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Metal and metalloid concentrations in wild mammals from SW Europe: European hedgehog (*Erinaceus europaeus*) and badger (*Meles meles*)

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1 **Metal and metalloid concentrations in wild mammals from SW Europe: European**  
2 **hedgehog (*Erinaceus europaeus*) and badger (*Meles meles*)**

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19 Abstract

20 In recent years, there have been increasing ecological and global concerns associated to  
21 Potentially Toxic Elements (PTEs). Thus, the relevance of wild mammals as biomonitors  
22 has been globally recognised. In the present study, Cd, Pb, Hg, Zn and As concentrations  
23 were quantified in European hedgehog and badger inhabiting SW Europe, and cumulative  
24 trends in relation to age and sex were evaluated. Liver and kidney samples were collected,  
25 mineralised and PTE content was determined by ICP-MS. Zn was the most abundant  
26 element quantified in both organs (239 and 89.8 mg kg<sup>-1</sup> for hedgehogs and 179 and 164  
27 mg kg<sup>-1</sup> dw for badgers). In hedgehogs, very high Hg concentration were quantified (4.35  
28 and 15.5 mg kg<sup>-1</sup> dw in liver and kidney), and Cd was the most abundant for badgers (4.70  
29 and 7.61 mg kg<sup>-1</sup> dw in liver and kidney). Positive correlations were observed for the  
30 concentrations of PTE in the organs of both species. Age-dependence increased only Cd  
31 concentration, with levels in adult kidneys being significantly higher. In this study,  
32 European hedgehog and badger were used as biomonitors for the determination of PTEs  
33 to provide current reference values in relatively non-polluted areas of SW Europe, and to  
34 enhance the use of these species for future ecotoxicological studies.

35 **Keywords:** Biomonitoring, potentially toxic elements, European hedgehog, European  
36 badger, liver, kidney.

## 38 1. Introduction

39 Among the environmental pollutants released into ecosystems, Potentially Toxic  
40 Elements (PTEs), otherwise known as heavy metals, are highly relevant and a matter of  
41 environmental and public health concern in the last decades. According to Ali & Khan  
42 (2018), “heavy metals are naturally occurring metals having atomic number greater than  
43 20 and an elemental density greater than  $5 \text{ g cm}^{-3}$ ”. Despite their natural and ubiquitous  
44 presence in the environment, they frequently occur at high concentrations as a result of  
45 human activities such as mining, farming, or industrial processing (Walker et al., 2012;  
46 Gall et al., 2015; Raj & Maiti, 2020). Among PTEs, some of these metals, such as zinc  
47 (Zn), are essential elements which can mediate biological functions (i.e., metabolic  
48 reactions, the synthesis of structural proteins, hormones, or enzymes), while other metals,  
49 such as cadmium (Cd), lead (Pb), mercury (Hg), or the metalloid arsenic (As), as non-  
50 essential elements, they are not involved in any organic functions (NRC, 2005;  
51 Kalisińska, 2019; Ali & Khan, 2019). When time of exposure and concentrations are  
52 exceeded, they constitute a potential health risk and a serious threat for the ecosystem and  
53 humans (Boening, 2000; Kosik-Bogacka & Łanocha-Arendarczyk, 2019; Tomza-  
54 Marciniak et al., 2019; Charkiewicz et al., 2020; Osuna-Martínez et al., 2021). In fact, the  
55 risk presented by these elements is mainly a result of sub-lethal effects (carcinogenicity,  
56 nephrotoxicity, mutagenicity, teratogenicity and neurotoxicity, as well as endocrine  
57 disruption), and the substance priority list recently published by the Agency for Toxic  
58 Substances and Disease Registry (ATSDR, 2022) has ranked As, Pb and Hg as the top  
59 three most hazardous substances, closely followed below by Cd. From an  
60 ecotoxicological perspective, the prevalence of these contaminants throughout the food  
61 chain, from lower to upper trophic levels, has been extensively studied (Hernández-  
62 Moreno et al., 2013; Dibbern et al., 2021; Yan et al., 2021; Zhang et al., 2021; Kalisińska  
63 & Łanocha-Arendarczyk, 2023). This has even led to the processed of bioaccumulation  
64 and biomagnification in wildlife (Chormare & Kumar, 2022). Nevertheless, the  
65 accumulation and transfer of these pollutants are dependent on species, metal(loid), and  
66 toxicokinetic. In a large amount of biomonitoring studies, the determination and  
67 quantification of these elements has primarily been carried out in soft tissues such as liver  
68 and kidney. These organs are of great relevance as they are involved in detoxification  
69 mechanisms and can therefore be used to obtain insights into the effects of short and long-  
70 term metal exposure (Kalisińska, 2019; Jota Baptista et al., 2022a). However,  
71 bioaccumulation trends depend on many factors, for example biological factors such as  
72 age, size, and sex, or external factors, including the degree of pollution of the site, diet,  
73 seasonal variation, or environmental factors (soil, pH, and redox conditions) (Talmage  
74 and Walton, 1991; Peakall and Burger, 2003; Burger, 2007; Brand et al., 2020). It  
75 therefore makes it difficult to interpret the results. When considering biological factors,  
76 multiple studies have observed a positive correlation between age and bioaccumulation  
77 rate, finding PTEs such as Cd or Pb in increasing concentrations with age in wild  
78 mammals (Komarnicki, 2000; Sánchez-Chardi & Nadal, 2007a; Pérez-López, et al.,  
79 2016). However, the influence of sex on the accumulation of these elements  
80 abovementioned, due to a lack of understanding, is not clear yet as a result of the  
81 differences in hormonal and reproductive state, gene expression and metabolic rate  
82 between males and females (Burger et al., 2007). In small and medium mammal, some

