Environmental Research 280 (2025) 121957

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Oxidative stress biomarkers, metal(loid)s and persistent organic pollutants in European hedgehogs: An integrated approach to environmental contamination

Javier García-Muñoz [a,*], Elsa Rodríguez Somoza [a], Irene Nuñez Zafra [a], David Fernández-Casado [a], Ángel Portillo-Moreno [a], María del Prado Míguez-Santiyán [a], Francisco Soler Rodríguez [a], Ana López-Beceiro [b], Luis Eusebio Fidalgo [b], Salomé Martínez-Morcillo [a], Marcos Pérez-López [a]

[a] Toxicology Area, Faculty of Veterinary Medicine (UEX), 10003, Caceres, Spain
[b] Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine (USC), 27003, Lugo, Spain

ARTICLE INFO

Keywords:
Biomonitoring
Bioaccumulation
Antioxidant response
Mammal
Xenobiotics

ABSTRACT

Environmental pollution is a growing concern, posing a significant threat to the ecosystem health. The European hedgehog (*Erinaceus europaeus*) is considered a suitable bioindicator for monitoring metal(loid)s and persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) or polycyclic aromatic hydrocarbons (PAHs). However, physiological responses to these pollutants remain understudied. In this study, oxidative stress biomarkers (catalase, glutathione S-transferase and malondialdehyde production) in liver and kidney of 70 European hedgehogs from Northwest Spain were assessed. Moreover, As, Hg, Cd, and Pb concentrations in hair, spines, liver and kidney were measured, and POPs including PCBs, OCPs and PAHs, were measured in adipose tissue. Metal(loid) interactions between tissues were estimated along with the relationships between oxidative stress biomarkers and endogenous factors such as age and sex. Mercury was the most abundant metal in all tissues, exceeding critical thresholds (8.09, 14.0, 5.3 and 3.1 mg kg^{-1} dw in hair, spines, kidney and liver). PAHs, PCBs and OCPs were detected in 57, 28 and 15 % of the samples, respectively. All metal(loid)s in hair and spines were significantly correlated with organs, as well as oxidative stress biomarkers with Cd, Hg, Pb, and POPs. Our results describe the oxidative status of hedgehogs, and the observed correlations suggest that the levels of contaminants found in the animals may be associated with oxidative stress responses. Moreover, the correlations observed between metal(loid) levels in hair and spines with internal organs suggest that these keratinized tissues serve as reliable indicators of internal metal burden.

1. Introduction

Although metal(loid)s are present in the environment at natural concentrations, their levels are increased by their widespread use in many anthropogenic activities, constituting a potential risk to ecosystems and human health (Tchounwou et al., 2012). Owing to their properties, some metal(loid)s exhibit high toxicity even at minimal concentrations, and their low degradation rate makes them highly persistent in the environment (Kalisińska et al., 2019). Consequently, they remain bioavailable to organisms, which can lead to bioaccumulation and biomagnification processes along the trophic chain (Ali et al., 2019). However, the health effects, accumulation, and transfer of these contaminants rely not only on the chemical, dose, or duration of exposure, but also on external and internal factors (season, food availability, age, sex, physiology and genetics of the individual) (Wren, 1986). Within the metal(loid) group, the most relevant from an

Abbreviations: 4,4′-DDD, 1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene; 4, 4′-DDE, 1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethenyl]benzene; 2,4′-DDT, 1-chloro-2-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]benzene; 4,4′-DDT, 1-chloro-4-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]benzene; HCH, hexachlorocyclohexane; POPs, persistent organic pollutants; PCB, polychlorinated biphenyls; PCB 28, 2,4,4′-trichlorobiphenyl; PCB 101, 2,2′,4,5,5′-pentachlorobiphenyl; PCB 138, 2,2′,3,4,4′,5-hexachlorobiphenyl; PCB 153, 2,2′,4,4′,5,5′-hexachlorobiphenyl; PCB 180, 2,2′,3,4,4′,5,5′-heptachlorobiphenyl.

* Corresponding author. Toxicology Area, Faculty of Veterinary Medicine (UEX), 10003, Cáceres, Spain.
E-mail address: jgarciamz@unex.es (J. García-Muñoz).

https://doi.org/10.1016/j.envres.2025.121957
Received 21 January 2025; Received in revised form 22 May 2025; Accepted 24 May 2025
Available online 25 May 2025

ecotoxicological point of view, particularly in terrestrial and aquatic mammals, are arsenic (As), mercury (Hg), lead (Pb) and cadmium (Cd). The Agency for Toxic Substances and Recorded Diseases (ATSDR) classifies them as the most hazardous because of the risks associated with lethal and sublethal effects (ATSDR, 2022). These elements are commonly called heavy metal(loid)s, and multiple studies have shown their neurotoxic, nephrotoxic, carcinogenic, endocrine disrupting, mutagenic, and teratogenic effects (Ali et al., 2019). On the other hand, while the use of persistent organic pollutants (POPs) has been banned for years, they are still employed in many countries. Their high persistence allows them to be transported over long distances, interfering with biological systems and accumulating in both wildlife and humans (Altshul et al., 2004; Alleva et al., 2006; Mateo et al., 2012; González-Gómez et al., 2021). They are still detected in several ecosystem compartments and tend to increase in concentration as they rise through food webs to potentially toxic levels (Weber and Goerke, 2003). Among POPs, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs) are the most widespread in the environment.

The use of living organisms as bioindicators has enabled the assessment of their exposure through the detection and quantification of pollutants (Sparling, 2016; Kalisińska, 2019). One of the fundamental tools are biomarkers of effects, especially those focused on oxidative stress. In the presence of xenobiotics, reactive oxygen species (ROS) are generated, including superoxide anion radicals (O^{2-}), hydroxyl radicals (OH^{-}), or hydrogen peroxides (H_2O_2), and an overproduction of these causes an imbalance in enzymatic and non-enzymatic antioxidants (Roméo et al., 2013). This antioxidant deficiency can lead to deleterious effects, where multiple studies have reported lipid peroxidation and alterations in several cellular structures and functions, such as alterations in DNA and proteins, inflammatory processes, cell damage, apoptosis, and even tissue injury (Halliwell and Gutteridge, 2007). The harmful effect of free radicals that can potentially cause biological damage is known as oxidative stress. Antioxidants are molecules responsible for maintaining redox balance and inhibiting the generation of free radicals, scavenging them, and reducing the oxidation caused by them (Isaksson, 2010; Kalinina et al., 2022). Among the biomarkers of oxidative stress, catalase (CAT), glutathione S-transferase (GST) and MDA are among the most widely used (Armstrong, 2002; Amiard--Triquet et al., 2012; Armstrong and Stratton, 2016; Costantini, 2014). Exposure to heavy metals and POPs is widely recognized as a potential inducer of oxidative stress, primarily through the overproduction of ROS (Swiergosz-Kowalewska et al., 2006; Regoli et al., 2011; Costantini, 2014). Based on previous studies on small mammals (Viegas-Crespo et al., 2003; Bonilla-Valverde et al., 2004; Ruiz et al., 2019), a commonly expected physiological response is that increased levels of exposure lead to increased oxidative damage, often measured through lipid peroxidation (e.g., MDA) and, as a compensatory mechanism, increased activity of antioxidant enzymes (such as CAT or GST). However, results from different studies show that these responses are not always linear or consistent (Sánchez-Chardi et al., 2008; Quina et al., 2023). This is probably due to several interacting physiological factors, such as the mode of action of the metal, tissue-specific detoxification mechanisms, bioaccumulation dynamics, and the general health and metabolic state of the organism (Harris, 1992; Lopes et al., 2002). Thus, although the overall prediction suggests a positive association between exposure and oxidative biomarkers, variations in this pattern may reflect complex regulatory mechanisms and adaptive physiological responses.

Literature on the use of keratinized samples as a tool for the quantification of metal(loid)s, such as hair, highlights them as a valuable alternative for non-destructive biomonitoring studies (Jota Baptista et al., 2022). Given the strong affinity between keratin sulfhydryl groups and metal(loid)s, hair serves as an effective removal pathway as it accumulates in this matrix during hair growth (Beernaert et al., 2007). This tissue is in close contact with detoxifying organs and the bloodstream, which facilitating the elimination of xenobiotics from the body (García-Muñoz et al., 2023). Metal(loid) levels in keratinized tissues, such as hair and spines, may reflect their internal concentrations, suggesting that they could serve as alternative bioindicators of chronic exposure to metal(loid)s or POPs (Vermeulen et al., 2009; Iatrou et al., 2019). Non-invasive sampling techniques minimize harm and stress, preserve ecosystem integrity, align with ethical principles, and enable continuous monitoring by providing data on pollutant dynamics (Jota Baptista et al., 2022).

In this context, the European hedgehog (*Erinaceus europaeus*) has been used as biomonitor of metal(loid) and pesticide pollution due to its high accumulation rate (Dowding et al., 2010; Vermeulen et al., 2010; Jota Baptista et al., 2021; Rasmussen et al., 2024; Valverde et al., 2024). This insectivore reflects a series of characteristics that make it a good bioindicator, including: its wide distribution; it inhabits urban, forest and agricultural environments; its key position in the food chain as insectivores present a high risk of metal high exposure; and behavioural habits closely tied to the ground (Nores, 2007; Wijnhoven et al., 2007). Many studies have quantified the levels of inorganic elements and POPs in different tissues of the European hedgehog, including those considered non-invasive (i.e. hair or spines) (D'Havé et al., 2006a, 2006b; Vermeulen et al., 2009; Rasmussen et al., 2024). Given the potential toxicity of these pollutants and their presence in the soil and water, it is important to investigate their occurrence and bioaccumulation in hedgehogs. However, health effects have not been associated with exposure to these pollutants. Only one study observed biliary hyperplasia in individuals with high metal concentrations (Jota Baptista et al., 2023).

In this study, we assessed oxidative stress biomarkers (CAT, GST, and MDA) in liver and kidney of European hedgehogs (*Erinaceus europaeus*) from Southern Europe. The concentrations of metal(loid)s (As, Hg, Cd and Pb) were determined in hair, spines, liver and kidney, whereas POPs (PAHs, PCBs and OCPs) were quantified in adipose tissue. A correlation analysis was performed to evaluate the relationship between oxidative stress levels and pollutant concentrations. The effectiveness of hair and spines as non-invasive matrices for metal(loid) quantification was also determined. Endogenous factors (age and sex) were considered in the assessment of oxidative stress. With this consideration, given the proximity of hedgehogs to areas with a high human activity with pollution sources such as mining, agricultural or peri-urban areas, high concentrations of metal(loid)s and POPs are expected to bioaccumulate in their tissues. This exposure is predicted to increase oxidative damage, subsequently triggering an increase in antioxidant defenses as a response to stress induce by exposure to pollutants.

2. Material and methods

2.1. Sample collection

European hedgehog specimens ($n = 70$) were collected during the period 2018–2022 in Galicia (NW Spain) (Fig. 1). All specimens came from different locations, mainly peri-urban, agricultural and forest areas. These specimens were found already dead, primarily due to traffic accidents or natural causes (diseases or non-toxicological reasons). Once collected, the corpses were transported to the Wildlife Recovery Centers in this region. To ensure the integrity of the samples, only specimens that were confirmed not to have been dead for more than 5 days prior to necropsy were included in this study. All dead specimens were stored at −80 °C until subsequent preparation for chemical analysis, which took place at the Veterinary Faculty of the University of Extremadura. The animals were sexed, and their age was estimated by general size, dental development and degree of sexual maturity (12 young males, 7 young females, 25 adult males, 23 adult females, 3 non-defined) as previously established for different wildlife species (Pérez-López et al., 2016). From each body, internal organs, adipose tissue, hair and spines were removed and stored in individual plastic bags, and frozen until metal(loid), POPs, and biochemical analysis. The hair was collected from the ventral

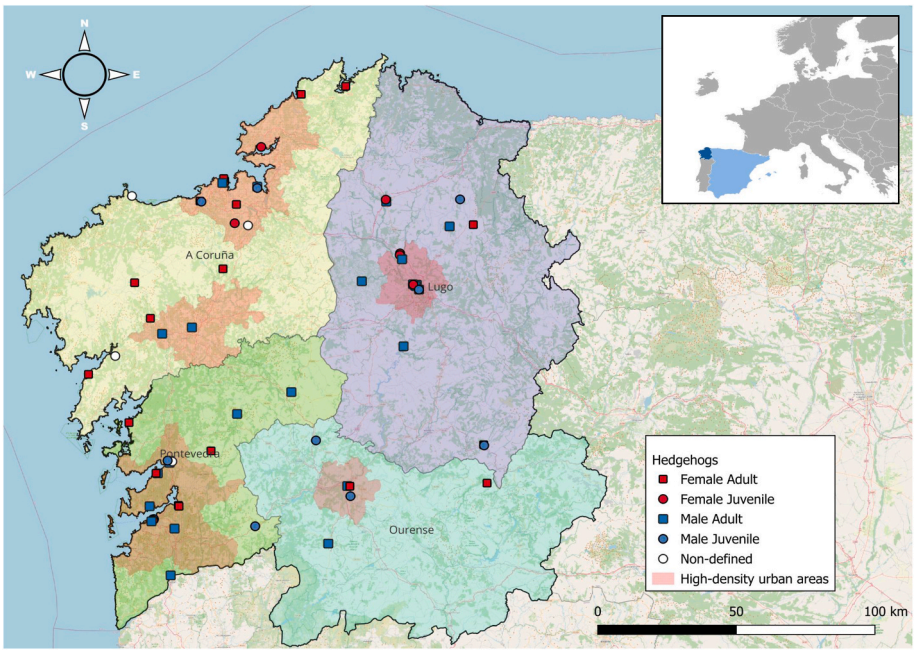

Fig. 1. Map of sample locations according to age, sex and distribution in NW Spain.

section of the carcass, while the spines were from the dorsal section. Samples were prepared to avoid metal contamination: plastic scalpels and surgical tools were cleaned or replaced for each individual, and disposable nitrile gloves were used. The working surface was cleaned after each operation. The samples were handled using plastic instruments following a procedure that avoided contact with external metal surfaces (Hernández-Moreno et al., 2013).

