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Black mambas (*Dendroaspis polylepis*) as novel bioindicators of urban heavy metal pollution☆

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ABSTRACT

Biomonitoring studies have documented the presence of contaminants in various urban wildlife species, but research has predominantly focused on avian and mammalian species. Despite snakes possessing several traits that make them excellent subjects for ecotoxicological studies, knowledge on their capacity to serve as bioindicators remains limited, particularly in African ecosystems where their utility has not previously been assessed. In this study, we investigated the use of black mambas (*Dendroaspis polylepis*), a large-bodied snake species native to sub-Saharan Africa, to serve as a bioindicator for heavy metal pollution within an urban environment. We examined concentrations of arsenic, cadmium, lead, and mercury in ventral scale clippings and compared these to concentrations in internal tissue samples from dead specimens opportunistically collected around the city of Durban, South Africa. Our findings demonstrated that black mambas are exposed to and accumulate heavy metals within their tissues. Metal concentrations tended to be higher in scales than liver and muscle tissue and displayed substantial variability, which was not strongly linked to body size or sex. We found a clear association between land use and heavy metal exposure in black mambas. Snakes living in connected green spaces around the city generally had significantly lower heavy metal concentrations in their scales compared to those in more industrial and commercial areas. Our findings indicate that black mambas can act as valuable sentinels of heavy metal pollution, enabling the detection of fine-scale local patterns. This suggests that snakes could be valuable models for environmental monitoring elsewhere in Africa.

1. Introduction

Urban environments present a complex mix of anthropogenic contaminants, which can pose significant threats to wildlife (e.g., Smith et al., 2021; Li et al., 2022). Bioaccumulation of these contaminants within urban wildlife populations is a critical concern, necessitating robust biomonitoring approaches to assess exposure levels and potential ecological impacts (e.g., Kumar, 2023; Morales-Zamudio et al., 2023; Parker et al., 2023). Biomonitoring studies have documented the presence of contaminants in various urban wildlife species, but research has predominantly focused on avian and mammalian species, with a significant lack of studies on the herpetofauna (Collins et al., 2021). Although research interest has grown in recent decades, studies on the use of snakes as bioindicators remain limited, despite the diverse ecological niches snakes occupy, their high trophic position, and the vital role they play in the structure and function of various aquatic and terrestrial ecosystems within urban environments. Recent reports (e.g., Weir et al., 2023; van den Brink et al., 2024) have highlighted the need for greater emphasis on reptilian ecotoxicology, citing the considerable gap in our knowledge regarding contaminant exposure and its impacts, as well as the unique characteristics of reptiles that make them excellent study organisms for ecotoxicological investigations.

Snakes exhibit a variety of traits that make them exceptional candidates for ecotoxicological studies and potentially good models for biomonitoring. Many snake species are dietary specialists and act as apex predators in the food web (Greene, 1997), increasing the likelihood

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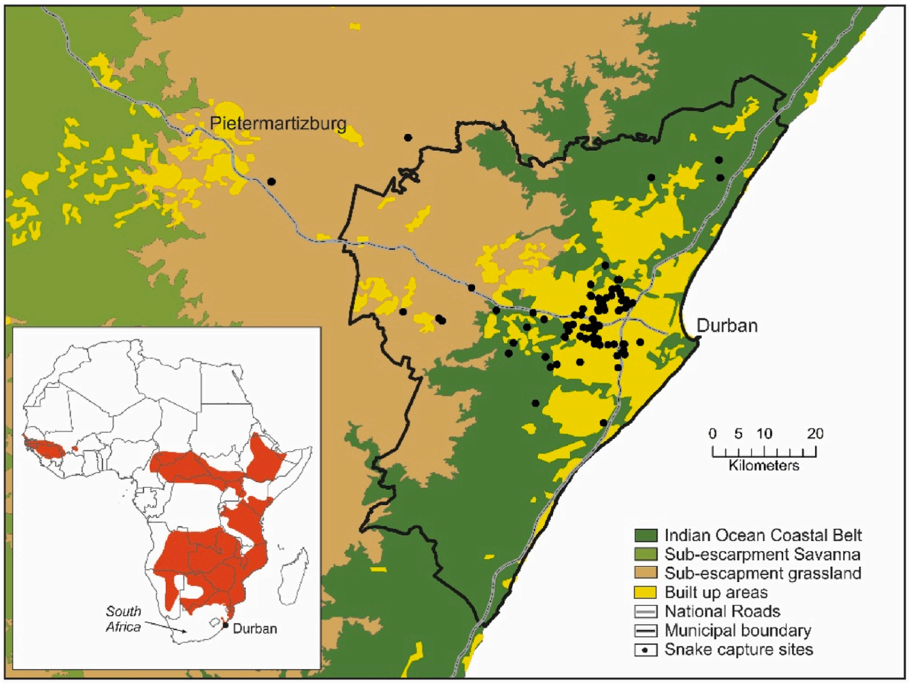

Fig. 1. A) The black mamba (*Dendroaspis polylepis*) inhabits a wide range in sub-Saharan Africa. In South Africa, its range is confined to the coastal regions of KwaZulu-Natal, and in the north-eastern parts of the country. Distribution map based on IUCN Red List spatial data (https://www.iucnredlist.org/). B) The study area located on the east coast of South Africa. Black dots show the location of where samples were collected. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

that they will be exposed to and accumulate pollutants. Furthermore, because snakes are non-migratory and have relatively small home ranges, they are thus likely to be particularly valuable biomarkers of local contamination (Burger, 1992; Lettoof et al., 2020). Available experimental (e.g., Hopkins et al., 2001; Frossard et al., 2019) and field studies (e.g., Haskins et al., 2021; Lettoof et al., 2021; Toledo et al., 2024) suggest that snakes can bioaccumulate substantial quantities of pollutants and potentially serve as useful indicators of ecosystem health. For example, several studies from the USA have shown that snakes can bioaccumulate significant amounts of mercury (see Haskins et al., 2019 for a review). However, our knowledge on the ability of snakes to act as novel bioindicators is limited, with most of this research conducted in North America (e.g., Rainwater et al., 2005; Burger et al., 2006, 2007; Wylie et al., 2009). The bioaccumulation of contaminants in snakes and their potential as bioindicators of ecosystem health have not yet been studied in Africa.