83 studies have shown higher PTEs concentration in males than in females (Beernaert et al.,  
84 2007; Zarrintab and Mirzaei, 2017), vice versa (Komarnicki, 2000; Scheirs et al., 2006;  
85 Millán et al., 2008; Fritsch et al., 2010; Jota Baptista et al., 2023) or no differences  
86 (González et al., 2008; Rautio et al., 2010; Pérez-López, et al., 2016).

87 Owing to their wide distribution, and food preferences, wild mammals are largely  
88 exposed to these chemical pollutants. Plenty of studies have shown their important role  
89 as bioindicators when considering the assessment of pollutants, including PTEs  
90 (Kalisińska et al., 2019). More specifically, small and medium-sized mammals have an  
91 elevated metabolic rate, making them highly sensitive to metal(loid)s accumulation. They  
92 are often regarded as representative prey items for top predators making their exposure to  
93 pollutants a major pathway for the entry of these chemicals into food chains. Thus, they  
94 have been considered as potential biomonitors of environmental contamination (Talmage  
95 & Walton, 1991). When considering terrestrial ecosystems, this approach is common  
96 worldwide, and the use of mammals has proven to be suitable for assessing environmental  
97 and biological predictors of PTEs exposure. For example, erinaceans such as the  
98 European hedgehog (*Erinaceus europaeus*), or the mustelid European badger (*Meles  
99 meles*), with a global distribution and key position in several trophic levels, have been  
100 used in ecotoxicological studies related to Cd, As, Pb, Hg, or Zn (Hernández et al., 1985;  
101 Van Den Brink & Ma, 1998; D'Havé et al., 2005 and 2006; Alleva et al., 2006; Millán et  
102 al., 2008; Rautio et al., 2010; Bilandžić et al., 2012; Ozimec et al., 2015 and 2017;  
103 Heimstad et al., 2019; Kalisińska et al., 2021; Mullineaux et al., 2021; Jota Baptista et al.,  
104 2023).

105 Some previous studies have suggested the relevance of both species as possible  
106 bioindicators due to their high exposure to PTEs. Hedgehogs are insectivores that prey  
107 on invertebrates (earthworms, slugs, earwigs, beetles, among others) (Shore, 1995;  
108 D'Havé et al., 2006). It can occupy several types of habitats, such as natural, rural and  
109 urban spaces, presents a limited home range, long life span, and its migration rated is  
110 reduced (Jota Baptista et al., 2021). Besides, badgers are mesocarnivores whose diet  
111 consists of 50-70% small rodents including hedgehogs, the rest feeding on invertebrates,  
112 grasses, or reptiles. Its habitat preference consists of a mixture of agricultural landscape,  
113 deciduous forest, or hedgerows. Moreover, both rely heavily on the soil for their  
114 grooming and burrowing habits (Jota Baptista et al., 2021; Mullineaux et al., 2021).  
115 Increased bioaccessibility can therefore be expected to affect PTE accumulation in  
116 hedgehog and badger, and the higher risk of exposure to those make them important  
117 candidates as biomonitors.

