

Assessing toxic element contamination in protected areas: A case study of the Sungun copper mine's impact on wildlife

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Abstract

Mining activities significantly contribute to the concentration of toxic elements (TEs) in protected areas (PAs), adversely affecting wildlife. The presence of the Sungun porphyry copper mine within the Arasbaran Biosphere Reserve (ABR) in East Azarbaijan, Iran, raises concerns about the potential impacts of TE contamination on the local ecosystem. This study pioneers the non-invasive monitoring of faecal toxic elements in Iran's protected areas, offering the first comparative analysis between the mining-impacted ABR and the relatively pristine Kiamaky Wildlife Refuge (KWR). We collected faecal samples from herbivorous (*Capra aegagrus*, *Lepus europaeus*) and carnivorous (*Canis lupus*, *Lynx lynx*) mammals across these sites and analyzed arsenic (As), lead (Pb), cadmium (Cd), and mercury (Hg) concentrations using atomic absorption spectrometry. Results showed significantly higher TE levels in ABR compared to KWR (eg PB median 17.7 ± 3.2 $\mu\text{g/kg}$ vs. 1.9 ± 0.5 $\mu\text{g/kg}$; $p < 0.01$). Carnivores exhibited elevated As (median 14.00 ± 2.1 $\mu\text{g/kg}$) and Cd (4.85 ± 1.1 $\mu\text{g/kg}$) relative to herbivores, while herbivores had higher Pb (11.20 ± 2.4 $\mu\text{g/kg}$) levels. Our findings underscore the value of faecal biomonitoring as a non-invasive, cost-effective tool for assessing toxic element exposure in wildlife, providing critical data for conservation management in mining-affected landscapes. Importantly, this study informs targeted monitoring and intervention strategies necessary to safeguard biodiversity in Iran's protected areas.

Keywords: Heavy elements; Non-invasive risk assessment; Conservation areas; Wildlife excretion

Introduction

Toxic elements (TEs) are non-biodegradable and can cause significant environmental contamination risks, affecting both the environment and living organisms (Jaishankar et al., 2014; Mitra et al., 2022; Subhanullah et al., 2024). Exposure to TEs from mining activities that are known to induce multiple organ damage, even at lower levels of exposure (Tchounwou et al., 2012), is common (Bussi res et al., 2004).

While TEs are naturally present in the environment due to geogenic process, human activities such as ore mining, mineral refining, and coal burning have significantly impacted the natural cycles and balance of these elements in the environment (Chen et al., 2022; Hoekstra et al., 2003). The availability of TEs varies at local, regional, and global scales due to differences in geochemical diversity, as well as variations in precipitation, vegetation, and climate (Webster et al., 2022).

The bioavailability of TEs in natural environments, which is influenced by both natural and anthropogenic processes, results in heterogeneity that affects the intake rate of them by wildlife species (van Beest et al., 2023). Wildlife can be exposed to TEs through environmental contamination from human activities such as mining (Pereira et al., 2006), transportation (Marcheselli et al., 2010), hunting (Arrondo et al., 2020), urbanization (Bauerov  et al., 2017), and agriculture (Badry et al., 2021; Evans et al., 2022). Studies have shown that the TE contents in wildlife species are significantly affected by their trophic level and habitat preference (Feng et al., 2020; Madgett et al., 2021). They are typically exposed to TE levels through dietary pathways, and these levels have been linked to trophic dynamics and bioaccumulation (Parker et al. 2023).

One effective approach for evaluating TEs in the environment and assessing the associated risks for organisms is biological monitoring (Markowski et al., 2014). Mammalian scats serve as a valuable non-invasive biomonitoring tool for assessing TE concentrations (Eeva et al., 2020; Imaeda et al., 2021), providing insights into the health of the protected area (Webster, Ganswindt, et al., 2021). Faecal element concentrations are closely related to dietary intake, which in turn reflects actual utilization of resources (B swald et al., 2018) making faeces a non-invasive matrix for assessment of TEs (Webster et al., 2022).

The spatial variation of TEs in wildlife species living in different habitat conditions is an important topic for environmental monitoring and wildlife health (Andersson Stavridis et al., 2024; Ethier et al., 2014; Gunn et al., 2019; Sun et al., 2022; van Beest et al., 2023). Several studies have investigated this phenomenon, such as the assessment of spatial variation in concentrations of TEs

in birds (Bala et al., 2021; Markowski et al., 2014; Martín-Vélez et al., 2021; Sun et al., 2022), fish (Gunn et al., 2019; Xia et al., 2019) and mammals (Andersson Stavridis et al., 2024; Ethier et al., 2014).