Given the occurrence of snakes within urban ecosystems (Alexander, 1990) and their potential to act as valuable sentinels of environmental pollution (Burger et al., 2007), incorporating snakes into urban biomonitoring programs may be effective in enhancing our understanding of contaminant exposure and its ecological consequences. In this study, we investigated the potential use of black mambas (*Dendroaspis polylepis*) as novel bioindicators of heavy metal pollution in an urban environment. As long-lived, active foragers (Branch, 1998) that specialise in endothermic prey (Evans and Alexander, 2021), black mambas are likely to be highly susceptible to exposure and bioaccumulation of pollutants, making them ideal indicators of environmental contamination. We examined heavy metal concentrations (arsenic, cadmium, lead, and mercury) in ventral scale clippings and internal tissue samples from individuals opportunistically collected in urban and semi-urban environments around the city of Durban, in KwaZulu-Natal Province of South Africa where black mamba abundance has risen in recent years (Alexander, 2023) (Fig. 1). Our primary objectives were to: 1) investigate the accumulation of heavy metal contaminants in black mamba tissues and evaluate the effectiveness of using non-lethal scale clippings for biomonitoring metal pollution, and 2) assess the relationship between element concentrations in scale clippings and potential pollution sources associated with different land uses. This study provides the first data on contaminant accumulation in this species, contributing to our limited knowledge of environmental contaminant exposure in snakes. Our findings have broader implications for the development and refinement of environmental monitoring programs in Africa.

2. Study species and study area

2.1. Study area

The city of Durban is located in the eThekwini Municipal Area in the province of KwaZulu-Natal on the east coast of South Africa (Fig. 1). The metropolitan municipality spans an area of 2556 km^2 and has a population of ~4.2 million people, making it the third-most populous city in South Africa (Stats SA, 2022). City growth originated historically around the Port of Durban, the largest and busiest shipping terminal in sub-Saharan Africa. Over time, development has expanded inland, primarily along two key national roads; one running north-south parallel to the coastline and another connecting Durban to the economic centre of Johannesburg.

Durban has a humid, subtropical climate and much of the metropolitan area falls within the Indian Ocean Coastal Belt biome, comprising dense bush and indigenous forest (Mucina and Rutherford, 2006). Further inland, thorny bushveld, savanna and sourveld grassland dominates. About 68 % of the municipal area is considered rural, with most people living in pockets of dense settlement (eThekwini Municipality, 2023a). A large proportion of eThekwini Municipality is rural in character and about 80 % of people live in densely populated urban areas. The vegetation has been drastically modified by urban development with the metropolitan area comprising a mosaic of mixed levels of urban developments (commercial, industrial, and residential) interspersed by green spaces (e.g., parks, conservancies, green belts and nature reserves). Natural vegetation persists in conservancies and nature reserves under the Durban Metropolitan Open Space System (D'MOSS) policy, a system of green spaces and corridors that link fragments of indigenous forest to facilitate passage of fauna and flora (Roberts, 1994). The D'MOSS network (95 000 ha) represents about one-third of the eThekwini Municipal Area and is managed by the eThekwini Metropolitan Municipality (eThekwini Municipality, 2023b). Several major rivers occur in the Durban region. These arise in the hills toward the west of the city and flow through extensively altered catchments, with multiple studies having reported the presence of elevated concentrations of heavy metals, among other pollutants, in these watercourses (e.g., Olaniran et al., 2014; Moodley et al., 2021; Ngubane et al., 2023).

The study area is situated within the Maputaland-Pondoland Albany biodiversity hotspot, recognised for its high levels of floral endemism. Twenty seven species of snake are known to occur in the Durban Municipality (Alexander, 1990), and black mamba abundance appears to have risen significantly in the province over the last four decades since the survey of Alexander (1990) (Alexander, 2023.).

2.2. Study species

The black mamba, belonging to the Elapidae family, is the largest venomous snake in Africa and is widely distributed across sub-Saharan Africa (Spawls & Branch, 2020; Tolley et al., 2025). Adults are usually between 2.4 and 3 m in body length, but large specimens exceeding 4 m have been recorded (Haagner and Morgan, 1993; Alexander and Marais, 2007). There is no size dimorphism between the sexes. In South Africa, the species is found along the coastal regions of KwaZulu-Natal and in the north-eastern parts of the country, where they typically occur in low population densities. The species occurs mainly in savanna (Tolley et al., 2025), and will typically select rocky areas that offer suitable retreats. Although black mambas have not been the subject of a formal telemetry study, observational data suggests they exhibit strong site fidelity, often utilizing the same retreat site for extended periods (Phelps, 2002).

Black mambas are primarily terrestrial and active foragers that specialise in small to medium-sized mammals, although they will also feed on birds (Broadley, 1983). Black mambas exhibit opportunistic feeding behaviour, also preying on domestic animals, such as cats (Marais, 2022). Black mambas experience rapid growth and have a lifespan of more than 20 years (Branch, 1998). The mating season typically starts in May, with males engaging in combat to establish dominance and gain access to receptive females. Females lay clutches of 6–17 eggs, with juveniles hatching around late February to early March (Haagner and Morgan, 1993).

3. Methods

3.1. Sampling and chemical analyses

Ventral scale clippings were collected from 61 live black mambas captured by *KwaZulu-Natal Amphibian and Reptile Conservation* in the Greater Durban area between October 2021 and June 2022. The snakes were removed following calls from local residents and businesses. Clippings were collected by restraining the snake in a clear plastic sampling tube and cutting a ~10 mm length of the posterior edge of a ventral scale using a pair of surgical scissors. Samples were transferred to a sterile 1.5 ml Eppendorf tube and stored frozen. The sex, total length, snout-vent length, and mass of each snake were recorded, as well as the GPS coordinates and private property addresses of the citizens who requested snake removal. Individuals were subsequently relocated and released into suitable habitats, such as nearby nature reserves or open land away from human activity.

Ventral scales and internal tissues were opportunistically sampled from 33 dead adult black mambas specimens collected over a four-year period (June 2019 to May 2023). Typically, these included snakes that had been hit by vehicles, intentionally killed by humans, or fatally injured by dogs. Where possible, the sex and total length of each specimen was recorded upon collection. Specimens were subsequently frozen and transported to the University of the Witwatersrand for chemical analysis. In the laboratory, specimens were dissected to remove ventral scales, liver and muscle tissue for analysis. In a few cases, soft tissue suitable for analysis could not be collected due to the advanced state of decay. Scale clippings were sonicated and rinsed in ultrapure Milli-Q water to remove potential surface contamination, before being dried in an oven at 60 °C.

Soft tissues (~0.5 g wet weight) and scale clippings (typically ~0.01 g dry weight) were digested in ultrapure nitric acid (65 % Suprapur, Sigma Aldrich) using a CEM Microwave Accelerate Reaction System (MARS) Xpress. The resulting digest was diluted to a final matrix of 5 % HNO_3, with 1 % HCl added for element stabilisation. Element concentrations were measured using an Agilent 7700 ICP-MS, with quantification performed against standard solutions prepared by gravimetric dilution from certified primary elemental solutions. The sample digestion and analysis procedures were validated using certified reference material NIST SRM 2976 (mussel tissue). Analytical results showed good agreement with the certified values with recoveries of 101 ± 2.2 % for lead, 104 ± 2.9 % for mercury, 104 ± 0.5 % for cadmium, and 116 ± 3.7 % for arsenic. Detection limits ranged between 0.005 ng g^{-1} (cadmium) and 0.04 ng g^{-1} (lead and mercury).