118 Both species are evaluated by the International Union for Conservation of Nature (IUCN)  
119 at the level of Least Concern (LC). Nonetheless, the badger is included in Annex III  
120 (Protected fauna species) of the “Convention on the conservation of European wildlife  
121 and natural habitats”, Bern Convention (ETS 104, 1979). In the Iberian Peninsula, they  
122 are found over a large extend and may constitute the prey for certain species, several of  
123 which are threatened or endangered such as Iberian wolf (*Canis lupus signatus*), lynx  
124 (*Lynx pardinus*), or brown bear (*Ursus arctos arctos*). The assessment of environmental  
125 contamination by PTEs through adequate monitoring programmes is therefore essential  
126 to preserve the value and biodiversity of Mediterranean ecosystems and to assess the need  
127 for possible corrective measures (Jota Baptista et al., 2022b).

128 For our knowledge, only three biomonitoring studies on these two species have been  
129 reported in this region, focussing exclusively on the Protected National Park of Doñana  
130 and Portugal. These studies provide metal(loid)s accumulation only in the hepatic tissue,  
131 leaving a gap in their status in other target organs such as kidney (Hernández et al., 1985;  
132 Millán et al., 2008; Jota Baptista et al., 2022b and 2023). Thus, little is known about the  
133 status of these two species in the rest of the Iberian Peninsula, and the extent to which  
134 they are affected by PTEs contamination. It should be highlight that no current references  
135 for Hg values in these two terrestrial mammals are available. The purposes of this study  
136 were therefore (i) to determine the current exposure of European hedgehog and European  
137 badger to PTEs, specifically Hg, Cd, Pb, As and Zn, from areas of NW Spain in order to  
138 fill the gap between current and nephritic values; (ii) to assess age- and sex-specific  
139 bioaccumulation trends in the concentrations of these elements; and (iii) to establish the  
140 current pollution degree cause by these elements in the terrestrial ecosystems of the  
141 Iberian Peninsula.

## 142 **2. Material and Methods**

143

### 144 *2.1. Sample collection*

145 European hedgehogs ( $n = 43$ ; body mass -BM- = 660-1210 g) and European badgers ( $n$   
146 = 20; BM = 4218-11000 g) were collected during the period 2020–2022 in Galicia (NW  
147 Spain) (Fig.1). All specimens came from different locations, mainly from agricultural and  
148 forestry areas with sparse human population. The collected specimens were found  
149 recently dead or had died naturally (i.e., via infectious diseases), or because of road traffic  
150 accidents. In all cases, these animals were referred to the Wildlife Recovery Centres in  
151 this region, and only animals that had been kept at the centre for less than 5 days before  
152 dying were considered for this study. The diet provided during this period was supposed  
153 to be free of environmental contaminants. All dead specimens were frozen and stored at  
154  $-80^{\circ}\text{C}$  until sample preparation for chemical analysis, which took place at the Veterinary  
155 Faculty. The animals were sexed (hedgehog: 24 males, 16 females, 3; badger: 9 males,  
156 11 females), and the age of the animal was estimated by the general size, the animal's  
157 dental development and the degree of sexual maturity (hedgehog: 31 adults, 9 young;  
158 badger: 4 young, 16 adults), as previously established for different wildlife species  
159 (Pérez-López et al., 2016). Regarding hedgehogs, the sex and age of 3 individual couldn't  
160 be determined. From each animal, internal organ samples were taken, placed in individual  
161 plastic bags and stored at  $-80^{\circ}\text{C}$ . Sample preparation was carried out taking care to avoid  
162 metal contamination and losses: plastic scalpels and surgical tools were cleaned or  
163 substituted for each animal, and disposable nitrile gloves were used. The working surface  
164 was also cleaned after each operation. Samples were handled in a way to avoid any  
165 contact with metallic external surfaces (Hernández-Moreno et al., 2013).

