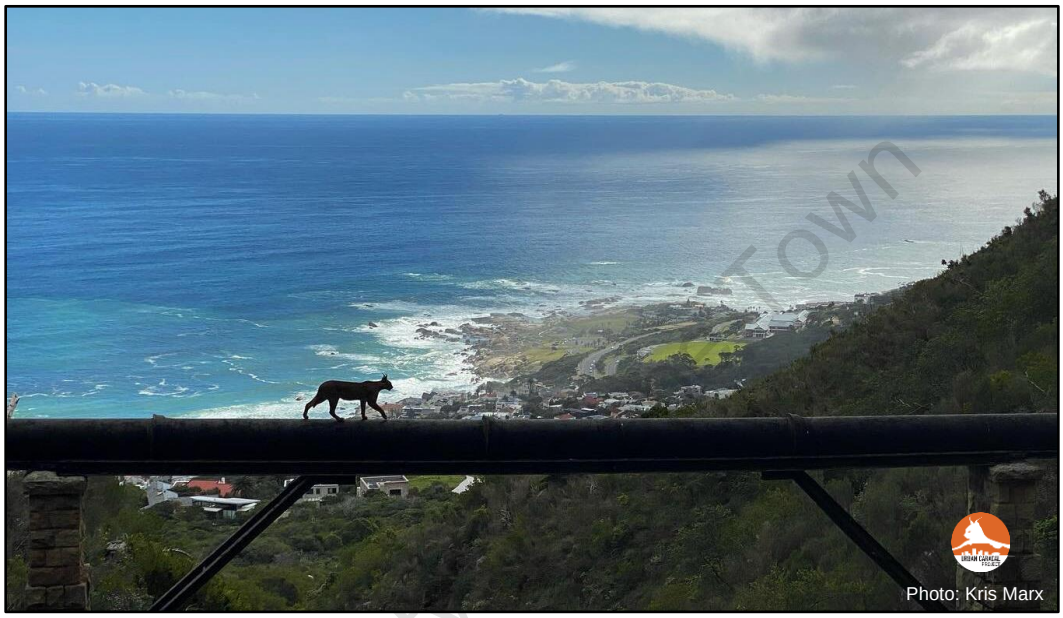
# Caracals as sentinels for metal exposure in a human-transformed landscape


## Kim Hélène Parker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# PLAGIARISM DECLARATION

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# ABSTRACT

Urbanisation and associated anthropogenic activity release large quantities of metallic elements and metalloids into the environment. Due to their toxicity, environmental persistence and bioaccumulative nature, these chemical pollutants threaten wildlife and human health. As urbanisation increases and top predators are forced to utilise more urban spaces, they will likely be exposed to more toxic heavy metals and metalloids. As secondary and tertiary consumers, terrestrial carnivores may be at increased risk of metal exposure through biomagnification effects. Relatively little is known about this emerging threat to terrestrial carnivores persisting in human-transformed landscapes, especially in the Global South. To better understand the extent of this issue, I quantified the level of metallic element and metalloid exposure in a sentinel top predator, the caracal (*Caracal caracal*), inhabiting the rapidly-urbanising, primarily coastal metropole of the Greater City of Cape Town, South Africa. Whole blood samples (n = 67) from GPS-collared (n = 25) and opportunistically sampled (n = 31) individuals were collected. Using Redundancy Analysis (RDA) and mixed-effect models, I explored the relative influence of caracal demography, landscape use, and diet on levels of individual exposure to 11 metallic elements and metalloids: Aluminium (Al), Arsenic (As), Cadmium (Cd), Chromium (Cr), Cobalt (Co), Copper (Cu), Mercury (Hg), Manganese (Mn), Lead (Pb), Selenium (Se), and Zinc (Zn). Mean metal concentrations (including Al, Cd, Co, Cu, Hg, Mn, Pb, Se and Zn) were generally found to be below toxic thresholds, however, As and Cr are present at potentially sublethal levels in certain individuals. The results suggest that increased use of human-transformed landscapes in home ranges (particularly urban areas, roads, and vineyards) was significantly associated with increased caracal exposure to Al ($P < 0.05$), Co ($P = 0.05$) and Pb ($P = 0.07$). Home ranges closer to the coast (RDA, $P < 0.05$) and feeding within aquatic food webs (marine and wetland-adapted prey) were associated with higher blood levels of Hg ($P < 0.05$), Se ($P < 0.01$) and As ($P < 0.05$). Further, increased predation on seabirds and aquatic birds likely facilitates the transfer of metals from aquatic to terrestrial food webs. Overall, these findings highlight the importance of urbanisation and anthropogenic activity as major environmental sources of metal exposure in terrestrial

wildlife, including more natural areas through long-distance transport of pollutants. Further, they contribute towards a growing global evidence base suggesting cities act as ecological traps for wildlife, threatening their long-term health and persistence in these landscapes. Therefore, it is crucial that cities work to reduce the large quantities of chemical pollutants released into their surrounding environment. In Cape Town, this effort could be focused on the urban edge, waste management, water treatments, roads and agricultural areas. Future research into a possible ecological trap for urban-adapted carnivores should focus on assessing metal exposure at different trophic levels and investigating the potential physiological responses of exposure in species. I argue that the caracal is a valuable sentinel for assessing metal exposure and should be used in tandem with other small and medium-sized carnivores and aquatic bird species in local and national pollutant monitoring programmes to mitigate further exposure and promote carnivore conservation in human landscapes. A charismatic species, with a well-established local platform of research and science communication, the caracal is centrally positioned to help promote greater public awareness and engagement in issues concerning environmental pollutant contamination and monitoring.

**Keywords:** *Caracal caracal*, ecotoxicology, heavy metals, metal exposure, sentinel species, urban wildlife

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# INTRODUCTION

The world is becoming increasingly urbanised (United Nations, 2018). Currently, approximately 55% of the world's population live or depend on urban areas, with an estimated increase to 68% by 2050 (United Nations, 2019). This global trend is responsible for rapidly transforming natural environments (Foley et al. 2005; Grimm et al. 2008), altering environmental processes, and reducing biodiversity through land transformation, the introduction of invasive species, over-exploitation of natural resources, and increasing pollution (Chown et al. 2010; Hill et al. 2020). As urban areas expand, wildlife (hereon specifically referring to animals) are increasingly forced to meet a highly novel set of challenges and threats (Ditchkoff et al. 2006; Baker & Harris, 2007; Lowry et al. 2013; Riley et al. 2014). Direct anthropogenic threats include vehicle collisions, hunting, retaliatory persecution, and disease spillover (Gantchoff et al. 2020). Wild species using urban areas also face challenges, such as restricted distributions and altered movement patterns due to habitat fragmentation and the presence of man-made physical barriers, as well as changes in resource availability (O'Donnell & delBarco-Trillo, 2020). Together, these threats and challenges have accelerated global biodiversity loss in and around urban centres (Ceballos et al. 2010; Ceballos et al. 2015). However, cities may also offer unique opportunities for species, such as access to anthropogenic food resources and reduced occurrence of indigenous top predators (Newsome et al. 2015; Wong & Candolin, 2015; Fleming & Bateman, 2018). As a result, some species persist and even thrive in urban landscapes by either adapting to or exploiting their changing environment (McKinney, 2006). Success or failure in the urban context depends largely on both behavioural flexibility (e.g., ability to change feeding patterns, habitat use, alert responses and nocturnal activity), and on the presence of certain innate personality traits (e.g., boldness), which may facilitate the continued ecological success of species (Lowry et al. 2013; Ritzel & Gallo, 2020). There is growing evidence to suggest that carnivores using urban areas possess many of these underpinning qualities and can therefore persist in changing landscapes (Bateman & Fleming, 2012; Franckowiak et al. 2019; Evans et al. 2021).

*Carnivores in an age of increasing urbanisation*

Terrestrial carnivores are increasingly forced to either adapt to human-transformed landscapes or face local extinction (Bouyer et al. 2015; Gantchoff et al. 2020, Hill et al. 2020). While large carnivores are generally highly sensitive to severe habitat fragmentation, which often results in local extirpation, many small and medium-sized carnivores (i.e., 'mesocarnivores') appear to be more resilient (Crooks, 2002; Bateman & Fleming, 2012; Streicher et al. 2021; Suraci et al. 2021). Species like coyote (*Canis latrans*; Lombardi et al. 2017), fox (*Vulpes vulpes*; Cypher, 2010), jackal (*Canis aureus*; Markov et al. 2016), bobcat (*Lynx rufus*; Riley et al. 2003), and caracal (*Caracal caracal*; Leighton et al. 2021) have successfully adapted to living in and around urban environments by adjusting aspects of their behavioural ecology to local conditions. For example, brown bears (*Ursus arctos*) shift activity patterns to maximise human avoidance (Ordiz et al. 2017). Human-transformed landscapes can also create opportunities for enhanced resource exploitation and habitat use (Fleming & Bateman, 2018; Larson et al. 2020). For example, coyotes have expanded their geographical range across North America by successfully utilising urban spaces (Hody & Kays, 2018; Wurth et al. 2020). Similarly, population densities of urban chacma baboon (*Papio ursinus*) have increased due to successful exploitation of anthropogenic food sources (Hoffman & O'Riain, 2012). Some species may even become synanthropic (i.e., forming a close association with humans) in urban areas, such as raccoon (*Procyon lotor*; Prange et al. 2003) and kit fox (*Vulpes macrotis*; Cypher, 2010), as the benefits they accrue influence their habitat selection. However, developing a preference for urban environments due to increased resource access may also expose species to greater risks, including human-wildlife conflict, and exposure to disease and harmful pollutants (Riley et al. 2007; Serieys et al. 2015; Moss et al. 2016; Schulte-Hostedde et al. 2018; Murray et al. 2019; Schell et al. 2021; Leighton et al. 2022).

*Urban landscapes as ecological traps for wildlife*

While wildlife may benefit from exploiting the urban edge, they may also be at risk of experiencing an "ecological trap" (Dwernychuk & Boag, 1972). Battin (2004) described ecological traps as "a low-quality habitat that animals prefer over other available habitats of higher quality".

Most wildlife use environmental cues to assess habitat suitability and maximise their survival potential (Hale & Swearer, 2016). However, human-induced environmental change may cause these cues to become unrepresentative of the true state of habitat quality. Alternatively, the animal may not be able to perceive cues for why the habitat is "bad" (Battin 2004; Smith et al. 2021). Rapid environmental change promotes the creation of ecological traps, as animals have less time to learn and adapt to changing cues, resulting in habitat selection errors (Kokko & Sutherland, 2001; Schlaepfer et al. 2002). Consequently, wildlife mistakenly opt to forage and reproduce in areas that are unfavourable for their long-term fitness (e.g., Hale et al. 2015; Vlaschenko et al. 2019; Kolenda et al. 2022). Within the urban context, ecological traps can occur when species perceive human-transformed habitats to be more favourable than natural ones (Schlaepfer et al. 2002). Accordingly, species choose to utilise these urban spaces, unknowingly exposing themselves to diverse anthropogenic threats. For example, the construction of stormwater wetlands in urban landscapes attract numerous aquatic and semi-aquatic biota (e.g., frogs and waterbirds) in search of natural refugia (Sievers et al. 2018). These systems, however, often become heavily polluted by chemicals from urban, agricultural, and vehicular sources, accumulating in wildlife (Xia et al. 2021). While the concept of ecological traps has been investigated for nearly fifty years, the geographical research focus remains limited. A recent review of available literature on urban ecological traps revealed a strong bias towards the Global North, with research focused in urban areas from North America and Europe (Zuñiga-Palacios et al. 2021). This excludes crucial, rapidly urbanising regions in Asia, Latin America and Africa, and which are generally more biodiverse (Brooks et al. 2002; Shackleton et al. 2021). There clearly remains large knowledge gaps in understanding the phenomenon of ecological traps in human transformed landscapes across the developing world, particularly in the Global South (Kong et al. 2021; Shackleton et al. 2021).

*Urban wildlife exposure to environmental pollutants*

An often-overlooked threat to both humans and wildlife is environmental pollutants (Newman & Schreiber, 1988; Riley et al. 2014; Landrigan et al. 2018). Pollution, in all its forms, is the greatest

environmental cause of disease and premature death in humans (reviewed in Landrigan et al. 2017). Nevertheless, this issue has repeatedly failed to receive appropriate levels of attention in global health agendas and international development plans (reviewed in Fuller et al. 2022). Environmental pollutants are harmful materials that degrade the chemical, physical and biological quality of the natural environment (Wasi et al. 2013). Chemical pollution is a growing global concern, occurring when chemical substances (both natural and synthetic) are released into the environment in excess by anthropogenic activity (Steffen et al. 2015; Persson et al. 2022). Cities and rapidly developing countries are disproportionately affected by chemical pollution due to high levels of industrial and anthropogenic activity (Landrigan et al. 2017). Environmental chemical pollutants are often not detectable by wildlife, and prolonged exposure results in their accumulation in bodily fluids and tissues (Ortiz-Santaliestra et al. 2015; Rodríguez-Estival et al. 2019) with potentially lethal and sublethal effects (Hale et al. 2015; Huang et al. 2018). Negative health impacts to wildlife include reduced body condition, neurological damage, reproductive impairment, immunosuppression, endocrine disruption, and increased susceptibility to infectious and non-infectious disease (Scheuhammer et al. 2007; Acevedo-Whitehouse & Duffus, 2009; Sánchez-Virosta et al. 2018; Murray et al. 2019). Some of the most well-studied environmental pollutants include pesticides, including rodenticides and several persistent organic pollutants (POPs), pharmaceuticals, personal care products (PPCPs) and metals (Rodríguez-Jorquera et al. 2017). Urban wildlife are at particular risk of exposure to these pollutants as they may feed within synanthropic (i.e., human-associated) food webs and utilise human-transformed habitats (Riley et al. 2007; Elliot et al. 2015; Guetté et al. 2017). While originating predominantly from urban and industrial centres, some chemical pollutants can be transported over long distances, for instance, through long-range atmospheric transport (Marx & Mcgowan, 2010). Therefore, wildlife living in less disturbed or remote locations may be similarly affected by these contaminants (Zhang et al. 2019b). For example, polar bears (*Ursus maritimus*) are among the many Arctic wildlife known to accumulate high concentrations of long-range pollutants such as POPs, perfluoroalkyl compounds (PFCAs) and mercury (Dietz et al. 2009; Routti et al. 2019).

Among environmental chemical pollutants, metals are among the most ubiquitous, toxic, and well-studied (Mitra et al. 2022). Heavy metals are high-density, metallic chemical elements or metalloids which may become toxic even at low concentrations (Duffus, 2001). Most heavy metals are naturally occurring in the Earth's crust, however, when they are released into the environment at unnaturally high quantities, they may become hazardous (Tchounwou et al. 2012). Certain anthropogenic activities (e.g., agriculture, industrial processing, mining, and poor waste disposal) enhance the release and bioavailability of heavy metals in the environment (Tchounwou et al. 2012; Briffa et al. 2020). Of all the chemical elements, heavy metals including Chromium (Cr), Mercury (Hg), Lead (Pb) and Cadmium (Cd), as well as certain metalloids like Arsenic (As) remain the most widely studied, as they are considered a substantial threat to human and animal health (ATSDR, 2019; Balali-Mood et al. 2021). Metals are a growing public health concern due to their toxicity, non-biodegradable chemistry, and ability to accumulate in plants, animals, and people (Jaishankar et al. 2014; Mitra et al. 2022). While humans become exposed through multiple pathways, including air pollution (Moja & Mnisi, 2013), contaminated drinking water (Custodio et al. 2020), agricultural crops (Okereafor et al. 2020), as well as through workplace exposure (e.g., in mines, coal-burning factories, smelters and landfills), wildlife are mostly exposed through dietary pathways (Gall et al. 2015; Treu et al. 2022). Toxic levels of metals have been linked to food webs that are connected to human activity (Mathee, 2014; Sabouhi et al. 2020).

Metallic elements and metalloids generally enter the terrestrial food web through soil (reviewed in Gall et al. 2015). Primary exposure pathways may include atmospheric deposition of metal-containing particles, runoff from industrial and urban activities and direct application of chemical products, like fertilisers and pesticides (Wuana & Okieimen, 2011). As a result, lower trophic level biota, such as plants, macroinvertebrates (e.g., earthworms, spiders, and insects) and small soil-dwelling organisms are usually the first to become contaminated (Heikens et al. 2001; Olowoyo et al. 2010; Verma et al. 2021). When birds, mammals, herpetofauna, amphibians and humans

feed on these lower trophic level species they too become exposed, bioaccumulating pollutants in their bodily fluids and tissues (Heikens et al. 2001). In this way, pollutants are biomagnified (defined as the enrichment of chemical compounds in the tissue of organisms) up the food chain (Gobas et al. 1993). Toxic levels (i.e., concentrations inducing adverse health effects) in terrestrial wildlife are generally recorded in species which utilise landscapes that have been contaminated by an anthropogenic point source. Point sources may include coal power plants, metal smelters, mines, oil spills and waste disposal sites (e.g., landfills or illegal dumping sites; Tersago et al. 2004; Hernández et al. 2017; Osibote & Oputu, 2020; Custodio et al. 2020). Further, Pb exposure in wildlife due to spent ammunition from hunting (Burco et al. 2012; North et al. 2015; Naidoo et al. 2017) and contamination through use of agricultural areas (Markov et al. 2016; Soliman et al. 2019; Evans et al. 2021) has also been widely reported. Even if a point source is removed from the environment, some metallic elements like Pb continue to persist for long periods of time (Scheifler et al. 2006). Moreover, the trophic transfer of metals means that species that are spatially removed from contaminated sites may still be impacted by metal contamination in terrestrial and aquatic ecosystems (Gall et al. 2015; Soliman et al. 2022).