To facilitate comparisons with other studies in the literature, selected liver and muscle subsamples (n = 10) were dried at 90 °C to measure tissue moisture contents. Calculated moisture contents for liver and muscle tissue were 69 ± 5.5 % and 72 ± 2.0 %, respectively. All liver and muscle tissue concentrations were subsequently converted to dry weight based on 70 % moisture content, and all concentrations are expressed in ng g^{-1} dry weight unless otherwise stated.

All snakes were removed by a professional herpetologist and trained venomous snake handler (Evans). The local wildlife and environmental authority (Ezemvelo KZN Wildlife) does not require any permitting to remove this species from potential conflict situations. This study was conducted with approval of the University of the Witwatersrand Animal Research Ethics Committee (number: 2013/32/01).

3.2. Statistical analysis

Statistical analyses were performed using Statistica 10 (StatsSoft, USA). Data were normalised by logarithmic transformation, and normality of residuals was confirmed by visual inspection of residuals and by Shapiro-Wilk test. Differences in heavy metal concentrations across tissues was assessed using a Student's t-test, while a one-way analysis of variance (ANOVA) with a Tukey post-hoc test was used for comparisons between sexes. Relationships between body size (length and mass) and tissue contaminant concentrations were examined using regression analysis. Relationships between heavy metal concentrations in scales and those in liver and muscle were examined using Spearman correlation tests. All statistical tests were considered significant when $p < 0.05$.

3.3. Spatial patterns

To investigate whether observed contaminant variability in scales (from both dead and live individuals) were associated with proximity to potential pollution sources, we compared spatial patterns in heavy metal concentrations to surrounding land use characteristics. To quantify the effect of land use, we grouped black mambas into three categories:1) those found within the D'MOSS network of conservancies, nature reserves and green spaces; 2) those found on the boundary/transition zone between D'MOSS areas and surrounding land uses, which typically included residential areas, and; 3) those found clearly outside the D'MOSS network.

Table 1
Metal concentrations (ng g^{-1} dry weight) measured in black mamba (*D. polylepis*) tissues. Results are expressed as the mean ± SE for each tissue. Concentration range in parenthesis.

Metal	Scales (n = 33)	Liver (n = 29)	Muscle (n = 32)
Arsenic	1960 ± 690 (168–22600)	600 ± 140 (72–3100)	775 ± 160 (52–3300)
Mercury	49 ± 8 (n.d – 164)	31 ± 7 (4–150)	107 ± 18 (9–434)
Cadmium	44 ± 15 (4–486)	35 ± 4 (8–87)	1.7 ± 0.2 (n.d – 6)
Lead	610 ± 105 (36–2790)	150 ± 23 (21–550)	53 ± 7 (n.d – 160)

n.d = not detected.
wet weight data reported in supplementary Table S1

4. Results

4.1. Heavy metal concentrations in tissues from dead specimens

Samples from a total of 33 dead individuals were collected for analysis. All snakes were adults and included 19 males (total length: 2220 ± 72 mm) and 14 females (total length: 2040 ± 65 mm). There was no significant difference ($p = 0.12$) in the size of males and females used in this study.

Heavy metal concentrations are reported in Table 1. Of the four elements examined in this study, As was found in highest concentration in all three tissue types. However, there was considerable variation in concentrations among different tissues. Mean As concentrations were significantly higher in scales compared to both liver ($p = 0.002$) and muscle ($p = 0.013$). Similarly, Pb concentrations were significantly elevated in scales compared to liver ($p < 0.001$) and muscle ($p < 0.001$). While scales also exhibited the highest Cd concentrations, significantly exceeding concentrations in muscle ($p < 0.001$), they were not significantly different from those in the liver ($p = 0.54$). Mercury concentrations were significantly higher in muscle tissue compared to both liver ($p < 0.001$) and scales ($p = 0.001$).

In general, we found no correlations between the concentrations of different heavy metals; individuals with high concentrations of one element did not necessarily have high concentrations of another element (Supplementary Table S2). Body length was also generally not correlated with heavy metal concentrations, apart from Cd in liver ($R^2 = 0.55$; $p < 0.001$). In addition, we found few differences in heavy metal concentrations across tissues between sexes. The only statistically significant variation was found for Cd in liver ($p = 0.020$, see Supplementary Table S3), where males showed higher concentrations.

To assess whether scales from black mambas could serve as nonlethal bioindicators of internal heavy metal contaminant burdens, we compared concentrations in scales with internal tissues. Concentrations