2.2. Heavy metal analysis

A significant amount of liver and kidney samples from each animal (3–4 g) was oven-dried for 72 h at 65 °C. Hair and spine samples were previously washed with tap water, distilled water, and acetone using a mixer (IKA® TRAYSTER digital) in a 5-min cycle, and each process was repeated three times in order to remove all surface contamination prior to digestion. The metal(loid) (As, Hg, Pb and Cd) levels were analysed at the Elemental and Molecular Laboratory of the Research Support Service (SAEM) (SAIUEX, accredited by ISO 9001:2008; University of Extremadura). For quantification, 0.7 g of hair, spines, liver and kidney were taken. Sample digestion was performed in closed PTFE Teflon tubes adding HNO3·H2O (3:1), following the protocol previously optimized in the laboratory for wildlife samples using an automatic microwave digester (Nardiello et al., 2019). Metal(loid) concentrations (As, Hg, Pb, and Cd) were determined using ICP-MS (7900) equipped with an autosampler (Agilent Technologies, Santa Clara, CA, USA). The limit of detection (LOD) for all metal(loid)s was 0.003 mg kg^{-1}, and the limit of quantification (LOQ) was 0.005 mg kg^{-1}. Each batch included blank and initial calibration standards, and a certified freeze-dried bovine liver sample (BCR®, ref 185R, Community Bureau of Reference, EU), was used as the reference material. The recovery yields ranged from 84 % for Cd to 92 % for Hg, and the coefficients of variation were consistently less than 10 %. Concentrations were expressed in mg kg^{-1} and dry weight (dw), given the recognized reliability and consistency of dry values compared to wet weight values (Adrian and Stevens, 1979). For an easier comparison with the existing literature reported on wet weight (ww), we adjusted the concentration by calculating the percentage moisture content of each sample during the drying process.

2.3. POPs analysis

A total of 63 POPs were analysed at the Analysis and Innovation in Animal Products Service (SiPA) (SAIUEX, University of Extremadura), concerning isomers and metabolites. The extraction protocol used in this study was adapted from the procedure described by Mateo et al. (2012). An adipose tissue amount of 1.0 g from abdomen was used for analysis. Once the sample was defrosted, it was mixed with 1 ml of n-hexane, and 5 ml of acetonitrile was added. Triphenyl phosphate (TPP) was added to control the extraction quality. The mixtures were centrifuged (2500g, 5 min, 4 °C) and a volume of 2.5 ml of supernatant was taken and frozen for 30 min at −20 °C. The samples were again centrifuged, and 1 mL was used for the extraction of POPs using the QuEChERS method. The supernatants were mixed with 80 mg of Primary/Secondary Amine, 50 mg of C18 and 70 mg of $MgSO_4$, and centrifuged at 9391g for 5 min. The obtained extract was subsequently evaporated under a stream of nitrogen, re-suspended in 200 μL n-hexane (cyclohexane and ethyl acetate, 9:1) and used for POPs concentration measurements. A Bruker Scion 456 triple quadrupole gas chromatograph mass spectrometer was used to analyse the samples. The results were analysed using specific GC-MS software. POPs quantification was performed using an external pattern in a matrix-mattach. Drift control was conducted every 20 injections in sequence and the recovery percentages for all samples were found to lie between 90 and 110 %. The limit of quantification (LOQ) was established as the lowest concentration level and validated with satisfactory recovery values (10 ng g^{-1}). The limit of detection (LOD) was not taken into account. Data are expressed in ng g^{-1}, lipid weight (lw).

2.4. Biochemical analysis

Oxidative stress biomarkers, enzymatic (glutathione-S-transferase -GST-, catalase -CAT-) and lipid peroxidation (malondialdehyde -MDA-), were analysed using a spectrophotometer (BioTek FL600). Liver and kidney tissue (0.5 g) were placed in glass tubes and a volume of 5 mL of phosphate buffer (0.1 M; pH 7.4) was added to each sample. Subsequently, each tube was mixed using a 20HS homogenizer (PCU Kinematica) and centrifuged at 4000 rpm for 5 min (Centronic S-577) to obtain the supernatant fraction. For enzymatic responses, the supernatants were centrifuged at 12000 rpm for 20 min at 4 °C, and a

subsequent supernatant was collected to determine the remaining oxidative stress biomarkers. CAT activity was analysed using the methodology described by Clairborne (1985), based on the reaction of H_2O_2 at 240 nm, and expressed as one enzyme unit (U) which is the amount of CAT capable of transforming 1.0 μmol of H_2O_2 in 1 min. GST activity was determined following procedure described by Habig et al. (1974), using 1-cloro-2,4-dinitrobenzene (CDNB) as substrate combined with GSH, for the change in the absorbance at 340 nm, and expressed as nmol min^{-1} mg^{-1} protein. For lipid peroxidation, MDA was measured using the thiobarbituric acid reactive substances (TBARS) reaction. Part of the supernatant was precipitated by adding 70 % perchloric acid and centrifuged at 4000 rpm for 15 min at 4 °C (DIGICEN 21R), following the colorimetric method described by Recknagel et al. (1982). MDA was expressed as nmol mg^{-1} protein. All biomarker determinations were assessed based on protein concentrations, which were analysed using a method adapted from Bradford (1976). Oxidative stress biomarker levels were expressed as a function of the protein content (in mg) of homogenates.

2.5. Statistical analysis

Statistical analysis was performed using GraphPad Prism 9.0.2 statistical software. Final concentrations are expressed as mean ± standard error of the mean (SEM), standard deviation (SD), median, and range. For statistical reasons, concentrations below the LOD (<LOD) were treated as zero values to obtain the least biased estimate of the reasonable maximum exposure. The assumptions of normality and homoscedasticity of the data were evaluated using the Shapiro-Wilk test, and a non-normal distribution was observed; therefore, a non-parametric study was subsequently applied. Therefore, the Kruskal-Wallis test was used to analyse the differences in metal(loid) accumulation between hair, spines, liver and kidney, as well as POP in adipose tissue, comparing the concentrations in the different tissue types. Spearman's test was used to assess the correlations among pollutant concentrations in all tissues and oxidative stress biomarkers. Finally, the Mann-Whitney *U* test was used to analyse the influence of intrinsic factors concerning age and sex in the oxidative stress parameters. The significance level was set at $p < 0.05$.

3. Results

3.1. Metal(loid) quantification and correlations among tissues

The main descriptive statistics related to metal(loid) concentrations in hair, spines and organs are shown in Table 1. All metal(loid)s were detected in all samples except Cd in three hair samples, the Kruskal-Wallis test revealed statistically significant differences among the tissues analysed ($p < 0.005$). Among the different tissues, Hg showed the highest concentration in hair and spines with mean concentrations of 8.09 and 5.31 mg kg^{-1} dw, and to a lesser extent Pb (0.497 and 0.464 mg kg^{-1} dw) and As (0.364 and 0.238 mg kg^{-1} dw) at the same levels. In both organs, Hg and Cd were the most accumulated elements, with the highest mean concentrations found in renal tissue (14.07 and 5.29 mg kg^{-1} dw, respectively). As was predominant in hair and spines (0.364 and 0.238 mg kg^{-1} dw) compared to the liver and kidney (0.177 and 0.176 mg kg^{-1} dw).

Significant relationships were found in metal(loid) concentrations among hair and spines with liver and kidney for all metal(loid)s (Table 2). Specifically, Spearman's test revealed significant positive associations between hair metal(loid) concentrations and both organs, except for As in liver tissue. Metal concentrations in spines were positively correlated with the Hg, Cd, and Pb in the liver and kidney. Concentrations in hair were positively correlated with those in spines for all metal(loid)s, as well as between the liver and kidney. When metal(loid) associations were analysed in each tissue, we observed statistically significant correlations in hair, which were negative for As/Cd and positive for Cd/Hg and Hg/Pb. In the spines, only a positive correlation was found between Cd and Pb. In the liver, positive associations were observed for As/Cd, As/Hg, Cd/Hg, Cd/Pb, and Hg/Pb, whereas in the kidneys, positive correlations were found for Cd/Hg and Cd/Pb.

Table 1
Metal(loid) concentrations in tissues of European hedgehog from Northern Spain. Data are expressed in mg kg^{-1} dw.

	Hair ($n = 70$)	Spines ($n = 67$)	Liver ($n = 70$)	Kidney ($n = 69$)
As				
Mean ±	0.364 ±	0.239 ±	0.177 ±	0.176 ±
SEM	0.046[a]	0.043[a]	0.028[b]	0.026[b]
Median	0.18	0.17	0.12	0.10
Min –	0.037–1.74	0.025–2.85	0.013–1.91	0.014–1.33
Max	0.51	0.099	0.16	0.11
IQR				
Cd				
Mean ±	0.016 ±	0.015 ±	1.49 ± 0.23[b]	5.29 ± 0.73[c]
SEM	0.001[a]	0.001[a]	0.78	3.29
Median	0.013	0.012	0.013–11.0	0.021–37.8
Min –	0.001–0.067	0.005–0.050	1.61	6.25
Max	0.012	0.009		
IQR				
Hg				
Mean ±	8.09 ± 1.0[ac]	5.31 ± 0.53[c]	3.06 ± 0.50[b]	14.1 ± 2.34[a]
SEM	5.22	4.53	1.45	6.61
Median	0.12–43.7	0.054–25.0	0.074–20.4	0.16–80.6
Min –	6.96	5.28	2.72	12.8
Max				
IQR				
Pb				
Mean ±	0.49 ± 0.062[a]	0.46 ± 0.062[a]	1.41 ± 0.31[b]	0.71 ± 0.083[b]
SEM	0.34	0.24	0.57	0.42
Median	0.019–3.07	0.029–2.12	0.075–16.4	0.074–3.84
Min –	0.61	0.54	0.97	0.74
Max				
IQR				

Standard error of mean (SEM) and minimum (Min) – maximum (Max). Mann-Whitney *U* test was performed to test differences between tissue concentrations for all metal(loid)s and was considered significant at $p < 0.005$. Means and SEM values for the respective metal(loid) followed by the same letter do not differ significantly between the tested tissues.

3.2. POPs

When considering POP's, the levels of OCPs, PCBs, including metabolites, and PAHs were determined in the adipose tissue of European hedgehogs from NW of Spain. The descriptive statistics are presented in Table 3. A concentration trend was observed, with the mean levels following the order from highest to lowest: ΣPAHs (1.63–214 ng g^{-1} lw) > ΣPCBs (1.01–2.28 ng g^{-1} lw) > ΣOCPs (0.006–7.08 ng g^{-1} lw). Among the analysed compounds, PAHs were dominant accounting for 67.4 % and 52.9 % of the total analysed POPs, respectively (Fig. 2). Naphthalene and phenanthrene were the dominant PAHs, followed by anthracene and fluorene. The percentages of PCBs and OCPs were lower, ranging from 5.88 % to 44.1 % and 2.94 %–23.5 %, respectively. PCB-138 and PCB-180 were detected in most of the individuals. In the OCPs group, 4,4′-DDT and 4,4′-DDE were dominant, and β-HCH was the most abundant isomer. The concentration of the α-HCH isomer was highest in this group (7.08 ng g^{-1} lw).

3.3. Oxidative stress biomarkers analysis

The main descriptive statistics concerning oxidative stress biomarkers, including antioxidant enzymes (CAT and GST), and lipid peroxidation (MDA), in both organs of hedgehogs are presented in Table 4. The CAT activity was significantly higher in the liver (0.294 ± 0.072 U mg^{-1} protein) than in the kidney (0.051 ± 0.009 U mg^{-1} protein) (Mann-Whitney *U* test: $p < 0.005$). In contrast, GST levels were higher in

Table 2
Spearman rank correlation (*r*) between metal(loid)s in the hair (H), spines (S), liver (L) and kidney (K) of the European hedgehog.