Within global metal contamination research, the focus has largely been on bioaccumulation of Hg, As and Pb in humans, birds and marine mammals (Kubota et al. 2001; Chen et al. 2008; Bossart, 2011; Summers et al. 2014; Naidoo et al. 2017). Aquatic environments generally act as sinks which accumulate and locally concentrate these metallic elements and metalloids (Weiss-Penzias et al. 2019). Due to anoxic conditions in water, metals may be converted into more toxic and bioavailable forms (e.g., mercury converted to methylmercury). Thereafter, they may be readily taken up by biota and biomagnified up and across food webs (Gobas et al. 1993; Scheuhammer et al. 2007). These findings suggest that aquatic species and those with a predominately aquatic-based diet may be at higher risk of exposure to certain metals (Ishii et al. 2017), where contamination may start within marine and freshwater food webs but can easily spill over into terrestrial food webs (Cristol et al. 2008). For example, marine-derived seabird guano, transported by breeding colonies of seabirds, has contaminated terrestrial plants and soils

309 with a variety of pollutants (Choy et al. 2010). The primary mechanism of pollutant transfer
310 between the aquatic and terrestrial realm, however, is diet, where terrestrial consumers feed on
311 aquatic species like fish, seabirds, waterbirds, aquatic insects, and amphibians (Rimmer et al.
312 2010; Becker et al. 2018). This phenomenon is well-documented in otters (*Lontra canadensis*)
313 and wild mink (*Mustela vison*), whose high Hg burdens have been linked to their predominately
314 fish-based diet (Yates et al. 2005; Josef et al. 2008; Haines et al. 2010). Similarly, humans have
315 been exposed to aquatic Hg and As by consuming contaminated fish and shellfish (Kar et al. 2011;
316 Sheehan et al. 2014; Petrova et al. 2020).
317
318 In South Africa, research into the presence of environmental pollutants reveals widespread
319 metallic element and metalloid contamination (especially Pb, Zinc (Zn), Copper (Cu), Cd, Cr, Hg
320 and As) in air (Moja & Mnisi, 2013), soil (Malan et al. 2014; Osibote & Oputu, 2020), plants (Krüger
321 et al. 2019; Ndlovu et al. 2019), freshwater (Jackson et al. 2009; Gilbert, 2015), marine systems
322 (Bosch et al. 2015; Sparks et al. 2017), agricultural crops (Meerkotter et al. 2012; Naicker et al.
323 2013), wildlife (Ansara-Ross et al. 2013; Pheiffer et al. 2014; North et al. 2015; Naidoo et al. 2017)
324 and people (Naicker et al. 2013). Despite these findings, metallic elements, and metalloids are
325 not currently included in South Africa's national air and water quality monitoring programmes
326 (Western Cape Department of Environmental Affairs and Development Planning, 2019; City of
327 Cape Town, 2020). Thus, the scale of metal contamination in the country and its potential risks
328 to human and animal health is likely underestimated. The Global One Health (OH) initiative aims
329 to achieve optimal global health by acknowledging the interconnectedness between humans,
330 animals and the environment (van Helden et al. 2013). This holistic framework includes
331 addressing chemically mediated health hazards such as emerging and infectious diseases (EIDs)
332 tracking (Gebreyes et al. 2014; Rabinowitz et al. 2013) and more recently, environmental
333 contaminant monitoring (Beasley, 2009). It advocates for the use of animal biomonitoring and
334 sentinel species to identify, manage, and mitigate risks posed by pollutants (Frazzoli et al. 2015;
335 Amadi et al. 2020). In this way, South Africa may benefit from adopting a One Health approach,
336 using sentinel species to monitor and assess pollutant contamination in its cities.

*Top predators as sentinels for metal exposure in transformed landscapes*

Sentinel species are defined as biological monitors that accumulate a contaminant in their tissues without significant adverse effect (reviewed by Beeby, 2001). These species accumulate and store pollutants from their surroundings (where exposure may be through air, water, soil, and diet) in their tissues (van der Schalie et al. 1999). Samples taken from these individuals can be analysed to measure the diversity, occurrence, and concentration of pollutants in an area (Bossart, 2011). Thus, sentinel species can act as early warning signs for environmental changes caused by pollutants (Stahl, 1997). Animal sentinels are important in the health sciences, providing crucial information on the presence, diversity, and level of pollutants in the environment (Basu et al. 2007; Frazzoli et al. 2015). Many different animal groups have been used as sentinels, including invertebrates (Heikens et al. 2001; Solé et al. 2009), birds (Grove et al. 2009; Gómez-Ramírez et al. 2014), fish (Bervoets et al. 2013; Fernández-Trujillo et al. 2021), marine mammals (Kubota et al. 2001; Bossart, 2011) and even domestic animals (van der Schalie et al. 1999; Esposito et al. 2019). Effective sentinels are usually positioned at the top of the food chain, have a relatively long lifespan, occur across large spatial areas and can bioaccumulate substances (O'Brien et al. 1993; Frazzoli et al. 2015). Terrestrial carnivores generally fulfil these requirements and are therefore considered ideal sentinels to assess environmental contamination in wildlife and humans (Espín et al. 2016; Harley et al. 2016; Rodríguez-Jorquera et al. 2017).

Carnivores play an integral role in ecosystem function, influencing prey abundances and assemblages in food webs (Ripple et al. 2014). Located at the top of the food web, these species are disproportionately exposed to pollutants through biomagnification (Gobas et al. 1993; Bateman & Fleming, 2012). Therefore, if pollutant exposure were to cause adverse population-level effects in carnivores, it may compromise the integrity of whole ecosystems (Rodríguez-Jorquera et al. 2017). Accordingly, carnivores are used to study bioaccumulation and trophic transfer of pollutants in terrestrial ecosystems (Yates et al. 2005; Ortiz-Santaliestra et al. 2015;

Badry et al. 2019). They have also been used to investigate human health risks, as humans generally share similar environments, biological systems, and food webs with terrestrial carnivores (van der Schalie et al. 1999; Basu et al. 2009; Esposito et al. 2019; Brand et al. 2020). For example, in southern Italy, an environmental biomonitoring programme uses stray domestic cats and dogs to assess metal contamination in its cities (Esposito et al. 2019). Wild carnivores like the red fox have fulfilled the same purpose in this region (Naccari et al. 2013). Recently, researchers have advocated for the use of raptors as sentinels to track pan-continental contaminant trends (Espín et al. 2016; Movalli et al. 2017). There is also increasing interest in using carnivores as indicators for anthropogenic chemicals, such as metals, in transformed and semi-transformed landscapes (Serieys et al. 2015; Markov et al. 2016; Rodríguez-Estival & Mateo, 2019; Evans et al. 2021). Specifically, they have been used to quantify the occurrence, level, and trophic transfer of metals, as well as to help elucidate possible exposure pathways. As urbanisation increases and terrestrial carnivores are forced to utilise these highly modified spaces, they will likely be exposed to more metallic elements and metalloids. This exposure threatens their persistence in these landscapes and it is therefore crucial to better understand the extent of this issue to conserve urban carnivores and establish strategies to mitigate pollutant exposure.

*Caracal in Cape Town: a sentinel for metal exposure in a rapidly expanding city?*

The caracal is a medium-sized felid with a large geographical range covering large portions of Africa, the Arabian Peninsula, and into central and southwest Asia (Avgan et al. 2016). Caracals are generalist predators preying on a wide variety of small to medium-sized mammals, birds, herpetofauna and insects (e.g., Grobler, 1981; Melville & Bothma 2006; Braczkowski et al. 2012). The species is found throughout southern Africa, occupying a diverse range of habitats (Avenant & Nel, 2002) including pristine natural (Melville et al. 2004), highly transformed urban areas (Serieys et al. 2019), and rural agricultural landscapes (Marker & Dickman, 2005; Ramesh et al. 2017). Their broad range, diverse prey base (Avenant & Nel, 2002) and high incidence of human-

392 wildlife conflict (Nattrass & O'Riain, 2020; Nieman et al. 2020) suggest that caracals may have a
393 generally higher tolerance for human disturbance (Avenant & Nel, 2002; Fraser, 2022).
394
395 In the city of Cape Town, South Africa, caracals have successfully adapted to living on the urban
396 edge, and in the absence of any larger carnivore species they are the *de facto* apex predator of
397 this rapidly transforming landscape. Leopards (*Panthera pardus*) were formerly present on the
398 Cape Peninsula but extirpated in the early 20th century (van Sittert, 1998). Since 2014, Cape
399 Town's caracals have been studied using a multi-disciplinary approach to better understand how
400 urbanisation threatens wildlife across South Africa's cities, and other parts of the world similarly
401 affected by urbanisation (see urbancaracal.org). Caracals around Cape Town face many novel
402 physiological stressors, including exposure to anticoagulant rodenticides (AR), parasites
403 (*Toxoplasma gondii*) and tick-borne blood pathogens, and organochlorine pollutants, largely
404 through dietary and landscape use pathways (Serieys et al. 2019; Viljoen et al. 2020; Leighton et
405 al. 2022). Analysis of organochlorines in caracal tissues reveals exposure to pollutants, such as
406 dichloro-diphenyl-42 trichloroethane (DDT) and polychlorinated biphenyls (PCBs), likely due to
407 an apparent selection by caracals to forage nearer to the urban edge when available (Leighton et
408 al. 2022). There is also evidence suggesting these contaminants may have important physiological
409 effects on individual caracals (Leighton et al. 2022). Widespread metal contamination via urban
410 point sources characterise many areas in South Africa (e.g., coal-fired power plant emissions,
411 Dabrowski et al. 2008; mining effluent, Ebenebe et al. 2017; contaminated indigenous forest
412 patches in Cape Town, Krüger et al. 2019), making it likely that caracals and other wildlife in and
413 around Cape Town may also be at risk of exposure to metallic elements and metalloids through
414 similar exposure pathways (Van Wyk et al. 2001; Ansara-Ross et al. 2013).
415
416 *Aims & Objectives*
417 The aims of this study are to (a) determine the presence and levels of metallic elements and
418 metalloids in Cape Town's caracal population, and (b) understand the relative roles of
419 demographics, diet, and landscape use in the spatial patterns of exposure. I address these aims

by answering three main questions: (i) how do demographic characteristics (age and sex) influence risk of caracal exposure to metallic elements and metalloids?, (ii) how does caracal land-use influence caracal risk of exposure? and, (iii) how do dietary pathways influence risk of caracal exposure to metallic elements and metalloids? To achieve this, levels of metallic elements and metalloids were quantified in caracal tissue samples using mass spectrometry. Metal concentrations were identified at lethal or sublethal levels based on thresholds reported in the literature. Potential risk factors (spatial, dietary and life history) for metal exposure were then explored using a model-based framework. The results are discussed in the context of other small-to-medium sized carnivores inhabiting human-transformed landscapes and known to become exposed to metals through similar pathways. Finally, I make suggestions for possible mitigation strategies and future research opportunities based on the exposure pathways identified.

# METHODS

*Study area*

Caracals occurring in and around the metropole of the City of Cape Town (hereafter referred to as the Greater City of Cape Town, GCT), Western Cape, South Africa (−33.942989, 18.630957) were sampled and tested for exposure to a suite of known metallic elements and metalloids associated with human-modified landscapes. The GCT spans approximately 470 $km^2$ with an estimated human population of 4.8 million, with ~ 1,530 people per $km^2$ (worldpopulationreview.com). The area comprises a mosaic of both human-transformed and natural areas (Fig. 1). The dominant land-use types include urban, industrial, agricultural, and informal settlements (townships). A significant portion (approximately 220 $km^2$) of the GCT's physical landscape includes Table Mountain National Park (TMNP), dominated by the Table Mountain Chain and a coastline of > 300 km (WCG, 2019). The Cape Peninsula is almost entirely bounded by the Atlantic Ocean and by urban sprawl to the east (Fig. 1). The TMNP is a natural protected area which aims to conserve the majority of the GCT's highly biodiverse fauna and flora (Holmes et al. 2012). The city also has a well-established Biodiversity Network (Bionet)

connecting indigenous biodiversity to urban 'green' open spaces via a network of ecological corridors (Bux et al. 2021) with the conservation management aim of enhanced movement and gene flow of wildlife (Holmes & Pugnalin, 2016).

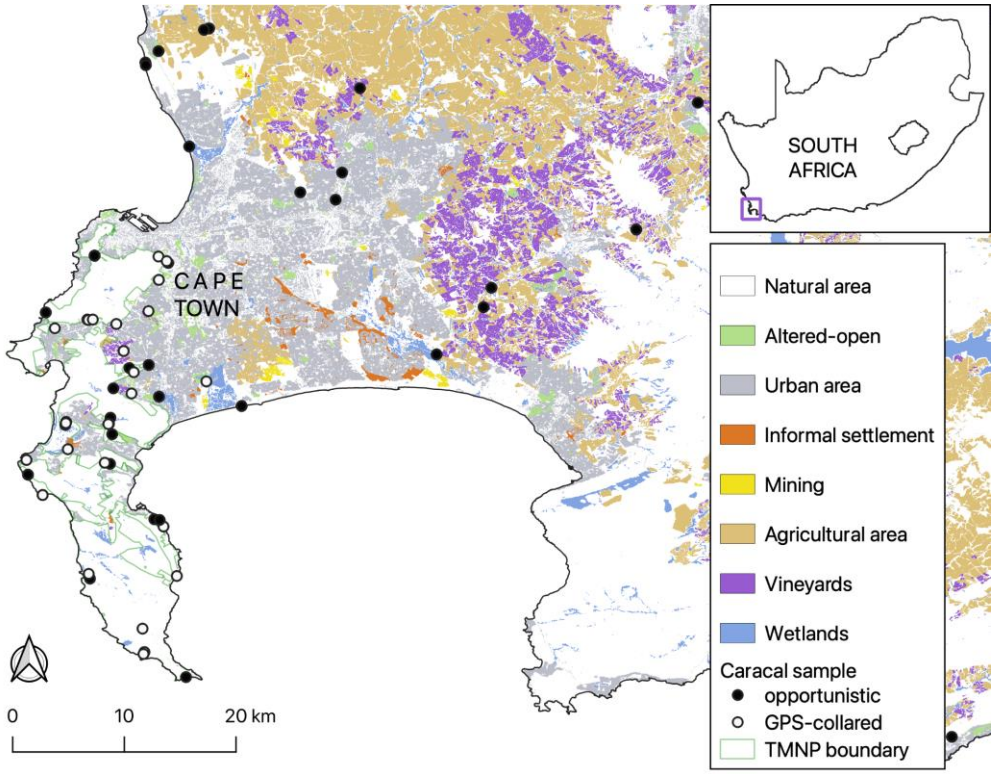



**Fig.1.** Map showing locations of individual caracals sampled for pollutants in the Greater Cape Town area, South Africa. White circles represent locations of caracal individuals that were trapped and monitored with GPS collars, and therefore have estimated home ranges. Black circles are opportunistically sampled individuals with buffers calculated around collection locations. Coloured areas correspond to different land-uses. The green line denotes the Table Mountain National Park (TMNP) boundary.

*Capture and sampling of caracals*

Standard cage-trapping techniques were used to humanely capture and GPS-collar caracals (n = 25) between 2014 – 2017 under veterinary supervision (detailed capture and collaring method described in Serieys et al. 2019 and Leighton et al. 2022). Individuals were fitted with GPS collars (Followit$^{TM}$ Tellus, Lindesberg, Sweden) which recorded their GPS locations at three-hour intervals. Collars had a drop-off mechanism that was activated within six months of collar fitting

and were also equipped with a rot-off cotton spacer to ensure eventual drop-off. Samples were also opportunistically collected from caracal carcasses (n = 31) collected from throughout the study area and necropsied. These individuals died from a variety of causes, including disease, poaching, conspecific and domestic dog attacks, as well as suspected poisoning, but most were from vehicle collisions. A total of 67 blood samples were taken during captures, recaptures, and necropsies of 53 unique individuals (Fig. 1). Animal capture, handling, and sampling protocols followed ethical guidelines approved by the American Society of Mammalogists and the University of Cape Town Animal Ethics Committee (2014/V20/LS). Samples were collected under permits from Cape Nature (AAA007-0147-0056), and South African National Parks (SERL/AGR/017–2014/V1).

*ICP-MS elemental analysis*

This study focuses on 11 metallic elements and metalloids, commonly analysed in wildlife studies: Aluminium (Al), As, Cd, Cr, Cobalt (Co), Cu, Hg, Manganese (Mn), Pb, Selenium (Se), and Zn. These elements include heavy metals (Cd, Cr, Hg and Pb), metallic elements (Al, Co, Cu, Mn, and Zn) and metalloids (As and Se). I selected metallic elements and metalloids based on the following criteria: (i) their relative level of toxicity and potential to cause adverse health effects in wildlife and humans at excess concentrations; (ii) those metals that are the focus of other relevant literature on metal exposure in carnivores and top predators; and (iii) metals with an established association with human-transformed landscapes.

Pollutants may enter the body through multiple pathways, such as ingestion, dermal contact, and inhalation. However, irrespective of exposure route, these pollutants are absorbed and distributed throughout the body in blood (Espín et al. 2016). Whole blood has been used to assess metal exposure in a variety of sentinel species (Van Wyk et al. 2001; Bauerová et al. 2020; Liu et al. 2020). In this study, metallic element and metalloid concentrations were determined in caracal whole blood using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) following methods described by Rodríguez-Estival et al. (2011). ICP-MS is used to measure elements at trace levels in biological fluids, and with its multi-element capability allows for multiple elements

503 to be measured simultaneously in a single analysis (Wilschefski & Baxter, 2019). Briefly, samples
504 were lyophilised (Christ Alpha 1–2, Braun Biotech) and then acid-digested in Pyrex tubes and
505 analysed for all 11 metallic elements and metalloids. Blood samples of < 100 mg were first
506 weighed and then added to pre-weighed tubes. These samples were digested with 1 ml of $HNO_3$,
507 1 ml of $H_2O_2$ and 11 ml of $H_2O$ (Milli-Q grade) and then placed in a microwave oven (Ethos E,
508 Milestone). The microwave conditions were as follows: for 1.5 minutes samples were ramped up
509 from ambient temperature to 85 ºC, for 2 minutes held at 135 ºC, then for another 1.5 minutes
510 at 230 ºC and held at this temperature for a further 15 minutes. Digested samples were then
511 diluted to a final volume of 50 ml with Milli-Q $H_2O$. Samples were analysed on an ICP-MS Agilent
512 A800 using an autosampler SPS-4. Finally, the samples and three replicates, were analysed with
513 ISIS (Integrated Sample Introduction System) at RF 1550 W at 100 sweeps/replicate. The limits of
514 detection (LOD, in µg/g dry weight) for all metallic elements and metalloids were as follows:
515 0.244 (Al), 0.002 (As), 0.001 (Cd), 0.026 (Cu), 0.002 (Co), 0.018 (Cr), 0.002 (Hg), 0.004 (Mn), 0.077
516 (Se), < 0.001 (Pb), and 0.019 (Zn). Final metal concentrations are reported in µg/g dry weight
517 (d.w.).
518
519 *Statistical Analysis*
520 *i. Landscape use and associated risk factors for metal exposure*
521 Individual caracals utilise habitats differently, which may in turn influence their exposure and
522 subsequent accumulation of metals (Serieys et al. 2019; Leighton et al. 2021). I investigated
523 whether land-use types (e.g., urban development, agriculture or mining; Appendix I: Table A)
524 were associated with variation in metallic element and metalloid concentrations in caracal blood.
525 For example, As, Pb, Zn, Cu and Cd are expected to be higher in agricultural areas and vineyards
526 due to current and historical use of agricultural products (e.g., fertilisers, pesticides and
527 herbicides) which are known to contain these metals (He et al. 2005; Markov et al. 2016;
528 Uwizeyimana et al. 2017). Metallic elements like Pb, Al, Zn, Cr and Mn are expected to be higher
529 in urban and industrial areas or close to roads. These metallic elements are commonly found in
530 urban and industrial waste and road and stormwater runoff from a variety of sources, such as

historic use of leaded fuel, petrol additives, brake abrasion and tyre wear (de Villiers et al. 2010; Apeagyei et al. 2011; Adamiec et al. 2016; Krüger et al. 2019). Proximity to the coast was explored as research suggests that terrestrial species may become exposed to metallic elements and metalloids like As, Hg, Se and Cd when feeding within marine food webs (Scheuhammer et al. 2007; Dietz et al. 2022).