Protected areas play a crucial role in maintaining essential habitats, offering refuge to various species, and contributing to the conservation of nature and its associated services. Additionally, protected areas are increasingly acknowledged for their potential in mitigating and adapting to climate change. Effectively managed systems of protected areas are essential for achieving the objectives of biodiversity conservation and the Sustainable Development Goals (IUCN, 2024). However, studies have shown that protected areas are exposed to environmental pollution (e.g. TEs (Alejandra Aguilar et al., 2021; Gupta & Bakre, 2012; Reglero et al., 2008; Sojka et al., 2022; Webster et al., 2022; Webster, Rossouw, et al., 2021) and pharmaceuticals (Kazakova et al., 2021)) originating from anthropogenic sources. TEs pollution compromises the ability of the protected areas to foster life and poses risks to animals, plants, and ecosystems through various pathways such as direct ingestion, absorption by plants, and consumption of contaminated water (Masindi & Muedi, 2018; Tchounwou et al., 2012).

Mining activities significantly contribute to the concentration of TEs in the environment, adversely affecting both wildlife and human health. The release of TEs from mining operations leads to contamination of soil, water, and air through tailings, dust, and wastewater discharge (Buch et al., 2023; Fashola et al., 2016; Ganjeizadeh Rohani & Mohamadi, 2022; Yaw Hadzi, 2022). The extraction process generates substantial waste, including tailings that often leach TEs into surrounding soils and water bodies, leading to elevated TE concentrations in nature. For instance, studies have shown that areas near copper mines, like the Bor area in Serbia (Filimon et al., 2016) and Dexing Mine in China (Ni et al., 2023), exhibit higher levels of TE contamination. While global studies have applied biological monitoring of TEs in wildlife, including non-invasive faecal analysis (Eeva et al., 2020; Imaeda et al., 2021), data remain scarce for terrestrial vertebrates in Iran's protected areas, especially where mining is active. The Arasbaran Biosphere Reserve (ABR) impacted by the Sungun porphyry copper mine, presents a unique case for assessing mining-related TE contamination; meanwhile, the nearby the Kiamaky Wildlife Refuge (KWR) offers a less disturbed comparison site. This study aims to fill this critical knowledge gap by comparing faecal TE concentrations in herbivorous (*Capra aegagrus*, *Lepus europaeus*) and carnivorous (*Canis*

lupus, *Lynx lynx*) mammals between ABR and KWR, thereby assessing environmental and trophic influences on TE exposure.

Material and methods

Study sites

In East Azarbaijan (Fig. 1), also known as Azerbaijan-e Sharghi, the presence of diverse climates and several mountains has significantly contributed to its high biodiversity. Additionally, the province's proximity to the Caucasian region and the Anatolian Plateau during different ice age periods has led to a greatly enriched biodiversity that has endured over time. The KWR and the ABR located in the north part of East Azarbaijan (Figure 1) are home to a broad spectrum of plant and animal species. The KWR covers some part of the Eastern Anatolian montane steppe. This region is known for its unique and diverse flora and fauna, characterized by its steppe habitats and mountainous landscapes. The ABR, which is a mountainous area and belongs to the Caucasus Iranian Highlands, has been recognized as a UNESCO-designated biosphere reserve since 1976. It encompasses a diverse landscape including high alpine meadows, semi-arid steppes, forests (Habibzadeh & Rafieyan, 2016) and is home to at least 48 mammal species (Darvishi et al., 2015). Based on topography and human impact, the general vegetation type of the ABR includes three zones (Ramezani et al., 2021): (1) low- to mid-elevation zone that consists of abandoned agricultural lands with secondary vegetation types of mostly Irano-Turanian origin. At elevations between 600 and 1250 m, secondary woodlands with dense stands of thorny shrubs are present, (2) Forest zone (mainly 1000 to 1800 m): This zone is the least impacted by human activity, and (3) Alpine zone (1800–2700 m) can be split into dwarf scrub grasslands and pure grasslands.