	As H	As S	As L	As K	Cd H	Cd S	Cd L	Cd K	Hg H	Hg S	Hg L	Hg K	Pb H	Pb S	Pb L	Pb K
As H		0.26*	ns	0.25*	−0.35**	−0.37**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
As S			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
As L				0.63***	ns	ns	0.46***	0.34**	ns	ns	0.40**	0.33**	ns	ns	ns	ns
As K					ns	ns	ns	ns	ns	0.33**	ns	ns	ns	ns	ns	ns
Cd H						0.49***	0.25*	0.29*	0.25*	ns	0.39**	ns	0.39**	ns	0.35**	ns
Cd S							0.30*	0.36**	ns	ns	0.32*	ns	0.30*	0.36**	0.40**	ns
Cd L								0.72***	0.35**	0.26*	0.57***	0.55***	0.39**	0.47***	0.36**	0.38**
Cd K									0.39**	0.25*	0.48***	0.62***	0.39**	0.47***	0.26*	0.40**
Hg H										0.64***	0.54***	0.67***	0.36**	0.45***	0.33*	ns
Hg S											0.38**	0.57***	0.33*	ns	ns	ns
Hg L												0.61***	ns	ns	0.41**	ns
Hg K														0.48***	ns	ns
Pb H														0.78***	0.47***	0.65***
Pb S															0.39**	0.66***
Pb L																0.44***
Pb K																

ns – not significant, *($p < 0.05$); **($p < 0.005$); ***($p < 0.0005$).

Table 3
Concentration of POPs in adipose tissue of European hedgehogs. Data are expressed in ng g^{-1} lw.

	N positive (%)	*Mean*	*SEM*	*Min-Max*
Organochlorine pesticides (OCPs)				
4, 4′-DDE	6 (17.7)	0.776	0.253	nd - 1.89
4,4′-DDD	1 (2.94)	0.008	–	–
2,4′-DDT	1 (2.94)	0.006		
4,4′-DDT	8 (23.5)	0.327	0.134	nd - 0.945
α-HCH	1 (2.94)	7.08	–	–
β-HCH	3 (8.82)	0.633	0.307	nd - 1.11
β-Heptachlor epoxide	2 (5.88)	0.095	0.002	nd - 0.097
Polychlorinated biphenyls (PCBs)				
PCB-28	2 (5.88)	1.18	0.109	nd - 1.29
PCB-101	3 (8.82)	1.12	0.092	nd - 1.24
PCB-138	14 (41.2)	2.28	0.497	nd - 6.37
PCB-153	6 (17.7)	1.01	0.417	nd - 2.88
PCB-180	15 (44.1)	1.89	0.605	nd - 7.91
Polycyclic aromatic hydrocarbons (PAHs)				
Acenaphthene	7 (20.6)	2.81	0.233	nd - 3.49
Anthracene	15 (44.1)	1.63	0.242	nd - 2.98
Fluoranthene	1 (2.94)	214	–	–
Fluorene	13 (38.2)	3.38	0.601	nd - 9.36
Naphthalene	23 (67.4)	5.03	0.239	nd - 7.80
Phenanthrene	18 (52.9)	2.76	0.785	nd - 14.1
Pyrene	5 (14.7)	21.7	1.21	nd - 24.4

SEM standard error of mean. nd (non-detected).

the kidney than in liver (211.9 and 168.8 nmol min^{-1} mg^{-1} protein, respectively) but the difference was not statistically significant ($p > 0.05$). When considering lipid peroxidation, the levels of MDA were higher in the kidney than in the liver (0.268 and 0.125 nmol mg^{-1} protein) ($p < 0.05$). Regarding endogenous factors, neither sex nor age showed a significant effect on the levels of the oxidative stress biomarkers analysed in this study ($p > 0.05$).

3.4. Effects of metals and POPs exposure on oxidative stress biomarkers

Spearman's test revealed a significant correlation in the levels of MDA in liver and kidneys ($r = 0.44$, $p < 0.05$). CAT activity was positively correlated with GST activity in the kidney ($r = 0.46$, $p < 0.005$), as well as CAT activity and MDA levels ($r = 0.36$, $p < 0.05$) (Fig. 3). Most correlations between metal(loid)s and oxidative stress biomarkers were negative (Fig. 4). Specifically, CAT was significantly negatively correlated with Cd ($r = -0.32$, $p < 0.05$), Hg ($r = -0.38$, $p < 0.005$) and Pb ($r = -0.41$, $p < 0.005$) in the liver. Additionally, CAT activity in the kidney was significantly correlated with Pb levels in the liver ($r = -0.34$, $p < 0.05$). GST activity in the liver was negatively correlated with Hg levels in the kidneys ($r = -0.32$, $p < 0.05$). MDA levels in the kidney were negatively correlated with Cd in liver ($r = -0.44$, $p < 0.005$) and kidney ($r = -0.36$, $p < 0.05$), and Hg in liver ($r = -0.35$, $p < 0.05$).

For POPs, most oxidative stress biomarkers showed a predominant correlation with PAHs, being significantly positive for naphthalene and those biomarkers in kidney ($r = 0.60$, $p < 0.005$ for CAT; $r = 0.61$, $p < 0.005$ for GST; $r = 0.58$, $p < 0.005$ for MDA), and negative for anthracene ($r = -0.70$, $p < 0.05$ for GST activity in liver) (Fig. 5). At last, for the rest of the xenobiotics, we only observe a positive correlation between MDA levels in liver with PCB-180 ($r = 0.63$, $p < 0.05$).

4. Discussion

4.1. Metal(loid)s

Hair is considered a suitable matrix for Hg determination due to its strong affinity for keratin, allowing it to reflect long-term exposure and chronic sublethal effects (Rashed and Soltan, 2005; Treu et al., 2018; García-Muñoz et al., 2023). Some authors have suggested that the normal Hg background level in terrestrial mammalian hair ranges from

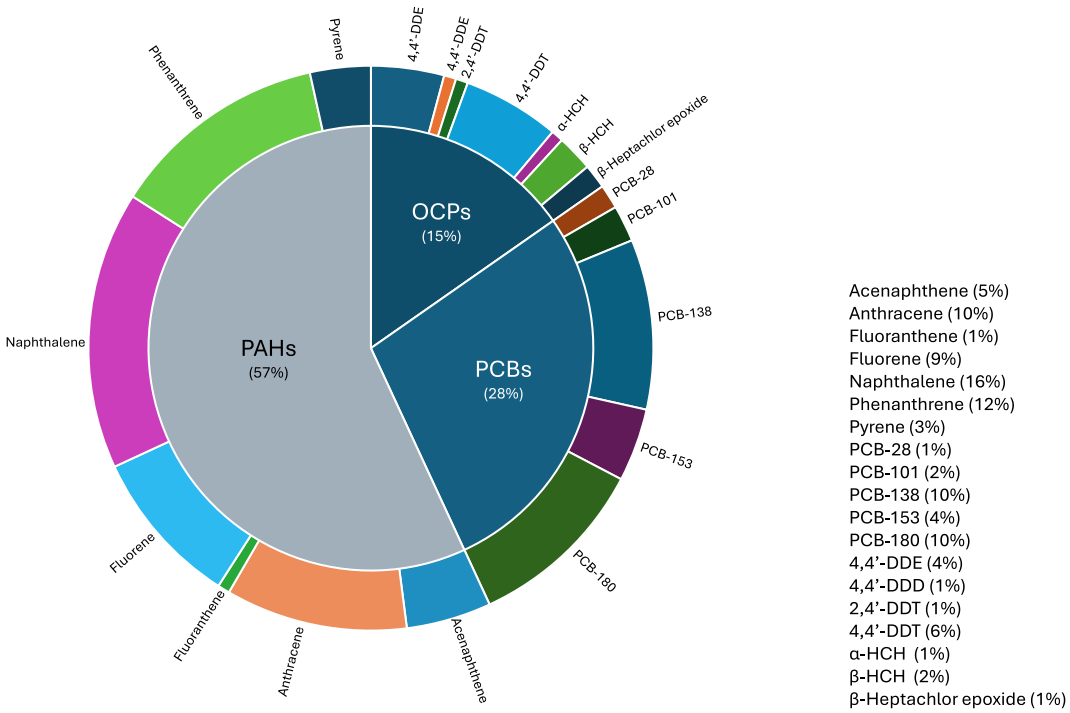

Fig. 2. Percentage of the different persistent organic compounds (POPs) corresponding to PAH, PCBs and OCPs detected in the adipose tissue samples of European hedgehogs. Percentages are expressed as an overall value of the graph.

Table 4
Main descriptive statistics corresponding to enzyme activity and lipid peroxidation in liver and kidney samples of European hedgehog from NW of Spain.

	Biomarker	Mean ± SEM	SD	Median	Range	IQR
Liver	CAT	0.294 ± 0.072	0.468	0.109	0.339–2.349	0.355
	GST	168.8 ± 30.15	195.4	89.64	12.24–884.1	189.7
	MDA	0.125 ± 0.013	0.084	0.109	0.025–0.383	0114
Kidney	CAT	0.051 ± 0.009	0.059	0.036	0.099–0.270	0.057
	GST	211.9 ± 23.24	150.6	222.8	4.002–474.5	267.7
	MDA	0.268 ± 0.044	0.286	0.138	0.032–1.410	0.246

Standard error of mean (SEM), standard deviation (SD).
CAT, U mg^{-1} protein.
GST, mU min^{-1} mg^{-1} protein.
MDA, nmol mg^{-1} protein.

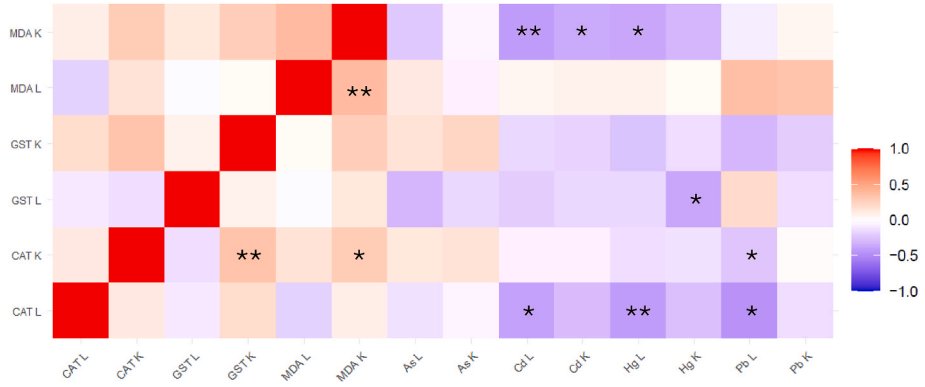

Fig. 3. Correlation matrix of the oxidative stress biomarkers and metal(loid)s analysed in the liver and kidney of European hedgehog. Blank square means no statistically significant correlation. Red colour represents positive correlation. Blue colour represents negative correlation. * ($p < 0.05$), ** ($p < 0.005$).

1 to 5 mg kg^{-1} (Sheffy and Amant, 1982). Above this range, subclinical neurological effects have been described in bears and bats at hair levels of 6–10 mg kg^{-1}, and 43 % of hedgehogs are above this threshold (Dietz et al., 2011; Ferrante et al., 2018). In addition, three individuals exceeded concentrations of 30 mg kg^{-1}, the lowest observed adverse effect level (LOAEL) established in the mammalian wildlife hair (43.66, 33.01 and 31.21 mg kg^{-1} dw) (Kalisińska et al., 2019). Only one study has reported Hg concentrations in hedgehog tissues, specifically in

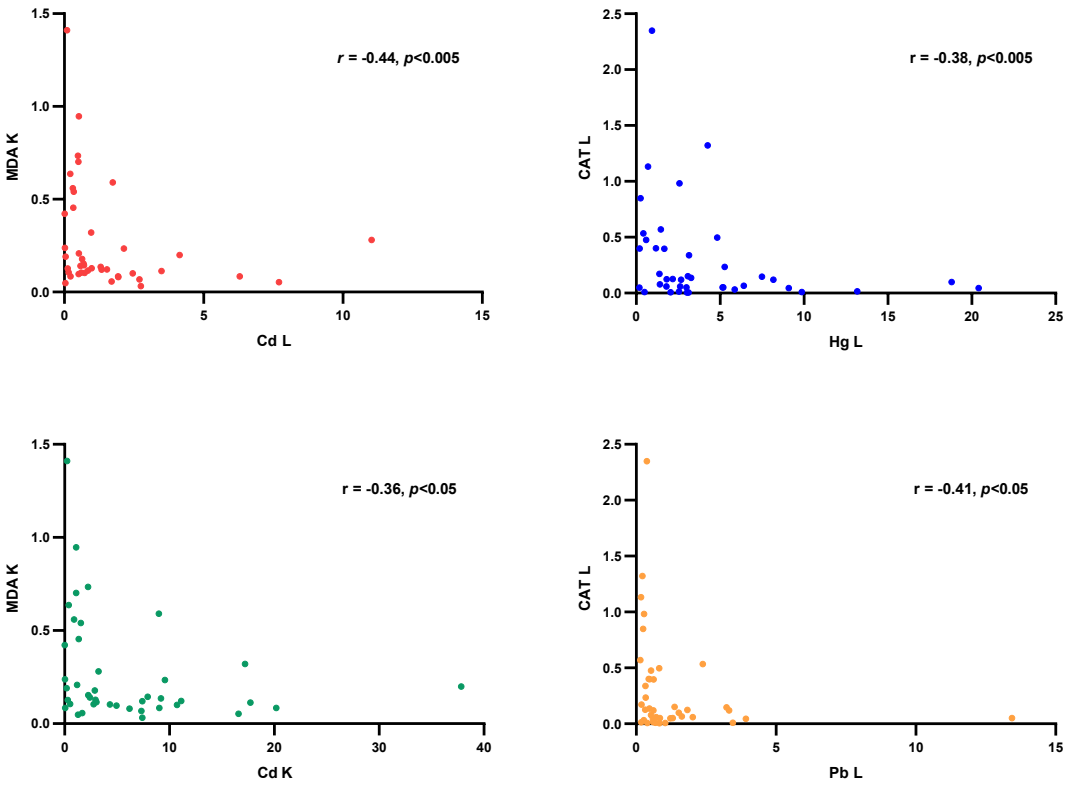

Fig. 4. Spearman correlation coefficients showing negative associations between oxidative stress biomarkers (MDA and CAT) and metal concentrations in the liver and kidneys of European hedgehogs. The r and p-values for each correlation are provided.