Caracal land-use was measured within 95% Local Convex Hull home ranges estimated using the spatial movement data (n = 36; see Leighton et al. 2022). For opportunistically sampled caracals (n = 31), land-use variables were calculated within a circular buffer around the GPS location where the individual was found, following Leighton et al. (2021). Previous research has found that caracal home ranges significantly varied amongst sex and age classes (Serieys et al. *in review*), therefore different buffer sizes were assigned for females (mean home range = 16.2 $km^2$), adult males (69.9 $km^2$) and subadult males (38.3 $km^2$). The land-use types considered were the proportion urban, agricultural, vineyard, wetland, informal settlement, mining, natural area, and altered-open area using the 2018 Department of Environmental Affairs (DEA) national land-cover raster. Altered-open areas included urban recreational fields, golf courses, fallow land, and old fields, abandoned quarries and landfill sites. Additionally, we included the mean distance to the coast, human population density, and road density (see Appendix I: Table A for details). Binary rasters were created for each land-use type, and density rasters for human population and roads (Appendix I: Table A). The proportion of each land-use variable and the mean distance to coast and the mean population and road density within individual caracal home range polygons or buffer circles was then calculated.

*ii. Dietary risk factors for metal exposure*

Diet is recognised as a major exposure pathway for metals and other pollutants in top predators (Millán et al. 2008; Kitowski et al. 2016; Garcia-Heras et al. 2018). A detailed dietary dataset (Appendix I: Table B; Leighton et al. 2020), which integrates both scat and caracal feeding sites located at GPS clusters of collared individuals, was used to investigate which dietary pathways

559 hold the highest metal exposure risks. Diet proportions were based on both biomass (BIO) and
560 frequency of occurrence (FO) of prey per caracal individual (Appendix I: Table B). Dietary patterns
561 were assessed by assigning prey into different groups and calculating their proportion in diet,
562 categorised as follows: broad food groups (proportion bird, mammal, reptile, insect), prey
563 functional groups (carnivore, insectivore, granivore, herbivore, omnivore and marine feeder),
564 prey foraging habitat type (i.e., in terrestrial, wetland, arboreal and marine environments) and
565 finally, an urban grouping of level of human association (i.e., wild, exotic or synanthropic prey).
566 Exotic prey included introduced species, domestic animals, and livestock. Synanthropic prey are
567 indigenous, wild species that live close to and benefit from humans (Guetté et al. 2017).
568
569 *iii. Correlations and associations among metal concentrations and potential source*
570 *identification*
571 I calculated descriptive statistics for the raw caracal whole blood metal concentration dataset
572 (Appendix II: Table A). I then performed a correlation analysis on this dataset to identify possible
573 associations among metallic elements and metalloids by constructing a Pearson's correlation
574 matrix (PCM). Correlation analysis is a relatively simple and convenient way of identifying
575 potential sources of pollutants like metals in the environment (Shan et al. 2013). It can provide
576 preliminary information about possible exposure pathways, as well as help confirm source
577 identification through other methods of analysis (Zhang et al. 2019a). Theoretically, if two metals
578 are highly significantly correlated, they likely originated from the same source. Similarly, if they
579 are negatively correlated, they likely originated from different sources (Chen et al. 2019).
580 However, it is important to note this type of correlation analysis has limitations. Most notably, it
581 assumes all relationships are linear and cannot distinguish between "cause" and "effect"
582 amongst variables (Aggarwal & Ranganathan, 2016). As metal concentrations were non-normal
583 (Shapiro-Wilk's $p < 0.001$), we natural log-transformed the data.

584 *iv. Redundancy analysis*

To assess the effects of land-use on metallic element and metalloid concentrations I first performed a detrended correspondence analysis (DCA) using the *vegan* package in R. This analysis revealed a gradient length of <3 for axis 1, thus, I used a model with a linear response curve (Ortiz-Santaliestra et al. 2015). Next, I conducted a redundancy analysis (RDA) to understand the broad influence of land-use explanatory variables on all caracal blood metal concentrations (ter Braak, 1994). Redundancy analysis (RDA) is a method of constrained ordination which aims to explain the variation in a set of response variables by a set of explanatory variables (ter Braak, 1994). Dietary variables were not included as this would significantly lower the sample size (n = 34) and thus the power of the model.

I assessed multicollinearity in all land-use explanatory variables (Appendix I: Table A) and kept only those considered independent (VIF < 8.5; Kim, 2019). Continuous explanatory variables were scaled and centred. I then performed a forward selection using the "ordistep" function in the *vegan* package to extract only those variables which were found to be significant predictor variables of metal concentrations. I then used these variables to build the final models which were selected using AICc scores. Significance testing of explanatory covariates was performed using a Monte Carlo Permutation test (1000 permutations).

*v. Linear and linear mixed-effects models*

*a) Demographic variables*

Leighton et al. (2022) found that juvenile male caracals had significantly higher blood organochlorine levels than other age classes, likely because they utilised more synanthropic prey. Therefore, it is likely that metal exposure may also vary with caracal demography. I therefore investigated the effects of demography (a composite of age and sex) on natural log-transformed metallic element and metalloid concentrations, using linear models (Fig. 2).

b) *Land-use, and dietary variables*

To investigate which variables best explained variation in the metallic elements and metalloids of interest, I ran linear mixed-effects models on land-use variables, and linear models on dietary

variables (Fig. 2). I then used AICc scores to identify the best model based on all combinations of the subset of relevant predictor variables we selected. To incorporate all caracal samples (those with known home ranges and opportunistically sampled) I included the spatial variable extraction method (home range area for collared individuals and buffer area for opportunistic mortalities) as a binary (home range = 1, buffer = 0) condition (random effect) in land-use RDA and linear models where necessary (i.e., for Cd, Hg, Mn and Se models). To avoid singularity issues, this random effect was not included in all models, as some datasets were unaffected (random effect variance = 0). All statistical analyses were performed in R version 4.1.0 (R Core Team, 2022) through the RStudio (version 1.1.453) interface.

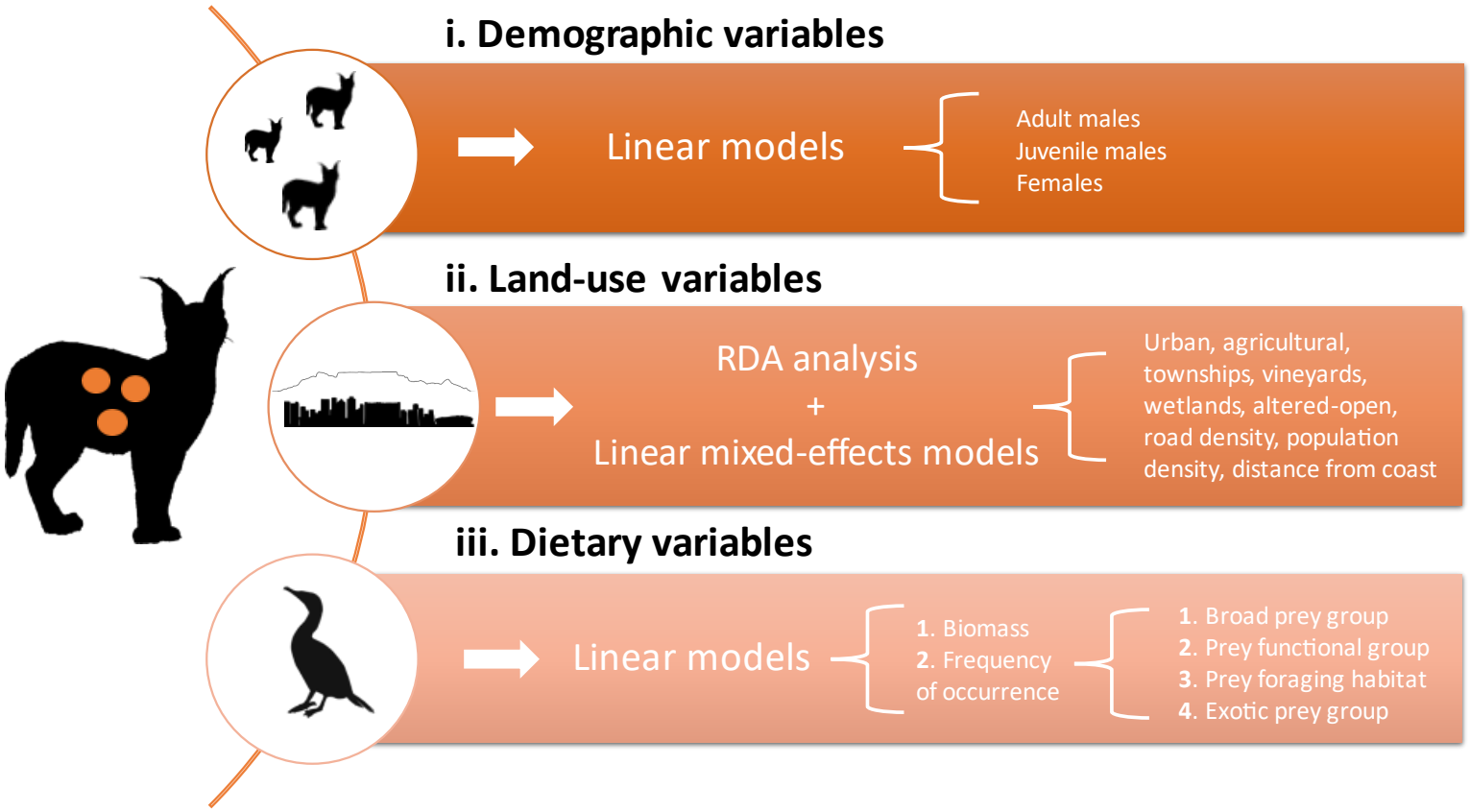



**Fig. 2.** Graphic demonstrating the method of statistical analysis used to assess i) demographic variables, ii) land-use variables, and iii) dietary variables influencing the presence of metallic elements and metalloids in caracals

## RESULTS

*Caracal exposure to metals in study area*

Metallic element and metalloid concentrations varied between individuals of the Greater City of Cape Town caracal population by as much as two orders of magnitude between means in certain metals (see additional descriptive statistics of raw data in Appendix II: Table A). Mean concentrations of the 11 focus metallic elements and metalloids in descending order were as follows: Zn > Al > Cu > Mn > As > Se > Hg > Cr > Cd > Pb > Co. Mean concentrations of metallic elements and metalloids with well-established biological functions (i.e., Co, Cu, Mn, Se, and Zn)

were, on average, more variable (based on standard deviations; refer to table of raw data in Appendix II: Table A) than those considered toxic heavy metals (i.e., Cd, Cr, Hg and Pb).

*Correlations and associations among metals*

A total of 38 significant and marginally significant ($P > 0.05$ with the potential of being of biological importance) positive associations were found among metals (Fig. 4). Highly significant positive correlations ($P \leq 0.001$) included: Al - Co (r = 0.61), Al - Pb (r = 0.68), Cd - Hg (r = 0.76), Co - Mn (r = 0.84), Co - Zn (r = 0.67), Cu - Mn (r = 0.80), Cu - Zn (r = 0.82) and Mn - Zn (r = 0.79). There was also a significant positive correlation between Hg - Se (r = 0.58; Fig. 5). No negative associations were found among metals. See all results of the PCM in Appendix III, Table A.

*Demographic risk factors*

Linear models (see full results in Appendix V: Table A) were used to explore the associations between metal concentrations and caracal demography. Linear models for demographic variables revealed that caracal demography significantly influenced the level of Al ($\beta = 0.68 \pm 0.32$, $P < 0.1$) and Mn ($\beta = 0.51 \pm 0.18$, $P < 0.05$) in caracal blood (Fig. 6). On average, juvenile males had a significantly higher level of Al and Mn than adult males and females (Fig. 6).

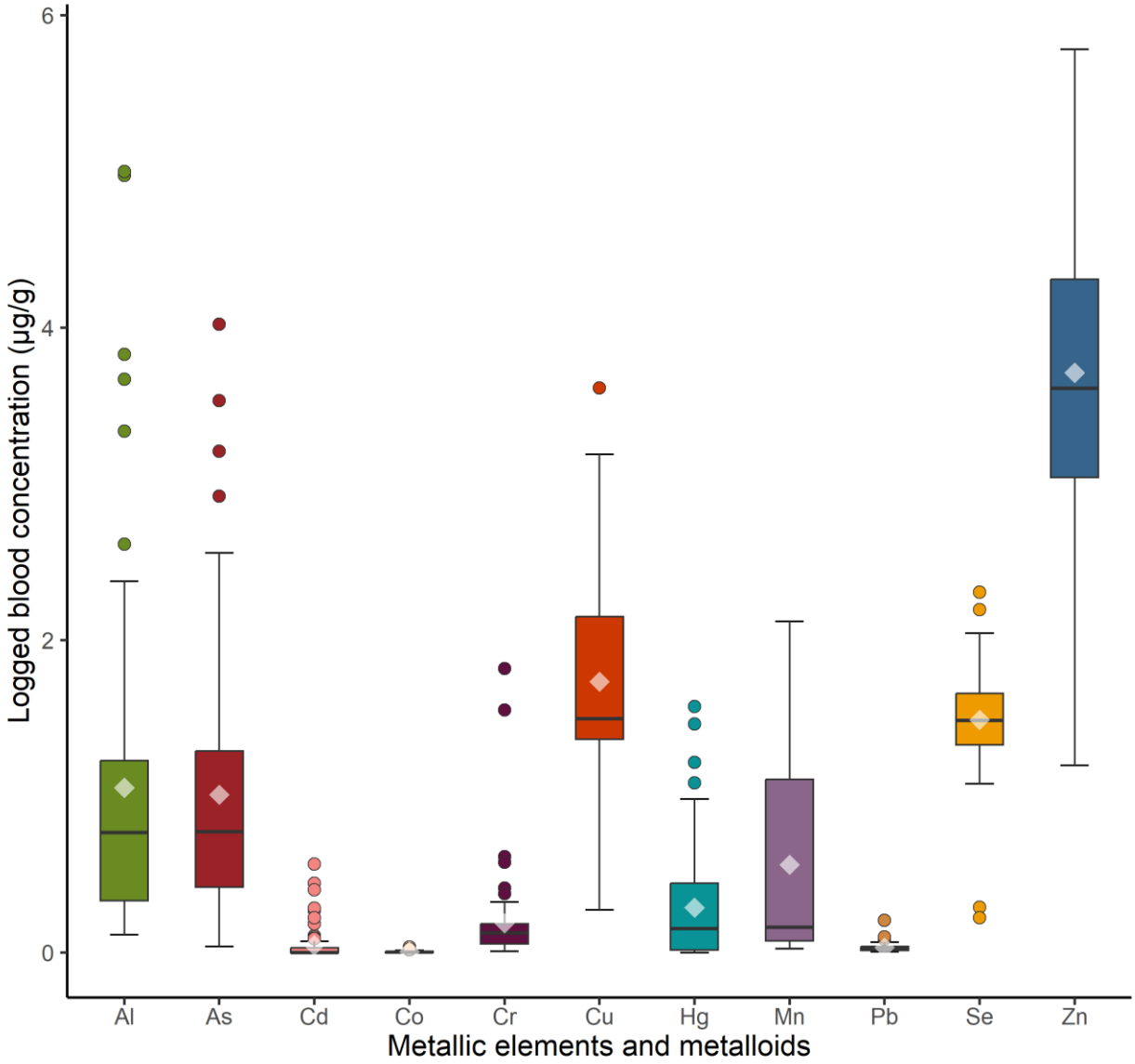
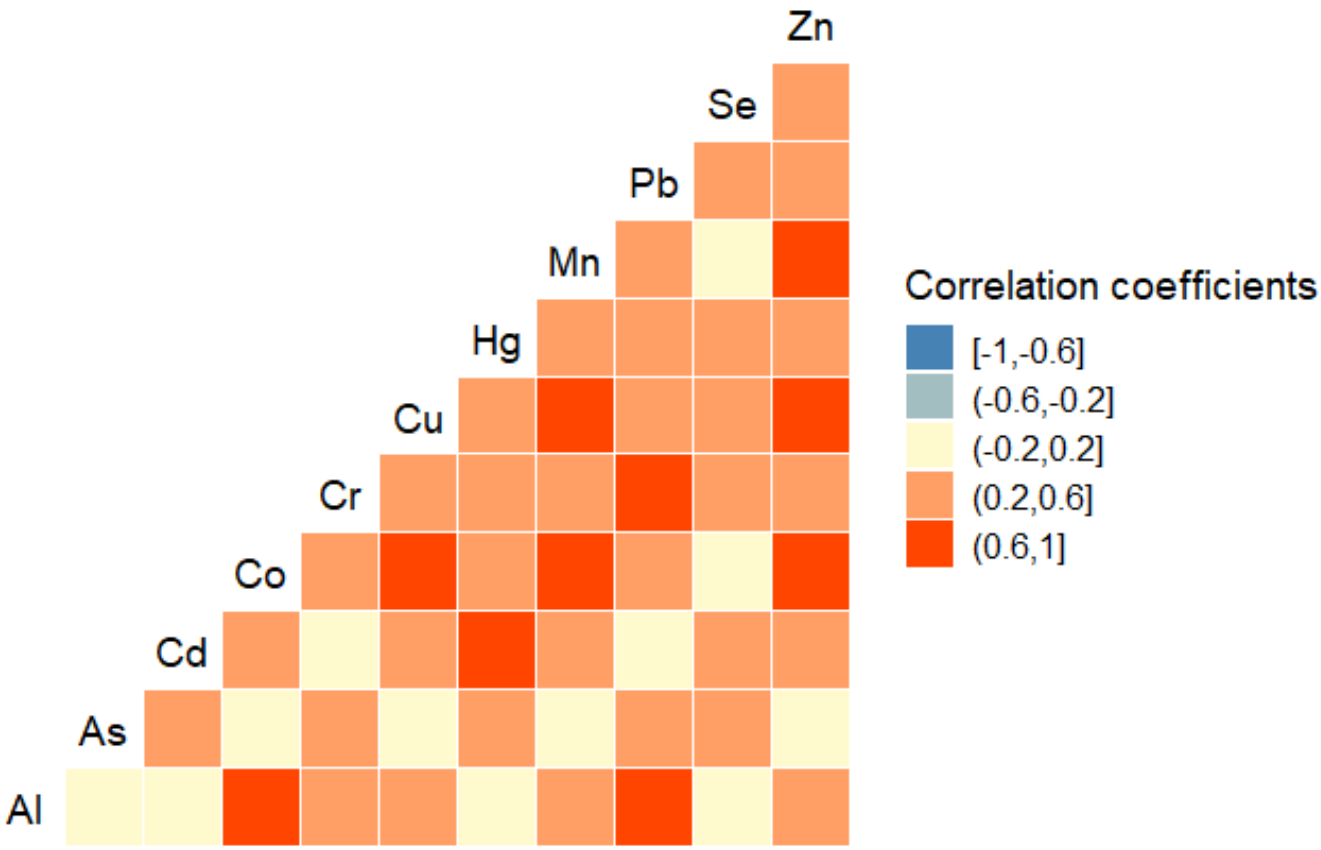