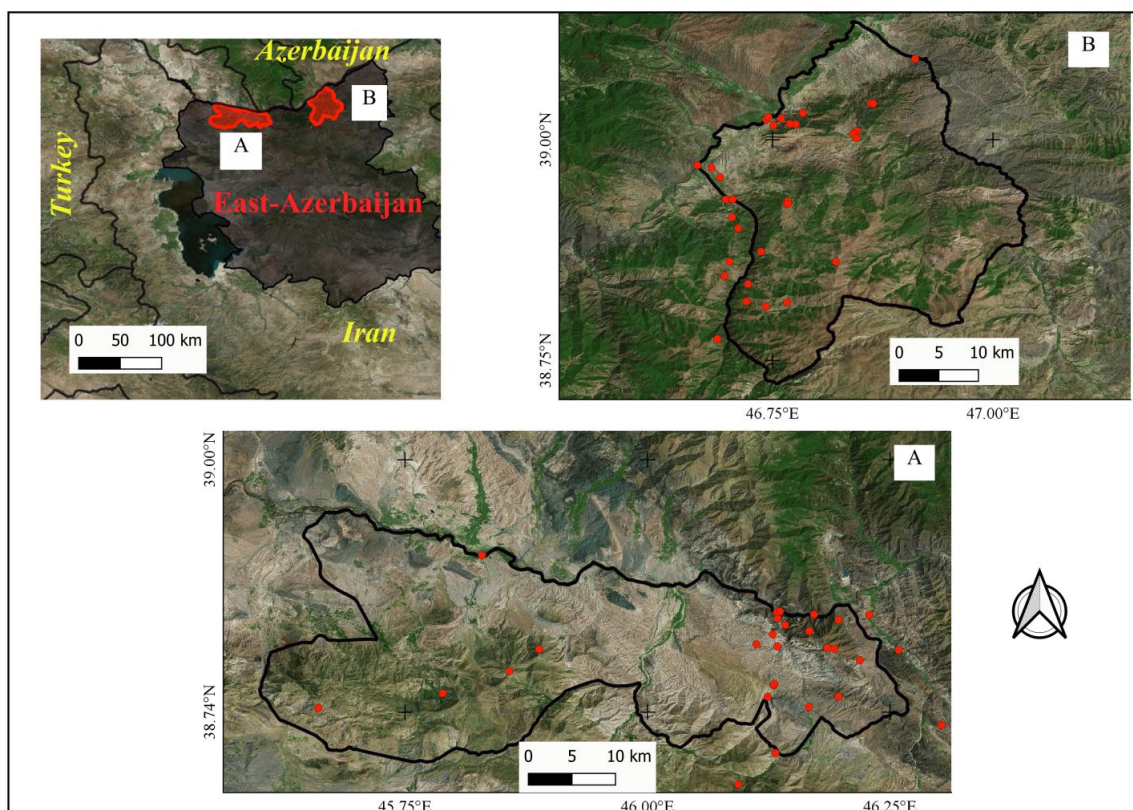


Figure 1. The Kiamaky Wildlife Refuge (A) and Arasbaran Biosphere Reserve (B) located in East-Azerbaijan province, Iran. Red dots indicate sample locations.

Despite the ABR's ecological significance, human activities, such as extensive mining, have negative impacts on its unique ecosystem. Mining operations encompass a variety of waste management units, such as tailings, waste rock depositories, and soils and sediments that have been contaminated due to their association with the extraction process (Ford & Beyer, 2014). The unregulated dissemination of mine tailings containing high levels of metal, through mechanisms such as dust entrainment, leaching, and erosion, persists as a significant hazard to wildlife (Rodríguez-Estival et al., 2011). Sungun porphyry copper mine, as the second and the tenth-largest copper mine in Iran and the world, respectively, is located within the Arasbaran dense forests. It is an open-pit mine that has been causing major negative impacts on the Arasbaran ecosystem (Aghili et al., 2018; Firozjaei et al., 2021; Hosseinpour et al., 2022; Moore et al., 2011) since its operation in 2008 (Nasrabadi et al., 2009). The minerals extracted from the mine not only contain copper (*Cu*) but also *As*, cobalt (*Co*), *Hg*, and nickel (*Ni*) (Firozjaei et al., 2021). They can lead to severe detrimental effects on water bodies due to acid mine drainage and the leaching of metals into stream and river ecosystems (Moore et al., 2011). The elevated concentrations of these TEs

in the sediments and water bodies pose a significant risk to the health of humans, plants, animals, and microorganisms (Nasrabadi et al., 2009).

Sample collection and preparation

We collected fresh scats from two carnivores ($n = 32$) and fresh dung from two herbivores ($n = 35$) across the KWR and ABR and their immediate surrounds (Table 1) during 2023-2024 (Fig. 1). Herbivore dung was collected in collaboration with trained wildlife rangers to verify species identity. Carnivore scats were opportunistically sampled during daily wardens' patrols with spatial-temporal controls to minimize pseudoreplication. No molecular confirmation was performed due to logistical constraints. Faecal material was thoroughly mixed in situ before obtaining sub-samples from animal excretions (Ganswindt et al., 2002). The droppings were carefully collected using gloves to prevent contamination of samples and to ensure the safety of researchers, labelled, deposited in the cooler bags contained ice bricks to keep samples cool until the end of the collection day, and preserved at -20°C before being transported to the laboratory for further analysis. Faecal samples underwent lyophilization at a temperature of -50°C for a few days. Subsequently, the dehydrated faecal samples from all species were subjected to mechanical pulverization utilizing a ceramic grater to disaggregate any residual indigestible matter. The resulting powdered faecal material was then sieved through a 37-micron plastic-mesh strainer to eliminate any large particles. Approximately 0.3 grams of faecal dry mass from individual herbivore and carnivore species were subjected to microwave-assisted acid digestion (MARS® –5) utilizing a mixture of 6.5 milliliters (65%) nitric acid (HNO_3) and 0.5 milliliters (30%) hydrochloric acid (HCl). Following the digestion process, deionized water was added to each sample to achieve a final volume of 50 milliliters (US.EPA, 1996).