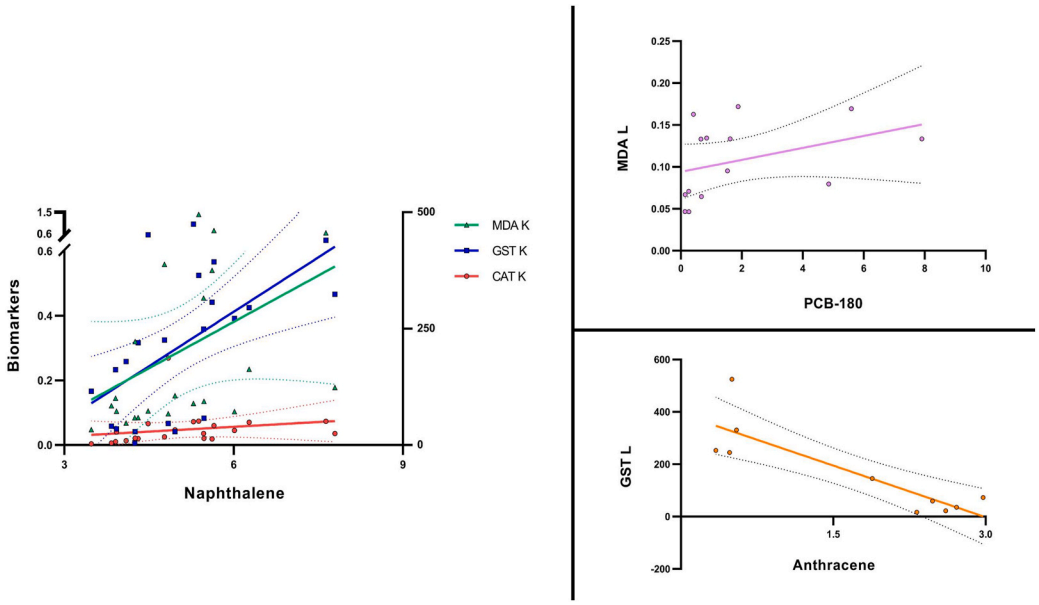

Fig. 5. Correlations between the levels of oxidative stress biomarkers in liver and kidney with POPs in adipose tissues of European hedgehogs.

Brandt's hedgehog (*Paraechinus hypomelas*), focusing mainly on spine assessment. The levels observed in that study were significantly lower compared to those found in our research (0.027, 0.066, 0.15 mg kg^{-1} dw in spines, liver and kidney) (Dahmardeh Behrooz et al., 2022). Despite the lack of studies measuring Hg in non-invasive samples, our research highlights the importance of this metal. When focusing on internal tissues, Hg was mainly accumulated in kidney tissues at high relevant concentrations, when compared to liver (14.07 and 3.06 mg kg^{-1} dw). In the kidney, 81 % of hedgehogs exceeded the Hg threshold for probable environmental mercury contamination (1.1 mg kg^{-1}), whereas in the liver 57 % of individuals surpassed this level (Eisler, 1987). In addition, two hedgehogs had a renal Hg concentration in the range associated with poisoning and mortality in wild mammals, and nine hedgehogs had a concentration above (25–30 mg kg^{-1}) (Shore et al., 2011). Although liver Hg concentrations were lower than those in the kidney, two individuals exceeded the LOAEL reporting clinical signs of intoxication and mortality (18.1 mg kg^{-1} in liver tissue) in terrestrial mammals (Wobeser et al., 1976). The presence of elevated Hg

concentrations can be attributed mainly to feeding and soil as the main sources of exposure, since hedgehogs forage close to the ground and consume invertebrates that can accumulate Hg. In addition, individual factors such as absorption and excretion rates, as well as behavioral habits, may contribute to variations among specimens (Talmage and Walton, 1993). Seasonality can also play a crucial role in small mammals that hibernate during winter, as they are forced to increase their intake during this period and are more susceptible to heavy metal(loid) exposure (Rautio et al., 2010). In all cases, elevated Hg concentrations in hedgehogs were consistently associated with proximity to urban areas, characterized by industrial activities and domestic emissions. This pattern is consistent with the findings of López-Alonso et al. (2007), who observed significantly higher Hg levels in urban dogs, attributing these differences to diffuse sources of pollution prevalent in urban environments. Galicia has several high-emission facilities, such as thermal power plants, aluminum and alumina refineries, and chlor-alkali industries, all of which are recognized sources of anthropogenic Hg. These activities have been associated with Hg emission levels that significantly exceed natural background levels (Nóvoa-Muñoz et al., 2008; Giráldez et al., 2022). Giráldez et al. (2022) corroborated that these industries were responsible for approximately 49 % of total Hg emissions in Galicia in 2019, highlighting their substantial contribution to regional environmental pollution.

The present study revealed low values of As and Pb in hair and spines compared to previous studies in hedgehogs (D'Havé et al., 2006a; Rautio et al., 2010; Valverde et al., 2024). Nevertheless, As and Pb concentrations are in agreement with those obtained in hedgehogs from unpolluted areas in Portugal and Belgium (Vermeulen et al., 2009; Jota Baptista et al., 2023). Keratinous tissues seem to be good biomarkers of chronic exposure to metal(loid)s, specifically As and Pb (Beernaert et al., 2007; Vermeulen et al., 2009; Tête et al., 2014; Binkowski, 2019). These elements bind to the sulfhydryl groups in keratin, and are incorporated into the hair follicle because of their distribution through the bloodstream (Tête et al., 2014; Squadrone et al., 2022). Similarly, the predominance of As in hair and spines was in accordance with D'Havé et al. (2006a), whose concentrations were 0.69 and 1.24 μg g^{-1} dw, as well as Jota Baptista et al. (2024) (0.22 mg kg^{-1} in spines). Although the thresholds for As and Pb in target organs such as the liver and kidney have been well described (Pereira et al., 2006; Ma, 2011), no data are available for hair or spines. The small size of hedgehogs, along with specific habits such as diet, is relevant due to their high metabolic rate, which increases their food intake and, consequently, their exposure to metal(loid)s (Wren, 1986). For instance, insectivores have a higher risk of metal(loid) intoxication during foraging than other small mammals (Talmage and Walton, 1993). Invertebrates such as earthworms, are able to accumulate high amounts of metals, mainly Cd or Pb (Al Sayegh Petkovšek et al., 2014; Beyer et al., 2018). Grooming and burrowing are habits closely linked to the soil fraction which, as exogenous sources, contribute to the exposure and accumulation of pollutants (D'Havé et al., 2005; Vermeulen et al., 2009). Significant linear relationships have been observed between As and Pb concentrations in spines, hair, and soil, in European hedgehogs from a non-ferrous smelter area in Belgium (D'Havé et al., 2005). In terrestrial mammals, As background levels in the liver and kidney do not exceed 0.5 mg kg^{-1} dw, and our results are in line with this finding (Binkowski, 2019). Other studies have considered 3 mg kg^{-1} as the background limit with no toxicological effects on the liver and kidney of mammals (Pereira et al., 2006). In the present study, none of Pb concentrations in kidneys reached the threshold associated with clinical signs of intoxication (25–35 mg kg^{-1} dw) (Ma, 2011). Even though all concentrations were below the renal critical value considered indicative of Pb toxicosis in mammals (15 mg kg^{-1} dw), some individual exceeded it in the liver (5 mg kg^{-1} dw) (Ma, 2011). Nine specimens exceeded the LOAEL for hepatic Pb concentration in small mammals, but none exceeded it in the kidney (2.7 and 5.9 mg kg^{-1} dw) (Shore and Douben, 1994). Based on previous studies, the normal background Pb concentration in both organs of mammals does not exceed 3–4 mg kg^{-1} dw, and our findings were within this range (Baranowska-Bosiacka et al., 2019). Nevertheless, the high Cd accumulation rate in target organs makes hair and spines unreliable indicators of Cd pollution. Our results align with those of Cooke (2011), indicating that Cd accumulates mainly in the kidneys and, once a threshold is exceeded, is stored in the liver. Therefore, our observed results for Cd concentrations in non-invasive matrices are as reported in the literature. Nevertheless, only one study has reported elevated Cd levels in hedgehog spines, attributing this to the high degree of urbanization in the area (Valverde et al., 2024).

4.1.1. Non-invasive vs invasive

To date, few studies have investigated the relationship between Hg concentrations in spines and internal tissues, and no such data are available for hair of European hedgehogs (García-Muñoz et al., 2023). Only one study was conducted on Brand's hedgehogs, in which positive correlations were observed between spine Hg levels and those in the kidney and muscle (Dahmardeh Behrooz et al., 2022). These findings are consistent with results from other mammalian studies, which suggest that Hg levels measured in keratinized tissues reflect those found in organs (Ferrante et al., 2018; Treu et al., 2018). Nevertheless, Pb and Cd levels in non-invasive matrices have been most frequently assessed in European hedgehogs. In accordance with our findings, D'Havé et al. (2006a) reported positive correlations between Cd and Pb levels in spines and hair and those in the liver, kidney and muscle. Positive relationships between hair and internal organ levels of these metals have been observed in other wild mammals, such as wood mice (*Apodemus sylvaticus*), bat (*Pipistrellus* sp.), European rabbit (*Oryctolagus cuniculus*), and Iberian wolf (*Canis lupus signatus*) (Beernaert et al., 2007; Hernández-Moreno et al., 2013; Tête et al., 2014; Hernout et al., 2016; Gil-Jiménez et al., 2020). Other studies have also found positive correlations between non-invasive tissues and internal organs for trace elements, such as zinc (Zn), cobalt (Co), and nickel (Ni) in European hedgehogs (Jota Baptista et al., 2024; Valverde et al., 2024). Although As levels measured in hair and spines were not correlated with those in the liver, a previous study reported positive relationship (D'Havé et al., 2006a). The absence of a significant correlation could be due to the role played by several factors such as sex, age, diet, sampling area, season, species-specific detoxification capacity of the hedgehog, environmental factors, or moulting (D'Havé et al., 2006a; Valverde et al., 2024). In view of these findings and consistent with previous studies, the keratin matrix seems to be suitable for metal binding due to its sulfhydryl groups. Thus, both matrices can be used for biomonitoring metals in the environment, serving as less invasive samples to predict internal concentrations (D'Havé et al., 2006b).

4.2. Persistent organic compounds

PAHs were the dominant compounds, with naphthalene being the most frequently detected, found in 67.4 % of the analysed samples. However, the highest PAH concentration was attributed to fluoranthene, with one individual showing 214 ng g^{-1} lw. Pyrene was the predominant compound in terms of concentration, detected in 14.7 % of the analysed samples. Except for individuals with excessive fluoranthene, PAH ranged from n.d. to 24.4 ng g^{-1} lw. To date, no studies on PAHs in European hedgehogs have been conducted. Quantification of PAHs in wildlife adipose tissue has not yet been described, with previous studies focusing only on the liver and hair (Iatrou et al., 2019; González-Gómez et al., 2021). PAHs have raised significant concern in recent years owing to their carcinogenic and mutagenic properties, posing a serious threat to both human and wildlife health (Abdel-Shafy and Mansour, 2016). Fluoranthene is used to manufacture of agrochemicals, dyes, and pharmaceuticals. The elevated concentration of fluoranthene can be attributed to its stability against oxidation, which explains its high levels in the soil (Douben, 2003). Additionally, the high concentrations found in this study may also be related to ingestion of contaminated soil through

feeding behavior. Experimental studies on the uptake of certain PAHs by earthworms, the main prey item of hedgehogs, showed that they were able to bioaccumulate large amounts of fluoranthene when exposed to polluted soils (Ma et al., 1995). In contrast to our findings, Iatrou et al. (2019) reported pyrene in a large percentage of the species but below the limit of quantification. Like the present study, fluorene and anthracene have also been quantified in the hair and liver in previous studies, but at higher mean concentrations in other wildlife species (Iatrou et al., 2019; González-Gómez et al., 2021).