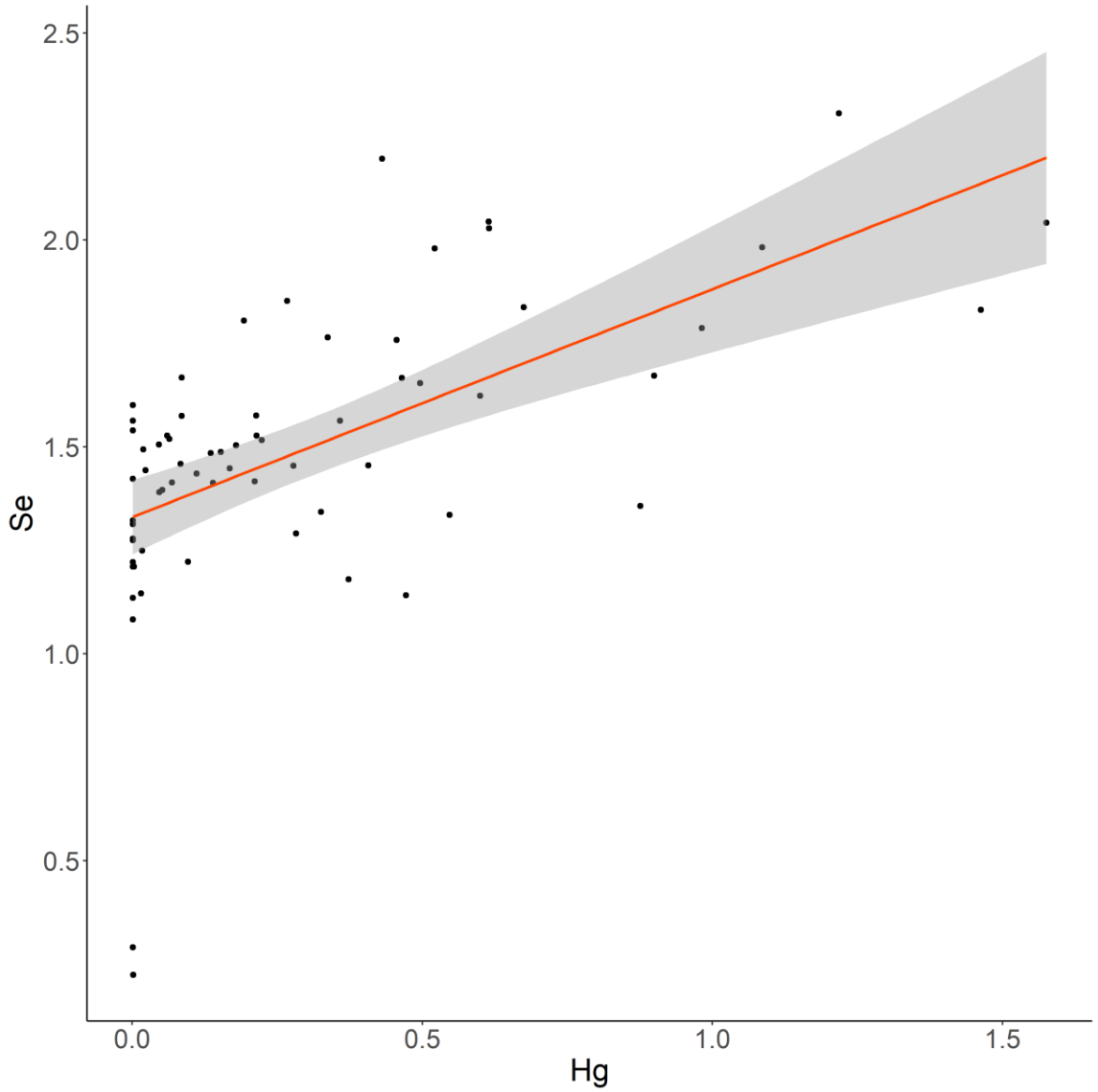

**Fig. 3.** Boxplots showing descriptive statistics (diamonds = means; bold lines = medians; circles = outliers) of logged metallic element and metalloid concentrations (µg/g, d.w) in whole blood samples taken from caracals in the Greater Cape Town area, South Africa. Metallic elements and metalloids are plotted in alphabetical order.




658 **Fig. 4.** Correlation matrix showing correlation coefficients between metallic elements and metalloids in
659 caracal blood samples. Correlation values are categorised, where significant positive correlations are
660 represented by darker shades of orange**.**

661

662

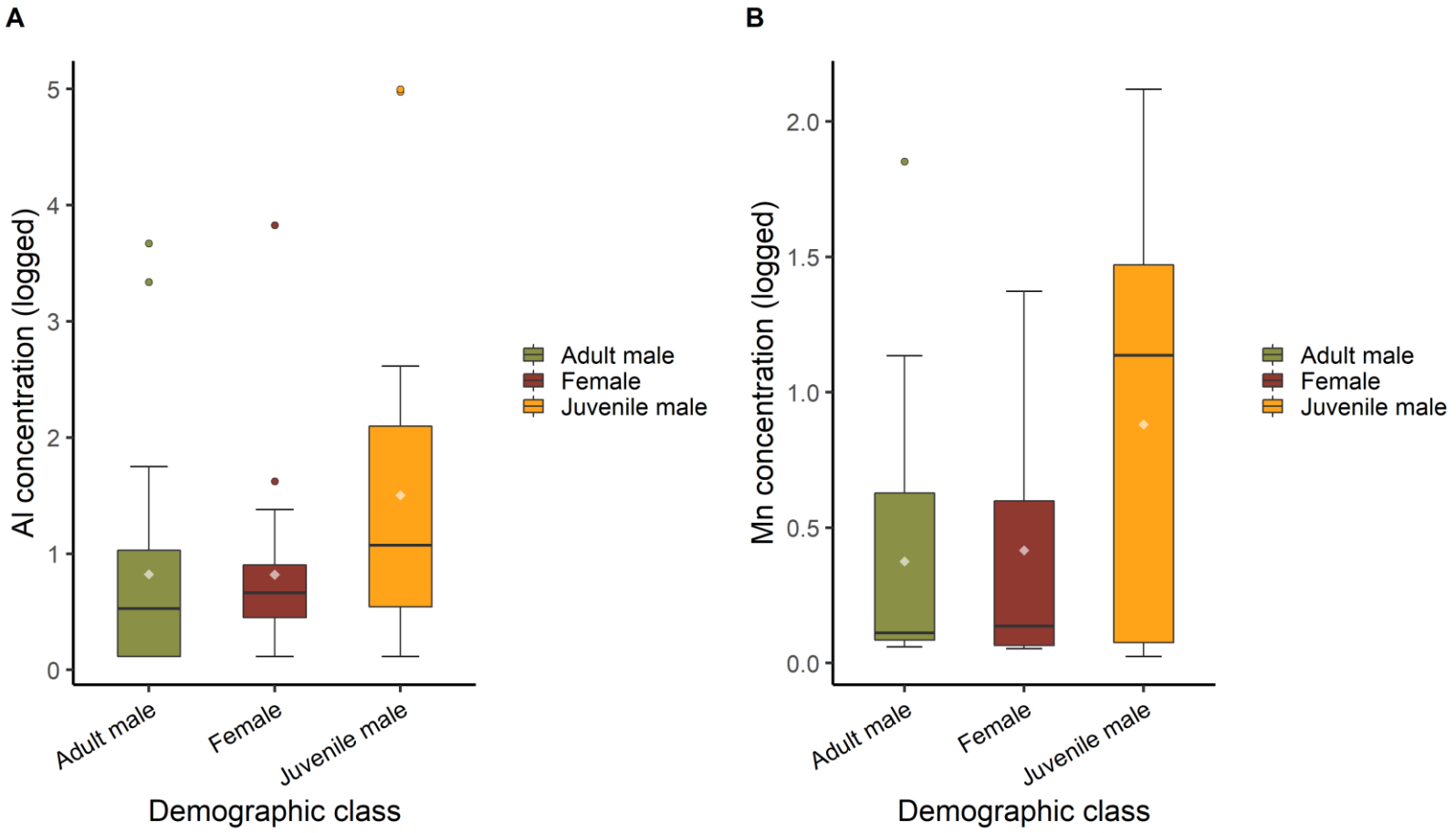



663 **Fig. 5**. Correlation biplot showing the association between Hg and Se concentrations (µg/g, d.w.) in
664 caracals. Grey area represents 95% confidence intervals. The plot depicts a significant positive relationship
665 between Hg and Se (r = 0.58; *P* < 0.01).

666

667

668




669

670 **Fig. 6.** Boxplots (diamonds = means; bold lines = medians; circles = outliers) showing significant variation
671 in A) Al and B) Mn concentrations (logged, µg/g) amongst adult male (n = 21), female (n = 23) and juvenile
672 male (n = 23) caracals.

673

674 *Land-use risk factors*

675 Together, land-use variables in the initial RDA model (see Appendix IV: Table A) explained
676 approximately 18.6% of the total variation in metal concentrations across caracal samples.
677 Forward selection of this model revealed "vineyards" and "distance from coast" were the top
678 predictor variables (Fig. 7), explaining a combined 11.5% of total variation. Therefore, we
679 selected these two variables to build our final RDA model (Appendix IV: Table A). The final model
680 was highly significant ($F_{2,63}$ = 4.11, $P$ < 0.05) with a total inertia of 3.73 (0.40 constrained, 3.3

unconstrained). The first RDA axis (RDA1) explained 8.87% of total variation and the second RDA axis (RDA2) explained 2.68% (Fig. 7). On RDA1, Al and Zn had the highest negative scores, while As and Hg had the highest positive scores amongst the response variables. On RDA2, Co and Mn had the only negative scores and Se, Hg and Zn had the highest positive scores.

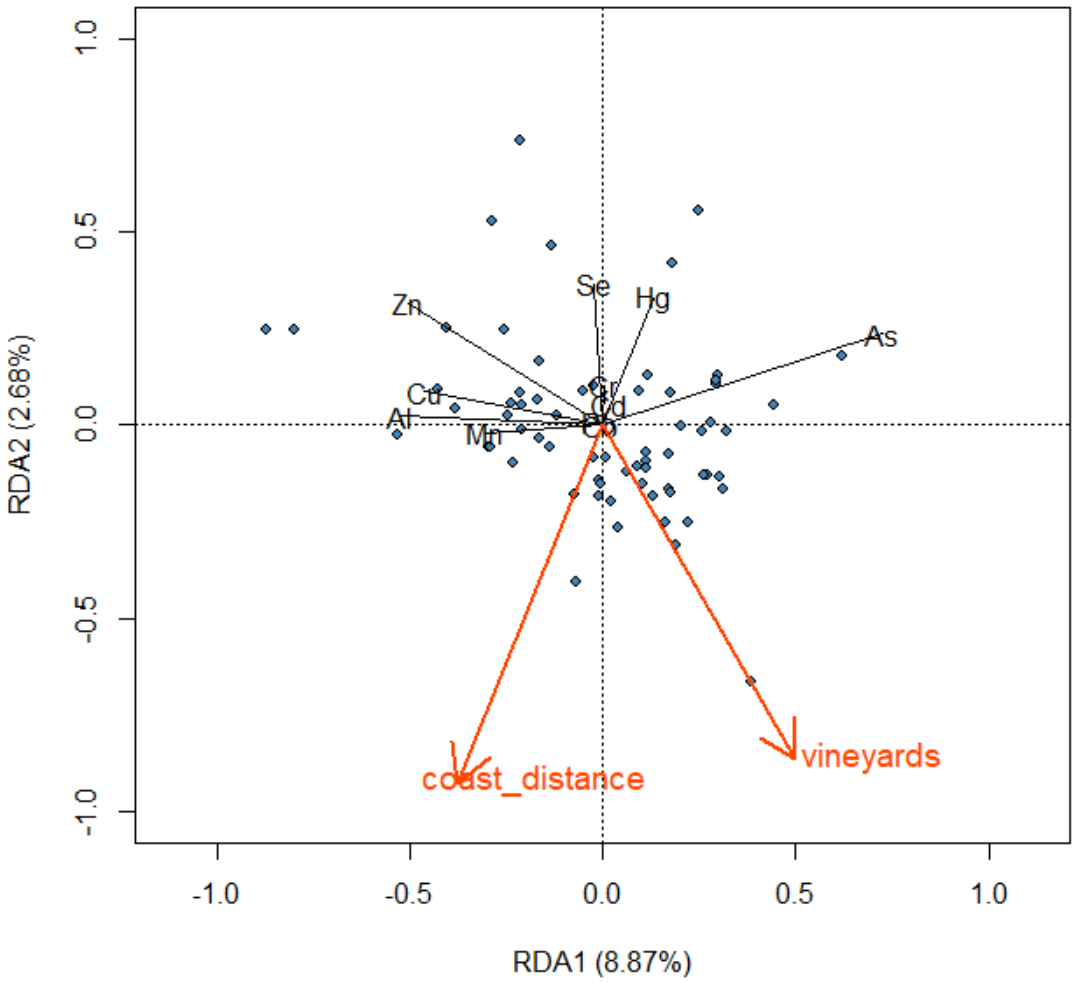



**Fig. 7.** Triplot showing results of RDA analysis and Monte Carlo permutation test to analyse the effect land-use variables (orange arrows) have on logged blood metallic element and metalloid concentrations (black lines) in caracals. Blue diamonds represent caracal samples. The first canonical axis (RDA1) with percentage variance explained, is shown on the x-axis and the second (RDA2), on the y-axis. Only significant variables shown.

Linear mixed-effects models were used to explore the associations between metal concentrations and different land-use types (see full model outputs in Appendix V: Table B). All significant variables are shown in Fig. 8. Nine (Al, As, Co, Cr, Cu, Hg, Pb, Se and Zn) of the 11 metallic elements and metalloids modelled showed a significant or marginally significant association with at least one land-use type.

With increasing proportion of urban land in home ranges and buffers, caracals had higher Al (β = 0.30 ± 0.13, $P < 0.05$; Fig. 8) levels in blood. Increased road density in home ranges and buffers was associated with a marginal increase in caracal blood levels of Co (β = 0.002 ± 0.001, $P = 0.05$) and Pb (β = 0.006± 0.0038, $P = 0.07$) and lower blood levels of As (β = -0.35 ± 0.11, $P < 0.05$). The use of agricultural land had a negative influence on levels of As (β = -0.34 ± 0.14, $P < 0.05$) and a significant negative influence on Se levels (β = -0.15 ± 0.03, $P < 0.01$). Increased use of vineyards elevated caracal blood levels of As (β = 0.28 ± 0.14, $P < 0.01$) and significantly lowered blood levels of Cu (β = -0.26 ± 0.07, $P < 0.01$) and Zn (β = -0.37 ± 0.11, $P < 0.01$). Caracals using areas further away from the coast had higher blood levels of Cu (β = 0.21 ± 0.07, $P < 0.05$), and Zn (β = 0.21 ± 0.11, $P = 0.06$) and lower levels of Hg (β = -0.12 ± 0.04, $P < 0.05$). A greater proportion of informal settlements in home ranges and buffers was marginally associated with elevated Co (β = 0.002 ± 0.001, $P < 0.05$), while human population density was positively associated with elevated levels of Cr (β = 0.07 ± 0.03, $P < 0.05$). The use of altered-open land had a negative influence on Se levels (β = -0.09 ± 0.04, $P < 0.05$). No significant relationships were found between any metallic element and metalloid and wetlands or mining land-uses. Cadmium was not significantly associated with any land-use type (Appendix V: Table B). Juvenile male caracals experienced elevated levels of As (β = 0.78 ± 0.25, $P < 0.05$) and Mn (β = 0.5 ± 0.18, $P < 0.05$), as well as Co (β = 0.004 ± 0.002, $P = 0.09$) and Cu (β = 0.31 ± 0.15, $P = 0.05$), although this was marginally significant.

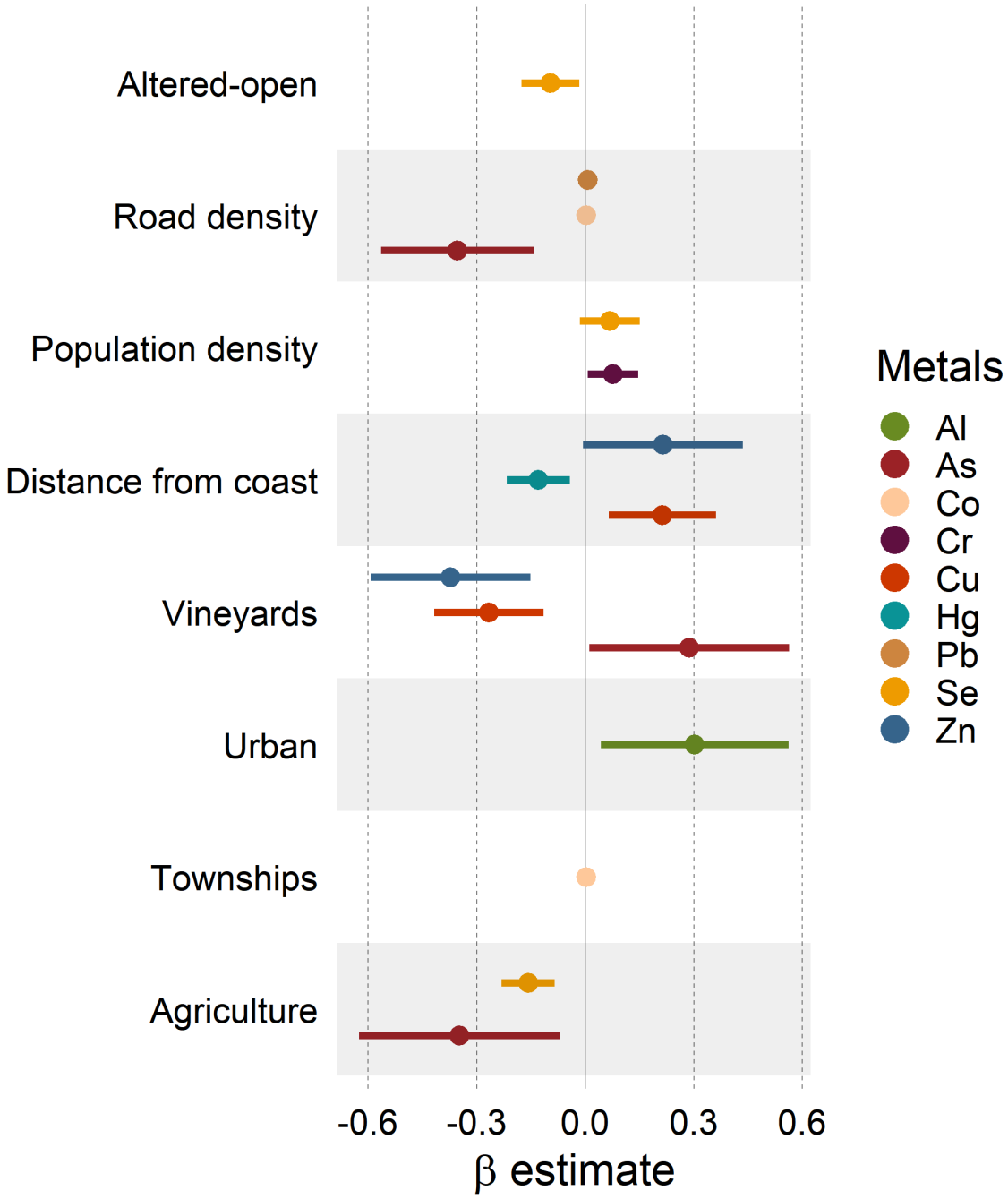



**Fig. 8.** Coefficient estimates and 95% confidence intervals for linear mixed-effects models of land-use variables (see Methods), with concentrations of metallic elements and metalloids in caracal whole blood as the response variable. Only significant top parameters (as determined by AICc) are shown. Metallic elements and metalloids reported in alphabetical order.

