mining on bats, using bats to monitor operational activities and potential mitigation strategies.

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Chapter 2: Bats and diamonds: bioindicators of disturbance due to an opencast diamond mine, Northern Limpopo, South Africa

Abstract

In general, bats are known to be indicators of general changes in the environment, as well as indicators of bioaccumulation of heavy metals. The identification of bat species as bioindicators has been sparsely investigated in South Africa, and focused on *Afronycteris nana* (Banana bat) foraging over waste water treatment plants in Kwa-Zulu Natal. Globally, there is scope for research to identify which particular species indicate a particular disturbance or disturbances on a fine scale. We address the impact of land degradation and importance of artificial resources created by an opencast diamond mine in northern Limpopo, South Africa, and which bat species indicate certain fine scale alterations in the environment. Approximately 22 bat species/species groups were recorded, with 18 species present on the Venetia Diamond Mine footprint. Mixed-effects models indicated that activity indices were significantly influenced by habitat type, (Limpopo Ridge Bushveld, Musina Mopane Bushveld, Venetia diamond mine waste water and Venetia diamond mine pit) ($P < 0.0001$), minimum temperature (T_{min}, $P = 0.0002$) and season with significantly lower activity during winter ($P < 0.0001$). Foraging activity was best explained by four models taking into consideration a combination of moon phase, T_{min}, foraging guild, season and habitat type. Species richness was significantly influenced by habitat type, T_{min} and season. The overall bat activity was highest at the tailings/waste water dam that was an important foraging and presumed drinking resource. Three species of bats have emerged as potential environmental and ecological indicators for the Venetia Diamond mine, *L. capensis* (Cape serotine), *A. nana* (Banana bat) and the species pair (that could not be separated acoustically) *Pipistrellus rusticus/N. anchietae* (Rusty bat/Anchieta's Pipistrelle). The majority of the species, particularly the molossid bats, exploit the resources provided by the mine regardless of the quality.

Keywords

Bioindicators, bats, opencast mining, diamond mining, environmental degradation, bat conservation

Introduction

As the global human population continues to increase, a negative global shift in ecosystem health and functionality has been hypothesised with detrimental consequences for species, including humans (Russo and Jones 2015). In response to changes in habitat due to climate change and other anthropogenic activities, species either avoid, utilise or adapt to anthropogenic habitats or become locally extinct, resulting in changes in the composition of communities, associated interspecific and intraspecific interactions, ecosystem functionality and ultimately conservation strategies (Mensing et al. 1998, Goddard et al. 2010, Concepción et al. 2015, Urban et al. 2016). Bat species occurring in current protected areas and those recognised as priorities for conservation actions are expected to decline as climate change and land use changes threaten bats globally (Jones et al. 2009, Ancillotto et al. 2016, Smith et al. 2016). Numerous studies have been undertaken to explain how bats respond to urbanisation (Legakis et al. 2000, Gehrt and Chelsvig 2003, Jung and Kalko 2011, Threlfall et al. 2012, Urban et al. 2012, Urban et al. 2013, Lenoir and Svenning 2014, Nagendra et al. 2014, Ancillotto et al. 2015, Caryl at el. 2015, Fischer et al. 2015, Stone et al. 2015, Ancillotto et al. 2016, Jung and Threlfall 2016, Rodriguez-Aguilar et al 2016), local land use and land use changes, including agricultural practices (Wickramasinghe et al. 2004, Park 2015, Treitler et al. 2016, Foord et al. 2018), and bioaccumulation of pesticides and heavy metal toxicity (Walker et al. 2007, Stechert et al. 2014, Zukal et al. 2015, Becker et al. 2018, Oliveira et al. 2021).

Being the second most species-rich mammalian order in the world (Fenton et al. 2020) representing approximately 23% of global mammal diversity, bats form a large component of global biodiversity and should be conserved as they deliver key services to both ecosystems and humans (Smith et al. 2016). Approximately 70% of bat species are insectivorous and provide insect population control, including significant crop pest control (Lehmkuhl Noer et al. 2012, Riccucci and Lanza 2014, Taylor et al. 2018). Nectarivorous and frugivorous bat species are important pollinators and seed dispersers aiding in forest regeneration (Hernandez-Montero et al. 2015, Tremlett et al. 2020). In conjunction with other taxa that are established bioindicators such as dragonflies (Bulánková 1997), amphibians (DeGarady and Halbrook 2006) and birds (Egwumah et al. 2017), bats are gaining more attention as bioindicators (Jones et al. 2009, Zukal et al. 2015, Mansour et al. 2016, Ferrante et al. 2018, Ramos-H et al. 2020).

Bats have been described as good bioindicators of ecosystem health and/or degradation as they are taxonomically stable, have a low reproductive output, long life expectancy, short and long-term effects on their populations that can be measured and monitored, they co-exist with humans thus are exposed to increased disease risk (e.g. white-nose syndrome) and pollution/contaminant levels, and they are globally distributed allowing for the effects of habitat change to be comparable (Jones et al. 2009, Jones et al. 2013, Russo and Jones 2015, Zukal et al. 2015, Frick et al. 2016, Smith et al. 2016). In addition, in order for bats to be used as bioindicators, their responses to environmental changes must reflect those of other taxa too (Park 2015) and since insectivorous bats occupy a high trophic level, changes in their activity may reflect changes in specific prey insect groups.

Acoustic monitoring of indicator bat species is important to minimise the need for invasive or lethal methods of bioindicator monitoring (Hernout et al. 2016a) although sometimes an invasive approach is unavoidable when the bat needs to be sacrificed for histological reasons (for example investigating the effects of toxicant accumulation in tissues) (see Zocche et al. 2010). Several studies on heavy metal toxicity in the urban adapter Banana bat (*Afronycteris nana*) have reported chronic health problems (e. g. organ damage and high levels of DNA damage) in bats foraging over waste water treatment works (Naidoo et al. 2013, 2015 and

2016). The identification of such impacts on individual health, together with additional expected impacts on community structure and activity of individual species has the potential to significantly enhance current environmental management plans for both mitigation of an anthropogenic activity as well as the conservation of local bat communities.

In South Africa, little research has focused on the potential of bat species as bioindicators. The opencast Venetia diamond mine was chosen as a unique case study to identify which species of bats would be good bioindicators for monitoring the health of the environment as the mine continues to operate. The Venetia diamond mine is unique in that it is situated in the north of the Limpopo Province, South Africa, in a semi-arid region and is surrounded by an expansive game reserve (~35 000 hectares) and numerous game farms. Although no data on bat species assemblages exists prior to the development of the mine, the extensive natural landscape around the mine could potentially provide an indication of natural species assemblages.

The current chapter focuses on the most obvious aspect of the opencast Venetia Diamond mining operation, habitat degradation. The hypothesis is that there is a significant difference in bat activity, composition and species richness between the open-cast mine and natural areas. The expectation is that species composition and activity is negatively impacted by habitat degradation on the mining footprint compared to the control areas neighbouring the mining operation. Alternatively, there is no significant difference. The aim is to identify species of bat that could be considered as bioindicators for monitoring the health of the ecosystem on and around the Venetia diamond mine, and species which readily exploit changes in the environment as a consequence of the mining activities regardless of the quality of the resource. Bats could play a crucial role in monitoring the environmental impact over time since mines are not always easily accessible and certain areas may not be safe to perform surveys on aquatic bioindicator taxa such as frogs and dragonflies due risks of human exposure to pollutants or contaminants and the risk of sinking into the accumulated fine substrate from the diamond extraction process in the waste water bodies.

Materials and Methods

Study Site

The study was conducted in northern South Africa, in the Limpopo Province, on the De Beers Venetia Diamond Mine (–22.435223°, 29.317562°) and two control plots of the same size situated west-south-west and east-north-east of the diamond mine. The control plots consisted of portions of the Venetia Game Reserve, Tranquil Nest Game Farm and Corea Game Farm. The Venetia diamond mine has been operational since 1992 (https://www.debeersgroup.com/the-group/our-history) in the Limpopo mobile belt, where a complex kimberlite pipe containing diamonds is situated (Brown et al. 2009).

The total study area covered 95.94km^2 and was divided up into three portions of approximately 31.5km^2 each. Four habitat types were covered namely: Musina Mopane Bushveld (MMB: west-south-west), Venetia Diamond Mine Pit (VDM-P), Venetia Diamond Mine Waste Water Dam (tailings dam) (VDM-W: centre) and Limpopo Ridge Bushveld (LRB: east-north-east) (Figure 3).

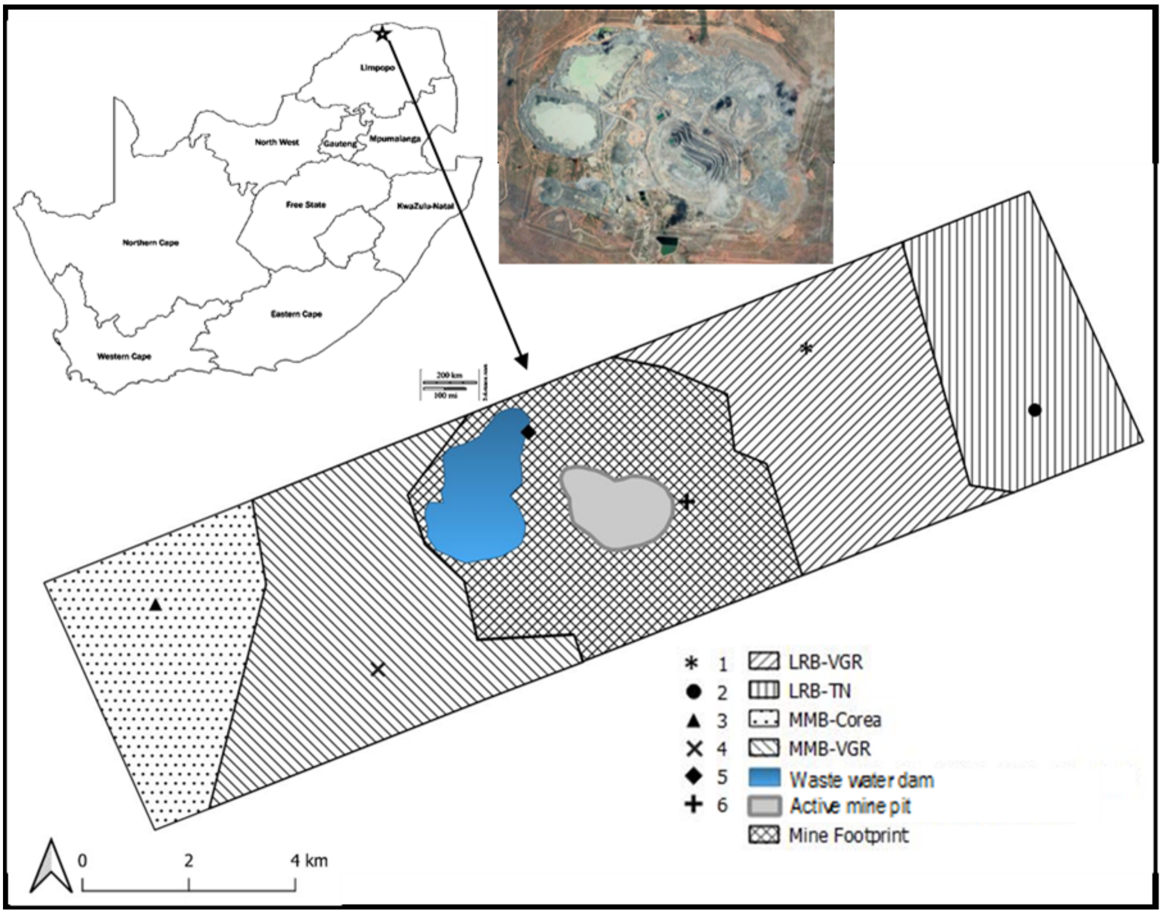

Figure 3. Layout of the study area indicating the main habitat types namely: MMB-Musina Mopane Bushveld (west-south-west), Venetia Diamond Mine (central) and Limpopo Ridge Bushveld (east-north-east) as well as the locations/farm portions within the natural habitat types namely: Corea Game Farm (Corea), Venetia Game Reserve (VGR) and Tranquil Nest Game Farm (TN). Arrangement of the six SM4BAT bat detectors (1 - 6) across the study site. The waste water (tailings) dam on the Venetia Diamond mine is indicated in blue and the active mine pit is indicated in grey. Insert of South Africa indicating the position of the study site in Northern Limpopo.

Two vegetation types dominate the area and are spatially complex, namely Musina Mopane Bushveld and Limpopo Ridge Bushveld. In general, the landscape of the study footprint was fairly flat dominated by sandy to clayey soils with the occasional basalt and sandstone rocky outcrop. The vegetation structure is open woodland to savanna on poorly developed soils, as well as moderately closed shrubveld. Although considered to be the most diverse mopaneveld type in South Africa, the Musina Mopane Bushveld together with the Limpopo Ridge Bushveld, is dominated by Mopane (*Colophospermum mopane*), Red Bushwillow (*Combretum apiculatum*) and Purple-pod Cluster-leaf (*Terminalia prunoides*) with a scattering of other iconic tree species such as Knobthorn (*Senegalia nigrescens*), Marula (*Sclerocarya birrea*) and Baobab (*Adansonia digitata*) (Mucina and Rutherford, 2011). The mine footprint itself is situated in the Limpopo Ridge Bushveld. The climate is characterised by very dry winters and hot summers with mean annual precipitation between 300-400mm (Mucina and Rutherford, 2011).

Active trapping

Two weeks of active trapping was conducted over the time period 1-13 December 2018 to validate call identification (Table 1**).** Active trapping was conducted on the mining footprint (Figure 4), on Corea Game Farm (Figure 5) and Tranquil Nest Game Farm (Figure 6). Free standing nylon monofilament mist nets (ECOTONE, Gdynia, Pomerania, Poland) and an

Austbank harp trap (Faunatech, Australia) were used to capture bats. Mist nets were placed strategically over water bodies and the harp trap was positioned along flight paths and a known bat roost in a building on the mine to increase the likelihood of bat capture. All mist net set ups were 9m and 12m wide and consisted of pairs of 9 m and 12 m mist nets respectively placed one above the other to have a resulting drop of ~5 m. The mist nets were supported by pairs of telescopic aluminium mist net poles (designed and fabricated by C. and D Cory-Toussaint) (Figure 6). Mist nets were opened shortly after sunset (~18:30, UTC + 02:00, Pretoria) and closed when bat activity notably lessened or when adverse weather conditions rendered capture conditions unfavourable. The harp trap was left in place from sunset to sunrise (~18:30–05:10). Mist net capture effort ≥ 2.5 hours was only possible on five out of 13 nights due to adverse weather conditions (Table 1). The harp trap was deployed even in unfavourable conditions as the bats were protected to a degree by the plastic inners of the harp trap bag. Each individual bat that was captured was processed: weighed, forearm measured, sexed and released.

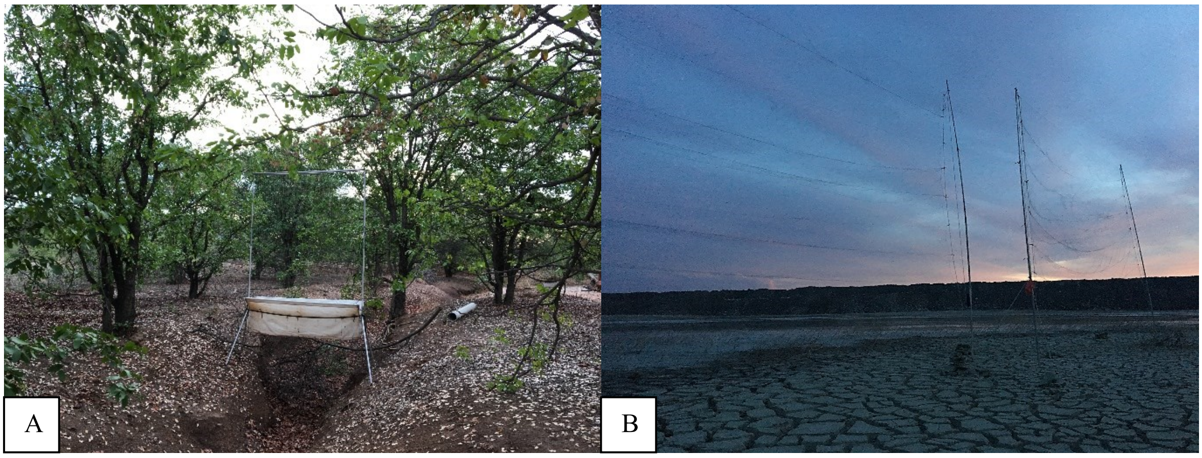

Figure 4. Two examples of the active trapping sites on the Venetia diamond mine with the Austbank harp trap placed in a potential flight path within the Musina Mopane Bushveld (A). Two sets of free standing nylon monofilament mist nets positioned along the edge of the waste water dam (B).

Figure 5. The active trapping site in natural Musina Mopane Bushveld on Corea Game Farm. Mist nets were extended over the temporary water pan to capture bats using the pan as a drinking and potential foraging area.

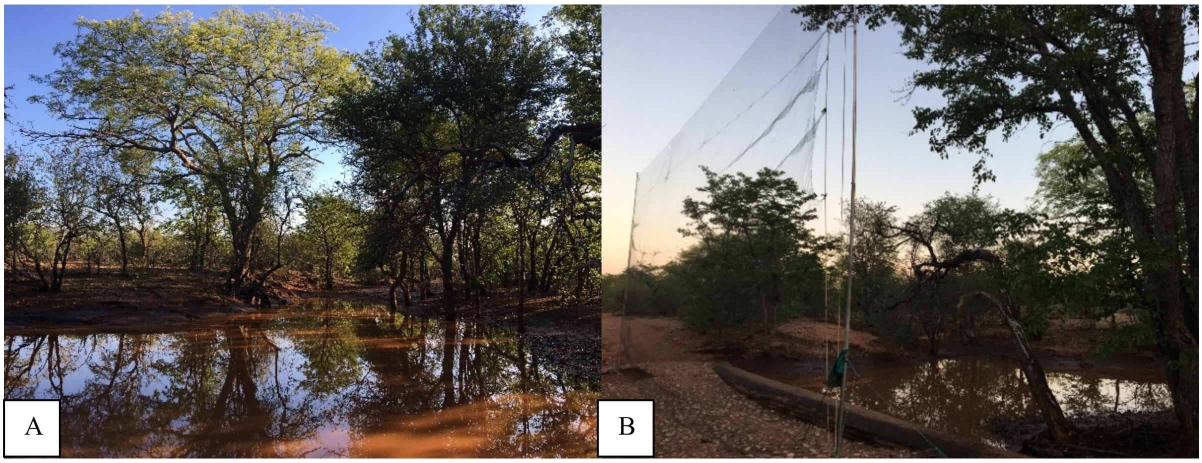

Figure 6. The active trapping site in the Limpopo Ridge Bushveld on Tranquil Nest Game Farm and an example of the associated mist net set up with two monofilament mist nest erected to create a system spanning from just above the ground to ~6 m high.

Table 1. Trapping effort over thirteen nights of active bat capture from 01–13 December 2018 indicating the trapping method, trapping effort, number of bats captured and prevailing weather conditions. Active capture was not possible on three nights due to thunderstorms and two nights were prematurely terminated after 30 minutes due to precipitating thunderstorms. Weather condition scores were as follows: 1 = wind still and clear skies, 2 = wind still and overcast, 3 = windy and clear skies, 4 = windy and overcast, 5 = windy and thunderstorm building and 6 = thunderstorm activity with wind and heavy rain.

Date	Location	Trapping Method	Start	End	Trapping Effort	# Bats captured	Weather condition score
12/1/2018	Corea Game Farm	Harp Trap only	18:30	5:10	22hr 40min	5	4
12/2/2018	N/A	Rainstorm	N/A	N/A	N/A	0	6
12/3/2018	Venetia Diamond Mine - Tailings Dam	Mist Net	18:30	21:00	2hr 30min	1	3
12/4/2018	Venetia Diamond Mine - Tailings Dam	Mist Net	18:30	~22:45	4hr 15min	1	5
12/4/2018	Venetia Diamond Mine - Sewage Works	Harp Trap	18:30	~22:45	4hr 15min	1	5
12/5/2018	Venetia Diamond Mine - Sewage Works	Mist Net	18:30	~20:00 Terminated	1hr 30min	0	6
12/6/2018	Venetia Diamond Mine - CNI Storeroom	Harp Trap only	18:30	5:10	22hr 40min	26	5
12/7/2018	Venetia Diamond Mine - Sewage Works	Mist Net	18:30	~19:00 Terminated	30min	0	6
12/7/2018	Venetia Diamond Mine - Sewage Works	Harp Trap	18:30	~19:00 Terminated	30min	0	6
12/8/2018	Corea Game Farm	Harp Trap only	18:30	5:10	22hr 40min	1	6
12/9/2018	N/A	Rainstorm	N/A	N/A	N/A	0	6
12/10/2018	N/A	Rainstorm	N/A	N/A	N/A	0	6
12/11/2018	Corea Game Farm - Pan	Mist Net	18:45	22:30	3hr 45min	8	1
12/12/2018	Tranquil Nest - Dam	Mist Net	18:45	22:00	3hr 15min	9	1
12/12/2018	Tranquil Nest - Dam	Harp Trap	18:30	22:00	3hr 15min	0	1
12/13/2018	Corea Game Farm - Pan	Mist Net	18:30	22:45	4hr 15min	8	1

Passive Acoustic Recording

Passive bat acoustic monitoring was conducted over two full seasons covering 186 nights; summer: December 2018–February 2019 and winter: June–August 2019. Six SM4BAT FS recorders (Wildlife Acoustics, Inc.) with SMM-U1 ultrasonic microphones mounted approximately 5m above the ground on fibreglass masts (Thorinbin Developments CC, Durban, South Africa) were deployed in pairs in the MMB, LRB and on aluminium masts on the Venetia Diamond Mine (Figure 3, Figure 7). Each SM4BAT was fitted with two 64GB SDXC cards (SanDisk Ultra, 80MB/s) and data downloaded on a monthly basis. Initially all six SM4BAT recorders were powered by four DD cell batteries each. During the winter 2019 data collection period, using the external power cables from Wildlife Acoustics, the four SM4BAT recorders in the natural areas were fitted with 12V, 7.0AH/20HR external batteries (Deltec Energy Solutions, Alrode, Johannesburg, South Africa). Several technical and biological issues resulted in unequal recording days of the systems. Table 2 indicates the bat detector deployment periods, number of nights that each detector actively recorded and significant issues that impacted on the recording period.

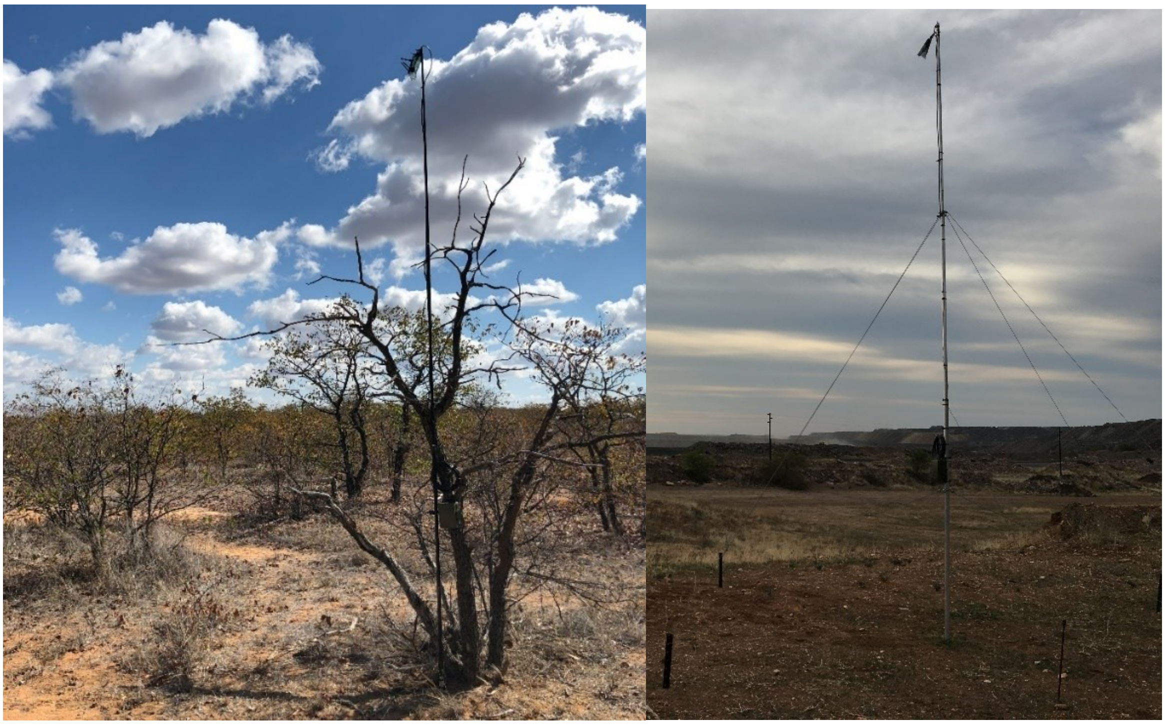

Figure 7. Typical set up of the Wildlife Acoustics SM4BAT recorders and ultrasonic microphones erected ~5m above the ground in the Limpopo Ridge and Musina Mopane Bushveld (left) and on the Venetia diamond mine (right).

Table 2. Passive acoustic recording deployment period covering 186 nights of the six SM4BAT detectors, number of nights recorded and issues associated with a bat detector that caused a disruption in data collection.

Detector	Deployment Period	# Nights Recorded	Notes
1	Summer: 02/12/2018–01/03/2019	82	None
	Winter: 31/05/2019–02/09/2019	83	None
2	Summer: 06/12/2018–03/03/2019	88	None
	Winter: 31/05/2019–02/09/2019	83	None
3	Summer: 03/12/2018–03/03/2019	91	None
	Winter: 31/05/2019–02/09/2019	86	Battery stolen on 10/08/2019. Replaced on 17/08/2019
4	Summer: 04/12/2018–01/03/2019	72	None
	Winter: 31/05/2019–02/09/2019	76	Elephants destroyed system on 21/06/2019. System reinstated on 10/07/2019. SM4BAT still functional.
5	Summer: 04/12/2018–04/03/2019	46	Bat activity extraordinarily high. SD cards filled very quickly.
	Winter: 31/05/2019–02/09/2019	86	Unexpected battery drain.
6	Summer: 04/12/2018–04/03/2019	41	Bat activity extraordinarily high. SD cards filled very quickly.
	Winter: 31/05/2019–02/09/2019	84	Unexpected battery drain.

Call analysis

Recorded bat calls were converted into zero-crossing (ZC) and sound files (.WAV) using Kaleidoscope (Wildlife Acoustics, Inc.) and analysed using a combination of AnalookW (Chris Corben) and BatSound (Pettersson Elektronik AB). Calls were initially filtered based on minimum pulses within a call (4 pulses per 10 seconds). Due to the number of species that occur in the area and the overlap in their echolocations calls (peak frequency overlaps and changes in frequencies depending on the behaviour of the bat) the calls were bulk classified to species level using self-designed filters in AnalookW based on call parameters from Taylor et al. 2013 and Monadjem et al. 2020 and refined according to the recorded calls from the study. Finally, all calls were manually checked and adjusted as necessary if the classifiers had incorrectly identified the calls. Additional call fragments/fragments of feeding buzzes/social calls were removed from the data as portions of calls without search calls included could not be reliably identified. Calls were also grouped into foraging guilds: open-air foragers (Molossidae and Emballonuridae), clutter-edge foragers (Vespertilionidae and Miniopteridae) and clutter foragers (Rhinolophidae and Hipposideridae). To lessen the bias of a single individual being recorded multiple times and over-representing activity, all bat passes were standardised to Activity Index (AI) based on Miller (2000), and thus AI was one call per species in each "active" minute interval. Detectability of the bats across the site does need to be considered as different species vary considerably in their detectability by bat detectors. A detectability correction factor was not determined during this study. Monadjem et al. (2017) attempted this in Swaziland. However, their proposed correction factor was not applied to the current data set due to several concerns. Until more research has been conducted in this field with updated technologies, we are hesitant to apply a correction factor because it could misrepresent the data.

A subset of 6155 individual calls from the entire data set covering 26-30 July 2019 and 07-11 December 2019 were filtered for foraging activity (feeding buzzes). A subsample of data was chosen for this particular analysis based on 10 consecutive nights of continuous recording that all six bat detectors were recording simultaneously during both winter and summer to analyse the importance of habitat type and site on the foraging activity of the bats. This subset was chosen based on the time consuming process of accurately identifying feeding buzzes which ideally would all have to be manually checked in Kaleidoscope Pro or BatSound to ensure the perceived feeding buzzes were not distorted calls. Additionally, the subset of calls recorded over exactly the same period eliminates the error that could otherwise be incurred based on the gaps in the recording period particularly for the detectors situated on the mining footprint that experienced some battery-life issues during winter and rapid filling of the SDXC cards during summer that may lead to an overestimation of foraging behaviour in the control areas than on the mining footprint. Foraging calls/passes were standardised to AI (Miller 200).

Acoustic monitoring transects
Driven transects through the control plots were conducted in conjunction with the passive acoustic monitoring, initiating at sunset after the first bat was recorded and covered as much of the control plots each night. The transects were confined to the Venetia Game Reserve due to the size of the area to be covered and the lack of access between the reserve and neighbouring farms. Twelve transects of ~46km each were driven: six transects during summer 2019 and six transects during winter 2019. Each night, the starting points of the transects were alternated between the beginning and end points to prevent a bias towards bat activity/preference for an area if there was a roost nearby.

Statistical Analysis
The statistical program R (version 3.6.3) was used to execute rarefaction models (iNext, ggplot2 and devtools), linear and generalised mixed-effects models (pscl, multicomp, lme4), hierarchical partitioning models (vegan, matrixStats) and permanovas (vegan and MASS) to perform a classical partitioning of species specific activity in relation to season and habitat type (Anderson 2017). Activity index was transformed using log (AI+1) as the data were not normally distributed. A linear mixed-effects model (lmer) was used. Species richness could not be transformed thus a generalised mixed-effects model (glmer, family Poisson) was used. We were primarily interested in the effect of habitat type on AI and species richness thus for the respective mixed effects models the random factors were site and date. Independent variables habitat type, season, minimum temperature (T_{min}, °C) and moon phase, were set as fixed factors. Concerning foraging activity a glmer (family Poisson) was used as the data was highly skewed and could not be transformed. Habitat type, foraging guild, season, T_{min}, and moon phase were factors of interest with site and date set as the random factors in the glmer.

The variation of inflation factor (VIF: "vif" function in R) was used to investigate collinearity between the fixed factors. Generalized VIF (GVIF) values > 3, 5 and 10 as a general rule of thumb indicate collinearity and redundant information or a lack of data (Imdadullah et al. 2016). VIF values > 10, one can either drop a collinear regressor or leave the regressor in the model (Imdadullah et al. 2016). We used the values of $GVIF^{1/2*df}$ instead of GVIF as suggested by Fox and Monette (1992). If the $GVIF^{1/2*df} < 5$, the association between the factors was deemed weak and were included in the models. Best-fit mixed-effects models were chosen based on the calculated corrected Akaike Information Criterion values (AICc) and associated delta AICc (ΔAICc) values <2. Hierarchical partitioning theory (Tuomisto 2010, Whittaker et al. 2001) was used to determine alpha diversity (effective number of species per sampling unit of mean species diversity) and beta diversity (turnover of species between bat detectors) and the factors that contribute to the observed beta diversity (Socolar et al 2016). The rarefaction models (iNext) determined 1) the sample coverage based on incidence frequency (the number of days that each species was recorded per habitat and per bat detector) was adequate as it neared and equalled 1.0 (or 100%) and 2) the potential or expected (extrapolated) number of species (species richness) that could occur in the study area. Extrapolated (effective) species richness was calculated using the number of species recorded per sampling units (habitat types). Both rarefaction model sets determined whether our sample coverage and species richness had reached "completeness" (Chao et al. 2014). The rarefaction models were important due to the sites being unequally sampled during the data collection period (Taylor et al. 2020). iNext expresses species diversity as Hill numbers $q = 0$ (species richness-taxonomic diversity), $q = 1$ (Shannon's diversity-phylogenetic diversity) and $q = 2$ (Simpson's diversity-functional diversity) (Taylor et al. 2020, Chao et al. 2014). The rarefaction models were important to show that in general, sampling effort was adequate.

To determine the similarity indices of activity index and species presence-absence between the six bat detectors, Bray-Curtis Similarity index was calculated using PAST 3.26 (Hammer 1999-2019). A seriation, was performed in PAST as an exploratory method to reorder the bat abundance data along a single continuum to reveal the pattern of occurrence of shared bat species across the site (Liiv 2010).

QGIS3.12.1 (Bucureşti) was used to determine areas of importance along the driven transects for foraging guilds and identified indicator species. Heat maps of the transects were produced using Kernel Density Estimates based on the activity of all foraging groups and the activity index of proposed key indicator species or species-pairs, *L. capensis/P. rueppellii, A. nana* and *P. rusticus/N. anchietae*. In QGIS, the GPS data were projected using AFRICA_ALBERS_EQUAL_AREA_conic. Due to the size of the area that the transects were conducted in and for the ease of visual presentation, the radius of the data points were set to 700m and pixel size was 100 to create smooth heat maps. Thereafter, the transect track was clipped with a buffer zone of 100m to accommodate the resulting Kernel density estimates although the actual detector distance would have been 30m on either side of the track (Weier et al. 2018).

Results

Active Trapping

Active trapping yielded 60 individual bats from 10 species. Each individual was measured and weighed for identification and call verification purposes (Table 3). The following species were confirmed in the control areas adjacent to the mine: *Tadarida aegyptiaca* (Egyptian free-tailed bat (E. Geoffroy Saint Hilaire 1818)), *Chaerephon pumilus* (Little free-tail (Cretzschmer 1826)), *Mops condylurus* (Angolan free-tailed bat (A. Smith 1833)), *Laephotis capensis* (Cape serotine (A. Smith 1829)), *N. zuluensis* (Zulu serotine (Roberts 1924)), *Scotophilus dinganii* (Yellow-bellied house bat (A. Smith 1833)), *Nycticeinops schlieffeni* (Schlieffen's twilight bat (Peters 1859)) and *Rhinolophus smithersi* (Smith's horseshoe bat (Taylor, Stoffberg, Monadjem, Schoeman, Bayliss and Cotterill 2012)). Only five species were captured on the mining footprint, namely: *C. pumilus*, *M. condylurus*, *Pipistrellus* (*Vansonia*) *rueppellii* (Rüppell's pipistrelle (Fisher 1829)), *N. schlieffeni* (Schlieffen's twilight bat (Peter 1859)) and *R. simulator* (Bushveld horseshoe bat (K. Anderson 1904)).

Table 3. Species list and associated measurements of the bats captured at various locations on the Venetia Diamond mine and control areas, Corea Game Farm and Tranquil Nest.

Location	**Species**	**Gender**	**Average Bat mass (g)**	**Average Forearm (mm)**	***N***
Corea Game Farm	*Chearephon pumilus*	Male	10.3 ± 0.42	38.6 ± 0.65	4
	Laephotis capensis	Female	6.8 ± 0.24	32.8 ± 1.54	3
	Nycticeinops schlieffeni	Male	4.5	30.1	1
	Mops condylurus	Female	23.0 ± 1.00	46.4 ± 0.52	2
	Scotophilus dinganii	Female	26.5 ± 1.50	54.3 ± 0.94	2
	Tadarida aegyptiaca	Female	21.0 ± 2.11	48.13 ± 0.88	9
	Tadarida aegyptiaca	Male	16.5	45.0	1
Tranquil Nest	*Neoromicia zuluensis*	Male	4.0	29.4	1
	Nycticeinops schlieffeni	Female	7.3 ± 0.25	30.9 ± 0.37	2
	Nycticeinops schlieffeni	Male	5.8 ± 0.62	30.9 ± 0.93	3
	Rhinolophus smithersi	Female	26.0	63.7	1
	Scotophilus dinganii	Female	32.5	55.5	1
	Scotophilus dinganii	Male	22.0	53.4	1
VDM- CNI Storeroom	*Chearephon pumilus*	Male	10.5 ± 0.50	37.1 ± 0.74	2
	Mops condylurus	Female	23.7 ± 4.24	46.0 ± 1.21	16
	Mops condylurus	Male	23.9 ± 1.76	47.6 ± 1.11	8
VDM-Sewage Works	*Rhinolophus simulator*	Male	8.5	45.9	1
VDM-Tailings Dam	*Nycticeinops schlieffeni*	Male	5.5	29.8	1
	Pipistrellus (*Vansonia*) *rueppellii*	Male	7.0	32.3	1

Passive Sample coverage and Species detected

A total of 129 988 bat passes were recorded and once converted to activity indices, the total activity index was 93 277. Sampling effort was adequate as sample coverage determined by the rarefaction for all four habitat types and the six bat detectors had reached 100% (i.e. was complete) by ~75 days which was well below the minimum number of sampling days (sampling units) of 125 sampled in the study (Figure 8). The Species diversity curves levelled off before the actual reference sample for LRB, MMB and VDM-P but only levelled off at the reference sample for VDM-W (Figure 9**,** *q* = 0, species richness). However, for *q* = 1 (phylogenetic diversity) and 2 (functional diversity), the curves had levelled off before the actual sample units (Figure 9). Thus, species diversity was satisfactorily sampled.

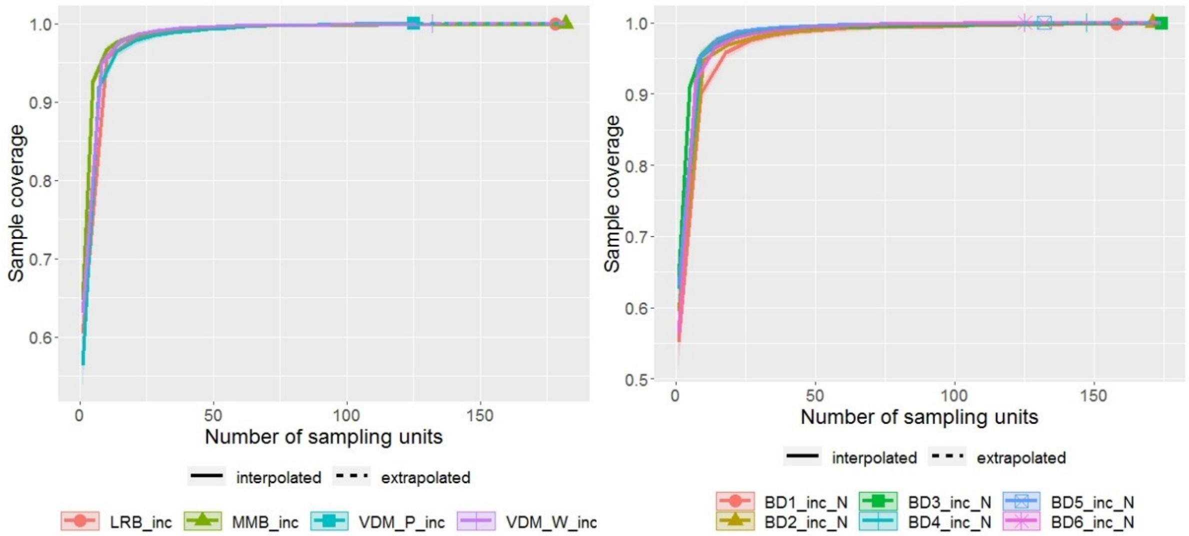

Figure 8. Sampling coverage for the four habitat types: Limpopo Ridge Bushveld (LRB), Musina Mopane Bushveld (MMB), Venetia Diamond Mine Pit (VDM_P) and Venetia Diamond Mine Waste Water (VDM_W), and for the six bat detectors (BD).

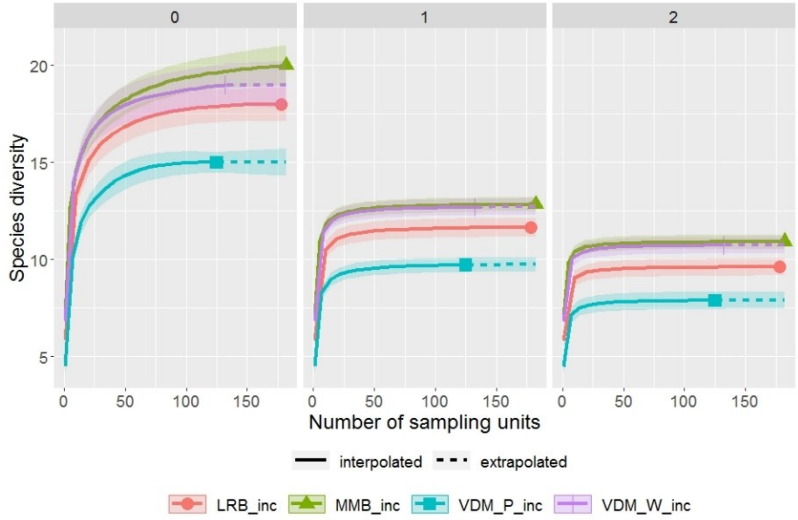

Figure 9. Species diversity recorded per habitat type: LRB = Limpopo Ridge Bushveld, MMB = Musina Mopane Bushveld, VDM_P = Venetia Diamond Mine Pit and VDM_W = Venetia Diamond Mine Waste Water. Species diversity is expressed in Hill numbers (q) where “0” is species richness (taxonomic diversity), “1” is phylogenetic diversity (Shannon’s diversity) and 2 is functional diversity (Simpson’s diversity).

Overall, 22 bat species/groups were acoustically identified representing a possible 26 species (Table A6): *T. aegyptiaca* (Egyptian free-tailed bat), *C. cf ansorgei*/Molossid 19kHz (Ansorge's free-tailed bat (Thomas 1913) and possibly *T. ventralis* (Giant free-tailed bat (Heuglin 1861)); see Taylor et al. 2015), *C. pumilus/M. condylurus* (Little free-tailed and Angolan free-tailed bats), *M. midas* (Midas free-tailed bat (Sundevall 1843)), *Sauromys petrophilus* (Roberts's flat-headed bat (Roberts 1917)), *Otomops martiensseni* (Large-eared giant mastiff bat (Matschie 1897)), *Taphozous mauritianus* (Mauritian tomb bat (E. Geoffroy Saint-Hilaire 1818)), *L capensis/P. (Vansonia) rueppellii* (Cape serotine and Rüppell's pipistrelle), *A. nana* (Banana bat (Peters 1852)), *N. zuluensis* (Zulu serotine), *P. hesperidus* (Dusky bat (Temminck 1840)), *P. rusticus/N. anchietae* (Rusty bat (Tomes 1861) and Anchieta's Pipistrelle (Seabra 1900)), *Eptesicus hottentotus* (Long-tailed serotine (A. Smith 1833)), *S. dinganii* (Yellow-bellied house bat), *N. schlieffeni* (Schlieffen's twilight), *Miniopterus fraterculus* (Lesser long-fingered bat (Thomas and Schwann 1906)), *M. natalensis* (Natal long-fingered bat (A. Smith 1833)), *R. simulator* (Bushveld horseshoe bat), *R. smithersi* (Smither's Horseshoe bat), *R. cf. blasii* (Blasius's Horseshoe bat (Peters 1867), this species is marked with a question mark as it is not known from the area; peak frequency: 84.24 kHz) and *Hipposideros caffer* (Sundevall's leaf-nosed bat (Sundevall 1846)). Three unknown species that have been noted as, Molossid 19kHz has been placed within the *C. cf. ansorgei* group due to overlapping call parameters, Bat 36kHz (which may be *P. rueppellii* or a lower call of *L. capensis* and have been placed together in the same group) and Rhinolophid 50kHz. In order of species diversity, the Musina Mopane Bushveld was the most diverse (~20 spp) followed by the Venetia Diamond mine waste water dam (~18 spp), the Limpopo Ridge Bushveld (18 spp) and Venetia Diamond mine pit (~15 spp) (Figure 9**).** (Approximately is used as some of the species were represented in groups due to overlapping echolocation call parameters as well as the inclusion of the unknown species). See Table A1 under appendices for call analysis parameters.

Bat community composition based on activity was most similar between bat detectors 3 and 4 (>0.825) in the MMB and detector 6 positioned at the VDM-P (0.75), the remaining bat detectors varying considerably with bat detector 2 positioned in the LRB being the least similar to any other (Figure 10a). Taking species presence-absence into account, all bat detectors were similar (>0.88). Two prominent groups were revealed with bat detectors 1 and 2 in the LRB most similar (>0.93) with VDM-P showing a similar community composition (>0.9) (Figure 10b). Similarly, detectors 3 and 4 in the MMB were most similar (>0.915) with the addition of the VDM-W (>0.9) (Figure 10b).

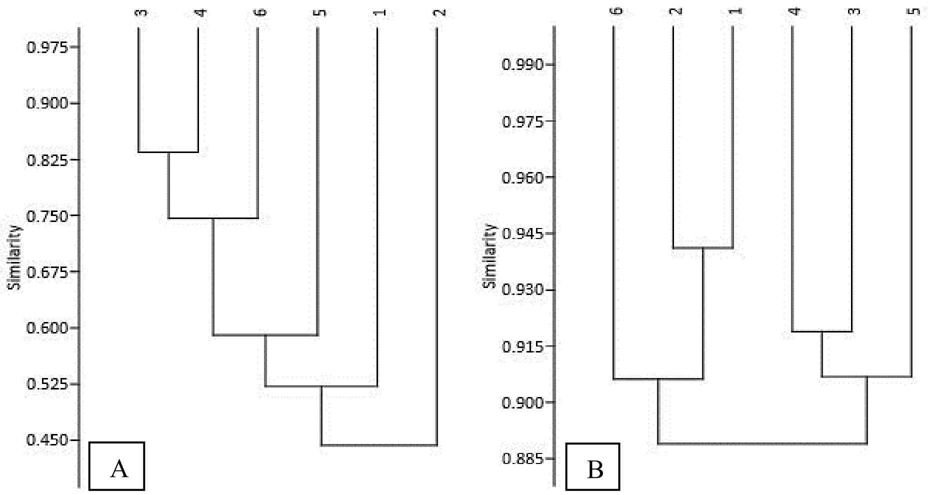

Figure 10. The Bray-Curtis Similarity Index based on the Activity Index (A) and species presence-absence (B) between the six bat detectors. Bat detectors 1 & 2 were in the Limpopo Ridge Bushveld (LRB), 3 & 4 were in the Musina Mopane Bushveld (MMB) and 5 & 6 were on the Venetia Diamond Mine (waste water and pit respectively). Bat activity was most similar between bat detectors 3 and 4 in the MMB and 6 at the pit of the mine. Species richness (B) was similar between all six bat detectors but more so between detectors in the respective habitats: 1 & 2 in the LRB and 3 & 4 in the MMB. Species composition over the waste water dam was most similar to the detectors in the MMB (3 & 4) and the species composition over the open pit of the mine was most similar to detectors positioned in the LRB (1 & 2).

The seriation (PAST) revealed that 14 of the 22 species/species groups were commonly shared between all six bat detectors with five species unique to a specific detector e.g. *H. caffer* only recorded at detector 3 and *R. cf. blasii* only recorded at bat detector 5 (Figure 11).