### 166 *2.2. Metal determination*

167 About 3-4 g of liver and kidney samples were dried in an oven for 72 h at  $65^{\circ}\text{C}$ . The  
168 PTEs levels were analysed at the Elemental and Molecular Laboratory of the Research  
169 Support Service (SAIUEX, accredited by ISO 9001:2008; University of Extremadura).  
170 0.7 g of liver and kidney was taken for the quantification of PTEs. Sample digestion was  
171 carried out in closed Teflon PTFE flaks, following the protocol previously optimised in

172 the laboratory for wildlife samples and using a microwave automatic digester (Nardiello  
173 et al., 2019). The concentrations of PTEs (Pb, Cd, Hg, As, Zn) were determined using an  
174 ICP-MS (7900) equipped with an autosampler (Agilent Technologies, Santa Clara, CA,  
175 USA). For all metals, the limit of detection (LOD) was 0.003 mg kg<sup>-1</sup>, and the limit of  
176 quantifications (LOQ) was 0.005 mg kg<sup>-1</sup>. All samples were run in batches that included  
177 blank and initial calibration standards. A certified sample of lyophilised bovine liver was  
178 used as reference material for quality control (BCR®, ref 185R, Community Bureau of  
179 Reference, EU). The recovery yields ranged from 84% for Cd to 96% for Zn, and the  
180 coefficients of variation were always below 10%. Final concentrations were expressed in  
181 milligrams per kilograms (mg kg<sup>-1</sup>) in dry weight (dw), since dry values are considered  
182 to be more reliable and consistent compared with wet weight values (Adrian and Stevens,  
183 1979). To compare PTEs concentrations measured in this study with earlier published  
184 data, other author's results reported on wet weigh (ww) were recalculated using an  
185 average dw value of 32% for liver and 25% for kidney, respectively (Pérez-López et al.,  
186 2016).

### 187 2.3. Statistical analysis

188 All data were analysed using GraphPad Prism 9.0.2 (GraphPad Software Inc., La Jolla,  
189 CA, USA). Final concentrations were expressed as mean ± standard error of mean (SEM),  
190 standard deviation (SD), median and range. Data normality was assessed by the Shapiro-  
191 Wilk test. As the data were not normally distributed, the non-parametric Kruskal-Wallis  
192 test was used to analyse the accumulation of inorganic elements in both organs. The  
193 Mann-Whitney *U* test was applied to analyse the influence of sex and age. To assess the  
194 different correlations among metal levels and tissues, Spearman's test was used.  
195 Interpretation of the correlation strength was made based on Kalisińska et al. (2023), with  
196 the following strengths: 0.8–1 very strong; 0.6–0.79 strong; 0.4–0.59 moderate, 0.2–0.39  
197 weak, 0–0.19 very weak. The significance level was established as  $p < 0.05$ .

198 Part of statistical analyses was performed using R (R Core Foundation, 2023). The  
199 Principal Component Analysis (PCA) was carried out using the R package FactoMineR  
200 (Lê et al., 2008) and plotted using ggplot2 (Wickham, 2016) and ggbiplot  
201 (<https://github.com/vqv/ggbiplot>). Rows (observations) with missing entries were  
202 dropped; remaining values were log-transformed and the PCA was centred. Finally, the  
203 correlations were investigated using the corrplot (Wei and Simko, 2021) R package.

## 204 3. Results and Discussion

205 In the present biomonitoring study, the concentrations of some PTEs (Pb, Cd, Hg, As,  
206 Zn) in European hedgehogs (*Erinaceus europaeus*) and European badgers (*Meles meles*)  
207 from NW Spain were assessed. The main statistical parameters related to metal  
208 concentrations (in terms of dw) in liver and kidney from both species are shown in Table  
209 1. Based on this information, the studied elements could be detected in all samples.

### 210 (Table 1)

#### 211 3.1. Liver and kidney global concentrations

212 Generally, PTEs concentrations were higher in hedgehog than in badger samples, with  
213 the exception of Cd. Overall, Zn was the most abundant element in the two species in