Table 1. Number of samples per species in herbivore and carnivore groups evaluated for potentially toxic element levels in Arasbaran Biosphere Reserve (ABR) and Kiamaky Wildlife Refuge (KWR), East Azerbaijan, Iran

IUCN status	Guild	Scientific name	Common name	Arasbaran Biosphere Reserve (n)	Kiamaky Wildlife Refuge (n)
Near threatened	Herbivore	<i>Capra aegagrus</i>	Wild goat	9	9
Least Concern	Herbivore	<i>Lepus europaeus</i>	European Hare	9	8
Least Concern	Carnivore	<i>Canis lupus</i>	Wolf	9	9

Least Concern	Carnivore	<i>Lynx lynx</i>	Eurasian lynx	10	4
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Atomic absorption spectrometry with graphite furnace was used to quantify the concentrations of elements in the samples in a certified laboratory (credit code: 11.8711.0105). The resulting concentrations of the analyzed constituents in faecal material were reported in micrograms per kilogram ($\mu\text{g/kg}$) of faecal dry mass.

Statistical Analyses

Descriptive statistics were computed for all measured TEs across samples from each trophic group and protected area. To enable a clearer visual comparison, the measurement scales for each TE were standardized by dividing each element's concentration by its mean value across protected areas and species. Subsequently, visual representations were generated using the ggplot2 R package (*ggplot2: Elegant Graphics for Data Analysis*, 2010) to compare the relative concentrations of elements among trophic groups and protected areas.

The normality of all variables was assessed using the Shapiro-Wilk test. In cases where normal distribution was not met, the optimal normalizing transformation functions were determined and implemented using the bestNormalize package in R (Peterson, Ryan, 2021). Bartlett's test was used to assess the homogeneity of the variances of the normalized variables. To examine the impact of trophic groups and protected areas on element concentrations in the faeces of species, we applied a linear regression model for normally distributed variables and a robust regression for variables that did not meet linear regression assumptions (Alma, 2011). Additionally, the relationships among TEs were examined through the calculation of Pearson's r correlation coefficients. The statistical significance of differences was determined by assessing p -values less than 0.05, and all statistical analyses were performed using the open-source software R version 4.2.2.

Results

Since species may experience varying levels of exposure to TEs based on their distinct dietary habits and physiological characteristics (Buzan et al., 2024; Kiabi, 1975; Santilli et al., 2023), we evaluated the differences in element concentrations among species before categorizing *C. aegagrus* and *L. europaeus* as herbivores, and *C. lupus* and *L. lynx* as carnivores. However, our analysis revealed no significant differences in the concentrations of toxic elements between the

herbivorous species (*As* ($t = 1.21$, $df = 33$, $p\text{-value} = 0.24$), *Pb* ($t = -1.59$, $df = 33$, $p\text{-value} = 0.13$), *Cd* ($W = 153.5$, $p\text{-value} = 0.99$), *Hg* ($W = 183$, $p\text{-value} = 0.30$)) and the carnivorous species (*As* ($t = -0.32$, $df = 30$, $p\text{-value} = 0.75$), *Pb* ($t = 0.67$, $df = 30$, $p\text{-value} = 0.51$), *Cd* ($W = 105$, $p\text{-value} = 0.44$), *Hg* ($W = 106.5$, $p\text{-value} = 0.46$)).

Influence of trophic groups and protected areas (PAs) on TEs concentrations

The intercept values for all TEs (*As*, *Pb*, *Cd*, *Hg*) were statistically significant ($p < 0.01$), indicating a baseline concentration of these elements in the faeces regardless of trophic levels and species occurrence in the PAs (KWR and ABR). The significant coefficients for trophic levels indicated that carnivores had higher concentrations of *As* (median = 14.00 ± 7.64 $\mu\text{g/kg}$) and *Cd* (median = 4.85 ± 2.93 $\mu\text{g/kg}$) compared to herbivores (*As* (median = 4.85 ± 16.30 $\mu\text{g/kg}$) and *Cd* (median = 3.7 ± 3.24 $\mu\text{g/kg}$)) (Table 2 and Fig. 2). Conversely, *Pb* levels were higher in herbivores (median = 11.20 ± 10.42 $\mu\text{g/kg}$) than carnivores (median = 8.65 ± 7.80 $\mu\text{g/kg}$). Additionally, there was no significant difference in *Hg* concentrations between herbivores (median = 0.16 ± 0.33 $\mu\text{g/kg}$) and carnivores (median = 0.21 ± 0.18 $\mu\text{g/kg}$) (Table 2 and Fig. 2).