	1	2	3	4	5	6
Rhino50		■				
Rsmith	■	■	■			
Hc			■			
Rsim	■	■	■	■	■	
Ca/Mol19	■	■	■	■	■	■
Sp	■	■	■	■	■	■
Mm	■	■	■	■	■	■
Cp/Mc	■	■	■	■	■	■
Ta	■	■	■	■	■	■
Nz	■	■	■	■	■	■
Ns	■	■	■	■	■	■
Sd	■	■	■	■	■	■
Eh	■	■	■	■	■	■
Pr/Na	■	■	■	■	■	■
An	■	■	■	■	■	■
Lc/Vr	■	■	■	■	■	■
Om	■	■	■	■	■	■
Tm	■	■	■	■	■	■
Mf			■	■	■	
Mn				■		
Ph		■	■	■	■	■
Rblas?					■	

Figure 11. Occurrence of all 23 species/species groups across the six sites indicating shared species. *Hc = Hipposideros caffer, Ca/Mol19 = Chaerephon cf ansorgei/Molossid 19kHz, Sp = Sauromys petrophilus, Mm = Mops midas, Cp/Mc = Chaerephon pumilus/Mops condylurus, Ta = Tadarida aegyptiaca, Nz = Neoromicia zuluensis, Lc/Pr = Laephotis capensis/Pipistrellus (Vansonia) rueppellii, Ns = Nycticeinops schlieffeni, An = A.*

nana, Sd = Scotophilus dinganii, Eh = Eptesicus hottentotus, Pr/Na = Pipistrellus rusticus/Neoromicia anchietae, Om = Otomops martiensseni, Tm = Taphozous mauritianus, Mf = Miniopterus fraterculus, Mn = M. natalensis, Ph = Pipistrellus hesperidus, Rsmith = Rhinolophus smithersi, Rsim = Rhinolophus simulator, Rblas?= Rhinolophus cf. blasii, Rhino50 = Rhinolophid 50kHz.

All $\text{GVIF}^{1/2df}$ values of the independent variables in the global lmer for AI were < 5 (Table A2) and were thus included in the model. The best-fit model (site: random factor) revealed that habitat type, T_{min} and season were significant factors contributing to the observed pattern in AI (Table 4 and Figure 12a). Habitat types MMB, VDM-P and VDM-W differed significantly from each other (all P values < 0.001) being highest over the VDM-W and lowest over the LRB (Figure 12a). AI was significantly higher during summer ($P < 0.001$) and at warmer T_{min} ($P < 0.001$) (Table 4, Figure 12a).

Table 4. Model selection table from the mixed-effects regression models to explain which factors had an effect on activity index (AI), and species diversity. Site and date were set as random effects to account for psuedoreplication due to the same sites being sampled twice and to account for any temporal variations. The fixed variables were habitat type, moon phase, T_{min} and season. The estimates are presented with associated significances from the cftests: $0 < Pr \leq 0.001$ = ***, $0.001 < Pr \leq 0.01$ = **, $0.01 < Pr \leq 0.05$ = *. Habitat types are as follows: MMB = Musina Mopane Bushveld, VDM-P = Venetia Diamond Mine pit, VDM-W = Venetia Diamond Mine waste water.

Model	**Habitat Type**	**Moon Phase**	**T_{min} (°C)**	**Season**	**AICc**
AI #1 (lmer)	MMB: 0.69 *** VDM-P: 1.00 *** VDM-W: 2.17 ***	x	x	Winter: - 1.70 ***	2222.2
Species Diversity (glmer)	MMB: 0.39 *** VDM-P: 0.13 NS VDM-W: 0.53 ***	x	0.08 *	Winter: - 0.37 ***	3763.5

Species richness (site and date: random factors) was best explained by the effects of habitat type, season, water and T_{min} (Table 4, Figure 12b). Species richness was significantly higher over the MMB ($P < 0.001$), followed by VDM-W ($P < 0.001$) and VDM-P ($P < 0.001$). Additionally, species richness was significantly lower during winter ($P < 0.001$) and and at warmer T_{min} ($P < 0.01$) (Table 4, Figure 12b).

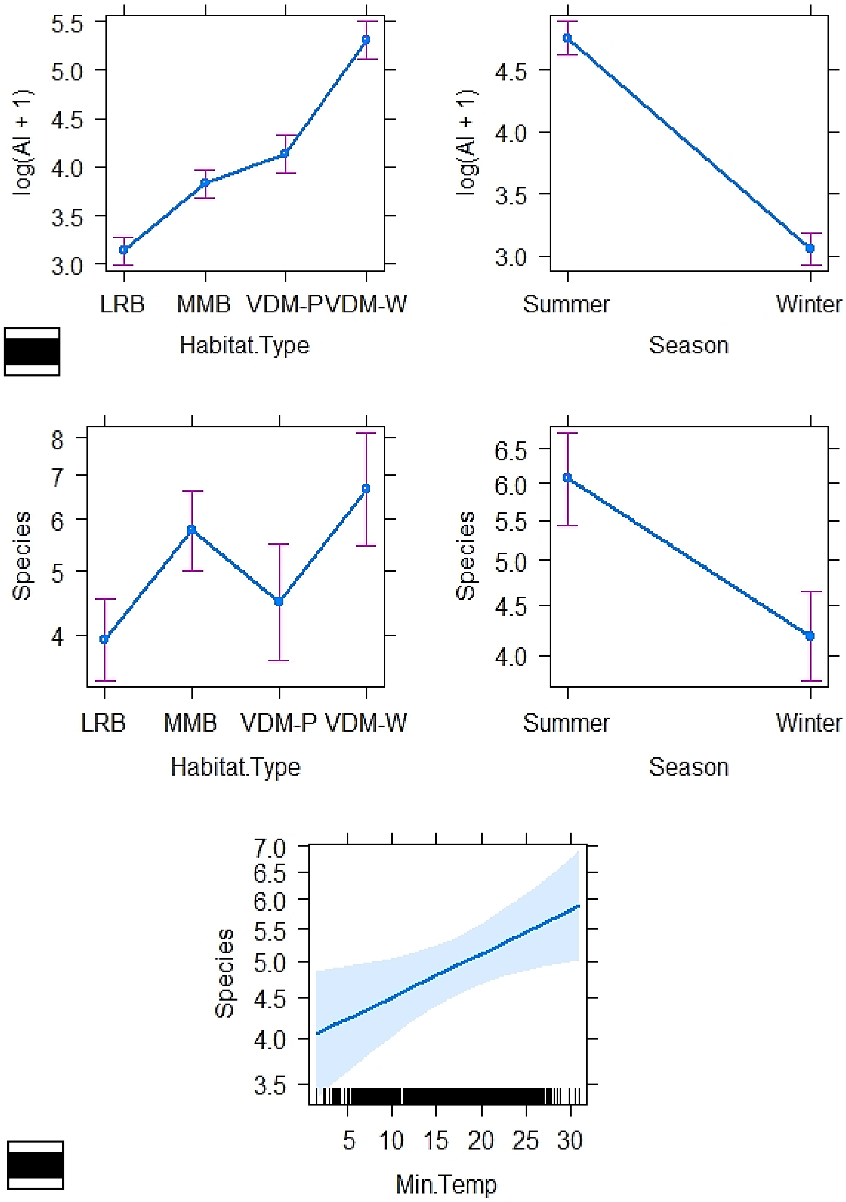

Figure 12. The best fit model outputs indicating the effect of habitat type and season on activity index AI (A), and the effect of habitat type, season and minimum temperature (Min. Temp) on species richness (B). Site and date were set as a random factors. Habitat types are as follows: LRB = Limpopo Ridge Bushveld, MMB = Musina Mopane Bushveld, VDM-P = Venetia Diamond Mine pit, VDM-W = Venetia Diamond Mine waste water.

The foraging calls (n = 1978) that were extracted from the subset of data had a resulting AI of 1007. The VIF test indicate that there was weak collinearity between the independent variables and all were retained in the glmer since all $GVIF^{1/2df}$ values < 5 (Table A2). Four best fit models were selected ($\Delta AICc < 2$) indicating that the most likely factors responsible for the observed foraging pattern were season, T_{min} and foraging guild (Table 5). Foraging guild and T_{min} were factors of significance in all four models with combinations of habitat type, season and moon phase in the remaining three models (Table 5). Foraging activity was significantly lower at low T_{min}, significantly lower in the OAF group, significantly higher during winter, significantly higher over the waste water dam on the mining footprint and significantly higher during the waning crescent moon phase (Table 5, Figure 13). Foraging behaviour was dominated by clutter-edge foragers: *A. nana* (n=436), *L. capensis/V. rueppellii* (n=142) and *P. rusticus/N. anchietae* (n=104).

Table 5. Model selection table of the four best-fit models (Δ AICc < 2.0) from the mixed-effects regression models to explain which factors had an effect on foraging activity. Site and date were entered as random variables. Habitat type, moon phase (WanCres = waning crescent, WaxCres = waxing crescent), minimum temperature (T_{min}), foraging guild (OAF = open-air and CEF = clutter-edge foragers) and season were fixed variables. “x” indicates the factors excluded based on the selection table for the best fit models. The estimates are presented with associated significances from the cftests: $0 < Pr \leq 0.001$ = ***, $0.001 < Pr \leq 0.01$ = **, $0.01 < Pr \leq 0.05$ = *, NS = not significant. Habitat types are as follows: MMB = Musina Mopane Bushveld, VDM-P = Venetia Diamond Mine pit, VDM-W = Venetia Diamond Mine waste water.

Model	**Habitat Type**	**Season**	**Moon Phase**	**T_{min} (°C)**	**Foraging Guild**	**AICc**
Foraging behaviour (AI) #1 (glmer)	x	Winter: 2.13 ***	x	0.22 ***	OAF: -0.24**	1058.9
Foraging behaviour (AI) #2 (glmer)	VDM-W: 1.97 ** VDM-P: -0.28 NS MMB: 0.32 NS	Winter: 2.11 ***	x	0.22 ***	OAF: -0.24 **	1059.9
Foraging behaviour (AI) #3 (glmer)	x	x	WanCres: 2.44 *** WaxCres: 0.37 NS	0.22 ***	OAF: -0.24 **	1060.7
Foraging behaviour (AI) #4 (glmer)	x	Winter: 2.44 ***	WaxCres: 0.37 NS	0.22 ***	OAF: -0.24 **	1060.7

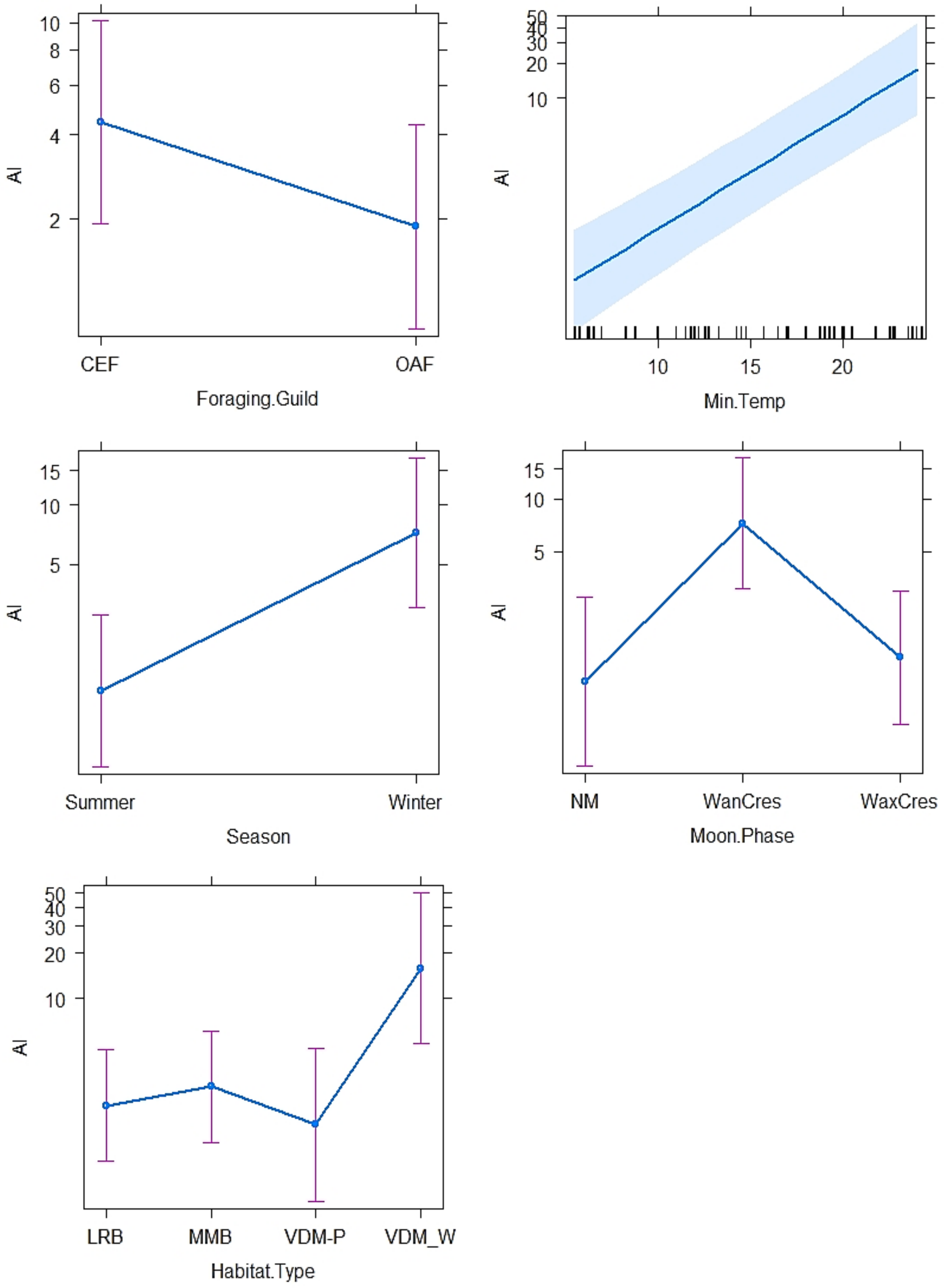

Figure 13. Best-fit model outputs (site and date: random variables) from all four best fit models (ΔAICc < 2) indicating the effect of the fixed variables: foraging guild (CEF = clutter-edge foragers, OAF = open-air foragers), minimum temperature (Min.Temp), season, moon phase (NM = new moon, WanCres = waning crescent, WaxCres = waxing crescent) and habitat type on foraging activity (AI). Habitat types are as follows: LRB = Limpopo Ridge Bushveld, MMB = Musina Mopane Bushveld, VDM-P = Venetia Diamond Mine pit, VDM-W = Venetia Diamond Mine waste water.

Of the total (gamma) richness, site richness (alpha richness) accounted for 60.00% and beta richness was 40.00%, of which turnover between seasons accounted for 68.75%, habitat type accounted for 25.00% and the remaining 6.25% was due to location of the bat detectors (Figure 14).

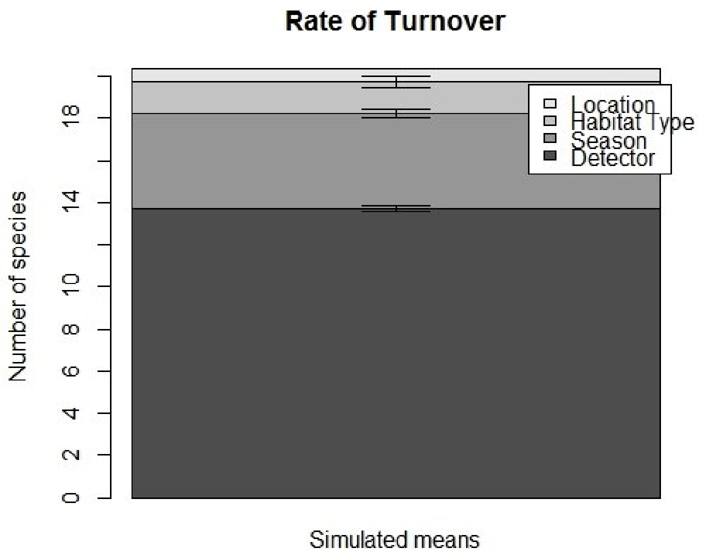

Figure 14. Factors responsible for the observed rate of turnover (Beta-diversity) on and around the Venetia Diamond mine in relation to season, habitat type and location. Detector represents alpha diversity across the study.

There was a significant difference between AI of the foraging guilds (Anova, $P = 7.72e^{-17}$) where open-air foragers showed significantly higher activity over the entire study site (control areas included) in comparison to the clutter-edge foragers (Figure 15a & b). The analysis of activity patterns for different foraging guilds (PERMANOVA) indicated significant effects of habitat type only on the activity of clutter-edge foragers (habitat type, p=0.035) (Figure 15a) (Table A3). The activity of open-air foragers was significantly impacted by habitat type and season (habitat type, p=0.0005; season, $p = 0.00001$) (Table A3, Figure 15b). Neither habitat type nor season were significant for clutter foragers (Figure 15c).

Clutter-edge forager activity was significantly higher over the VDM-W during winter, dominated by *A. nana* (habitat type: $P < 0.0001$ and season: $P < 0.0001$) and *P. rusticus/N. anchietae* (habitat type: $P = 0.001$ and season: $P = 0.001$) (Figure 16a & 16b). Over the control sites, although not significant, the activity of the clutter-edge foragers decreased in summer over the LRB and increased over the MMB. *Laephotis capensis* dominated the bat community over the MMB (Figure 14c). Habitat type and season had significant effects on the activity of certain species of clutter-edge foragers that had a notably high activity index. The bat species or species-pairs of importance were *L. capensis, A. nana* and *P. rusticus/N. anchietae* as they were present year round and exhibited a high affiliation with the VDM-W (Figure 16).

Conversely to the clutter-edge foragers, open-air forager activity was significantly higher over the VDM-W during the summer period with activity of *C. cf ansorgei/Molossid 19kHz* and *T. aegyptiaca* prolific over the waste water site (season: $P = 0.02$ and 0.01 respectively, Figure 17a & b). The only open-air foragers that showed a significant strong seasonal and habitat association were *S. petrophilus* ($p < 0.001$), *O. martiensseni* ($P < 0.01$) and *M. midas* ($P < 0.01$) (Table A3). *S. petrophilus* was absent from the study area during winter (Figure 17c) and similarly *O. martiensseni* was essentially absent with only one call per habitat type recorded during winter (Figure 17d). Both of these species had a strong preference for the VDM-W during summer. The only Emballonuridae species recorded on site was *T. mauritianus* (open-air forager) that also showed a significantly strong seasonal activity effect with a high prevalence during summer and a preference for the MMB followed by the VDM-W.

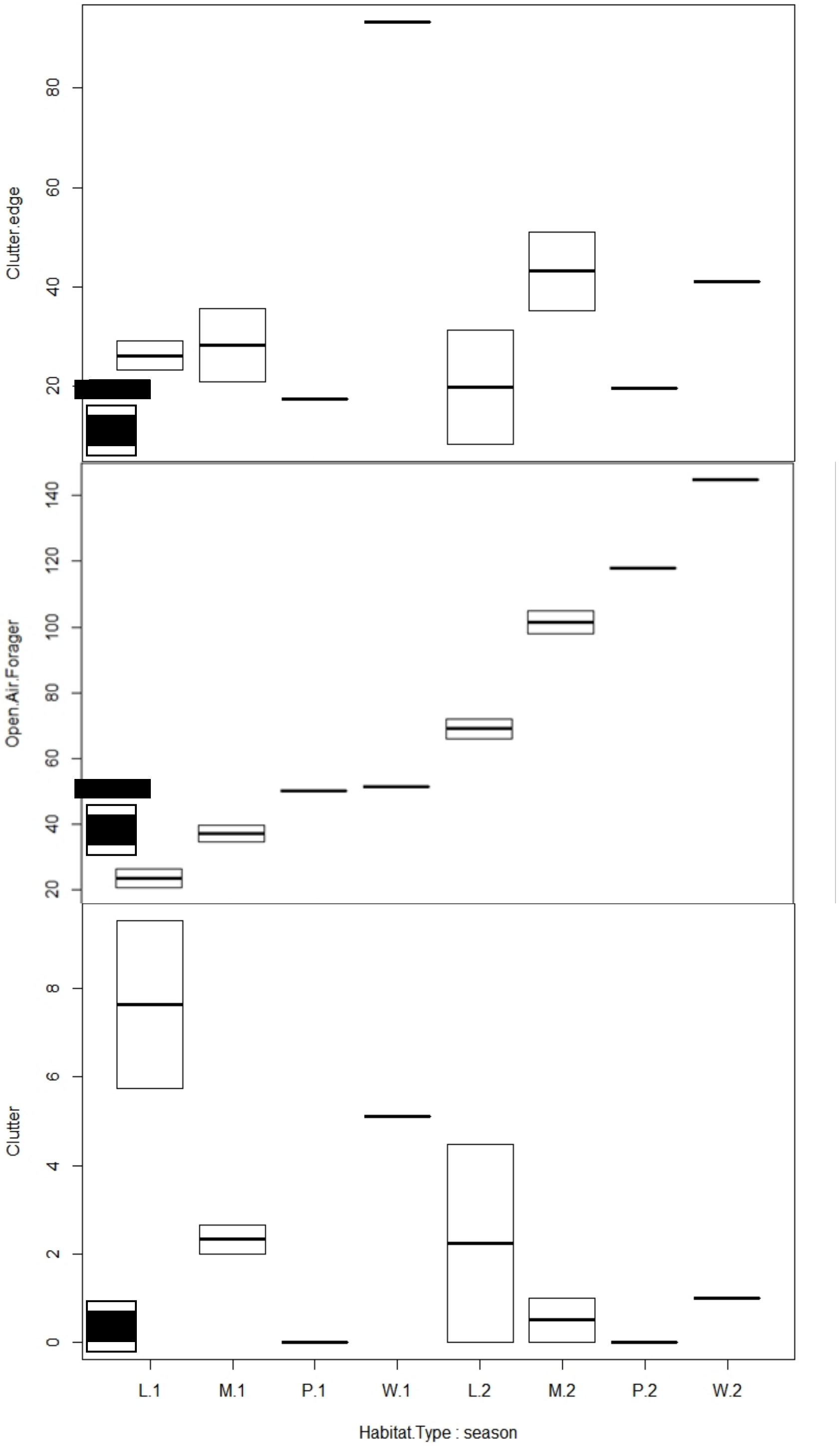

Figure 15. Activity indices of A) clutter-edge foragers (Vespertilionidae), B) open-air foragers (Molossidae and Emballonuridae) and C) clutter foragers (Rhinolophidae and Hipposideridae) over the four habitat types and two seasons. L = Limpopo Ridge Bushveld, M = Musina Mopane Bushveld, P = Venetia Diamond Mine Pit, W = Venetia Diamond Mine Waste Water, 1 = winter and 2 = summer. Significant effects of both season and habitat are indicated by "***" and the effect of habitat only is indicated by "*".

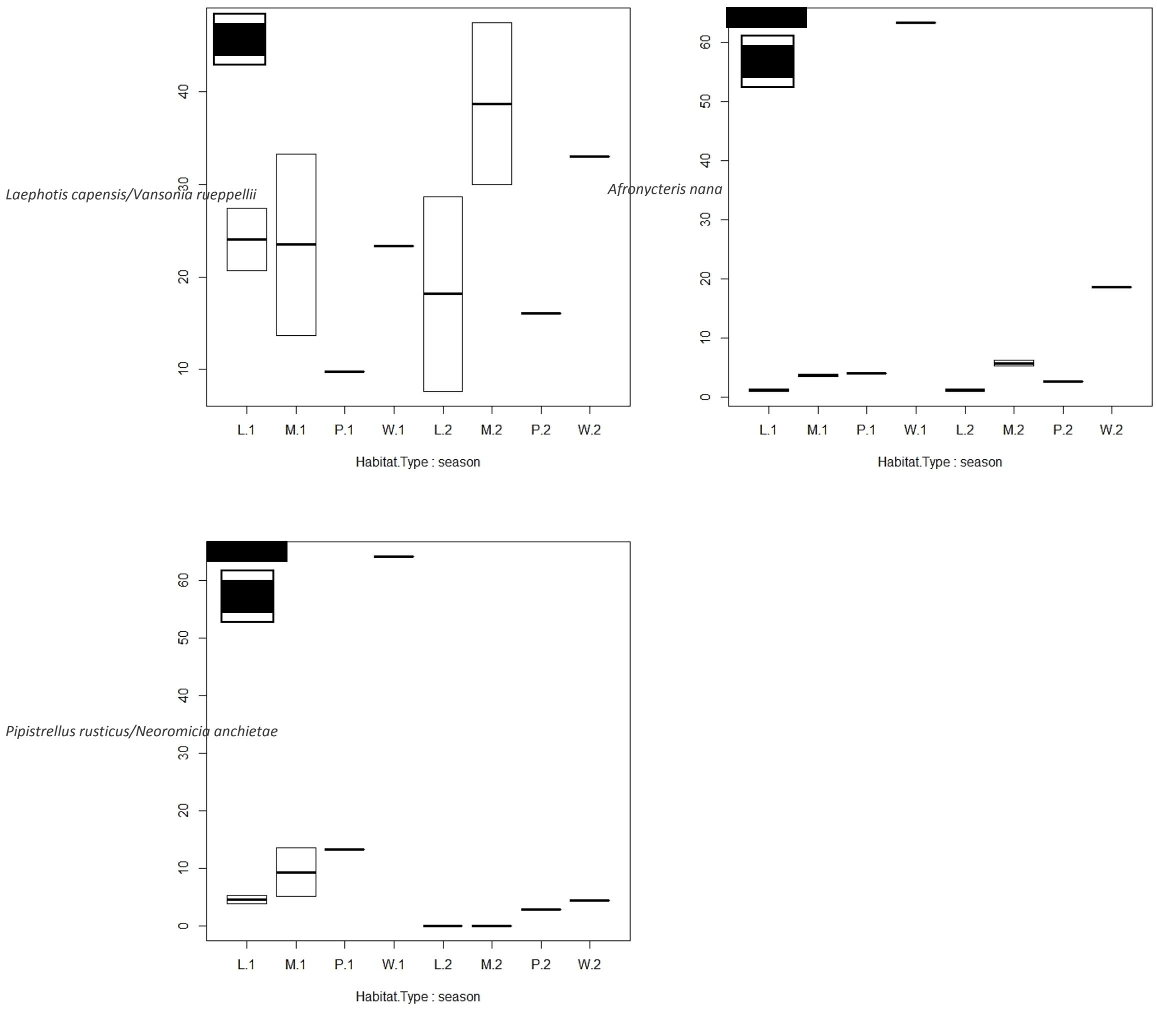

Figure 16. Seasonal and habitat type influences on the change in activity pattern of *Laephotis capensis/Vansonia rueppellii* (A), *Afronycteris nana* (B) and *Pipistrellus rusticus/N. anchietae* (C) in relation to habitat type and season. L = Limpopo Ridge Bushveld, M = Musina Mopane Bushveld, P = Venetia diamond mine pit, W = Venetia diamond mine waste water, 1 = winter and 2 = summer. Significant effect of both season and habitat indicated by “***”.

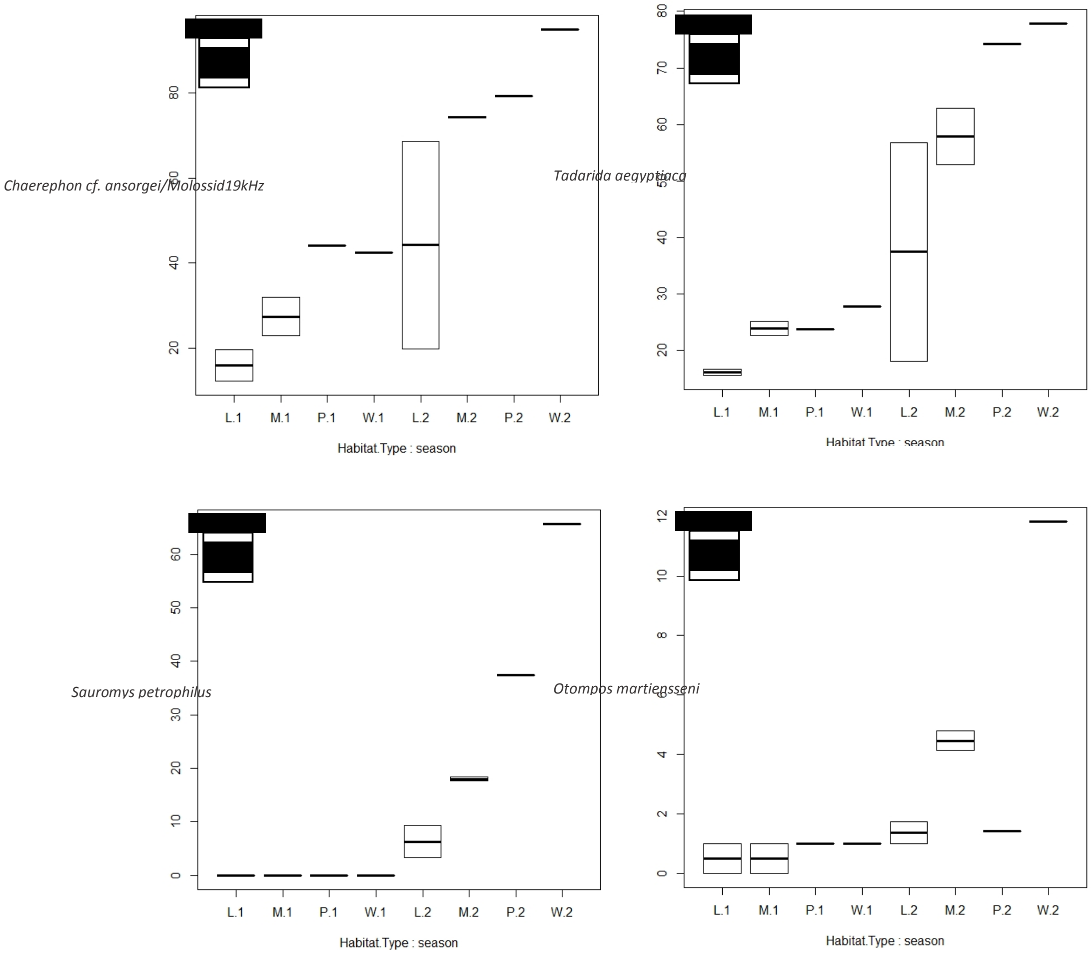

Figure 17. Impacts of habitat type and season on the activity of *Chaerephon cf. ansorgei/Molossid 19kHz* (A), *Tadarida aegyptiaca* (B), *Sauromys petrophilus* (C) and *Otomops martiensseni* (D) . L = Limpopo Ridge Bushveld, M = Musina Mopane Bushveld, P = Venetia diamond mine pit, W = Venetia diamond mine waste water, 1 = winter and 2 = summer. Significant effect of both season and habitat indicated by "***" and season only indicated by "*".

Transects

The Kernel Density Estimate (KDE) analysis indicated that along the transect (*n* = 12 nights), bat activity was concentrated to the west of the mine near the Kolope River and along the northern border of the Venetia Diamond Mine where a small scale diamond mining operation is active (Figure 18a). Activity was dominated by open-air foragers accounted for the majority of the activity. Due to the low number of calls recorded from *A. nana* and *P. rusticus/N. anchietae* (*n* = 27) a KDE was not run for them. However, *L. capensis* was recorded 132 times and the KDE indicated that*L. capensis* had a preference for areas along the transect that passes through watercourses with riparian vegetation (Figure 18b).

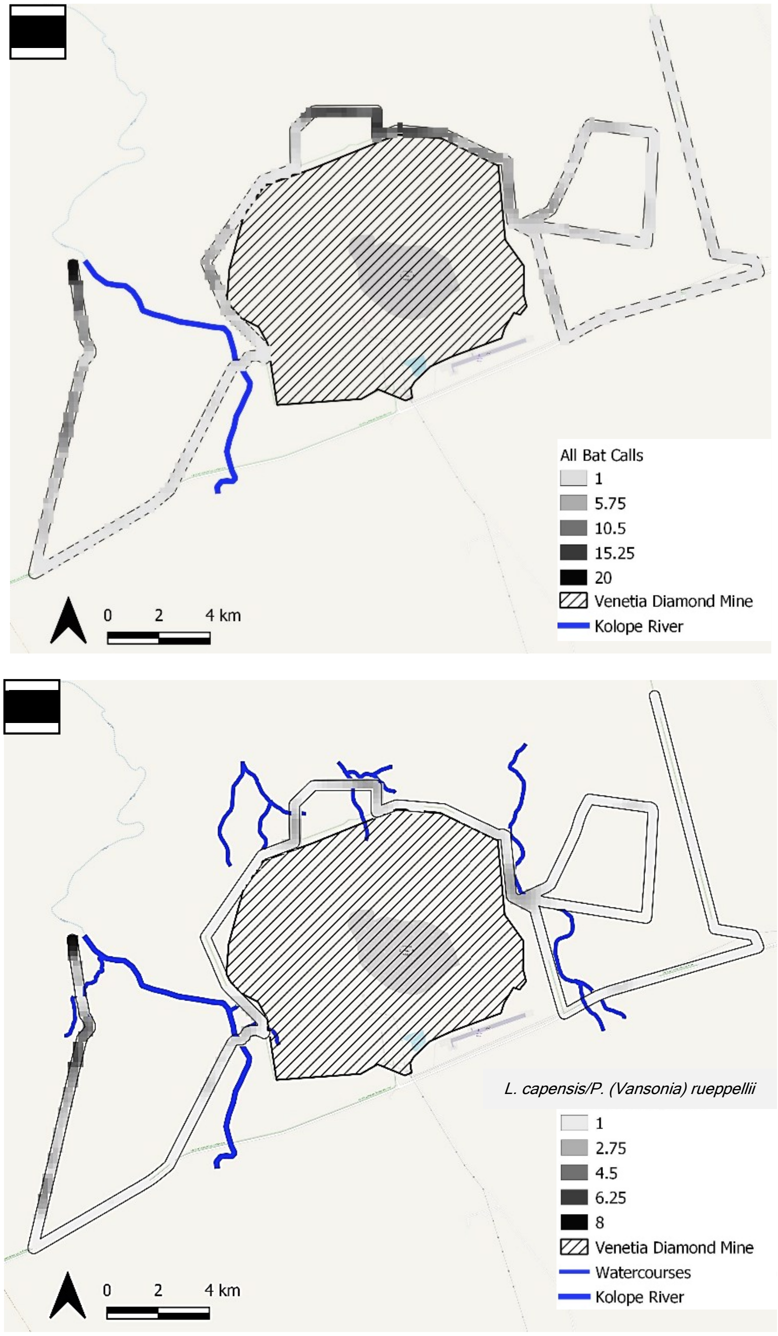

Figure 18. The areas of importance to bats based on A) all bat calls recorded along the transect with the activity concentrated near the Kolope and along the northern boundary of the mine and B) the activity of a key indicator, *Laephotis capensis/P. (Vansonia) rueppellii*. Areas of greatest activity are indicated in dark grey and black.

Discussion

The Venetia diamond mine appears to provide an important resource for bats in the hot, semi-arid Limpopo River Valley. Numerous studies have shown how important open water resources are for bats in arid environments, where the availability and structure of the water resources seem to affect bat distribution and activity in these landscapes (Rabe and Rosenstock 2005, Razgour et al. 2011, Korine et al. 2015, Taylor et al. 2020). Greenfeld et al. (2017) highlighted the challenges when native bats, particularly in desert regions, are faced with competition and displacement by other species at artificial water resources created by anthropogenic developments. Specifically to the Limpopo River Valley, a study conducted by Taylor et al. (2020), illustrated the importance of water and riparian vegetation in the semi-arid environment with higher bats activity, species richness and diversity at sites closer to the Limpopo River than in areas further away from the river. On the Venetia diamond mine, the main determinant preventing the displacement of other species of the same foraging guild, for example the clutter-edge foragers, could be the significant expanse of the waste water dam that covered an area of approximately 2.7km^2 and provided an important foraging and drinking resource for a high AI of bats.

Ecosystems are complex and intricate, and identifying and understanding how all levels of biodiversity in an ecosystem respond to anthropogenic activities is essentially impossible (Starik et al. 2018). It is important to identify specific taxa that can be used to measure and describe anthropogenic impacts on ecosystems. Of the 22 species or species-pairs recorded in the area, *L. capensis/P. (Vansonia) rueppellii*, *A. nana* and *P. rusticus/N. anchietae* (Vespertilioniformes) are proposed to be potential bioindicators, due to clearcut responses (avoidance or attraction) in their activity to the mining footprint (notably the waste water dam).

It is important not to dismiss the prospect of common, adaptable bat species as potential bioindicators. *Laephotis capensis* is a common and widespread bat across South Africa that thrives in arid environments and is a known urban adapted bat and aridity indicator (Monadjem et al. 2018, Taylor et al. 2020, Monadjem et al. 2020) and has potential to be an ecological indicator of habitat quality (or structural heterogeneity) (Table 6), particularly concerning rehabilitation efforts of the mine. *Laephotis capensis/P. (Vansonia) rueppellii* was the dominant species of Vespertilionidae bat and clutter-edge forager species over the control plots and yet only accounted for 3.88% of the total bat activity on the Venetia Diamond mine. Over the control plots, *L. capensis/P. (Vansonia) rueppellii* had a greater presence in the MMB on Corea Game Farm, 105.64% higher than at the Venetia diamond mine waste water dam. A potential reason for this observation was that this site was in close proximity (~250m) to a drainage line and associated riparian vegetation. The lack of vegetation around the waste water dam appears to not suit *L. capensis* opposed to being outcompeted by other bats species of the same foraging guild, such as *A. nana* and *P. rusticus/N. anchietae*. It appears that *L. capensis* in this instance has a closer association with vegetation structure than with water. This notion is supported by Taylor et al. (2020) where *L. capensis* dominated the bat community in the MMB of the Venetia Game Reserve and had a marked decrease in number of calls closer to the Limpopo River in Mapungubwe National Park. Although the PERMANOVA did not indicate that habitat was an important factor determining the AI of *L. capensis/P. (Vansonia) rueppellii,* a standalone ANOVA did indicate significant differences in AI in relation to habitat type and site ($P < 0.0001$). A similar pattern was recorded during the driven transects, where the highest *L. capensis/P. (Vansonia) rueppellii* AI was recorded near riparian vegetation of various watercourses (Figure 18b). Riparian vegetation structures were absent from the other sites. Bat habitat selection and vegetation dynamics (age of trees, vertical structure and species composition) particularly in forest habitats, have been shown to be strongly related (Monadjem and Reside 2008, Vasko et al. 2020). *Laephotis capensis/P. (Vansonia) rueppellii* may be responding to 1) the lack of preferred foraging habitat structure (or habitat complexity) and 2) potentially the absence of preferred insects associated with habitat complexity. Some bats and their prey are sensitive to differences in habitat quality influenced by anthropogenic activities or management practices (Starik et al. 2018). Higher bat species diversity and richness was shown to be positively influenced by the increase in vegetation structural complexity (particularly in the understorey) and the associated increase in insect abundance (Starik et al. 2018). A similar correlation was shown by Kusch et al. (2004) where in a low mountain range mixed forest, bat activity was significantly influenced by insect abundance and structure of the forest canopy.

Conversely, *A. nana* and *P. rusticus/N. anchietae* seem to benefit greatly from the Venetia Diamond mine and their activity on the Venetia Diamond mine is noteworthy, particularly at the waste water dam (alkaline: pH > 7) where during winter, these two species accounted for 71% of the bat activity where the otherwise prolific free-tailed bats, *C. ansorgei/Molossid 19kHz, C. pumilus/M. condylurus* and *T. aegyptiaca* only accounted for 23% of the activity. Surprisingly, *A. nana* and *P. rusticus/N. anchietae* almost disappear during the summer time with a reduction in activity of 96%, whereas free-tailed bats accounted for 90% of the observed activity and *N. nana* and *P. rusticus/N. anchietae* accounted for less than 2% of the total activity. It has been previously noted that *A. nana* in natural environments had preference to forage below the canopy of trees opposed to out in the open (Fenton 1977) and have been associated with well wooded habitats (Monadjem et al. 2020) and within clearings along the roads in forest in Uganda (Monadjem et al. 2010). In contrast, this behaviour was not recorded during the study as *A. nana* activity was by far higher over the open waste water dam than in the natural environment during both winter and summer. Large open water bodies have three essential characteristics that benefit foraging activity of some species bats namely: an acoustic environment that is uncluttered and facilitates echolocation, acoustic landmarks (banks and edges) for orientation over a large scale and most importantly, insect availability that is relatively high (Kusch et al. 2004). At the large open waste water dam on the Venetia Diamond mine, the subset sample of 10 days indicated that *A. nana* were predominantly foraging over the waste water dam (69.6% of calls were foraging calls). *Afronycteris nana* are of importance because they are known for being attracted to areas where chironomid midges swarm (Naidoo et al. 2013) and being of the same size and foraging guild, we speculate a similar diet for *P. rusticus/N. anchietae* but literature on their diet is scant. Swarms of chironomid midges would be an easily meal for these small species of bats. Biscardi et al. (2007) noted that the diet of *Myotis capaccinii* (although larger than *A. nana* and *P. rusticus/N. anchietae*) that was foraging over water of varying quality, was dominated by *Chironomus* midges. Although abundant over the waste water dam, a subset of 10 days of *P. rusticus/N. anchietae* calls showed that only 22.7% were foraging calls, with the remaining 77.3% search calls. The high activity of *A. nana* and *P. rusticus/N. anchietae* over the waste water dam, particularly during winter, is potentially an indirect indicator of poor water quality through the anticipated abundance of invertebrates tolerant of poor water quality**.** *Afronycteris nana* have gained increased attention since the publication of several papers by Naidoo et al. (2013, 2015 and 2016) that highlighted the adaptability and risk that these bats are exposed to in urban and peri-urban environments while foraging over waste water treatment works. The reduction in summer activity of these two species is presumably due to food abundance increasing during summer in natural habitats. The high activity of *A. nana* and *P. rusticus/N. anchietae* over the waste water dam on the mine could potentially expose these species to elements (heavy metals and trace elements) and other pollutants associated with the mining activity. *Afronycteris nana* and *P. rusticus/N. anchietae* are good candidates for investigating the biological effects of these contaminants associated with diamond mining.

The open-air forager group, which was made up predominantly of molossid bat species, dominated by *C. ansorgei/Molossid19kHz* and *T. aegyptiaca*, appeared to benefit from the Venetia Diamond mine with their activity being highest over the mining footprint during both summer and winter compared to the control plots. *Tadarida aegyptiaca* are tolerant of a wide range of habitats and climatic conditions, and are known to be strongly associated with water bodies in arid regions (Monadjem et al. 2020). Thus, the high activity of this species over the waste water dam was not surprising.

Table 6. List of proposed indicator species for the Venetia diamond mine detailing what type of indicator they could be and the type of impact they potentially identify. Environmental indicators respond to environmental disturbances in predictable ways e.g. bats responses to habitat removal or alterations. Ecological indicators respond to environmental stressors and reflect the response of other taxa in the habitat e.g. changes in water quality and the associated insect species that tolerate poor water quality.

Species	**Indicator Type**	**Type of Impact**	**Reason**
Cape serotine (*Laephotis capensis*)	Environmental	Habitat degradation	Virtually absent from mine footprint. Associated with natural vegetation structure and wooded areas.
Banana bat (*Afronycteris nana*)	Environmental & Ecological	Potentially indicate water quality and potential change in invertebrate abundance.	Highest activity at waste water dam.
Rusty bat/Anchieta's Pipistrelle (*Pipistrellus rusticus/Neoromicia anchietae*)	Environmental & Ecological	Potentially indicate water quality and potential change in invertebrate abundance.	Highest activity at waste water dam.

Although the open-air foragers were present in comparatively high numbers all year round, there were significant differences between summer and winter activity, with activity being highest during the summer months. In general, habitat only marginally influenced the activity of the open-air foragers. South African molossid bats are well known for making use of roosts in anthropogenic structures where they can choose different microclimates within the roosts to benefit thermoregulation and reproduction (Bouchard 1998, Bronner et al 1999, Vivier and van der Merwe 2007, Cory Toussaint et al. 2010, Lehmkuhl Noer et al. 2012, Monadjem et al. 2020). The available roosting spaces in the mine infrastructure and the available surface water on the Venetia Diamond mine year round, appears to greatly benefit *T. aegyptiaca, C. cf ansorgei* and *C. pumilus/M. condylurus*, making these species true exploiters as opposed to good indicator species (Table 7). In general, as seen in some studies, anthropogenic degradation of the landscape seems to benefit certain species in some instances, not only in distribution expansions, but also in the alteration of community structure, as adaptable species, like those molossids mentioned above, may outcompete more sensitive species or species with similar biology and behaviour (Ancillotto et al. 2016, Urban et al. 2012, Hersteinsson and MacDonald 1992).

Three notable exceptions to the molossids were *S. petrophilus, M. midas* and *O. martiensseni*. *Sauromys petrophilus* was absent from the control plots and mining footprint during the entire winter monitoring period, and was present only during summer, accounting for 9% of the observed activity. *Mops midas* and *O. martiensseni* showed a marked seasonal fluctuation with a decrease in activity between summer and winter of 98%. As such, we have considered them to be seasonal exploiters (Table 7). They do not seem to make use of the available roosting opportunities in the mine infrastructure as none were captured from a large mixed roost of *C. pumilus* and *M. condylurus* bats. Perhaps they could be outcompeted by *T. aegyptiaca*, *C. pumilus* and *M. condylurus* bats during winter. Possibly *S. petrophilus, M. midas* and *O. martiensseni* are not as tolerant of night-time low temperatures over an extended period of time (i.e. winter). During winter, *S. petrophilus* was absent, and *M. midas* and *O. martiensseni* were virtually absent (n = 5 and n= 4 respectively) during the winter study period, the reason for which is unknown.as they were virtually absent from the area during the winter monitoring period. In a study conducted by Cory Toussaint and McKechnie (2012), *S. petrophilus* did not exhibit a clear thermoneutral zone in a temperature chamber and allowed their body temperatures to fall below 32°C at ambient temperatures between and equal to 10–25°C. At ambient temperatures < 10°C their body temperature fell below 25°C which indicates that these bats are capable of torpor (Cory Toussaint and McKechnie 2012), but for what length of time is unknown. At ambient temperatures below 15°C over several days, free-ranging *T. aegyptiaca* have been shown to hibernate, albeit with a very small sample size of individuals (Cory Toussaint et al. 2010) and *M. condylurus* readily enter torpor at low ambient temperatures (Vivier and van der Merwe 2007, Bronner et al. 1999). This may account for the reduction in activity of these two species.