214 both hepatic and renal tissues (mean: 239 and 89.8 mg kg<sup>-1</sup> dw, respectively, for  
215 hedgehogs; 179 and 164 mg kg<sup>-1</sup> dw, for badger), being mainly stored in the hepatic tissue  
216 only for hedgehogs ( $p < 0.0001$ ). Although Zn is excreted through external tissues such as  
217 hair or spines (Vermeulen et al., 2009; Hernández-Moreno et al., 2013), the relevance of  
218 soft tissues in detoxification and storage processes makes the study of such internal tissues  
219 highly relevant. Zn is an essential element and takes part in a wide variety of physiological  
220 functions, but above the threshold values of 465 and 274 mg kg<sup>-1</sup> dw for liver and kidney,  
221 respectively, in mammals, Zn becomes toxic, with several negative effects on the  
222 organism (Eisler, 1993). The mean values determined in this study were lower for liver  
223 and kidney than the threshold values. Nevertheless, in badger samples, maximum Zn  
224 concentrations exceeded those values for liver (537.2 mg kg<sup>-1</sup> dw) and even three times  
225 for kidney (845.7 mg kg<sup>-1</sup> dw). Kosik-Bogaacka & Lanocha-Arendarzyk (2019) reported  
226 the optimal Zn level in the liver and kidney of mustelid mink ranging between 83-333,  
227 and 90-100 mg kg<sup>-1</sup> dw. We do not therefore dismiss the possibility that high Zn  
228 concentrations could be related to the death of these two individuals, but it may also be  
229 due to a physiological origin, such as stress, or even a response to other pollutants.

230 When data obtained in the present study are compared with the scientific literature, it can  
231 be concluded that Zn concentrations in hedgehog organs from NW Spain are in the range  
232 previously reported in liver (52.14 to 371 mg kg<sup>-1</sup> dw) and kidney (103.2 to 135 mg kg<sup>-1</sup>  
233 dw), respectively (Table 2). Our results were similar to those obtained in a hedgehog  
234 population living in unpolluted area from Finland (228.9 and 103.2 mg kg<sup>-1</sup> dw) (Rautio  
235 et al., 2010). When comparing our results with previous studies performed on  
236 insectivorous small mammals in our study area, Zn levels in liver were slightly higher  
237 than those reported in the liver of wood mice (*Apodemus sylvaticus*) (158-216 mg kg<sup>-1</sup>  
238 dw), Spanish shrew (*Sorex granarius*) (109 mg kg<sup>-1</sup> dw), and greater white-toothed  
239 shrews (*Crocidura russula*) (229 mg kg<sup>-1</sup> dw), while kidney levels were lower than the  
240 range quantified in these three small mammals (96.9 to 168, 144 and 205 mg kg<sup>-1</sup> dw)  
241 (González et al., 2008). A similar reasoning could be made when comparing with the  
242 results of studies carried out with different species from the Iberian Peninsula, such as  
243 greater white-toothed shrews (167.26-232.26 mg kg<sup>-1</sup> dw) (Sánchez-Chardi et al., 2007a,  
244 2007b). The increase of Zn in liver can be attributed to physiological regulations such as  
245 protection and/or detoxification, and elevated concentrations in mammals may be a  
246 consequence of disrupted metabolism or high metal intakes. Regarding badger samples,  
247 our own results exceeded the range for liver (<144.3 mg kg<sup>-1</sup> dw) and kidney (<156.89  
248 mg kg<sup>-1</sup> dw) reported in earlier studies (Table 2). In addition, liver and kidney  
249 concentrations were greater than those measured in other terrestrial wild mammals living  
250 in the same study area, such as Iberian wolf (*Canis lupus signatus*) (77.9 and 25.81 mg  
251 kg<sup>-1</sup> dw) (Hernández-Moreno et al., 2013), red fox (*Vulpes vulpes*) (77 and 17 mg kg<sup>-1</sup>  
252 dw) (Pérez-López et al., 2016) and wild boar (*Sus scrofa*) (56.9 mg kg<sup>-1</sup> dw for liver)  
253 (Neila et al., 2017). Although pollution episodes have not been reported in the sampling  
254 area, high levels of this metal in both species may not just be caused by bioaccumulation  
255 from plant and prey intake, but can also be due to several natural factors such as  
256 mineralogical soil composition, pH, or redox condition, or anthropogenic factor (local  
257 point source). In recent years, 57% of Zn emissions from point sources in Galicia come  
258 from thermal power plants, and small industrial developments (Giráldez et al., 2022).

259 **(Table 2)**



260 Regarding non-essential metals, and when considering mean values, Hg was the main  
261 element accumulated in European hedgehogs in both organs (4.35 and 15.5 mg kg<sup>-1</sup> dw  
262 for liver and kidney samples, respectively), being mostly stored in renal tissue  
263 ( $p < 0.0001$ ). However, when considering the badger tissues (0.77 and 1.16 mg kg<sup>-1</sup> dw for  
264 liver and kidney), no significant differences were observed between organs ( $p > 0.05$ ). It  
265 must be noted that Hg, as a potentially toxic element, is released into the natural  
266 environment via industrial and agricultural activities, being highly present in many  
267 ecosystems (Boening, 2000). In this research, we measured total Hg, but the most toxic  
268 and bioavailable form of this element is methylmercury (MeHg), which under the right  
269 conditions is methylated and therefore able to biomagnifies its concentration with  
270 increasing trophic position (Peterson et al., 2021).