*Dietary exposure risks*

Using detailed dietary datasets, we explored caracal metal exposure risk through different dietary pathways. We explored i) broad prey groups, ii) prey functional groups, iii) prey foraging habitats and iv) levels of human association (see full model outputs in Appendix V: Table C – J). All significant variables shown in Fig. 9 – 12.

***i. Broad prey groups***

Caracals with a greater proportion of bird prey in their diet had marginally higher concentrations of As (β = 0.25 ± 0.13 biomass, *P* = 0.05) and Hg (β = 0.09 ± 0.05 biomass, *P* = 0.09; β = 0.09 ± 0.05 FO, *P* = 0.08) in their blood (Fig. 9). Most frequently consumed bird species included Cape cormorants (*Phalacrocorax capensis*), Guinea fowl (*Numida meleagris*) and Egyptian geese (*Alopochen aegyptiaca*). Bird species made up a relatively large proportion of total caracal diet, averaging 46% in terms of biomass and 51% in terms of FO (Appendix I: Table B). An increase in the proportion of insects in diet marginally increased caracal exposure to Al (β = 0.37 ± 0.19 biomass, *P* = 0.06) and influenced exposure to Co (β = 0.002 ± 0.0009 biomass, *P* < 0.05; β = 2.079e-03 ± 9.536e-04 FO, *P* = 0.01) and when juvenile males (β = 0.047 ± 0.002 biomass, *P* < 0.05). However, insects made up the smallest proportion of all prey groups (0.08% biomass; 0.96% FO).

***ii. Prey functional groups***

An increase in the proportion of carnivorous species in caracal diet elevated the level of Al (β = 0.35 ± 0.19 biomass, *P* = 0.07; β = 0.45 ± 0.19 FO, *P* < 0.05) in the blood of juvenile males (β = 1.05 ± 0.46 FO, *P* < 0.05). An increase in the proportion of carnivore (β = -0.27 ± 0.13 biomass, *P* < 0.05) and herbivore (β = -0.35 ± 0.13 biomass, *P* < 0.01) prey functional groups, decreased exposure to As (Fig. 10). Herbivores made up the largest proportion of prey consumed by caracals, on average (51.5% biomass; 30.2% FO; Appendix I: Table B), while carnivorous prey was far lower (9.6% biomass; 8.2% FO). An increase in preying on marine-feeders (β = 0.07 ± 0.04 biomass, *P* = 0.06; β = 0.08 ± 0.05 FO, *P* < 0.05) increased exposure

770 to Se (Fig. 11). The majority of marine prey species were seabirds (Marine feeder: 11.14%
771 biomass, 13.27% FO; Appendix I: Table B), mainly Cape cormorants and to a lesser degree,
772 kelp gulls (*Larus dominicanus*). An increase in the frequency of occurrence of herbivorous
773 prey species was associated with a decrease in caracal blood exposure to Hg (β = -0.13 ± 0.05
774 FO, $P < 0.01$) and As (β = -0.35 ± 0.13 biomass, $P < 0.01$). Most common herbivorous species
775 included the vlei rat (*Otomys irroratus)*, rock hyrax *(Procavia capensis)* and Egyptian geese.
776 The most common carnivorous species included the domestic cat (*Felis catus*), large spotted
777 genet (*Genetta tigrina*) and Cape grey mongoose (*Galerella pulverulenta*).

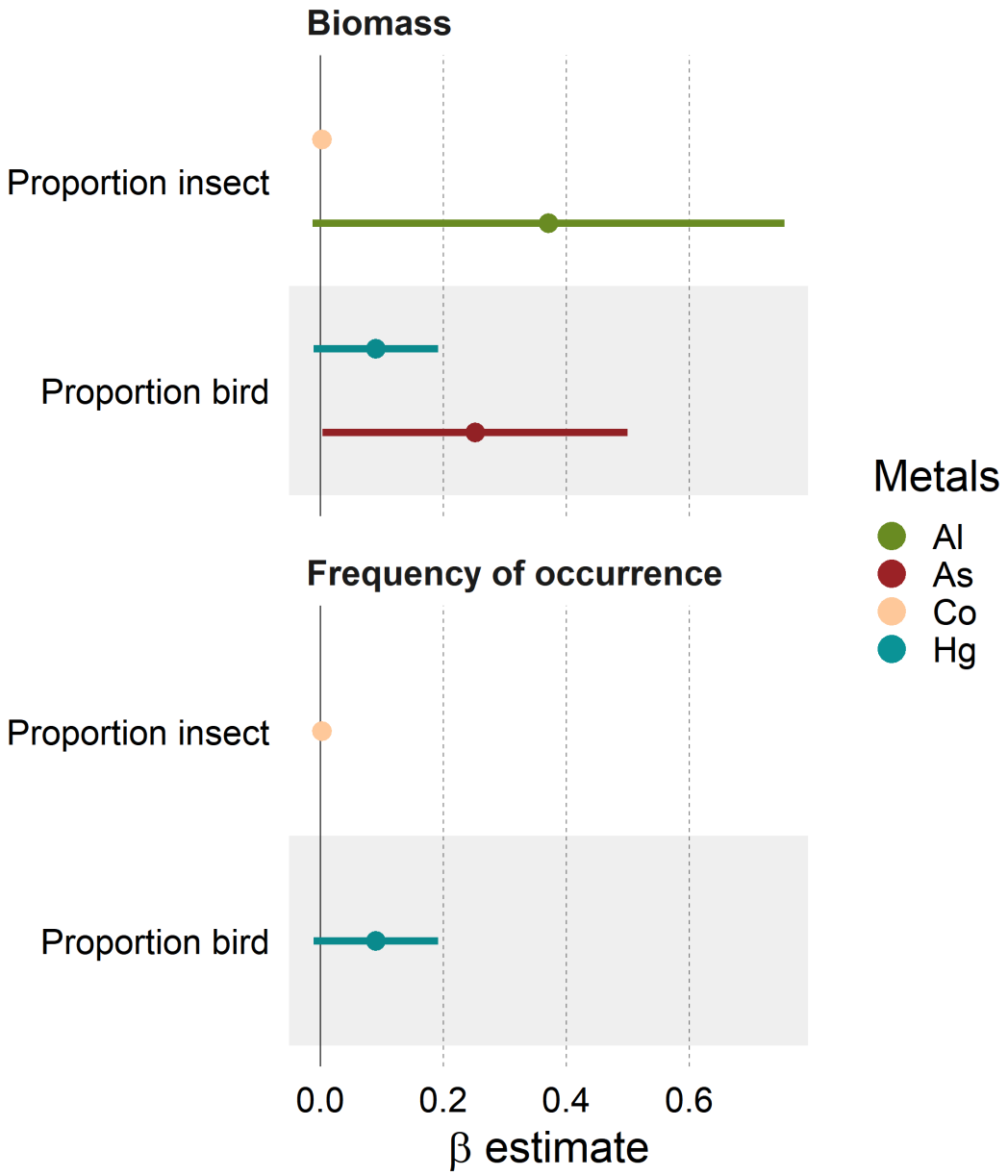
778 ***iii. Prey foraging habitat***

779 An increase in the proportion of wetland-adapted species in diet marginally increased caracal
780 blood exposure to As (β = 0.27 ± 0.13 biomass, $P < 0.05$), Hg (β = 0.16 ± 0.05 biomass, $P <$
781 0.05, β = 0.11 ± 0.05 FO, $P < 0.05$) and Se (β = 0.09 ± 0.04 biomass, $P < 0.01$; β = 0.08 ± 0.03
782 FO, $P < 0.05$). There was also a marginally significant increase in the concentration of exposure
783 to Cu (β = 0.18 ± 0.09 biomass, $P = 0.06$), Mn (β = 0.16 ± 0.09 biomass, $P = 0.07$) and Zn (β =
784 0.21 ± 0.13 biomass, $P = 0.09$) in the blood of caracals feeding on more wetland-adapted
785 species (Fig. 12). Wetland-adapted species mainly included Egyptian geese, African sacred
786 ibis (*Threskiornis aethiopicus)*, and vlei rat (Appendix I: Table B: 16.98% biomass; 27.91% FO).
787 Caracal risk of exposure to Hg (β = 0.09 ± 0.04 biomass, $P < 0.05$, β = 0.13 ± 0.05 FO, $P < 0.05$)
788 and Se (β = 0.09 ± 0.04 biomass, $P < 0.01$, β = 0.11 ± 0.03 FO, $P < 0.05$) increased when feeding
789 on marine-adapted species (Table S2:11.14% biomass; 13.27% FO). The main marine-adapted
790 species included seabirds like Cape Cormorants and kelp gulls. No significant associations
791 were found for arboreal prey groups.

792 ***iv. Exotic prey group***

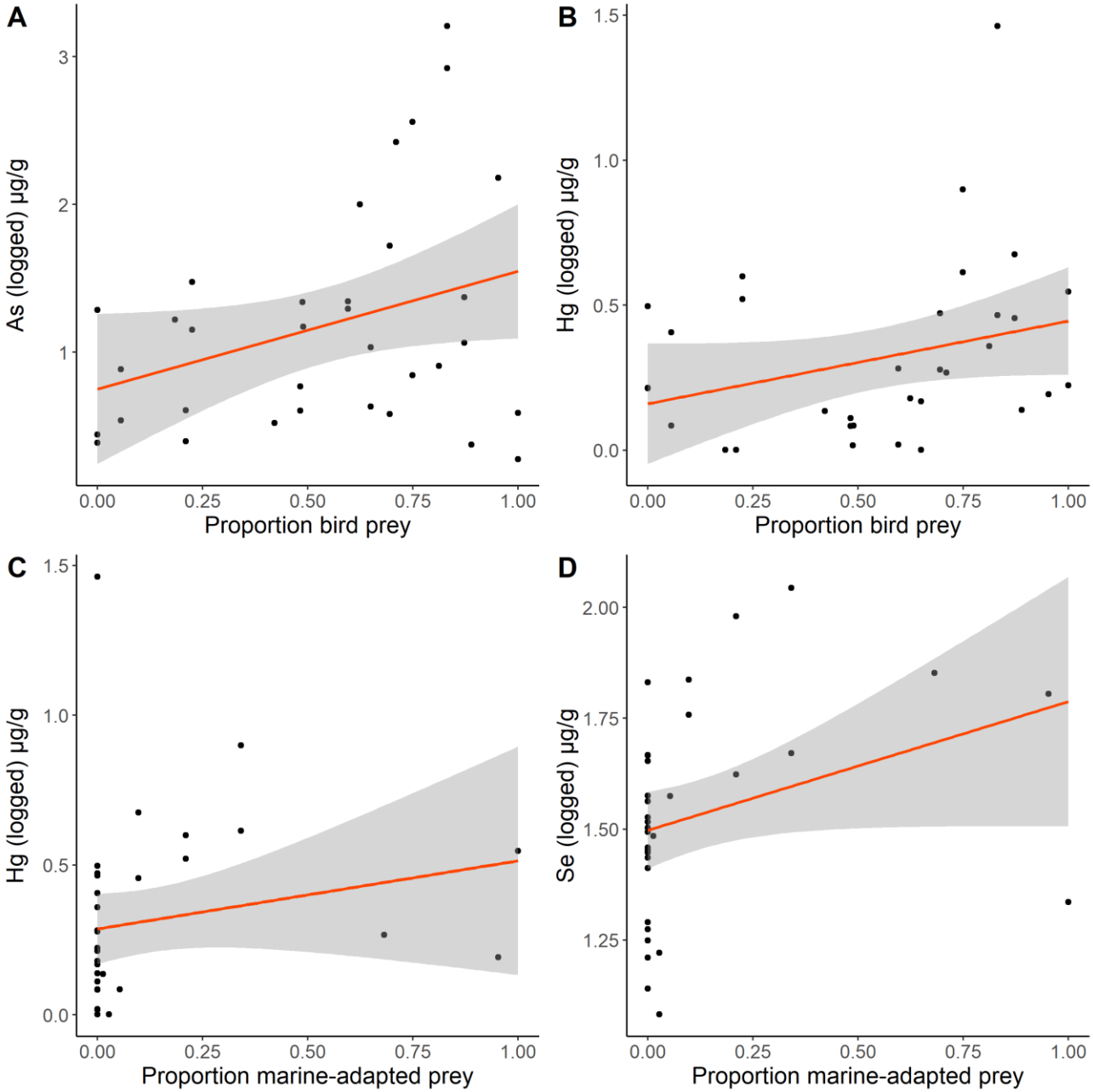
793 Caracals had marginally lower levels of As (β = -0.30 ± 0.12 biomass, $P < 0.05$; β = -0.22 ± 0.12
794 FO, $P = 0.07$) and Se (β = -0.08 ± 0.04 biomass, $P < 0.05$; β = -0.06 ± 0.08 FO, $P = 0.08$) when
795 feeding on synanthropic prey species. Synanthropic prey species were mainly Guinea fowl

796 and Egyptian geese (Appendix I: Table B; 27.3% biomass; 25%:FO). No significant associations
797 were found for exotic prey groups.




798

799 **Fig. 9.** Coefficient estimates and 95% confidence intervals for dietary linear models of proportion of broad
800 prey groups and concentrations of metallic elements and metalloids in caracal whole blood as the
801 response variable. Only significant top model parameters (as determined by AICc) are shown. Metallic
802 elements and metalloids reported in alphabetical order.

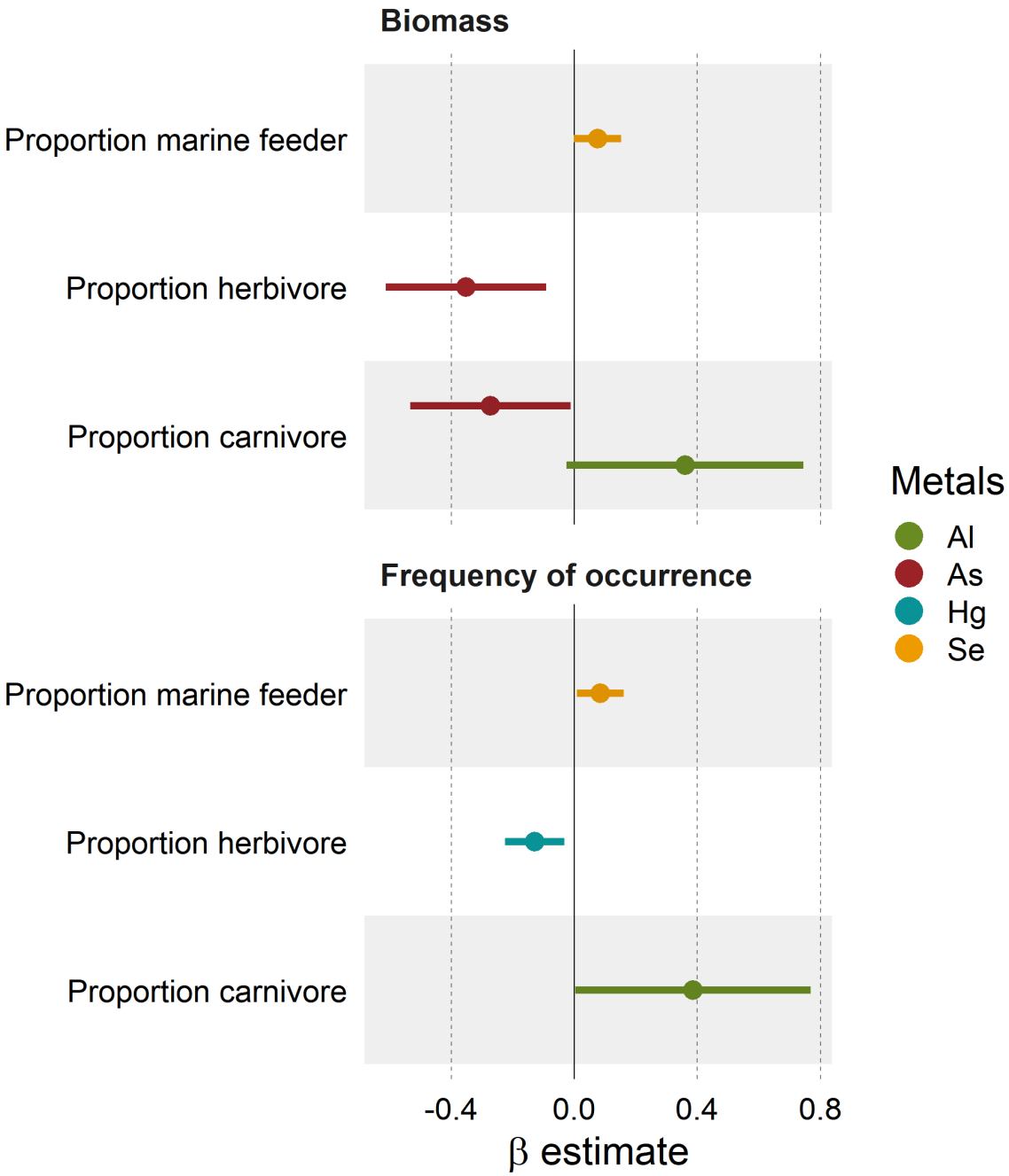



803

804 **Fig. 10.** Associations between logged metallic element and metalloid concentrations in Cape Town caracal
805 whole blood (n = 34) and A – B) proportion bird prey (biomass) and C – D) proportion marine-adapted
806 prey (biomass). Grey area represents 95% confidence intervals.