PCB concentrations ranged from n.d. to 7.91 ng g^{-1} lw, with PCB 138 and PCB 180 being the most frequently detected in hedgehog samples (41.2 and 44.1 %, respectively). PCB 138, 153, and 180 are the most frequently reported congeners of PCBs in small mammalian tissues. The predominance of these congeners in different tissues has been previously observed in European hedgehogs (D'Havé et al., 2006b; 2007; Rasmussen et al., 2024). These authors reported higher PCB concentrations in the adipose tissue of hedgehogs from Belgium and the Netherlands (340 ng g^{-1} ww) (D'Havé et al., 2006b), or from Italy (6.43 mg kg^{-1}) (Alleva et al., 2006) compared to our study. In all cases, our mean PCB concentrations were below 17 μg g^{-1} lw, which is the threshold that causes adverse biological effects, and did not exceed the hepatic threshold associated with mortality (4–5 μg g^{-1} ww) and reproductive impairment (10 μg g^{-1} lw) in aquatic mammals such us European otter (*Lutra lutra*) (Kannan et al., 2000). Higher PCB levels have been found in the hair and muscle of other hedgehog species, such as *Erinaceus roumanicus* (7.54 and 36.41 ng g^{-1}) and *Erinaceus concolor* (7.38 and 28.10 ng g^{-1}) from Turkey (Arıkan et al., 2018). Vermeulen et al. (2010) found that PCB concentrations in earthworm were correlated with those in hedgehog hair, ranging from 0.62 to 13.53 ng g^{-1}, although these relationships were not significant due to the low PCB concentration. When compared to wildlife from the southwest Spain, mean PCB concentrations were relatively lower than those measured in the liver and muscle of hedgehogs and other wild carnivorous species (Hernández et al., 1985; Mateo et al., 2012). Nevertheless, our results are in the range found in the adipose tissue from other wildlife species, such as brown bear (*Ursus arctos*) and grey wolf (*Canis lupus*) (<LOD to 14.4 ng g^{-1} lw) from Croatia (Herceg Romanić et al., 2015).

When considering OCPs, 4,4′-DDT and 4,4′-DDE were the major contributors, found in 23.5 % and 17.7 % of adipose tissue samples, respectively, with the concentrations ranging from n.d. to 1.89 ng g^{-1} lw. The highest level was detected for the α-HCH isomer, however it was only found in one hedgehog. Compared with previous studies, HCH levels in adipose tissue were higher than those measured in hedgehogs from central Europe (1.6 ng g^{-1} ww) (D'Havé et al., 2006b). Moreover, the presence of α-HCH in adipose tissue has been observed in other small mammals such as the Arctic ground squirrel (*Spermophilus parryii*) (Allen-Gil et al., 1997). According to Willett et al. (1998), α-HCH is commonly found in soil, water or air samples, and high concentrations of this isomer suggest recent exposure. In contrast, β-HCH is less metabolizable and more lipophilic, tending to persist in the environment, and biomagnifies with the higher trophic level (Walker et al., 1999). Therefore, as hedgehogs occupy lower trophic levels than carnivores, β-HCH levels were expected to be lower than α-HCH levels. However, the unexpectedly elevated levels of α-HCH found in this individual may suggest exposure through soil contamination, indicating a potential risk of intoxication. DDE and DDT levels were much lower than those from central Europe (65 ng g^{-1} ww), and Italian hedgehogs (0.52–4.91 mg kg^{-1} in Italy) (Alleva et al., 2006). Quantification of these compounds has also been performed in other matrices such as blood, hair, liver, kidney and muscle, showing elevated concentrations in comparison to our study (Hernández et al., 1985; Rasmussen et al., 2024). Owing to their highly lipid-soluble properties, they tend to be stored in adipose tissue where the highest concentrations are reached. Once mobilized, they can be redistributed to target organs via the bloodstream, which can lead to toxic effects (Walker, 2001).

4.3. Oxidative stress

Oxidative stress biomarkers are commonly used in ecotoxicology as indicators of biological effects. Their activation is influenced by environmental conditions, especially exposure to pollutants such as metal (loid)s and POPs, which can alter the oxidative balance and cause stress (Halliwell and Gutteridge, 2007; Walker et al., 2012; Costantini, 2014). In the present study, CAT activity was higher in the liver than in the kidney. Although the liver is particularly rich in GST enzymes, GST activity was higher in kidney samples. CAT is present in all organs but is especially concentrated in the liver, which is the main organ responsible for detoxifying harmful metabolites and pollutants (Lentini et al., 2017). As a result, antioxidant defences are generally found at much higher concentrations in the liver than in other organs (Buhler and Williams, 1988). This enzyme inhibits the production of hydroxyl radicals by catalysing the conversion of H_2O_2 into H_2O and O_2 (Amiard-Triquet et al., 2012). GST is a multifunctional enzyme with broad and overlapping substrate specificity, which catalyzes the conjugation of reduced glutathione to various xenobiotics (Mulder et al., 1996). Several endogenous compounds such as products of lipid peroxidation and steroids are also substrates for GST, and an exposure to pollutants leads to an increase in the levels of this enzyme due to its sensitivity (Boyer, 1989). Both enzymes play key roles in the detoxification of oxidant species. CAT was positively correlated with GST in the kidneys, suggesting that hedgehogs were subjected to oxidative stress from different aspects, while the synergistic increase in different antioxidants could provide protection against oxidative stress. Significant negative correlations were observed between oxidative stress biomarkers measured in the liver and kidney, and metal(loid)s. CAT activity was negatively correlated with Cd, Hg and Pb in liver and kidney, and similar trend was also observed in GST activity for Hg levels in kidney. This may be due to inhibition of these antioxidant enzymes by metal exposure and toxicity. However, metal-induced toxicity may also be managed by other biochemical pathways. Alterations in antioxidant defences have been well-documented in laboratory and wild mammals exposed to trace elements (Li et al., 2006; Pinheiro et al., 2001; Reynolds et al., 2006).

Given the results from other research on the effects of inorganic elements on oxidative stress biomarkers determined in mammals, the low levels of CAT in European hedgehogs were not surprising. In other small mammals such as the Algerian mouse (*Mus spretus*), it has been observed that CAT activity in liver and kidney was significantly higher in mice from areas affected by mining spill (Aznalcóllar, Southern Spain) (320–402.4 U mg^{-1} protein) when compared with unaffected reference areas (263.5–333.1 U mg^{-1} protein) (Bonilla-Valverde et al., 2004). Moreover, this author reported a negative correlation between the activity of superoxide dismutase (SOD), and Pb and Cd levels in mouse kidneys, but CAT and GST were positively associated with Cd and As. They suggested that the increase of CAT activity, as well as other antioxidant defences, was an expected response to heavy metal(loid) exposure in those animals living in the surrounding areas affected by the mine spill. In a more recent study, significantly lower CAT levels were observed in the red blood cells of a Finnish population of Daubenton's bat (*Myotis daubentonii*) from a smelter where a metal discharge occurred (70.69–95.66 U mg^{-1} protein), compared to bats from unpolluted areas (96.42–97.68 U mg^{-1} protein) (Ruiz et al., 2019). Thus, negative correlations between CAT and copper (Cu), manganese (Mn) and Ni were observed. In view of this inhibition, the authors hypothesised that a combined effect between metal exposure and parasite may have contributed to the decrease in CAT levels. The exposure to pollutants not only challenges the antioxidant defence system of the organism to cope with the prooxidant species, but also other factors such as food scarcity, parasites, reoxygenation after hypoxia during hibernation or predation (Costantini, 2014). The low CAT activity in response to metal(loid) exposure has also been described in other small mammals (Ossola et al., 1997). In contrast to our study, Kalinina et al. (2022) showed higher CAT activity in kidney compared to liver (14.61 and

10.77 U mg^{-1} protein) of wild boar (*Sus scrofa*) from natural habitats in NW Russia. In line with these results, Quina et al. (2023) observed lower muscle CAT activities in greater white-toothed shrew (*Crocidura russula*) populations from mining areas (1.87–2.94 nmol min^{-1} mg^{-1} protein) versus the reference area (4.01 nmol min^{-1} mg^{-1} protein). Nevertheless, these results are much lower in comparison to the present study. It is well known that metal exposure can cause alterations of GST activity (Swiergosz-Kowalewska et al., 2006). Previous studies have demonstrated that enzyme activities within detoxification systems in small mammals, such as the short-tailed shrew (*Blarina brevicauda*), can be influenced by their physiological conditions (Stewart et al., 2005). Differences have also been observed between species, with Lopes et al. (2002) reporting higher GST levels in the liver of Algerian mice compared to wood mice (147.3 and 94.3 U g^{-1} wet liver, respectively). Viegas-Crespo et al. (2003) reported a significant increase in hepatic GST activity in Algerian mice from Portugal. Specifically, individuals from the polluted mining area showed higher GST activity (187.8 U g^{-1} wet liver) compared to those from the reference area (147.3 U g^{-1} wet liver). In line with our findings, similar trends were observed in the correlations of SOD and GST with essential metals such as Mn, iron (Fe), Cu or Zn in mice from polluted areas. This increase may reflect the response of the antioxidant system to metal exposure; however, it cannot be ruled out that postmortem oxidative changes over time are a contributing factor. This trend was corroborated in further studies. For instance, Bonilla-Valverde et al. (2004) reported an increased GST activity in the livers of mice living near mining areas from SW Spain, as well as a positive correlation between GST levels and Cd and As concentrations. Similarly, Marques et al. (2007) observed higher GST activity in greater white-toothed shrews from mining regions in Southern Portugal (1.18 U mg-1 protein) compared to those in reference areas (0.93 U mg-1 protein). Nevertheless, a later study conducted in the same area found lower GST activity in the liver of shrews from the abandoned mine site (685.96 and 986.76 μmol min^{-1} g^{-1} protein from mine and reference specimens, respectively), contrasting with previous findings (Sánchez-Chardi et al., 2008). Thus, the antioxidant enzyme glutathione peroxidase (GPx) was correlated with hepatic concentrations of Pb, Cu, Mn, and chromium (Cr). In accordance with these findings, Quina et al. (2023) also reported reduced GST activity in the muscle of shrews from the same polluted mining area (2.92 nmol min^{-1} mg^{-1} protein) compared to individuals from reference sites (4.24 nmol min^{-1} mg^{-1} protein).

When assessing lipid peroxidation, despite the fact that the liver contains more lipids, MDA levels were higher in the kidneys and showed a positive correlation between both organs. It is well established that exposure to metals can result in the overproduction of ROS, leading to increased lipid damage. MDA is a polar molecule with a small mass, and is one of the final products of lipid peroxidation (Amiard-Triquet et al., 2012). The correlation observed in MDA levels between the liver and kidneys may be attributed to the transfer of metabolites through the bloodstream between the two organs. However, it is also possible that post-mortem oxidation contributed to this pattern. We observed a positive correlation between MDA and CAT activity in the kidneys. Increased lipid peroxidation may induce the activation of antioxidants to scavenge ROS (Halliwell and Gutteridge, 2007). Nevertheless, antioxidant enzymes are activated by increasing oxidative stress to a certain threshold. When the oxidant/antioxidant balance is disrupted, these enzymes are inhibited because of oxidative stress, as suggested by the increase in MDA. For instance, Bonilla-Valverde et al. (2004) observed a threefold rise in MDA levels in the livers of Algerian mice residing near mining spill sites (13.19–15.35 arbitrary fluorescence units, AU), where antioxidant defences were decreased, compared to mice from reference areas (4.45–5.47 AU). Similar trends were observed in the liver and kidney of hares (*Lepus europaeus*) from Croatia, with MDA levels ranging from 23.73 to 26.09 nmol g^{-1} tissue in the liver and 24.40–27.50 nmol g^{-1} tissue in the kidney in polluted areas, compared to reference areas (20.15–20.82 nmol g^{-1} tissue in the liver, and 16.92–22.28 nmol g^{-1} tissue in the kidney) (Linšak et al., 2014). A recent study by Quina et al. (2023) corroborated earlier findings, showing that shrews from mining sites exhibited higher lipid peroxidation levels (312.97–398.73 nmol TBARS g^{-1} wet tissue), compared to reference shrews, which had lower values (287.21 nmol TBARS g^{-1} wet tissue). Lipid peroxidation could play a significant role in the decline of cellular function during oxidative stress, and is considered one of the most valuable indicators of oxidative damage to cellular components. It is possible that the high concentrations of metal(loid)s measured in liver and kidney of European hedgehogs has enhanced the ROS production in both organs, and the antioxidant defences were not able to effectively scavenge them.

When considering POPs, few studies have assessed the relationship between these xenobiotics and oxidative stress biomarkers in terrestrial wild mammals, with most research focused on marine organisms (Sinaei and Zare, 2019; Labrada-Martagón et al., 2025). However, POPs are known to have detrimental effects on physiological and biochemical parameters (Sonne et al., 2020). In this study, we observed significant positive correlations between naphthalene with CAT and GST activity in kidney tissues. Several studies have shown that both antioxidant enzymes are involved in the detoxification of many chemical pollutants (Amiard-Triquet et al., 2012). In fact, the modulation of antioxidant activity is dependent on the degree of contamination and chemical forms (Costantini, 2014). We suppose that an increase in the levels of these enzymes may result from modulation to enhance the elimination of oxidant compounds generated by POPs, which could explain the association with naphthalene. This may therefore be a response to a possible adaptation of hedgehogs to a stressful polluting environment. In this study, the toxicity of naphthalene and PCBs-180 is evidenced by their positive correlation with MDA levels in both organs, where the presence of these compounds increases free radical concentrations, leading to lipid peroxidation (Newman, 2009). Although we did not observe any correlation between enzymatic antioxidants and organochlorine compounds such as DDTs or PCBs, other researchers have found negative associations between GST activity and these POPs accumulated in the liver of wild boar (Tomza-Marciniak et al., 2014). In contrast, a negative correlation was found between anthracene and GST in the liver. The significant negative correlation could be explained by the toxicity of this xenobiotic, since it can alter the structure or function of GST, causing a decrease or inhibition of its activity (Vieira et al., 2008). In addition, the accumulation of anthracene may overload the antioxidant system, reducing the capacity of GST to neutralize free radicals.