Concerning species of Rhinolophidae, they are often considered 'sensitive' as this is a family of bats that often have very specific habitat (they are adapted to foraging in dense vegetation) and roosting requirements. Dramatic changes in the environment can impact heavily on these species and in some areas they can be used as bioindicators (at a genetic level) of habitat fragmentation/degradation (Dool et al. 2016). In a study conducted by Schoeman (2015), *R. simulator* was categorised as an urban avoider and did not make use of the light at stadiums for foraging. *Rhinolophus hipposideros* (Britain) has a similar aversion to lit habitats and is known to experience a significant reduction in activity in lit areas as well as commuting routes to potential foraging areas were disrupted by light (Stone et al. 2009). On the well-lit Venetia Diamond mine, particularly in the vicinity of the waste water dam where there is little vegetation structure, the same species had an activity index of 20 that was half the activity index recorded at a site in the Limpopo Ridge Bushveld ~5km away (straight line distance). Elsewhere in the study area, *R. simulator* had a very low activity index ranging from 2–5. In contrast to *R. simulator*, *R. smithersi* was not recorded on the mine footprint and was recorded predominantly in the LRB (n = 101) with a single call recorded in the MMB. There was a significant difference in *R. simulator* activity between summer and winter with an increase in activity from 17 calls during summer to 84 calls recorded in winter. Surprisingly the activity index of *R. simulator* and *R. smithersi* across the study site during summer was only 2 and 17 respectively but during winter, it increased to 65 and 84 respectively. *Rhinolophus cf. blasii* was only recorded at the VDM-W (n = 6). The waste water dam on the Venetia Diamond mine appeared to be important to *R. simulator* during winter, perhaps available drinking water was more important than habitat degradation to this "sensitive" species. *Rhinolophus simulator* in this context has thus been classified as an adapter species whereas *R. smithersi* has been classified as an avoider species (Table 7). Detectability of bat species with high frequency and highly directional calls, such as rhinolophids, are reported to have a lower detectability by bat detectors since the calls do not refract well around a bat detector (Waters and Walsh 1994, Monadjem et al. 2017 (n=1)), therefore, the activity of horseshoe bat species may be under-represented.

Table 7. Bat species qualifying as exploiters (bat species that benefit greatly from the mining activities including making use of the infrastructure as roosting sites), adapters (species that were present in the natural landscape, but made use of the resources on the mining footprint), avoiders of the Venetia opencast diamond mine and those species generally present in very low numbers, thus indicated as such (*n* = total AI). Unknown calls not included.

Species	**Common Name**	**Foraging guild**	**Category & Notes**
Tadarida aegyptiaca	Egyptian free-tailed bat	Open-air	Exploiter
Chaerephon pumilus	Little free-tailed bat	Open-air	Exploiter
Chaerephon cf. ansorgei	Ansorge's free-tailed bat	Open-air	Exploiter
Mops condylurus	Angolan free-tailed bat	Open-air	Exploiter
Mops midas	Midas free-tailed bat	Open-air	Seasonal Exploiter (Low presence in winter n = 5)
Otomops martiensseni	Large-eared giant mastiff bat	Open-air	Seasonal Exploiter (Low presence in winter n = 4)
Sauromys petrophilus	Roberts's flat-headed bat	Open-air	Seasonal Exploiter (Present in summer only)
Taphozous mauritianus	Mauritian tomb bat	Open-air	Seasonal Exploiter (Low presence in winter n = 12)
Pipistrellus hesperidus	Dusky Pipistrelle	Clutter-edge	Adapter (Present at on the mine during winter and summer, elsewhere in the landscape, present only in summer)
Eptesicus hottentotus	Long-tailed serotine	Clutter-edge	Adapter Had a preference for Musina Mopane Bushveld.
Scotophilus dinganii	Yellow-bellied house bat	Clutter-edge	Adapter Had a preference for Musina Mopane Bushveld furthest from the mine.
Laephotis capensis/Pipistrellus (Vansonia) rueppellii	Cape serotine/ Rüppell's pipistrelle	Clutter-edge	Avoider
Neoromicia zuluensis	Zulu serotine	Clutter-edge	Adapter
Afronycteris nana	Banana bat	Clutter-edge	Adapter
Pipistrellus rusticus/Neoromicia anchietae	Rusty bat/Anchieta's Pipistrelle	Clutter-edge	Adapter
Nycticeinops schlieffeni	Schlieffen's Twilight bat	Clutter-edge	Adapter
Rhinolophus simulator	Bushveld horseshoe bat	Clutter	Adapter Was most common in study site during winter (*n*=65), hardly a presence during summer (*n*=1). Made use of the waste water dam during winter (*n*=19).
Rhinolophus smithersi	Smith's Horseshoe bat	Clutter	Avoider Higher activity during winter. Avoided the mine footprint.
Rhinolophus cf. blasii	Blasius's horseshoe bat	Clutter	Low presence (*n*=6), only recorded at the mine waste water dam.
Miniopterus natalensis	Natal long-finger bat	Clutter-edge	Low presence (*n*=3)
Miniopterus fraterculus	Lesser long-finger bat	Clutter-edge	Low presence (*n*=8)
Hipposideros caffer	Sundevall's leaf-nose bat	Clutter	Low presence (*n*=4)

Secondary habitats created by mining (underground) have been shown to contributed to freshwater biodiversity conservation concerning dragonflies, that had a high species richness, including a high proportion of habitat specialists (Dolný and Harabiš 2012). The use of bats as bioindicators in areas already degraded by anthropogenic activities could provide us with much needed insight to the requirements of bats in semi-arid landscapes and the implications of these areas for bat conservation. There will always be a trade-off since those species that greatly benefit from anthropogenic activities and seem to use man-made resources to the fullest, create a new set of challenges and management implications. Large colonies of bats are unwelcome in infrastructure (personal communications with mine staff, Bouchard 1998), as they cause damage to ceilings in infrastructure and they potentially outcompete other bat species (Biscardi et al 2007). Even though the creation of favourable resources for bats by the Venetia diamond mine may seem to greatly benefit bats, the exposure to high concentrations of elements and other potential pollutants suspended in the waste water sources from mining operations may have detrimental impacts on bats (e.g. coal mining: Zocche et al. 2010). These potential effects of environmental contaminants on bats is poorly understood and documented (Zukal et al. 2015).

Lastly, between the bat detectors, species turnover (beta-diversity) was very high and this is evident concerning the preference of certain species (including the molossids) for available resources at a finer scale as opposed to general habitat type. As an index, beta-diversity is better than alpha diversity to explain the observed differences/compositional turnover between bat local assemblages between detectors (sites) (Whittaker et al. 2001, Socolar et al. 2016). This can be important when selecting bioindicators as species turnover at such a fine scale may lend towards to the identification of those species sensitive to anthropogenic changes in the environment at a very fine scale. For example, the sensitivity of *A. nana* and *P. rusticus/N. anchietae* to prey species attracted to stagnant/pollutant waste water bodies and *L. capensis* sensitivity to habitat degradation between comparatively close sites.

Limitations and constraints

Some days of sampling were missed on the Venetia Diamond Mine due to the sheer volume of bat calls filling up the SD cards much sooner than anticipated. There are some gaps in data from the 3rd and 4th bat detectors due to theft of batteries and elephant damage respectively (Table 2). However, we do not believe that this in any way would influence the results.

Active capture for validation of calls on the mining footprint was virtually impossible due to the light pollution across the entire footprint and often capture was terminated due to thunderstorms that rolled in every evening. There were very few dark zones (if any) on the mining footprint, with no dark zones at the waste water dams where trapping was focused.

Multiple years of monitoring data is essential to show if bat populations are stable and to determine the seasonal variability (interannual variability) of bat species as species with stable populations and low seasonal variability are preferred indicators (Starik et al. 2018). Species that show a strong seasonal response that are absent from the area during winter are probably not good candidates as environmental impacts during those periods will not be able to be monitored e.g. *S. petrophilus.* There are no data concerning bat activity over the footprint of the mine pre-development and a requirement of an indicator species is that it should be sensitive to changes in the environment and have a graded response over long periods of time so that the severity of the disturbance can be determined and remediated (Jones et al. 2009, Starik et al. 2018). Additional years of data will allow the proposed species of bats as indicators and potentially others to either strengthen or be rejected or be added as the seasonal fluctuations of bat populations over time many indicate temporal stability.

Certain species showed considerable overlap in call parameters depending on what the individual bat was doing, thus even though Egyptian, Little and Angolan free-tailed bats could be distinguished by the author with a degree of certainty, calls that were suspicious were assigned to the Egyptian free-tailed group. Additionally, the Cape serotine allowed their peak frequency (~38kHz) to fall to around 35-36kHz in certain call sequences. Uncertainty arose with full calls with peak frequencies between 35–36kHz and were thus classified as Bat 35–36kHz but were possibly *P.* (*Vansonia*) rueppellii. Certain bat species such as the Common slit-faced bat (*Nycteris thebaica*) were not recorded by the bat detectors based on their very soft calls. However, they are also well known to commonly occur in the area (Monadjem et al. 2020).

Conclusion

Responses of bat species to anthropogenic changes in the landscape differ greatly with some species of urban-adapters proving to be exploiters of anthropogenic resources opposed to reliable indicators of ecosystem health, while others are potentially suited as bioindicators to indicate habitat degradation and environmental health (water quality) based on their diet (Jones et al. 2009). Species that are even considered to be avoiders of anthropogenic disturbances, such as *R. simulator* (Schoeman 2015), can be classified as an adapter in this instance (Table 9). When determining which species of bat will be best suited as a bioindicators, it is imperative that it is specific to the study area/site or land use that the anthropogenic activity is occurring in.

In the instance of the Venetia Diamond Mine, the availability of surface water created by the waste water dam, irrespective of habitat degradation and quality, provides an important resource for bats not only for directly drinking but also for foraging. The direct implications of the water quality on the bats needs to be investigated. A potential bioindicator for this specific area is *L. capensis* (environmental indicator of habitat degradation) (Table 6). *Afronycteris nana* and *P. rusticus/N. anchietae* could potentially be indirect indicators of water quality based on the change in insect abundance and composition, however, detailed dietary studies are needed to clarify if these two species are consuming pollutant-tolerant invertebrate species and could thus be reliable bioindicators of water quality. Alternatively, they may just be adapters to an environment altered by anthropogenic activities.

The response of bats to water quality needs to be further explored over a much wider range of species than what has currently been represented in the literature, especially in South Africa. Bat species that respond to water quality changes and those with more specialised diets and specific habitat requirements could be more valueable as bioindicators as they could reflect the impact of environmental quality on their specific prey preferences and associated vegetation and or water quality. It has been shown in a review by Salvarina (2016) that not only are aquatic resources important for foraging and drinking, but the responses of bats to aquatic resources and quality of the resource are diverse and vary from region to region. Some bat species activity has been found to negatively correlate with poor water quality (*Lasiurus cinereus* and *Eptesicus fuscus*), others favour poor water quality (*Lasiurus borealis* and *Perimyotis subflavus*) while in others water quality seems to be of no consequence (*Lasionycteris noctivagans*) (Li and Kalcounis-Rueppell 2017). It is however, of utmost importance to understand how bats respond to anthropogenic activities, water quality (Laverty and Berger 2020) and climate change (Adams 2010) as well as monitor bat populations in our continually changing landscapes. These aspects are crucial for conservation planning and promoting ecosystem service provision that benefit humans too (e.g. bats as pest control agents in Macadamia orchards, Linden et al. 2019, Taylor et al. 2018).

From my study, it has proven difficult to identify additional species that can be used to monitor environmental health as the generalist species of bats, such as molossids, appear to benefit from the artificial resources and possibly have had enough time over which to adapt to the disturbances. Perhaps, initially, there may have been a response by bats to the development of the Venetia Diamond Mine, but without initial data collection at this time, it is superfluous to hypothesise the kind of initial response by bats. Since bats respond fairly quickly to changes in their environment, are long-lived, slow reproducers, are fairly easy to monitor, globally they have been regarded as suitable bioindicators (Li and Kalcounis-Rueppell 2017). However, the use of bat species suitable as bioindicators from an acoustic monitoring perspective, particularly in South Africa, has not been investigated and is more complex than perhaps initially envisioned. There is a need for studies to provide baseline data for future studies on which to build. There is a need to investigate how bats respond to these comparatively small areas of development that appear to be crucial for certain bat species living in arid environments in South Africa.

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NOTE: We strongly caution against the citation of this paper in support of proposed mining developments. This study is unique to the Venetia Diamond Mine and should be kept in context. Mining practices vary according to the resource that is being mined with their own suite of environmental contamination and biodiversity impacts.

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Chapter 3: Artificial lighting, anthropogenic noise and vegetation cover impacts bat communities on opencast mines in sub-tropical regions: a case study in Africa.

Abstract

Bats are known to be sensitive to changes in their environment. The development of large-scale mining operations are being encouraged in Africa, threatening important bat habitat. With the looming risk of industrial (including mining) development in the fairly pristine sub-tropical northern Limpopo Province, the impact of natural vegetation cover, artificial light intensity and noise (dBA) were investigated on the bat community on the opencast Venetia diamond mine. Mixed-effect models revealed that natural vegetation was the most important factor impacting species richness and bat activity (total activity index (AI), foraging guild and behaviour). Species richness and total bat activity were highest where natural vegetation cover was ≥25%. Species richness was significantly affected by minimum temperature (T_{min}) in addition to natural vegetation, with highest species richness recorded at warmer temperatures. In conjunction with natural vegetation and T_{min} were significant for AI, open-air foragers and searching/commuting behaviour. Artificial light only significantly affected foraging activity with less foraging activity in the lit areas. In the third best-fit model for AI and the second best-fit model for searching/commuting behaviour, anthropogenic light was identified as an important factor although not significant. Season and moon phase were only significant in the best-fit models for foraging activity with the lowest foraging activity recorded during early spring and during the first quarter and waxing/waning gibbous phases. Noise (dB) was only selected as an important factor in the second best-fit model for open-air foragers, however, it was not significant. Our study highlights the importance of vegetation cover and the complexity of the interaction between bats and the environment incorporating anthropogenic factors (artificial lighting, continuous noise and habitat degradation) and natural factors such as T_{min}, moon phase and season that confound trends in bat species richness and responses in response to opencast mining. Understanding how African bats respond to these significant alterations in the landscape will be crucial in determining mitigation and remediation strategies for large-scale mining operations that are a looming threat to bat biodiversity and conservation in sub-Saharan Africa.

Keywords: Chiroptera, artificial light, anthropogenic noise, bat behaviour, opencast mining, bat ecology

Introduction

The expansion of urbanization and economic development over the centuries has resulted in the expansion and intensification of associated artificial lighting and anthropogenic noise in habitats that were previously unlit and quiet (Davies et al. 2012, Stone et al. 2015, Brumm and Horn 2019).

With a global annual increase in artificial lighting of approximately 6% (Davies et al. 2012, Stone et al. 2015, Lacoeuilhe et al. 2014), the impact of ecological light pollution has become a significant global concern (Davies et al. 2012, Gaston and Bennie 2014). A variety of artificial lighting has been employed globally. Low-pressure sodium lamps (LPS) and high-pressure sodium lamps (HPS) have highest luminous efficiency, do not emit ultraviolet (UV) light and are predominantly used for street lighting (Lewanzik 2017, Wakefield et al. 2017, Stone et al. 2015). LPS lights produce a monochrome orange light that peaks at 589.3nm and hardly allows the human eye to identify colour, however HPS lights produce a range of wavelengths, thus allowing for a degree of colour identification and are used more often than LPS lights for street lighting (Lewanzik 2017). High-pressure mercury (MV) lights and metal-halide (MH) lights emit a multitude of wavelengths that allows for good colour perception, but also emit UV and a significant amount of waste light into the environment (Lewanzik 2017). Ecological light pollution has largely been under-emphasized concerning anthropogenic developments (e.g. mining, urban and rural planning) and the resulting implications for conservation strategies and environmental management (including biodiversity management) poorly understood (Owens et al. 2019).

Ecological light pollution is waste light from anthropogenic developments that changes and or disrupts the natural light-dark regime and a large percentage of ecological light pollution is as a result of street lighting (Lewanzik 2017, Gaston and Bennie 2014). As underlined by many studies, ecological light pollution disrupts time measurement by interference with circadian, seasonal and lunar cycles (Longcore and Rich 2004), impacts physiological function recovery and repair (cellular level) (see Longcore and Rich 2016), alters the recognition of resources and predators (Gaston et al. 2013), affects temporal niche portioning (diurnal animal activity extending into the night) (see Longcore and Rich 2016, Spoelstra et al. 2017), impairs navigation and even leads to disorientation (Poot et al. 2008). These impacts affect a variety of taxa including, plants (ffrench-Constant et al. 2016, Meravi and Prajapati 2020), invertebrates (Davies et al. 2012, Wakefield et al. 2017, Owens et al. 2019), fish (Riley et al. 2013), crustaceans (Navarro-Barranco and Hughes 2015), birds (Da Silva et al. 2015), amphibians (Dias et al. 2019), reptiles (Perry et al. 2008) and mammals (Duffy et al. 2015, Robert et al. 2015, Le Tallec et al 2016, Haddock et al. 2019) that could lead to detrimental effects on biodiversity and ecosystem functioning over time.

The responses to ecological light pollution vary between species. A few examples will be explored. Some species are light-distracted, typically insects that often arrive in large numbers through some form of disorientation that distracts them from movements that are normally conducted in darkness (Gaston and Bennie 2014, Bailey at el. 2019, Owens et al. 2019). Especially in urban developments, some lighting is more detrimental than others based on the wavelengths of light energy emitted, the amount of waste light and their desirability to insects (Eisenbeis and Hänel 2009, Firebaugh and Haynes 2019, Wakefield et al. 2017, Owens et al. 2019). Ecological light pollution has been shown to cause behavioural and physiological changes that impact on insect physiology (development suppression and acceleration), fitness, migration (including local daily movements), reproductive success, and predation (Davies et al. 2012, Owens et al. 2019).

Anthropogenic noise is an emerging pollutant that is gaining more attention in terms of different species sensitivity to noise, the physiological and ecological impacts of anthropogenic noise and the implications for conservation of biodiversity (Bunkley and Barber 2015) and has been studied in a variety of species of birds, frogs, mammals (terrestrial and aquatic), insects and other invertebrates such as mussels and hermit crabs (Hotchkin and Parks 2013, Kruger and Du Preez 2013, Shannon et al. 2016, Nagelkerken et al. 2019, Tidau and Briffa 2019, Wale et al. 2019, Eastcott et al. 2020, Halfwerk and van Oers 2020, Sathyan and Couldridge 2020). Anthropogenic noise is often loudest at low frequencies and has in instances resulted in individuals avoiding acoustic masking by shifting their signal band upwards and or increasing the signal amplitude of their call to improve the signal-to-noise ratio, such as common blackbirds (*Turdus merula*). This is known as the Lombard effect (Hotchkin and Parks 2013, Gallego-Abenza et al. 2019).

Acoustic masking has the potential to disrupt information transmission between individuals resulting in fitness consequences for the sender and receiver when the detection and interpretation of the auditory cues are erroneous (Hotchkin and Parks 2013). Stress, perceived predation threat, missed mating opportunities, selection of lower quality mates, unnecessary aggressive behaviour, missed foraging opportunities (including prey escape due to delayed attack or not noticing a prey item) and reduction in attention caused by noise can result in severe fitness consequences (Brown et al. 2012, Hage and Metzner 2013, Hotchkin and Parks 2013, Luo et al. 2015, Manson et al. 2016, Gomes et al. 2016, Geipel et al. 2019, Gallego-Abenza et al. 2019, Eastcott et al. 2020, Halfwerk and van Oers 2020).

Predators that hunt by passive listening to sounds emitted by potential prey items can be negatively impacted by anthropogenic noise by the decreased success of detecting and striking prey and an overall poorer hunting success (Gomes et al. 2016, Manson et al. 2016). A good example is how saw-whet owls (*Aegolius acadius*) experienced an overall decrease in hunting success when exposed to compressor station noise associated with natural gas extraction (Manson et al. 2016). Even for predators that rely on vision for hunting Halfwerk and van Oers 2020 showed that great tits (*Parus major*) took longer to approach and attack artificial targets as noise levels increased. Noise significantly impacted on the birds' ability to process visual information associated with more complex tasks especially where targets were cryptic, increased noise levels resulted in attack latencies significantly increasing (Halfwerk and van Oers 2020). The birds habituated over time and could perform simple tasks, such as identifying conspicuous and colour matched targets, but not the cryptic targets (Halfwerk and van Oers 2020).

Another response to anthropogenic noise is to either reduce or change acoustic activity to avoid fitness costs. Field crickets, *Gryllus bimaculatus*, exposed to traffic noise were studied in the field by Gallego-Abenza et al. (2019). They showed that male crickets adjusted their singing behaviour depending on the level of anthropogenic noise. Males that were chirping closer to the road and exposed to high traffic noise reduced their chirp rate, maintained a stable number of pulses per chirp, and could dissociate the noise of a car from a potential predator thus they recovered faster after a noisy period, making the most of the intermittent quiet periods between passing cars to attract females. Males further away from the road took longer to recover and continue chirping after a car had passed possibly based on anti-predatory behaviour (Gallego-Abenza 2019). Sathyan and Couldridge (2020) showed that the bladder grasshopper (*Bullacris unicolor*) lowered their call peak frequency and increased the inter-call interval as noise levels increased and even shifted the calling period to later at night, possibly to avoid the higher noise levels and take advantage of the quieter period. Eastcott et al. (2020) showed in a field-based study that dwarf mongooses exhibited intrapopulation responses to traffic noise that was dependent on individual mongoose behaviour and age. Individuals that were foraging showed the strongest immediate response to traffic noise and pups fled more quickly, increased their vigilance and resumed foraging slower than adults (Eastcott et al. 2020). Lastly, Kruger and Du Preez (2013) reported temporal and spectral changes in the calls of male Pickersgill's Reed frog (*Hyperolius pickersgilli*) calling at a site impacted by airplane noise. The frogs called at higher pitches and increased their calling effort during and immediately after intense airplane noise compared to calls prior to airplane noise disturbance.

Anthropogenic developments expose bats to habitat alterations and a range of pollutants, to which bats are known to be sensitive (Jones et al. 2009, Naidoo et al. 2016, Frick et al. 2020). Opencast mining presents an interesting opportunity to study the effect of habitat degradation from the physical removal of natural habitat to the introduction of continual noise and night time light pollution from mining operations. Bats responses to these environmental changes appear to be from a morphological perspective due to the evolution of particular wing shapes and echolocation calls specific to certain habitat types (Aldridge and Rautenbach 1987) and possibly from a physiological perspective due to the sensitivities of their audiological and visual systems. Bat habitat preference can be inferred from wing morphology and echolocation call structure even though diet may differ between species of similar morphology (Brigham et al. 1997, Monadjem et al. 2020). In general bats that prefer to forage above vegetation in open spaces (open-air foragers) have long, narrow wings with aspect ratios >10.9 and high wing loading and produce narrowband, long duration and low frequency calls (Norberg and Rayner 1987, Monadjem et al. 2020). Species which prefer to forage along the edge of vegetation and within semi-open habitats (clutter-edge foragers) have aspect ratios between 7 and 10.9 and intermediate wing loading with associated broad- and narrowband calls of intermediate duration and intermediate frequencies (Norberg and Rayner 1987, Monadjem et al. 2020). Broad and relatively short wings with low wing loading and aspect ratios <7 with either long duration, high-duty constant frequencies or short duration, low-duty frequency modulated calls are typically characteristic of bat species that forage within cluttered habitats (clutter foragers) (Norberg and Rayner 1987, Monadjem et al. 2020). Alterations to habitats creating unsuitable foraging and roosting habitats for any of the abovementioned foraging groups, results in shifts in bat behaviour and community structure (Bader et al. 2015).

Many studies have focused on the impacts of noise and light individually on bat behaviour predominantly in a laboratory setting, focusing on a single or select few species. There are exceptions that have investigated these impacts in the field in habitats that are naturally dark and lit for experimental purposes (Stone et al. 2009, Minnaar et al. 2014, Bailey et al. 2019) or areas that experience temporary lighting depending on human activities e.g. at stadiums (Schoeman 2015). Consequently, all these studies show that the response of bats to light and noise in their environment is species specific (Schaub et al 2008, Stone et al. 2009). Below, we briefly discuss some of the impacts firstly of artificial lighting and secondly of noise.

Research has shown that all types of conventional lighting can impact bats over large and fine spatial scales (Stone et al. 2009, Minnaar et al. 2014, Rowse et al. 2016, Lewanzik 2017). There is evidence that bat eyes function best under low light conditions as the eye predominantly contains rods, therefore as ambient light increases, bats eyes experience a decrease in visual sensitivity (Fure 2006, Gorresen et al. 2015, Liu et al. 2015). Bats can exploit low light conditions due to this specific physiology and may avoid bright light (Fure 2006, Liu et al 2015). Artificial night time lighting delays and reduces the number of bats emerging from their roosts (Downs et al. 2003, Boldogh et al. 2007), can result in the abandonment of roosts (Rydell et al. 2017), influences foraging behaviour (Minnaar et al. 2014, Stone et al. 2015, Lewanzik 2017, Voigt et al. 2020), affects commuting behaviour (Stone et al. 2009, Gaston et al. 2013, Stone et al. 2015, Lewanzik 2017) and possibly interferes with navigation (Mathews et al. 2015). If adults have to forage further afield from maternity roosts, the resulting higher energetic cost and decreased foraging time could negatively impact the growth rates of young bats (Boldogh et al. 2007, Stone et al. 2015). Artificial lighting can thus create "barriers" that may limit the effective dispersal of species, separating habitat patches and populations from immigration and reduce the connectivity of habitats in the landscape e.g. *Rhinolophus hipposideros* (Stone et al. 2009, Gaston and Bennie 2014). Many bat species appear to be intolerant of light and avoid lit areas, particularly slow-flying highly manoeuvrable species that feed within cluttered spaces (clutter foragers) (Schoeman 2015, Haddock et al. 2019). In general they are accepted to be intolerant of light and avoid lit areas due to the sensitivity of their eyes to light (and ultraviolet) that may lead to potential vision impairment in lit areas (Jones et al. 2009, Lewanzik 2017). Bats may even perceive a higher predation risk in illuminated habitats (Minnaar et al. 2014).

Although some bat species avoid artificially lit areas, some bat species are light-tolerant and appear to benefit from artificial light, regardless of possible negative impacts, by quickly identifying and exploiting insects swarming around lights, thus increasing their foraging efficiency (Rowse et al. 2016, Lewanzik 2017, Lewanzik and Voigt 2017, Azam et al. 2018, Cravens and Boyles 2019). Insects are attracted to all types of conventional lighting, although they are attracted to certain types of lighting more so than others, resulting in the observed swarming behaviour (Gaston et al. 2013, Rowse et al. 2017). Light-tolerant bat species are often open-air and clutter-edge foragers, fast flyers with echolocation calls that are adapted for open and semi-open habitats respectively (Rowse et al. 2016). Interspecific competition at lights for food resources can arise by the extension of foraging activity into the night-time period of diurnal species (e.g. birds). Bailey et al. (2019) recorded shifts in the diet of generalist feeders, *Pipistrellus hesperidus*, to include more abundant insects attracted to lights. The colour of streetlamps have also been shown to alter the behavior of insects and associated bat activity. In 1994, Blake et al. showed that white streetlamps attracted more insects than orange lights as well as three times more foraging *P. pipistrellus* than orange streetlamps and unlit roads. *Plecotus* and *Myotis* species appear to be more sensitive to white and green light than red light and the opposite true for species of *Pipistrellus* (Spoelstra et al. 2017). There are those species that appear to not be affected by light spectra and have been recorded foraging around lights such as *Eptesicus serotinus* and *Nyctalus noctula* (Azam et al. 2016, Spoelstra et al. 2017). Nevertheless, for those species that are attracted to street lamps as foraging opportunities, there could be an increased risk for collisions with vehicles (Rowse et al. 2016, Stone et al. 2015). Artificial lighting could alter the stability of communities through competitive exclusion where light-tolerance species may become abundant in the environment by outcompeting intolerant species (Stone et al. 2015). For example, artificial lighting may have resulted in the competitive exclusion of *R. hipposideros* by *P. pipistrellus* in Switzerland as shown by Arlettaz et al. (2000).

Soundscapes are the acoustic perception of a landscape that encompass the complete set of noises in a given environment that originate from biological (biotic), geophysical (non-biological, abiotic) and anthropogenic sources (Pavan 2017). Natural biotic (created by conspecifics, heterospecifics or potential predators and prey) and abiotic (weather, vegetation, moving wateretc.) noises are important auditory cues for animals (Farina 2019, Gallego-Abenza et al. 2019). For example, the sound of rain is an important cue for bats *Micronycteris microtus* and *Molossus molossus* (Geipel et al. 2019). The sound of rain (together with increased humidity, decrease in barometric pressure and odour compositional changes) provides information to bats concerning the cost and benefits of foraging in the rain and for *M. microtus* and *M. molossus* resulted in a delay in their emergence (Geipel et al. 2019). In naturally noisy environments, animals exhibit flexibility in their signal production and responses (flexible adaptation) (Gallego-Abenza et al. 2019). Anthropogenic noise is an emerging pollutant that is gaining more attention in terms of different species' sensitivity to noise, the physiological and ecological impacts of anthropogenic noise and the implications for conservation of biodiversity (Bunkley and Barber 2015). Increased anthropogenic alterations of soundscapes have been studied in a variety of species of birds, frogs, mammals (terrestrial and aquatic), insects and other invertebrates such as mussels and hermit crabs (Halfwerk and Oers van 2020, Sathyan and Couldridge 2020, Eastcott et al. 2020, Wale et al. 2019, Tidau and Briffa 2019, Nagelkerken et al. 2019, Shannon et al. 2016, Kruger and Du Preez 2013, Hotchkin and Parks 2013). The World Health Organisation (WHO) lists outdoor noise levels between 50–55 dBA to be moderately to seriously annoying and can disrupt sleep at continuous noise levels > 30 dBA and > 45 dBA for individual noise events (Berglund et al. 2000).

Bats are known auditory specialists (Lattenkamp et al. 2020), with excellent hearing over several octaves, with the greatest sensitivity in species-specific echolocation call spectral ranges, although this is not true for all bat species e.g. *R. ferrumequinum* (Bohn et al. 2006). Foraging success can be impeded by auditory conflicts resulting from anthropogenic or natural noise that has spectral ranges similar to that of any given species of echolocating bat or species that rely on the sounds produced by prey (mating calls and movement) (Simmons et al. 1978 , Schaub et al. 2008, Luo et al. 2015, Gomes et al. 2016). The response of bats to noise seems to be not only species and individual specific but is also dependent on the behavioural context (Schaub et al. 2008, Luo et al. 2014, Luo et al. 2015). Noise did not cause acoustic masking or reduce attention in *M. daubentonii* but, under laboratory conditions, foraging success was reduced due to avoidance of noise (Luo et al. 2015). *Antrozous pallidus* has been shown to increase their searching time under anthropogenic noise conditions that may, in the field, result in decreased fitness or survival as energy budgets might not be met during the night-time foraging period (Bunkley and Barber 2015). Under laboratory conditions *M. myotis* avoided the playback sound stimulus and actively avoided foraging areas that were heavily impacted by noise (Schaub et al. 2008). Conversely, during rest (torpid period), *M. myotis* quickly habituated to anthropogenic noise exposure (Luo et al. 2014). Species that rely on passive listening to locate prey are expected to avoid foraging habitats degraded by anthropogenic noise such as *M. blythii, M. evotis, M. septentrionalis, Euderma maculatum,* and species from the genera *Plecotus* and *Corynorhinus* (Schaub et al. 2008, Bunkley and Barber 2015). Overall bat activity and foraging behaviour of free-living bat communities in England showed a marked decrease when exposed to playback of traffic noise in areas that previously did not have noise (Finch et al. 2020). These studies are important to provide an expected initial response to proposed and approved anthropogenic developments. Furthermore, follow up monitoring after the development (e.g. road building, mining developments etc.) can be used to address the rate of habituation of certain species to the changes in soundscapes.

Studies on the effects of large scale opencast mining activities on bat communities in tropical regions is scant and few exist for temperate regions. In Southwest Britain the Drakelands opencast mine was shown to have impacts extending up to 900m from the mining activity (Theobald et al. 2020). The main factors influencing total bat activity and species richness were distance from the boundary of the mine, woodland cover and climatic variables (Theobald et al. 2020). In the desert region of Western Australia, near Marble Bar, the short-term impacts of exploration drilling (vibrations and noise) on colonies of *Macroderma gigas* and *Rhinonicteris aurantia* were shown to not be a significant disturbance to bats, provided drilling activities were not within 85 m of a roost or within 25m of the mine entrance (Armstrong 2010). The noise from machinery associated with opencast iron ore mining in Brazil was shown to have a significant negative impact on the biological soundscape complexity of neighbouring Atlantic forest (Duarte et al. 2015). At sites close to the opencast mining operation the observed decrease in complexity is suggested to be due to lower species richness closer to the mine, animal community structure changes, call spectral characteristic differences between the forest and mine areas and /or the adaptive responses of animals to noise (Duarte et al. 2015).

In the fairly pristine, sub-tropical and semi-arid northern Limpopo River Valley, bat communities are faced with various survival challenges from natural climatic conditions, availability of surface water for drinking (Taylor et al. 2020) and looming anthropogenic developments (future mega industrial zone developments: Coal mining projects and associated processing plants and power stations, Digby and Wells Environmental 2019, Munnik 2020). We investigated the impact of artificial lighting, noise and natural vegetation cover on bat activity and behaviour in relation to opencast mining. Our study is novel as we investigate the effects of vegetation structure, continuous light and noise together in the field, taking moon phase, minimum temperature and season into consideration where most studies have investigated the impact of light and noise on bats separately. We hypothesise that along a transect impacted by ecological light pollution, anthropogenic noise and varying natural vegetation cover that there will be a difference in species richness and activity (including foraging behaviour). Alternatively, there may be no difference in bat species richness and activity in areas affected by ecological light and noise pollution, and reduced vegetation cover. We predict that 1) bat activity (total activity and foraging guild activity) and 2) bat species richness will be significantly lower in areas affected by ecological light pollution, high noise levels and reduced vegetation cover than the dark/dimly lit, quiet, vegetated areas. Additinally, we expect that foraging activity will be higher under lit conditions due to the expected increase in insect activity around light sources compared to unlit conditions. Alternatively, foraging activity will not be higher under lit conditions.

Materials and Methods

Study site

The study was conducted on the footprint of the opencast Venetia diamond mine (-22.427708°, 29.324158°) during the periods 04–10 March 2019 (early autumn) and 02–16 September 2019 (early spring) (21 nights) (Figure 19). The Venetia diamond mine is situated in the northern Limpopo River Valley, approximately 60km north of the Soutpansberg mountain range (Figure 19). Mining related activities began in 1984 and the mine was fully operational since 1992. (https://www.debeersgroup.com/the-group/our-history). Mining operations are active 24 hours a day. The mine is located in the Limpopo Ridge Bushveld (Mucina and Rutherford, 2011) and is restricted to a kimberlite pipe containing the diamonds (Brown et al. 2009). The Limpopo Ridge Bushveld is dominated by Mopane (*Colophospermum mopane*), Red Bushwillow (*Combretum apiculatum*) and Purple-pod Cluster-leaf (*Terminalia prunoides*) with a handful of other iconic tree species such as Knobthorn (*Senegalia nigrescens*), Marula (*Sclerocarya birrea*) and Baobab (*Adansonia digitata*) (Mucina and Rutherford, 2011). The area is considered subtropical and semi-arid. The climate is characterized by warm dry winters and hot summers with mean annual precipitation between 300-400mm falling predominantly during the summer period (Mucina and Rutherford, 2011) in the form of terrific thunderstorms (pers. Obs.).

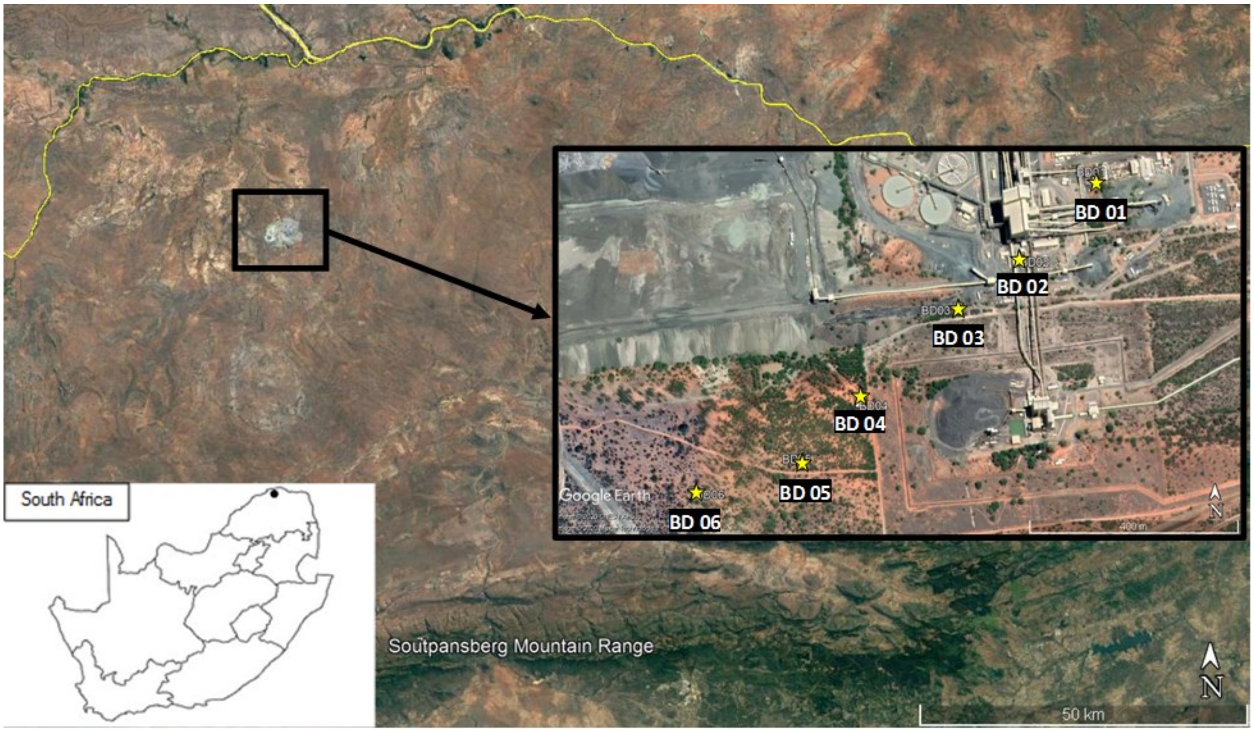

Figure 19. The location of the study site in the northern Limpopo River Valley, South Africa and the placement of the bat detectors (★) on the Venetia diamond mine. BD01 was situated at a workshop, BD02 was at the processing plant itself, BD03 was on the edge of a laydown (open-air storage) area, BD04 was placed along the edge of a road opposite the sorting area, BD05 and BD06 were place furthest from the noise and light of the active mining areas in natural vegetation.

Light transect location and luminosity measurements

As the study is part of a larger case study, the *in-situ* lighting on the mine was used to identify the impact of light along a gradient. Six SM4BAT FS recorders (Wildlife Acoustics, Inc.) with SMM-U1 ultrasonic microphones mounted approximately 6m above the ground and fitted with two 64GB SDXC cards were placed approximately 100m apart along a light gradient. The transect began from the floodlights near several workshops and the processing plant and extended in a straight line way from the mine into darker areas (Figure 19). The specific positioning of the transect ensured that the effect of water was eliminated to prevent an over-representation of activity at a given site since bats are known to be attracted to artificial water points in semi-arid regions in the absence of larger, natural water sources (Taylor et al. 2020). Figure 19 indicates the placement of the six detectors which avoided water bodies. Bat detector 01 was placed in an area that was exposed directly to a harsh white light from the workshop buildings as well as an orange floodlight from a nearby conveyer belt system (~36m away). Bat detector 02 was positioned in an area that was exposed to direct light from an orange floodlight at the processing area. Bat detector 03 was placed on the edge of the processing plant in a laydown area (open-air storage space). Bat detector 04 was placed at the edge of the mining footprint to the southwest of the processing plant. Bat detectors 05 and 06 were placed furthest away from the processing plant extending into natural vegetation (Figure 19). Bat detectors 03–06 were exposed to the spill over of light from orange floodlights at the processing plant, office buildings and sorting area.

A handheld digital lux meter (ME-GM1020 Digital Lux Meter) held ~ 2m above the ground with the light-sensitive sphere pointing towards the light source was used to determine a proxy for maximum luminosity at each site and was presented as the measurement lux. Maximum luminosity was recorded as this would in effect be what the bats are exposed to when flying through lit patches. At bat detectors 04, 05 and 06, spill over light was measured in lit areas within the vegetation as the vegetation had effectively created dark spots where luminosity was recorded as 0.0 lux. Due to logistic constraints, light measurements could not be taken each night. Light intensity readings were recorded on the initial nights of the transect installations during March and September 2019 after the sun had set and the horizon no longer had the glow of the setting sun. Thus, lux measurements were taken twice and the maximum lux reading per bat detector was used for analysis.

Noise frequencies and sound pressure levels

The primary source of noise that bats would be exposed to was the constant noise from the processing plant (crusher and conveyor systems). There would also be intermittent noise from trucks and earth moving plant (engine noises, reverse alarms) and loading and offloading of material. From the recorded bat call files, the continuous noise levels that the bats would have been exposed to at each bat detector per night was extracted from all the files. The Noise Analysis tool in Kaleidoscope Pro (www.wildlifeacoustics.com) was used to determine the maximum sound pressure level (SPL) of the mine at each bat detector. The analysis was followed in accordance with the guidelines suggested by Wildlife Acoustics (www.wildlifeacoustics.com/resources/video-tutorials/kaleidoscope-pro-software-noise-level-analysis-english). We selected the standard A-weighted frequency band (covering the audible frequency range from 20 Hz–20 kHz) to be analysed, as this would provide a frequency response curve typical to how a human ear would perceive the ambient noise of the mine and is considered ideal for bat hearing ranges (Bunkley and Barber 2015). We required the scale of the SPL output results to be in relation to the international reference pressure (auditory threshold) of 0 dB (SPL) = 20 μPa (sound pressure), where 1 Pa is equal to 94 dB relative to 20 μPa, thus 94 dB was entered in the dB adjustment field (Bruneau 2006). Taking into account the microphone sensitivity gain of +12 dB entered into the settings of the SM4BAT, the software applied a correction factor of 81 dB. From here on, SPL will be referred to as noise (dBA). The noise frequency along the transect that the bats were exposed to was measured in BatSound from sound files with a timestamp as close to 19:00 as possible on each night. The frequency of the background noise was determined over a 2000 ms period and the mean and standard deviation was calculated.

To determine the acoustic intensity ratio (z) between the "quietest" and "loudest" points along the transect, the equation $\Delta L = 10\log_{10}(z)$ was used. ΔL is the difference between two relative intensities and z is the ratio of one sound to another, thus $z = 10^{\Delta L/10}$. To calculate the perceived change in loudness or level change (x), the equation $\Delta L = 10\log2(x)$ was used, thus $x = 2^{\Delta L/10}$ (equations by Sengpeil Audio).

Percentage of natural vegetation estimation

Natural vegetation cover was visually estimated and recorded as a percentage: bat detectors 01 and 02: 0% (completely devoid of vegetation: no trees and no grass), bat detector 03: 25% (in a cleared lay-down area: on the edge of a stand of trees but no grass), bat detector 04: 50% (on the edge of a wooded area bordered by a road and open grassy area) and bat detectors 05 and 06: 100% (unaltered natural vegetation). For the mixed-effects model analysis and resulting graphical outputs, each percentage was assigned a letter to ensure it was treated as a categorical factor: A = 0%, B = 25%, C = 50%, D = 100%.

Call analysis

Kaleidoscope (Wildlife Acoustics, Inc.) was used to convert sound files (.WAV) into zero-crossing (ZC) and associated .WAV files. AnalookW (Chris Corben) and BatSound (Pettersson

Elektronik AB) were used to identify all bat calls. A minimum of four pulses per 15 seconds was initially filtered from the data set. The filtered sound files were then bulk sorted to species level using filters designed in AnalookW based on call parameters from Taylor et al. (2013) and Monadjem et al. (2020) and were refined using bat calls recorded on site. Due to the overlap in call parameters (particularly peak frequencies, durations and bandwidths), all calls were manually checked and adjusted as necessary if the filters had incorrectly identified the calls. Three categories of bat behaviour namely searching/commuting, foraging attempts (feeding buzzes) and socializing, were manually identified from the ZC files and were validated using BatSound. The identification of each echolocation call to bat behaviour was important to determine if the artificial lighting on the mine provided a foraging opportunity. All calls were organized into foraging guilds according to Monadjem et al. (2020): open-air foragers (OAF) that fly and forage above the vegetation (Molossidae and Emballonuridae), clutter-edge foragers (CEF) that forage near/along the edge of vegetation (Vespertilionidae and Miniopteridae) and clutter foragers (CF) that forage within cluttered spaces, often close to the ground (Rhinolophidae and Hipposideridae). All bat passes were standardised to Activity Index (AI) based on Miller (2000). Activity Index was thus represented as one call per specific species over a one-minute interval. The same was done for behavioural calls with special attention paid in instances where conspecifics were performing two types of behaviours during the same one-minute interval, thus no identified calls were lost.

Detectability of the bats across the site does need to be considered. However, the proposed correction factor by Monadjem et al. (2017) will not be applied to the current data set due to several concerns around sample size, bat detector brand and methodology. Until more research has been conducted in this field with new technologies, we are hesitant to apply a correction factor to the current data as it will undoubtedly distort the data.

Statistical Analysis

R version 3.6.3 and packages "car", "pscl", "lme4", "MuMIn", "multcomp" and "mgcv" were used to perform the statistical analyses. Significant differences in artificial light and noise (dBA) between six bat detectors were explored using one-way ANOVAs since these main independent (light and noise) variables of particular interest were expected to be significantly different between the bat detectors. Linear mixed-effects models (lmer) and a generalized mixed-effects model (glmer) were used to determine which factors and associated models were most likely responsible for the observed differences in AI (total AI, AI per foraging guild and AI associated with specific behaviours) and species richness along the transect. Bat detector was set as the random factor to account for the pseudo replication as the transect was performed twice. The fixed factors were light intensity (lux), noise (dBA), minimum temperature (T_{min}, °C), percentage natural vegetation cover, moon phase and season. All AI data were log-transformed to normalize the data suitable for lmer with the exception of foraging activity as it could not be log transformed. In this instance, a glmer (family Poisson) was used to determine the best fit model. Best-fit models were selected based on the calculated corrected Akaike Information Criterion values (AICc) and associated delta AICc (ΔAICc) values < 2. Collinearity between the fixed factors was tested using the variation of inflation factor (VIF) function in R. The general rule of thumb is that generalized VIF (GVIF) values > 3, 5 and 10 indicate collinearity and redundant information or a lack of data (Imdadullah et al. 2016). If VIF values are above 10, one can either drop a collinear regressor or leave the regressor in the model (Imdadullah et al. 2016). As suggested by Fox and Monette (1992), we used $GVIF^{1/2*df}$ instead of GVIF. If the $GVIF^{1/2*df} < 5$, the association between the factors was deemed weak and were included in the mixed-effects models.

Type II Wald Chi-square tests (Anova: lme4) were used on each mixed-effects model (lmer and glmer) to determine any significant differences in the means of the independent variables: moon phase, T_{min} (°C), maximum luminosity (lux), noise (dBA) and percentage of natural vegetation cover in relation to the dependent variables: AI, species richness across the six bat detectors. The Wald Chi-square test was chosen as it is not bound by a specific distribution and is thus a suitable non-parametric test that can be used for non-normal variable distributions in mixed-effects models.