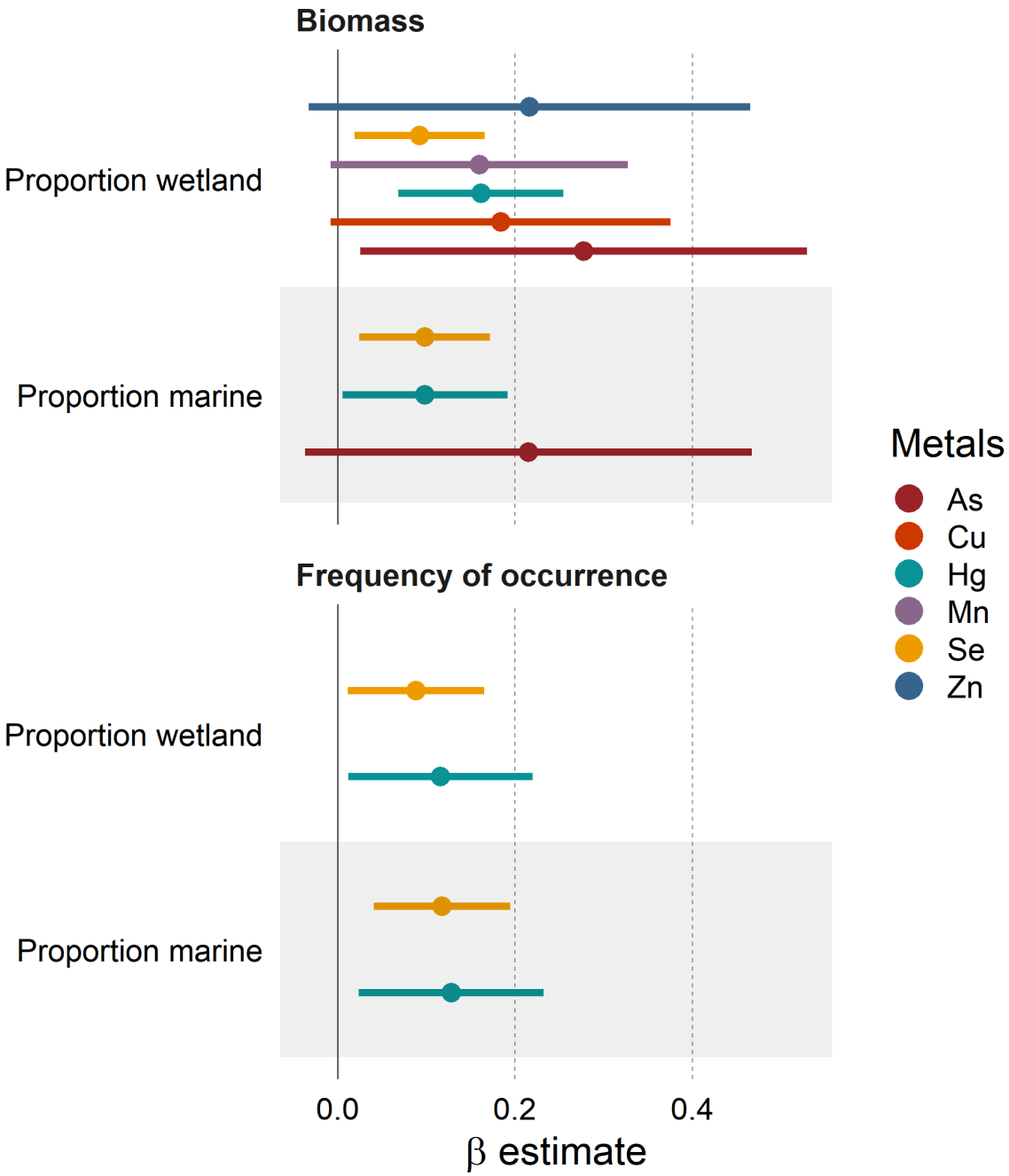
807

808




809

810 **Fig. 11.** Coefficient estimates and 95% confidence intervals for dietary linear models of proportion of prey
811 functional groups and concentrations of metallic elements and metalloids in caracal whole blood as the
812 response variable. Only significant top model parameters (as determined by AICc) are shown. Metallic
813 elements and metalloids reported in alphabetical order.




814

815 **Fig. 12.** Coefficient estimates and 95% confidence intervals for dietary linear models of proportion of prey
816 foraging habitat groups and concentrations of metallic elements and metalloids in caracal whole blood as
817 the response variable. Only significant top model parameters (as determined by AICc) are shown. Metallic
818 elements and metalloids reported in alphabetical order.

# DISCUSSION

Urbanisation and associated anthropogenic activities release large quantities of potentially toxic metallic elements and metalloids into the surrounding environment (Hu et al. 2013; Steffen et al. 2015). Relatively little is known about this emerging threat to terrestrial carnivores persisting in human-transformed landscapes, especially in the Global South (Rodríguez-Estival et al. 2019; Shackleton et al. 2021). This study quantified the level of metallic elements and metalloids in the blood of caracals inhabiting the Greater City of Cape Town, South Africa, a rapidly expanding metropole in a primarily coastal landscape. Further, it explored the possible influence of demographics (age and sex), landscape use and dietary exposure pathways on metal exposure in caracal blood. Due to a lack of species-specific thresholds for metal concentrations in caracals, findings are compared to those of other terrestrial carnivores in the literature. Most metallic elements and metalloids were detected below established toxic concentrations in mammals, with the exception of As and Cr, which may represent toxic levels in certain individuals. Demography, landscape use and diet significantly influenced metal levels in caracals and these exposure pathways likely vary between metals. Increased use of human-transformed landscapes increased caracal exposure to Al, As, Co and Pb, while exploiting aquatic food webs (both marine and terrestrial) increased caracal exposure to As, Hg and Se. The blood metallic element and metalloid concentration dataset reported here can be used as a baseline to test for future fluctuations in metal exposure in caracals and other co-occurring terrestrial carnivores.

*Caracal metal exposure in context*

The majority (> 80%) of mean caracal metallic element and metalloid concentrations (i.e., see Al, Cd, Co, Cu, Hg, Mn, Pb, Se and Zn) were comparable to those of published studies on terrestrial carnivores in natural and urban landscapes (Appendix VI: Table A) and below levels considered lethal or sublethal (Pandey & Madhuri, 2014; Sattar et al. 2016; Gupta et al. 2021). However, As and Cr appear to be present at levels with lethal and/or sublethal effects. This is concerning, given that caracals are also exposed to anticoagulant rodenticides (Serieys et al. 2019) and organochlorines (Leighton et al. 2022) which may act in concert with high metal burdens to exert

847 negative health impacts (Murray et al. 2019). For example, urban bobcats in southern California
848 that were more exposed to rodenticides, experienced increased susceptibility to severe mange
849 (Riley et al. 2007; Serieys et al. 2018). Mean arsenic concentrations in caracal blood were as much
850 as 12 times higher than in other terrestrial carnivores studied (see Appendix VI: Table A), notably
851 the Eurasian otter (*Lutra lutra*; Brand et al. 2020), raccoon (Souza et al. 2013), wolf (*Canis lupus*;
852 Squadrone et al. 2022) and fox (Squadrone et al. 2022). Although caracal sensitivity to this
853 exposure is not known, these findings suggest Cape Town's caracals may be at risk of As toxicity.
854 Arsenic is an especially toxic metalloid, ranked first on the 2019 Substance Priority List created
855 by the Agency for Toxic Substances and Disease Registry (ATSDR, 2019). Acute toxicity can result
856 in death, DNA damage, immune dysfunction, cancer or other sublethal effects in wildlife (Sattar
857 et al. 2016; Gupta et al. 2021). For example, Great tit (*Parus major*) nestlings exposed to As at
858 levels well below here, experienced reduced reproductive success and signs of oxidative stress
859 (Sánchez-Virosta et al. 2018). Humans consuming As-contaminated drinking water above levels
860 recommended by the World Health Organisation were also more exposed to cancer risks (Guo,
861 2007).
862
863 Even at low levels, metal exposure may be concerning for human and animal health. Chromium
864 is one of the most environmentally persistent heavy metals and is capable of inducing toxicity at
865 low concentrations (ATSDR, 2019). While mean caracal Cr concentrations (Fig. 3; Appendix II:
866 Table A) were within range of other carnivores like the Arctic fox (*Vulpes lagopus*; Filistowicz et
867 al. 2012) and crab-eating fox (*Cerdocyon thous*; Curi et al. 2012), and far below those reported
868 for arsenic poisoning in the African white-backed vulture (*Pseudogyps africanus*; Van Wyk et al.
869 2001; refer to Appendix II: Table A), certain individuals may be at risk of sublethal exposure. Evans
870 et al. (2021) found that elevated levels of Cr in Malay civet (*Viverra tangalunga*) hair correlated
871 with haematological parameters indicating the onset of regenerative anaemia. Two individuals
872 from the Cape Town caracal population had Cr concentrations above this level. Further
873 investigation should assess potential health impacts by exploring physiological responses in
874 caracal bloodwork. This is especially important, as evidence already suggests organochlorine

875 accumulation, through similar exposure pathways, may elicit adverse physiological effects
876 suggestive of enhanced immune responses in caracals (Leighton et al. 2021).
877
878 Several important caveats should be highlighted when considering these concentrations and
879 thresholds. First, the lack of any previously published, baseline caracal metallic element and
880 metalloid concentration data makes drawing accurate comparisons difficult. As the species
881 sensitivity to metals remains largely unknown, this study only makes inferences regarding
882 possible metal toxicity or lack thereof by drawing on comparisons from other terrestrial
883 mammals for which lethal and sublethal thresholds have been determined. Second, studies
884 assessing metal concentrations in terrestrial carnivores using whole blood samples are scarce,
885 while the use of hair, liver and kidney tissue is far more common (refer to Appendix VI: Table A).
886 As pollutants may accumulate at different rates between tissues (Crafford & Avenant-Oldewage,
887 2010; Vizuete et al. 2022) direct comparisons of metal concentrations amongst different tissue
888 types is problematic. Third, most mean metal concentrations are below toxic levels at present,
889 they may cross these thresholds over time through bioaccumulation. If bioaccumulation was a
890 significant predictor of metal exposure in caracals one would expect adult caracals to have
891 elevated metal levels, however, this analysis reveals that juvenile males have the highest levels
892 of exposure. It is possible that juvenile males sampled in the study had been more recently
893 exposed to metallic elements and metalloids and not enough time had passed for storage in
894 tissues (e.g., in adipose or liver). Alternatively, caracals, like some other mammals, may be able
895 to expel metal contaminants to some degree through perspiration, urine, faeces, hair and other
896 excretory mechanisms (Chaturvedi et al. 2021). For example, during pregnancy and lactation
897 female grey seals (Vanden Berghe et al. 2012), domestic cows (Swarup et al. 2005) and Croatian
898 brown bears (Lazarus et al. 2018) pass on contaminants to their young through placental and
899 milk-transfer, reducing a small portion of their metal burden (Borrell et al. 1995). Additionally,
900 Esposito et al. (2019) suggests that sexual dimorphism may characterise in the capacity to store
901 metallic elements and metalloids in the organs of domestic dogs and cats. This may contribute

902 to our understanding of lower mean blood metal concentrations in female caracals relative to
903 juvenile and adult males.
904
905 *Increased metal exposure through human-associated landscape use*
906 Urban environments support multiple anthropogenic point sources of pollution, and accordingly,
907 are generally more heavily contaminated than natural ones (Grimm et al. 2008; Riley et al. 2014).
908 Consistent with expectations that increased anthropogenic activity may influence wildlife
909 exposure to metallic elements and metalloids, caracals using human-transformed landscapes
910 generally had higher metal burdens. Similar patterns of metal exposure in wildlife utilising urban
911 spaces have been reported in Bonelli's eagles (*Aquila fasciata*) in southern Portugal (Badry et al.
912 2019), flying foxes (*Pteropus spp.*) in Australia (Sánchez et al. 2022), and nine-banded armadillos
913 (*Dasypus novemcinctus*; Jarvis et al. 2013) and bobcats in North America (Thomason et al. 2016).
914 Notably, Cape Town's remaining indigenous forest patches are also contaminated by metals
915 sourced from a combination of vehicular traffic emissions and air pollution from urban and
916 industrial activity (Krüger et al. 2019). Caracal use of urban areas was strongly associated with Al
917 concentrations, a metallic element which may be acutely toxic and whose main urban sources
918 include emissions from coal combustion, waste incinerators and automobiles (Alexandrino et al.
919 2020; Alasfar & Isaifan, 2021). Further, Cr exposure is associated with higher human population
920 density, and Co with greater proportion of informal settlements (i.e., areas of extremely dense
921 informal housing) in caracal home ranges. Another important landscape feature associated with
922 elevated metal levels, particularly Pb and Co, was road density. Roads are impervious surfaces
923 known to accumulate a wide variety of toxic metallic elements (e.g., Cu, Pb, Fe, Cd, Co, Zn, Al and
924 Cr), mostly through vehicle exhaust and non-exhaust emissions (i.e., brake pad and tyre wear;
925 Okonkwo et al. 2006; Apeagyei et al. 2011; Wicke et al. 2012; Adamiec et al. 2016; Ferreira et al.
926 2016). While leaded petrol was banned in South Africa in 2006, Pb is environmentally persistent
927 with a half-life of over 20 years and continues to be recorded in South African ecosystems and
928 humans (Mathee et al. 2006; Ansara-Ross et al. 2013; Naicker et al. 2013; Krüger et al. 2019).
929

In this study, agricultural areas, particularly vineyards, are generally located at the interface between urban and natural areas, and therefore attract adaptable carnivores able to persist in these transition zones (Bateman & Flemming, 2012; Humphries et al. 2016). Caracals, like other generalists, frequent agricultural areas to exploit the often widely available synanthropic food resources (Braczkowski et al. 2012; Ramesh et al. 2017; Leighton et al. 2021). Accordingly, agricultural landscape use has been associated with metal exposure in carnivores such as the golden jackal (Markov et al. 2016) and Malay civet (Evans et al. 2021). The analysis revealed that elevated concentrations of As were associated with vineyard use. Notably, previously published studies on this population also report a relationship between vineyard use and increased exposure to anticoagulant rodenticides (Serieys et al. 2019) and organochlorines (Leighton et al. 2022). Vineyards are clearly resource-rich areas, but their use also increases exposure to important environmental contaminants in caracals and other wildlife in Cape Town. Agricultural-related sources of As include arsenic-based pesticides, organic and inorganic chemical fertilisers, livestock dips and contaminated irrigation groundwater (Roychowdhury et al. 2005; Casentini et al. 2011; Malan et al. 2014). While South Africa has one of the highest application rates of pesticides in Africa (Quinn et al. 2011), use of As has been banned by the national government since 1983 (DEA, 2017). Although some evidence suggests continued use of banned agrochemicals (both As- and Hg-based) in the GCT (Dalvie & London, 2001), a more likely source may be contaminated groundwater (Abiye & Bhattacharya, 2019; Irunde et al. 2022). On vineyards, agricultural activities including ploughing and cultivating can increase natural rates of weathering and mineralisation of As-containing rocks, causing excess quantities of As to enter groundwater supplies and contaminate terrestrial food chains (Roychowdhury et al. 2005). Together, these findings suggest that use of urban areas, especially those with high road density and vineyards, represent important exposure pathways for metal exposure in caracals. Additionally, they likely contribute to a growing trend that urban wildlife are disproportionately exposed to harmful chemical pollutants (Newman & Schreiber 1988; Riley et al. 2014; Murray et al. 2019). It is clear that urban planners, viticulturalists, conservation practitioners and local government need to work together to develop a robust chemical remediation plan to reduce

958 harmful pollutants generated from these point sources that are undoubtably 'spilling over' into
959 surrounding natural systems.
960
961 *Trophic transfer of metals through aquatic food webs*
962 While using human-transformed landscapes may expose wildlife to harmful pollutants,
963 interestingly, even selecting to forage in less-disturbed habitats may equally pose risks due to
964 long-distance transfer of pollutants. Accordingly, caracals with home ranges located closer to the
965 coast and that consumed more marine-adapted and wetland-adapted prey, experienced
966 elevated blood levels of Hg, As and Se. Similar patterns were observed in wild canids, which
967 accumulated high levels of Hg when foraging in coastal habitats and adopting a primarily fish-
968 based diet (Gamberg & Braune, 1999; Kalisińska et al. 2009). The endangered Florida panther
969 (*Felis concolor coryi*) accumulated high Hg body burdens by preying on Hg-contaminated
970 raccoons feeding within aquatic food webs (Jordan, 1990; Roelke et al. 1991). Additionally,
971 marine fog contributed towards elevated methylmercury body burdens in pumas inhabiting
972 coastal regions of California, United States (Weiss-Penzias et al. 2019). Aquatic environments
973 across the land-ocean continuum (Billen et al. 1991; Bouwman et al. 2013) are important
974 environmental sinks, accumulating large quantities of pollutants (Gani et al. 2021; Miraji et al.
975 2021; Tang et al. 2021). The bioaccumulation and biomagnification of certain metals like Hg
976 through aquatic food webs, is well-established (Clayden et al. 2013; Lavoie et al. 2013; Chiang et
977 al. 2021; Córdoba-Tovar et al. 2022). An emerging global trend is the transfer of aquatic
978 pollutants and metals to terrestrial food chains (Cristol et al. 2008; Rimmer et al. 2010; Noël et
979 al. 2014; Becker et al. 2018; Huang et al. 2018). In general, terrestrial food webs become exposed
980 to aquatic metals when terrestrial species consume metal-contaminated aquatic biota (Cristol et
981 al. 2008). Trophic transfer increases these metal concentrations from one level to the next,
982 elevating the relative toxicity and retention time of these substances in terrestrial species (Baby
983 et al. 2010). The dietary results reported here suggest caracals are likely exposed to toxic heavy
984 metals and metalloids by exploiting contaminated aquatic food webs, mostly from water- and
985 seabird prey (Fig. 9 & 10). These findings may have implications for other adaptable species

inhabiting the world's cities and displaying aquatic (mostly marine) foraging behaviour. For example, several species co-occurring with caracals in Cape Town also forage along the coast, such as chacma baboon (Lewis, 2015), Cape clawless otter (*Aonyx capensis*; Okes, 2017), and the African Fish Eagle (*Haliaeetus vocifer*). Other coastal foragers include black-backed jackal (*Lupulella mesomelas*; Nel et al. 1997) in Namibia, wolves (Darimont et al. 2004) in British Columbia, coyotes (Reid et al. 2018) in North America, pumas (*Puma concolor*; Rau & Jiménez, 2002; Weiss-Penzias et al. 2019) in South America, and several species of Arctic (Roth, 2003; Bocharova et al. 2013) and Australian fox (Kimber et al. 2020). Furthermore, globally, humans may also be at risk of exposure through consumption of freshwater and marine species, especially fish (Chen et al. 2008; Petrova et al. 2020).

To protect wildlife and humans from potentially toxic aquatic heavy metals and metalloids (especially Hg and As), it is crucial to better understand their environmental sources. While exposure may be at the marine interface, the original point sources of Hg and As include coal combustion, domestic fuel burning, natural fires and wastewater from cities (Wiedinmyer & Friedli, 2007; Pandey et al. 2011; Driscoll et al. 2013; Bundschuh et al. 2021). Badry et al. (2019) reported elevated levels of Hg in Bonelli's eagles that were located closer to coal-fired power plants. In South Africa, coal-fired power plants are major contributors to Hg emissions (approximately 78% of national Hg emissions) at levels well above the global average (Masekoameng et al. 2010). While all coal-power stations in South Africa are located in provinces outside our study area (i.e., in Limpopo, Mpumalanga and Gauteng provinces), Hg and As are capable of long-distance transport (Marx & McGowan, 2010; Wai et al. 2016). Therefore, it is probable that emissions from coal combustion and subsequent environmental deposition is a major source of aquatic Hg and As in Cape Town. Further, domestic fuel burning for cooking, heating (Buthelezi et al. 2019) and waste disposal (Haywood et al. 2021), especially from informal settlements, is a significant source of local air pollution in Cape Town and a likely a source of toxic metallic elements and metalloids (Bagula et al. 2021). Additionally, fires may be natural sources of atmospheric Hg emissions (Wiedinmyer & Friedli, 2007). The GCT is a fire-prone and fire-

adapted landscape and indigenous vegetation (mainly fynbos) burns periodically in fire cycles of 10-15 years (van Wilgen et al. 2010). For example, Brunke et al. (2001) reported that a large veld fire at Cape Point was responsible for releasing large amounts of Hg into the atmosphere which was subsequently deposited in the area. Caracals with elevated Hg concentrations selected to forage in coastal areas in this region, suggesting natural fires may play a role in caracal Hg exposure. Further, anthropogenic activity is increasing the frequency of fire in Cape Town, which will likely increase future Hg release and deposition in the region (Forsyth & van Wilgen, 2008). Although more detailed research is required to better understand this association, these results suggest a combination of both local and long-distance transport of metals likely influences metal exposure in Cape Town's terrestrial wildlife (Treu et al. 2022).