271 The mean Hg levels detected in hedgehogs were above the established threshold value  
272 for serious health effects (1.1 mg kg<sup>-1</sup>) in liver and kidney, but below the range associated  
273 with poisoning and death in wild mammals (25-30 mg kg<sup>-1</sup> for both organs) (Shore et al.,  
274 2011). Nevertheless, 6 hedgehogs were above the toxic threshold by far. The lowest  
275 observed adverse effect level (LOAEL) established for terrestrial mammals (18.1 mg kg<sup>-1</sup>  
276 in liver tissue from mink in an experimental setting) reports clinical signs and death, and  
277 the hepatic Hg concentration in two hedgehogs exceeded it (Wobeser et al., 1975). These  
278 isolated cases of excessive Hg concentrations in these organs of hedgehogs could be  
279 attributed to their individual characteristics, such as problems in metal metabolism and  
280 excretion, or exposure to extreme conditions of metal pollution. When compared with  
281 similar studies, Hg concentrations detected in this study were markedly higher than those  
282 measured in hedgehog liver samples in Italy (<0.2 mg kg<sup>-1</sup> dw) (Alleva et al., 2006) or in  
283 liver and kidney of Brandt hedgehogs (*Paraechinus hypomelas*) from Iran (0.066 and  
284 0.15 mg kg<sup>-1</sup> dw) (Dahmardeh Behrooz et al., 2022). To our knowledge, no information  
285 is available about the levels of Hg in terrestrial wild mammals from our reference area,  
286 as most studies have focused on Galician estuaries and coast (De La Peña et al., 2019).  
287 However, and taking in consideration that hedgehogs are often linked to urban  
288 landscapes, sharing the same environment as pets such as dogs, comparative studies with  
289 this terrestrial species are adequate. Likewise, Hg concentrations measured in hedgehogs  
290 were markedly higher than the results reported by López-Alonso et al. (2007) in dog liver  
291 and kidney from this region, which in all cases were below 0.25 mg kg<sup>-1</sup> dw. According  
292 to Eisler (2004), it seems that size is related to resistance to Hg pollution, and elevated  
293 Hg levels in hedgehogs could be associated to dietary and behavioural habits increasing  
294 the degree of exposure to Hg. But the possibility that these concentrations have been  
295 influenced by a local point or diffuse source of pollution should not be excluded, and  
296 further analyse of these results including spatial trends must be performed. In 2019, the  
297 Hg levels were above natural background levels, with industrial activity accounting for  
298 49% of Hg emission in Galicia (Giráldez et al., 2022).

299 The average Hg concentrations in the liver and kidney of badgers were 3 to 8 times higher  
300 than those obtained for the same species from different areas of Europe (ranging between  
301 0.09-0.55 mg kg<sup>-1</sup> dw) (Alleva et al., 2006; Millán et al., 2008; Bilandžić et al., 2012,  
302 Ozimec et al., 2017; Heimstad et al., 2019; Kalisińska et al., 2021). Only highlights the  
303 study by Bukovjan et al. (2014), where Hg levels quantified in this species from the Czech  
304 Republic were twice as high as in the present study (aprox. 1.53 and 2.68 mg kg<sup>-1</sup> dw in  
305 liver and kidney). In short, in some individuals, the concentration which cause serious

306 health effects was exceeded, however, mean concentrations determined in our study are  
307 in accordance with the normal values established for mustelids in liver and kidney (1.0  
308 and 0.2-0.7 mg kg<sup>-1</sup> ww) (Kalisíńska et al., 2021). Findings of elevated mercury (Hg)  
309 concentrations in the liver and kidneys of European hedgehogs in the NW Iberian  
310 Peninsula raise important concerns. They may indicate possible contamination of water  
311 and soil in the region. Identification of sources and assessment of water and soil quality  
312 are critical steps to address this concern.