Results

A total of 35 327 files recorded over the 21 nights were identified (species level and activity type) using a combination of AnalookW and BatSound resulting in a total of 42 028 bat passes. The resulting total bat activity index was 31 563 (Table 8).

Species richness and activity index

Overall, 20 (potentially 24) bat species were acoustically identified (Figure 20). Bat species richness varied along the transect (Table 8). The highest species richness was recorded at the end of the transect (21 species) and the lowest species richness was recorded at the beginning of the transect (seven species). For all analyses, 20 species or species-groups were used as listed in Figure 20 and are as follows:

> Open-air foragers: *cf. Chaerephon ansorgei/Molossid 19kHz* (Ansorge's free-tailed bat and an unknown Molossid, possibly *Tadarida ventralis* (Giant free-tailed bat: see Taylor et al. 2015)*, C. pumilus/Mops condylurus* (Little free-tail and Angolan free-tailed bats)*, Mops midas* (Midas free-tailed bat)*, Otomops martiensseni* (Large-eared giant mastiff bat)*, Sauromys petrophilus* (Roberts's flat-headed bat)*, Tadarida aegyptiaca* (Egyptian free-tailed bat) and *Taphozous mauritianus* (Mauritian tomb bat).
>
> Clutter-edge foragers: *Eptesicus hottentotus* (Long-tailed serotine)*, Miniopterus fraterculus* (Lesser long-fingered bat)*, Miniopterus natalensis* (Natal long-fingered bat)*, Myotis tricolor* (Temminck's Myotis)*, Laephotis capensis/Pipistrellus rueppellii* (Cape serotine/Rüppell's pipistrelle)*, Afronycteris nana* (Banana bat)*, Neoromicia zuluensis* (Zulu serotine)*, Nycticeinops schlieffeni* (Schlieffen's twilight bat)*, Pipistrellus hesperidus* (Dusky pipistrelle)*, Pipistrellus rusticus/Neoromicia anchietae* (Rusty bats/Anchieta's pipistrelle) and *Scotophilus dinganii* (Yellow-bellied house bat).
>
> Clutter foragers: *Rhinolophus simulator* (Bushveld Horseshoe bat) and *Hipposideros cafer* (Sundevall's leaf-nosed bat).

Caution was exercised when differentiating bat species based on echolocation calls due to the degree of overlap in call parameters. *C. pumilus* and *M. condylurus* are known to occur on the mine, even sharing the same roosts (pers. Obs.) but could not be reliably distinguished from each other acoustically and thus were placed in the same call group but considered as one species for the analyses. *Cf. C. ansorgei* and *Molossid 19kHz* (*T. ventralis*) exhibit overlapping call parameters and were grouped as one species. The same procedure was followed for *P. rusticus* and *N. anchietae*, and *L. capensis* and *P. rueppellii* with each species group counted as a single species for the analysis although *P. rueppellii* was captured on the mining footprint as part of another study thus its presence could be validated.

Total AI, AI per foraging guild and behaviour (foraging attempts, searching/commuting and social) were all significantly different along the transect (all $P < 0.0001$ and $P = 0.002$ for social AI). Overall, AI per foraging guild was significantly different ($P < 0.0001$). Open-air foragers accounted for the highest AI (24664), social (29), foraging (2594) and searching/commuting

(22041) activity (Table 8, Figure A1). Clutter-edge foragers accounted for the second-highest AI (6896), social (13), foraging (887) and searching/commuting (5996) activity (Table 8, Figure A1). Finally, clutter foragers were poorly represented with only 3 recorded bat passes (Table 8) one of two individuals of *R. simulator* and a single *H. cafer*.

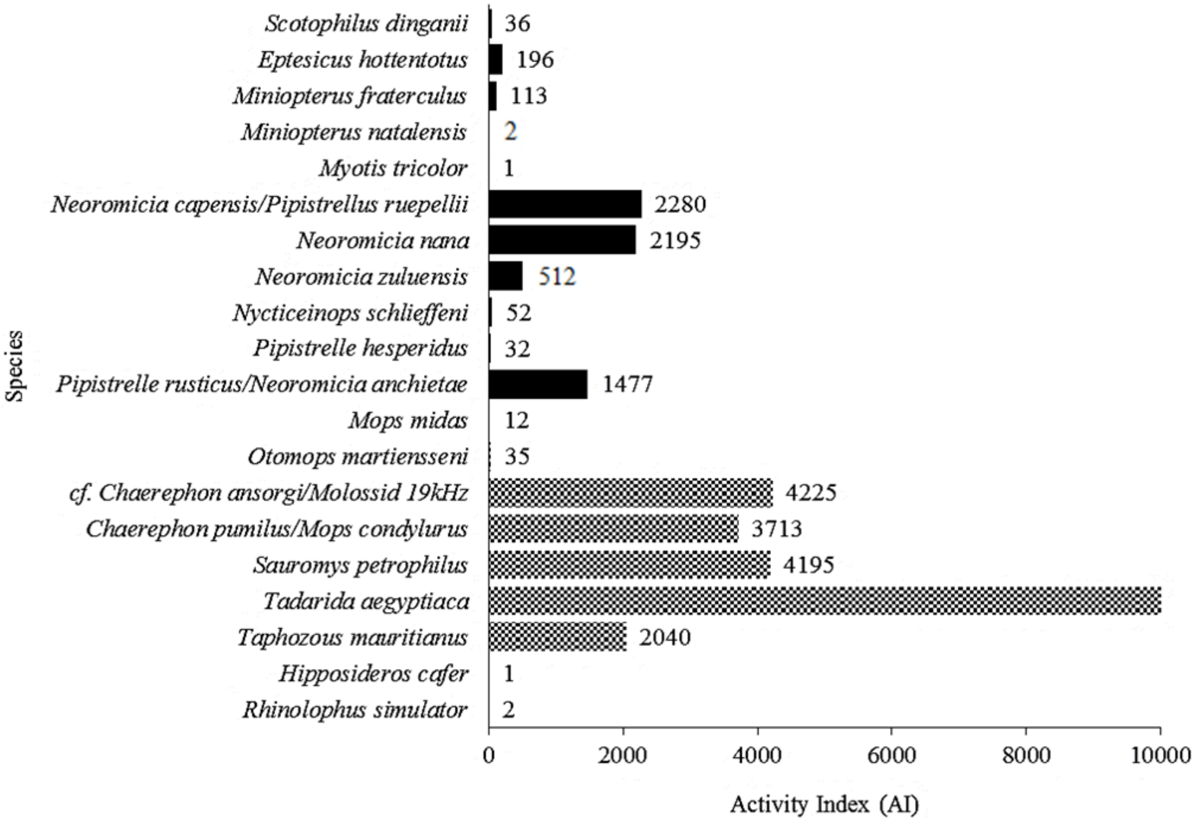

Figure 20. Species identified with the associated sum of behavioural AI categories. Solid filled bars indicate clutter-edge foragers and the checkered bars indicate open-air foragers. *Rhinolophus simulator* and *Hipposideros cafer* are clutter foragers but were represented in such low numbers that a fill is not visible.

Artificial light and anthropogenic noise

Light intensity and noise (dBA) were significantly different along the transect ($P < 0.0001$). As expected, light intensity and noise (dBA) was higher at the beginning of the transect. All noise (dBA) fell into the range that would be considered to be moderately to seriously annoying, particularly to humans (Berglund et al. 2000). The averages and standard deviations of maximum luminosity (lux), noise frequency (kHz) (including the maximum) and noise level (dBA) recorded at each bat detector are detailed in Table 8.

Analysis of variance and mixed-effects models

The results of the one-way analysis of variance (Anova: lme4) are presented in Table A4 indicating the effect of light intensity (lux), noise (dBA), T_{min} (°C), percentage of natural vegetation cover (% Nat. veg), moon phase and season on the dependent variables. Percentage of natural vegetation cover was the only factor that had a significant effect on all dependent variables: activity index, species richness, OAF and CEF activity, and foraging and searching/commuting behaviour with all $Pr < 0.05$. Light intensity only significantly impacted foraging attempts ($Pr < 0.05$). Noise (dBA) had no significant impact on the dependent variables. T_{min} was significant for total AI, species richness, OAF and searching/commuting behaviour (all

Pr < 0.01) whereas moon phase was significant for species richness, OAF activity and foraging attempts (*Pr* < 0.005), and season was only significant for foraging activity (*Pr* < 0.001).

The tests of collinearity on each linear mixed-effect regression model (Lmer) showed that all factors had fairly weak associations when considering GIVF$^{1/2*df}$ since all values were < 5 (Table A4). The best fit model selection and associated cftest results are presented in Table A5. The percentage of natural vegetation cover was significantly important for all 13 best-fit models accounting for some of the observed differences, with significantly higher AI, species richness, forager guild activity and behaviour over 25%, 50% and 100% natural vegetation cover than areas devoid of natural vegetation (Table A5). Differences in AI were best explained by three best-fit models in addition to percentage of vegetation cover, light intensity, T_{min}, moon phase and season (Figure 21A). Species richness was best explained in two best-fit models by T_{min}, percentage vegetation cover (25-100%) and moon phase, with the addition of season in the first best-fit model (Table A5, Figure 21B). Noise (dBA) was only indicated as potentially important in the second best-fit model for OAF, although not significant (Table A5).

Table 8. Average and standard deviation of maximum luminosity (lux), noise frequency (kHz) and sound pressure level (SPL, dB) recorded at each bat detector. The maximum noise frequency (kHz) at each site is indicated in brackets below the average value. Distribution of activity index (AI) of all bats across the transect indicating behaviours and foraging guilds with the AI per behaviour and foraging guild presented in brackets.

Total AI: 31 563						Behaviour (AI)			Foraging Guild (AI)		
Bat Detector	Luminosity$_{max}$ (lux±SD)	Noise Frequency (kHz±SD) (Max. kHz)	SPL (dB±SD)	# of nights (n)	# Species	Foraging (3481)	Commuting/searching (28040)	Social (42)	Clutter forager (3)	Clutter-edge forager (6896)	Open-air forager (24664)
BD01	6.31 ± 0.60	8.36 ± 2.24 (11.6)	55.22 ± 4.74	21	11	30	869	0	0	123	776
BD02	14.19 ± 0.25	8.40 ± 0.70 (9.8)	61.04 ± 3.87	21	7	18	233	0	0	120	131
BD03	2.26 ± 0.09	8.25 ± 2.24 (13.6)	52.07 ± 2.35	21	15	620	6233	6	0	622	6237
BD04	2.58 ± 1.77	4.15 ± 4.54 (13.5)	52.07 ± 5.41	21	18	1517	10681	25	1	4385	7837
BD05	0.47 ± 0.82	3.80 ± 4.95 (16.3)	47.62 ± 6.28	21	16	725	5930	8	0	856	5807
BD06	1.12 ± 4.56	3.23 ± 4.01 (10.6)	44.04 ± 5.83	21	20	571	4094	3	2	790	3876

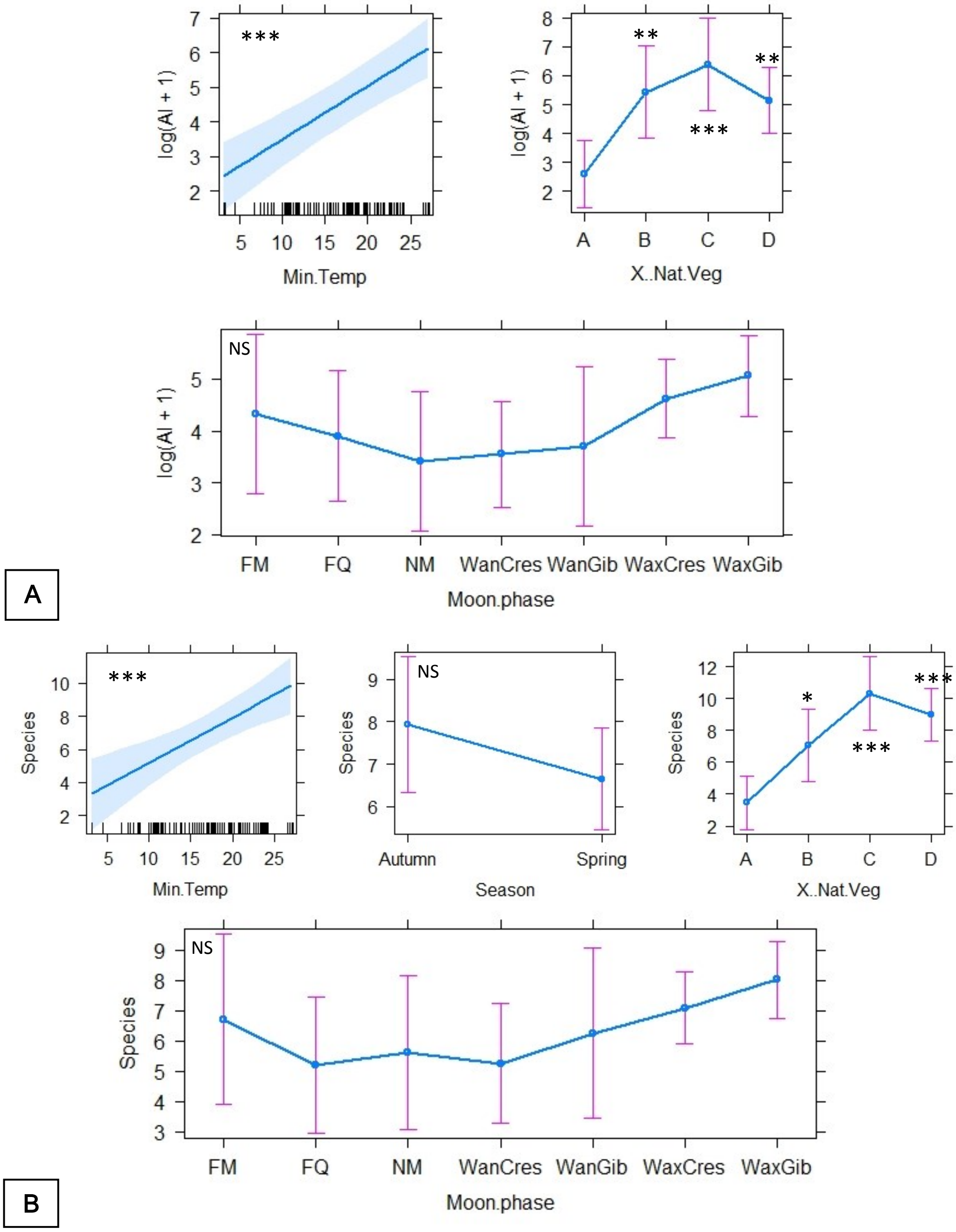

Figure 21. The best fit model #1 outputs for total AI (A) and species richness (B) showing the influence of minimum temperature, percentage of natural vegetation cover, moon phase and season. Bat detector and date were set as the random factors. Codes in the graphs are as follows: A = 0%, B = 25%, C = 50%, D = 100%. FM = full moon, FQ = first quarter, NM = new moon, WanCres = waning crescent, WanGib = waning gibbous, WaxCres = waxing crescent and WaxGib = waxing gibbous.

Open-air forager activity was best explained by T_{min}, natural vegetation cover ≥25% and moon phase in both models with the addition of noise (dBA) and season in the second model (Table A5, Figure 22A). Clutter-edge forager activity was shown to be only significantly affected by percentage of natural vegetation cover in both best-fit models although season was included in the first model from the best-fit selection table (Table A5, Figure 22B).

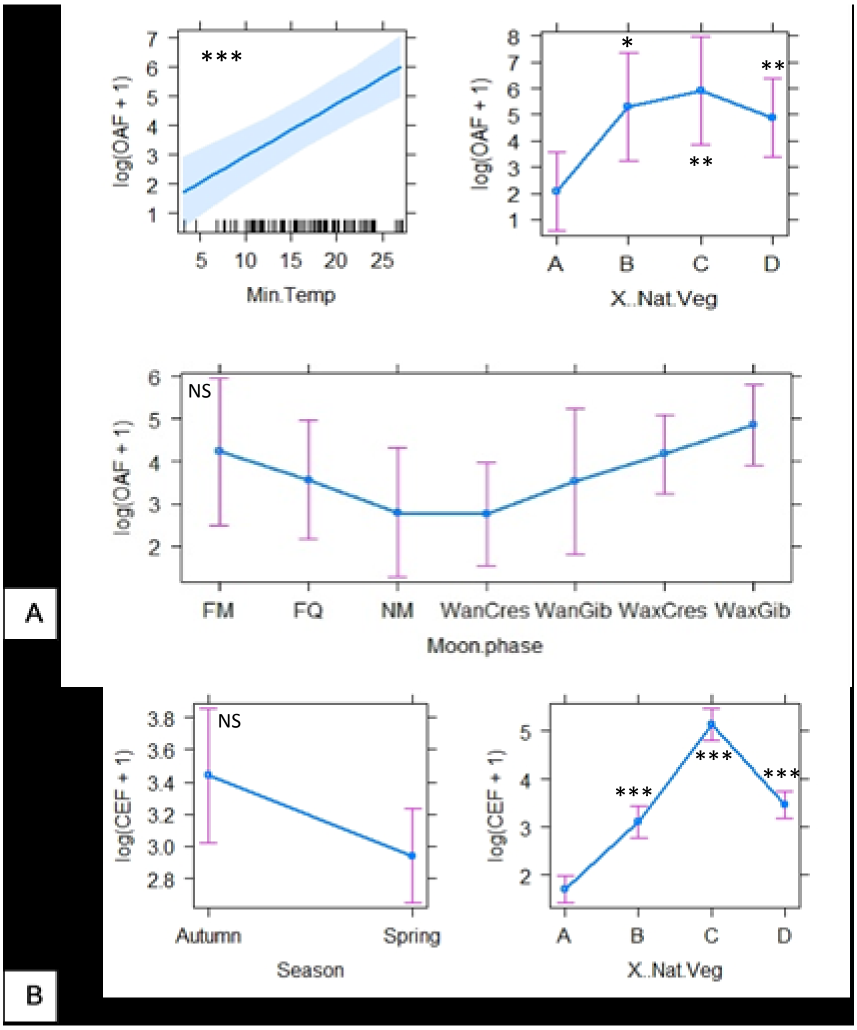

Figure 22. The best fit model #1 outputs indicating the factors responsible for the observed pattern of activity of open-air foragers (A) and clutter-edge foragers (B). Bat detector and date were set as the random factors. Codes in the graphs are as follows: A = 0%, B = 25%, C = 50%, D = 100%. FM = full moon, FQ = first quarter, NM = new moon, WanCres = waning crescent, WanGib = waning gibbous, WaxCres = waxing crescent and WaxGib = waxing gibbous.

Light intensity, percentage natural vegetation cover, moon phase and season were all significant factors describing the observed pattern in foraging attempts by the first best-fit model (Table A5, Figure 23A). Lastly, three best-fit models indicated that T_{min} and percentage natural vegetation cover were significant factors in all best-fit models for bats searching/commuting behaviour with moon phase in the first and second although not significant, season in the second, also not significant and light intensity in the third as a significant factor (Table A5, Figure 23B). Season was a non-significant factor in the second best-fit model for searching/commuting behaviour (Table A5).

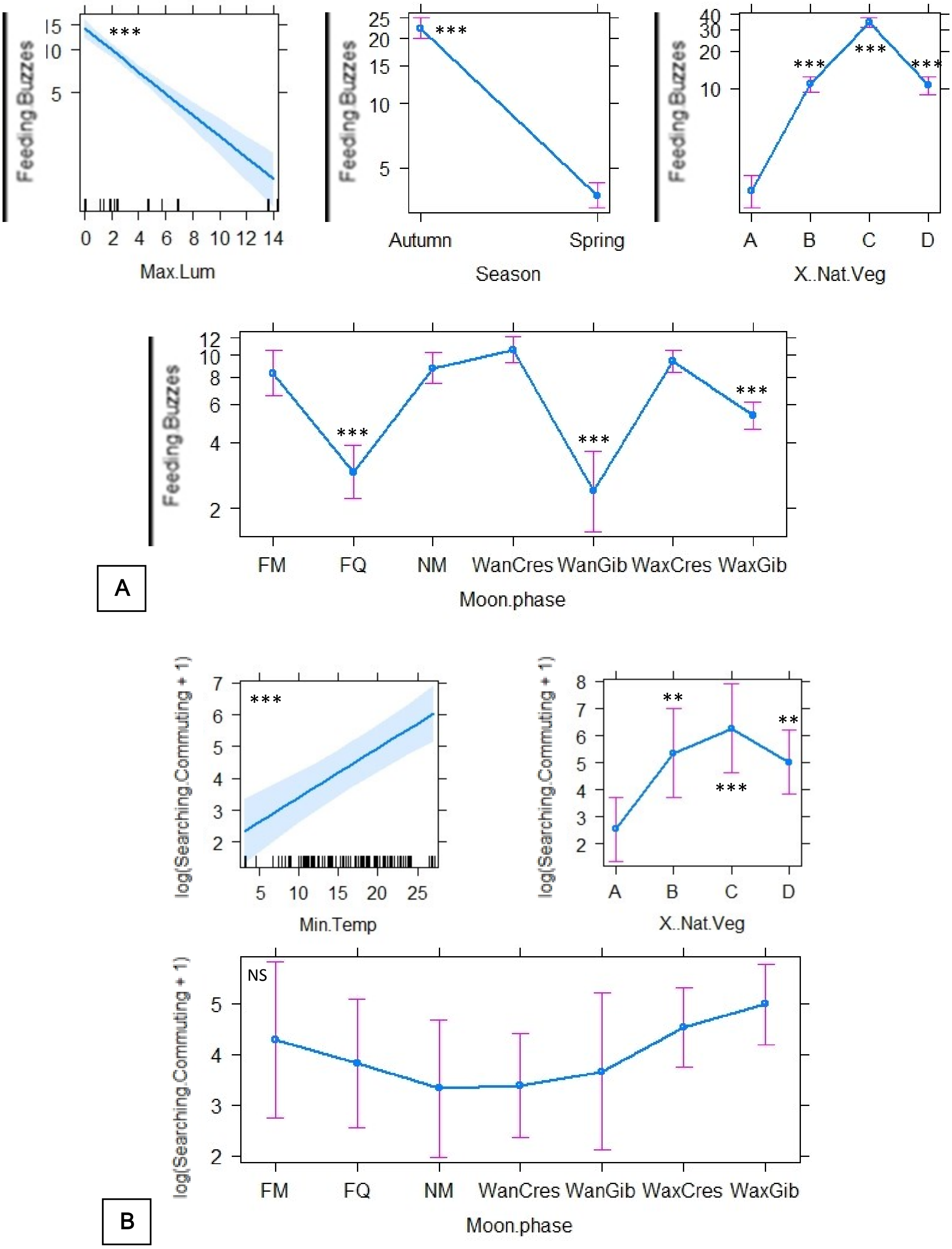

Figure 23. The factors to best explain the observed patterns of foraging (A) and commuting/searching (B) activity by the best-fit models #1. Bat detector and date were set as the random factors. Codes in the graphs are as follows: A = 0%, B = 25%, C = 50%, D = 100%. FM = full moon, FQ = first quarter, NM = new moon, WanCres = waning crescent, WanGib = waning gibbous, WaxCres = waxing crescent and WaxGib = waxing gibbous.

Discussion

Percentage vegetation cover was highlighted in our study as the most important factor in all mixed-effects models with T_{min} playing an important role in some of the lmer and glmer models and to a lesser extent, light intensity, noise (dBA), moon phase and season. Light intestiy was a significant factor in feeding and possibly AI and searching/commuting behaviour.Foraging activity in general was higher during early autumn over the dimly lit areas that were exposed to spill-over from the lights of the mine, had lower noise (dBA) levels, over areas of natural vegetation cover and during periods of darker moon phases (Table A5, Figure 23A). Species richness recorded on the Venetia diamond mine is comparable with the species richness recorded by Taylor et al. (2020) from the surrounding area. It is assumed that organisms can adapt to a particular disturbance regime (Grindal and Brigham 1998). Since the Venetia diamond mine has been operational since 1992 (27 years at the time of data collection), we assume that the bats inhabiting and utilizing the resources of the Venetia Diamond mine have possibly adapted to a degree to changes in the landscape (including habitat clearing) and possibly habituated to the nightly artificial light and the persistent anthropogenic noise from the mining activities (earth moving equipment, processing plant, conveyor belt systems etc.). This is indicated by the general lack of impact of noise (dBA) on total AI (including foraging guilds and behaviour) and species richness and the apparent disinterest of bats to make use of light for foraging opportunities. In the absence of pre-operational bat data, an inference to the level of adaption of the bat species recorded on the mine is not possible.

It is not surprising that overall the greatest number of bat passes belonged to the open-air forager guild (predominantly Molossidae) as they were very common on the mining footprint, particularly *C. pumilus* and *M. condylurus*, which made use of the infrastructure as roosting opportunities. The most notable foraging activity of open-air foragers was recorded over a habitat that was on the edge of natural vegetation (50% natural vegetation), was exposed to 2.6 lux spill over light from the diamond sorting area to the east. There is very little evidence from the current study indicating that the open-air foragers made use of the flood lights as foraging opportunities. Some species of fast-flying bat species belonging to the genera *Tadarida, Myotis, Eptesicus, Pipistrellus,* and *Vespertilio* to name a few, benefit greatly from increased foraging opportunities around street lamps as the bats predated on insects that were attracted by shortwave light (Schoeman et al. 2015, Stone et al. 2015, Stone et al. 2009).

The low number of open-air forager AI, particularly from the family Molossidae, recorded over the active mining area was unexpected as we had anticipated a higher number of calls as bats left their roosts and commuted to foraging habitats. Perhaps the lack of calls could have been due to the bats navigating visually using the artificially illuminated areas of the mine. Orientation by sight is not implausible since molossids generally commute and forage in the open, fairly high above the ground where the risk of collision with stationary objects is low. It has been shown that vision takes priority over echolocation when bats are travelling far distances (commuting or migrating) and in instances where a bat may be receiving conflicting information from its sight and echolocation calls (Rowse et al. 2016, Gorresen et al. 2015, Liu et al. 2015, Eklöf 2003). Most bats' eyes are adapted to low light conditions with a dominance of rod-based retinas as opposed to cones, with large lenses and surfaces of the cornea in relation to the size of the eye (Eklöf 2003). This physiology should be sufficient for open-air foragers to visually orientate and commute to respective foraging grounds. It is known that the emballonurids (also open-air foragers) have large eyes, are alert, very tolerant of daylight and opportunistically forage during the day (Eklöf 2003, Monadjem et al. 2020), thus visual orientation while commuting may not be improbable for this family too. The increase in searching/commuting activity of open-air foragers mirrors the increase of foraging calls along the transect indicating a potentially suitable or preferred foraging area above the semi-transformed mining footprint followed by areas with natural vegetation.

A similar pattern was observed for the clutter-edge foragers with a distinct preference for the semi-transformed habitat (Figure A2). *Laephotis capensis*, *A. nana* and *P. rusticus/N. anchietae* were the three dominant species in this habitat and had a higher searching/commuting activity than foraging activity (Figure A2). At a finer scale, some features may have been of importance to clutter-edge foraging bat species. The semi-transformed habitat bordered an open grassy area and had a tall stand of mopane trees that allowed for a light mosaic environment with dark spaces and dappled light. Clutter-edge foragers are known for their preference to hunt insects along the edge of bush clumps, forest edges and gaps within dense vegetation (Monadjem et al. 2020). The structure of the habitat provided distinct edges along which the clutter-edge foragers may have easily commuted, foraged, and found refuge from potential predators, opposed to the natural habitats that lacked a distinct edge and suitable vegetation structure (either too open or too closed) (Figure 19). Thus, *L. capensis*, *A. nana* and *P. rusticus/N. anchietae* used the habitat for foraging and commuting and may not have been perturbed by the constant drone of the mine nor the patchy light mosaic. It is difficult to determine why the remaining Vespertilionidae bat species were present in such low numbers (Figure 20). We can only assume that the combination of anthropogenic factors (habitat degradation, light and noise) have created an unfavourable habitat or that their natural prevalence in the area is generally low (a similar trend of low prevalence is noted in Taylor et al. 2020) (Figure 23). Further investigation is required.

The incidence of clutter foragers was very low indicating that *R. simulator* and *H. cafer* may have avoided the lit areas possibly due to a physiological sensitivity to light (Figure 20). It is known that Rhinolophidae and some Vespertilionidae are sensitive to light (Schoeman 2015, Stone et al. 2015, Rowse et al. 2016, Azam et al. 2018). Stone et al. (2009) showed that artificial lighting negatively affected *R. hipposideros* (lesser horseshoe bats) and disrupted commuting routes when hedges were lit (53.09 lux). The unlit side of the hedge (4.17 lux) was also avoided and 0.45 lux was considered to be too bright. Thus the range of light along the gradient may have been too bright for the clutter foragers. Taylor et al. (2020) recorded low numbers of *R. simulator* and no *H. cafer* in the surrounding natural areas, thus their prevalence in the landscape could be naturally low and compounded by the anthropogenic effects of the mine.

Noise is another factor that exacerbates challenges experienced by organisms caused by anthropogenic development and activity, such as habitat fragmentation and degradation (Bunkley and Barber 2015). Acoustics or sound is a wave (a “perturbation of a steady state”) and how an ear perceives a sound (e.g. intensity and loudness) follows a logarithmic scale (Rienstra and Hirschberg 2018, Rowley 1966, Fletcher and Munson 1933). The intensity of a sound relates to the power or energy of a sound, and loudness relates to the perceived volume of a sound or magnitude of the auditory sensation (Fletcher and Munson 1933, Rowley 1966). For example, between the “quietest” and “loudest” points along the transect, there was a 17 dB difference which equates to an acoustic intensity of 50.12 times and a perceived increase in loudness of 3.25 times. Sounds that overlap with bats auditory cues, and sounds with properties (including loudness and intensity) that fall within the sensitive auditory range of bats could be intolerable to bats and are avoided. These sounds can cause stress, reduce attention, disrupt biological processes (e.g. communication) and mask auditory perceptions (acoustic masking) (Lou et al. 2015, Gomes et al. 2016, Geipel et al. 2019). Ambient noise can provide a challenge for most insectivorous bats which echolocate and rely on hearing the returning echo of their call to orientate, forage and communicate (Rowse et al. 2016).

The potential of the noise frequencies produced by the mine to cause acoustic masking in this instance would be slim. Acoustic masking occurs when the background noise (noise of the mine) is at the same or similar frequency to the acoustic signals associated with prey detection that causes interference, whether it be interference with the sound of the shuffling of an insect or the returning echo of a bat’s call (Luo et al. 2014). Noise frequencies that were recorded at the beginning of the transect at and near the processing plant could have only overlapped with

Otomops martiensseni, which is known to produce a narrow bandwidth (6.4 ± 2.3 kHz) and long duration (24 ± 14.8 ms) echolocation call with a peak frequency of 10.8 ± 2 kHz (Monadjem et al. 2020). With only 35 calls recorded of *O. martiensseni*, noise frequency was not used in any of the analyses since the chance of acoustic masking of the remaining species of bats would be negligible to absent.

Though many studies have focused on acoustic communication, showing how animals altered the frequency range and amplitude of their calls to avoid a strong overlap with anthropogenic noise (Schaub et al. 2008, Barber et al. 2010), how bats deal with noise in the field is still not well known. There are few field studies that show how traffic-noise impacts bat ecology (Luo et al. 2014, Rowse et al. 2016, Finch et al. 2020). Bat communities in England showed a reduction in overall activity and foraging behaviour (particularly for *P. pipistrellus* and *P. pygmaeus*) when exposed to playback of traffic noise in the field (Finch et al. 2020). Under laboratory conditions *R. ferrumequinum* was shown to reduce their call rate and produced calls with shortened durations when exposed to playback of loud, low frequency noises. (Hage and Metzner 2013). Similarly, *T. brasiliensis* altered the structure of their call and produced longer duration and more intense calls under noisy conditions (Simmons et al 1978). Identifying the specific impact that noise has on foraging bats in the field is a challenge because there is often more than one factor impacting on the activity and behaviour of bats.

Due to the observed roosting habits of *M. condylurus* and *C. pumilus* within the mining buildings they are exposed daily to noise from the processing plant and earth moving vehicles in operation throughout the day. Noise (dBA) was indicated as potentially a factor that could have impacted open-air forager activity although not significantly so. As observed by Luo et al. (2014), the bats roosting on the mine footprint have the potential to become habituated to noise over time due to continual exposure, but it may impact on them during certain activities e.g. in our study, OAF activity was significantly higher over areas with low levels of noise (dBA). The addition of a radar study would be beneficial to determine if this is indeed the case specifically if bats have perhaps developed a preference for visual orientation and thus fewer echolocation calls could have been recorded closer to the beginning of the transect.

Clutter-edge foragers' activity was not impacted by noise. Since they rely more on echolocation to detect and capture prey on the wing as opposed to listening for acoustic cues from prey, noise possibly did not impact them in the same manner that it would affect clutter-edge gleaning bat species. For example, Jones (2008) and Schaub et al. (2008) showed that in a laboratory setting, playback of traffic noise reduced time and effort of foraging activity in the gleaner, the Greater mouse-eared bat (*M. myotis*). The explanations for the observed behaviour was acoustic masking of prey sounds by loud ambient noise and potential difficulties that bats may experience in processing auditory information from multiple streams (Jones 2008, Rowse et al. 2016). Conversely, *M. myotis* is known to roost near high levels of anthropogenic noise and torpid bats were unperturbed by traffic noise and were more responsive to conspecific noises and vegetation noise (Schaub et al. 2008, Luo et al. 2014), although their foraging efficiency decreased when exposed to traffic noise (Schaub et al. 2008). Noise also interferes with passive listening of bats where playback noise that masked mating calls of frogs resulted in delayed attacks by *Trachops cirrhosus* (Gomes et al. 2016).

The observed pattern of activity in response to T_{min} and moon phase is well known and well represented in the literature (Ciechanowski et al. 2007, Appel et al. 2017, Pech-Canché et al. 2018, Pretorius et al. 2020). Reduced bat activity has often been associated with the energetic costs associated with flight, reduced prey availability and maintaining stable body temperatures during cooler temperatures and unfavourable weather conditions (Erickson and West 2002, Bender and Hartman 2015). In general, activity was significantly lower at cool temperatures during early spring (average 14.3 ± 4.4 °C) compared to early autumn when T_{min} were still warm (average 22.3 ± 2.5 °C). On bright moon nights, the general school of thought is that bats experience lunar-phobia and are less active during bright moon nights as bats are more susceptible to predation risk from visually orientated birds of prey and carnivorous bats but this varies greatly between species, foraging guilds and regions (Eklöf 2003, Appel et al. 2017, Musila et al. 2019). Musila et al. (2019) showed that lunar-phobia was not evident in clutter-edge foragers from the genera *Scotophilus* and *Scotoecus* but showed that habitat, season and time of night affected foraging activity, which partially supports the pattern of observed clutter-edge forager activity explained by vegetation cover and possibly season. Moon phase was shown to be important in our best-fit models but only significantly so for foraging activity where foraging activity was significantly lower during the first quarter, waning gibbous and waxing gibbous (Figure 23A).

To our knowledge, no study has incorporated the impact of ecological light pollution and continuous noise from mining operations on bat species using a mining footprint for foraging and or roosting opportunities. The study of bats on the Venetia diamond mine highlights the anthropogenic factors (artificial lighting, noise and habitat degradation) and natural factors that influenced bat activity. Different bat species respond to anthropogenic impacts in different manners as adapters, exploiters or avoiders (Schoeman 2015, Gaston and Bennie 2014). Even these categories assigned to certain genera or species may not always hold true, one can expect exceptions. Bats responses to anthropogenic disturbances may be a function of echolocation type and flight morphology, for example, OAFs with long-range echolocation calls may forage in the open spaces under and around lights to exploit insects that are attracted to light (Stone et al. 2015, Rydell 1992), which was not observed by the OAF group on the Venetia diamond mine. This may have been exacerbated by the lack of vegetation and noise levels of the processing plant. Perhaps from a seasonal perspective, the swarms of insects that conceivably occur during the peak of summer may have been missed during the early autumn and early spring sampling periods of this study. Open-air foragers have also been shown to alter their call frequencies and durations when exposed to anthropogenic noise (Simmons et al. 1978), which was beyond the scope of this study. As with moonlight, bats may avoid artificially lit areas as the actual or perceived risk of predation is generally higher for slow-flying bats adapted to forage within cluttered environments (Stone et al. 2015), and possibly those that forage in open spaces and over water bodies (Musila et al. 2019). In the presence of anthropogenic noise, CF bats may well adapt their echolocation calls too (Hage and Metzner 2013). The lack of activity of CF bat activity in this study in the altered and natural sites unfortunately provide no support for the impact of light and noise on clutter foragers on the Venetia diamond mine.

In conclusion, percentage of natural vegetation cover was the main factor (present in all mixed-effect models) responsible for the observed patterns in bat activity, community composition and behaviour. We partially accept the first hypothesis that bat activity will be significantly lower in areas affected by ecological light pollution, high noise levels and reduced vegetation cover has partially been met as artificial light was shown to have no impact on AI. Mixed-effects models revealed that total bat activity, open-air forager activity, foraging attempts and searching/commuting activity were significantly lower in areas affected by high noise levels and reduced vegetation cover, and higher in the quieter, vegetated areas that were dimly lit by spill over light from the Venetia diamond mine floodlights. Light intensity was only a significant factor influencing foraging attempts, which were lower in the brightly lit areas and higher in the dimly lit areas. Species richness was shown not to be influenced by ecological light pollution or noise by the mixed-effects model thus we in part accept the alternative hypothesis in this study since there was no difference in species richness in relation to light and noise pollution but species richness was lower in areas devoid of vegetation cover. Based on our data, we reject the third hypothesis that foraging activity will be higher under lit conditions as we could provide no evidence of this during the sampling period. However, this may differ during summer. We also raise caution concerning the generalization of anthropogenic impacts on bats as different aspects of their activity and species richness are effected by a combination of anthropogenic and natural factors that are not mutually exclusive from each other. As the first case study of the impacts of opencast diamond mining in South Africa (and globally), we are hoping that the study highlights the need for scientific rigorous studies and that it inspires students, researchers and consultants alike to investigate the impacts of large-scale developments, particularly mines, to better understand how African bats respond or adapt to mining developments in the field.

Bat habitats in tropical Africa are under threat by large-scale mining operations, including opencast mining operations, e.g. there is great interest in the Simandou Range, Guinea, for high grade iron to be mined (Fahr and Ebigbo 2003). As one of the last areas of protected and fairly undisturbed habitat, open cast iron-ore mining is predicted to have a “devastating” impact on remaining habitat and the bat diversity in Guinea (Fahr and Ebigbo 2003). New species records and new species of bats are often discovered during bat surveys in this area of economic importance (Fahr and Ebigbo 2003, Dechner et al. 2015).West Africa is known for its high bat biodiversity with ~65 bat species currently recorded for the Simandou Range alone (Dechner et al. 2015). The African Mining Vision (African Union, 2009) has illustrated the potential development of mines and associated corridor networks that are largely focused on sub-Saharan Africa. With the encouragement of foreign investment for the development of mining infrastructure and mineral extraction, there is a significant concern over environmental threats and social risks such as community displacements and exclusion of local participation in these projects (Edwards et al. 2014, Dauda 2020). The impact of mining operations on the environment and bat conservation in sub-Saharan Africa could be devastating if environmental protection policies (including offset areas and protection of natural habitats) are not put in place and adhered to. Conservation efforts are dependent on bat taxonomy and ecology but importantly, how they react to anthropogenic disturbances (Armstrong 2010). We concur with other authors that studies concerning bat responses to anthropogenic developments are imperative to inform decisions for environmental mitigation strategies and management (Armstrong 2010, Duarte et al. 2015, Stone et al. 2015). Mitigation strategies that can be implemented are the creation of bat habitats in areas disturbed by mining (rehabilitation efforts are critical during and post mining operations), development of corridors connecting habitat patches for bat movement, installation of artificial roosts for displaced bats and lighting regimes that are tolerable for bats (e.g. Theobald et al. 2020). Most importantly, proactive conservation of African bats is essential where stakeholders (including Governments) understand and support the importance of bats for ecosystem functionality.

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Chapter 4: Non-invasive sampling of bats reflects their potential as ecological indicators of heavy metal and trace metal contamination due to open cast diamond mining.

Abstract

Bats have been proposed as reliable bioindicators for monitoring bioaccumulation of elements and chemicals in natural and transformed ecosystems. Non-invasive methods are becoming more popular as research moves away from destructive methodologies. We present the first concentrations of 23 elements in Angolan and Egyptian free-tailed bat fur and blood from an opencast diamond mine and control site using inductively coupled plasma mass spectrometry (ICP-MS). Fifteen of the 22 elements (excluding barium (Ba)) were significantly higher in the fur samples than in the blood due to elements being incorporated over time into the fur as it grows, whereas blood reveals short-term exposure to elements. Iron (Fe) was the only element to be significantly higher in blood than in the fur ($P < 0.05$). Concentrations of boron (B), potassium (K), rubidium (Rb) and cadmium (Cd) in the fur of bats were significantly higher in bats from the opencast diamond mine compared with the control site ($P < 0.05$). Zinc (Zn) and mercury (Hg) were significantly higher in the blood of bats from the mining footprint than the control area, whereas manganese (Mn) was significantly higher in the blood of bats from the control area. Concentrations of most of the elements were reasonably low except Al, Fe and Zn. Although weak, significant correlations between fur and blood element concentrations were found for Hg and strontium (Sb) ($P < 0.05$; rho = 0.69 and 0.75 respectively). In general, the element concentrations particularly in the fur samples were comparable with other international studies reporting elemental fur concentrations from anthropogenically impacted and natural areas. Pending further research on toxic thresholds and physiological and ecological unknowns around element concentrations in bat tissues and organs, fur has the potential to be a viable indicator of toxicity. Fur and blood have the potential to be viable indicators of environmental toxicity, but research is required on toxic thresholds and physiological and ecological unknowns around concentrations of elements in bat tissues and organs.

Key-words. Bioaccumulation, heavy metals, Chiroptera, ecotoxicology, biomarkers, opencast mining.

Introduction

Heavy metals and trace elements are naturally occurring minerals present in the earth's crust, which under natural conditions, leach into water from the surrounding rock and soil, and are taken up by plants and animals (Garrett 2000; Nagajyoti et al. 2010; Flache et al. 2015). The general accepted definitions of heavy metals based on the density and/or specific gravity of elements are "hopelessly imprecise, leads to confusion and is useless to describe toxic properties" (Smith and Norberg 2015). We consider three main groups of elements pertinent to our study in line with Smith and Norberg (2015); main metal groups, transition metals and metalloids. Based on the confusion and multiple re-definitions of heavy metals, from this point forward, we will simply refer to heavy metals, trace elements, macronutrients and micronutrients using the term "elements".

Elemental bioaccumulation, toxicity and resulting effects have been a subject of interest for many years (Jakimska et al. 2011, Bat et al. 2020). The concentrations of elements released from natural sources depends on release process (natural versus anthropogenic), geochemical properties of parent rock material, environmental conditions and the weathering process (Garret 2000, Tchounwou et al. 2012). Natural releases of elements are exacerbated by anthropogenic activities that results in the accumulation of high concentrations of these elements in the soil often near the source of emission (Nagajyoti et al. 2010, Flache et al. 2015, Obrist et al. 2018). Sources of anthropogenic element releases are in the form of fertilizers (organic and inorganic), pesticides, agricultural practices (including continued irrigation of agricultural crops resulting in leaching and surface runoff), mining activities and associated transport and processing of ore (dust fallout, smelting, amalgamation processes), paper and plastic processing, wood preservation, waste water treatment plants and urban storm water runoff (Nagajyoti et al. 2010, Naidoo et al. 2016, Moreno-Brush et al. 2018, Carrasco-Rueda et al. 2020). But, even remote areas do not escape metal contamination as some metals emitted through anthropogenic activities and volcanic eruptions, like mercury that linger in the atmosphere up to a year, are able to be transported over vast distances in the atmosphere and contaminate areas far from the point of emission (Nagajyoti et al. 2010, Flache et al. 2018).

Bioavailability of these elements is complex and consists of three parts as detailed in Kim et al. (2015). Firstly, the total concentrations of elements in the soil that is referred to as "environmental availability". Secondly, the fraction of the dissolved elements in pore water (water in the spaces between the soil particles) is called the "environmental bioavailability". These dissolved elements are thus available to be taken up by soil organisms and plant roots. And lastly, "toxicological bioavailability", is accumulation of element/s in an organism or the physiological effect induced but elements. Bioavailability depends on several factors: temperature, adsorption and sequestration (removal and storage), absorption, phase association, chemical factors (the state of the element: solid, liquid or gas and how they interact with other elements/molecules, lipid solubility etc.) and biological factors (trophic interactions, characteristics of species, physiological or biochemical adaptations etc.) (Tchounwou et al. 2012). Bioaccumulation of elements in an organism is generally influenced by specific location, age of the organism and its interactions with the environment (Sánchez-Chardi and Nadal 2007) and can enter into the food chain directly from sediment into organisms, by drinking from contaminated water sources or feeding on contaminated plants or prey items, inhalation from the air/dust and dermal exposure (Reis et al. 2010, Gall et al 2015, Hernout et al. 2016b). For example, heavy metals are known to accumulate in marine sediments, affect benthic life and become a risk for humans consuming marine organisms such as sea cucumbers (Bat et al. 2020). There are a number of organisms ranging from invertebrates, such as sea cucumbers, to vertebrates such as fish, birds, shrews and rodents that are suggested as suitable bioindicators for heavy metal and trace element exposure (Dallinger 1994, Sánchez-Chardi et al. 2007, Egwumah et al. 2017, Bat et al. 2020). Data on element toxicity effects in wild animals is limited and it is challenging to provide a concentration range of heavy metal and trace elements that reflects "normal" conditions in organs and tissues (Sánchez-Chardi et al. 2007).

The effects and impacts of heavy metal, chemical pollutants and other elements that bats have been and are currently exposed to is gaining increased attention (Zocche et al. 2010, Griffiths et al. 2014, Lovett and McBee 2015, Naidoo et al. 2016, Carrasco-Rueda et al. 2020). Heavy metal pollution has been shown to affect bat ecology, genetics, physiology and behaviour. Effects of element pollution include but are not limited to changes relating to bat diversity, alteration of relative abundances, population structure changes, negative impacts on flight activity, disruption of plasma glucocorticoids, central nervous system alterations (causing a general lack of coordination, loss of movement, tremors, paralysis), damage to internal organs (renal inclusion bodies), hemochromatosis ("iron overload"), immunosuppression and mortality (Zocche et al. 2010, Zukal et al. 2015, Naidoo et al. 2016, Mina et al. 2019).