Cities are major contributors of chemical pollution to their immediate and surrounding aquatic systems (Dudgeon et al. 2006; Wu et al. 2014). Accordingly, another potential source of metal pollution in aquatic systems is urban wastewater. The City of Cape Town, mandated by the Department of Environmental Affairs (DEA), releases large quantities of wastewater into its freshwater and marine systems (CSIR, 2017). This wastewater is comprised of stormwater and urban runoff, sewage, agricultural waste and overflows from wastewater treatment works (Petrik et al. 2017; Zackon, 2021). Following release from these point sources, polluted water travels through urban wetlands and river systems into the sea (Brown & Magoba, 2009). Evidence suggests limited treatment of this wastewater has resulted in chemical and microbial contamination of local marine systems and biota (Petrik et al. 2017; Ojemaye & Petrik, 2019; Zackon, 2021). Urban wetlands become natural reservoirs for pollutants which, in turn, accumulate in resident biota (Dudgeon et al. 2006; Levengood & Heske, 2008; Le et al. 2018; Sievers et al. 2018; Xia et al. 2021). For example, in the Great Lakes basin in Canada, wild mink experience elevated levels of mercury when utilising more riverine wetlands (Martin et al. 2011). In South Africa, aquatic and semi-aquatic birds inhabiting anthropogenically polluted wetlands, accumulate pollutants like PCBs, DDTs and Hg in their feathers and eggs (Van Eeden, 2004; Bouwman et al. 2008; van der Schyff et al. 2016). These pollutants are also documented in marine

and freshwater fish species in this region (Crafford & Avenant-Oldewage, 2010; Papu-Zamxaka et al. 2010; Pheiffer et al. 2014; Bosch et al. 2015; Ojemaye & Petrik, 2019; Erasmus et al. 2022). Therefore, it is likely that in the GCT aquatic food webs are contaminated by Hg-enriched city wastewater which then accumulate in prey species and consequently their predators.

*Seabirds and waterbirds as dietary sources of aquatic metals*

Diet is a major exposure pathway of metal contamination in caracals (Fig. 13). Dietary model results suggest exposure to multiple elements, including Hg, As, Al and Se from consuming various prey types. Specifically, consumption of seabirds and waterbirds represent important exposure pathways of metallic elements and metalloids in caracals. This prey group likely facilitates the transfer of aquatic metals to terrestrial food webs in which caracals feed. In their review, Cristol et al. (2008) argue that as top consumers in aquatic food chains, seabirds and waterbirds are ideal candidates for studying metal exposure in aquatic environments as they are disproportionately exposed through biomagnification effects while herbivorous bird species (e.g., Egyptian geese and Guinea fowl) have a lower metal magnification potential (Pérez-López et al. 2006; Ishii et al. 2017). For this reason, seabirds are used as sentinels to monitor marine pollutants, including POPs, organochlorines, plastics and metals (Burger et al. 1992; Furness et al. 1997; Summers et al. 2014). Research suggests carnivorous and piscivorous aquatic birds have higher metal burdens (Ackerman et al. 2016). Caracals exploit numerous seabird species including Cape cormorants and kelp gulls, while a high proportion of wetland-adapted prey species included aquatic birds like Egyptian geese and the African sacred ibis. The Cape cormorant is a piscivore, mostly feeding on pelagic prey species and is therefore likely an important source of marine metals in caracals (Williams et al. 1984; Ryan et al. 2010). Burger & Gochfeld (2001) assessed metal accumulation in seabirds off the coast of Namibia, reporting that Cape cormorants recorded the highest metal levels (Pb, Cd, Cr and Mn) in all cases, except for Hg. Although in this case higher Hg levels in omnivorous species like Hartlaub's gull (*Larus hartlaubii*), and the lesser flamingo (*Phoeniconaias minor*) was attributed to their access to large volumes of pelagic fish waste from fishing operations in the area. Several pelagic fish species off

the coast of South Africa, which form the prey base of cormorants, have reportedly high levels of Hg and other metallic elements and metalloids (Furness & Cooper, 1982; Bosch et al. 2016). This may also have important human health implications for local fishing communities and wider seafood consumers (Papu-Zamxaka et al. 2010; Bosch et al. 2015; Erasmus et al. 2022).

*Does urban landscape use increase the risk of an ecological trap for wildlife?*

Caracals, like other urban adapters, may be at risk of an ecological trap by selecting to forage in and around urban landscapes. Urban areas, roads and vineyards may present as attractive foraging areas (Fleming & Bateman, 2018); however, this study reveals that by exploiting these areas caracals are exposed to potentially harmful metals. Together, with the findings of Serieys et al. (2019) and Leighton et al. (2022), these results reveal a complex system of trade-offs for the species. Animals rely on environmental cues to make decisions (i.e., regarding habitat and prey selection) which directly affect their survival and reproduction potential (Hale & Swearer, 2016). Both anthropogenic activity and habitat alteration can change or disrupt these cues, resulting in animals making habitat-selection errors (Delibes et al. 2009; Smith et al. 2021). Leighton et al. (2021) suggest that caracals foraging predominately at the urban edge may be at risk of an ecological trap, where they unwittingly expose themselves to multiple harmful pollutants (e.g., rodenticides and organochlorines) while benefitting from prey resource availability. This study provides further evidence: caracals utilising human-transformed landscapes (i.e., urban areas, roads and vineyards) are more likely to be exposed to harmful metallic elements and metalloids (Fig. 13). Therefore, a possible 'ecotoxicological' trap for wildlife foraging at Cape Town's urban edge includes exposure to multiple pollutants, including rodenticides, organochlorines and metals (Serieys et al. 2019; Leighton et al. 2022). Interestingly, caracals using coastal areas and feeding within marine food webs are also at risk of metal exposure. Caracals in and around the Cape Point area, who are less habituated to urbanisation and forage extensively on seabirds on the coastal margin, are at risk of exposure to a different set of toxic metallic elements and metalloids, despite occurring in far less disturbed habitats. This suggests the potential ecotoxicological trap around Cape Town may be linked to edge effects

(Ries & Fagan, 2003), including both the urban and the coastal edge. This phenomenon has been reported in birds attracted to reproduce at forest edges where prey abundances are often higher, however, they also suffer reduced breeding success due to higher rates of predation and parasitism (Gates & Gysel, 1978; Flaspohler et al. 2001). In the same way, despite high seabird abundances, the coastal margin may be a sub-optimal foraging area for caracals, increasing their exposure to marine metals like Hg.

Ecological traps are fundamentally characterised by negative fitness effects due to suboptimal habitat selection (Schlaepfer et al. 2002). Wildlife faced with high metal burdens reportedly suffer severe health implications, including reduced reproductive success, growth deformities, neurological damage and lower survival rates (Kunito et al. 2008; Tartu et al. 2013). For example, metal-induced endocrine disruption can reduce reproductive success in wildlife (Georgescu et al. 2011; Paschoalini et al. 2019). In response, some species have developed physiological mechanisms to counteract metal toxicity in their bodies (Ikemoto et al. 2004). For example, the co-accumulation of Se with toxic heavy metals and metalloids like Hg and As, mostly in a molar ratio of 1:1, is well-documented in fish (Ponton et al. 2022), birds (Tartu et al. 2013), marine mammals (Ikemoto et al. 2004), terrestrial mammals (Ralston et al. 2008; Haines et al. 2010; Kosik-Bogacka et al. 2020) and even humans (Chen et al. 2007); this may also occur in caracals. Selenium is known to bind to a number of toxic metals, via compounds known as selenomethionines and selenites, a mechanism reported to detoxify these metals (Levander, 1977; Chen et al. 2007; Gailer, 2007; Berry & Ralston, 2009; Ponomarenko et al. 2017). It is possible that Cape Town caracals also utilise this physiological response to counteract As and Hg toxicity. Exposure to As and Hg may ultimately lead to caracal population declines as is suspected in the Florida panther (Barron et al. 2004) and in southern African vultures (Naidoo et al. 2017). Further research is still required to understand how best to test for immune, neurological, endocrine and stress effects linked to pollutant exposure in caracals. Although ecological traps are difficult to demonstrate and require a rigorous set of criteria be met, such as demonstrating fitness effects and population-level consequences (Robertson & Hutto, 2006), this study

contributes to a growing global evidence base suggesting that cities may become ecological traps for urban-adapters (Johnson et al. 2012; Huang et al. 2018; Sievers et al. 2018; Murray et al, 2019; Vlaschenko et al. 2019; Zuñiga-Palacios et al. 2021).

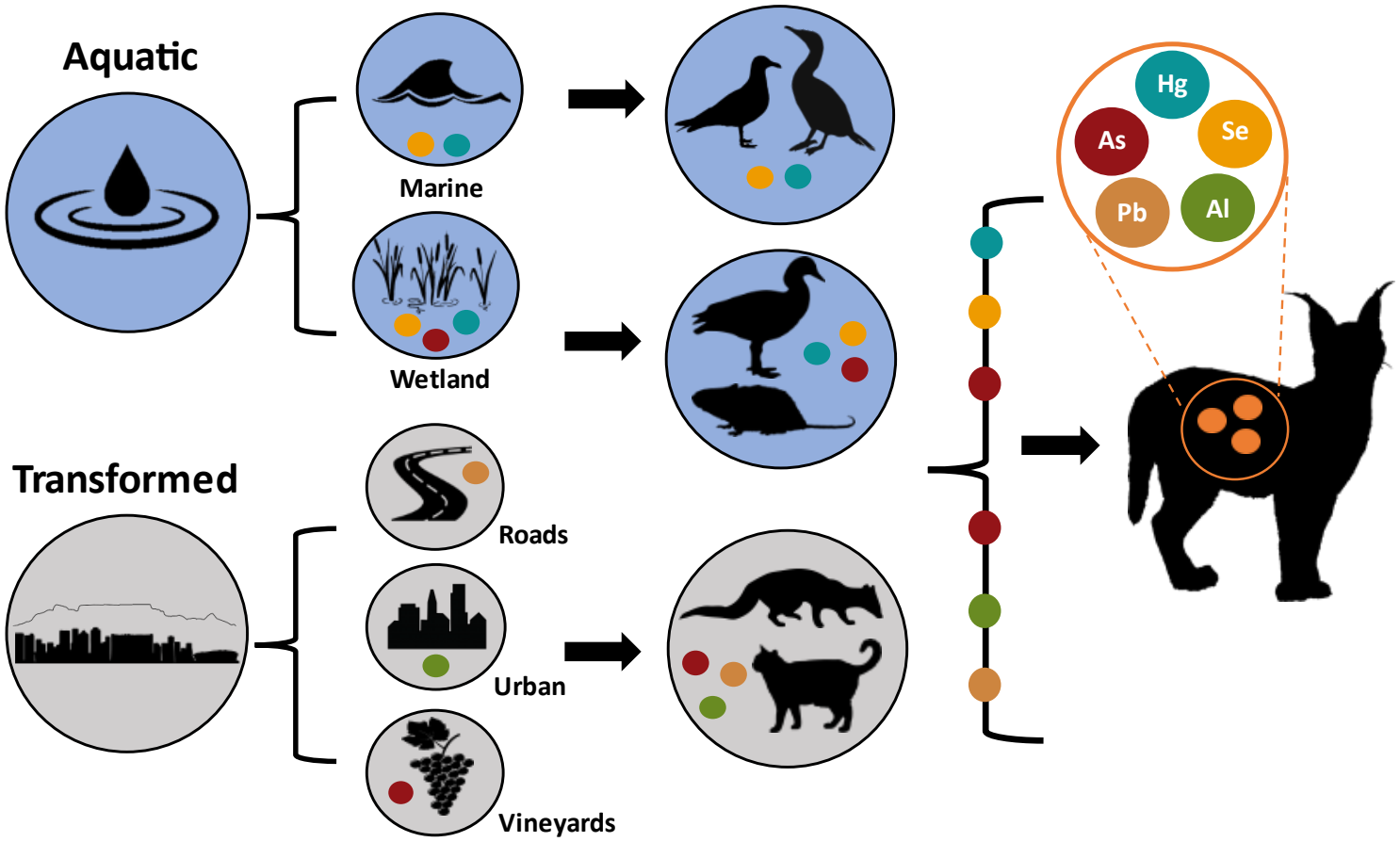



**Fig. 13.** The landscape use and dietary exposure pathways of metal exposure in caracals inhabiting the Greater City of Cape Town, South Africa, a rapidly expanding, primarily coastal metropole. Blue circles represent potential metal exposure through aquatic-associated dietary pathways (marine and wetland-adapted prey) and grey circles represent exposure through human-transformed (roads, urban areas and vineyards) landscape use pathways. Metallic elements and metalloids are represented by smaller coloured circles (green = Al; red = As; blue = Hg, Pb = brown; yellow = Se).

## CONCLUSIONS AND RECOMMENDATIONS

This study is the first to report whole blood metallic element and metalloid concentrations in the caracal, and the first for an African terrestrial mammalian carnivore. In the city of Cape Town,

caracals are exposed to a suite of metallic elements and metalloids that have known health effects in both humans and wildlife. Although most metals assessed were below established toxic thresholds, As and Cr may be present at toxic levels in several individuals. Metal exposure may represent an important threat to the persistence of caracals and other terrestrial carnivores inhabiting the rapidly urbanising, coastal landscape of Cape Town. Demography, landscape use and dietary pathways successfully explain variation in metallic element and metalloid exposure patterns in caracals. Overall, these findings highlight the importance of urbanisation and anthropogenic activity as major environmental sources of metal exposure in terrestrial wildlife, including more natural areas through long distance transport. Further, they contribute towards a growing global evidence base suggesting that cities, with their unique attributes and resources, may ultimately act as ecological traps for wildlife. For the well-being of both humans and wildlife in urban areas, it is crucial that cities work to reduce the large quantities of chemical pollutants released into their surrounding environment. In Cape Town, this could be focused on the urban edge, waste management, water treatments, roads, and agricultural areas. Available mitigation options include bioremediation of contaminated wetlands and freshwater systems, stricter regulations on treatment of city wastewater, a better understanding and monitoring of both legal and illegal pesticide use and reducing road runoff by installing porous pavements (i.e., permeable paving surfaces allowing precipitation to seep through them (Brattebo & Booth, 2003; Schieritz, 2016). More broadly, it should involve the introduction of stricter regulations that reduce fuel-burning emissions and the direct release of city wastewater into marine systems.

To better understand how metallic elements and metalloids enter and move between Cape Town's wildlife, future research should focus on assessing metal exposure at different trophic levels. This research could be complemented by examining additional environmental variables and substrates characterising caracal home ranges such as soil, surface water, groundwater, and air to help elucidate exposure pathways. Further, determining measures of potential physiological responses (immune, stress, endocrine and neurological) to metal exposure in caracals could be achieved by using various biochemical and haematological biomarkers. Lastly,

in line with the One Health approach (Frazzoli et al. 2015), it is crucial to develop a robust local, provincial, and national pollutant monitoring programme using a variety of sentinel species, including small and medium-sized carnivores and aquatic bird species.

*The caracal: a successful sentinel for metal contamination in cities*

The caracal is an effective sentinel for metallic element and metalloid exposure in a human-transformed landscape. Importantly, this species can act as an early warning sign for As and Cr toxicity in other terrestrial wildlife exposed to these metals through similar pathways. This finding has implications for future pollutant risk assessments and monitoring efforts, both locally and nationally. Furthermore, the continued use of the caracal as a sentinel species is beneficial to pan-continental contaminant tracking as they fulfil similar ecological niches as several other mesocarnivores being studied in North and South America, Europe and Australia (Bateman & Fleming, 2012; Jooste et al. 2014). For example, they share similar behavioural and feeding ecologies with bobcats, coyotes, pumas and foxes which make for valid comparisons. Additionally, Cape Town's caracal population provides a valuable model to understand the impacts of urbanisation on carnivores in the Global South, a crucial yet under-studied area of research.

In today's rapidly changing world Marneweck et al. (2022) argue that smaller carnivores may be more appropriate sentinels than large-to-medium sized carnivores. Located lower in the food chain, smaller carnivores are more sensitive to environmental change, have shorter lifespans and higher reproduction rates, and therefore may respond more rapidly to changes. In the GCT, smaller mammalian and avian carnivores should be incorporated into future multi-level pollutant biomonitoring programmes, alongside the caracal. Co-occurring species include the Cape Clawless otter, large-spotted genet, water mongoose (*Atilax paludinosus*), small grey mongoose, Cape Eagle owl (*Bubocapensis*) and jackal buzzard (*Buteo rufofuscus*). Given their role in facilitating aquatic transfer of metals and other pollutants to terrestrial food webs, marine and freshwater bird species such as Cape cormorants, kelp gulls, Egyptian geese, and the African

sacred ibis are also valuable targets for monitoring. The caracal is a charismatic species which has garnered extensive local and international public interest in Cape Town through the work of the Urban Caracal Project (urbancaracal.org). This platform is centrally positioned to harness and promote greater public awareness and engagement in issues involving environmental pollutant contamination and monitoring in South Africa.