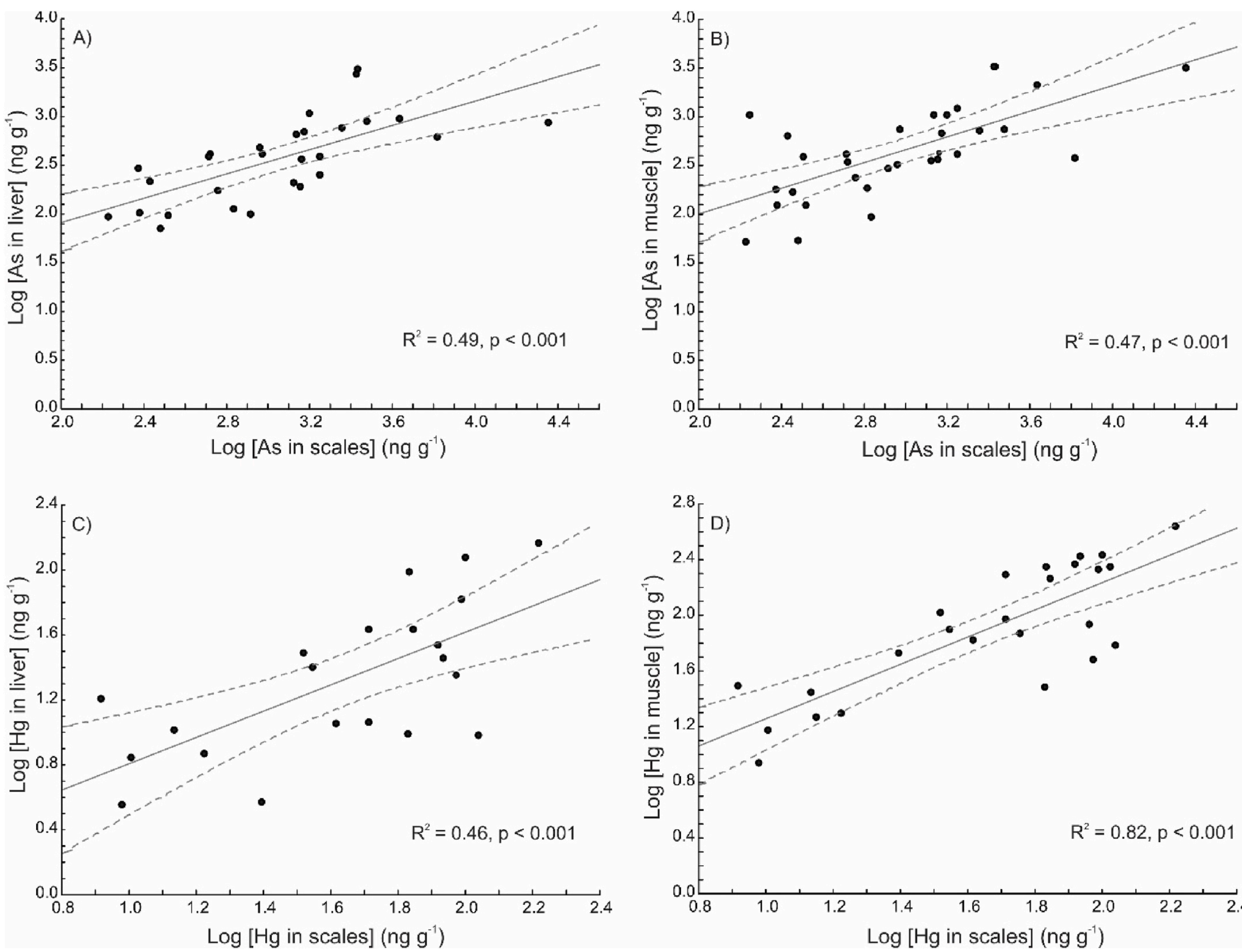

Fig. 2. Correlation between heavy metal concentrations in scales versus internal tissues, showing that scales effectively predicted As and Hg concentrations in both liver and muscle tissue.

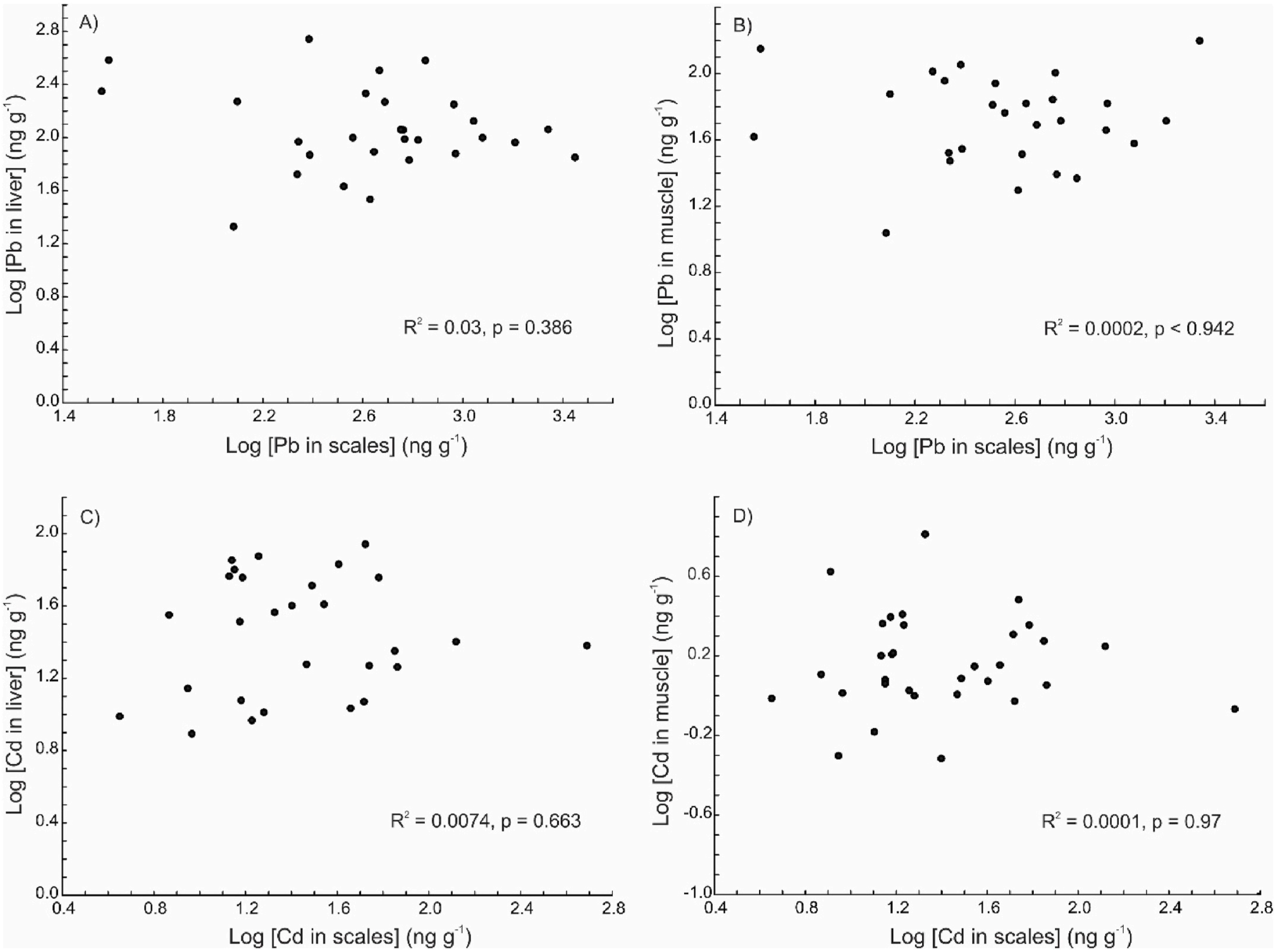

Fig. 3. Correlation between Pb and Cd concentrations in scales versus internal tissues. None of the comparisons were significant.