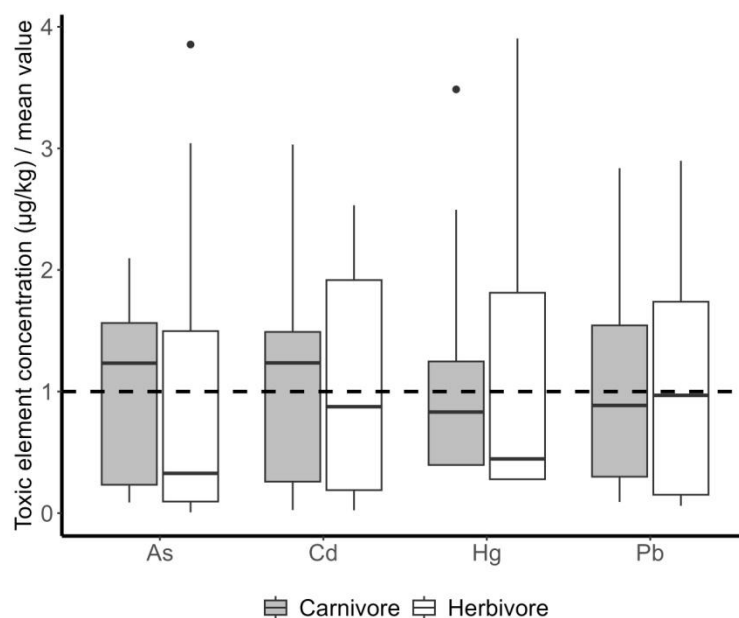


Fig. 2. Comparative analysis of potentially toxic element concentrations in herbivorous (white) and carnivorous (dark grey) mammals relative to the mean for both protected areas (dashed line). The dark lines in the boxes depict median values, with the box indicating the upper (3rd) and lower (1st) quartiles. Whiskers show the range of minimum to maximum values, excluding

outliers, while points represent any outliers. Concentrations are normalized and expressed as $\mu\text{g/kg}$ of fecal dry mass.

Animals living in the mining-impacted ABR had significantly higher levels of all TEs (*As* (median = $18.10 \pm 11.20 \mu\text{g/kg}$), *Pb* (median = $17.70 \pm 7.40 \mu\text{g/kg}$), *Cd* (median = $6.50 \pm 2.00 \mu\text{g/kg}$), and *Hg* (median = $0.38 \pm 0.28 \mu\text{g/kg}$)) compared to animals in the less disturbed KWR (*As* (median = $1.60 \pm 3.36 \mu\text{g/kg}$), *Pb* (median = $1.90 \pm 2.10 \mu\text{g/kg}$), *Cd* (median = $0.67 \pm 0.94 \mu\text{g/kg}$), and *Hg* (median = $0.10 \pm 0.04 \mu\text{g/kg}$)) (Table 2 and Fig. 3).

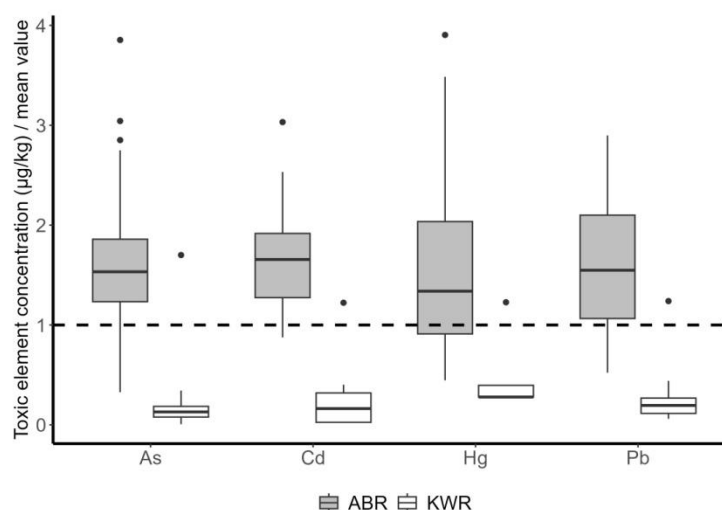


Fig. 3. Comparative analysis of potentially toxic element concentrations in wild mammals living in Arasbaran Biosphere Reserve (ABR: dark grey) and Kiamaky Wildlife Refuge (KWR; white) relative to the mean for both protected areas (dashed line). The dark lines in the boxes depict median values, with the box indicating the upper (3rd) and lower (1st) quartiles. Whiskers show the range of minimum to maximum values, excluding outliers, while points represent any outliers. Concentrations are normalized and expressed as $\mu\text{g/kg}$ of fecal dry mass.