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# APPENDICES

## Appendix I: Details on land-use and dietary variables

**Table A**: Land-use covariates considered for models of metallic element and metalloid exposure in caracals in the Greater Cape Town area, South Africa

| Covariates | Type | Range | Source | Description |
|---|---|---|---|---|
| **Urban** | Binary | 0 – 1 | South African Department of Environmental Affairs (DEA) Land-cover 2018 raster (egis.environment.gov.za/gis_data_downloads) | 20 m resolution. 73 categories simplified to 2 (1 = urban, 0 = non-urban) |
| **Agricultural** | Binary | 0 – 1 | South African Department of Environmental Affairs (DEA) Land-cover 2018 raster (egis.environment.gov.za/gis_data_downloads) | 20 m resolution. 73 categories simplified to 2 (1 = agricultural, 0 = non-agricultural) |
| **Vineyards** | Binary | 0 – 1 | South African Department of Environmental Affairs (DEA) Land-cover 2018 raster (egis.environment.gov.za/gis_data_downloads) | 20 m resolution. 73 categories simplified to 2 (1 = vineyard, 0 = non-vineyard) |
| **Wetlands** | Binary | 0 – 1 | South African Department of Environmental Affairs (DEA) Land-cover 2018 raster (egis.environment.gov.za/gis_data_downloads) | 20 m resolution. 73 categories simplified to 2 (1 = wetland, 0 = non-wetland) |
| **Township** | Binary | 0 – 1 | South African Department of Environmental Affairs (DEA) Land-cover 2018 raster (egis.environment.gov.za/gis_data_downloads) | 20 m resolution. 73 categories simplified to 2 (1 = township, 0 = non-township) |
| **Altered-open** | Binary | 0 – 1 | South African Department of Environmental Affairs (DEA) Land-cover 2018 raster (egis.environment.gov.za/gis_data_downloads) | 20 m resolution. 73 categories simplified to 2 (1 = altered-open, 0 = non- altered-open) |
| **Distance from coast** | Binary | 0 – 1 | GEBECO, 2003. South African coastline. (omap.africanmarineatlas.org/BASE/pages/coastline.htm.) | 30 m resolution |
| **Road density** | Continuous | 0 – 50 | CNGI, 2020. Western Cape Transportation Network. | 50 m resolution |

| | | | | |
|---|---|---|---|---|
| | | | (cdngiportal.co.za/cdngiportal/) | |
| **Human population density** | Continuous | 0 – 65 | A dataset generated by machine learning to identify buildings overlaid with census data created in May 2019. Facebook Connectivity Lab and Center for International Earth Science Information Network - CIESIN - Columbia University. 2016. High Resolution Settlement Layer (HRSL). Source imagery for HRSL © 2016 DigitalGlobe. https://data.humdata.org/dataset/highresolutionpopulationdensitymaps-zaf | 30 m resolution |
| **Demographic** | Categorical | 0.01 – 77 | This study | 1 = adult male, 2 = juvenile male, 3 = female |

**Table B.** Diet proportions in several prey groupings for caracals around Cape Town, South Africa as determined through integrating diet datasets from scat and prey remains found at GPS clusters (reproduced from Leighton et al. 2022)

| Prey group | Prey category | n | % diet (FO) | % diet (biomass) |
|---|---|---|---|---|
| **Broad prey group** | bird | 376 | 51.44 | 46.22 |
| | insect | 7 | 0.96 | 0.08 |
| | mammal | 327 | 44.73 | 53.25 |
| | reptile | 21 | 2.87 | 0.45 |
| **Functional group** | carnivore | 60 | 8.21 | 9.63 |
| | granivore | 77 | 10.53 | 3.08 |
| | herbivore | 221 | 30.23 | 51.58 |
| | insectivore | 37 | 5.06 | 0.81 |
| | marine feeder | 97 | 13.27 | 11.14 |
| | omnivore | 239 | 32.69 | 23.77 |

| | | | | |
|---|---|---|---|---|
| **Prey foraging habitat** | arboreal | 29 | 3.97 | 0.78 |
| | marine | 97 | 13.27 | 11.14 |
| | terrestrial | 401 | 54.86 | 71.10 |
| | wetland | 204 | 27.91 | 16.98 |
| **Urban prey groups** | exotic | 72 | 9.95 | 13.76 |
| | synanthropic | 183 | 25.03 | 27.32 |
| | wild | 476 | 65.12 | 58.93 |

## Appendix II: Descriptive statistics for caracal whole blood metallic element and metalloid concentrations

**Table A:** Summary of whole blood metallic element and metalloid concentration (mean ± SD; µg/g; raw dataset) of caracals living in the Greater City of Cape Town area, South Africa

| Metallic elements and metalloids | Mean | Median | Range |
|---|---|---|---|
| **Al** | 7.70 ± 25.57 | 1.16 | 0.12 - 147.08 |
| **As** | 3.74 ± 8.38 | 1.17 | 0.04 - 54.82 |
| **Cd** | 0.06 ± 0.14 | 0.001 | 0.001 - 0.76 |
| **Co** | 0.01 ± 0.01 | 0.003 | 0.001 - 0.04 |
| **Cr** | 0.29 ± 0.76 | 0.14 | 0.01 - 5.16 |
| **Cu** | 5.88 ± 5.66 | 3.47 | 0.31 - 36.08 |
| **Hg** | 0.45 ± 0.74 | 0.17 | 0.001 - 3.84 |
| **Mn** | 3.84 ± 1.79 | 0.18 | 0.02 - 7.33 |
| **Pb** | 0.03 ± 0.03 | 0.02 | 0.01 - 0.23 |
| **Se** | 3.69 ± 1.55 | 3.41 | 0.25 - 9.03 |
| **Zn** | 56.55 ± 54.18 | 35.99 | 2.31 - 322.79 |

## Appendix III: Correlation matrix results

**Table A.** Correlation matrix showing correlation coefficients (*P*-values) between metallic element and metalloid concentrations (logged) in caracal whole blood samples. Significant correlations in bold and highly significant correlations in orange.

**Blood metallic element and metalloid concentrations (logged)**

| | Al | As | Cd | Co | Cr | Cu | Hg | Mn | Pb | Se | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Al** | | | | | | | | | | | |
| **As** | 0.101 (.415) | | | | | | | | | | |
| **Cd** | 0.112 (.365) | 0.357 (.003) | | | | | | | | | |
| **Co** | **0.608 (<.001)** | 0.038 (.759) | 0.361 (.003) | | | | | | | | |
| **Cr** | **0.460 (<.001)** | 0.220 (.074) | 0.133 (.285) | 0.375 (.002) | | | | | | | |
| **Cu** | **0.533 (<.001)** | 0.123 (.321) | **0.488 (<.001)** | **0.657 (<.001)** | 0.248 (.043) | | | | | | |
| **Hg** | 0.180 (.146) | **0.500 (<.001)** | **0.764 (<.001)** | 0.287 (.019) | 0.331 (.006) | 0.387 (.001) | | | | | |
| **Mn** | **0.447 (<.001)** | 0.075 (.545) | **0.507 (<.001)** | **0.842 (<.001)** | 0.232 (.059) | **0.808 (<.001)** | **0.400 (.001)** | | | | |
| **Pb** | **0.628 (<.001)** | 0.222 (.072) | 0.149 (.228) | 0.401 (.001) | **0.648 (<.001)** | **0.528 (<.001)** | 0.303 (.013) | 0.399 (.001) | | | |
| **Se** | 0.043 (.730) | **0.528 (<.001)** | 0.370 (.002) | 0.155 (.209) | 0.213 (.083) | 0.256 (.037) | **0.582 (<.001)** | 0.155 (.209) | 0.221 (.072) | | |
| **Zn** | **0.434 (<.001)** | 0.117 (.344) | **0.488 (<.001)** | **0.672 (<.001)** | 0.365 (.002) | **0.827 (<.001)** | **0.456 (<.001)** | **0.799 (<.001)** | **0.490 (<.001)** | 0.337 (.005) | |

## Appendix IV: Redundancy analysis results

**Table A**. Eigenvalues and percentage of variance explained by the redundancy analysis (RDA) calculated on selected land-use predictor variables. Results of the Monte Carlo permutations tests to check the significance of the full model and the first canonical axis are also shown (*$P < 0.1$; **$P < 0.05$; ***$P < 0.01$).

| **Selected predictor variables** | Vineyards<br>Distance from coast | |
|---|---|---|
| | **RDA 1** | **RDA 2** |
| **Eigenvalues** | 0.33 | 0.10 |
| **Proportion explained** | 0.88 | 0.26 |
| | | |
| **Monte Carlo test** | **F-statistic** | ***P*-value** |
| **Significance of first canonical axis** | 6.31 | $P < 0.05$ |
| **Significance of full model** | 4.11 | $P < 0.05$ |

## Appendix V: Linear and linear mixed-effects model results

**Table A**. Estimates (SE) for top linear models and tests for significance, for demographic variables in whole blood metallic element and metalloid concentrations (logged) in caracals (n = 67) in the Greater Cape Town area, South Africa *(*P* < 0.1*; **P* < 0.05*; ***P* < 0.01)

| | Al | As | Cd | Cr | Cu | Hg | Mn | Pb | Se | Zn |
|---|---|---|---|---|---|---|---|---|---|---|
| **Female** | | | | | | | | | | |
| **Subadult male** | 0.68* (0.32) | | | | | | 0.51** (0.18) | | | |

**Table B.** Estimates (SE) for top linear mixed-effects models and tests for significance of metallic element and metalloid concentrations (logged) and land-use variables in caracals (n = 67) in the Greater Cape Town area, South Africa (**P* < 0.1; ***P* < 0.05; ****P* < 0.01)

| Explanatory variables | Al | As | Cd | Co | Cr | Cu | Hg | Mn | Pb | Se | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Juvenile male** | | 0.78** (0.25) | | 0.004 (0.002) | | 0.31 (0.15) | | 0.5** (0.18) | | | |
| **Agriculture** | | -0.34*** (0.14) | | | | | | | | -0.15*** (0.03) | |
| **Altered open** | | | | | | | | | | -0.09** (0.04) | |
| **Distance from coast** | | | | | | 0.21** (0.07) | -0.12** (0.04) | | | | 0.21 (0.11) |
| **Human population density** | | | | | 0.07** (0.03) | | | | | | |
| **Road density** | | -0.35** (0.11) | | 0.002 (0.001) | | | | | 0.006 (0.003) | | |
| **Townships** | | | | 0.002** (0.001) | | | | | | | |
| **Urban** | 0.30** (0.13) | | | | | | | | | | |
| **Vineyards** | | 0.28** (0.14) | | | | -0.26*** (0.07) | | | | | -0.37*** (0.11) |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Constant** | 1.06*** (0.13) | 0.61*** (0.17) | 0.04*** (0.01) | 0.005** (0.001) | 0.18 (0.03) | 1.62*** (0.11) | 0.12 (0.07) | 0.41** (0.13) | 0.032*** (0.003) | 1.49*** (0.03) | 3.72*** (0.09) |

**Table C.** Estimates (SE) for top linear models and tests for significance of diet (biomass) for broad prey groups and metallic element and metalloid concentrations (logged) in caracals (n = 34) in the Greater Cape Town area, South Africa (*$P$ < 0.1; **$P$ < 0.05; ***$P$ < 0.01)

| Explanatory variables | Al | As | Cd | Co | Cr | Cu | Hg | Mn | Pb | Se | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Juvenile male** | | | | 0.0047** (0.002) | | | | | | | |
| **Bird** | | 0.25 (0.13) | | | | | 0.09 (0.05) | | | | |
| **Insect** | 0.37 (0.19) | | | 0.002** (0.0009) | | | | | | | |
| **Constant** | 1.07*** (0.19) | 1.17*** (0.12) | 0.02 (0.01) | 0.002 (0.001) | 0.17** (0.05) | 1.62*** (0.09) | 0.31*** (0.05) | 0.16 (0.13) | 0.03 (0.006) | 1.53*** (0.04) | 3.58*** (0.12) |

**Table D.** Estimates (SE) for top linear models and tests for significance of diet (FO) for broad prey groups and metallic element and metalloid concentrations (logged) in caracals (n = 34) in the Greater Cape Town area, South Africa (*$P$ < 0.1; **$P$ < 0.05; ***$P$ < 0.01).

| Explanatory variables | Al | As | Cd | Co | Cr | Cu | Hg | Mn | Pb | Se | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Juvenile male** | | | | 5.418e-03* (2.310e-03) | | | | | | | |
| **Bird** | | | | | | | 0.09 (0.05) | | | | |
| **Insect** | | | | 2.079e-03 ***(9.536e-04) | | | | | | | |
| **Constant** | 1.07*** (0.19) | 0.78*** (0.21) | 0.02 (0.01) | 0.003*** (0.001) | 0.17** (0.05) | 1.52*** (0.17) | 0.31*** (0.05) | 0.15 (0.14) | 0.03*** (0.006) | 1.53*** (0.04) | 3.58*** (0.12) |

**Table E.** Estimates (SE) for top linear models and tests for significance of diet (biomass) for prey functional groups and metallic element and metalloid concentrations (logged) in caracals (n = 34) in the Greater Cape Town area, South Africa (*$P$ < 0.1; **$P$ < 0.05; ***$P$ < 0.01)

| Explanatory variables | Al | As | Cd | Co | Cr | Cu | Hg | Mn | Pb | Se | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Carnivore** | 0.35 (0.19) | -0.27** (0.13) | | | | | | | | | |
| **Herbivore** | | -0.35*** (0.13) | | | | | | | | | |
| **Marine feeder** | | | | | | | | | | 0.07 (0.04) | |
| **Constant** | 1.07*** (0.19) | 1.17*** (0.11) | 0.02 (0.01) | 0.001 (0.001) | 0.17** (0.05) | 1.52*** (0.17) | 0.31*** (0.05) | 0.14 (0.14) | 0.03*** (0.006) | 1.53*** (0.03) | 3.58*** (0.12) |

**Table F.** Estimates (SE) for top linear models and tests for significance of diet (FO) for prey functional groups and metallic element and metalloid concentrations (logged) in caracals (n = 34) in the Greater Cape Town area, South Africa (*$P$ < 0.1; **$P$ < 0.05; ***$P$ < 0.01)

| Explanatory variables | Al | As | Cd | Co | Cr | Cu | Hg | Mn | Pb | Se | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Juvenile male** | 1.05** (0.46) | 0.99** (0.31) | | | | | | | | | |
| **Carnivore** | 0.45** (0.19) | | | | | | | | | | |
| **Herbivore** | | | | | | | -0.13*** (0.05) | | | | |
| **Insectivore** | | -0.25 (0.13) | | | | | | | | | |
| **Marine feeder** | | | | | | | | | | 0.08** (0.05) | |
| **Constant** | 0.64 (0.32) | 0.71** (0.20) | 0.02 (0.01) | 0.001 (0.001) | 0.17** (0.05) | 1.52*** (0.17) | 0.31*** (0.04) | 0.14 (0.14) | 0.03*** (0.006) | 1.53*** (0.03) | 3.58*** (0.12) |

**Table E.** Estimates (SE) for top linear models and tests for significance of diet (biomass) for prey foraging habitat groups and metallic element and metalloid concentrations (logged) in caracals (n = 34) in the Greater Cape Town area, South Africa (*$P$ < 0.1; **$P$ < 0.05; ***$P$ < 0.01)

| Explanatory variables | Al | As | Cd | Co | Cr | Cu | Hg | Mn | Pb | Se | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Marine-adapted** | | | | | | | 0.09** (0.04) | | | 0.09*** (0.04) | |
| **Wetland-adapted** | | 0.27** (0.13) | | | | 0.18 (0.09) | 0.16** (0.05) | 0.16 (0.09) | | 0.09*** (0.04) | 0.21 (0.13) |
| **Constant** | 1.07*** (0.19) | 1.17*** (0.12) | 0.02 (0.01) | 0.001 (0.001) | 0.17** (0.05) | 1.62*** (0.09) | 0.31*** (0.04) | 0.30** (0.08) | 0.03*** (0.006) | 1.53*** (0.03) | 3.58*** (0.12) |

**Table H.** Estimates (SE) for top linear models and tests for significance of diet (FO) for prey foraging habitat groups and metallic element and metalloid concentrations (logged) in caracals (n = 34) in the Greater Cape Town area, South Africa (*$P$ < 0.1; **$P$ < 0.05; ***$P$ < 0.01)

| Explanatory variables | Al | As | Cd | Co | Cr | Cu | Hg | Mn | Pb | Se | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Marine-adapted** | | | | | | | 0.13** (0.05) | | | 0.11** (0.03) | |
| **Wetland-adapted** | | | | | | | 0.11** (0.05) | | | 0.08** (0.03) | |
| **Constant** | 0.79* (0.34) | 0.79*** (0.21) | 0.02 (0.01) | 0.001 (0.001) | 0.17** (0.05) | 1.52*** (0.17) | 0.31*** (0.04) | 0.15 (0.14) | 0.03*** (0.006) | 1.53*** (0.03) | 3.58*** (0.12) |

**Table I.** Estimates (SE) for top linear models and tests for significance of diet (biomass) for exotic prey groups and metallic element and metalloid concentrations (logged) in caracals (n = 34) in the Greater Cape Town area, South Africa (*$P$ < 0.1; **$P$ < 0.05; ***$P$ < 0.01)

| Explanatory variables | Al | As | Cd | Co | Cr | Cu | Hg | Mn | Pb | Se | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Female** | | 0.72* (0.30) | | | | | | | | 0.23* (0.09) | |
| **Juvenile male** | | 0.97** (0.29) | | | | | | | | | |
| **Synanthropic** | | -0.30** (0.12) | | | | | | | | -0.08** (0.04) | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Constant** | 1.07*** (0.19) | 0.6** (0.21) | 0.02 (0.01) | 0.001 (0.001) | 0.17** (0.05) | 1.52*** (0.17) | 0.31*** (0.05) | 0.16 (0.15) | 0.03*** (0.006) | 1.42*** (0.06) | 3.58*** 0.12 |

**Table J.** Estimates (SE) for top linear models and tests for significance of diet (FO) for exotic prey groups and metallic element and metalloid concentrations (logged) in caracals (n = 34) in the Greater Cape Town area, South Africa (*$P$ < 0.1; **$P$ < 0.05; ***$P$ < 0.01)

| **Explanatory variables** | **Al** | **As** | **Cd** | **Co** | **Cr** | **Cu** | **Hg** | **Mn** | **Pb** | **Se** | **Zn** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Female** | | 0.51 (0.29) | | | | | | | | | |
| **Juvenile male** | | 0.81** (0.29) | | | | | | | | | |
| **Synanthropic** | | -0.22 (0.12) | | | | | | | | -0.06 (0.08) | |
| **Constant** | 1.07*** (0.19) | 0.73** (0.2) | 0.02 (0.01) | 0.001 (0.001) | 0.17** (0.05) | 1.52*** (0.17) | 0.31*** (0.05) | 0.14 (0.14) | 0.03*** (0.006) | 1.53*** (0.03) | 3.58*** (0.12) |

**Appendix VI: Table A**. Summary of published mean metallic element and metalloid concentrations (µg/g, d.w.) in tissues of terrestrial carnivores taken from peer-reviewed literature

| **Species** | **Sample type** | **Al** | **As** | **Cd** | **Co** | **Cr** | **Cu** | **Hg** | **Mn** | **Pb** | **Se** | **Zn** | **Study** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Caracal, *Caracal caracal*** | **Blood** | **7.70** | **3.74** | **0.06** | **0.01** | **0.29** | **5.88** | **0.45** | **3.84** | **0.03** | **3.69** | **56.55** | **This study** |
| Arctic Fox, *Vulpes lagopus* | Hair | - | - | - | - | 0.14 | 3.76 | - | - | 0.54 | - | 58.6 | Filistowicz et al. 2012 |
| Eurasian otter, *Lutra lutra* | Liver | - | 0.27 | 0.44 | 0.07 | 0.25 | 35.7 | 6.82 | 9.43 | 0.57 | 6.76 | 118 | Brand et al. 2020 |
| Malay civet, *Viverra tangalunga* | Hair | 114 | 0.11 | 0.01 | 0.16 | 3.50 | 10.10 | 2.39 | 14.6 | 0.52 | - | 191 | Evans et al. 2021 |
| Domestic cat, *Felis catus* | Kidney | - | - | 0.10 | - | - | - | - | - | 0.26 | - | - | Esposito et al. 2019 |
| Golden Jackal, *Canis aureus* | Kidney | - | - | 1.41 | 0.52 | - | 17.6 | - | - | 4.06 | - | 58.52 | Markov et al. 2016 |
| Iberian lynx, *Lynx pardinus* | Liver | - | - | 0.12 | - | - | 18.2 | 0.23 | - | 0.04 | 1.54 | 114.70 | Millán et al. 2008 |
| Coyote, *Canis latrans* | Liver | - | - | 0.23 | - | - | 90.1 | - | - | 0.30 | - | 139 | Hough et al. 2020 |
| River otter, *Lontra canadensis* | Brain | - | - | - | - | - | - | 0.3 - 18 | - | - | 1.0 – 7.8 | - | Haines et al. 2010 |
| Ocelot, *Felis pardalis* | Hair | - | - | - | - | - | - | 0.5 - 1.25 | - | 0.56 - 26.8 | 1.5 - 3.48 | - | Mora et al. 2000 |
| Bonelli's eagle, *Aquila fasciata* | Blood | - | 0.10 | - | - | - | - | 0.04 | - | 0.09 | - | 23.30 | Ortiz-Santaliestra et al. 2015 |
| African white-backed vulture, *Pseudogyps africanus* | Blood | 46.74 | - | - | 1.62 | 17.7 | 8.36 | - | 2.21 | 7.81 | - | 16.51 | van Wyk et al. 2001 |