It is important to recognize that this study establishes correlations between metal(loid) and POPs concentrations with biomarkers of oxidative stress, but does not demonstrate a causal relationship. Although some studies have demonstrated the potential of roadkill samples for the quantification of accumulated contaminants or the viability of DNA (Rautio et al., 2010; Arıkan et al., 2018; Coba-Males et al., 2023), in this study correlation analyses reveal associations but do not confirm causality on effects on biomarkers of oxidative stress, which would require controlled experiments with exposure to xenobiotics. In addition, the sample used in this study may not be fully representative of the entire hedgehog population, as it consists mainly of recently dead individuals. These physiological conditions could influence oxidative stress biomarker levels independently of contaminant exposure.

5. Conclusions

Oxidative stress biomarkers (CAT, GST and MDA) were assessed, and metal(loid)s (As, Hg, Cd and Pb) and POP levels were quantified in different tissues of European hedgehogs. The significant correlations observed between biomarkers of oxidative stress and concentrations of metals(loids) and POPs in liver and kidney could indicate their potential role as early indicators of chronic exposure to hazardous compounds in terrestrial mammals. Our results suggest that hedgehogs inhabiting anthropogenically modified ecosystems may exhibit variations in detoxification and antioxidation capacity. However, more research is

needed to determine whether these changes represent adaptive responses to environmental pollution. Our results provide valuable insights into the use of these biomarkers in small mammals, thereby addressing the current gap in research on this group of species. However, no influence of age or sex on oxidative stress biomarkers was observed in the present study, highlighting the need for further research to better understand these potential factors.

Elevated Hg concentrations have been found in different tissues of European hedgehogs, and this study provides the first data on Hg quantification in non-invasive tissues. The relationships observed between them (hair and spines) and internal metal(loid) concentrations reflect the suitability of these tissues as effective and ethical tools for ongoing environmental assessment. Although many POPs have been banned in the EU for many years, our findings in adipose tissue of hedgehogs showed that these compounds are still detected in relevant concentrations, with PAHs being the most frequently quantified compounds, which could pose a risk to wildlife and human health. In line with "One Health" concept, these results emphasize the importance of monitoring environmental contaminants, as wildlife health serves as an indicator of broader ecological impacts and potential risks to human health, reinforcing the need for integrated approaches to pollution management and public health.

CRediT authorship contribution statement

Javier García-Muñoz: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Elsa Rodríguez Somoza:** Methodology, Investigation. **Irene Nuñez Zafra:** Methodology, Investigation. **David Fernández-Casado:** Methodology, Data curation. **Ángel Portillo-Moreno:** Methodology, Data curation. **María del Prado Míguez-Santiyán:** Visualization, Software, Formal analysis. **Francisco Soler Rodríguez:** Resources, Funding acquisition, Formal analysis, Conceptualization. **Ana López-Beceiro:** Resources, Methodology. **Luis Eusebio Fidalgo:** Resources, Methodology. **Salomé Martínez-Morcillo:** Writing – review & editing, Validation, Supervision, Conceptualization. **Marcos Pérez-López:** Writing – review & editing, Validation, Supervision, Conceptualization.

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.

Acknowledgements

The authors wish to thank the Galician Wildlife Recovery Centers and the Dirección Xeral de Patrimonio Natural (Consellería de Medio Ambiente y Ordenación do Territorio) of the Xunta de Galicia, for authorizing the use and transfer of wildlife specimens used in this work. We would like to thank the Research Support Services of the University of Extremadura (SAIUEx) for their technical assistance in the pollutant analysis. This work has been possible thanks to the European Regional Development Fund (ERDF), and to the Junta de Extremadura (GR21118).

Data availability

Data will be made available on request.

References

Abdel-Shafy, H.I., Mansour, M.S., 2016. A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. Egypt. J. Pet. 25 (1), 107–123. https://doi.org/10.1016/j.ejpe.2015.03.011.

Adrian, W.J., Stevens, M.L., 1979. Wet versus dry weights for heavy metal toxicity determinations in duck liver. J. Wildl. Dis. 15 (1), 125–126. https://doi.org/10.7589/0090-3558-15.1.125.

Al Sayegh Petkovšek, S., Kopušar, N., Kryštufek, B., 2014. Small mammals as biomonitors of metal pollution: a case study in Slovenia. Environ. Monit. Assess. 186 (7), 4261–4274. https://doi.org/10.1007/s10661-014-3696-7.

Ali, H., Khan, E., Ilahi, I., 2019. Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. J. Chem. https://doi.org/10.1155/2019/6730305.

Allen-Gil, S.M., Landers, D.H., Wade, T.L., Sericano, J.L., Lasorsa, B.K., Crecelius, E.A., Curtis, L.R., 1997. Heavy metal, organochlorine pesticide and polychlorinated biphenyl contamination in arctic ground squirrels (*Spermophilus parryi*) in Northern Alaska. Arctic 323–333.

Alleva, E., Francia, N., Pandolfi, M., De Marinis, A.M., Chiarotti, F., Santucci, D., 2006. Organochlorine and heavy-metal contaminants in wild mammals and birds of Urbino-Pesaro Province, Italy: an analytic overview for potential bioindicators. Arch. Environ. Contam. Toxicol. 51 (1), 123–134. https://doi.org/10.1007/s00244-005-0218-1.

Altshul, L., Covaci, A., Hauser, R., 2004. The relationship between levels of PCBs and pesticides in human hair and blood: preliminary result. Environ. Health Perspect. 112 (11), 1193–1199. https://doi.org/10.1289/ehp.6916.

Amiard-Triquet, C., Amiard, J.C., Rainbow, P.S. (Eds.), 2012. Ecological Biomarkers: Indicators of Ecotoxicological Effects. CRC press.

Arıkan, K., Arıkan, Z.Y., Turan, S.L., 2018. Persistent organochlorine contaminant residues in tissues of hedgehogs from Turkey. Bull. Environ. Contam. Toxicol. 100 (3), 361–368. https://doi.org/10.1007/s00128-018-2272-1.

Armstrong, D. (Ed.), 2002. Oxidative Stress Biomarkers and Antioxidant Protocols, vol. 186. Humana Press, Totowa, NJ, USA.

Armstrong, D., Stratton, R.D. (Eds.), 2016. Oxidative Stress and Antioxidant Protection: the Science of Free Radical Biology and Disease. John Wiley & Sons.

Agency for Toxic Substances and Disease Registry (ATSDR), 2022. Substance Priority List. Agency for Toxic Substances and Disease. Registry. http://www.atsdr.cdc.gov/SPL/index.html. (Accessed 23 July 2024).

Baranowska-Bosiacka, I., Korbecki, J., Marchlewicz, M., 2019. Lead, Ld. In: Kalisińska, E. (Ed.), Mammals and Birds as Bioindicators of Trace Element Contaminations in Terrestrial Environments: an Ecotoxicological Assessment of the Northern Hemisphere. Springer Nature, Switzerland, AG, Berlin, pp. 563–592. https://doi.org/10.1007/978-3-030-00121-6_16.

Beernaert, J., Scheirs, J., Leirs, H., Blust, R., Verhagen, R., 2007. Non-destructive pollution exposure assessment by means of wood mice hair. Environmental Pollution (Barking, Essex: 1987) 145 (2), 443–451. https://doi.org/10.1016/j.envpol.2006.04.025.

Beyer, W.N., Codling, E.E., Rutzke, M.A., 2018. Anomalous bioaccumulation of lead in the earthworm *Eisenoides lonnbergi* (Michaelsen). Environ. Toxicol. Chem. 37 (3), 914–919. https://doi.org/10.1002/etc.4031.

Binkowski, L.J., 2019. Arsenic, as. In: Kalisińska, E. (Ed.), Mammals and Birds as Bioindicators of Trace Element Contaminations in Terrestrial Environments: an Ecotoxicological Assessment of the Northern Hemisphere. Springer Nature, Switzerland, AG, Berlin, pp. 463–482. https://doi.org/10.1007/978-3-030-00121-6_13.

Bonilla-Valverde, D., Ruiz-Laguna, J., Muñoz, A., Ballesteros, J., Lorenzo, F., Gómez-Ariza, J.L., López-Barea, J., 2004. Evolution of biological effects of Aznalcóllar mining spill in the Algerian mouse (*Mus spretus*) using biochemical biomarkers. Toxicology 197 (2), 123–138. https://doi.org/10.1016/j.tox.2003.12.010.

Boyer, T.D., 1989. The glutathione S-transferases: an update. Hepatology (Baltimore, Md 9 (3), 486–496. https://doi.org/10.1002/hep.1840090324.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254. https://doi.org/10.1006/abio.1976.9999.

Buhler, D.R., Williams, D.E., 1988. The role of biotransformation in the toxicity of chemicals. Aquat. Toxicol. 11 (1–2), 19–28.

Clairborne, A., 1985. Catalase activity. In: Greenwald, R.A. (Ed.), Handbook of Methods in Oxygen Radical Research. CRC Press, Boca Raton, Floride (USA), pp. 283–284.

Coba-Males, M.A., Medrano-Vizcaíno, P., Enríquez, S., Brito-Zapata, D., Martin-Solano, S., Ocaña-Mayorga, S., Carrillo-Bilbao, G.A., Narváez, W., Salas, J.A., Arrivillaga-Henríquez, J., González-Suárez, M., Poveda, A., 2023. From roads to biobanks: roadkill animals as a valuable source of genetic data. PLoS One 18 (12), e0290836. https://doi.org/10.1371/journal.pone.0290836.

Cooke, J.A., 2011. Cadmium in small mammals. In: Beyer, W.N., Meador, J.P. (Eds.), Environmental Contaminants in Biota. Interpreting Tissue Concentrations. CRC Press, New York, pp. 609–624. https://doi.org/10.1201/b10598.

Costantini, D., 2014. Oxidative stress and hormesis in evolutionary ecology and physiology. A Marriage Between Mechanistic and Evolutionary Approaches. Springer, Berlin. https://doi.org/10.1007/978-3-642-54663-1.

Dahmardeh Behrooz, R., Poma, G., Barghi, M., 2022. Non-destructive mercury exposure assessment in the Brandt's hedgehog (*Paraechinus hypomelas*): spines as indicators of endogenous concentrations. Environ. Sci. Pollut. Res. Int. 29 (37), 56502–56510. https://doi.org/10.1007/s11356-022-19926-0.

D'Havé, H., Scheirs, J., Mubiana, V.K., Verhagen, R., Blust, R., De Coen, W., 2005. Nondestructive pollution exposure assessment in the European hedgehog (*Erinaceus europaeus*): I. Relationships between concentrations of metals and arsenic in hair, spines, and soil. Environ. Toxicol. Chem. 24 (9), 2356–2364. https://doi.org/10.1897/04-597r.1.