313 For the current study, a significant difference was observed in Cd levels between kidney  
314 and hepatic tissues in both hedgehog and badger. Specifically higher Cd concentrations  
315 were detected in the kidney tissues, with values of 5.89 mg kg<sup>-1</sup> dw for hedgehogs and  
316 7.61 mg kg<sup>-1</sup> dw for badgers. The biological half-life of Cd lies in the range of about 25-  
317 30 years, and it primarily accumulates in the kidney, an organ often first affected by its  
318 toxicity (Cooke, 2011). Over time, when renal thresholds are surpassed, Cd can also  
319 accumulate in the liver (Goretti et al., 2018). Notably, detrimental effects on mammals  
320 have been reported at Cd concentrations exceeding 105 mg kg<sup>-1</sup> dw in renal tissue  
321 (Tomza-Marciniak et al., 2019). Still, the concentrations found in our study remain below  
322 this toxic threshold. A recent study developed by Jota Baptista et al. (2023) suggested  
323 that in the liver of small mammals with a predominant insectivorous diet, the average Cd  
324 concentration ranged from 0.2 to 1.5 mg kg<sup>-1</sup> dw. Our study found elevated levels,  
325 possibly indicating hedgehogs varied diet which might expose them to more potential  
326 sources of Cd (Jota Baptista et al., 2021). In comparison, badgers from NW Spain  
327 presented higher Cd concentration than those recorded in previous studies (from 0.017 to  
328 3.5 mg kg<sup>-1</sup> dw), but were consistent with kidney values reported in other region like  
329 Netherlands (Table 2). This discrepancy might highlight the influence of local  
330 anthropogenic activities such as mining, farming, and industrial processes on Cd levels  
331 in our study area. Interestingly, when our findings are contrasted with other wildlife  
332 studies from the same region badgers showed higher Cd concentrations than other  
333 omnivorous species such as wild boar, red fox and European wolf (Hernández-Moreno et  
334 al., 2013; Pérez-López et al., 2016; Neila et al., 2017). Therefore, the reason why the  
335 levels determined in badgers were higher than in hedgehogs may be due to food  
336 bioavailability which relies on seasonal variation. In the Mediterranean region,  
337 earthworms can be an important part of badger diets, particularly in areas with moist soils,  
338 such as northern Spain, which has a prominent Atlantic climate with consistent wetness.  
339 However, the presence of a high concentration of Cd in the environment makes  
340 earthworms a significant source of input into the local food web, as they readily  
341 bioaccumulate this element (Al Sayegh Petkovšek et al., 2014).

342 The mean Pb levels for kidney samples from hedgehogs and badgers were slightly similar  
343 (0.6731 and 0.4477 mg kg<sup>-1</sup> dw), whereas in the liver were markedly higher in hedgehogs  
344 than in badgers (1.660 and 0.3084 mg kg<sup>-1</sup> dw), indicating that Pb is significantly  
345 accumulated in the liver ( $p < 0.001$ ). Earthworms are one of the primary components of  
346 the hedgehog diet, which bioaccumulate significantly high levels of Pb and represent a  
347 potential pathway for Pb perpetuation in the trophic webs (Beyer et al., 2018). The kidney  
348 is a significant target organ and suitable bioindicator of Pb exposure, and the  
349 concentrations in soft tissues of mammals generally decrease from the kidney to the liver  
350 (Ma, 2011), which has been observed for the badger samples in this study. However, it  
351 should not be ignored that 90% of the total body burden of Pb is found in bone (Talmage

352 & Walton, 1991). In all cases, the risk of exposure to wild mammals to Pb present in soils  
353 depends on the bioavailability of Pb to plants and invertebrate soil organisms used as  
354 food.