Bats may be directly exposed to elements through drinking from contaminated water sources or through the accumulation of elements in prey items that breed in polluted waterbodies (Pikula et al. 2010, Mansour et al. 2016, Mina et al. 2019). Bats are also indirectly exposed to pollutants through human activities that may change the physical habitat and alter availability and quality of resources (food and water) (Zocche et al. 2010, Gall et al. 2015). Even at low concentrations, lead (Pb), Fe, Cu, Mn, cadmium (Cd), Ni and aluminium (Al) are known to be toxic, damaging DNA, causing gene mutations and cancers (Beyersmann and Hartwig 2008, Naidoo et al. 2013) but the concentration at which these metals are toxic and or fatal to bats is unknown. Zocche et al. (2010) reported preliminary results that the exposure to and resulting uptake of elements associated with coal mining such as Al, silicon (Si), Mn, Fe, Cr, Cd, Zn, Pb, Cu and Ni, resulted in DNA damage in *Eptesicus diminutus* and heavy metal accumulation in *Molossus molossus* and *Tadarida brasiliensis.* They suggested that the above bat species occurring in the Carboniferous Basin of Santa Catarina could be used as bioindicators species for ecosystems that are directly and indirectly affected by coal mines to detect bioaccumulation of heavy metals (Zocche et al. 2010).

Persistent environmental pollutants are an underrated threat to bats and the manner in which contaminants transfer, bio-magnify through trophic levels and accumulate within an organism (in tissues and organs) is fairly complex (Clarke et al. 1986, Flache et al. 2018, Mina et al. 2019). Different bat species may show specific trace element concentrations in their tissues and organs associated with variations in exposure within different foraging habitats, dietary guilds and physiological regulation of elements (Karouna-Renier et al. 2014, Zukal et al. 2015, Flache et al. 2015, Hernout et al. 2016b, Becker et al. 2018, Flache et al. 2018, Moreno-Brush et al. 2018, Carrasco-Rueda et al 2020, de Souza et al. 2020). There could be numerous instances where high levels of metals may not be due to the contamination of the environment, but may be an artefact of the bat's diet. E.g. *Myotis myotis* has been reported to contain high levels of Mn that may come from their predominant carabid beetle diet which reportedly strengthen their mandibles with Mn (Flache et al. 2015). It is evident from the literature, that different species of bats are exposed to different types of contaminants based on their dietary guild, sex, age and seasonality (Clark et al. 1986, Naidoo et al. 2013, Hernout et al. 2016b, de Souza et al. 2020). However, this differs depending on the element and possibly species for example: no differences in concentrations of mercury in the fur of *Carollia perspicillata* and *Phyllostomus elongatus* were found to be attributed to sex and age, which indicated that Hg was not accumulated over time (Moreno-Brush et al. 2018).

Bats are good potential bioindicators for ecosystem health due to their small size, high mobility, high metabolic rates and associated high prey intake of between 40-100% of their body weight each night, global distribution and coexistence with humans thus increasing their exposure to a range of contaminants (Hickey et al. 2001, Jones et al. 2009, Russo and Jones 2015, Zukal et al. 2015). Bat species of particular interest are those that feed on emerging insects such as Trichoptera which have an aquatic larval stage, typically live in sediment, accumulate metals, and thus provide a pathway for contaminants to pass from aquatic to terrestrial ecosystems (Zukal et al. 2015, Mina et al. 2019). Being the second highest species-rich mammalian order in the world representing approximately 20% of global mammal diversity, bats form a large component of global biodiversity and deliver key services to both ecosystems and humans (pest control, pollination, seed dispersal and forest regeneration) (Jones et al. 2009, Kasso and Balakrishnan 2013, Bayat et al. 2014, Riccucci and Lanza 2014, Taylor et al. 2018).

The acquisition of biological material to use as biomarkers from wildlife is moving towards non-invasive and non-destructive methods mainly from an animal ethical and conservation perspective (Hernout et al. 2016b, Powolny et al. 2019). Brain, blood, liver, kidney and whole animal sampling for the determination of biomarkers and elemental contamination in bats are highly-invasive (e.g. liver, kidney biopsies) or destructive sampling methodologies (e.g. Smith and Rongstad 1982, Naidoo et al. 2013, Flache et al. 2018). The liver, kidneys and other internal organs are known organs for deposition of ingested heavy metals and the concentrations of heavy metals in these organs reflect the level of exposure and accumulation of these elements over a prolonged period (Naidoo et al. 2013). It is for these reasons that these organs are such good biomarkers and have been widely used (e.g. livers of fish: Dragun et al. 2019, kidneys and or liver of bats: Zocche et al. 2010 and Hernout et al. 2016a, kidneys of mink and otters: Harding et al. 1998). Using fur could be a good biomarker as the roots are constantly in contact with bloodstream thus metals may be incorporated into the fur during growth and additionally fur also stores external airborne particles, thus external exposure and ingestion of elements could be investigated (de Souza et al. 2020). Fur provides an indication of the long-term exposure to elements and provides information concerning the exposure of an animal at the time of the tissue formation (Fraser et al. 2013, Hernout et al. 2016b). E.g. Flache et al. 2015 used bat fur to monitor bat's exposure to potentially toxic metals in their foraging habitat and reported trace metal concentrations of Cd, Cu, Mn, Pb and Zn in fur samples collected from *Myotis bechsteinii, M. daubentonii, M. myotis* and *Pipistrellus pipistrellus.*

Metal concentration in fur varies at different times of the year e.g. fur collected prior to the annual moult cycle may contain higher metal concentrations than those during or after the moult therefore the moult cycle must be taken into consideration when collecting samples (Fraser et al. 2013, Flache et al. 2015, Hernout et al. 2016a). The moult cycles of bats has not been well studied (particularly in South Africa) and Fraser et al. (2013) provided a summary of the moult cycles of ~27 bat species and highlighted that timing and pattern of the moult differed between species, sex, reproductive status and age. Blood, on the other hand, provides information concerning a more recent exposure as it is gradually replaced (Fraser et al. 2013). Powolny et al. (2019) showed that in wood mice (*Apodemus syl*vaticus) sampled along a pollution gradient, blood could be a good indication of internal organ levels of Se, Pb and Thallium (Tl). On the contrary, blood concentrations of titanium (Ti), Cd, Fe, Cu, Mo and Zn were not good indicators of internal organ concentrations (Powolny et al. 2019).

We investigated the elemental concentrations in blood and fur of two species of open-air foragers: *Mops condylurus* (Angolan free-tailed bat: A. Smith 1833) and *Tadarida aegyptiaca* (Egyptian free-tail bat: É. Geoffroy Saint-Hilaire 1818) collected during summer. Our aim was to determine if bats would be suitable bioindicators for the monitoring of ecosystem health over and near opencast diamond mining operations using non-lethal and non-invasive methods. We expect that bioaccumulation of heavy metals in bats fur and blood is significantly higher over mining areas than the adjacent control area. Alternatively we may find no difference in heavy metal concentration in the fur and blood of bats active over the opencast mine and adjacent control area. We also determined if there were any correlations between concentrations of elements between fur and blood to investigate if fur could be a reliable indicator of internal element concentrations.

Materials and methods

Study site

The study was conducted on the De Beers Venetia diamond mine, in the Limpopo Province (-22.449593°, 29.319494°) and Corea Game Farm (-22.462280°, 29.256442°) (Figure 24). The Venetia diamond mine has been in operation since 1992 (https://www.debeersgroup.com/the-group/our-history) in the Limpopo mobile belt, where a complex kimberlite pipe containing diamonds is situated (Brown et al. 2009). A temporary water pan on Corea Game Farm was used as the control site that was situated ~5km in a straight line from the large wastewater dam on the western side of the mining footprint and ~6.5km from the bat roosting site (Figure 24). Corea Game Farm is situated within the diverse Musina Mopane Bushveld vegetation unit dominated by mopane trees (*Colophospermun mopane*) on poorly developed soils (Mucina and Rutherford, 2011). The mine footprint is situated in the Limpopo Ridge Bushveld, which is also dominated by mopane trees. The climate of the area is characterised by very dry winters and hot summers with mean annual precipitation between 300-400mm (Mucina and Rutherford, 2011).

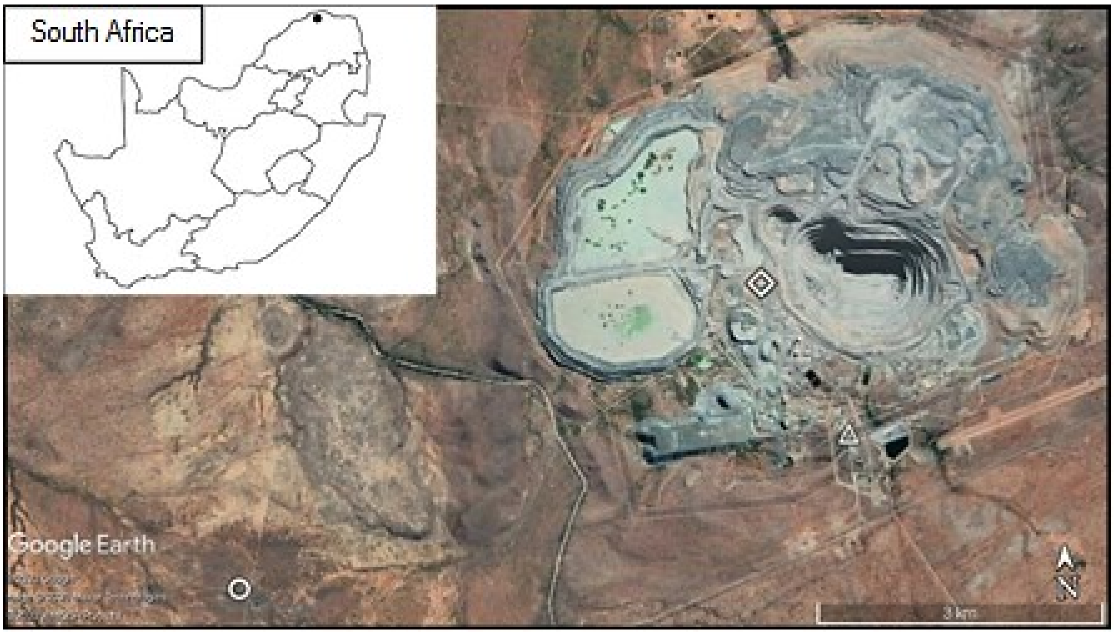

Figure 24. The layout of the study site and capture sites: the Venetia Diamond Mine (diamond shape) footprint and associate capture site (white triangle) and the control area capture site at a temporary water pan (open circle) on Corea Game Farm. The solid fill dot in the insert map of South Africa indicates the approximate location of the Venetia diamond mine in northern Limpopo Province.

Bat capture

Bat capture was conducted over five nights (3–7 December 2018) on the Venetia diamond mine and seven nights (1–2, 8–11 & 13 December 2018) on Corea Game Farm. Free standing nylon monofilament mist nets (ECOTONE, Gdynia, Pomerania, Poland) and an Austbank harp trap (Faunatech, Australia) were used to capture bats. On the mine, free standing mist nets of 9 m and 12 m were extended parallel to edge of waste water dams. Due to the tempestuous summer weather and resulting billowing motion of the free-standing mist nets, capture success in the mist nets was poor on the mine. An Austbat two-bank harp trap was placed at the entrance/exit of a known roost of free-tailed bats (Molossidae) in an unused building on the Venetia diamond mine to capture individuals from the roost. Trapping on Corea game farm consisted of two sets of 9 m and 12 m mist nets strategically placed across temporary water pans. Two mist nets were placed one above the other to have a resulting drop of ~5 m. All captured bats were initially held in cotton bags, processed and identified to species level. *Mops condylurus* were captured on the mining footprint and *T. aegyptiaca* dominated the control site. Female bats dominated over males during the active capture sessions particularly over the control area, with only a single male captured on Corea Game Farm. Four males and seven females were selected from the captured individuals. It would have been ideal to only have males representing the sample as in some instances, sex and reproductive status effects element concentrations in some bat species e.g. females eliminate metals better than males through lactation (Hernout et al. 2016b).

Faecal pellet preparation and insect reference sampling.
Each cotton holding bag was inspected and faecal pellets were collected to identify the orders of insects consumed. A light trap consisting of an 11 watt (600 lum) warm-white light bulb suspended over a container of water and powered by a portable power unit (EcoBoxx Qube 160, South Africa) was used to collect a representative sample of arthropods available to bats foraging over the mining footprint and over the temporary water pan on Corea Game Farm. The light trap was regularly checked and insects were collected. Insects were sorted predominantly to order level and where possible to family or species. Representative individuals of each order/family were crushed into fine pieces with a pestle to simulate the grinding action of a bat's teeth and mounted between two microscope slides (Lasec Laboratory & Scientific Equipment Company (pty) Ltd.). Each insect order was weighed to calculate percentage abundance to determine what was available to foraging bats during the sample period. Faecal pellets were softened using 98% ethanol and spread between two slides. The faecal pellets and insect remains were inspected and photographed using a Zeiss Stemi 508 microscope (Karl Zeiss, Germany) fitted with a 4mp Axiocam ERc 5s (Rev. 2.0) camera. Arthropod remains in the faecal pellets were identified to order and where possible family using the arthropod key in Kunz and Parsons (2009) and the study reference samples identified using Picker et al. (2004). Percentage frequency for each arthropod order was visually estimated in accordance with Kunz and Whitaker (1983).

Fur and blood collection
Since the collection period was during December, we assume that the bats had already had their annual summer moult (Fraser et al. 2013). Fur was collected using a pair of sharp surgical scissors (Lasec, Laboratory & Scientific Equipment Company (pty) Ltd.). Fur was carefully clipped from the dorsal side of the bat, as close to the skin as possible, starting from the pelvic region and ending between the scapulars. Individual fur samples were immediately placed into small zip-lock bags and marked.

Blood was only taken from males and reproductively inactive females that were not pregnant nor lactating. Blood samples were taken from each bat in line with Smith et al. (2010). Each bat was gently restrained in the left hand. The left wing was carefully extended to expose the inner upper arm which was then sanitised using an alcohol swab. Slight pressure was applied to the brachial vein near the base of the upper arm and the tip of a sterile 24 gauge needle was used to puncture the vein. The resulting bead of blood was sampled using 125 µL heparinised clinitubes (Radiometer, Denmark). Acceptable volumes of blood between 62.5 µL–120 µL were taken (2.9 $\mu L.g^{-1}$–5.5 $\mu L.g^{-1}$ respectively) (Smith et al. 2010). A small butane torch (Zengaz, ZT-50) was used to melt the ends of the capillary tubes and with a quick twist of the melted glass, the ends were sealed. The blood samples were immediately refrigerated. After the blood sample was taken, slight pressure was applied to the puncture site to encourage blood clotting at the site of the puncture. Once the bleeding had stopped (this happened quickly) the bats were returned to their respective cloth bags, placed in a cool, quiet room and held until dusk to be released.

Fieldwork was conducted with approval of the animal ethics clearance by Research and Innovation, Office of the Director, University of Venda, Project No: SMNS/19/ZOO/02/0307. All bats sampled survived and were successfully released at their respective sites of capture. Bats captured on Corea Game Farm were processed the same night of capture and immediately released. Only the bats captured from the Venetia diamond mine using the harp trap were held for ~12 hours due to mine security access and were offered mealworms and water prior to release back on the mine the following evening.

Trace elements and heavy metals by ICP-MS

Eleven fur and blood samples were tested for heavy metals antimony (Sb), arsenic (As), barium (Ba), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni) tin (Sn), vanadium (V) and zinc (Zn), and trace elements aluminum (Al), boron (B), calcium (Ca), potassium (K), rubidium (Rb), selenium (Se) and strontium (Sr) (Table 1). The elemental analysis was conducted by the Central Analytical Facility (CAF), University of Stellenbosch, Western Cape using an Agilent 7900 quadrupole inductively coupled plasma mass spectrometer (ICP-MS). Biological samples were weighed directly into 15 ml acid cleaned Falcon® tubes. 0.25 ml Ultra-pure Nitric and 0.25 ml Ultra-pure Hydrogen peroxide was added to each tube. The tubes were then placed in an oven at 60 °C for 30 minutes. After samples were digested, 2 ml of Ultra-pure de-ionised water. Detail on analytical conditions for the ICP-MS is included in the Appendices under *Supplementary Material: ICP-MS Analysis Detail from CAF, University of Stellenbosch*. Concentrations of metals in fur and blood were reported as $\mu g.g^{-1}$ and $\mu g.ml^{-1}$ and for the statistical analysis, were converted to parts per million (ppm).

For comparative purposes, dry weight element concentrations reported in the literature were converted to wet weight by dividing the values by four (see Hernout et al. 2016a and Ferrante et al. 2018) and are presented in Table A7 in the appendices.

Table 9. The classification (indicated by "X") of elements tested for in the blood and fur of bats based on Smith and Norberg (2015). Elements marked with "*" indicate their presence in animal and plant cells but their biological importance is largely unknown (see Bánfalvi 2011)

Element	Metalloid	Main Group Metal	Transition Metal
Antimony (Sb)	X		
Aluminum (Al)*		X	
Arsenic (As)*	X		
Barium (Ba)*		X	
Boron (B)	X		
Calcium (Ca)		X	
Cadmium (Cd)*			X
Chromium (Cr)			X
Cobalt (Co)			X
Copper (Cu)			X
Iron (Fe)			X
Lead (Pb)*		X	
Manganese (Mn)			X
Mercury (Hg)*			X
Molybdenum (Mo)			X
Nickel (Ni)			X
Potassium (K)		X	
Rubidium (Rb)*		X	
Selenium (Se)	X		
Strontium (Sr)*		X	
Tin (Sn)		X	
Vanadium (V)			X
Zinc (Zn)			X

Statistical analysis

Data was analysed in R (Version 1.1.456, RStudio, Inc.). Results of element concentrations in fur and blood are presented as median and range as the values in many instances varied considerably. T-tests assuming equal variances was used to test if there was a significant morphological difference between *M. condylurus* and *T. aegyptiaca* that may affect the results of the element analysis.

Each element concentration data set of the bat fur and blood were tested individually for normality using Shapiro tests ($P < 0.05$ not-normally distributed and $P > 0.05$ normal distribution) to make comparisons between the mining footprint and reference site, and between the total fur and blood samples. Where data were normally distributed, Levene's tests were used to determine the homogeneity of variances (unequal variance: $P < 0.05$, equal variance: $P > 0.05$). A two-sample t-test was used when comparing two data sets with a normal and non-normal distribution assuming equal or unequal variances based on the results from Levene's tests. Anovas (anova(aov)) were run where data had a normal distribution. Nonparametric Mann-Whitney U tests (wilcox.test, conf.int = TRUE) were used where data did not have a normal distribution. Paired Wilcoxon tests were run when comparing the fur and blood concentrations of elements that were not normally distributed.Spearman's correlation coefficient in R (cor.test) was used to determine if there were any correlations between the element concentrations in blood and fur.

Results

Dietary analysis

Insects collected represented 11 orders: Coleoptera, Lepidoptera, Hemiptera, Hymenoptera, Blattodea, Trichoptera, Mantodea, Diptera, Orthoptera, Isoptera and Dermaptera (Figure 25). The relative abundance of each order is represented in Figure 25. Isoptera were present in 20 of the 22 faecal pellets with evidence of Coleoptera, Hymenoptera and Lepidoptera in one faecal pellet and Hemiptera present in two faecal pellets (Table 10). Coleoptera dominated the light trap samples on both the mining and control sites comprising 53.92% and 37.74% of the sample respectively (Figure 25). Isoptera were the second dominant insect order comprising 17.16% of the sample collected in the control site but their presence was negligible on the mine area (Figure 25).

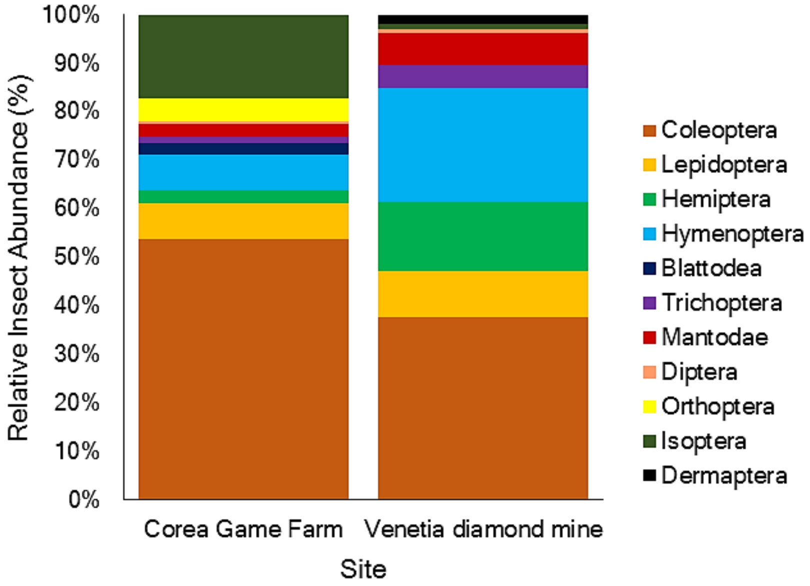

Figure 25. Relative abundance of insect orders sampled using a light trap on Corea Game Farm (n = 2 nights) and Venetia diamond mine (n = 2 nights) where Coleoptera were dominant on both sites comprising 53.92% and 37.74% of the sample respectively.

Table 10. Selected individuals of *Mops condylurus* and *Tadarida aegyptiaca* captured during summer (December 2018) from the Venetia diamond mine (VDM) and Corea Game Farm (CGF) with their associated measurements and faecal pellet analysis data.

Location (Bat Code)	Gender	Forearm	Mass	Species (# of faecal pellets)	Percentage volume of insect order (Family/Suborder)				
					Isoptera (Termitidae)	Hymenoptera (Formicidae)	Coleoptera	Lepidoptera	Hemiptera (Heteroptera)
VDM (DCT024)	Male	47.11	21.50	*M. condylurus* (n=1)	100	-	-	-	-
VDM (DCT025)	Male	47.13	23.00	*M. condylurus* (n=2)	100	-	-	-	-
VDM (DCT029)	Female	43.99	18.00	*M. condylurus* (n=2)	100	-	-	-	-
VDM (DCT033)	Male	46.90	23.00	*M. condylurus* (n=2)	100	-	-	-	-
VDM (DCT034)	Female	46.22	20.00	*M. condylurus* (n=1)	100	-	-	-	-
VDM (DCT035)	Male	49.82	26.00	*M. condylurus* (n=3)	100	-	-	-	-
CGF (DCT049)	Female	46.58	18.00	*T. aegyptiaca* (n=4)	99	1	-	-	-
CGF (DCT051)	Female	47.91	19.50	*T. aegyptiaca* (n=4)	100	-	-	-	-
CGF (DCT052)	Female	48.54	18.50	*T. aegyptiaca* (n=1)	100	-	-	-	-
CGF (DCT066)	Female	45.83	22.00	*M. condylurus* (n=1)	-	-	-	90	10
CGF (DCT070)	Female	48.06	24.50	*T. aegyptiaca* (n=1)	-	-	99.5	-	0.5

Trace elements by ICP-MS

Tadarida aegyptiaca and *M. condylurus* belong to the family Molossidae and are similar from an ecological and morphological perspective (masses and forearm lengths not significantly different: $P = 0.30$ and $P = 0.21$ respectively) thus we do not expect a phylogenetic effect of heavy metal and trace element concentrations of the fur and blood. Body size and mass probably did not play a role in the resulting differences in elemental concentrations between the individuals captured on the Venetia diamond mine and Corea Game Farm.

Six *M. condylurus* from the Venetia diamond mine and four *T. aegyptiaca* and one *M. condylurus* were selected from the control area (Corea Game Farm) for the analysis (Table 10). Blood concentrations of elements Al, Cd, Co, Cr, Mo, Ni and Sb were below detection limits in 63.64%, 45.45%, 9.09%, 36.36%, 9.39%, 0.09% and 54.54% of the samples respectively, thus half the detection limits provided by CAF was used in the analysis (Hickey et al. 2001, Andreani et al. 2019). Barium was only detectable in three out of 11 fur samples with concentrations ($\mu g.g^{-1}$w.w) of 1.07, 1.51, and 2.26. In all blood samples, Ba was below the detection limit and was therefore not included in the statistical analyses. Table 11 shows the detail of the statistical analyses used based on the distributions of the data set pairs that were analysed using Mann-Whitney U tests, Anovas and t-tests assuming either equal or unequal variances.

Table 11. Summary of the statistical analyses *P*—values indicating significant ($P < 0.05$) and non-significant ($P > 0.05$) results in elements comparing element concentrations in fur and blood between sites and the total fur and blood concentrations. CGF = control site: Corea Game Farm: VDM = Venetia Diamond Mine

	Fur	Blood	Fur~Blood
Element	CGF~VDM	CGF~VDM	CGF~VDM
B	$P < 0.05^{a}$	$P > 0.05^{b}$	$P < 0.05^{c2}$
Al	$P > 0.05^{c1}$	$P > 0.05*^{a}$	$P < 0.05^{d}$
K	$P < 0.05^{b}$	$P > 0.05^{b}$	$P > 0.05^{b}$
Ca	$P > 0.05^{c1}$	$P > 0.05*^{c1}$	$P < 0.05^{d}$
V	$P > 0.05*^{b}$	$P > 0.05^{a}$	$P < 0.05^{d}$
Cr	$P > 0.05^{b}$	$P > 0.05*^{c1}$	$P < 0.05^{d}$
Mn	$P > 0.05^{b}$	$P < 0.05*^{c1}$	$P < 0.05^{d}$
Fe	$P > 0.05^{c1}$	$P > 0.05^{b}$	$P < 0.05^{c1}$
Co	$P > 0.05*^{b}$	$P > 0.05*^{c1}$	$P < 0.05*^{c2}$
Ni	$P > 0.05^{c1}$	$P > 0.05*^{a}$	$P < 0.05^{d}$
Cu	$P > 0.05^{a}$	$P > 0.05^{b}$	$P < 0.05^{c1}$
Zn	$P > 0.05^{c1}$	$P < 0.05^{c1}$	$P > 0.05^{b}$
As	$P > 0.05^{b}$	$P > 0.05*^{b}$	$P < 0.05^{c2}$
Se	$P > 0.05^{b}$	$P > 0.05*^{b}$	$P > 0.05^{b}$
Rb	$P < 0.05^{c1}$	$P > 0.05^{c1}$	$P > 0.05^{d}$
Sr	$P > 0.05^{b}$	$P > 0.05*^{c1}$	$P < 0.05^{d}$
Mo	$P > 0.05^{c1}$	$P > 0.05^{b}$	$P > 0.05*^{b}$
Cd	$P < 0.05*^{c1}$	$P > 0.05^{c1}$	$P < 0.05^{c2}$
Sn	$P > 0.05*^{b}$	$P > 0.05*^{a}$	$P > 0.05*^{c1}$
Sb	$P > 0.05*^{b}$	$P > 0.05*^{b}$	$P < 0.05*^{c2}$

Hg	$P > 0.05$ [c2]	$P < 0.05$* [c1]	$P < 0.05$ [c2]
Pb	$P > 0.05$ [c1]	$P > 0.05$ [c1]	$P < 0.05$* [c2]

Since the data distributions were not all normally distributed: where both data sets were not normally distributed, Mann-Whitney U-test was performed ([a]) where both data sets were not normally distributed, anovas were used ([b]) where both data sets were normally distributed, t-tests assuming equal ([c1]) and unequal variances ([c2]) were run where one data set was normally distributed and the other was not and a paired Wilcoxon test were run ([d]) when comparing the fur and blood concentrations of elements that were not normally distributed.

*cannot compute excat *P*-values with ties (data with the same values)

Concentrations of elements varied greatly for most elements (Table 12). Overall, fur and blood concentrations for most of the heavy metals and trace elements were fairly low except for fur concentrations of Al, Ca, Fe and Zn with overall median concentrations (µg.g^{-1} w.w) of 104.88, 396.52, 120.24 and 121.68 respectively (Figure 26, Table 4). With the exception of Fe where a higher concentration was recorded in blood than fur ($P < 0.0005$), concentrations of elements were generally higher in fur than blood, and significantly so for elements Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sb, Sr, and V ($P < 0.005$, Table 11, Figure 26).

The bats from the mining footprint had significantly higher fur concentrations of B, Cd, K and Rb ($P \leq 0.03$) than those from the control site (Table 11). The maximum concentrations (µg.g^{-1} w.w) were 18.9, 7.30, 2.96 and 0.171 respectively from the mine. Although not statistically significant, it is interesting to note that bats fur from the control site had higher maximum concentrations of Al (558.06 µg.g^{-1} w.w), V (1.24 µg.g^{-1} w.w), Cr (2.71 µg.g^{-1} w.w), Mn (32.61 µg.g^{-1} w.w), Fe (814.00 µg.g^{-1} w.w), Co (0.673 µg.g^{-1} w.w), Sr (3.651 µg.g^{-1} w.w) and Pb (1.465 µg.g^{-1} w.w) than the fur from bats roosting on the mine.

Blood element concentrations for the most part were comparable between the two sites except for Mn, Zn, and Hg. These three elements were significantly higher ($P \leq 0.05$) in the blood of the bats from the Venetia diamond mine with maximum concentrations (µg.ml^{-1}) of 0.43, 7.04, and 0.05 recorded from the blood of bats roosting on the mine (Table 11 and 12). The highest concentration of a heavy metal was recorded for Fe in blood that ranged from a minimum concentration of 484.83 µg.ml^{-1} from the control site to 1026.21 µg.ml^{-1} from the mine. Statistically, there was no difference in blood and fur Fe concentrations between the control and mining footprint.

The only significant correlations between fur and blood element concentration was for Hg: $P < 0.05$, rho = 0.69 and Sb: $P < 0.05$, rho = 0.75 (Table 13). There may well be other correlations between element concentration in fur and blood but a larger sample size is needed to determine any potential correlations.

Table 12. Medians and ranges of the concentration of heavy metals and trace elements in the fur (µg.g^{-1} w.w) and blood (µg.ml^{-1} w.w) of molossid bats sampled on the Venetia Diamond Mine and Corea Game Farm (reference area). n = number of samples per tissue type and per site. The limit of quantification (LOQ) of elements in blood is indicated in brackets next to each relevant element symbol.

	Fur (n=11)						Blood (n=11)					
Element	Corea Game Farm (n=5)			Venetia Diamond Mine (n=6)			Corea Game Farm (n=5)			Venetia Diamond Mine (n=6)		
(LOQ)	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max
B (0.16)	3.380	1.643	3.914	7.440	6.791	18.900	1.330	1.250	2.321	2.162	1.250	3.015
Al (0.21)	102.620	51.437	558.062	109.380	43.969	185.393	0.11	0.105	1.999	0.11	0.105	1.380
K (12.7)	1330.06	769.00	2137.00	3310.00	2536.00	7298.00	2390.00	1882.00	3416.00	2450.00	1882.00	3379.00
Ca (0.2)	380.00	271.00	822.00	420.00	315.00	834.00	60.00	46.00	82.00	69.00	46.00	220.00
V (0.0004)	0.520	0.339	1.236	0.280	0.240	0.402	0.001	0.001	0.004	0.001	0.001	0.013
Cr (0.014)	1.070	0.369	2.708	0.990	0.545	1.471	0.060	0.014	0.099	0.007	0.007	0.409
Mn (0.011)	13.330	6.189	32.612	4.750	3.232	8.010	0.150	0.080	0.347	0.052	0.030	0.425
Fe (0.092)	147.64	79.05	814.00	101.41	67.54	181.25	629.47	484.83	861.64	690.91	631.09	1026.21
Co (0.0006)	0.240	0.166	0.673	0.130	0.096	0.199	0.001	0.0003	0.004	0.002	0.0003	0.004
Ni (0.004)	1.380	0.766	2.575	1.130	0.790	2.970	0.010	0.003	0.014	0.009	0.002	0.100
Cu (0.011)	6.090	5.343	17.413	8.430	5.686	26.106	0.380	0.261	0.665	0.731	0.261	0.968
Zn (0.0089)	97.700	37.775	241.865	147.090	117.801	346.409	1.900	1.376	3.589	4.040	1.376	7.044
As (0.0015)	0.260	0.184	0.320	0.720	0.369	1.201	0.005	0.003	0.009	0.006	0.003	0.023
Se (0.0009)	4.030	1.948	6.396	5.480	3.645	8.214	0.600	0.570	0.731	0.822	0.570	1.052
Rb (0.0006)	0.890	0.628	1.055	1.260	1.114	2.958	1.020	0.826	2.363	0.893	0.977	1.570
Sr (0.0009)	1.710	1.369	3.651	1.440	1.018	1.854	0.060	0.027	0.078	0.079	0.027	0.383
Mo (0.0012)	0.720	0.518	0.817	0.850	0.586	0.917	0.003	0.002	0.006	0.003	0.0006	0.007
Cd (0.0002)	0.020	0.016	0.097	0.090	0.031	0.171	0.0003	0.0001	0.003	0.001	0.0001	0.004
Sn (0.0012)	0.060	0.033	0.164	0.090	0.062	0.183	0.003	0.002	0.003	0.004	0.002	0.722
Sb (0.0006)	0.040	0.019	0.074	0.180	0.094	0.223	0.0003	0.0003	0.0003	0.001	0.0003	0.003
Hg (0.0005)	0.600	0.386	1.112	1.270	0.578	1.947	0.011	0.006	0.026	0.029	0.006	0.045
Pb (0.0017)	0.480	0.420	1.465	0.450	0.156	0.936	0.011	0.008	0.038	0.033	0.008	0.169

Table 13. Spearman’s Correlation analysis between total fur and blood element concentrations.

Element	rho	*P* - value	S
B	0.40	0.23	132
Al	0.11	0.76	196.84
K	-0.45	0.17	318
Ca	0.23	0.50	170
V	-0.40	0.23	308
Cr	-0.06	0.86	233.31
Mn	0.13	0.71	192
Fe	-0.06	0.86	234
Co	-0.47	0.14	324.24
Ni	-0.08	0.82	238
Cu	0.09	0.80	200
Zn	0.55	0.09	100
As	0.50	0.12	110
Se	0.01	0.99	218
Rb	-0.29	0.39	284
Sr	-0.50	0.12	330
Mo	0.47	0.15	116
Cd	0.05	0.89	209.51
Sn	0.30	0.37	154
Sb	0.75	0.008	55.34
Hg	0.69	0.02	68
Pb	0.02	0.97	216

Discussion

We provide the first data for a range of 23 elements using fur and blood as biomarkers in two open-air forager bat species, *M. condylurus* and *T. aegyptiaca* from northern Limpopo Province, South Africa. *Tadarida aegyptiaca* predominantly feeds on Coleoptera (including water beetles), Lepidoptera, Orthoptera, Hymenoptera, Isoptera, Diptera and Arachnids (spiders) (Taylor et al. 2019, Monadjem et al. 2020). *Mops condylurus* feeds mainly on Coleoptera, Hemiptera, Diptera and Lepidoptera (Taylor et al. 2019, Monadjem et al. 2020) and during sample collection, fed mostly on Isoptera that emerged in response to the onset of the rainfall season. This suggests that the bats roosting on the mine were not foraging in the vicinity of the mist net capture sites on the mine where the insect sampling took place but possibly over the natural areas adjacent to the mine. It is known that elements biomagnify through trophic levels (Pikula et al. 2010, Ali et al. 2019) but element concentrations in bat diets is largely unknown. In general the diet of different bat species has been shown in the literature to be an important route through which heavy metals and trace elements could accumulate in tissues and organs of bats (Karouna-Renier et al. 2014, Becker et al. 2018, Carrasco-Rueda et al. 2020). Idowu et al. (2014) and Denloye et al. (2015) presented fairly low concentrations of heavy metal accumulation in mound termites (Termitidae) that are fondly consumed by people in Africa. The latter authors cautioned that even though the concentrations of heavy metals in the different species and castes were low, the different species and castes of termite may differ in their ability to accumulate heavy metals based on their physiological needs and could pose a risk to humans who regularly consume them. Termites may provide a seasonal source of heavy metal and trace element ingestion in molossid bats and other bat species that opportunistically feed on them during the summer emergence.

Sixteen of the 22 elements investigated (excluding Ba) showed significant differences between fur and blood, with 15 elements being reported higher in fur than blood (Figure 26). Only Fe was found to be higher in blood than in the fur with approximately a seven fold difference in the medians between the two sites (Figure 26). Correlations between tissues and internal organs are not standard and cannot be generalised. In our study, a novel finding was that only Hg and Sb had a significant (although weak) correlation between fur and blood concentrations that is supported by a similar finding by Karouna-Renier et al. (2014). They found that Hg concentrations in the blood of *M. lucifugus* were highly predictive by fur Hg concentrations (Karouna-Renier et al. 2014). There are currently no toxic thresholds of heavy metals and trace elements for fur concentrations and internal organs/tissues in bats. For all intents and purposes, until the toxic thresholds for heavy metals and trace elements in various bat species has been determined, we may refer to toxic thresholds of elements reported for other mammalian species as a reference, although we acknowledge that this may significantly differ for bats. Clark et al. (1986) suggested that heavy metal accumulation in the kidneys of bats should be compared with that of shrews, as they have a similar diet opposed to mice. However, toxic thresholds of heavy metals and trace elements is also lacking for shrews. In the available literature, reported element concentrations range from means, to geometric means and medians and thus without the raw data it is not always easily comparable across studies (for example: Hickey et al. 2001, Becker et al. 2018, Mina et al. 2019 and the current study). Comparative data for the 23 elements investigated in our study is scant and limited to a handful of studies that will be discussed below to place our findings into a global context (see Table A7).

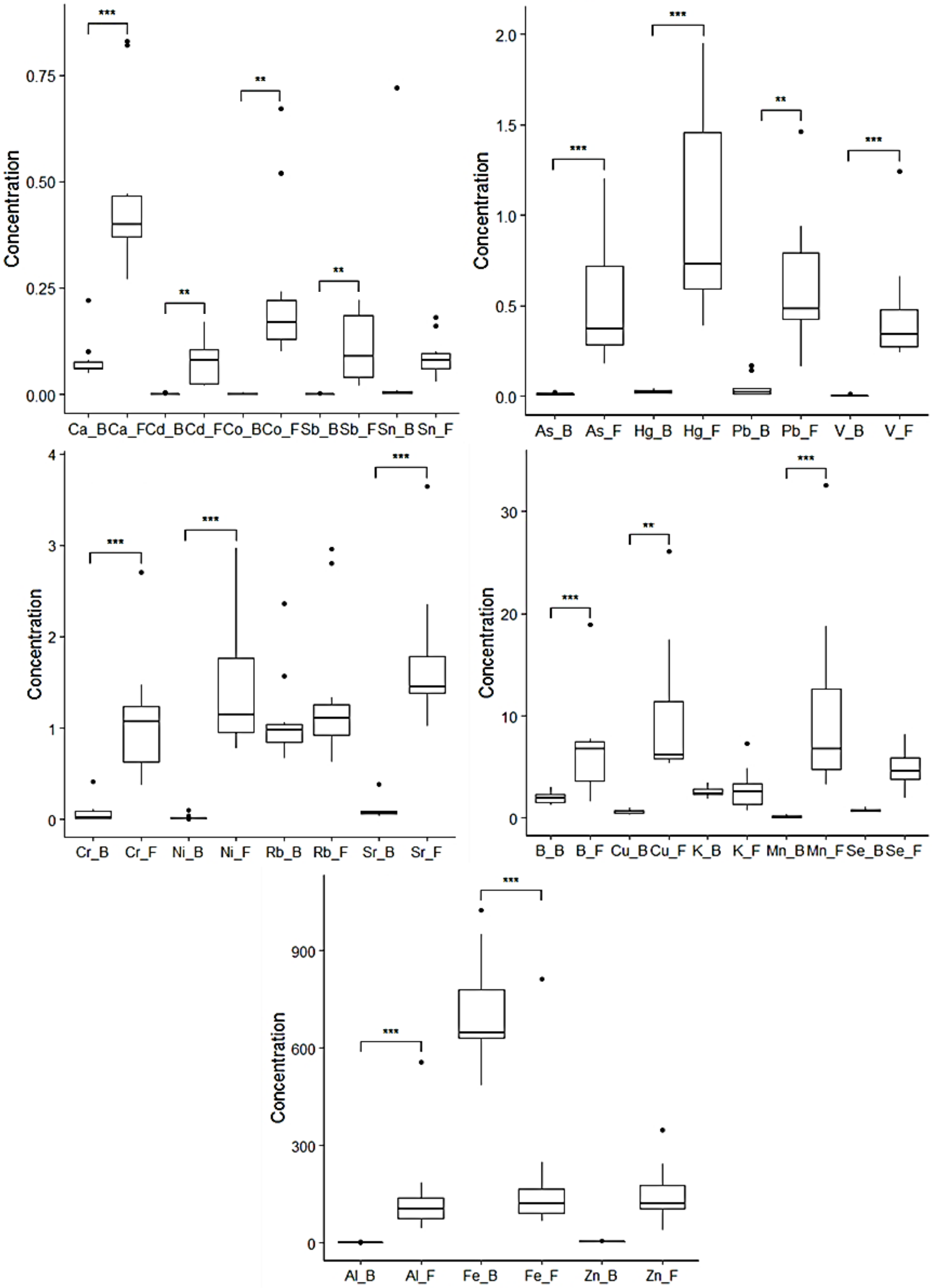

Figure 26. Box and whiskers plots indicating the median, the 25th and 75th percentiles and range of the concentrations of heavy metals and trace elements (open circles) in relation to tissue type (F = fur, B = blood). Statistically significant differences between heavy metal and trace element concentrations in fur and blood are indicated as follows: *= $P < 0.05$, **=$P < 0.005$, and ***$P < 0.0005$. Wilcoxon tests were used to compare Al, Ca, V, Cr, Mn, Ni, Rb and Sr: Anovas were used to compare K, Zn, Se: t-tests assuming unequal variances were used to compare B, Co, As, Cd, Sb, Hg and Pb and t-tests assuming equal variances were used to compare Fe, Cu and Sn.

Mercury has been reported to have a toxic threshold of ≥10 ppm (or 10 µg.g^{-1}) in hair that indicates adverse health effects such as neurochemical effects and functional behaviour changes in wild mice and captive mink (Wobeser et al. 1976, Burton et al. 1977) and has been accepted to have potential health effects in bats (Becker et al. 2018, Moreno-Brush et al. 2018, Carrasco-Rueda et al. 2020). Mercury contamination in the fur of comparatively few bat species from areas impacted by anthropogenic activities has been investigated. For example, 10 µg.g^{-1} total Hg in the fur of *Myotis lucifugus* indicates neurological alterations but lower concentrations of Hg correlate to innate immunity in *Desmodus rotundus* (Becker et al. 2018). The median levels of Hg in bat fur reported in our study for molossid bats of 1.27 µg.g^{-1} (range 0.58–1.95µg.g^{-1}) on the Venetia diamond mine and 0.60 µg.g^{-1} (range: 0.39–1.11 µg.g^{-1}) on Corea Game Farm are well below the concentrations responsible for neurological alterations in *M. lucifugus* (Becker et al. 2018). Mercury concentrations in the molossid fur in our study were generally lower than those reported in insectivorous bats by Hickey et al. (2001), Carrasco-Rueda et al. (2020) and Becker et al. (2018), higher than those reported in skin-fur samples by Andreani et al. (2019) and comparable with the ranges reported by Ferrante et al. (2018) and Carrasco-Rueda et al. (2020) as a consequence of a variety of anthropogenic activities. In south-eastern Amazon, Peru, *Carollia perspicillata* and *Phyllostomus elongatus* foraging in the vicinity of artisanal gold mines were shown to have fur Hg concentration medians of 0.66 µg.g^{-1} and 1.90 µg.g^{-1} respectively (Moreno-Brush et al. 2018). Becker et al. (2018) reported dietary guild influenced Hg concentrations in 22 species of bat from a reserve and forest patch surrounded by agriculture ranging from 0.03 µg.g^{-1} in the frugivore *Artibeus intermedius* to 145.27 µg.g^{-1} in the carnivorous (predominantly fish eating) bat *Noctilio leporinus*.

Carrasco-Rueda et al. (2020) reported Hg contamination in 30 bat species from various dietary guilds impacted by small-scale gold mining with mean Hg concentrations in all fur samples of 0.36 ± 0.47 µg.g^{-1}. Mercury concentration in fur was shown to be lowest for frugivorous and nectivorous bat species from the family Phyllostomidae (mean range: 0.00–0.58 µg.g^{-1}), followed in increasing concentrations of Hg in fur by omnivores (0.10–0.26 µg.g^{-1}), gleaning insectivores (0.01–0.73 µg.g^{-1}) and carnivores (0.35–0.84 µg.g^{-1}) (Carrasco-Rueda et al. 2020). Aerial insectivores (Vespertilionidae, Molossidae and Emballonuridae) had the highest concentration of Hg in their fur with mean ranges between 0.42–1.97 µg.g^{-1}. At these concentrations, the authors suggested that the levels may not pose a health risk and could be due to background concentrations in the environment and not as a result of proximity to gold mining activities or agricultural practices (Carrasco-Rueda et al. 2020). Conversely, Hg levels in the skin-fur of *T. teniotis* and *Miniopterus schreibersii* from the African Quarter of Rome (Italy) reported a low mean concentration of 0.065 ± 0.031 µg.g^{-1} (Andreani et al. 2019). In Canada (Ontario and Quebec), Hickey et al. (2001) reported high and significantly different concentrations of Hg in the fur of four species of bats with values that could reflect biomagnification from aquatic prey that develop in Hg contaminated sediments into top predators (bats). The four bat species were: *M. lucifugus* (1.3–7.6 µg.g^{-1}), *M. septentrionalis* (geometric mean of 4.4 µg.g^{-1}–max. concentration of 10.2 µg.g^{-1})*, M. leibii septentrionalis* (geometric mean of 5.3 µg.g^{-1}–max. concentration of 76.2 µg.g^{-1}) and *Eptesicus fuscus* (geometric mean of 1.5 µg.g^{-1}–max. concentration of 15.4 µg.g^{-1}) (Hickey et al. 2001). Wieringa et al. (2020) reported a lower mean Hg concentration in bat fur than our study, reporting 0.005 µg.g^{-1} in the fur of *Lasiurus borealis* from across its range in North America which was similar to the concentrations reported for Phyllostomidae bats in Carassco-Rueda et al. (2020). Mean mercury concentrations in *M. lucifiugus* fur from two contaminated sites in north-western Virginia (USA) and a reference site in Moscow reported much higher mean concentrations of 118.4 µg.g^{-1} and 3.3 µg.g^{-1} respectively, than that observed in the molossids from our study (Karouna-Renier et al. 2014). The mean concentration observed in the molossids from the mine were appromiately 93 times and 2.6 times lower than the contaminated and reference sites (Karouna-Renier et al. 2014).

Lead in the blood of one individual molossid roosting on the mine had a blood concentration of 0.17 µg.ml^{-1} which is hazardously close to the level of 0.2 ppm Pb contamination in the blood of an animal (cattle) considered to be lead poisoned (Reis et al. 2010). Additionally, if an animal is suffering from Pb poisoning, the animal should also be deficient in Ca, Fe and Zn, however, the individual bat of concern had the highest blood concentrations of: Fe (1026.21 µg.ml^{-1}), Ca (220 µg.ml^{-1}) and Zn (7.04 µg.ml^{-1}) compared to all the other individuals. The implications of this observation is unknown. Elevated levels of Pb in the fur of bats could be due to Pb contamination of prey and the physical exposure of bats to vehicular traffic continually entering and exiting the mine throughout the day in close proximity to their roost as seen in *P. pipstrellus* (Flache et al. 2015). In our study, the median Pb concentrations in the fur of the bats from both sites were similar (control: 0.48 µg.g^{-1}, mine: 0.45 µg.g^{-1}) (Table 12) and were comparable with the ranges of fur Pb concentrations of *M. myotis* in Flache et al. (2015) and Ferrante et al. (2018) of 0.004–1.68 µg.g^{-1} and 0.04–1.52 µg.g^{-1} respectively in mixed environments. Our study presents Pb concentrations much lower than skin-fur concentrations reported by Andreani et al. (2019) of 36.9 ± 18.4 µg.g^{-1} in *T. teniotis* and *M. schreibersii* from Italy. Similarly, the maximum concentrations of Pb reported in our study of 0.936 µg.g^{-1} (mine) and 1.465 µg.g^{-1} (control) were considerably lower than that reported for *A. nana* foraging over a reference site of 6.65 µg.g^{-1} (Hill et al. 2017).