The interaction between trophic levels and PAs significantly influenced the concentrations of TEs in species. Notably, the relationship between carnivores and herbivores from different PAs revealed a complex dynamic that warrants careful examination. In the mining-impacted ABR, herbivores had significantly higher concentrations of all measured TEs (*As* (median = $21.95 \pm 13.83 \mu\text{g/kg}$), *Pb* (median = $20.10 \pm 6.40 \mu\text{g/kg}$), *Cd* (median = $8.10 \pm 1.99 \mu\text{g/kg}$), and *Hg* (median = $0.65 \pm 0.30 \mu\text{g/kg}$)) than carnivores, which had *As* (median = $17.40 \pm 3.30 \mu\text{g/kg}$), *Pb* (median = $10.80 \pm 6.89 \mu\text{g/kg}$), *Cd* (median = $5.30 \pm 1.74 \mu\text{g/kg}$), and *Hg* (median = $0.28 \pm 0.17 \mu\text{g/kg}$)) (Table 2 and Fig. 4). This finding suggests that herbivores are more adversely affected by the

contaminated environment in the ABR, potentially due to their direct exposure to contaminated vegetation or soil. Conversely, in the KWR, carnivores had higher concentrations of *As* (median = 1.90 ± 4.71 $\mu\text{g/kg}$) and *Pb* (median = 2.40 ± 2.80 $\mu\text{g/kg}$) than herbivores (*As* (median = 1.30 ± 1.08 $\mu\text{g/kg}$) and *Pb* (median = 1.60 ± 1.08 $\mu\text{g/kg}$)), indicating a different exposure dynamic in this less disturbed area (Table 2 and Fig. 4).

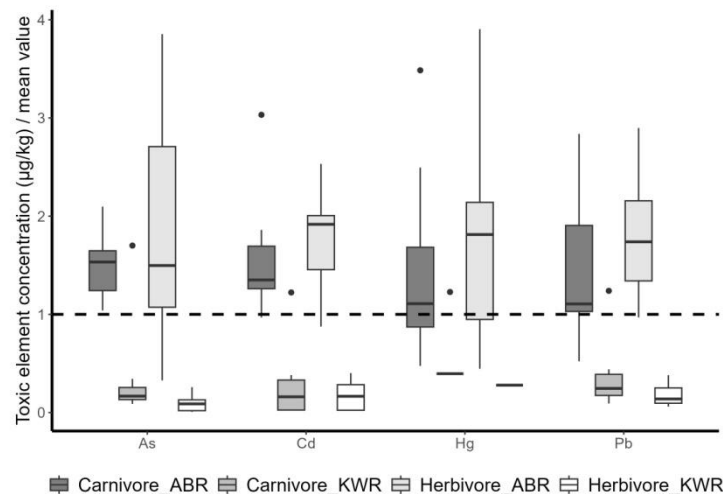


Figure 4. Differences in measured potentially toxic element concentrations between protected areas (Arasbaran Biosphere Reserve: ABR and Kiamaky Wildlife Refuge: KWR) in herbivorous and carnivorous mammals relative to the mean for both protected areas (dashed line). The dark lines in the boxes depict median values, with the box indicating the upper (3rd) and lower (1st) quartiles. Whiskers show the range of minimum to maximum values, excluding outliers, while points represent any outliers. Concentrations are normalized and expressed as $\mu\text{g/kg}$ of fecal dry mass.

The adjusted *R*-squared values indicated that the models explained a substantial portion of the variability in TEs exposure, ranging from 0.66 for *As* and *Pb* to 0.89 for *Cd* (Table 2).

Table 2. Estimated parameters for the impact of trophic levels and protected areas on toxic element concentrations in herbivorous and carnivorous mammalian feces using linear and robust regression models in Arasbaran Biosphere Reserve (ABR) and Kiamaky Wildlife Refuge (KWR), East Azerbaijan, Iran. Single and double star symbols denote significance levels of $p < 0.05$ and $p < 0.01$, respectively. The values in parentheses represent the standard deviation.