Table 2
Body measurements and mean metal concentrations (±SE) quantified in ventral scale clippings from black mambas. Concentration range in parenthesis.

	n	TL (mm)	Mass (g)	As (ng g^{-1})	Cd (ng g^{-1})	Hg (ng g^{-1})	Pb (ng g^{-1})
All samples	61	2264 ± 32	1388 ± 83	1880 ± 230 (337–9660)	73 ± 11 (n.d – 519)	165 ± 27 (n.d – 750)	1200 ± 175 (n.d – 6420)
Males	29	2283 ± 49 (1600–2780)	1545 ± 120 (400–2750)	1590 ± 330 (337–9660)	79 ± 20 (n.d – 519)	110 ± 28 (n.d – 485)	860 ± 150 (276–3650)
Females	32	2245 ± 43 (1670–2630)	1247 ± 110 (550–2900)	2140 ± 310 (503–8010)	67 ± 11 (n.d – 310)	227 ± 45 (n.d – 750)	1710 ± 330 (n.d – 6240)
Detection frequency (%)				100	93.4	24.6	49.2

n.d = not detected.

of As and Hg in muscle and liver were significantly correlated with those in scales (Fig. 2). This relationship was strongest for Hg in muscle. However, scales proved to be a poor predictor for Pb and Cd in internal tissues, with no correlational pattern for either liver or muscle (Fig. 3).

4.2. *Heavy metal concentrations in scale clippings from live snakes*

Ventral scale clippings were collected from a total of 61 live individuals, which included 29 adult males (2283 ± 49 mm) and 32 adult females (2245 ± 43 mm) (Table 2). Arsenic and Cd were detected in the vast majority of samples, whereas Pb and Hg were detected less frequently (49.2 % and 24.6 %, respectively). As observed in scales analysed from dead specimens, As exhibited highest concentrations in scale clippings from live snakes, followed by Pb. Overall, there was a high degree of variability in the concentrations measured in the sampled population. For example, As concentrations ranged from 340 ng g^{-1} to 9660 ng g^{-1}, while Pb concentrations varied from 100 ng g^{-1} to 6420 ng g^{-1}. Although body length was highly correlated with body mass ($y = 3E\text{-}09 \times ^{3.475}$; $R^2 = 0.72$; $p < 0.001$), neither measure of size proved to be a good predictor of heavy metal concentration (Supplementary Table S4). There were also no significant differences in heavy metal concentrations between sexes (Supplementary Table S5).

4.3. *Spatial patterns*

In general, black mambas captured in less anthropized, outlying

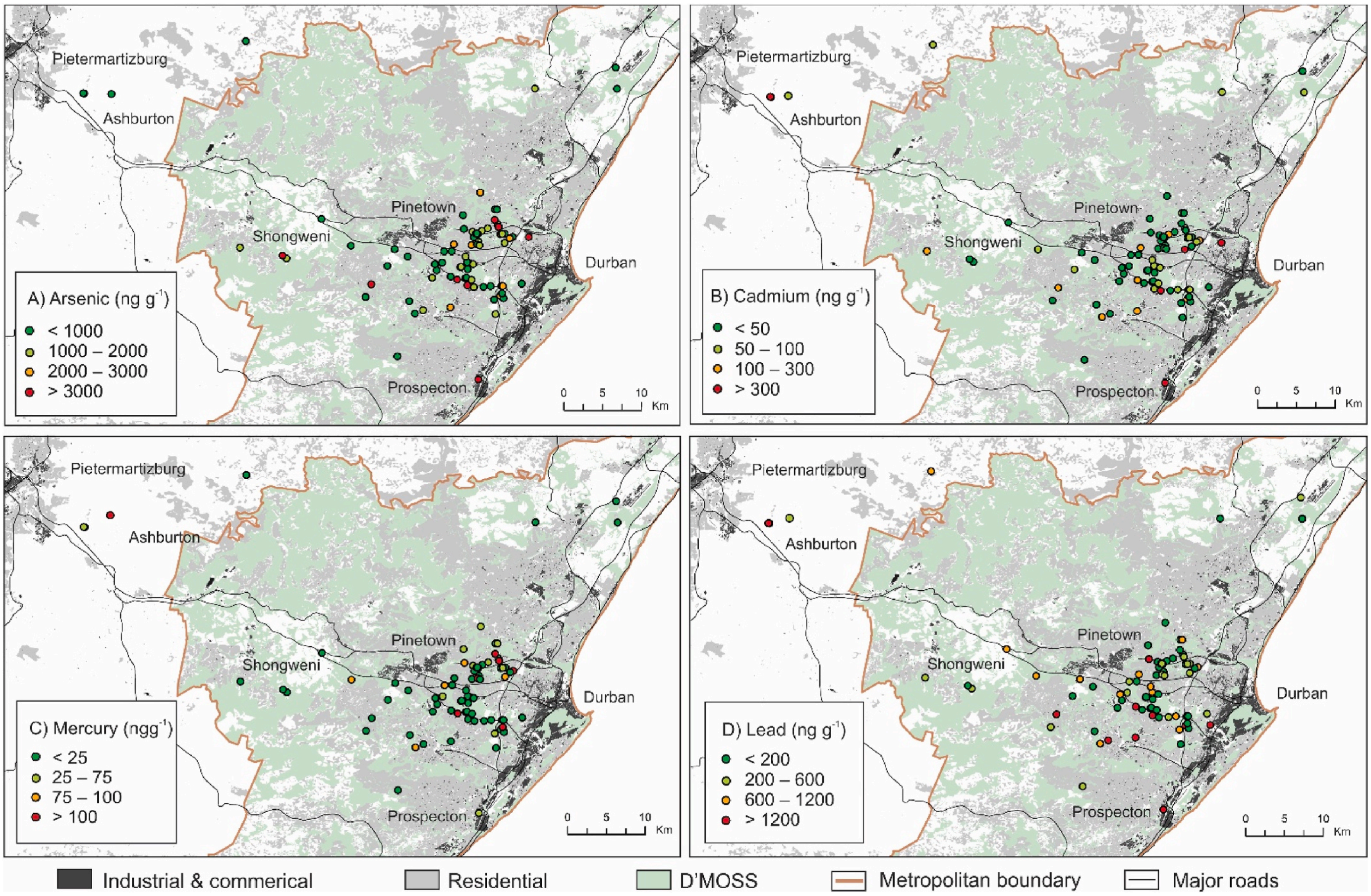

Fig. 4. Spatial variation in heavy metal concentrations in ventral scales of black mambas (*D. polylepis*) sampled in the Greater Durban area in relation to major land use types. Durban Metropolitan Open Space System (D'MOSS) represents a network of open spaces that incorporates areas of high biodiversity value. Land use data obtained from the South African National Land-Cover 2020 database. D'MOSS data obtained from the eThekwini Municipality Open GIS Database (https://gis-ethekwini.opendata.arcgis.com/).

areas (e.g., Shongweni and northern coast) exhibited lower metal concentrations compared to those captured around Durban City and suburban areas (Fig. 4). Industrial and commercial activities located around Durban City appear to be particularly important point sources of pollution exposure. For example, an individual captured from the Prospecton industrial area, located just south of Durban Harbour, exhibited the highest As (9660 ng g^{-1}) and Cd (520 ng g^{-1}) concentrations, as well as one of the highest Pb concentrations (3650 ng g^{-1}). Prospecton is characterised by heavy industrial activity, including metal fabrication, vehicle manufacturing, and paint production. Notably, it is also the site of Sapref refinery, which was the largest crude oil refinery in southern Africa before it closed in 2022.Lead contamination showed a more scattered pattern, but the most elevated levels were typically found around industrial zones in Durban South (e.g., Queensmead and Escombe) and nearby suburbs, including Queensburgh (recording the highest Pb concentration at 6420 ng g^{-1}) and Chatsworth (with the second highest Pb concentration at 4330 ng g^{-1}).