Of greater concern was the Al recorded in the fur of the molossids in our study were ~1.6 up to 23 times higher than the concentrations of Al in fur of *M. lucifugus* (27–70 µg.g^{-1}) and *E. fuscus* (4.8 µg.g^{-1}) that comparatively were very low (Hickey et al. 2001). Some of the Al values for *M. lucifugus* fell within the range of values from the mine (43.97–185.39 µg.g^{-1}). Andreani et al. (2019) reported a mean concentration of 306 ± 75_µg.g^{-1} in skin-fur samples of *T. teniotis* and *M. schreibersii* from a polluted area in Italy (African Quarter of Rome). This mean concentration was higher than the median recorded in our study in bat fur of 102.62 µg.g^{-1} from the control site but lower than the maximum concentration of 558.06 µg.g^{-1} reported at the same site. The median and minimum fur Al level were slightly elevated in the control area but maximum concentration recorded from the control area was much higher than in the fur from the mine (Table 12). Aluminum is a non-essential element (Reis et al. 2010) and the range of concentrations in blood and fur of the molossids raises a cause of concern. In rodents, bone concentration of Al >10 µg.g^{-1} has been considered to indicate a reduction in the ability of the animal to excrete Al or an indication of exposure to high concentrations of Al (Scheuhammer 1987). Aluminium is known to be toxic to invertebrates and freshwater fishes (van Dam et al. 2018, AL-Taee et al. 2020), neurotoxic to humans as well as causing disturbances in cellular growth, communication and secretory functions, and reductions in bone tissue (Barabasz et al. 2002). There is concern that important metabolic processes in birds and mammals could be negatively affected by Al uptake in environments containing high concentrations of Al (Rosseland et al. 1990) since Al has been shown to negatively affect phosphorous (P) and Ca metabolism resulting in bone abnormalities, weakness of muscle and decreased growth rates (Scheuhammer 1987). Aluminium is a common element in soils (Rosseland et al. 1990) and has been shown to be present in soil dust (8.2% according to Friedlander 1973) but the percentage that Al contributes to soil dust on the Venetia Diamond Mine and surrounding area is unknown. Future research should investigate whether these concentrations measured in the molossid fur indicate 1) the background levels, 2) a contamination event or 3) have any health implications.

Levels of Fe reported in the fur for the control and mine bats (147.64 µg.g^{-1} and 101.41 µg.g^{-1} respectively) (Table 12) were comparable with the concentration observed in *E. fuscus* (100.00 µg.g^{-1}) in Hickey et al. (2001) but were highr than the ranges reported for *A. nana* (5.63–33.52 µg.g^{-1} and 5.95–42.63 µg.g^{-1} foraging over wastewater treatment works, and 6.16–31.33 µg.g^{-1} from reference sites) (Hill et al. 2017). The maximum concentration of 814.00 µg.g^{-1} from an individual from the control site was the highest level reported in fur compared to available literature. Median fur Zn concentrations of 147.09 and 97.7 µg.g^{-1} (Table 12) were similar to the medians and ranges for *P. pipistrellus* but higher than *M. nattereri, M. bechsteinii, M. daubentonii* and *Plectus auritus* studied by Flache et al. (2015 and 2018), and comparable with *M. lucifugus, M. septentionalis* and *E. fuscus* Hickey et al. (2001). Flache et al. (2015) had noted that *M. daubentonii* (32.25 µg.g^{-1} Zn in fur) is known to forage on chironomid midges emerging from water bodies with contaminated sediment. Perhaps a similar occurrence took place with the molossids opportunistically feeding on emerging adult insects from the mine waste water dam. Perhaps this could explain the observed high Zn concentrations in the fur of the bats from the mine (117.80 – 346.4 µg.g^{-1}). The ranges of Zn concentrations in our study were higher than with concentrations reported in Hill et al. (2017) of 61.81–97.84 µg.g^{-1}, 51.65–138.60 µg.g^{-1} (wastewater treatment works) and 40.48–89.49 µg.g^{-1} (reference sites).

The median As fur concentrations of 0.72 µg.g^{-1} (mine) and 0.26 µg.g^{-1} (control) (Table 12) were comparable to *T. teniotis* and *M. schreibersii* skin-fur from a polluted urban area in Italy of 0.29 ± 0.07 µg.g^{-1} (Andreani et al. 2019), higher than *M. myotis* roosting near a petrochemical plant as well as in an uncontaminated area with As fur concentrations of 0.11 µg.g^{-1} and 0.14 µg.g^{-1} respectively (Ferrante et al. 2018). The concentrations of As from the fur of the molossids from the mine were comparable with the range of concentrations reported in fur from *A. nana* foraging over wastewater treatment works (0.15–0.64 µg.g^{-1} and 0.10–0.49 µg.g^{-1}) (Hill et al. 2017). Arsenic is an element of concern as it can crosses the blood-brain barrier and is implicated in neurogenerative diseases (Escudero-Lourdes 2016). Elevated concentrations in bat fur may indicate negative neurological effects in the long-term (Hill et al. 2017). Although significantly different between our two sites, low concentrations of fur Cd were reported for the molossids on the mine and in the control area of 0.09 and 0.02 µg.g^{-1} respectively. The control area concentrations of Cd were comparable with Cd medians of 0.02 µg.g^{-1} for *Hypsugo savii/Nyctalus leisleiri/Pipistrellus pipistrellus/P. pygmaeus* (Mina et al. 2019) and mean concentrations of 0.02 µg.g^{-1}, 0.03 µg.g^{-1}, 0.01 µg.g^{-1} in fur of *A. nana* foraging over two wastewater treatments works and a reference area respectively (Hill et al. 2017) and 0.04 ± 0.008 µg.g^{-1} in skin-fur of *T. teniotis* and *M. schreibersii* (Andreani et al. 2019). The median Cd concentration in the fur of the molossids on the mine were comparable with the maxiumum concentrations reported in Flache et al. (2015, 2018), Mine et al. (2019) amd Hill et al. (2017). Overall, the values reported in our study overlap with the ranges reported in Hernout et al. (2016b), Flache et al. (2018) and Flache et al. (2015) from a variety of land uses excluding mines. The concentration of Sb in the bat fur from the mine was slightly elevated above the control area (medians 0.18 µg.g^{-1} vs 0.04 µg.g^{-1}) and the opposite was observed for V (0.28 µg.g^{-1} vs 0.52 µg.g^{-1}) (Table 12). Both elements were higher than median concentrations recorded for *M. myotis* in Sicily from a polluted area near a petrochemical plant and a control area in Pantalica: Sb: 0.01 µg.g^{-1} and 0.02 µg.g^{-1}, V: 0.07 µg.g^{-1} and 0.11 µg.g^{-1} respectively for the two sites (Ferrante et al. 2018). But were lower than the mean concentration of Sb recorded in skin-fur of *T. teniotis* and *M. schreibersii* of 1.98 ± 1.95 µg.g^{-1} (Andreani et al. 2019).

The median concentrations of Co (mine: 0.13 µg.g^{-1} and control: 0.24 µg.g^{-1}), Ni (mine: 1.13 µg.g^{-1} and control: 1.38 µg.g^{-1}), Se (mine: 5.48 µg.g^{-1} and control: 4.03 µg.g^{-1}) and Mn (mine:4.75 µg.g^{-1} and control: 13.33 µg.g^{-1}) in the fur of molossids from both sites in our study were higher than median fur concentrations reported in Mina et al. (2019) of Co (0.09 µg.g^{-1}), Ni (0.65 µg.g^{-1}), Se (0.86 µg.g^{-1}) and Mn (2.84 µg.g^{-1}) in *Hypsugo savii/Nyctalus leisleiri, Pipistrellus pipistrellus/P. pygmaeus*. Similarly, the maxiumum concentrations on the mine and control area respectively of Co (0.20 µg.g^{-1}–0.67 µg.g^{-1}), Ni (2.97 µg.g^{-1}–2.58 µg.g^{-1}), Se (8.21 µg.g^{-1} and 6.40 µg.g^{-1}) and Mn (8.01 µg.g^{-1}–32.61 µg.g^{-1}) recorded in our study were higher than the maxium values reported in Ferrante et al. (2018). Manganese concentrations in the fur of the molossids also fell within the ranges reported for *A. nana* foraging over wastewater treatments works but the maximum concentration reported from the mine in our study (32.61 µg.g^{-1}) was two times higher than the maximum concentration recorded from the reference sites in Hill et al. (2017) of 16.80 µg.g^{-}1. Median Cu concentrations in the molossids fur at both sites of 8.43 µg.g^{-1} and 6.09 µg.g^{-1} were higher than the values in *M. myotis* of 1.30 µg.g^{-1} and 1.84 µg.g^{-1} (Ferrante et al. 2018). The ranges of the molossid fur Cu concentrations fell within the ranges reported by Flache et al. (2015), Hernout et al. (2016b), Hill et al. (2017), Flache et al. (2018) and Mina et al. (2019). The median fur concentrations of 0.85 µg.g^{-1} (mine) and 0.72 µg.g^{-1} (control area) of Mo in the molossids (Table 12) were lower than that reported in *M. bechsteinii, M. nattereri* and *P. auritus* (Flache et al. 2018). The only comparative data for fur Sn and Rb concentrations in the literature have recently been published by Wieringa et al. (2020). Mean fur concentrations of Sn (4.23 ppb) and Rb (0.65 ppb) (Wieringa et al. 2020) were lower than the minimum values recorded from the mine and control area of 0.09 µg.g^{-1} and 0.06 µg.g^{-1} respectively for Sn and 0.63 µg.g^{-1} and 1.11 µg.g^{-1} for Rb. Comparative concentrations of Sr are presented in Andreani et al. (2019) with a skin-fur mean concentration of 9.14 ± 0.60 µg.g^{-1} that is 2.5 and 5 times higher than the maximum concentrations reported in our study in the molossid bats fur from the control site (3.65 µg.g^{-1} (median: 1.71 µg.g^{-1})) and mine area (1.85 µg.g^{-1} (median: 1.44 µg.g^{-1})). The concentration range of Ba in the fur of the molossids in our study (mine: 0.06 µg.g^{-1}–1.51 µg.g^{-1}: control area: 0.06 µg.g^{-1}–2.26 µg.g^{-1}) was lower than the range of 0.28 – 59.5 µg.g^{-1} in all types of tissue of insectivorous bats summarised in Zukal et al. (2015) but higher than the mean concentration of 0.005 µg.g^{-1} in lactant *T. teniotis* (Andreani et al. 2019).

Finally, Cr levels in the molossids in our study of 0.99 µg.g^{-1} in bats from the control area and 1.07 µg.g^{-1} from the diamond mine were higher than the median concentration of 0.63 µg.g^{-1} (range: 0.21–2.17 µg.g^{-1}) in *Hypsugo savii/Nyctalus leisleiri/Pipistrellus pipistrellus/P. pygmaeus* on wind farms in Portugal (Mina et al. 2019) and higher than the median concentrations of and 0.26 µg.g^{-1} and 0.29 µg.g^{-1} in *M. myotis* respectively from a control area and an area near a petrochemical plant, Sicily (Ferrante et al. 2018). Similarly, the molossid fur concentrations of Cr were higher than the ranges reported for *A.* nana captured over wastewater treatment works and reference sites in South Africa (Hill et al. 2017). The concentration of Cr that would be considered harmful in bats is unknown but elevated levels of Cr and other heavy metals (Cu, Cd, Zn and Pb) in the liver and kidneys in the shrew *Crocidura russula* from a landfill site in Garraf (Spain) of 3.49 ± 0.45 µg.g^{-1} and 5.40 ± 0.59 µg.g^{-1} respectively were suggested to have negative health implications (Sánchez-Chardi et al. 2007). Currently, there are no comparable studies for the bat fur concentrations of B and K with other bat or small mammal species, thus the interpretation and potential health impacts of these element concentrations remains unknown.

Data are scant concerning heavy metal and trace element levels in small mammal blood for an adequate comparison with the current data. Karouna-Renier et al. (2014) investigated the effects of Hg concentrations on mtDNA in *M. lucifugus* collected from contaminated sites from South (Grottoes) and North (Mt. Sydney) Rivers in north-western Virginia, USA and a reference site in Moscow, Russia. *Myotis lucifugus* from the contaminated sites (particularly Mt. Sydney) revealed significantly higher levels of mtDNA damage than those from the reference area. The mean Hg concentration in the blood from the molossids from the mine in our study was comparable with the concentration reported from Moscow by Karouna-Renier et al. (2014) of 0.03ppm (our study: 0.03µg.ml^{-1}, Moscow: 0.03µg.g^{-1}). Mean blood Hg concentrations from the molossids collected from our control site was notably lower than the reference and contaminated sites reported by Karouna-Renier et al. (2014). Thus the health effects of the concentrations of Hg recorded in our study are probably negligible. A study on whole bat samples (Smith and Rongstad 1982) investigating Zn, Cu, Cd, Pb and Ni concentrations in bats from a proposed mining site and an active mine near Timmins, Ontario, Canada revealed heavy metal concentrations much higher than those recorded in the blood from the molossids in our study (See Table A7). Heavy metals are known to accumulate in internal organs and tissues (Naidoo et al. 2016) thus the whole bat sample would have much higher concentrations than the blood samples, but we use it as a proxy none-the-less. With the exception of Fe and Zn, the reported heavy metal and trace element values in the blood of the molossids may reflect the background conditions, future research in this area is critical to establish baseline reference data in South Africa.

The only available literature concerning heavy metals in South African bats was published by Naidoo et al (2013, 2014, 2016) and Hill et al. (2017). Naidoo et al. (2013, 2015 and 2016) reported that an urban adapter bat species, the Banana bat (*Afronycteris nana*), foraging over waste water treatment works may suffer from chronic health problems associated with bioaccumulation of heavy metals. Bioaccumulation of heavy metals in *A. nana* could have been through the consumption of Diptera (possibly chironomid midges that are tolerant of the polluted water bodies) swarming over the waste water sites (Naidoo et al. 2013). The heavy metal concentrations reported could have been responsible for the observed damage to the kidneys (including enlarged kidneys that indicated nephrotoxicity) and liver that are responsible for detoxifying pollutants (Naidoo et al. 2016). Even at low concentrations, Ni, Cd and Pb inhibit proteins that are involved in DNA repair which may have accounted for the significant DNA damage noted in *A. nana* (Naidoo et al. 2015). Additionally, Hill et al. (2017) provided evidence of significantly higher concentrations of As in the brains and fur of *A. nana* foraging over waste water treatment works (WWTW) compared to reference sites. Overall, the antioxidant capacity in the brains of bats captured over WWTW was significantly lower than those from reference sites. The potential implications of long-term exposure to pollutants could affect the protection mechanisms and cellular processes in the brains of *A. nana* (Hill et al. 2017).

Implications for bat conservation

In addition to the global stressors that bats already face, namely habitat loss, changes in available resource quantity and quality, climate change, increasing number of wind turbines, disease pressure (de Souza et al. 2020, Lawson et al. 2020, Flache et al. 2018, Hernout et al. 2016a) and environmental pollution including organophosphates (Bayat et al. 2014), it is vital that both the origin of elements and the toxicological response of bats is understood. This knowledge will assist conservation authorities and specialists to make informed decisions concerning bat conservation and mitigation strategies within the context of looming anthropogenic developments and conservation areas. For example, we may find that in accordance with legislation and mine protocols that the current specifications for the containment of waste water can still expose ecosystems to elevated concentrations of elements. Bat fur used as a biomarker could indicate bioaccumulation of elements either through directly drinking from the waste water dams or feeding on emerging adult insects with an aquatic development phase within the waste water dams. The shortfall with the current available literature is that we still do not know 1) the specific toxicological thresholds for numerous bat species, 2) the physiological and resulting ecological effects of these elements and 3) whether there are phylogenetic responses to elements (e.g. Total mercury concentrations in fur has been shown to have a strong phylogenetic signal as reported by Becker et al. (2018)). Southern hemisphere bat species have been poorly studied in this regard. Additionally, there is a lack of long-term and recapture studies on the impact of heavy metals and trace elements on bat populations that include the effects of age and gender (Hernout et al. 2016b).

Conclusion

Our study is the first in South Africa to provide some data on heavy metal and trace element concentrations in blood and fur of *M. condylurus* and *T. aegyptiaca* roosting on the Venetia Diamond Mine and in a control area. Bats do demonstrate some potential as suitable bioindicators for the monitoring of ecosystem health over and near opencast mining operations using non-lethal and non-invasive methods. Only six out of the 23 elements tested in the fur and blood were significantly higher in the bats roosting on the mine compared to those from the reference area namely: B, K, Rb and Cd (fur) and Zn and Hg (blood). Manganese (blood) was significantly higher in the bats from the reference area than those roosting on the mine. Overall, 16 elements significantly differed between fur and blood, with most elements except Fe present in higher concentrations in fur than blood. Only Sb and Hg concentrations had significant correlations between fur and blood providing support that for at least these two elements, fur could provide a reliable indication of internal element concentrations.

The concentrations of the elements reported in our study could reflect the natural background levels and may not necessarily be a cause of concern at this point in time. However, this may fluctuate during the year and the impacts of these elements on the organs and bones of bats may reveal another story. We could not investigate the heavy metal and trace element contamination of other bat species, as comparative individuals were not captured, thus we do not know the impact of the mining activity on many other bat species known to occur in the area. Future investigation is required in this regard.

We have but a mere glimpse into how acute and chronic exposure of heavy metals and trace elements affect bats. There is an exciting opportunity for much needed research to bridge the gap in our knowledge with regards to collecting baseline levels of elements in bat fur, investigating correlations between internal and external tissue/organ elemental concentrations, understanding how these elements physiologically interact in different bat species and the bat specific toxic thresholds of these elements. Significantly higher levels of elements in fur than blood (as reported in our study) may not necessarily indicate that a bat is suffering from toxicological effects of the elements. Using bat fur as a reliable less-invasive biological marker has potential to indicate a degree of environmental contamination (Hernout et al. 2016a & b). A cautionary proposal can only be given at this point in time that the elevated concentrations of some elements in the fur samples could indicate which elements persist in the environment. These elements may be a cause of concern for the local bat communities and their consequent conservation. The data presented here should be used with caution and can be regarded as the first baseline data for two species of molossid bats in northern Limpopo, South Africa.

As anthropogenic activities, particularly mining operations continue to liberate metals, trace elements and chemicals into the environment, these substances become available for assimilation into the food chain, where individual animal health and ecosystem resilience can be negatively impacted, especially when physiological damage is incurred. Understanding how elements and chemicals are made available during opencast diamond mining and the impacts of such will determine how these impacts could potentially be managed and how these impacts will affect local animal populations and their inherent conservation.

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Chapter 5: General discussion and conclusions

Global bat population declines are attributed to habitat loss, changes in available resource quantity and quality, climate change, increasing number of wind turbines, disease pressure and exposure to persistent chemical and elemental pollutants in the environment (Barre et al. 2018, Frick et al. 2020, Oliveira et al. 2021, Hopkins et al. 2021). Bat species communities (species richness and abundance) are sensitive to changes in the landscape and have a fairly low resilience to changes and or reduction in natural habitats (Muylaert et al. 2016). Responses to anthropogenic impacts is known to be species specific and that even within the same foraging guild the abundances of different species are affected by habitat types and transformed landscapes (mixture of natural vegetation patches surrounded gradual vegetation changes: low contrast matrixes) (Rodríguez-San Pedro and Simonetti 2015, Muylaert et al. 2016).

Bioindicator species can convey invaluable information about the impact of the Venetia diamond mine on the environment. Bioindicators provide an indication of the quality of the environment, biotic elements or humans in a given ecosystem. Bioindicators fall into three general categories based on what changes they indicate 1) environmental indicators, 2) ecological indicators and 3) biodiversity indicators (Jones et al. 2009). Bats meet the criteria that have been suggested for choosing bioindicators in that they are easy to sample (using acoustic methodologies), are exposed to a range of stressors (e.g. land use change, climate change and disease), they provide important ecosystem services (e.g. pest control) and are taxonomically stable (Park 2015, Zukal et al. 2015, Frick et al. 2016). Bats have been proposed as reliable bioindicators for monitoring bioaccumulation of heavy metals, elements and chemicals in natural and transformed ecosystems (Zocche et al. 2010, Russo and Jones 2015, Mansour et al. 2016, Ferrante et al. 2018). There is a deficiency of knowledge concerning bat biological use of heavy metals and trace elements, the biochemical interactions of these elements, bat specific toxicity thresholds and ultimately how concentrations of elements in fur correlate with internal tissues and organs.

Species diversity recorded on the Venetia diamond mine and in the surrounding natural control sites has provided a list of potentially 25 bat species based predominantly on echolocation calls and validation of some species through active trapping. Table A6 lists all the species (validated and expected) with accompanying profiles and habitat type in which they were recorded. The Venetia diamond mine is in a unique situation as it completely surrounded by minimally altered natural landscapes which is a highly probable reason why it supported such a diverse bat community.

Habitat degradation, anthropogenic effects (artificial light and noise) and elemental concentrations in bat fur and blood

Three species/species groups emerged as potential environmental and ecological indicators for the Venetia diamond mine namely: *L. capensis/P. (Vansonia) rueppellii, A. nana* and *P.rusticus/N. anchietae.* The clutter forager, *R. simulator* was identified as an adapter as it made use of the wastewater dam during winter which is in contradiction to its general acceptance as an avoider (Schoeman 2015). The activity of *R. simulator and R. smithersi* is perplexing as they were recorded in higher numbers during winter than the summer period. Overall, all bat activity indices were significantly influenced by season (summer) and habitat type with the Venetia diamond mine waste water (tailings dam) supporting the highest total and foraging activity. The Venetia diamond mine appeared to provide an important resource for bats in the semi-arid landscape of northern Limpopo.

The majority of the species recorded, particularly the molossid bats, exploit the resources provided by the mine regardless of the quality.

Especially in South Africa, the response of bats to water quality needs to be further explored over a much wider range of species than what has currently been represented in the literature. Bat species that respond to water quality changes and those with more specialised diets and specific habitat requirements could be more valuable as bioindicators as they could reflect the impact of environmental quality on their specific prey preferences and associated vegetation and or water quality. Salvarina (2016) showed that not only are aquatic resources important for foraging and drinking, but bat's responses to aquatic resources and quality are diverse and vary from region to region. The activity of *Lasiurus cinereus* and *E. fuscus* have been found to negatively correlate with poor water quality, conversely other species such as *L. borealis* and *Perimyotis subflavus* favour poor water quality, while water quality appears to be of no consequence for *Lasionycteris noctivagans* (Li and Kalcounis-Rueppell 2017). Available surface water in semi-arid and arid regions is a resource that bats readily make use of with increased activity around these "scarce" resources (Taylor et al. 2020, Korine et al. 2015). It appears that few bat species are associated with water quality, with some species showing a preference for better quality water bodies such as *N. zuluensis, N. thebaica, R. hipposideros and R. clivosus* (Korine et al 2015, Laverty and Berger 2020). The surface area of a given water body is also an important factor influencing bat activity in arid environments (Razgour et al. 2010, Laverty and Berger 2020). In the hot dry arid northern Limpopo, the waste water dams on Venetia diamond mine are potentially an important resource for foraging and drinking bats and could be a source of exposure of bats to heavy metals and trace elements. As they currently monitor water quality of all the waste water dams, priority should be given to heavy metal and trace element concentrations, even if the waste water is not discharged into the environment.

Even in the presence of artificial light and anthropogenic noise, natural vegetation cover was revealed to be the most important factor influencing all bat behaviour (including activity of foraging guilds, foraging and searching/commuting behaviour) and species richness which is supported by numerous studies (Adams et al. 2009, Jung et al. 2012, Rocha et al. 2016). Anthropogenic noise (dBA) from the mining activities had more of an impact on overall bat activity (excluding CEF actvitiy) that artificial light. Light intensity was only significant concerning foraging activity. There was no evidence of bats using the artificially lit areas for foraging as reported in other studies (Blake et al. 1994, Minnaar et al. 2014). However this may have been an artefact of the early spring and late summer sampling periods. In this regard, T_{min} was significant for all activity except the activity of clutter-edge foragers which is well supported by literature as cool temperatures are known to be thermo-challenging to bats and reduce insect prey activity, thus bats incur energetic costs associated with maintaining high body temperatures and flight (Erickson and West 2002, Bender and Hartman 2015). There are studies focusing on the impacts of artificial light and anthropogenic noise on bats but are often tested separately and under laboratory conditions (Schaub et al. 2008, Hage and Metzner 2013, Gomes et al. 2016). A few studies have been conducted in the field under experimentally lit and noisy conditions (Stone et al. 2009, Minnaar et al. 2014, Schoeman 2015, Bailey et al. 2019, Geipel et al. 2019). To my knowledge, this is the first study incorporating the impacts of natural vegetation cover, artificial light and anthropogenic noise on an opencast diamond mine (or any situation) that bats have been exposed to over a long period of time as the mine has been in operation for more than 20 years.

The ease of fur collection and lower impact on an individual bat makes it an attractive alternative biomarker to invasive, lethal and non-lethal methodologies that have been the main focus of determining ecotoxicological responses of animals to heavy metal and trace element accumulation (Hernout et al. 2016). We present the first concentrations of 23 elements in *T. aegyptiaca* and *M. condylurus* fur and blood from an opencast diamond mine and control site using inductively coupled plasma mass spectrometry (ICP-MS). In general the concentrations of elements in the bat fur was higher than concentrations reported in the blood. This was expected as elements are incorporated over time into the fur as it grows, whereas blood reveals short-term exposure to elements (Hernout et al. 2016). Generally, the elemental concentrations particularly in the fur samples were comparable with other international studies reporting concentrations in fur from anthropogenically impacted and natural areas (see Table A7 for comparative concentrations of elements in fur of global bat species). Pending further research on toxic thresholds and physiological and ecological unknowns around element concentrations in bat tissues and organs, fur has the potential to be a viable indicator of toxicity. High concentrations of elements in fur can indicate potential health risks of elevated elements within the bats that could lead to a variety of health issues, from central nervous system issues to DNA damage and detoxification organ damage (Zocche et al. 2010, Karouna-Renier et al. 2014, Hill et al. 2017, Mina et al. 2019). These levels could indicate contamination of water bodies and or prey items that develop/feed in contaminated water bodies or soils (Zocche et al. 2010, Becker et al. 2018)Even with the physiological and ecological implication unknowns of element concentrations in bat organs and tissues, bat fur has the potential to be a viable indicator of toxicity in relation to opencast diamond mining in Northern Limpopo.

Bats as bioindicators in Limpopo

There is a need to investigate how bats respond to these comparatively small areas of development that appear to be crucial for certain bat species living in arid environments in South Africa. Passive acoustic sampling provides opportunities to monitor and evaluate the consequences of land use decisions particularly on mines as access and monitor using conventional methods is often difficult (Duarte et al. 2015). The development of large-scale mining operations are being encouraged in Africa, threatening important bat habitat (Edwards et al. 2014, Dauda 2020). The impact of mining operations on the environment and bat conservation in sub-Saharan Africa could be devastating if environmental protection policies (including offset areas and protection of natural habitats) are not put in place and adhered to. Conservation efforts are dependent on bat taxonomy and ecology but importantly, how they respond to anthropogenic disturbances (Armstrong 2010). Studies concerning bat responses to anthropogenic developments are imperative to inform decisions for environmental mitigation strategies, management and remediation (Armstrong 2010, Duarte et al. 2015, Stone et al. 2015). With the looming risk of industrial development in the fairly pristine sub-tropical northern Limpopo Province (future mega coal mining projects and associated processing plants and power stations, Digby and Wells Environmental 2019, Munnik 2020), understanding the impacts of habitat degradation, artificial light intensity and anthropogenic noise on the bat communities in northern Limpopo Province is imperative and this study has provided the first glimpse into these impacts.

The long-term vision is that the monitoring of bats on Venetia diamond mine has great potential to 1) provide valuable insight into trends in the bat population (including species richness) that correlate with environmental factors and impacts of opencast mining operations, 2) collect valuable data that can be used to monitor the current environmental impacts of the mine and, 3) monitor and assist rehabilitation efforts to be undertaken. A long-term bat monitoring program will provide much needed data to strengthen the use of identified bats species as bioindicators, and may reveal more species that may qualify. It is suggested that in addition to the current mine water analysis element list, arsenic, mercury, tin, antimony and selenium should also be tested as these elements, particularly mercury and arsenic, are of great concern when liberated into the environment from their natural sources (e.g. rock and soils). The same set of elements should be tested for in the undisturbed soils, fine particulate waste material (tailings) present in the waste water bodies. The elemental list would then be as follows: magnesium (Mg), sodium (Na), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), molybdenum (Mo), cadmium (Cd), tin (Sn), mercury (Hg) and lead (Pb), and trace elements boron (B), aluminum (Al), potassium (K), calcium (Ca), selenium (Se), rubidium (Rb), strontium (Sr), silver (Ag), Lithium (Li), Barium (Ba), silicon (Si) and antimony (Sb).

Potential mitigation and remediation of mining activities

At this point, one should rather be cautious and implement strategies that could benefit the greater bat community based on available research (e.g. bat responses to light, noise, blasting, habitat alterations) (Torres-Flores and Santos-Moreno 2017). The creation of bat habitats in areas disturbed by mining (during and post mining operations), the development of corridors connecting habitat patches for bat (and other fauna) movement, the installation of artificial roosts for displaced bats (particularly species of conservation concern) and bat tolerable lighting regimes will all assist in conserving and promoting bat communities in mining areas (Mering and Chambers 2014, Theobald et al. 2020).

Bats are known to occupy abandoned mines, particularly mines that have microclimates suited to bats that may provide alternative roosting sites for species in areas where natural vegetation has been heavily altered (López-González and Torres-Morales 2004, Derusseau and Huntley 2012). In the case of open cast mining, there are few to no abandoned tunnels for bat occupy. Opencast mining (including quarries such as gravel-sand pits) profoundly alter landscapes and often cause irreversible damage that is sometimes impossible to remediate (Kerbiriou and Parisot-Laprun 2020). If inactive mines are left to naturally rehabilitate, the resulting biodiversity will be significantly different due to the physical and chemical changes of the landscape (Kerbiriou et al. 2018). The rehabilitation of gravel-sand pits in France demonstrated that the length of time (> 10 years) and the surrounding habitat played significant roles in rehabilitating pits to be “attractive” to bats (Kerbiriou et al. 2018, Kerbiriou and Parisot-Laprun 2020). Similarly, in a review on the rehabilitation of mine in Australia, Cristescu et al. (2012) showed that the rehabilitation method and the length of time an area had been rehabilitated impacted the diversity of fauna that recolonized the area.

A significant benefit to the Venetia diamond mine during its operational phase and for the future rehabilitation phase is the surrounding natural landscape that rehabilitation efforts could be modelled on. The natural vegetation and landscape could provide a sound preparatory point for rehabilitation although there is a significant amount of stockpiled waste rock that would have to be rehabilitated (vegetated) and the hydrology on the footprint has most probably been disrupted. Using bats to monitor the change in species richness and abundance over time could provide support for the rehabilitation progress or even guide any changes that may need to be made. For example, as vegetation complexity increases, one would anticipate an increase in arthropod diversity that could possibly be reflected in the change in bat community with an increase in clutter-edge and clutter foragers.

Monitoring of these mitigation and remediation strategies will provide valuable data to enhance these strategies. Most importantly, a proactive approach to the conservation of African bats is essential where stakeholders (including Governments) understand and support the importance of bats for ecosystem functionality and the benefits they provide to humans.

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Appendices

Table A1. Echolocation call parameters of recorded bat calls and the associated species identification. The frequency characteristic of the call (Fc), duration (Dc), maximum frequency (F_{max}), minimum frequency (F_{min}), mean frequency (F_{mean}), frequency at the point at which the call slope sharply changes: “knee” (Fk) and the bandwidth are presented for 23 species/groups. Where two species could not be reliably distinguished, they were placed into a species group. *N* = number of calls.

Species	**Fc (kHz)**	**Dc (ms)**	**F_{max} (kHz)**	**F_{min} (kHz)**	**F_{mean} (kHz)**	**Fk (kHz)**	**Bandwidth (kHz)**	***N***
Neoromicia zuluensis	47.31±0.66	3.87±0.79	52.77±2.90	44.34±3.87	47.26±1.80	48.53±0.84	8.43±4.23	15
Afronycteris nana	68.20±1.86	2.83±0.28	77.73±5.25	66.95±2.48	69.70±1.92	69.55±1.86	10.77±5.70	15
Laephotis capensis/Vansonia rueppellii	37.64±1.67	3.35±0.51	43.92±5.06	36.70±2.21	38.62±1.77	39.41±1.69	7.22±5.33	30
Pipistrellus rusticus/Neoromicia anchietae	54.93±0.53	3.34±0.58	62.46±3.90	52.09±3.01	55.48±1.88	57.91±0.47	10.38±5.16	15
Pipistrellus hesperidus	50.28±1.10	3.35±0.51	62.43±6.49	49.13±2.15	52.50±1.80	52.53±1.32	13.30±6.87	15
Nycticeinops schlieffeni	42.45±0.43	3.62±1.17	49.89±3.83	38.49±4.40	42.20±2.58	44.62±0.69	11.40±5.72	15
Scotophilus dinganii	33.30±0.82	4.241.06	38.28±3.11	32.23±1.78	34.19±1.13	35.03±0.80	6.05±3.30	11
Eptesicus hottentotus	31.23±0.65	4.01±1.01	40.77±4.95	30.84±0.70	33.57±1.31	33.87±0.86	9.93±4.98	15
Miniopterus fraterculus	63.43±1.90	3.14±0.73	70.57±3.36	61.22±2.88	64.23±1.52	65.56±1.07	9.35±6.01	5
Miniopterus natalensis	48.95±0.24	3.91±0.66	65.05±11.73	48.76±0.31	51.93±1.93	50.90±0.74	16.29±12.02	3
Myotis bocagii	44.53	2.46	62.88	44.35	48.61	47.95	18.53	1
Rhinolophus simulator	79.31±0.48	11.83±5.77	79.93±0.44	77.86±2.07	79.33±0.40	79.26±0.45	2.07±2.07	15
Rhinolophus smithersi	46.29±0.78	18.74±10.58	46.69±0.74	43.74±3.77	46.01±1.29	46.33±0.78	2.95±3.70	15
Rhinolophus cf. blasii	84.24±0.67	23.48±3.20	84.79±0.61	83.19±1.08	84.17±0.63	84.08±0.59	1.60±0.54	4
Rhinolophid 50kHz	48.89±0.33	23.48±3.20	49.17±0.35	48.08±0.53	48.86±0.32	48.93±0.26	1.09±0.64	3
Hipposideros cafer	141.76±0.93	4.93±0.38	143.50±0.39	101.99±12.46	142.19±0.35	142.24±1.34	41.51±12.78	4
Chaerephon cf. ansorgei/Molossid19kHz	18.50±0.84	6.88±1.66	20.09±1.93	17.74±1.19	18.76±1.02	19.42±0.95	2.35±1.63	30
Tadarida aegyptiaca	21.34±0.77	6.70±1.94	23.88±1.81	21.28±0.77	22.11±0.96	22.84±1.19	2.61±1.49	15
Otomops mart	11.63±0.62	7.57±2.16	12.03±0.80	11.54±0.65	11.74±0.69	11.92±0.82	0.49±0.22	17
Taphozous mauritianus	25.19±0.71	6.13±1.57	25.92±0.93	25.07±0.68	25.43±0.75	25.76±0.89	0.85±0.51	15
Sauromys petrophilus	28.85±0.44	7.06±1.11	29.37±0.52	28.70±0.44	29.68±2.72	29.19±0.47	0.67±0.28	15
Mops midas	15.03±0.62	7.43±1.98	16.52±0.80	14.67±1.19	15.34±0.70	16.06±0.64	1.85±1.58	15
Chaerephon pumilus/Mops condylurus	26.14±0.77	5.79±1.10	30.31±2.64	25.99±0.80	27.32±1.07	28.04±1.15	4.31±2.49	15

Table A2. Resulting GVIF and GVIF$^{1/2*df}$ values of the variation of inflation analysis and analysis of variance (Type II Wald Chi-square test) of the global mixed-effects regression models for total activity (AI: lmer), species diversity (glmer) and foraging activity (glmer) indicating weak associations between factors where GVIF$^{1/2*df}$ < 5. Factors not included in a specific global model are indicated with a "/". Significant codes are: $0 < Pr \leq 0.001$ = ***, $0.001 < Pr \leq 0.01$ = **, $0.01 < Pr \leq 0.05$ = *, NS = not significant.

Factor	AI (Site & Date: random)			Species Diversity (Site & Date: random)			Foraging Activity (AI) (Site & Date: random)		
	GVIF	**GVIF$^{1/2*df}$**	***P* - value**	**GVIF**	**GVIF$^{1/2*df}$**	***P* - value**	**GVIF**	**GVIF$^{1/2*df}$**	***P* - value**
Habitat Type	1.30	1.20	***	1.05	1.00	***	1.02	1.00	**
Season	2.91	2.04	***	3.71	1.93	***	3.70	1.92	***
Moon Phase	1.05	1.00	NS	1.05	1.00	NS	3.04	1.74	NS
T_{min} (°C)	3.18	2.07	NS	3.78	1.94	*	1.43	1.19	***
Foraging Guild	/	/	/	/	/	/	1.01	1.00	**

Table A3. PERMANOVA results and total AI (n) of all species and foraging guilds in order of abundance recorded during the study. Significant codes are as follows: $0 < Pr \leq 0.001$ = ***, $0.001 < Pr \leq 0.01$ = **, $0.01 < Pr \leq 0.05$ = *. "N/A" indicates sample too small to run meaningful PERMANOVA.

Foraging Group/Species	**Total AI**	**Habitat Type *P* - value**	**Season *P* - value**	**Habitat Type:Season *P* - value**
Open-air Foragers	74041	***	***	*
Clutter-edge Foragers	19053	*	NS	NS
Clutter Foragers	183	NS	NS	NS
Chaerephon cf. ansorgei/Mol 19kHz	37322	NS	*	NS
Tadarida aegyptiaca	24895	NS	*	NS
Laephotis capensis/Vansonia rueppellii	8477	NS	NS	NS
Sauromys petrophilus	6461	***	***	***
Pipistrellus rusticus/Neoromicia anchietae	4555	***	***	**
Afronycteris nana	4464	***	***	***
Chaerephon pumilus/Mops condylurus	2747	NS	**	NS
Taphozous mauritianus	2096	NS	**	NS
Neoromicia zuluensis	583	NS	NS	NS
Nycticeinops schlieffeni	424	NS	NS	NS
Eptesicus hottentotus	421	NS	*	NS
Mops midas	330	***	***	**
Otomops martiensseni	190	**	***	**
Rhinolophus smithersi	102	NS	NS	NS
Rhinolophus simulator	67	NS	*	NS
Pipistrellus hesperidus	60	NS	NS	NS
Scotophilus dinganii	58	NS	NS	NS
Miniopterus fraterculus	8	N/A	N/A	N/A
Rhinolophus cf. blasii	6	N/A	N/A	N/A
Hipposideros caffer	4	N/A	N/A	N/A
Miniopterus natalensis	3	N/A	N/A	N/A
Rhinolophid 50kHz	3	N/A	N/A	N/A
Rhinolophid 70kHz	1	N/A	N/A	N/A

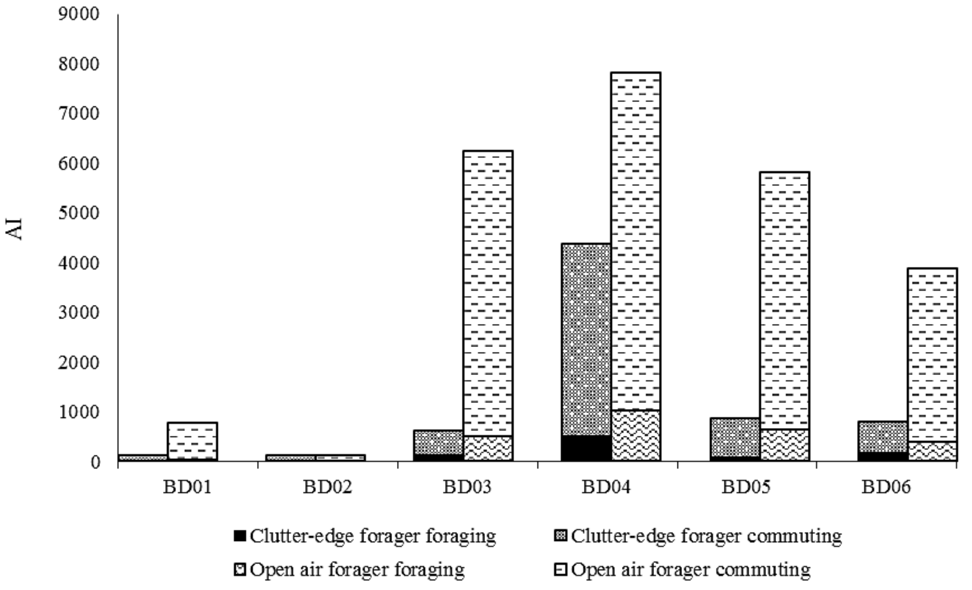

Figure A1. AI across the transect for the two main foraging guilds, open-air foragers (right bar) and clutter-edge foragers (left bar), and for two different behaviours, foraging (lower bars) and commuting (upper bars). The activity index of the clutter foragers and social activity were excluded from the current plot as clutter forager activity social calls were minute in comparison to the other two foraging guilds and activity types (see Table 8 for detail).

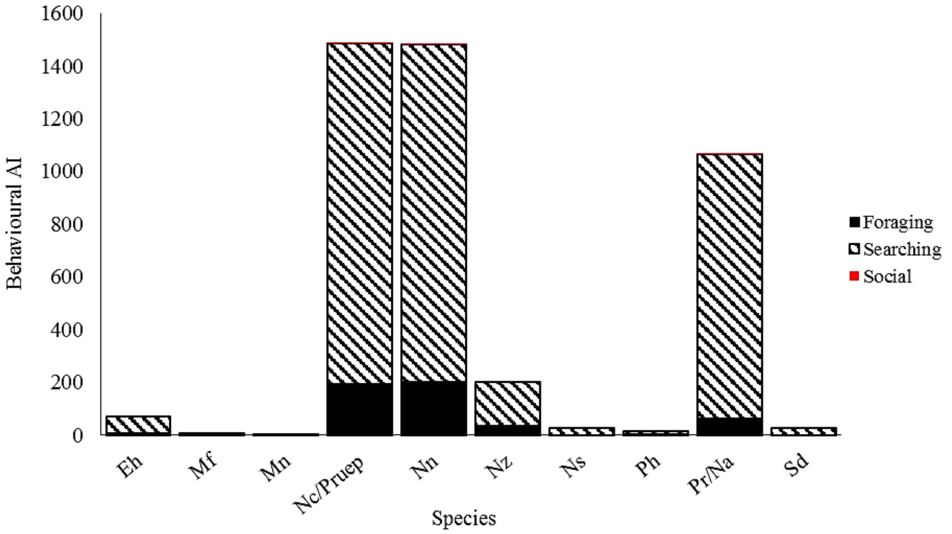

Figure A2. Number of passes in relation to activity of clutter-edge forager species in the semi-transformed natural habitat where percentage natural vegetation cover was 50%. Species present were *Eptesicus hottentotus* (*Eh*), *Miniopterus fraterculus* (*Mf*), *M. natalensis* (*Mn*), *Laephotis capensis/Pipistrellus rueppellii* (*Lc/Pruep*), *A. nana* (*An*), *N. zuluensis* (*Nz*), *Nycticeinops schlieffeni* (*Ns*), *P. hesperidus* (Ph), *P. rusticus/Neoromicia anchietae* (*Pr/Na*), *Rhinolophus simulator* (*Rs*) and *Scotophilus dinganii* (*Sd*).

Table A4. Analysis of variance (Type II Wald Chi-square test) and associated GVIF and GVIF$^{1/2*df}$ values of the five global linear mixed-effects regression models and one generalised mixed-effects model (*) indicating weak associations between factors/regressors since all GVIF < 10 and all GVIF$^{1/2*df}$ < 5. Site and date are random factors. Significance codes are as follows: $0 < Pr \leq 0.001$ = ***, $0.001 < Pr \leq 0.01$ = **, $0.01 < Pr \leq 0.05$ = *, NS = not significant.

Factor	**Log (AI+1)**			**Species**			**Log (OAF+1)**			**Log (CEF+1)**			***Foraging (glmer)**			**Log (searching/commuting+1)**		
	***Pr* value**	**GVIF**	**GVIF$^{1/2*df}$**	***Pr* value**	**GVIF**	**GVIF$^{1/2*df}$**	***Pr* value**	**GVIF**	**GVIF$^{1/2*df}$**	***Pr* value**	**GVIF**	**GVIF$^{1/2*df}$**	***Pr* value**	**GVIF**	**GVIF$^{1/2*df}$**	***Pr* value**	**GVIF**	**GVIF$^{1/2*df}$**
Light Intensity (Lux$_{max}$)	NS	2.89	1.70	NS	2.06	1.44	NS	2.53	1.59	NS	5.14	2.27	***	6.49	2.55	NS	2.84	1.69
Noise (dB)	NS	1.57	1.25	NS	1.49	1.22	NS	1.50	1.22	NS	4.63	2.15	NS	2.24	1.50	NS	1.57	1.25
T$_{min}$	***	1.98	1.41	***	2.16	1.47	***	2.01	1.42	NS	3.26	1.81	NS	2.69	1.64	***	1.99	1.41
% Nat. veg	**	2.65	1.18	*	1.69	1.09	*	2.21	1.14	***	7.68	1.40	***	6.71	1.37	**	2.59	1.17
Moon phase	NS	2.00	1.06	*	2.26	1.07	*	2.10	1.06	NS	1.92	1.06	***	3.59	1.11	NS	2.02	1.06
Season	NS	2.26	1.50	NS	2.48	1.57	NS	2.32	1.52	NS	2.58	1.61	***	2.20	1.48	NS	2.28	1.51

Table A5. Model selection table from the linear mixed effects regression models and a generalized mixed-effects model ($^{\lozenge}$glmer) to explain which factors had an effect on activity index (AI), species richness per bat detector, open-air foragers (OAF), clutter-edge foragers (CEF) foraging activity and searching/commuting behaviour. Bat detector and date were entered as the random variables with maximum luminosity (lux), noise (dB), minimum temperature (T_{min}), percentage vegetation cover, moon phase and season as fixed variables. Asterisks indicate significant *Pr* values according to the cftest: $0 < Pr \leq 0.001$ = ***, $0.001 < Pr \leq 0.01$ = **, $0.01 < Pr \leq 0.05$ = *, NS = not significant. The values associated with each model are the estimates.