D'Havé, H., Scheirs, J., Mubiana, V.K., Verhagen, R., Blust, R., De Coen, W., 2006a. Non-destructive pollution exposure assessment in the European hedgehog (*Erinaceus europaeus*): II. Hair and spines as indicators of endogenous metal and as

concentrations. Environmental Pollution (Barking, Essex: 1987) 142 (3), 438–448. https://doi.org/10.1016/j.envpol.2005.10.021.
D'Havé, H., Scheirs, J., Covaci, A., Schepens, P., Verhagen, R., De Coen, W., 2006b. Nondestructive pollution exposure assessment in the European hedgehog (*Erinaceus europaeus*): III. Hair as an indicator of endogenous organochlorine compound concentrations. Environ. Toxicol. Chem. 25 (1), 158–167. https://doi.org/10.1897/05-208r.1.
D'Havé, H., Scheirs, J., Covaci, A., van den Brink, N.W., Verhagen, R., De Coen, W., 2007. Non-destructive pollution exposure assessment in the European hedgehog (Erinaceus europaeus): IV. Hair versus soil analysis in exposure and risk assessment of organochlorine compounds. Environmental Pollution (Barking, Essex: 1987) 145 (3), 861–868. https://doi.org/10.1016/j.envpol.2006.05.013.
Dietz, R., Born, E.W., Rigét, F., Aubail, A., Sonne, C., Drimmie, R., Basu, N., 2011. Temporal trends and future predictions of mercury concentrations in Northwest Greenland polar bear (*Ursus maritimus*) hair. Environ. Sci. Technol. 45 (4), 1458–1465. https://doi.org/10.1021/es1028734.
Douben, P.E.T., 2003. Pahs: an Ecotoxicological Perspective. John Wiley & Sons, Ltd. https://doi.org/10.1002/0470867132.
Dowding, C.V., Shore, R.F., Worgan, A., Baker, P.J., Harris, S., 2010. Accumulation of anticoagulant rodenticides in a non-target insectivore, the European hedgehog (*Erinaceus europaeus*). Environmental Pollution (Barking, Essex: 1987) 158 (1), 161–166. https://doi.org/10.1016/j.envpol.2009.07.017.
Eisler, R., 1987. Mercury hazards to fish, wildlife, and invertebrates: a synoptic review. U S Fish Wildl. Serv. Biol. Rep. 85 (1.10). https://pdfs.semanticscholar.org/ff3d/225a1e5b134937f88d340a2756c21a6ac4d4.pdf.
Ferrante, M., Spena, M.T., Hernout, B.V., Grasso, A., Messina, A., Grasso, R., Agnelli, P., Brundo, M.V., Copat, C., 2018. Trace elements bioaccumulation in liver and fur of *Myotis myotis* from two caves of the eastern side of Sicily (Italy): a comparison between a control and a polluted area. Environ. Pollut. (Barking, Essex:1987) 240, 273–285. https://doi.org/10.1016/j.envpol.2018.04.133.
García-Muñoz, J., Pérez-López, M., Soler, F., Prado Míguez-Santiyán, M., Martínez-Morcillo, S., 2023. Non-Invasive Samples for Biomonitoring Heavy Metals in Terrestrial Ecosystems. IntechOpen. https://doi.org/10.5772/intechopen.1001334.
Gil-Jiménez, E., Mateo, R., de Lucas, M., Ferrer, M., 2020. Feathers and hair as tools for non-destructive pollution exposure assessment in a mining site of the Iberian Pyrite Belt. Environmental Pollution (Barking, Essex: 1987) 263 (Pt B), 114523. https://doi.org/10.1016/j.envpol.2020.114523.
Giráldez, P., Crujeiras, R.M., Fernández, J.Á., Aboal, J.R., 2022. Establishment of background pollution levels and spatial analysis of moss data on a regional scale. Sci. Total Environ. 839, 156182. https://doi.org/10.1016/j.scitotenv.2022.156182.
González-Gómez, X., Cambeiro-Pérez, N., Figueiredo-González, M., Martínez-Carballo, E., 2021. Wild boar (*Sus scrofa*) as bioindicator for environmental exposure to organic pollutants. Chemosphere 268, 128848. https://doi.org/10.1016/j.chemosphere.2020.128848.
Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249 (22), 7130–7139.
Halliwell, B., Gutteridge, J., 2007. Free Radicals in Biology and Medicine, fourth ed. Oxford University Press, New York.
Harris, E.D., 1992. Regulation of antioxidant enzymes. FASEB J. : Official Publication of the Federation of American Societies for Experimental Biology 6 (9), 2675–2683. https://doi.org/10.1096/fasebj.6.9.1612291.
Herceg Romanić, S., Klinčić, D., Kljaković-Gašpić, Z., Kusak, J., Reljić, S., Huber, Đ., 2015. Organochlorine pesticides and polychlorinated biphenyl congeners in wild terrestrial mammals from Croatia: interspecies comparison of residue levels and compositions. Chemosphere 137, 52–58. https://doi.org/10.1016/j.chemosphere.2015.05.026.
Hernández, L.M., González, M.J., Rico, M.C., Fernández, M.A., Baluja, G., 1985. Presence and biomagnification of organochlorine pollutants and heavy metals in mammals of Doñana National Park (Spain), 1982-1983. J. Environ. Sci. Health Part B Pestic. Food Contam. Agric. Wastes 20 (6), 633–650. https://doi.org/10.1080/03601238509372501.
Hernández-Moreno, D., de la Casa Resino, I., Fidalgo, L.E., Llaneza, L., Soler Rodríguez, F., Pérez-López, M., López-Beceiro, A., 2013. Noninvasive heavy metal pollution assessment by means of Iberian wolf (*Canis lupus* signatus) hair from Galicia (NW Spain): a comparison with invasive samples. Environ. Monit. Assess. 185 (12), 10421–10430. https://doi.org/10.1007/s10661-013-3341-x.
Hernout, B.V., McClean, C.J., Arnold, K.E., Walls, M., Baxter, M., Boxall, A.B., 2016. Fur: a non-invasive approach to monitor metal exposure in bats. Chemosphere 147, 376–381. https://doi.org/10.1016/j.chemosphere.2015.12.104.
Iatrou, E.I., Tsygankov, V., Seryodkin, I., Tzatzarakis, M.N., Vakonaki, E., Barbounis, E., Zakharenko, A.M., Chaika, V.V., Sergievich, A.A., Tsatsakis, A.M., Golokhvast, K., 2019. Monitoring of environmental persistent organic pollutants in hair samples collected from wild terrestrial mammals of Primorsky Krai, Russia. Environ. Sci. Pollut. Res. Int. 26 (8), 7640–7650. https://doi.org/10.1007/s11356-019-04171-9.
Isaksson, C., 2010. Pollution and its impact on wild animals: a metaanalysis on oxidative stress. EcoHealth 7 (3), 342–350. https://doi.org/10.1007/s10393-010-0345-7.
Jota Baptista, C.V., Seixas, F., Gonzalo-Orden, J.M., Oliveira, P.A., 2021. Can the European hedgehog (*Erinaceus europaeus*) be a sentinel for one Health concerns? Biologics 1 (1), 61–69. https://doi.org/10.3390/biologics1010004.
Jota Baptista, C., Seixas, F., Gonzalo-Orden, J.M., Oliveira, P.A., 2022. Biomonitoring metals and metalloids in wild mammals: invasive versus non-invasive sampling. Environ. Sci. Pollut. Res. Int. 29 (13), 18398–18407. https://doi.org/10.1007/s11356-022-18658-5.
Jota Baptista, C., Seixas, F., Gonzalo-Orden, J.M., Patinha, C., Pato, P., Ferreira da Silva, E., Casero, M., Brazio, E., Brandão, R., Costa, D., Mateus, T.L., Coelho, A.C., Oliveira, P.A., 2024. The first full study of heavy metal(loid)s in western-European hedgehogs (Erinaceus europaeus) from Portugal. Environ. Sci. Pollut. Res. Int. 31 (8), 11983–11994. https://doi.org/10.1007/s11356-024-31877-2.
Jota Baptista, C., Seixas, F., Gonzalo-Orden, J.M., Patinha, C., Pato, P., Ferreira da Silva, E., Casero, M., Brazio, E., Brandão, R., Costa, D., Mateus, T.L., Oliveira, P.A., 2023. High levels of heavy Metal(loid)s related to biliary hyperplasia in hedgehogs (*Erinaceus europaeus*). Animals 13 (8), 1359. https://doi.org/10.3390/ani13081359.
Kalinina, S., Panchenko, D., Ilyukha, V., Canfield, A., Baishnikova, I., Antonova, E., Nikerova, K., 2022. Elements and antioxidants in wild boar from northwestern Russia. Eur. J. Wildl. Res. 68 (2), 22. https://doi.org/10.1007/s10344-022-01570-1.
Kalisińska, E., 2019. Mammals and Birds as Bioindicators of Trace Element Contaminations in Terrestrial Environments: an Ecotoxicological Assessment of the Northern Hemisphere. Springer Nature, Switzerland, AG, Berlin. https://doi.org/10.1007/978-3-030-00121-6.
Kalisińska, E., Lanocha-Arendarczyk, N., Kosik-Bogacka, D.I., 2019. Mercury, Hg. In: Kalisińska, E. (Ed.), Mammals and Birds as Bioindicators of Trace Element Contaminations in Terrestrial Environments: an Ecotoxicological Assessment of the Northern Hemisphere. Springer Nature, Switzerland, AG, Berlin, pp. 593–654. https://doi.org/10.1007/978-3-030-00121-6_17.
Kannan, K., Blankenship, A.L., Jones, P.D., Giesy, J.P., 2000. Toxicity reference values for the toxic effects of polychlorinated biphenyls to aquatic mammals. Human and Ecological Risk Assessment 6 (1), 181–201.
Labrada-Martagón, V., Cúmez-Caté, B.L., Yáñez-Estrada, L., Rodríguez-Salazar, C.L., Delgado, L., Maldonado, G., Zenteno-Savín, T., Solé, M., 2025. Inter- and intraspecific blood-related biomarkers and chemical exposure in confined and free-living sea turtles. Environ. Res. 264 (Pt 2), 120385. https://doi.org/10.1016/j.envres.2024.120385.
Lentini, P., Zanoli, L., Granata, A., Signorelli, S.S., Castellino, P., Dell'Aquila, R., 2017. Kidney and heavy metals - the role of environmental exposure. Mol. Med. Rep. 15 (5), 3413–3419. https://doi.org/10.3892/mmr.2017.6389 (Review).
Li, G., Sang, N., Guo, D., 2006. Oxidative damage induced in hearts, kidneys and spleens of mice by landfill leachate. Chemosphere 65 (6), 1058–1063. https://doi.org/10.1016/j.chemosphere.2006.02.056.
Linšak, D.T., Linšak, Z., Spirić, Z., Srebočan, E., Glad, M., Cenov, A., Jakovac, H., Milin, C., 2014. Influence of cadmium on metallothionein expression and products of lipid peroxidation in the organs of hares (*Lepus europaeus Pallas*). J. Appl. Toxicol. : JAT 34 (3), 289–295. https://doi.org/10.1002/jat.2880.
Lopes, P.A., Viegas-Cresp, A.M., Nunes, A.C., Pinheiro, T., Marques, C., Santos, M.C., Mathias, M.deL., 2002. Influence of age, sex, and sexual activity on trace element levels and antioxidant enzyme activities in field mice (*Apodemus sylyaticus* and *Mus spretus*). Biol. Trace Elem. Res. 85 (3), 227–239. https://doi.org/10.1385/bter:85:3:227.
López-Alonso, M., Miranda, M., García-Partida, P., Cantero, F., Hernández, J., Benedito, J.L., 2007. Use of dogs as indicators of metal exposure in rural and urban habitats in NW Spain. Sci. Total Environ. 372 (2–3), 668–675. https://doi.org/10.1016/j.scitotenv.2006.10.003.
Ma, W.C., 2011. Lead in mammals. In: Beyer, W.N., Meador, J.P. (Eds.), Environmental Contaminants in Biota. Interpreting Tissue Concentrations. CRC Press, New York, pp. 609–624. https://doi.org/10.1201/b10598.
Ma, W.C., Immerzeel, J., Bodt, J., 1995. Earthworm and food interactions on bioaccumulation and disappearance in soil of polycyclic aromatic hydrocarbons: studies on phenanthrene and fluoranthene. Ecotoxicol. Environ. Saf. 32 (3), 226–232. https://doi.org/10.1006/eesa.1995.1108.
Marques, C.C., Sánchez-Chardi, A., Gabriel, S.I., Nadal, J., Viegas-Crespo, A.M., da Luz Mathias, M., 2007. How does the greater white-toothed shrew, *Crocidura russula*, responds to long-term heavy metal contamination? – A case study. Sci. Total Environ. 376 (1–3), 128–133. https://doi.org/10.1016/j.scitotenv.2007.01.061.
Mateo, R., Millán, J., Rodríguez-Estival, J., Camarero, P.R., Palomares, F., Ortiz-Santaliestra, M.E., 2012. Levels of organochlorine pesticides and polychlorinated biphenyls in the critically endangered Iberian lynx and other sympatric carnivores in Spain. Chemosphere 86 (7), 691–700. https://doi.org/10.1016/j.chemosphere.2011.10.037.
Mulder, G.J., Peters, W.H.M., Vermeulen, N.P., van Bladeren, P.J. (Eds.), 1996. Glutathione S-Transferases: Structure, Function and Clinical Implications. CRC Press.
Nardiello, V., Fidalgo, L.E., López-Beceiro, A., Bertero, A., Martínez-Morcillo, S., Míguez, M.P., Soler, F., Caloni, F., Pérez-López, M., 2019. Metal content in the liver, kidney, and feathers of Northern gannets, *Morus bassanus*, sampled on the Spanish coast. Environ. Sci. Pollut. Res. Int. 26 (19), 19646–19654. https://doi.org/10.1007/s11356-019-05356-y.
Newman, M.C., 2009. Fundamentals of Ecotoxicology, 3rd ed. CRC Press https://doi.org/10.1201/9781439883129.
Nores, C., 2007. *Erinaceus europaeus* Linnaeus, 1758. In: Palomo, L.J., Gisbert, J., Blanco, J.C. (Eds.), Atlas Y Libro Rojo De Los Mamíferos Terrestres De España. Dirección General Para la Biodiversidad -SECEM-SECEMU, Madrid, pp. 80–82.
Nóvoa-Muñoz, J.C., Pontevedra-Pombal, X., Martínez-Cortizas, A., García-Rodeja Gayoso, E., 2008. Mercury accumulation in upland acid forest ecosystems nearby a coal-fired power-plant in southwest Europe (Galicia, NW Spain). Sci. Total Environ. 394 (2–3), 303–312. https://doi.org/10.1016/j.scitotenv.2008.01.044.
Ossola, J.O., Groppa, M.D., Tomaro, M.L., 1997. Relationship between oxidative stress and heme oxygenase induction by copper sulfate. Arch. Biochem. Biophys. 337 (2), 332–337. https://doi.org/10.1006/abbi.1996.9788.
Pereira, R., Pereira, M.L., Ribeiro, R., Gonçalves, F., 2006. Tissues and hair residues and histopathology in wild rats (*Rattus rattus* L.) and Algerian mice (*Mus spretus* Lataste) from an abandoned mine area (Southeast Portugal). Environ. Pollut. (Barking, Essex: 1987) 139 (3), 561–575. https://doi.org/10.1016/j.envpol.2005.04.038.
Pérez-López, M., Rodríguez, F.S., Hernández-Moreno, D., Rigueira, L., Fidalgo, L.E., Beceiro, A.L., 2016. Bioaccumulation of cadmium, lead and zinc in liver and kidney

of red fox (*Vulpes vulpes*) from NW Spain: influence of gender and age. Toxicol. Environ. Chem. 98 (1), 109–117. https://doi.org/10.1080/02772248.2015.1107065.