Grouping black mambas based on their proximity to the D'MOSS network revealed a clear link between land use on heavy metal exposure (Fig. 5). Snakes found within D'MOSS conservancies, nature reserves and green spaces around Durban had significantly lower scale concentrations of As, Cd and Pb compared to those sampled from outside D'MOSS areas. Snakes sampled near the boundary of D'MOSS areas typically showed lower average heavy metal concentrations compared to those from more distant areas, although this difference was only statistically significant for Cd (Fig. 5). No significant trends were found for Hg.

5. Discussion

Our findings demonstrated that black mambas inhabiting the Greater Durban area are exposed to and accumulate heavy metals within their tissues. Overall, As was present in highest concentrations in the sampled black mambas, with significant differences in heavy metal concentrations across tissue types. Notably, As, Pb, and to a lesser extent, Cd, accumulated to higher concentrations in scales compared to liver and muscle tissues, likely due to the strong binding affinity of these metals to keratin, the primary component of scales (Toni and Alibardi, 2007). Concentrations of As and Hg in scales effectively predicted levels in muscle and liver tissues, but this relationship was not observed for Pb and Cd. Geographic patterns of metal accumulation across the study site revealed a link between the degree of urban development and the accumulation of metals in the samples, revealing that the relatively low vagility of snakes facilitates the detection of local patterns in heavy metal pollution. Thus, our findings support the hypothesis that snakes such as black mambas make ideal bioindicators for detecting patterns of heavy metal pollution in the environment and they can reveal local patterns in pollution levels.

Metal concentrations in black mamba scales exhibited a high degree of variation, which was not significantly correlated with sex or body size. Body size has been identified as a key factor in contaminant accumulation, particularly Hg, with previous studies documenting a positive correlation between body size and Hg concentrations in various snake species, including watersnakes (*Nerodia fasciata, N. floridana, N. sipedon*), cottonmouths (*Agkistrodon piscivorous*), ribbon snakes (*Thamnophis sauritus*), and Burmese pythons (*Python bivittatus*) (Haskins et al.,

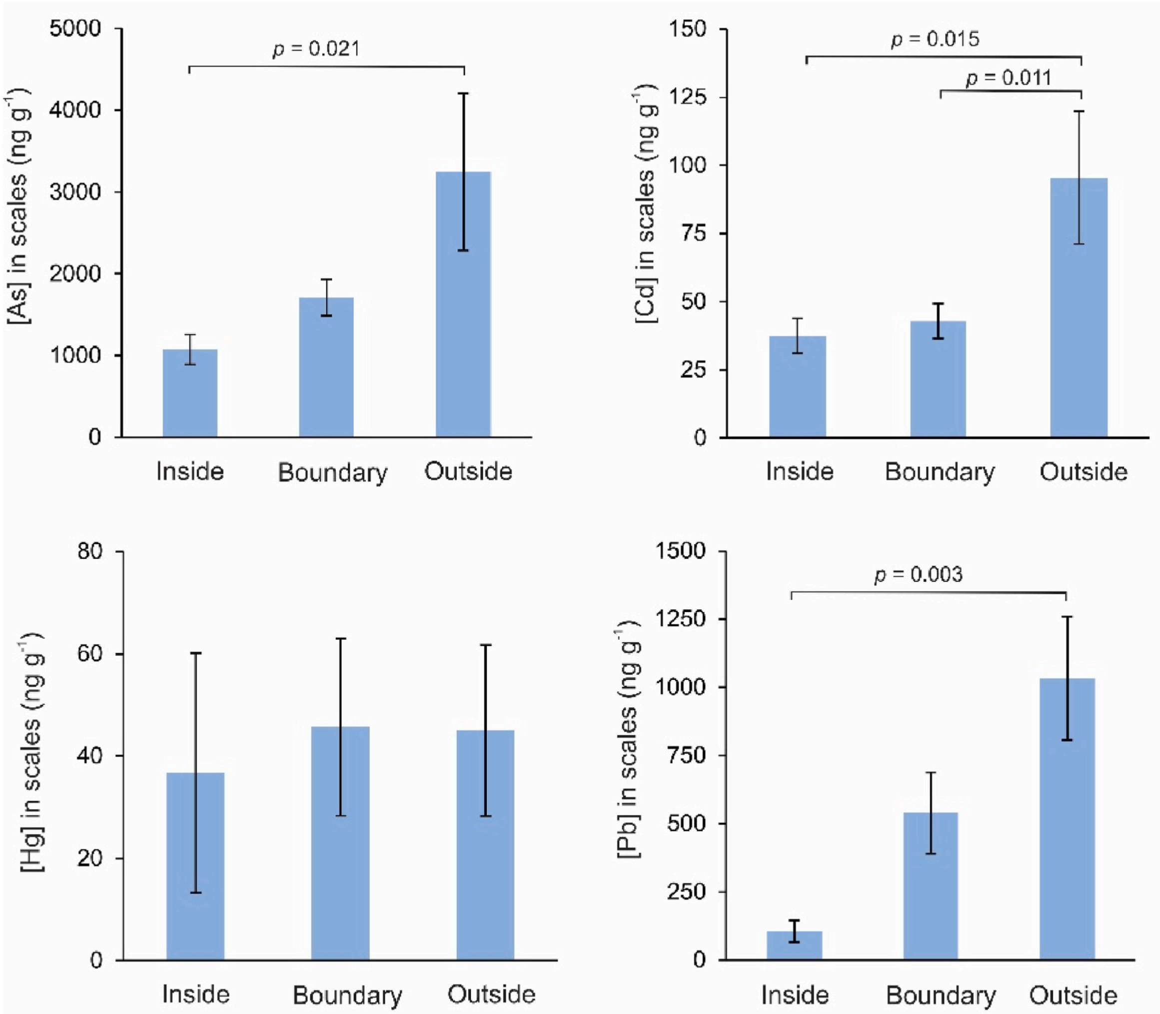

Fig. 5. Effect of land use on heavy metal concentrations in ventral scales of black mambas. Groupings based on if the snake was found 1) inside the D'MOSS network of conservancies, nature reserves and green spaces; 2) on the boundary/transition zone between D'MOSS areas and surrounding land uses; or 3) clearly outside the D'MOSS network. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2021; Rumbold and Bartoszek, 2019; Lemaire et al., 2018; Albrecht et al., 2007; Rainwater et al., 2005). In many of these studies, the sampled population was exposed to the same pollutant source, allowing for the detection of bioaccumulation patterns linked to body size. In contrast, because the snakes in this study were randomly sampled across a landscape of varied land use, the measured heavy metal concentrations likely reflect exposure to different pollutant sources related to each snake's home range and foraging habits.