Model	**Lum.$_{max}$ (lux)**	**Noise (dBA)**	**T_{min} (°C)**	**% Veg. cover**	**Moon phase**	**Season**	**AICc**
AI #1	-	-	0.16 ***	2.83 (25%) ** 3.79 (50%) *** 2.54 (100%) **	NS	-	291.1
AI #2	-	-	0.13 ***	2.77 (25%) ** 3.67 (50%) *** 2.42 (100%) **	NS	NS	291.3
AI #3	-0.17***	-	0.14***	1.50 (25%) ** 2.42 (50%) *** 1.03 (100%) *	-	-	292.6
OAF #1	-	-	0.18 ***	3.25 (25%) * 3.82 (50%) ** 2.81 (100%) **	NS	-	314.6
OAF #2	-	NS	0.16 ***	3.16 (25%) * 3.69 (50%) ** 2.63 (100%) *	NS	NS	314.8
CEF #1	-	-	-	1.41 (25%) *** 3.43 (50%) *** 1.76 (100%) ***	-	NS	280.1
CEF #2	-	-	-	1.41 (25%) *** 3.43 (50%) *** 1.76 (100%) ***	-	-	280.5
Species #1	-	-	1.48 ***	3.58 (25%) * 6.84 (50%) *** 5.52 (100%) ***	NS	NS	480.3
Species #2	-	-	1.81 ***	3.72 (25%) ** 7.11 (50%) *** 5.80 (100%) ***	NS	-	481.3
$^{\lozenge}$Feeding #1 (glmer)	-0.17 ***	-	-	2.02 (25%) *** 3.18 (50%) *** 1.99 (100%) ***	-1.04 (FQ) *** -1.25 (WanGib) *** -0.44 (WaxGib) ***	-1.79 (early spring) ***	958.6
Searching/ commuting #1	-	-	0.15 ***	2.81 (25%) ** 3.72 (50%) *** 2.48 (100%) **	NS	-	958.6
Searching/ commuting #2	-	-	0.13 ***	2.75 (25%) ** 3.61 (50%) *** 2.36 (100%) **	NS	NS	293.3
Searching/ commuting #3	-0.17 ***	-	0.14 ***	1.42 (25%) ** 2.30 (50%) ***	-	-	295.3

Table A6. Bat diversity of the Venetia diamond mine and surrounding habitat types: Limpopo Ridge Bushveld (LRB) and Musina Mopane Bushveld (MMB). "X" indicates the habitat types that the bats were recorded in. Bat species with no asterisks behind the name were captured during the active trapping sessions. "*" indicates bat species that are expected to occur in the area based on their distinct echolocation calls recorded during the study and have been caught in northern Limpopo by D. Cory Toussaint and others outside of the current study. Bat species that could occur in the area based on their echolocation calls are indicated by "**". Unknown bats have not been included in the table below such as *Rhinolophid_50kHz.*

Family and Species	Profile	VDM	LRB	MMB
Molossidae				
Chaerephon pumilus - Little free-tail bat		X	X	X
*Chaerephon ansorgei** - Ansorge's free-tail bat		X	X	X
Mops condylurus - Angolan free-tail bat		X	X	X
*Mops midas** - Midas free-tail bat		X	X	X
*Otomops martiensseni*** - Large-eared giant mastiff bat		X	X	X
*Sauromys petrophilus** - Roberts' free-tail bat		X	X	X

Tadarida aegyptiaca - Egyptian free-tail bat		X	X	X
*Tadarida ventralis*** (Molossid 19kHz) © P. J. Taylor - Transvaal free-tail bat		X	X	X
Vespertilionidae				
*Eptesicus hottentotus** - Long-tailed serotine		X	X	X
Laephotis capensis - Cape serotine		X	X	X
*Afronycteris nana** - Banana bat (Image taken in Kwa-zulu Natal)		X	X	X
Neoromicia zuluensis - Zulu serotine		X	X	X

Nycticeinops schlieffeni - Schlieffen's twilight bat		X	X	X
Vansonia rueppellii - Rüppell's bat		X	X	X
*Pipistrellus hesperidus** - Dusky pipistrelle		X	X	X
Pipistrellus rusticus/Neoromicia anchietae*** - Rusty bat*/Anchieta's pipistrelle**		X	X	X
Scotophilus dinganii - Yellow-bellied house bat		X	X	X
*Myotis tricolor** Temminck's myotis		X		
Miniopteridae				
*Miniopterus fraterculus** - Lesser long-fingered bat		X		X

*Miniopterus natalensis** - Natal long-fingered bat				X
Emballonuridae				
*Taphozous mauritianus** - Mauritian tomb bat		X	X	X
Hipposideridae				
*Hipposideros caffer** - Sundeval's leaf-nose bat				X
Rhinolophidae				
Rhinolophus cf. blasii - Blasius's horseshoe bat	No image available.	X		
Rhinolophus simulator - Bushveld horseshoe bat		X	X	X
Rhinolophus smithersi - Smithers' horseshoe bat		X	X	

Table A7. Literature summary of heavy metal and trace element concentrations (ppm, W.W) in bat fur from various bat species in relation to diet, foraging guild, region, land use and biological material. The values have been grouped according to element followed by biological material. The values reported in the current study are highlighted in green. Foraging guilds are represented as follows: CEF = clutter-edge forager, CF = clutter forager, OAF = open-air forager. Diets are represented as: I = insectivore, C = carnivore, F = frugivore, N = nectarivore, S = sanguivore, O = omnivore and U = unknown. The references are included in the reference list of Chapter 4.

Species	Foraging Guild	Diet	Region	Sector/Feature	Biological Material	Element	Mean W.W	Median W.W	Geometric Mean	Min	Max	Reference
Mops condylurus	OAF	I	Northern Limpopo, SA	Diamond Mining	Blood	Al		0.001		0.11	1.38	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, SA	Game Farm	Blood	Al		0.11		0.11	2.00	Current study
Mops condylurus	OAF	I	Northern Limpopo, SA	Diamond Mining	Blood	As		0.01		0.003	0.02	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, SA	Game Farm	Blood	As		0.01		0.003	0.01	Current study
Mops condylurus	OAF	I	Northern Limpopo, SA	Diamond Mining	Blood	B		2.16		1.25	3.01	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, SA	Game Farm	Blood	B		1.33		1.25	2.32	Current study
Mops condylurus	OAF	I	Northern Limpopo, SA	Diamond Mining	Blood	Ba		0.0005		0.0005	0.0005	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, SA	Game Farm	Blood	Ba		0.0005		0.0005	0.0005	Current study
Mops condylurus	OAF	I	Northern Limpopo, SA	Diamond Mining	Blood	Ca		0.07		0.05	0.22	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, SA	Game Farm	Blood	Ca		0.06		0.05	0.08	Current study
Mops condylurus	OAF	I	Northern Limpopo, SA	Diamond Mining	Blood	Cd		0.001		0.0001	0.004	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, SA	Game Farm	Blood	Cd		0.0003		0.0001	0.003	Current study
Mops condylurus	OAF	I	Northern Limpopo, SA	Diamond Mining	Blood	Co		0.002		0.0003	0.004	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, SA	Game Farm	Blood	Co		0.001		0.0003	0.004	Current study
Mops condylurus	OAF	I	Northern Limpopo, SA	Diamond Mining	Blood	Cr		0.01		0.01	0.41	Current study

Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, SA	Game Farm	Blood	Cr		0.06		0.01	0.10	Current study
Mops condylurus	OAF	I	Northern Limpopo, SA	Diamond Mining	Blood	Cu		0.73		0.26	0.97	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, SA	Game Farm	Blood	Cu		0.38		0.26	0.66	Current study
Mops condylurus	OAF	I	Northern Limpopo, SA	Diamond Mining	Blood	Fe		690.91		631.09	1026.21	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, SA	Game Farm	Blood	Fe		629.47		484.83	861.64	Current study
Mops condylurus	OAF	I	Northern Limpopo, SA	Diamond Mining	Blood	Hg		0.03		0.01	0.04	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, SA	Game Farm	Blood	Hg		0.01		0.01	0.03	Current study
Myotis lucifugus	CEF	I	Moscow, Russia	Uncontaminated	Blood	Hg	0.03			0.01	0.61	Karouna-Renier et al. 2014
Myotis lucifugus	CEF	I	Northwestern Virginia, USA	Contaminated (Grottoes & Mt. Sydney	Blood	Hg	0.74			0.01	3.80	Karouna-Renier et al. 2014
Myotis lucifugus	CEF	I	Northwestern Virginia, USA	Contaminated (Grottoes)	Blood	Hg	1.39			0.08	3.76	Karouna-Renier et al. 2014
Myotis lucifugus	CEF	I	Northwestern Virginia, USA	Contaminated (Mt. Sydney	Blood	Hg	0.11			0.01	0.92	Karouna-Renier et al. 2014
Eptesicus fuscus	CEF	I	Northeast United States	Point source and non-point source	Blood	Hg	16.64	9.59			200.00	Yates et al. 2014
Lasionycteris noctivagans	CEF	I	Northeast United States	Point source and non-point source	Blood	Hg	7.96	7.89			14.23	Yates et al. 2014
Lasiurus borealis	CEF	I	Northeast United States	Point source and non-point source	Blood	Hg	4.03	2.73			25.54	Yates et al. 2014
Lasiurus cinereus	CEF	I	Northeast United States	Point source and non-point source	Blood	Hg	1.33	1.34			3.61	Yates et al. 2014
Myotis grisescens	CEF	I	Northeast United States	Point source and non-point source	Blood	Hg	18.61	5.37			84.50	Yates et al. 2014
Myotis leibii	CEF	I	Northeast United States	Point source and non-point source	Blood	Hg	12.88	15.70			18.83	Yates et al. 2014
Myotis lucifugus	CEF	I	Northeast United States	Point source and non-point source	Blood	Hg	29.22	5.39			707.64	Yates et al. 2014
Myotis septentionalis	CEF	I	Northeast United States	Point source and non-point source	Blood	Hg	26.89	7.37			480.00	Yates et al. 2014
Myotis sodalis	CEF	I	Northeast United States	Point source and non-point source	Blood	Hg	10.58	10.35			18.30	Yates et al. 2014
Perimyotis subflavus	CEF	I	Northeast United States	Point source and non-point source	Blood	Hg	40.77	15.30			255.00	Yates et al. 2014

Mops condylurus	OAF	I	Northern Limpopo, SA	Diamond Mining	Blood	K		2.45		1.88	3.38	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, SA	Game Farm	Blood	K		2.39		1.88	3.42	Current study
Mops condylurus	OAF	I	Northern Limpopo, SA	Diamond Mining	Blood	Mn		0.05		0.08	0.42	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, SA	Game Farm	Blood	Mn		0.15		0.08	0.35	Current study
Mops condylurus	OAF	I	Northern Limpopo, SA	Diamond Mining	Blood	Mo		0.003		0.00	0.01	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, SA	Game Farm	Blood	Mo		0.003		0.00	0.01	Current study
Mops condylurus	OAF	I	Northern Limpopo, SA	Diamond Mining	Blood	Ni		0.01		0.00	0.10	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, SA	Game Farm	Blood	Ni		0.01		0.00	0.01	Current study
Mops condylurus	OAF	I	Northern Limpopo, SA	Diamond Mining	Blood	Pb		0.03		0.01	0.17	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, SA	Game Farm	Blood	Pb		0.01		0.01	0.04	Current study
Mops condylurus	OAF	I	Northern Limpopo, SA	Diamond Mining	Blood	Rb		0.89		0.98	1.57	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, SA	Game Farm	Blood	Rb		1.02		0.83	2.36	Current study
Mops condylurus	OAF	I	Northern Limpopo, SA	Diamond Mining	Blood	Sb		0.001		0.0003	0.003	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, SA	Game Farm	Blood	Sb		0.0003		0.0003	0.0003	Current study
Mops condylurus	OAF	I	Northern Limpopo, SA	Diamond Mining	Blood	Se		0.82		0.57	1.05	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, SA	Game Farm	Blood	Se		0.60		0.57	0.73	Current study
Mops condylurus	OAF	I	Northern Limpopo, SA	Diamond Mining	Blood	Sn		0.004		0.002	0.72	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, SA	Game Farm	Blood	Sn		0.003		0.002	0.003	Current study
Mops condylurus	OAF	I	Northern Limpopo, SA	Diamond Mining	Blood	Sr		0.08		0.03	0.38	Current study

Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, SA	Game Farm	Blood	Sr		0.06		0.03	0.08	Current study
Mops condylurus	OAF	I	Northern Limpopo, SA	Diamond Mining	Blood	V		0.00		0.001	0.01	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, SA	Game Farm	Blood	V		0.00		0.001	0.004	Current study
Mops condylurus	OAF	I	Northern Limpopo, SA	Diamond Mining	Blood	Zn		4.04		1.38	7.04	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, SA	Game Farm	Blood	Zn		1.90		1.38	3.59	Current study
Miniopterus schreibersii	CEF	I	Italy	African Quarter of Rome	Bone	Al	4.22	2.96				Andreani et al. 2019
Tadarida teniotis	OAF	I	Italy	African Quarter of Rome	Bone	Al	6.34	3.74				Andreani et al. 2019
Tadarida teniotis (lactant)	OAF	I	Italy	African Quarter of Rome	Bone	Al	4.94	4.14				Andreani et al. 2019
Miniopterus schreibersii	CEF	I	Italy	African Quarter of Rome	Bone	As	0.03	0.01				Andreani et al. 2019
Tadarida teniotis	OAF	I	Italy	African Quarter of Rome	Bone	As	0.02	0.01				Andreani et al. 2019
Tadarida teniotis (lactant)	OAF	I	Italy	African Quarter of Rome	Bone	As	0.05	0.04				Andreani et al. 2019
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Bone	As		0.01		0.003	0.07	Mina et al. 2019
Miniopterus schreibersii	CEF	I	Italy	African Quarter of Rome	Bone	Ba	17.20	4.62				Andreani et al. 2019
Tadarida teniotis	OAF	I	Italy	African Quarter of Rome	Bone	Ba	9.01	3.10				Andreani et al. 2019
Lasiurus borealis	CEF	I	Italy	African Quarter of Rome	Bone	Ba	23.40					Wieringa et al. 2020
Miniopterus schreibersii	CEF	I	Italy	African Quarter of Rome	Bone	Cd	0.08	0.10				Andreani et al. 2019
Tadarida teniotis	OAF	I	Italy	African Quarter of Rome	Bone	Cd	<LOQ	<LOQ				Andreani et al. 2019
Tadarida teniotis (lactant)	OAF	I	Italy	African Quarter of Rome	Bone	Cd	<LOQ	<LOQ				Andreani et al. 2019
Pipistrellus sp.	CEF	I	England & Wales	Mixed	Bone	Cd	0.0001 - 6.00	0.06				Hernout et al. 2016a
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Bone	Cd		0.003		0.00	0.02	Mina et al. 2019

Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Bone	Co		0.01		0.003	0.67	Mina et al. 2019
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Bone	Cr		0.14		0.03	1.03	Mina et al. 2019
Pipistrellus sp.	CEF	I	England & Wales	Mixed	Bone	Cu	0.05 - 6.25	0.90				Hernout et al. 2016a
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Bone	Cu		0.31		0.02	1.08	Mina et al. 2019
Miniopterus schreibersii	CEF	I	Italy	African Quarter of Rome	Bone	Hg	0.01	0.01				Andreani et al. 2019
Tadarida teniotis	OAF	I	Italy	African Quarter of Rome	Bone	Hg	<LOQ	<LOQ				Andreani et al. 2019
Tadarida teniotis (lactant)	OAF	I	Italy	African Quarter of Rome	Bone	Hg	0.02	0.01				Andreani et al. 2019
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Bone	Mn		0.4125		0.16	1.45	Mina et al. 2019
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Bone	Ni		0.09		0.01	9.58	Mina et al. 2019
Miniopterus schreibersii	CEF	I	Italy	African Quarter of Rome	Bone	Pb	0.72	0.62				Andreani et al. 2019
Tadarida teniotis	OAF	I	Italy	African Quarter of Rome	Bone	Pb	168.00	52.90				Andreani et al. 2019
Tadarida teniotis (lactant)	OAF	I	Italy	African Quarter of Rome	Bone	Pb	35.50	56.50				Andreani et al. 2019
Pipistrellus sp.	CEF	I	England & Wales	Mixed	Bone	Pb	0.001 - 177.00	13.29				Hernout et al. 2016a
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Bone	Pb		0.12		0.03	3.72	Mina et al. 2019
Miniopterus schreibersii	CEF	I	Italy	African Quarter of Rome	Bone	Sb	<LOQ	<LOQ				Andreani et al. 2019
Tadarida teniotis	OAF	I	Italy	African Quarter of Rome	Bone	Sb	<LOQ	<LOQ				Andreani et al. 2019
Tadarida teniotis (lactant)	OAF	I	Italy	African Quarter of Rome	Bone	Sb	<LOQ	<LOQ				Andreani et al. 2019
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Bone	Se		0.06		0.03	0.11	Mina et al. 2019
Miniopterus schreibersii	CEF	I	Italy	African Quarter of Rome	Bone	Sr	190.00	56.90				Andreani et al. 2019

Tadarida teniotis	OAF	I	Italy	African Quarter of Rome	Bone	Sr	68.30	16.90				Andreani et al. 2019
Tadarida teniotis (lactant)	OAF	I	Italy	African Quarter of Rome	Bone	Sr	99.30	58.10				Andreani et al. 2019
Miniopterus schreibersii	CEF	I	Italy	African Quarter of Rome	Bone	Th	<LOQ	<LOQ				Andreani et al. 2019
Tadarida teniotis	OAF	I	Italy	African Quarter of Rome	Bone	Th	<LOQ	<LOQ				Andreani et al. 2019
Tadarida teniotis (lactant)	OAF	I	Italy	African Quarter of Rome	Bone	Th	<LOQ	<LOQ				Andreani et al. 2019
Miniopterus schreibersii	CEF	I	Italy	African Quarter of Rome	Bone	Tl	<LOQ	<LOQ				Andreani et al. 2019
Tadarida teniotis	OAF	I	Italy	African Quarter of Rome	Bone	Tl	0.11	0.09				Andreani et al. 2019
Tadarida teniotis (lactant)	OAF	I	Italy	African Quarter of Rome	Bone	Tl	0.11	0.15				Andreani et al. 2019
Pipistrellus sp.	CEF	I	England & Wales	Mixed	Bone	Zn	0.18 - 257.50	68.90				Hernout et al. 2016a
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Bone	Zn		16.97		6.97	26.72	Mina et al. 2019
Afronycteris nana	CEF	I	Kwa-Zulu Natal, South Africa	Verlam wastewater treatment works	Brain	As	0.10			0.02	0.44	Hill et al. 2017
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Reference Sites	Brain	As	0.03			0.01	0.06	Hill et al. 2017
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Brain	As		0.05		0.01	0.40	Mina et al. 2019
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Verlam wastewater treatment works	Brain	Cd	0.01			0.0003	0.03	Hill et al. 2017
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Reference Sites	Brain	Cd	0.003			0.0005	0.01	Hill et al. 2017
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Brain	Cd		0.01		0.003	0.03	Mina et al. 2019
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Brain	Co		0.02		0.01	2.16	Mina et al. 2019
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Verlam wastewater treatment works	Brain	Cr	0.02			0.01	0.07	Hill et al. 2017
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Reference Sites	Brain	Cr	0.01			0.01	0.01	Hill et al. 2017
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Brain	Cr		0.26		0.06	1.01	Mina et al. 2019

Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Verlam wastewater treatment works	Brain	Cu	0.34			0.01	2.64	Hill et al. 2017
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Reference Sites	Brain	Cu	1.00			0.01	5.94	Hill et al. 2017
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Brain	Cu		3.36		1.96	7.19	Mina et al. 2019
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Verlam wastewater treatment works	Brain	Fe	29.93			19.63	45.59	Hill et al. 2017
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Reference Sites	Brain	Fe	26.33			23.43	28.94	Hill et al. 2017
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Verlam wastewater treatment works	Brain	Mn	0.52			0.31	0.79	Hill et al. 2017
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Reference Sites	Brain	Mn	0.59			0.37	1.13	Hill et al. 2017
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Brain	Mn		0.54		0.25	1.31	Mina et al. 2019
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Brain	Ni		0.19		0.03	2.17	Mina et al. 2019
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Verlam wastewater treatment works	Brain	Pb	0.29			0.01	0.20	Hill et al. 2017
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Reference Sites	Brain	Pb	0.18			0.01	1.02	Hill et al. 2017
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Brain	Pb		0.16		0.04	0.55	Mina et al. 2019
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Brain	Se		0.31		0.19	0.51	Mina et al. 2019
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Verlam wastewater treatment works	Brain	Zn	25.38			14.30	48.46	Hill et al. 2017
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Reference Sites	Brain	Zn	24.17			17.80	39.55	Hill et al. 2017
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Brain	Zn		14.13		8.42	32.79	Mina et al. 2019
Eptesicus fuscus	CEF	I	Cornwall, Ontario, Canada	Mixed Urban	Fur	Al	4.80					Hickey et al. 2001
Myotis lucifugus	CEF	I	Fly Creek, Ontario, Canada	Mixed Urban	Fur	Al	27.00					Hickey et al. 2001
Myotis lucifugus	CEF	I	Lafleche 2 Cave, Quebec, Canada	Cave	Fur	Al	70.00					Hickey et al. 2001
Myotis lucifugus	CEF	I	Lafleche-1 Cave, Quebec, Canada	Cave	Fur	Al	67.00					Hickey et al. 2001

Myotis lucifugus	CEF	I	Tyendinaga, Ontario, Canada	Mixed Urban	Fur	Al	41.00					Hickey et al. 2001
Lasiurus borealis	CEF	I	North America	Across range	Fur	Al	0.02					Wieringa et al. 2020
Mops condylurus	OAF	I	Northern Limpopo, South Africa	Diamond Mining	Fur	Al		109.38		43.97	185.39	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, South Africa	Game Farm	Fur	Al		102.62		51.44	558.06	Current study
Mops condylurus	OAF	I	Northern Limpopo, South Africa	Diamond Mining	Fur	As		0.72		0.37	1.20	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, South Africa	Game Farm	Fur	As		0.26		0.18	0.32	Current study
Myotis myotis	CEF	I	Sicily	Petrochemical Plant	Fur	As	0.64	0.11		<0,01	1.02	Ferrante et al. 2018
Myotis myotis	CEF	I	Sicily	Pipistrelli Cave	Fur	As	0.68	0.14		<0,01	0.55	Ferrante et al. 2018
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Verlam wastewater treatment works	Fur	As	0.38			0.15	0.64	Hill et al. 2017
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Umbilo wastewater treament works	Fur	As	0.20			0.10	0.49	Hill et al. 2017
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Reference Sites	Fur	As	0.10			0.01	0.29	Hill et al. 2017
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Fur	As		0.22		0.05	1.91	Mina et al. 2019
Mops condylurus	OAF	I	Northern Limpopo, South Africa	Diamond Mining	Fur	B		7.44		6.79	18.90	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, South Africa	Game Farm	Fur	B		3.38		1.64	3.91	Current study
Tadarida teniotis (lactant)	OAF	I	North America	Across range	Fur	Ba	0.01	0.003				Andreani et al. 2019
Mops condylurus	OAF	I	Northern Limpopo, South Africa	Diamond Mining	Fur	Ba		0.0005		0.0005	1.51	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, South Africa	Game Farm	Fur	Ba		0.0005		0.0005	2.26	Current study
Mops condylurus	OAF	I	Northern Limpopo, South Africa	Diamond Mining	Fur	Ca		0.42		0.32	0.83	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, South Africa	Game Farm	Fur	Ca		0.38		0.27	0.82	Current study
Mops condylurus	OAF	I	Northern Limpopo, South Africa	Diamond Mining	Fur	Cd		0.09		0.03	0.17	Current study

Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, South Africa	Game Farm	Fur	Cd		0.02		0.02	0.10	Current study
Myotis myotis	CEF	I	Sicily	Petrochemical Plant	Fur	Cd	0.02	0.004		<0,01	0.02	Ferrante et al. 2018
Myotis myotis	CEF	I	Sicily	Pipistrelli Cave	Fur	Cd	0.02	0.004		<0,01	0.01	Ferrante et al. 2018
Myotis bechsteinii	CEF	I	Middle Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Cd		0.05		0.0001	0.15	Flache et al. 2015
Myotis daubentonii	CEF	I	Middle Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Cd		0.01		0.0001	0.09	Flache et al. 2015
Myotis myotis	CEF	I	Middle Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Cd		0.07		0.02	0.36	Flache et al. 2015
Pipistrellus pipistrellus	CEF	I	Middle Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Cd		0.20		0.0001	61.25	Flache et al. 2015
Myotis bechsteinii	CEF	I	Central Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Cd		0.05		0.00	0.15	Flache et al. 2018
Myotis nattereri	CEF	I	Central Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Cd		0.05		0.00	0.10	Flache et al. 2018
Plecotus auritus	Clutter	I	Central Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Cd		0.05		0.00	0.13	Flache et al. 2018
Pipistrellus sp.	CEF	I	England & Wales	Mixed	Fur	Cd	0.001 - 53.00	0.03				Hernout et al. 2016a
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Verlam wastewater treatment works	Fur	Cd	0.02			0.004	0.07	Hill et al. 2017
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Umbilo wastewater treament works	Fur	Cd	0.03			0.004	0.20	Hill et al. 2017
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Reference Sites	Fur	Cd	0.01			0.00	0.04	Hill et al. 2017
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Fur	Cd		0.02		0.01	0.14	Mina et al. 2019
Lasiurus borealis	CEF	I	North America	Across range	Fur	Cd	0.0003					Wieringa et al. 2020
Mops condylurus	OAF	I	Northern Limpopo, South Africa	Diamond Mining	Fur	Co		0.13		0.10	0.20	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, South Africa	Game Farm	Fur	Co		0.24		0.17	0.67	Current study
Myotis myotis	CEF	I	Sicily	Petrochemical Plant	Fur	Co	0.03	0.01		0.003	0.02	Ferrante et al. 2018
Myotis myotis	CEF	I	Sicily	Pipistrelli Cave	Fur	Co	0.09	0.02		0.003	0.22	Ferrante et al. 2018
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Fur	Co		0.09		0.02	1.96	Mina et al. 2019

Mops condylurus	OAF	I	Northern Limpopo, South Africa	Diamond Mining	Fur	Cr		0.99		0.55	1.47	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, South Africa	Game Farm	Fur	Cr		1.07		0.37	2.71	Current study
Myotis myotis	CEF	I	Sicily	Petrochemical Plant	Fur	Cr	1.05	0.22		0.07	1.27	Ferrante et al. 2018
Myotis myotis	CEF	I	Sicily	Pipistrelli Cave	Fur	Cr	1.14	0.25		0.12	0.80	Ferrante et al. 2018
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Verlam wastewater treatment works	Fur	Cr	0.14			0.01	0.84	Hill et al. 2017
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Umbilo wastewater treament works	Fur	Cr	0.06			0.01	0.27	Hill et al. 2017
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Reference Sites	Fur	Cr	0.06			0.01	0.14	Hill et al. 2017
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Fur	Cr		0.63		0.21	2.17	Mina et al. 2019
Lasiurus borealis	CEF	I	North America	Across range	Fur	Cs	0.001					Wieringa et al. 2020
Mops condylurus	OAF	I	Northern Limpopo, South Africa	Diamond Mining	Fur	Cu		8.43		5.69	26.11	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, South Africa	Game Farm	Fur	Cu		6.09		5.34	17.41	Current study
Myotis myotis	CEF	I	Sicily	Petrochemical Plant	Fur	Cu	5.47	1.30		0.36	2.37	Ferrante et al. 2018
Myotis myotis	CEF	I	Sicily	Pipistrelli Cave	Fur	Cu	7.76	1.84		0.95	3.13	Ferrante et al. 2018
Myotis bechsteinii	CEF	I	Middle Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Cu		7.80		1.43	31.25	Flache et al. 2015
Myotis daubentonii	CEF	I	Middle Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Cu		17.55		7.43	121.50	Flache et al. 2015
Myotis myotis	CEF	I	Middle Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Cu		5.23		0.78	23.70	Flache et al. 2015
Pipistrellus pipistrellus	CEF	I	Middle Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Cu		9.05		3.65	44.00	Flache et al. 2015
Myotis bechsteinii	CEF	I	Central Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Cu		7.50		1.25	17.50	Flache et al. 2018
Myotis nattereri	CEF	I	Central Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Cu		18.75		5.50	22.75	Flache et al. 2018
Plecotus auritus	Clutter	I	Central Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Cu		6.50		0.00	26.00	Flache et al. 2018
Pipistrellus sp.	CEF	I	England & Wales	Mixed	Fur	Cu	2.20 - 103.00	3.74				Hernout et al. 2016a
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Verlam wastewater treatment works	Fur	Cu	1.31			0.01	11.68	Hill et al. 2017

Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Umbilo wastewater treament works	Fur	Cu	1.48			0.01	8.64	Hill et al. 2017
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Reference Sites	Fur	Cu	9.42			0.01	47.07	Hill et al. 2017
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Fur	Cu		3.14		1.72	12.41	Mina et al. 2019
Lasiurus borealis	CEF	I	North America	Across range	Fur	Cu	0.01					Wieringa et al. 2020
Mops condylurus	OAF	I	Northern Limpopo, South Africa	Diamond Mining	Fur	Fe		101.41		67.54	181.25	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, South Africa	Game Farm	Fur	Fe		147.64		79.05	814.00	Current study
Eptesicus fuscus	CEF	I	Cornwall, Ontario, Canada	Mixed Urban	Fur	Fe	100.00					Hickey et al. 2001
Myotis lucifugus	CEF	I	Fly Creek, Ontario, Canada	Mixed Urban	Fur	Fe	72.00					Hickey et al. 2001
Myotis lucifugus	CEF	I	Lafleche 2 Cave, Quebec, Canada	Cave	Fur	Fe	220.00					Hickey et al. 2001
Myotis lucifugus	CEF	I	Lafleche-1 Cave, Quebec, Canada	Cave	Fur	Fe	220.00					Hickey et al. 2001
Myotis lucifugus	CEF	I	Tyendinaga, Ontario, Canada	Mixed Urban	Fur	Fe	79.00					Hickey et al. 2001
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Verlam wastewater treatment works	Fur	Fe	14.87			5.63	33.52	Hill et al. 2017
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Umbilo wastewater treament works	Fur	Fe	21.14			5.95	42.63	Hill et al. 2017
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Reference Sites	Fur	Fe	15.32			6.16	31.33	Hill et al. 2017
Lasiurus borealis	CEF	I	North America	Across range	Fur	Fe	0.07					Wieringa et al. 2020
Artibeus intermedius		F	Belize	Gold minining & Agriculture	Fur	Hg	0.03	0.01				Becker et al. 2018
Artibeus jamaicensis	/	F	Belize	Gold minining & Agriculture	Fur	Hg	0.04	0.01				Becker et al. 2018
Artibeus lituratus	/	F	Belize	Gold minining & Agriculture	Fur	Hg	0.04	0.02				Becker et al. 2018
Bauerus dubiaquercus	CEF	I	Belize	Gold minining & Agriculture	Fur	Hg	1.05	0.21				Becker et al. 2018
Carollia sowelli	/	F	Belize	Gold minining & Agriculture	Fur	Hg	0.11	0.10				Becker et al. 2018
Chrotopterus auritus	Clutter	C	Belize	Gold minining & Agriculture	Fur	Hg	0.53	0.13				Becker et al. 2018
Dermanura phaeotis	/	F	Belize	Gold minining & Agriculture	Fur	Hg	0.05	0.002				Becker et al. 2018

Dermanura watsoni	/	F	Belize	Gold minining & Agriculture	Fur	Hg	0.10	-				Becker et al. 2018
Desmodus rotundus	/	S	Belize	Gold minining & Agriculture	Fur	Hg	0.05	0.02				Becker et al. 2018
Eptesicus furinalis	CEF	I	Belize	Gold minining & Agriculture	Fur	Hg	6.46	3.20				Becker et al. 2018
Glossophaga soricina	/	N/O	Belize	Gold minining & Agriculture	Fur	Hg	0.10	0.04				Becker et al. 2018
Molossus rufus	OAF	I	Belize	Gold minining & Agriculture	Fur	Hg	2.91	0.90				Becker et al. 2018
Myotis elegans	CEF	I	Belize	Gold minining & Agriculture	Fur	Hg	4.39	1.99				Becker et al. 2018
Noctilio leporinus	/	C	Belize	Gold minining & Agriculture	Fur	Hg	145.27	-				Becker et al. 2018
Pteronotus davyi	CEF	I	Belize	Gold minining & Agriculture	Fur	Hg	3.92	3.08				Becker et al. 2018
Pteronotus mesoamericanus	CEF	I	Belize	Gold minining & Agriculture	Fur	Hg	10.19	10.69				Becker et al. 2018
Rhogeessa aeneus	OAF	I	Belize	Gold minining & Agriculture	Fur	Hg	4.87	-				Becker et al. 2018
Rhynchonycteris naso	OAF	I	Belize	Gold minining & Agriculture	Fur	Hg	24.85	5.66				Becker et al. 2018
Saccopteryx bilineata	Clutter	I	Belize	Gold minining & Agriculture	Fur	Hg	6.44	2.69				Becker et al. 2018
Sturnira lilium	/	F	Belize	Gold minining & Agriculture	Fur	Hg	0.51	0.75				Becker et al. 2018
Trachops cirrhosus	/	C	Belize	Gold minining & Agriculture	Fur	Hg	1.71	0.42				Becker et al. 2018
Uroderma bilobatum	/	F	Belize	Gold minining & Agriculture	Fur	Hg	0.07	0.02				Becker et al. 2018
Artibeus lituratus	/	F	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	0.003	0.01				Carrasco-Rueda et al. 2020
Artibeus lituratus	/	F	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	-	-				Carrasco-Rueda et al. 2020
Artibeus obscurus	/	F	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	0.01	0.03				Carrasco-Rueda et al. 2020
Artibeus obscurus	/	F	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	-	-				Carrasco-Rueda et al. 2020
Artibeus planirostris	/	F	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	0.003	0.01				Carrasco-Rueda et al. 2020

Artibeus planirostris	/	F	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	0.005	0.02				Carrasco-Rueda et al. 2020
Carollia benkeithi	/	F	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	-	-				Carrasco-Rueda et al. 2020
Carollia benkeithi	/	F	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	0.25	-				Carrasco-Rueda et al. 2020
Carollia brevicauda	/	F	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	0.24	1.18				Carrasco-Rueda et al. 2020
Carollia brevicauda	/	F	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	-	-				Carrasco-Rueda et al. 2020
Carollia perspicillata	/	F	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	0.27	0.22				Carrasco-Rueda et al. 2020
Carollia perspicillata	/	F	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	0.58	0.88				Carrasco-Rueda et al. 2020
Chrotopterus auritus	/	C	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	0.35	0.09				Carrasco-Rueda et al. 2020
Chrotopterus auritus	/	C	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	-	-				Carrasco-Rueda et al. 2020
Eptesicus brasiliensis	CEF	I	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	-	-				Carrasco-Rueda et al. 2020
Eptesicus brasiliensis	CEF	I	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	1.56	-				Carrasco-Rueda et al. 2020
Eptesicus furinalis	CEF	I	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	1.25	1.36				Carrasco-Rueda et al. 2020
Eptesicus furinalis	CEF	I	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	1.15	0.73				Carrasco-Rueda et al. 2020
Glossophaga soricina	/	N/O	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	0.22	0.11				Carrasco-Rueda et al. 2020
Glossophaga soricina	/	N/O	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	0.38	-				Carrasco-Rueda et al. 2020
Lonchophylla thomasi	/	N	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	0.02	-				Carrasco-Rueda et al. 2020

Lonchophylla thomasi	/	N	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	-	-				Carrasco-Rueda et al. 2020
Lophostoma silvicolum	Clutter	I	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	0.27	0.48				Carrasco-Rueda et al. 2020
Lophostoma silvicolum	Clutter	I	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	0.01	-				Carrasco-Rueda et al. 2020
Mesophylla macconnelli	/	F	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	-	-				Carrasco-Rueda et al. 2020
Mesophylla macconnelli	/	F	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	-	-				Carrasco-Rueda et al. 2020
Micronycteris minuta	Clutter	I	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	0.35	-				Carrasco-Rueda et al. 2020
Micronycteris minuta	Clutter	I	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	-	-				Carrasco-Rueda et al. 2020
Mimon crenulatum	Clutter	I	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	0.73	-				Carrasco-Rueda et al. 2020
Mimon crenulatum	Clutter	I	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	-	-				Carrasco-Rueda et al. 2020
Molossus cf. rufuc	OAF	I	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	-	-				Carrasco-Rueda et al. 2020
Molossus cf. rufuc	OAF	I	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	0.42	-				Carrasco-Rueda et al. 2020
Molossus molossus	OAF	I	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	-	-				Carrasco-Rueda et al. 2020
Molossus molossus	OAF	I	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	0.83	-				Carrasco-Rueda et al. 2020
Phyllostomus elongatus	Clutter	O	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	0.26	0.30				Carrasco-Rueda et al. 2020
Phyllostomus elongatus	Clutter	O	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	-	-				Carrasco-Rueda et al. 2020
Phyllostomus hastatus	Clutter	O	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	0.10	0.33				Carrasco-Rueda et al. 2020

Phyllostomus hastatus	Clutter	O	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	0.28	-				Carrasco-Rueda et al. 2020
Platyrrhinus incarum	/	F	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	0.00	-				Carrasco-Rueda et al. 2020
Platyrrhinus incarum	/	F	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	-	-				Carrasco-Rueda et al. 2020
Rhinophylla pumilio	/	F	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	-	-				Carrasco-Rueda et al. 2020
Rhinophylla pumilio	/	F	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	0.00	-				Carrasco-Rueda et al. 2020
Saccopteryx bilineata	CEF	I	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	-	-				Carrasco-Rueda et al. 2020
Saccopteryx bilineata	CEF	I	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	1.96	-				Carrasco-Rueda et al. 2020
Sphaeronycteris toxophyllum	/	F	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	0.00	-				Carrasco-Rueda et al. 2020
Sphaeronycteris toxophyllum	/	F	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	0.00	-				Carrasco-Rueda et al. 2020
Sturnira lilium	/	F	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	-	-				Carrasco-Rueda et al. 2020
Sturnira lilium	/	F	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	0.00	-				Carrasco-Rueda et al. 2020
Tonatia suarophila	Clutter	I	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	0.68	-				Carrasco-Rueda et al. 2020
Tonatia suarophila	Clutter	I	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	-	-				Carrasco-Rueda et al. 2020
Trachops cirrhosus	/	C	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	0.43	0.69				Carrasco-Rueda et al. 2020
Trachops cirrhosus	/	C	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	0.84	-				Carrasco-Rueda et al. 2020
Trinycteris nicefori	Clutter	I	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	0.01	0.04				Carrasco-Rueda et al. 2020

Trinycteris nicefori	Clutter	I	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	-	-				Carrasco-Rueda et al. 2020
Uroderma bilobatum	/	F	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	0.002	0.01				Carrasco-Rueda et al. 2020
Uroderma bilobatum	/	F	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	-	-				Carrasco-Rueda et al. 2020
Uroderma magnirostrum	/	F	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	0.00	-				Carrasco-Rueda et al. 2020
Uroderma magnirostrum	/	F	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	-	-				Carrasco-Rueda et al. 2020
Vampyriscus bidens	/	F	Peru	Small-scale gold mining - Papya plantation	Fur	Hg	0.00	-				Carrasco-Rueda et al. 2020
Vampyriscus bidens	/	F	Peru	Small-scale gold mining - Cattle pasture	Fur	Hg	-	-				Carrasco-Rueda et al. 2020
Mops condylurus	OAF	I	Northern Limpopo, South Africa	Diamond Mining	Fur	Hg		1.27		0.58	1.95	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, South Africa	Game Farm	Fur	Hg		0.60		0.39	1.11	Current study
Myotis myotis	CEF	I	Sicily	Petrochemical Plant	Fur	Hg	2.37	0.46		0.16	2.35	Ferrante et al. 2018
Myotis myotis	CEF	I	Sicily	Pipistrelli Cave	Fur	Hg	2.74	0.61		0.04	2.57	Ferrante et al. 2018
Eptesicus fuscus	CEF	I	Canada	-	Fur	Hg			1.50	15.40		Hickey et al. 2001
Eptesicus fuscus	CEF	I	Cornwall, Ontario, Canada	Mixed Urban	Fur	Hg	4.60					Hickey et al. 2001
Myotis leibii	CEF	I	Canada	-	Fur	Hg			5.30	76.20		Hickey et al. 2001
Myotis lucifugus	CEF	I	Canada	-	Fur	Hg			1.50	2.50		Hickey et al. 2001
Myotis lucifugus	CEF	I	Fly Creek, Ontario, Canada	Mixed Urban	Fur	Hg	3.90					Hickey et al. 2001
Myotis lucifugus	CEF	I	Lafleche 2 Cave, Quebec, Canada	Cave	Fur	Hg						Hickey et al. 2001
Myotis lucifugus	CEF	I	Lafleche-1 Cave, Quebec, Canada	Cave	Fur	Hg	2.00					Hickey et al. 2001
Myotis lucifugus	CEF	I	Tyendinaga, Ontario, Canada	Mixed Urban	Fur	Hg						Hickey et al. 2001

Myotis septentionalis	CEF	I	Canada	-	Fur	Hg			4.40	10.20		Hickey et al. 2001
Myotis lucifugus	CEF	I	Moscow, Russia	Uncontaminated	Fur	Hg	3.30			0.60	14.90	Karouna-Renier et al. 2014
Myotis lucifugus	CEF	I	Northwestern Virginia, USA	Contaminated (Grottoes & Mt. Sydney	Fur	Hg	118.40			2.00	707.60	Karouna-Renier et al. 2014
Myotis lucifugus	CEF	I	Northwestern Virginia, USA	Contaminated (Grottoes)	Fur	Hg	189.10			2.90	707.60	Karouna-Renier et al. 2014
Myotis lucifugus	CEF	I	Northwestern Virginia, USA	Contaminated (Mt. Sydney	Fur	Hg	28.90			2.40	320.80	Karouna-Renier et al. 2014
Lasiurus borealis	CEF	I	North America	Across range	Fur	Hg	0.005					Wieringa et al. 2020
Eptesicus fuscus	CEF	I	Northeast United States	Point source and non-point source	Fur	Hg	0.10	0.06			0.89	Yates et al. 2014
Lasionycteris noctivagans	CEF	I	Northeast United States	Point source and non-point source	Fur	Hg	-	-			-	Yates et al. 2014
Lasiurus borealis	CEF	I	Northeast United States	Point source and non-point source	Fur	Hg	0.05	0.03			0.22	Yates et al. 2014
Lasiurus cinereus	CEF	I	Northeast United States	Point source and non-point source	Fur	Hg	0.02	0.01			0.03	Yates et al. 2014
Myotis grisescens	CEF	I	Northeast United States	Point source and non-point source	Fur	Hg	0.12	0.02			0.46	Yates et al. 2014
Myotis leibii	CEF	I	Northeast United States	Point source and non-point source	Fur	Hg	-	-			-	Yates et al. 2014
Myotis lucifugus	CEF	I	Northeast United States	Point source and non-point source	Fur	Hg	0.28	0.04			3.76	Yates et al. 2014
Myotis septentionalis	CEF	I	Northeast United States	Point source and non-point source	Fur	Hg	0.60	0.12			3.70	Yates et al. 2014
Myotis sodalis	CEF	I	Northeast United States	Point source and non-point source	Fur	Hg	-	-			-	Yates et al. 2014
Perimyotis subflavus	CEF	I	Northeast United States	Point source and non-point source	Fur	Hg	0.74	0.42			2.75	Yates et al. 2014
Mops condylurus	OAF	I	Northern Limpopo, South Africa	Diamond Mining	Fur	K		3.31		2.54	7.30	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, South Africa	Game Farm	Fur	K		1.33		0.77	2.14	Current study
Lasiurus borealis	CEF	I	North America	Across range	Fur	Mg	0.05					Wieringa et al. 2020
Mops condylurus	OAF	I	Northern Limpopo, South Africa	Diamond Mining	Fur	Mn		4.75		3.23	8.01	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, South Africa	Game Farm	Fur	Mn		13.33		6.19	32.61	Current study
Myotis myotis	CEF	I	Sicily	Petrochemical Plant	Fur	Mn	2.91	0.55		0.13	4.66	Ferrante et al. 2018

Myotis myotis	CEF	I	Sicily	Pipistrelli Cave	Fur	Mn	3.39	0.73		0.13	2.78	Ferrante et al. 2018
Myotis bechsteinii	CEF	I	Middle Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Mn	8.48			0.00	18.78	Flache et al. 2015
Myotis daubentonii	CEF	I	Middle Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Mn	5.88			0.00	12.38	Flache et al. 2015
Myotis myotis	CEF	I	Middle Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Mn	16.38			7.98	35.25	Flache et al. 2015
Pipistrellus pipistrellus	CEF	I	Middle Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Mn	6.70			0.31	23.20	Flache et al. 2015
Myotis bechsteinii	CEF	I	Central Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Mn		7.50		0.00	19.00	Flache et al. 2018
Myotis nattereri	CEF	I	Central Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Mn		0.00		0.00	3.00	Flache et al. 2018
Plecotus auritus	Clutter	I	Central Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Mn		10.25		0.00	10.88	Flache et al. 2018
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Verlam wastewater treatment works	Fur	Mn	2.41			0.78	6.71	Hill et al. 2017
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Umbilo wastewater treament works	Fur	Mn	3.00			0.79	7.83	Hill et al. 2017
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Reference Sites	Fur	Mn	7.67			2.42	16.80	Hill et al. 2017
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Fur	Mn		2.84		0.93	27.75	Mina et al. 2019
Lasiurus borealis	CEF	I	North America	Across range	Fur	Mn	0.001					Wieringa et al. 2020
Mops condylurus	OAF	I	Northern Limpopo, South Africa	Diamond Mining	Fur	Mo		0.85		0.59	0.92	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, South Africa	Game Farm	Fur	Mo		0.72		0.52	0.82	Current study
Myotis bechsteinii	CEF	I	Central Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Mo		0.19		0.00	0.38	Flache et al. 2018
Myotis nattereri	CEF	I	Central Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Mo		0.35		0.16	0.76	Flache et al. 2018
Plecotus auritus	Clutter	I	Central Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Mo		0.21		0.10	0.55	Flache et al. 2018
Lasiurus borealis	CEF	I	North America	Across range	Fur	Mo	0.002					Wieringa et al. 2020
Mops condylurus	OAF	I	Northern Limpopo, South Africa	Diamond Mining	Fur	Ni		1.13		0.79	2.97	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, South Africa	Game Farm	Fur	Ni		1.38		0.77	2.58	Current study
Myotis myotis	CEF	I	Sicily	Petrochemical Plant	Fur	Ni	0.43	0.06		<0,02	0.86	Ferrante et al. 2018