Pinheiro, T., Rocha, P., Lopes, P.A., Viegas-Crespo, A.M., Santos, M.C., Mathias, M.L., 2001. Laboratory testing of biochemical effects of Mn, Fe and Se in the Algerian mouse (*Mus spretus*). Ecotoxicology and Environmental Restoration 4, 60–63.

Quina, A.S., Rodrigues, A.C.M., Soares, A.M.V.M., da Luz Mathias, M., Gravato, C., 2023. The use of muscle biomarkers for assessing physiological effects of heavy metal pollution in the greater white-toothed shrew (*Crocidura russula*). Sci. Total Environ. 888, 164162. https://doi.org/10.1016/j.scitotenv.2023.164162.

Rashed, M.N., Soltan, M.E., 2005. Animal hair as biological indicator for heavy metal pollution in urban and rural areas. Environ. Monit. Assess. 110 (1–3), 41–53. https://doi.org/10.1007/s10661-005-6288-8.

Rasmussen, S.L., Pertoldi, C., Roslev, P., Vorkamp, K., Nielsen, J.L., 2024. A Review of the occurrence of metals and xenobiotics in European hedgehogs (*Erinaceus europaeus*). Animals 14 (2), 232. https://doi.org/10.3390/ani14020232.

Rautio, A., Kunnasranta, M., Valtonen, A., Ikonen, M., Hyvärinen, H., Holopainen, I.J., Kukkonen, J.V., 2010. Sex, age, and tissue specific accumulation of eight metals, arsenic, and selenium in the European hedgehog (*Erinaceus europaeus*). Arch. Environ. Contam. Toxicol. 59 (4), 642–651. https://doi.org/10.1007/s00244-010-9503-8.

Recknagel, R.O., Gelende, E.A.Jr., Waller, R.L., Lowrey, K., 1982. Lipid peroxidation: biochemistry. Measurement and significance in liver cell injury. In: Plaa, G.L., Hewitt, W.R. (Eds.), Toxicology of the Liver. Raven Press, New York, pp. 213–241.

Regoli, F., Giuliani, M.E., Benedetti, M., Arukwe, A., 2011. Molecular and biochemical biomarkers in environmental monitoring: a comparison of biotransformation and antioxidant defense systems in multiple tissues. Aquat. Toxicol. 105 (3–4 Suppl. l), 56–66. https://doi.org/10.1016/j.aquatox.2011.06.014.

Reynolds, K.D., Schwarz, M.S., McFarland, C.A., McBride, T., Adair, B., Strauss, R.E., Cobb, G.P., Hooper, M.J., McMurry, S.T., 2006. Northern pocket gophers (*Thomomys talpoides*) as biomonitors of environmental metal contamination. Environ. Toxicol. Chem. 25 (2), 458–469. https://doi.org/10.1897/05-130r1.1.

Roméo, M., Giambérini, L., Amiard-Triquet, C., Amiard, J., 2013. History of biomarkers. Ecological Biomarkers, Indicators of Ecotoxicological Effects. CRC Press Taylor and Francis Group, Boca Raton London, New York. https://doi.org/10.1201/b13036-6.

Ruiz, S.R., Eeva, T., Kanerva, M., Blomberg, A., Lilley, T.M., 2019. Metal and metalloid exposure and oxidative status in free-living individuals of *Myotis daubentonii*. Ecotoxicol. Environ. Saf. 169, 93–102. https://doi.org/10.1016/j.ecoenv.2018.10.083.

Sánchez-Chardi, A., Marques, C.C., Gabriel, S.I., Capela-Silva, F., Cabrita, A.S., López-Fuster, M.J., Nadal, J., Mathias, M.L., 2008. Haematology, genotoxicity, enzymatic activity and histopathology as biomarkers of metal pollution in the shrew *Crocidura russula*. Environmental Pollution (Barking, Essex: 1987) 156 (3), 1332–1339. https://doi.org/10.1016/j.envpol.2008.02.026.

Sheffy, T.B., St Amant, J.R., 1982. Mercury burdens in furbearers in Wisconsin. J. Wildl. Manag. 46 (4), 1117–1120. https://doi.org/10.2307/3808255.

Shore, R.F., Douben, P.E., 1994. The ecotoxicological significance of cadmium intake and residues in terrestrial small mammals. Ecotoxicol. Environ. Saf. 29 (1), 101–112. https://doi.org/10.1016/0147-6513(94)90035-3.

Shore, R.F., Pereira, M.G., Walker, L.A., Thompson, D.R., 2011. Mercury in nonmarine birds and mammals. In: Beyer, W.N., Meador, J.P. (Eds.), Environmental Contaminants in Biota. Interpreting Tissue Concentrations. CRC Press, New York, pp. 609–624. https://doi.org/10.1201/b10598.

Sinaei, M., Zare, R., 2019. Polycyclic aromatic hydrocarbons (PAHs) and some biomarkers in the green sea turtles (Chelonia mydas). Mar. Pollut. Bull. 146, 336–342. https://doi.org/10.1016/j.marpolbul.2019.06.024.

Sonne, C., Siebert, U., Gonnsen, K., Desforges, J.P., Eulaers, I., Persson, S., Roos, A., Bäcklin, B.M., Kauhala, K., Tange Olsen, M., Harding, K.C., Treu, G., Galatius, A., Andersen-Ranberg, E., Gross, S., Lakemeyer, J., Lehnert, K., Lam, S.S., Peng, W., Dietz, R., 2020. Health effects from contaminant exposure in Baltic Sea birds and marine mammals: a review. Environ. Int. 139, 105725. https://doi.org/10.1016/j.envint.2020.105725.

Sparling, D.W., 2016. Ecotoxicology Essentials: Environmental Contaminants and their Biological Effects on Animals and Plants. Academic Press.

Squadrone, S., Robetto, S., Orusa, R., Griglione, A., Falsetti, S., Paola, B., Abete, M.C., 2022. Wildlife hair as bioindicators of metal exposure. Biol. Trace Elem. Res. 200 (12), 5073–5080. https://doi.org/10.1007/s12011-021-03074-6.

Stewart, J.M., Woods, A.K., Blakely, J.A., 2005. Maximal enzyme activities, and myoglobin and glutathione concentrations in heart, liver and skeletal muscle of the Northern short-tailed shrew (Blarina brevicauda; Insectivora: soricidae). Comp. Biochem. Physiol. B Biochem. Mol. Biol. 141 (3), 267–273. https://doi.org/10.1016/j.cbpc.2005.02.016.

Swiergosz-Kowalewska, R., Bednarska, A., Kafel, A., 2006. Glutathione levels and enzyme activity in the tissues of bank vole *Clethrionomys glareolus* chronically exposed to a mixture of metal contaminants. Chemosphere 65 (6), 963–974. https://doi.org/10.1016/j.chemosphere.2006.03.040.

Talmage, S.S., Walton, B.T., 1993. Food chain transfer and potential renal toxicity of mercury to small mammals at a contaminated terrestrial field site. Ecotoxicology (Lond.) 2 (4), 243–256. https://doi.org/10.1007/BF00368533.

Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K., Sutton, D.J., 2012. Heavy metal toxicity and the environment. In: Luch, A. (Ed.), Molecular, Clinical and Environmental Toxicology. Springer, Basel, pp. 133–164. https://doi.org/10.1007/978-3-7643-8340-4_6.

Tête, N., Afonso, E., Crini, N., Drouhot, S., Prudent, A.S., Scheifler, R., 2014. Hair as a noninvasive tool for risk assessment: do the concentrations of cadmium and lead in the hair of wood mice (*Apodemus sylvaticus*) reflect internal concentrations? Ecotoxicol. Environ. Saf. 108, 233–241. https://doi.org/10.1016/j.ecoenv.2014.07.010.

Tomza-Marciniak, A., Marciniak, A., Pilarczyk, B., Drozd, R., Ligocki, M., Prokulewicz, A., 2014. Wild boar (*Sus scrofa*) as a bioindicator of organochlorine compound contamination in terrestrial ecosystems of West Pomerania Province, NW Poland. Environ. Monit. Assess. 186 (1), 229–238. https://doi.org/10.1007/s10661-013-3368-z.

Treu, G., Krone, O., Unnsteinsdóttir, E.R., Greenwood, A.D., Czirják, G.Á., 2018. Correlations between hair and tissue mercury concentrations in Icelandic arctic foxes (*Vulpes lagopus*). Sci. Total Environ. 619–620, 1589–1598. https://doi.org/10.1016/j.scitotenv.2017.10.143.

Valverde, I., Defosseux, I., Le Bot, T., Jouvion, G., Le Barzic, C., Arné, P., Gasparini, J., 2024. Effect of urbanization on the trace element concentrations in the kidney, liver and spines of the European hedgehog (*Erinaceus europaeus*). Sci. Total Environ. 914, 169944. https://doi.org/10.1016/j.scitotenv.2024.169944.

Vermeulen, F., D'Havé, H., Mubiana, V.K., Van den Brink, N.W., Blust, R., Bervoets, L., De Coen, W., 2009. Relevance of hair and spines of the European hedgehog (*Erinaceus europaeus*) as biomonitoring tissues for arsenic and metals in relation to blood. Sci. Total Environ. 407 (5), 1775–1783. https://doi.org/10.1016/j.scitotenv.2008.10.039.

Vermeulen, F., Covaci, A., D'Havé, H., Van den Brink, N.W., Blust, R., De Coen, W., Bervoets, L., 2010. Accumulation of background levels of persistent organochlorine and organobromine pollutants through the soil-earthworm-hedgehog food chain. Environ. Int. 36 (7), 721–727. https://doi.org/10.1016/j.envint.2010.05.006.

Viegas-Crespo, A.M., Lopes, P.A., Pinheiro, M.T., Santos, M.C., Rodrigues, P.D., Nunes, A.C., Marques, C., Mathias, M.L., 2003. Hepatic elemental contents and antioxidant enzyme activities in Algerian mice (*Mus spretus*) inhabiting a mine area in central Portugal. Sci. Total Environ. 311 (1–3), 101–109. https://doi.org/10.1016/S0048-9697(03)00136-0.

Vieira, L.R., Sousa, A., Frasco, M.F., Lima, I., Morgado, F., Guilhermino, L., 2008. Acute effects of benzo[a]pyrene, anthracene and a fuel oil on biomarkers of the common goby *Pomatoschistus microps* (Teleostei, Gobiidae). Sci. Total Environ. 395 (2–3), 87–100. https://doi.org/10.1016/j.scitotenv.2008.01.052.

Walker, C.H., 2001. Organic Pollutants. an Ecotoxicological Perspective. Taylor and Francis, London, UK.

Walker, C.H., Sibly, R.M., Sibly, R.M., Peakall, D.B., 2012. Principles of Ecotoxicology, fourth ed. CRC Press. https://doi.org/10.1201/b11767.

Walker, K., Vallero, D.A., Lewis, R.G., 1999. Factors influencing the distribution of lindane and other hexachlorocyclohexanes in the environment. Environ. Sci. Technol. 33 (24), 4373–4378.

Weber, K., Goerke, H., 2003. Persistent organic pollutants (POPs) in antarctic fish: levels, patterns, changes. Chemosphere 53 (6), 667–678. https://doi.org/10.1016/S0045-6535(03)00551-4.

Wijnhoven, S., Leuven, R.S., van der Velde, G., Jungheim, G., Koelemij, E.I., de Vries, F.T., Eijsackers, H.J., Smits, A.J., 2007. Heavy-metal concentrations in small mammals from a diffusely polluted floodplain: importance of species- and location-specific characteristics. Arch. Environ. Contam. Toxicol. 52 (4), 603–613. https://doi.org/10.1007/s00244-006-0124-1.

Willett, K.L., Ulrich, E.M., Hites, R.A., 1998. Differential toxicity and environmental fates of hexachlorocyclohexane isomers. Environ. Sci. Technol. 32 (15), 2197–2207.

Wobeser, G., Nielsen, N.O., Schiefer, B., 1976. Mercury and Mink. II. Experimental methyl mercury intoxication. Can. J. Comp. Med.: Rev. Can. Med. Comp. 40 (1), 34–45.

Wren, C.D., 1986. Mammals as biological monitors of environmental metal levels. Environ. Monit. Assess. 6 (2), 127–144. https://doi.org/10.1007/BF00395625.