	Linear regression model		Robust regression	
	As	Pb	Hg	Cd
Intercept	0.48 (0.13) **	0.43 (0.13) **	0.26 (0.03) **	5.64 (0.28) **
Trophic group	0.50 (0.20) *	0.55 (0.19) **	0.01 (0.02)	2.30 (0.74) **

Protected areas	-0.99 (0.21) **	-1.10 (0.21) **	-0.17 (0.03)**	-5.06 (0.32) **
Trophic group × Protected areas	-1.03 (0.29)**	-0.95 (0.29) **	-0.11 (0.05) **	-2.15 (0.75) **
Adjusted <i>R</i> -squared	0.66	0.66	0.83	0.89

Comparison of TE associations in trophic groups within PAs

Pearson's correlation between TEs for each trophic group in the ABR and KWR is shown in Table 3. In carnivores at the ABR, there was evidence of a mutual correlation between *Cd*, *Pb*, and *Hg* (minimum association: $r = 0.47$). The highest correlation for TEs measured in the excretions of carnivores of the ABR was between *Cd* and *Pb* ($r = 0.76$; $p < 0.01$). In herbivores at the ABR, *Hg* had a significant correlation with *As* ($r = 0.67$) and *Cd* ($r = 0.65$). At the KWR, whilst mutually significant correlations between all studied TEs (minimum association: $r = 0.86$) were evident in carnivores with *As* and *Hg* ($r = 0.98$; $p < 0.01$) being highest, it was only a significant correlation between *Cd* and *Pb* ($r = 0.81$; $p < 0.01$) in herbivores.

Table 3. Correlations of toxic elements in two trophic groups at the Arasbaran Biosphere Reserve (ABR) and Kiamaky Wildlife Refuge (KWR). Significant associations are denoted by bold numbers with single and double stars, indicating significant associations at 0.05 and 0.01 levels, respectively.

ABR									
Carnivore					Herbivore				
	<i>As</i>	<i>Cd</i>	<i>Hg</i>	<i>Pb</i>		<i>As</i>	<i>Cd</i>	<i>Hg</i>	<i>Pb</i>
<i>As</i>	-				<i>As</i>	-			
<i>Cd</i>	0.08	-			<i>Cd</i>	0.27	-		
<i>Hg</i>	0.31	0.67**	-		<i>Hg</i>	0.67**	0.65**	-	
<i>Pb</i>	0.47*	0.76**	0.71**	-	<i>Pb</i>	- 0.37	0.23	-0.12	-
KWR									
Carnivore					Herbivore				
	<i>As</i>	<i>Cd</i>	<i>Hg</i>	<i>Pb</i>		<i>As</i>	<i>Cd</i>	<i>Hg</i>	<i>Pb</i>
<i>As</i>	-				<i>As</i>	-			
<i>Cd</i>	0.88**	-			<i>Cd</i>	0.49	-		
<i>Hg</i>	0.98**	0.90**	-		<i>Hg</i>	0.21	0.07	-	
<i>Pb</i>	0.95**	0.86**	0.92**	-	<i>Pb</i>	0.45	0.81**	0.04	-

Discussion

The presence of non-biodegradable TEs poses significant environmental contamination risks, impacting both ecosystems and wildlife (Lima et al., 2022). The long-term exposure to these

elements threatens wild populations, affecting communities and ecosystem integrity (Mitra et al., 2022). Human activities like mining and mineral refining have disrupted natural element cycles, leading to environmental imbalances. Protected areas, vital for biodiversity conservation and climate change mitigation, are not immune to pollution, including TEs originating from anthropogenic sources. TE pollution jeopardizes the ability of protected areas to sustain life, endangering animals, plants, and ecosystems through various pathways such as direct ingestion and absorption by plants (Hasan et al., 2022). The study's focus on terrestrial mammals in South Africa highlighted the importance of assessing TEs in wildlife species using non-invasive methods like faecal analysis (Webster et al., 2022).

While studies have shed light on TE contamination in wild mammal species (Gupta & Bakre, 2012; Webster et al., 2022), research focusing on the terrestrial wildlife of Iran remains limited. Mining activities in Iran pose a substantial risk of TE exposure due to widespread mining operations. The presence of TEs like *As*, *Cd*, *Hg*, and *Pb* across terrestrial and aquatic environments underscores the need for comprehensive assessment and monitoring (Hama Aziz et al., 2023; Tchounwou et al., 2012). Our study marked a crucial step in quantitatively evaluating multiple TEs in herbivorous and carnivorous terrestrial mammals within protected areas of Iran, emphasizing the importance of understanding TE dynamics in different ecosystems to mitigate environmental risks (Kim H. Parker et al., 2023).

The faecal samples of wild mammals inhabiting the ABR exhibited elevated concentrations of TEs compared to those from the KWR. This disparity likely reflects the higher levels of TE contamination within the non-biological constituents (soil, water) and vegetation of the ABR ecosystem, which can be attributed to the influence of mining activities in this area. The mining operations have introduced increased TE loads into various environmental matrices within the ABR (Aghili et al., 2018; Nasrabadi et al., 2009), consequently leading to the accumulation of these elements in the bodies of wild mammals, as evidenced by the elevated TE concentrations detected in their faecal matter. Several studies have reported concentrations of metals in wild mammals living in highly contaminated areas near mines (Andrews et al., 1984; Robert & Johnson, 1978; Roberts et al., 1978). The age-dependent accumulation of *Cd* within the kidney, liver and muscle of elk living near the ore smelters at Sudbury, Ontario has been documented by Parker and Hamr (2001). A study by Hort et al. (2017) compared the levels of TEs in faecal samples of red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*) living in an area influenced by a paper

mill industry. They found that over the year potassium (*K*), chromium (*Cr*), iron (*Fe*), barium (*Ba*), and *Pb* were present in higher concentrations in red deer than in roe deer, possibly due to differences in their diet and metabolism (Hort et al., 2017).