We hypothesise that the spatial distribution of black mambas and their proximity to localised sources of pollution may be the primary determinants of metal concentrations within the individuals studied. Black mambas inhabiting urban areas around Durban are likely exposed to environmental contaminants primarily through their diet. These snakes predominantly prey on rodents, which are abundant in human-altered environments where they are attracted to food waste. Rodents have been well-established as valuable indicator species in toxicological studies, particularly within urban environments, as they have small home ranges and readily accumulate metals, notably in their liver tissues, through the ingestion of contaminated food and soil, as well as via self-grooming behaviours (e.g., Way and Schroder, 1982; Ceruti et al., 2002). While dietary exposure is likely the primary route of contaminant exposure for most snakes, including black mambas, direct contact with contaminated soils through burrowing, nesting, and general movement represents a potential exposure pathway. Studies have suggested that dermal absorption of contaminants from soil may be an underappreciated exposure route in some reptiles, particularly lizards (e.g., Talent, 2005; Weir et al., 2010; Weir et al., 2016). However, the extent to which dermal exposure contributes to the overall contaminant burden in reptiles is largely unknown. The permeability and thickness of the keratinized epidermis in snakes likely significantly limits or prevents dermal absorption of contaminants.

Heavy metal exposure in black mambas was clearly associated with land use. Snakes within D'MOSS conservancies, nature reserves, and Durban's green spaces accumulated significantly lower concentrations of As, Cd, and Pb in their scales compared to those in industrial and commercial areas outside D'MOSS. The absence of a similar relationship for Hg is likely due to its infrequent detection in scales, combined with its volatile nature and capacity for long-range transport (Gworek et al., 2020). Despite this exception, the observed spatial patterns of heavy metal accumulation suggest that black mamba scales serves as a reliable indicator of environmental contamination levels and thus provide a valuable tool for biomonitoring heavy metals of ecological and toxicological interest.

Limited research has investigated the impacts of contaminant accumulation on snake populations (Campbell and Campbell, 2002), and toxicity thresholds are lacking for most reptile species and contaminants (Hopkins, 2005). The lack of data regarding pollutant exposure and its effects on reptiles has long been recognised as a significant knowledge

Table 3
Metal concentrations (ng g^{-1} dw) measured in liver and muscle tissues of snakes from other locations. Concentrations are given as the mean ± SE where available.

Species and location	Tissue	*n*	Arsenic	Cadmium	Mercury	Lead	Source
Black mamba (*Dendroaspis polylepis*)							
Durban, South Africa	Liver	29	600 ± 140	35 ± 4	31 ± 7	150 ± 23	This study
	Muscle	32	775 ± 160	1.7 ± 0.2	107 ± 18	53 ± 7	
Western tiger snake (*Notechis scutatus occidentalis*)							
Perth, Australia (urban wetlands)							
Herdsman Lake	Liver	5	1300 ± 77	77 ± 13	550 ± 30	23 ± 13	Lettoof et al. (2020) [a]
Bibra Lake	Liver	5	320 ± 40	50 ± 13	200 ± 67	n.d	
Lake Joondalup	Liver	5	630 ± 270	67 ± 20	210 ± 47	n.d	
Loch McNess	Liver	5	920 ± 160	190 ± 43	970 ± 230	160 ± 93	
Annulated sea snake (*Hydrophis cyanocinctus*)							
Persian Gulf, Iran	Muscle	13		72 ± 29		309 ± 29	Rezaie-Atagholipour et al. (2012)
	Liver	13		339 ± 120		390 ± 64	
Common water snake (*Nerodia sipedon*)							
New Jersey, USA (urban/suburban)	Liver	18	310 ± 90	220 ± 70	1000 ± 300	210 ± 40	Burger et al. (2007)
	Muscle	18	200 ± 50	40 ± 13	1200 ± 160	340 ± 70	
Tennessee, USA (Dept. of Energy site)	Liver	47	310 ± 90	250 ± 30	3400 ± 380	120 ± 13	Burger et al. (2005)
	Muscle	46	120 ± 20	50 ± 7	2200 ± 130	200 ± 33	
Cottonmouth (*Agkistrodon piscivorus*)							
Texas, USA (contaminated military site)	Liver	19			2500		Rainwater et al. (2005) [a]
South Carolina, USA (contaminated nuclear site)	Muscle	13	7000 ± 90		900 ± 100	3000 ± 1000	Burger et al. (2006)
Banded water snake (*Nerodia fasciata*)							
South Carolina, USA (reference site)	Liver	5	230	120			Hopkins et al. (1999)
South Carolina, USA (contaminated coal ash site)	Liver	5	134000	500			
South Carolina, USA (contaminated nuclear site)	Muscle	47	400 ± 40		600 ± 50	2000 ± 200	Burger et al. (2006)
Brown water snake (*Nerodia taxispilota*)							
South Carolina, USA (contaminated nuclear site)	Muscle	10	700 ± 90		700 ± 100	32000 ± 500	Burger et al. (2006)
Shreve's keelback (*Helicops pastazae*)							
Santa María, Colombia	Liver	28		21 ± 6.5		420 ± 124	Hurtado-Morales et al. (2022)
	Muscle	28		2.1 ± 1.1		1930 ± 1220	
South American rattlesnake (*Crotalus durissus*)							
Lagoa Santa Karst, Brazil	Liver	28	260 ± 160	220 ± 135	1020 ± 640	260 ± 410	Quintela et al. (2024)
	Muscle	30	270 ± 210	230 ± 190	1050 ± 820	210 ± 175	

[a] Reported data converted to dry mass based on 70 % moisture content; n.d = not detected.

gap in the scientific literature, posing a considerable challenge to researchers for decades (Hopkins, 2000; Grillitsch and Schiesari, 2010). Consequently, an assessment of the potential chemical-specific ecological risks posed by pollutants to snake populations is beyond the scope of this study. While it is difficult to assess the ecotoxicological significance of the metal concentrations observed in the studied black mamba population, the sequestration of a large proportion of the contaminant burden in scales is presumably relatively benign from an ecotoxicological perspective. The preferential sequestration of metals within scales may represent a detoxification mechanism, analogous to the role of feathers in metal sequestration in birds (e.g., Tsipoura et al., 2011; López-Berenguer et al., 2021). Experimental evidence indicates that although snakes can excrete heavy metals during skin shedding (Hopkins et al., 2001; Jones and Holladay, 2006), this typically represents a very small proportion (<0.1 %) of the contaminants ingested by a snake. Skin shedding is therefore unlikely to be major depuration mechanism through which mambas can reduce their contaminant body burden.