Myotis myotis	CEF	I	Sicily	Pipistrelli Cave	Fur	Ni	0.56	0.11		0.01	0.77	Ferrante et al. 2018
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Fur	Ni		0.65		0.15	11.22	Mina et al. 2019
Lasiurus borealis	CEF	I	North America	Across range	Fur	Ni	0.002					Wieringa et al. 2020
Mops condylurus	OAF	I	Northern Limpopo, South Africa	Diamond Mining	Fur	Pb		0.45		0.16	0.94	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, South Africa	Game Farm	Fur	Pb		0.48		0.42	1.47	Current study
Myotis myotis	CEF	I	Sicily	Petrochemical Plant	Fur	Pb	0.76	0.06		0.01	1.52	Ferrante et al. 2018
Myotis myotis	CEF	I	Sicily	Pipistrelli Cave	Fur	Pb	0.43	0.07		0.01	0.43	Ferrante et al. 2018
Myotis bechsteinii	CEF	I	Middle Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Pb		1.29		0.45	2.16	Flache et al. 2015
Myotis daubentonii	CEF	I	Middle Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Pb		1.07		0.005	5.15	Flache et al. 2015
Myotis myotis	CEF	I	Middle Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Pb		0.10		0.004	1.68	Flache et al. 2015
Pipistrellus pipistrellus	CEF	I	Middle Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Pb		8.55		0.004	129.75	Flache et al. 2015
Myotis bechsteinii	CEF	I	Central Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Pb		1.25		0.45	2.20	Flache et al. 2018
Myotis nattereri	CEF	I	Central Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Pb		1.50		0.28	3.40	Flache et al. 2018
Plecotus auritus	Clutter	I	Central Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Pb		0.70		0.00	3.20	Flache et al. 2018
Pipistrellus pipistrellus	CEF	I	England & Wales	Mixed	Fur	Pb	71.50					Hernout et al. 2016a
Pipistrellus pygmaeus	CEF	I	England & Wales	Mixed	Fur	Pb	1389.50					Hernout et al. 2016a
Pipistrellus sp.	CEF	I	England & Wales	Mixed	Fur	Pb	0.01 - 5099.75	7.20				Hernout et al. 2016a
Eptesicus fuscus	CEF	I	Canada	-	Fur	Pb			ND-6.1			Hickey et al. 2001
Eptesicus fuscus	CEF	I	Cornwall, Ontario, Canada	Mixed Urban	Fur	Pb	8.80					Hickey et al. 2001
Myotis leibii	CEF	I	Canada	-	Fur	Pb			-			Hickey et al. 2001
Myotis lucifugus	CEF	I	Canada	-	Fur	Pb			ND-11.3			Hickey et al. 2001
Myotis lucifugus	CEF	I	Fly Creek, Ontario, Canada	Mixed Urban	Fur	Pb	6.20					Hickey et al. 2001

Myotis lucifugus	CEF	I	Lafleche 2 Cave, Quebec, Canada	Cave	Fur	Pb	3.00					Hickey et al. 2001
Myotis lucifugus	CEF	I	Lafleche-1 Cave, Quebec, Canada	Cave	Fur	Pb	2.50					Hickey et al. 2001
Myotis lucifugus	CEF	I	Tyendinaga, Ontario, Canada	Mixed Urban	Fur	Pb	1.60					Hickey et al. 2001
Myotis septentionalis	CEF	I	Canada	-	Fur	Pb			-			Hickey et al. 2001
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Verlam wastewater treatment works	Fur	Pb	0.46			0.01	1.55	Hill et al. 2017
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Umbilo wastewater treament works	Fur	Pb	0.39			0.01	1.74	Hill et al. 2017
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Reference Sites	Fur	Pb	1.49			0.01	6.65	Hill et al. 2017
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Fur	Pb		0.63		0.30	14.39	Mina et al. 2019
Mops condylurus	OAF	I	Northern Limpopo, South Africa	Diamond Mining	Fur	Rb		1.26		1.11	2.96	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, South Africa	Game Farm	Fur	Rb		0.89		0.63	1.06	Current study
Lasiurus borealis	CEF	I	North America	Across range	Fur	Rb	0.001					Wieringa et al. 2020
Mops condylurus	OAF	I	Northern Limpopo, South Africa	Diamond Mining	Fur	Sb		0.18		0.09	0.22	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, South Africa	Game Farm	Fur	Sb		0.04		0.02	0.07	Current study
Myotis myotis	CEF	I	Sicily	Petrochemical Plant	Fur	Sb	0.06	0.01		<0,01	0.04	Ferrante et al. 2018
Myotis myotis	CEF	I	Sicily	Pipistrelli Cave	Fur	Sb	0.07	0.01		<0,01	0.05	Ferrante et al. 2018
Mops condylurus	OAF	I	Northern Limpopo, South Africa	Diamond Mining	Fur	Se		5.48		3.65	8.21	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, South Africa	Game Farm	Fur	Se		4.03		1.95	6.40	Current study
Myotis myotis	CEF	I	Sicily	Petrochemical Plant	Fur	Se	0.81	0.19		0.08	0.42	Ferrante et al. 2018
Myotis myotis	CEF	I	Sicily	Pipistrelli Cave	Fur	Se	1.08	0.27		0.13	0.43	Ferrante et al. 2018
Eptesicus fuscus	CEF	I	Canada	-	Fur	Se			-			Hickey et al. 2001
Eptesicus fuscus	CEF	I	Cornwall, Ontario, Canada	Mixed Urban	Fur	Se	9.50					Hickey et al. 2001

Myotis leibii	CEF	I	Canada	-	Fur	Se			-			Hickey et al. 2001
Myotis lucifugus	CEF	I	Canada	-	Fur	Se			ND-26.90			Hickey et al. 2001
Myotis lucifugus	CEF	I	Fly Creek, Ontario, Canada	Mixed Urban	Fur	Se	69.00					Hickey et al. 2001
Myotis lucifugus	CEF	I	Lafleche 2 Cave, Quebec, Canada	Cave	Fur	Se	13.00					Hickey et al. 2001
Myotis lucifugus	CEF	I	Lafleche-1 Cave, Quebec, Canada	Cave	Fur	Se	17.00					Hickey et al. 2001
Myotis lucifugus	CEF	I	Tyendinaga, Ontario, Canada	Mixed Urban	Fur	Se	22.00					Hickey et al. 2001
Myotis septentionalis	CEF	I	Canada	-	Fur	Se			-			Hickey et al. 2001
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Fur	Se		0.86		0.24	5.48	Mina et al. 2019
Mops condylurus	OAF	I	Northern Limpopo, South Africa	Diamond Mining	Fur	Sn		0.09		0.06	0.18	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, South Africa	Game Farm	Fur	Sn		0.06		0.03	0.16	Current study
Lasiurus borealis	CEF	I	North America	Across range	Fur	Sn	0.004					Wieringa et al. 2020
Mops condylurus	OAF	I	Northern Limpopo, South Africa	Diamond Mining	Fur	Sr		1.44		1.02	1.85	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, South Africa	Game Farm	Fur	Sr		1.71		1.37	3.65	Current study
Mops condylurus	OAF	I	Northern Limpopo, South Africa	Diamond Mining	Fur	V		0.28		0.24	0.40	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, South Africa	Game Farm	Fur	V		0.52		0.34	1.24	Current study
Myotis myotis	CEF	I	Sicily	Petrochemical Plant	Fur	V	0.29	0.07		<0,01	0.18	Ferrante et al. 2018
Myotis myotis	CEF	I	Sicily	Pipistrelli Cave	Fur	V	0.45	0.11		0.02825	0.29	Ferrante et al. 2018
Lasiurus borealis	CEF	I	North America	Across range	Fur	Y	0.0004					Wieringa et al. 2020
Mops condylurus	OAF	I	Northern Limpopo, South Africa	Diamond Mining	Fur	Zn		147.09		117.80	346.41	Current study
Tadarida aegyptiaca/Mops condylurus	OAF	I	Northern Limpopo, South Africa	Game Farm	Fur	Zn		97.70		37.78	241.87	Current study
Myotis bechsteinii	CEF	I	Middle Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Zn		25.75		13.25	36.50	Flache et al. 2015

Myotis daubentonii	CEF	I	Middle Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Zn		32.25		23.38	35.50	Flache et al. 2015
Myotis myotis	CEF	I	Middle Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Zn		22.13		15.33	29.75	Flache et al. 2015
Pipistrellus pipistrellus	CEF	I	Middle Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Zn		95.75		70.50	288.75	Flache et al. 2015
Myotis bechsteinii	CEF	I	Central Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Zn		26.50		14.00	36.00	Flache et al. 2018
Myotis nattereri	CEF	I	Central Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Zn		23.44		14.00	31.69	Flache et al. 2018
Plecotus auritus	Clutter	I	Central Hesse, Germany	Mixed: Urban, agriculture, forest	Fur	Zn		32.75		24.94	43.7	Flache et al. 2018
Pipistrellus sp.	CEF	I	England & Wales	Mixed	Fur	Zn	2.95 - 144.50	18.24				Hernout et al. 2016a
Eptesicus fuscus	CEF	I	Canada	-	Fur	Zn			101.30	154.80		Hickey et al. 2001
Eptesicus fuscus	CEF	I	Cornwall, Ontario, Canada	Mixed Urban	Fur	Zn	160.00					Hickey et al. 2001
Myotis leibii	CEF	I	Canada	-	Fur	Zn			314.60	5523.00		Hickey et al. 2001
Myotis lucifugus	CEF	I	Canada	-	Fur	Zn			101.40	110.10		Hickey et al. 2001
Myotis lucifugus	CEF	I	Fly Creek, Ontario, Canada	Mixed Urban	Fur	Zn	130.00					Hickey et al. 2001
Myotis lucifugus	CEF	I	Lafleche 2 Cave, Quebec, Canada	Cave	Fur	Zn	200.00					Hickey et al. 2001
Myotis lucifugus	CEF	I	Lafleche-1 Cave, Quebec, Canada	Cave	Fur	Zn	140.00					Hickey et al. 2001
Myotis lucifugus	CEF	I	Tyendinaga, Ontario, Canada	Mixed Urban	Fur	Zn	130.00					Hickey et al. 2001
Myotis septentionalis	CEF	I	Canada	-	Fur	Zn			107.60	121.60		Hickey et al. 2001
Afronycteris nanananana	CEF	I	Kwa-Zulu Natal, South Africa	Verlam wastewater treatment works	Fur	Zn	74.68			61.81	97.84	Hill et al. 2017
Afronycteris nanananana	CEF	I	Kwa-Zulu Natal, South Africa	Umbilo wastewater treament works	Fur	Zn	77.15			51.65	138.60	Hill et al. 2017
Afronycteris nanananana	CEF	I	Kwa-Zulu Natal, South Africa	Reference Sites	Fur	Zn	63.95			40.48	89.49	Hill et al. 2017
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Fur	Zn		59.83		37.30	206.79	Mina et al. 2019
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Heart	As		0.04		0.003	0.76	Mina et al. 2019
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Heart	Cd		0.04		0.003	0.26	Mina et al. 2019

Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Heart	Co		0.05		0.01	1.95	Mina et al. 2019
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Heart	Cr		0.24		0.02	1.41	Mina et al. 2019
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Heart	Cu		5.46		2.18	12.62	Mina et al. 2019
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Heart	Mn		1.62		0.53	3.92	Mina et al. 2019
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Heart	Ni		0.15		0.01	44.94	Mina et al. 2019
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Heart	Pb		0.13		0.02	0.75	Mina et al. 2019
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Heart	Se		0.36		0.25	0.85	Mina et al. 2019
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Heart	Zn		14.78		8.23	26.05	Mina et al. 2019
Tadarida teniotis	OAF	I	Italy	African Quarter of Rome	Kidney	Al	0.55	2.95				Andreani et al. 2019
Tadarida teniotis (lactant)	OAF	I	Italy	African Quarter of Rome	Kidney	Al	<LOQ	<LOQ				Andreani et al. 2019
Tadarida teniotis	OAF	I	Italy	African Quarter of Rome	Kidney	As	0.01	0.01				Andreani et al. 2019
Tadarida teniotis (lactant)	OAF	I	Italy	African Quarter of Rome	Kidney	As	0.01	0.01				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	African Quarter of Rome	Kidney	Ba	0.17	0.22				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	African Quarter of Rome	Kidney	Ba	0.14	0.05				Andreani et al. 2019
Tadarida teniotis	OAF	I	Italy	African Quarter of Rome	Kidney	Cd	<LOQ	<LOQ				Andreani et al. 2019
Tadarida teniotis (lactant)	OAF	I	Italy	African Quarter of Rome	Kidney	Cd	0.01	0.01				Andreani et al. 2019
Myotis austroriparius	CEF	I	Gainesville, Florida	Battery salvage plant	Kidney	Cd	0.41	0.57	0.27	0.15	2.10	Clark et al. 1986

Myotis austroriparius	CEF	I	Judges Cave, Florida	Battery salvage plant	Kidney	Cd	1.13	0.73	0.89	0.22	2.90	Clark et al. 1986
Pipistrellus sp.	CEF	I	England & Wales	Mixed	Kidney	Cd	0.001 - 0.20	0.01				Hernout et al. 2016a
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Umbilo River - Sewage Tank	Kidney	Cd	0.66			0.20	0.92	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Umbilo River - Downstream	Kidney	Cd	0.44			-	-	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Little Amanzimtoti - Upstream	Kidney	Cd	0.001			-	-	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Little Amanzimtoti - Tank	Kidney	Cd	0.23			0.001	0.65	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Little Amanzimtoti - Downstream	Kidney	Cd	0.07			-	-	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Mdloti - Upstream	Kidney	Cd	0.02			0.001	0.03	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Mdloti - Tank	Kidney	Cd	0.15			0.03	0.24	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Mdloti - Downstream	Kidney	Cd	0.06			0.03	0.10	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Umbilo River - Sewage Tank	Kidney	Cr	0.002			0.002	0.002	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Umbilo River - Downstream	Kidney	Cr	0.002			-	-	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Little Amanzimtoti - Upstream	Kidney	Cr	0.002			-	-	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Little Amanzimtoti - Tank	Kidney	Cr	0.002			-	-	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Little Amanzimtoti - Downstream	Kidney	Cr	0.002			-	-	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Mdloti - Upstream	Kidney	Cr	0.002			0.002	0.002	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Mdloti - Tank	Kidney	Cr	0.002			0.002	0.002	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Mdloti - Downstream	Kidney	Cr	0.03			0.002	0.171	Naidoo et al. 2013
Pipistrellus sp.	CEF	I	England & Wales	Mixed	Kidney	Cu	0.01 - 33.50	3.22				Hernout et al. 2016a
Tadarida teniotis	OAF	I	Italy	African Quarter of Rome	Kidney	Hg	0.01	0.01				Andreani et al. 2019
Tadarida teniotis (lactant)	OAF	I	Italy	African Quarter of Rome	Kidney	Hg	0.11	0.07				Andreani et al. 2019
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Umbilo River - Sewage Tank	Kidney	Ni	0.004			0.00	0.00	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Umbilo River - Downstream	Kidney	Ni	0.004			-	-	Naidoo et al. 2013

Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Little Amanzimtoti - Upstream	Kidney	Ni	0.004			-	-	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Little Amanzimtoti - Tank	Kidney	Ni	0.004			-	-	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Little Amanzimtoti - Downstream	Kidney	Ni	0.004			-	-	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Mdloti - Upstream	Kidney	Ni	0.004			0.004	0.00	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Mdloti - Tank	Kidney	Ni	0.34			0.004	2.01	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Mdloti - Downstream	Kidney	Ni	0.82			0.004	4.91	Naidoo et al. 2013
Tadarida teniotis	OAF	I	Italy	African Quarter of Rome	Kidney	Pb	5.67	2.93				Andreani et al. 2019
Tadarida teniotis (lactant)	OAF	I	Italy	African Quarter of Rome	Kidney	Pb	0.21	0.67				Andreani et al. 2019
Pipistrellus sp.	CEF	I	England & Wales	Mixed	Kidney	Pb	0.001 - 91.75	0.18				Hernout et al. 2016a
Tadarida teniotis	OAF	I	Italy	African Quarter of Rome	Kidney	Sb	0.01	0.01				Andreani et al. 2019
Tadarida teniotis (lactant)	OAF	I	Italy	African Quarter of Rome	Kidney	Sb	<LOQ	<LOQ				Andreani et al. 2019
Tadarida teniotis	OAF	I	Italy	African Quarter of Rome	Kidney	Sr	0.24	0.08				Andreani et al. 2019
Tadarida teniotis (lactant)	OAF	I	Italy	African Quarter of Rome	Kidney	Sr	0.29	0.09				Andreani et al. 2019
Tadarida teniotis	OAF	I	Italy	African Quarter of Rome	Kidney	Th	<LOQ	<LOQ				Andreani et al. 2019
Tadarida teniotis (lactant)	OAF	I	Italy	African Quarter of Rome	Kidney	Th	<LOQ	<LOQ				Andreani et al. 2019
Tadarida teniotis	OAF	I	Italy	African Quarter of Rome	Kidney	Tl	0.13	0.09				Andreani et al. 2019
Tadarida teniotis (lactant)	OAF	I	Italy	African Quarter of Rome	Kidney	Tl	0.09	0.17				Andreani et al. 2019
Pipistrellus pipistrellus	CEF	I	England & Wales	Mixed	Kidney	Zn	7.75					Hernout et al. 2016a
Pipistrellus pygmaeus	CEF	I	England & Wales	Mixed	Kidney	Zn	19.00					Hernout et al. 2016a
Pipistrellus sp.	CEF	I	England & Wales	Mixed	Kidney	Zn	0.33 - 88.50	4.51				Hernout et al. 2016a
Tadarida teniotis	OAF	I	Italy	African Quarter of Rome	Liver	Al	0.27	0.76				Andreani et al. 2019
Tadarida teniotis (lactant)	OAF	I	Italy	African Quarter of Rome	Liver	Al	0.55	0.48				Andreani et al. 2019
Eptesicus diminutus	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Al	8.78					Zocche et al. 2010

Molossus molossus	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Al	≤3.98					Zocche et al. 2010
Molossus molossus	OAF	I	Santa Catarina, Southern Brazil	Control area	Liver	Al	≤3.90					Zocche et al. 2010
Tadarida brasiliensis	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Al	4.75					Zocche et al. 2010
Tadarida brasiliensis	OAF	I	Santa Catarina, Southern Brazil	Control area	Liver	Al	6.95					Zocche et al. 2010
Tadarida teniotis	OAF	I	Italy	African Quarter of Rome	Liver	As	0.01	0.01				Andreani et al. 2019
Tadarida teniotis (lactant)	OAF	I	Italy	African Quarter of Rome	Liver	As	0.02	0.01				Andreani et al. 2019
Myotis myotis	CEF	I	Sicily	Petrochemical Plant	Liver	As	0.05	0.05		<0,01	0.53	Ferrante et al. 2018
Myotis myotis	CEF	I	Sicily	Pipistrelli Cave	Liver	As	0.03	0.03		<0,01	0.18	Ferrante et al. 2018
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Liver	As		0.08		0.02	1.71	Mina et al. 2019
Miniopterus schreibersii	CEF	I	Italy	African Quarter of Rome	Liver	Ba	0.18	0.05				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	African Quarter of Rome	Liver	Ba	0.17	0.21				Andreani et al. 2019
Tadarida teniotis	OAF	I	Italy	African Quarter of Rome	Liver	Cd	<LOQ	<LOQ				Andreani et al. 2019
Tadarida teniotis (lactant)	OAF	I	Italy	African Quarter of Rome	Liver	Cd	0.01	0.01				Andreani et al. 2019
Myotis austroriparius	CEF	I	Gainesville, Florida	Battery salvage plant	Liver	Cd	0.30	0.18	0.26	0.14	0.75	Clark et al. 1986
Myotis austroriparius	CEF	I	Judges Cave, Florida	Battery salvage plant	Liver	Cd	0.63	0.15	0.61	0.36	0.85	Clark et al. 1986
Myotis myotis	CEF	I	Sicily	Petrochemical Plant	Liver	Cd	0.34	0.34		0.01	1.33	Ferrante et al. 2018
Myotis myotis	CEF	I	Sicily	Pipistrelli Cave	Liver	Cd	0.24	0.24		<0,01	1.50	Ferrante et al. 2018
Pipistrellus sp.	CEF	I	England & Wales	Mixed	Liver	Cd	0.0004 - 3.25	0.01				Hernout et al. 2016a
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Liver	Cd		0.15		0.04	0.44	Mina et al. 2019
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Umbilo River - Sewage Tank	Liver	Cd	0.47			0.10	0.72	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Umbilo River - Downstream	Liver	Cd	0.14			-	-	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Little Amanzimtoti - Upstream	Liver	Cd	0.001			-	-	Naidoo et al. 2013

Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Little Amanzimtoti - Tank	Liver	Cd	0.02			0.00075	0.1025	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Little Amanzimtoti - Downstream	Liver	Cd	0.001			-	-	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Mdloti - Upstream	Liver	Cd	0.001			0.001	0.001	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Mdloti - Tank	Liver	Cd	0.02			0.001	0.07	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Mdloti - Downstream	Liver	Cd	0.001			0.001	0.001	Naidoo et al. 2013
Eptesicus diminutus	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Cd	0.90					Zocche et al. 2010
Molossus molossus	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Cd	1.00					Zocche et al. 2010
Molossus molossus	OAF	I	Santa Catarina, Southern Brazil	Control area	Liver	Cd	1.20					Zocche et al. 2010
Tadarida brasiliensis	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Cd	≤1.01					Zocche et al. 2010
Tadarida brasiliensis	OAF	I	Santa Catarina, Southern Brazil	Control area	Liver	Cd	≤0.75					Zocche et al. 2010
Myotis myotis	CEF	I	Sicily	Petrochemical Plant	Liver	Co	0.10	0.10		0.03	0.23	Ferrante et al. 2018
Myotis myotis	CEF	I	Sicily	Pipistrelli Cave	Liver	Co	0.09	0.09		0.04	0.24	Ferrante et al. 2018
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Liver	Co		0.03		0.01	0.26	Mina et al. 2019
Myotis austroriparius	CEF	I	Gainesville, Florida	Battery salvage plant	Liver	Cr	0.05, 0.06					Clark et al. 1986
Myotis myotis	CEF	I	Sicily	Petrochemical Plant	Liver	Cr	0.98	0.98		<0,07	2.71	Ferrante et al. 2018
Myotis myotis	CEF	I	Sicily	Pipistrelli Cave	Liver	Cr	0.88	0.88		0.18	2.39	Ferrante et al. 2018
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Liver	Cr		0.10		0.03	0.20	Mina et al. 2019
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Umbilo River - Sewage Tank	Liver	Cr	0.002			0.002	0.002	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Umbilo River - Downstream	Liver	Cr	0.002			-	-	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Little Amanzimtoti - Upstream	Liver	Cr	0.002			-	-	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Little Amanzimtoti - Tank	Liver	Cr	0.002			-	-	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Little Amanzimtoti - Downstream	Liver	Cr	0.002			-	-	Naidoo et al. 2013

Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Mdloti - Upstream	Liver	Cr	0.002			0.002	0.002	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Mdloti - Tank	Liver	Cr	0.002			0.002	0.002	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Mdloti - Downstream	Liver	Cr	0.002			0.002	0.002	Naidoo et al. 2013
Eptesicus diminutus	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Cr	≤1.55					Zocche et al. 2010
Molossus molossus	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Cr	1.43					Zocche et al. 2010
Molossus molossus	OAF	I	Santa Catarina, Southern Brazil	Control area	Liver	Cr	≤2.38					Zocche et al. 2010
Tadarida brasiliensis	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Cr	≤2.73					Zocche et al. 2010
Tadarida brasiliensis	OAF	I	Santa Catarina, Southern Brazil	Control area	Liver	Cr	≤1.33					Zocche et al. 2010
Myotis myotis	CEF	I	Sicily	Petrochemical Plant	Liver	Cu	10.30	10.30		4.83	48.74	Ferrante et al. 2018
Myotis myotis	CEF	I	Sicily	Pipistrelli Cave	Liver	Cu	9.26	9.26		4.83	22.76	Ferrante et al. 2018
Pipistrellus sp.	CEF	I	England & Wales	Mixed	Liver	Cu	0.0004 - 3.25	0.01				Hernout et al. 2016a
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Liver	Cu		4.56		2.84	8.29	Mina et al. 2019
Eptesicus diminutus	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Cu	7.20					Zocche et al. 2010
Molossus molossus	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Cu	6.83					Zocche et al. 2010
Molossus molossus	OAF	I	Santa Catarina, Southern Brazil	Control area	Liver	Cu	5.08					Zocche et al. 2010
Tadarida brasiliensis	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Cu	5.80					Zocche et al. 2010
Tadarida brasiliensis	OAF	I	Santa Catarina, Southern Brazil	Control area	Liver	Cu	3.88					Zocche et al. 2010
Eptesicus diminutus	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Fe	585.00					Zocche et al. 2010
Molossus molossus	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Fe	264.75					Zocche et al. 2010
Molossus molossus	OAF	I	Santa Catarina, Southern Brazil	Control area	Liver	Fe	418.25					Zocche et al. 2010
Tadarida brasiliensis	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Fe	258.00					Zocche et al. 2010
Tadarida brasiliensis	OAF	I	Santa Catarina, Southern Brazil	Control area	Liver	Fe	229.50					Zocche et al. 2010
Tadarida teniotis	OAF	I	Italy	African Quarter of Rome	Liver	Hg	0.02	0.01				Andreani et al. 2019

Tadarida teniotis (lactant)	OAF	I	Italy	African Quarter of Rome	Liver	Hg	0.14	0.10				Andreani et al. 2019
Myotis myotis	CEF	I	Sicily	Petrochemical Plant	Liver	Hg	1.79	1.79		0.18	10.18	Ferrante et al. 2018
Myotis myotis	CEF	I	Sicily	Pipistrelli Cave	Liver	Hg	1.50	1.50		0.04	11.81	Ferrante et al. 2018
Myotis myotis	CEF	I	Sicily	Petrochemical Plant	Liver	Mn	10.24	10.24		2.36	22.04	Ferrante et al. 2018
Myotis myotis	CEF	I	Sicily	Pipistrelli Cave	Liver	Mn	10.02	10.02		3.16	24.45	Ferrante et al. 2018
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Liver	Mn		2.06		0.34	5.53	Mina et al. 2019
Eptesicus diminutus	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Mn	14.88					Zocche et al. 2010
Molossus molossus	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Mn	3.40					Zocche et al. 2010
Molossus molossus	OAF	I	Santa Catarina, Southern Brazil	Control area	Liver	Mn	8.70					Zocche et al. 2010
Tadarida brasiliensis	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Mn	7.60					Zocche et al. 2010
Tadarida brasiliensis	OAF	I	Santa Catarina, Southern Brazil	Control area	Liver	Mn	6.58					Zocche et al. 2010
Myotis myotis	CEF	I	Sicily	Petrochemical Plant	Liver	Ni	0.08	0.08		<0,02	0.45	Ferrante et al. 2018
Myotis myotis	CEF	I	Sicily	Pipistrelli Cave	Liver	Ni	0.04	0.04		<0,02	0.26	Ferrante et al. 2018
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Liver	Ni		0.13		0.04	0.86	Mina et al. 2019
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Umbilo River - Sewage Tank	Liver	Ni	0.004			0.004	0.004	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Umbilo River - Downstream	Liver	Ni	0.004			-	-	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Little Amanzimtoti - Upstream	Liver	Ni	0.004			-	-	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Little Amanzimtoti - Tank	Liver	Ni	0.004			-	-	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Little Amanzimtoti - Downstream	Liver	Ni	0.004			-	-	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Mdloti - Upstream	Liver	Ni	0.004			0.004	0.004	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Mdloti - Tank	Liver	Ni	0.004			0.004	0.004	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Mdloti - Downstream	Liver	Ni	0.004			0.004	0.004	Naidoo et al. 2013

Eptesicus diminutus	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Ni	2.15					Zocche et al. 2010
Molossus molossus	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Ni	1.08					Zocche et al. 2010
Molossus molossus	OAF	I	Santa Catarina, Southern Brazil	Control area	Liver	Ni	≤2.26					Zocche et al. 2010
Tadarida brasiliensis	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Ni	≤1.40					Zocche et al. 2010
Tadarida brasiliensis	OAF	I	Santa Catarina, Southern Brazil	Control area	Liver	Ni	1.05					Zocche et al. 2010
Tadarida teniotis	OAF	I	Italy	African Quarter of Rome	Liver	Pb	65.70	26.80				Andreani et al. 2019
Tadarida teniotis (lactant)	OAF	I	Italy	African Quarter of Rome	Liver	Pb	1.20	1.18				Andreani et al. 2019
Myotis austroriparius	CEF	I	Gainesville, Florida	Battery salvage plant	Liver	Pb	0.05, 0.26, 0.29, 0.41, 0.58					Clark et al. 1986
Myotis austroriparius	CEF	I	Judges Cave, Florida	Battery salvage plant	Liver	Pb	0.15, 0.17, 0.23, 0.23					Clark et al. 1986
Myotis myotis	CEF	I	Sicily	Petrochemical Plant	Liver	Pb	0.10	0.10		<0,02	0.85	Ferrante et al. 2018
Myotis myotis	CEF	I	Sicily	Pipistrelli Cave	Liver	Pb	0.06	0.06		<0,02	0.31	Ferrante et al. 2018
Pipistrellus sp.	CEF	I	England & Wales	Mixed	Liver	Pb	0.001 - 1260.00	0.08				Hernout et al. 2016a
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Liver	Pb		0.07		0.04	0.25	Mina et al. 2019
Eptesicus diminutus	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Pb	1.35					Zocche et al. 2010
Molossus molossus	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Pb	1.45					Zocche et al. 2010
Molossus molossus	OAF	I	Santa Catarina, Southern Brazil	Control area	Liver	Pb	4.59					Zocche et al. 2010
Tadarida brasiliensis	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Pb	≤1.83					Zocche et al. 2010
Tadarida brasiliensis	OAF	I	Santa Catarina, Southern Brazil	Control area	Liver	Pb	≤0.98					Zocche et al. 2010
Tadarida teniotis	OAF	I	Italy	African Quarter of Rome	Liver	Sb	0.02	0.02				Andreani et al. 2019
Tadarida teniotis (lactant)	OAF	I	Italy	African Quarter of Rome	Liver	Sb	<LOQ	<LOQ				Andreani et al. 2019

Myotis myotis	CEF	I	Sicily	Petrochemical Plant	Liver	Sb	<0,01	<0,01		<0,01	0.02	Ferrante et al. 2018
Myotis myotis	CEF	I	Sicily	Pipistrelli Cave	Liver	Sb	<0,01	<0,01		<0,01	0.02	Ferrante et al. 2018
Myotis myotis	CEF	I	Sicily	Petrochemical Plant	Liver	Se	0.82	0.82		0.27	1.87	Ferrante et al. 2018
Myotis myotis	CEF	I	Sicily	Pipistrelli Cave	Liver	Se	0.99	0.99		0.34	2.65	Ferrante et al. 2018
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Liver	Se		0.68		0.33	1.04	Mina et al. 2019
Eptesicus diminutus	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Si	6.65					Zocche et al. 2010
Molossus molossus	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Si	1.40					Zocche et al. 2010
Molossus molossus	OAF	I	Santa Catarina, Southern Brazil	Control area	Liver	Si	1.20					Zocche et al. 2010
Tadarida brasiliensis	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Si	1.95					Zocche et al. 2010
Tadarida brasiliensis	OAF	I	Santa Catarina, Southern Brazil	Control area	Liver	Si	1.90					Zocche et al. 2010
Tadarida teniotis	OAF	I	Italy	African Quarter of Rome	Liver	Sr	0.48	0.53				Andreani et al. 2019
Tadarida teniotis (lactant)	OAF	I	Italy	African Quarter of Rome	Liver	Sr	0.39	0.13				Andreani et al. 2019
Tadarida teniotis	OAF	I	Italy	African Quarter of Rome	Liver	Th	<LOQ	<LOQ				Andreani et al. 2019
Tadarida teniotis (lactant)	OAF	I	Italy	African Quarter of Rome	Liver	Th	<LOQ	<LOQ				Andreani et al. 2019
Tadarida teniotis	OAF	I	Italy	African Quarter of Rome	Liver	Tl	0.22	0.18				Andreani et al. 2019
Tadarida teniotis (lactant)	OAF	I	Italy	African Quarter of Rome	Liver	Tl	0.11	0.13				Andreani et al. 2019
Myotis myotis	CEF	I	Sicily	Petrochemical Plant	Liver	V	0.05	0.05		0.014	0.11	Ferrante et al. 2018
Myotis myotis	CEF	I	Sicily	Pipistrelli Cave	Liver	V	0.05	0.05		<0,01	0.13	Ferrante et al. 2018
Myotis austroriparius	CEF	I	Gainesville, Florida	Battery salvage plant	Liver	Zn	28.50	1.28	28.50	26.00	30.00	Clark et al. 1986
Myotis austroriparius	CEF	I	Judges Cave, Florida	Battery salvage plant	Liver	Zn	31.10	2.66	31.00	27.00	35.00	Clark et al. 1986
Pipistrellus sp.	CEF	I	England & Wales	Mixed	Liver	Zn	0.20 - 1301.25	4.70				Hernout et al. 2016a
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Liver	Zn		19.29		9.82	53.11	Mina et al. 2019

Eptesicus diminutus	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Zn	28.45					Zocche et al. 2010
Molossus molossus	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Zn	19.90					Zocche et al. 2010
Molossus molossus	OAF	I	Santa Catarina, Southern Brazil	Control area	Liver	Zn	29.05					Zocche et al. 2010
Tadarida brasiliensis	OAF	I	Santa Catarina, Southern Brazil	Coal mining	Liver	Zn	35.35					Zocche et al. 2010
Tadarida brasiliensis	OAF	I	Santa Catarina, Southern Brazil	Control area	Liver	Zn	33.08					Zocche et al. 2010
*Afronycteris nanana*na	CEF	I	Kwa-Zulu Natal, South Africa	Umbilo River - Sewage Tank	Muscle	Cd	0.01			0.001	0.03	Naidoo et al. 2013
*Afronycteris nanana*na	CEF	I	Kwa-Zulu Natal, South Africa	Umbilo River - Downstream	Muscle	Cd	0.001			-	-	Naidoo et al. 2013
*Afronycteris nanana*na	CEF	I	Kwa-Zulu Natal, South Africa	Little Amanzimtoti - Upstream	Muscle	Cd	0.001			-	-	Naidoo et al. 2013
*Afronycteris nanana*na	CEF	I	Kwa-Zulu Natal, South Africa	Little Amanzimtoti - Tank	Muscle	Cd	0.006			0.001	0.03	Naidoo et al. 2013
*Afronycteris nanana*na	CEF	I	Kwa-Zulu Natal, South Africa	Little Amanzimtoti - Downstream	Muscle	Cd	0.001			-	-	Naidoo et al. 2013
*Afronycteris nanana*na	CEF	I	Kwa-Zulu Natal, South Africa	Mdloti - Upstream	Muscle	Cd	0.001			0.001	0.001	Naidoo et al. 2013
*Afronycteris nanana*na	CEF	I	Kwa-Zulu Natal, South Africa	Mdloti - Tank	Muscle	Cd	0.001			0.001	0.001	Naidoo et al. 2013
*Afronycteris nanana*na	CEF	I	Kwa-Zulu Natal, South Africa	Mdloti - Downstream	Muscle	Cd	0.02			0.001	0.10	Naidoo et al. 2013
*Afronycteris nanana*na	CEF	I	Kwa-Zulu Natal, South Africa	Umbilo River - Sewage Tank	Muscle	Cr	0.002			0.002	0.002	Naidoo et al. 2013
*Afronycteris nanana*na	CEF	I	Kwa-Zulu Natal, South Africa	Umbilo River - Downstream	Muscle	Cr	0.002			-	-	Naidoo et al. 2013
*Afronycteris nanana*na	CEF	I	Kwa-Zulu Natal, South Africa	Little Amanzimtoti - Upstream	Muscle	Cr	0.002			-	-	Naidoo et al. 2013
*Afronycteris nanana*na	CEF	I	Kwa-Zulu Natal, South Africa	Little Amanzimtoti - Tank	Muscle	Cr	0.002			0.002	0.002	Naidoo et al. 2013
*Afronycteris nanana*na	CEF	I	Kwa-Zulu Natal, South Africa	Little Amanzimtoti - Downstream	Muscle	Cr	0.002			-	-	Naidoo et al. 2013
*Afronycteris nanana*na	CEF	I	Kwa-Zulu Natal, South Africa	Mdloti - Upstream	Muscle	Cr	0.002			0.002	0.002	Naidoo et al. 2013
*Afronycteris nanana*na	CEF	I	Kwa-Zulu Natal, South Africa	Mdloti - Tank	Muscle	Cr	0.30			0.002	0.24	Naidoo et al. 2013
*Afronycteris nanana*na	CEF	I	Kwa-Zulu Natal, South Africa	Mdloti - Downstream	Muscle	Cr	0.002			0.002	0.002	Naidoo et al. 2013
*Afronycteris nanana*na	CEF	I	Kwa-Zulu Natal, South Africa	Umbilo River - Sewage Tank	Muscle	Ni	0.01			0.004	0.034	Naidoo et al. 2013
*Afronycteris nanana*na	CEF	I	Kwa-Zulu Natal, South Africa	Umbilo River - Downstream	Muscle	Ni	0.31			-	-	Naidoo et al. 2013

Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Little Amanzimtoti - Upstream	Muscle	Ni	0.003			-	-	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Little Amanzimtoti - Tank	Muscle	Ni	0.004			0.004	0.004	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Little Amanzimtoti - Downstream	Muscle	Ni	0.004			-	-	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Mdloti - Upstream	Muscle	Ni	0.004			0.004	0.004	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Mdloti - Tank	Muscle	Ni	0.004			0.004	0.004	Naidoo et al. 2013
Afronycteris nananana	CEF	I	Kwa-Zulu Natal, South Africa	Mdloti - Downstream	Muscle	Ni	0.004			0.004	0.004	Naidoo et al. 2013
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Skin-fur	Al	306.00	75.00				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Skin-fur	As	0.29	0.07				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Skin-fur	Ba	30.00	16.70				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Skin-fur	Cd	0.04	0.01				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Skin-fur	Hg	0.07	0.03				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Skin-fur	Pb	36.90	18.40				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Skin-fur	Sb	1.98	1.95				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Skin-fur	Sr	9.14	0.60				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Skin-fur	Th	0.27	0.04				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Skin-fur	Tl	0.07	0.04				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Skinned body	Al	8.69	4.56				Andreani et al. 2019

Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Skinned body	As	0.01	0.01				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Skinned body	Ba	4.28	0.72				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Skinned body	Cd	<LOQ	<LOQ				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Skinned body	Hg	0.01	0.003				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Skinned body	Pb	49.50	6.20				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Skinned body	Sb	0.03	0.03				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Skinned body	Sr	11.80	1.30				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Skinned body	Th	0.01	0.01				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Skinned body	Tl	0.06	0.04				Andreani et al. 2019
Pipistrellus sp.	CEF	I	England & Wales	Mixed	Stomach	Cd	0.0004 - 0.50	0.01				Hernout et al. 2016a
Pipistrellus sp.	CEF	I	England & Wales	Mixed	Stomach	Cu	0.13 - 60.00	0.18				Hernout et al. 2016a
Pipistrellus sp.	CEF	I	England & Wales	Mixed	Stomach	Pb	0.001 - 33.50	0.21				Hernout et al. 2016a
Pipistrellus sp.	CEF	I	England & Wales	Mixed	Stomach	Zn	0.28 - 334.25	4.76				Hernout et al. 2016a
Miniopterus schreibersii	CEF	I	Italy	African Quarter of Rome	Whole animal	Al	36.90	16.10				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Whole animal	Al	47.95	16.43				Andreani et al. 2019
Miniopterus schreibersii	CEF	I	Italy	African Quarter of Rome	Whole animal	As	0.08	0.03				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Whole animal	As	0.05	0.01				Andreani et al. 2019

Bat species unknown	U	U	Kenting National Park, Taiwan	Nature Reserve	Whole animal	As	0.34			0.20	0.77	Hsu et al. 2006
Miniopterus schreibersii	CEF	I	Italy	African Quarter of Rome	Whole animal	Ba	1.38	0.12				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Whole animal	Ba	5.75	0.95				Andreani et al. 2019
Miniopterus schreibersii	CEF	I	Italy	African Quarter of Rome	Whole animal	Cd	0.04	0.01				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Whole animal	Cd	0.01	0.002				Andreani et al. 2019
Bat species unknown	U	U	Kenting National Park, Taiwan	Nature Reserve	Whole animal	Cd	0.15			0.01	0.34	Hsu et al. 2006
Bat species unknown	U	U	Kenting National Park, Taiwan	Nature Reserve	Whole animal	Cu	36.25			2.33	190.50	Hsu et al. 2006
Bat species unknown	U	U	Kenting National Park, Taiwan	Nature Reserve	Whole animal	Fe	190.00			103.25	285.00	Hsu et al. 2006
Miniopterus schreibersii	CEF	I	Italy	African Quarter of Rome	Whole animal	Hg	0.08	0.03				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Whole animal	Hg	0.01	0.01				Andreani et al. 2019
Bat species unknown	U	U	Kenting National Park, Taiwan	Nature Reserve	Whole animal	Hg	0.25			0.10	0.49	Hsu et al. 2006
Bat species unknown	U	U	Kenting National Park, Taiwan	Nature Reserve	Whole animal	Mg	507.75			137.75	1585.00	Hsu et al. 2006
Bat species unknown	U	U	Kenting National Park, Taiwan	Nature Reserve	Whole animal	Mn	4.73			0.60	20.43	Hsu et al. 2006
Miniopterus schreibersii	CEF	I	Italy	African Quarter of Rome	Whole animal	Pb	0.31	0.12				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Whole animal	Pb	39.38	5.39				Andreani et al. 2019
Bat species unknown	U	U	Kenting National Park, Taiwan	Nature Reserve	Whole animal	Pb	1.13			0.65	2.49	Hsu et al. 2006
Miniopterus schreibersii	CEF	I	Italy	African Quarter of Rome	Whole animal	Sb	<LOQ	<LOQ				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Whole animal	Sb	0.08	0.03				Andreani et al. 2019
Bat species unknown	U	U	Kenting National Park, Taiwan	Nature Reserve	Whole animal	Sn	1.99			0.11	7.20	Hsu et al. 2006
Miniopterus schreibersii	CEF	I	Italy	African Quarter of Rome	Whole animal	Sr	28.20	5.30				Andreani et al. 2019

Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Whole animal	Sr	9.80	1.25				Andreani et al. 2019
Miniopterus schreibersii	CEF	I	Italy	African Quarter of Rome	Whole animal	Th	<LOQ	<LOQ				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Whole animal	Th	0.04	0.01				Andreani et al. 2019
Miniopterus schreibersii	CEF	I	Italy	African Quarter of Rome	Whole animal	Tl	<LOQ	<LOQ				Andreani et al. 2019
Tadarida teniotis/Miniopterus schreibersii	OAF/CEF	I	Italy	Mixed	Whole animal	Tl	0.06	0.04				Andreani et al. 2019
Bat species unknown	U	U	Kenting National Park, Taiwan	Nature Reserve	Whole animal	Zn	24.53			7.53	82.75	Hsu et al. 2006
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Wing	As		0.11		0.01	0.53	Mina et al. 2019
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Wing	Cd		0.02		0.01	0.06	Mina et al. 2019
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Wing	Co		0.08		0.03	0.81	Mina et al. 2019
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Wing	Cr		1.72		0.32	11.00	Mina et al. 2019
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Wing	Cu		4.51		0.60	13.69	Mina et al. 2019
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Wing	Mn		1.96		0.62	6.77	Mina et al. 2019
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Wing	Ni		1.94		0.36	80.82	Mina et al. 2019
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Wing	Pb		0.88		0.21	5.47	Mina et al. 2019
Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Wing	Se		0.71		0.18	2.74	Mina et al. 2019

Hypsugo savii/Nyctalus leisleri /Pipistrellus pipistrellus/P.pygmaeus	CEF	I	North and central Portugal	Wind farm	Wing	Zn		23.21		9.27	107.24	Mina et al. 2019

Supplementary Material

ICP-MS Analysis Detail from CAF, University of Stellenbosch.

Trace element analysis was performed on an Agilent 7900 quadrupole ICP-MS instrument equipped with a High Matrix Introduction (HMI) system to minimize matrix loading during sample introduction. This reduces the effect of high matrix ions on analyte measurement by first minimizing instrument drift over time (resulting from salt deposition on the interface parts), while the ionization efficiency in the plasma is also increased through reduction of easily ionized matrix elements.

The sample is introduced through a ~0.2ml/min concentric nebulizer into a Peltier cooled spray chamber, after which the argon dilution gas from the HMI configuration is added before introduction into the high temperature plasma. A 4th generation Octopole Reaction System (ORS), with He as collision gas and H2 as reaction gas is used to remove polyatomic interferences from the analytes of interest. Instrument conditions are summarized below in Table 1.

The instrument was optimized daily for sensitivity and low oxide ratios (CeO/Ce < 0.3%). B, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Sr, Mo, Cd, Sn, Sb, Ba, Hg and Pb were measured in He collision mode, while H_2 reaction gas was used for Se.

Calibration

NIST traceable multi-element stock solutions supplied by Inorganic Ventures (INORGANIC VENTURES, 300 Technology Drive, Christiansburg VA 24073) were used to prepare instrument calibration standards in 2% HNO_3 ranging from 1ppb–1000ppb. Single element Hg standards ranging fron 0.5ppb–5ppb were preprared in 2% HNO_3 + 2% HCl. Suprapur (65%) double distilled nitric acid (HNO_3) and Suprapur (30%) hydrochloric acid (HCl) were purchased from Merck KGaA, Darmstadt, Germany. Ultra-pure de-ionised water (18 MΩcm-1) used for dilution was produced by a Milli-Q® IQ Element (Merck KGaA, Darmstadt, Germany).

Initial calibration verification standards to confirm accuracy and calibration linearity was analysed directly after instrument calibration. NIST traceable multi-element standards and single element Hg standard from De Bruyn Spectroscopic Solutions, Bryanston, South Africa was used for this purpose.

Instrument drift and matrix effects were monitored and corrected by internal standard elements (^{45}Sc, ^{89}Y, ^{115}In, ^{72}Ge, ^{103}Rh) added automatically from a multi-element mixture in 2% HNO_3 to each sample and standard before introduction into the ICP-MS instrument.

Method validation

In order to evaluate if the selected digestion methods were efficient in collecting the extractable mineral content from the samples and can be accurately and reproducibly measured by ICP-MS, suitable reference materials are processed in the same way as the samples. The accuracy of the method is reported along with the results. Sample duplicates are included when enough material is available.

For the samples in this study, the following reference materials were used (Blood: Seronorm L2)

Table 1: Agilent 7900 optimised instrument parameters for trace element analysis in faeces and sediment samples

RF Power (W)	1600
Robustness mode	4x HMI
Carrier gas (L/min)	0.83
Dilution gas (L/min)	0.15
Sample depth (mm)	10
Micromist nebulizer flow (ml/min)	0.2
Oxide ratio	< 0.3%
ORS settings	
- He flow (ml/min)	4.8
- H2 flow (ml/min)	6
ICP-MS Software	Mass Hunter V 4.1