The interaction effects between trophic levels and PAs provided a complex understanding of TE accumulation in our studied wild mammals. In the ABR, herbivores were at an increased risk of accumulating harmful levels of TEs compared to carnivores, suggesting that direct exposure to contaminated vegetation is possibly a significant contributing factor. Conversely, in the KWR, carnivores exhibited higher concentrations of TEs than herbivores, indicating that the dynamics of exposure vary considerably between the two PAs. These findings highlight the necessity of considering trophic interactions and environmental influences when evaluating TE levels in wildlife populations.

Human activities such as mining operations have been identified as significant sources of TE pollution, impacting biodiversity and ecosystem functions. The negative effects of the unregulated mining activities surrounding the ABR exemplify the challenges posed by human on protected areas. Consuming water from polluted sources represents a significant pathway of exposure for wildlife species (Webster et al., 2022). Approximately 70% to 90% of inorganic *As* is assimilated via the gastrointestinal tract, with predominant distribution to the liver, kidneys, bladder, and lungs, followed by secondary accumulation in muscle and nerve tissues (Palma-Lara et al., 2020). Long-term exposure to *As* can result in pregnancy-related issues in animals, such as miscarriages, low fetal birth weight, and fetal deformities. These complications impact the survival and reproductive capacity of carnivore populations (Agency for Toxic Substances and Disease Registry, 2007). Lead is recognized as a systemic toxic substance, and an increasing body of evidence indicates that even minimal environmental exposure from diverse origins can detrimentally affect renal function and neurological maturation in young individuals, potentially leading to enduring neurological impairments, disturbances in endocrine function, and reproductive inadequacies (Agency for Toxic Substances and Disease Registry, 2020). Cadmium finds application across various industrial sectors and is also utilized in agricultural fertilizers and when associated with fine particulate matter, it can undergo atmospheric transport (Burger, 2008). Plants possess the capacity to amass *Cd* within their roots and shoots at concentrations that prove toxic to a majority of other organisms (Godinho et al., 2018). Organisms that ingest *Cd*-laden forage are prone to accumulating this element in their liver and kidneys (Webster et al., 2022).

Mercury is a significant global pollutant with implications for human and ecosystem health. It is recognized as a prominent contaminant of health significance, ranking among the top ten pollutants of concern (Driscoll et al., 2013). It is mobilized into ecosystems through various human activities like mining, industrial processes, and waste-related activities, leading to its widespread distribution globally (Driscoll et al., 2013).

The comprehensive assessment of TEs in terrestrial wildlife within PAs of Iran sheds light on the significant environmental risks posed by TE contamination. The study emphasized the importance of understanding TE dynamics in different ecosystems to mitigate environmental threats. The observed variations in TE concentrations among trophic groups within and between PAs underscore the need for targeted conservation efforts and monitoring strategies. Human activities, particularly mining operations, are identified as significant sources of TE pollution, emphasizing the challenges faced by PAs. Understanding the pathways of TE contamination and their effects on wildlife populations is crucial for effective conservation management practices. Faecal biomonitoring provides valuable, non-invasive insights into wildlife contaminant exposure but bears inherent limitations since faecal element concentrations are influenced by recent diet and excretion rates and may not correlate perfectly with tissue bioaccumulation (Eeva et al., 2020; Webster, Ganswindt, et al., 2021). Therefore, faecal data should complement tissue analyses in comprehensive risk assessments. Additionally, further research into the sources, non-biological pathways, and impacts of TE contamination in these PAs is essential for developing sustainable conservation strategies and safeguarding wildlife and ecosystems from the detrimental effects of TE pollution.

Conclusion

Our study demonstrates significantly elevated faecal TE concentrations in wildlife residing near the Sungun copper mine within ABR compared to the less disturbed KWR, highlighting the environmental impact of mining on protected areas in Iran. Elevated TEs in herbivores in ABR suggest direct exposure through contaminated vegetation and soil, whereas carnivores' exposure dynamics likely vary with prey and trophic level. By filling a regional knowledge gap, our findings underscore the necessity for targeted monitoring and mitigation efforts in mining-affected reserves and recommend adopting faecal biomonitoring as a practical conservation tool. Further research is warranted to understand long-term ecological impacts and to expand monitoring across diverse taxa and contaminant types.

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