To provide context for the metal concentrations measured in black mambas, we compared our findings with data reported for other snake species (Table 3). The heavy metal concentrations measured in black mambas were similar to those reported in Western tiger snakes (*Notechis scutatus occidentalis*) inhabiting urban wetlands in Perth, Australia (Lettoof et al., 2020). This observation might be expected, given the potential for comparable urban pressures on snake populations across two major cities. Arsenic concentrations in black mamba livers were comparable to those found in tiger snakes, while Cd and Hg concentrations were generally higher in tiger snakes (Table 3). Conversely, black mambas generally exhibited higher levels of Pb. Lettoof et al. (2022) found that tiger snakes with higher heavy metal burdens

Table 4
Metal concentrations (ng g^{-1} dw) measured in ventral scale clippings taken from live snake species. Concentrations are given as the mean ± SE.

Species and location	*n*	Arsenic	Cadmium	Mercury	Lead	Source
Black mambas (*Dendroaspis polylepis*)						
Durban, South Africa	61	1880 ± 230	73 ± 11	165 ± 27	1200 ± 175	This study
Western Tiger snakes (*Notechis scutatus occidentalis*)						
Perth, Australia						Lettoof et al. (2021)
Captive	9	47 ± 13	5 ± 1	228 ± 58	22 ± 9	
Herdsman Lake	30	1052 ± 144	10 ± 4	290 ± 49	183 ± 73	
Bibra Lake	28	273 ± 50	6 ± 1	225 ± 48	75 ± 25	
Joondalup	29	381 ± 59	4 ± 1	204 ± 15	70 ± 13	
Loch McNess	26	1525 ± 157	7 ± 1	317 ± 28	74 ± 10	
Viperine snakes (*Natrix maura*)						
Brenne, France	11			145 ± 71		Lemaire et al. (2018)
Cébron, France	22			289 ± 218		
Fontenille, France	27			439 ± 261		
Moëze, France	26			183 ± 159		
Tour du Valat, France	29			220 ± 153		
Ons, Spain	13			438 ± 241		

(including As, Cd, Pb and Hg) exhibited poor body condition and proposed that the combined bioaccumulation of these pollutants might have a synergistic negative impact on snakes physiology and metabolism. Given the similarity in heavy metal concentrations measured in black mambas, there is potential for toxicological impacts and these should be explored in future studies.

Much of the data generated for snakes in the US has focused on heavily contaminated sites, such as army ammunition plants, coal-burning power plants, and former nuclear production sites. For instance, Hopkins et al. (1999) and Burger et al. (2006) investigated heavy metal concentrations in banded water snakes (*Nerodia fasciata*) inhabiting the Savannah River Site in South Carolina, a former nuclear weapons production facility severely impacted by chemical and radiological releases from the 1950s to the 1980s. Similarly, highly elevated levels of Hg have been measured in water snakes (*Nerodia sipedon* and *Nerodia taxispilota*) and cottonmouths (*Agkistrodon piscivorus*) inhabiting severely contaminated sites (Rainwater et al., 2005; Burger et al., 2006; Burger et al., 2007; Haskins et al., 2021). Predictably, heavy metal concentrations in the liver and muscle tissues of these snakes were considerably higher than those observed in our study of black mambas. In South America, several snake species have been shown to accumulate heavy metals in their tissues. For example, Hurtado-Morales et al. (2022) found elevated concentrations of Pb and Cd in Shreve's keelback (*Helicops pastazae*) living in urban areas of Colombia. Similarly, Quintela et al. (2024) reported high levels of Cd, Hg and Pb in various snake species, including South American rattlesnakes (*Crotalus durissus*) from areas in Brazil affected by mining and urbanisation in Brazil. The average heavy metal concentrations measured in internal tissues of black mambas in our study are generally lower than those reported in these South American studies, except for As.

Only two previous studies (Lemaire et al., 2018; Lettoof et al., 2021) have investigated metal concentrations in snake ventral scale clippings (Table 4). Lettoof et al. (2021) examined metal concentrations in tiger snake scales from four wetlands along an urban gradient in Perth and found that inter-site differences in mean metal concentrations in scales mirrored patterns observed in sediment. Overall, As, Cd and Pb concentrations in black mamba scales were substantially higher than those observed in wild tiger snakes. Conversely, Hg concentrations were slightly lower in black mamba scales compared to those reported in tiger snakes from Perth and those measured in viperine snake (*Natrix maura*) from France and Spain by Lemaire et al. (2018).

6. Conclusions

Black mambas from urban environments around Durban accumulate heavy metals within their tissues. Several of the metals investigated in this study (As, Cd, and Pb) had higher accumulation within scales, suggesting that valuable data regarding pollutants of environmental and toxicological interest can be acquired through a straightforward, non-lethal scale sampling technique. Metal accumulation in scales was found to be independent of sex and body size, suggesting that contaminant levels primarily reflect localised environmental exposure rather than individual characteristics. A clear association existed between land use and heavy metal exposure in black mambas. Snakes living in conservancies, nature reserves, and green spaces generally had significantly lower heavy metal concentrations in their scales compared to those in more industrial and commercial areas. These findings suggest that black mambas can serve as valuable bioindicators of environmental contamination. The study also highlights the ecological benefit of connected open spaces, which appear to buffer snakes from the negative impacts of contaminant exposure associated with extensive urban development.

Black mambas have a wide geographic distribution and appear to be increasing in abundance within urban environments surrounding Durban. The inevitability of human-snake interactions presents a unique opportunity for long-term biomonitoring studies within this urban environment. Recognising snakes as indicator organisms capable of providing valuable insights into potential human exposure risks could benefit conservation efforts. Increased public tolerance may arise if snakes are perceived as valuable tools for monitoring human well-being, thereby promoting more effective environmental management within large urban areas. The broader potential of using snakes for environmental monitoring in Africa warrants further investigation, and future research should focus on a wider range of snake species across diverse ecological niches to better understand their potential as bioindicators of environmental pollution.

CRediT authorship contribution statement

Marc Humphries: Writing – original draft, Investigation, Formal analysis, Conceptualization. **Nick Evans:** Writing – review & editing, Investigation. **Cormac Price:** Writing – original draft, Investigation. **Graham J. Alexander:** Writing – original draft, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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Data availability

Data will be made available on request.

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