355 Thus, Pb is highly present in the environment due to its widespread and long-term use by  
356 humans, with lead smelters and hunting being the major sources. In general, mean  
357 concentrations did not reach the threshold which shows clinical signs of poisoning (25–  
358 35 mg kg<sup>-1</sup> dw), nor that resulting in toxicosis in mammals (15 and 5 mg kg<sup>-1</sup> dw in kidney  
359 and liver), but just in a couple of individuals it was clearly exceeded (13.44 and 16.44 mg  
360 kg<sup>-1</sup> dw in liver) (Ma, 1996). Moreover, these mean concentrations are in the normal range  
361 established for mammals in liver and kidney (<3-4 mg kg<sup>-1</sup> ww) (Baranowska-Bosiacka  
362 et al., 2019). On the other hand, our results showed that 6 specimens of hedgehogs have  
363 hepatic concentration higher than the LOAELs levels in small mammals, defined in 2.7  
364 and 5.9 mg kg<sup>-1</sup> dw for liver and kidney metal burden, respectively (Shore and Douben,  
365 1994). Pb concentrations measured in the selected tissues of the European hedgehog were  
366 in the ranges quantified in other areas of Europe (aprox. 2.47 to 10.9 mg kg<sup>-1</sup> dw in liver,  
367 0.95 to 5.2 mg kg<sup>-1</sup> dw in kidney), and similar results were obtained in badgers (<10 mg  
368 kg<sup>-1</sup> dw in liver, 15 mg kg<sup>-1</sup> dw in kidney) (Table 2). When focusing on the Mediterranean  
369 region, especially in the natural park of Doñana, these results were slightly greater than  
370 hedgehogs from this area (1.46 mg kg<sup>-1</sup> dw in liver), but similar for badger (0.46 mg kg<sup>-1</sup>  
371 dw) (Hernández et al., 1985; Millán et al., 2008). We assume a hypothetical reason that  
372 in this protected park, where hunting is forbidden, hedgehogs are not as exposed to  
373 ingestion of Pb through hunting bullets as in our area. In addition, given that the corpses  
374 come from road accidents, it is also possible that the concentration has been partly  
375 influenced by the high-density traffic of combustion vehicles. Furthermore, our results  
376 were higher than other polluted areas measured in small mammals with insectivore diets,  
377 such as shrews, living surrounding of an old lead/zinc mine in southern Portugal (1.17  
378 mg kg<sup>-1</sup> dw in the liver) (Marques et al., 2007). In contrast, our results are not in  
379 accordance with Sánchez-Chardi et al. (2007a) who quantified 1.93 and 5.37 mg kg<sup>-1</sup> dw  
380 in the liver and kidney of shrews from free-pollutant area in NE Spain. When comparing  
381 with other mesocarnivores, Pb concentrations in badger were lower than those of the red  
382 fox from NW Spain (0.81 mg kg<sup>-1</sup> dw in the liver), or Egyptian mongoose and common  
383 genet from Southern Spain (1.753 and 0.652 mg kg<sup>-1</sup> dw in the liver) (Millán et al., 2008;  
384 Pérez-López et al., 2016). Pb quantification in bones could have been relevant to assess  
385 whether the presence of this metal in these two species is due to temporary or permanent  
386 exposure, however, limitations in collecting samples did not allow us to perform it.

387 Of the non-essential PTEs, As was the least quantified and no statically significant  
388 differences between the tissues were observed, suggesting that this metalloid is equally  
389 accumulated at low levels in the liver and kidney of European hedgehog (0.1941 and  
390 0.2040 mg kg<sup>-1</sup> dw,  $p>0.05$ ) and European badger (0.138 and 0.081 mg kg<sup>-1</sup> dw,  $p>0.05$ ).  
391 Despite the kidney being the major organ for As elimination from the body, previous  
392 studies have shown its preference to bioaccumulate in the liver (Binkowski et al., 2019),  
393 although the underlying mechanism is still unclear. Moreover, the As accumulation  
394 pattern in mammals varies among species, organs, and locations, rendering all  
395 biomonitoring studies with this PTE of high relevance. In this study, with no exception,  
396 the levels of As found for both tissue types and both species were below 3 mg kg<sup>-1</sup>, the  
397 limit considered as background and with no toxicological effect on living organisms

398 (Pereira et al., 2006). Furthermore, low levels of As were quantified in comparison to  
399 previous studies carried out in European hedgehogs. For instance, the observed values  
400 were four and three times lower than those found for hedgehog populations living in  
401 polluted areas in Flanders, Belgium (0.69 and 0.58 mg kg<sup>-1</sup> dw in liver and kidney), and  
402 two times lower than those for hedgehogs from non-polluted areas in Finland (0.45 and  
403 0.47 mg kg<sup>-1</sup> dw in liver and kidney) (D'Havé et al., 2006; Rautio et al., 2010).  
404 Nevertheless, it should be noted that our results are slightly greater than those obtained  
405 in the liver of hedgehogs from Portugal (0.13 mg kg<sup>-1</sup> dw). In addition, when compared  
406 with other small mammals such as wild rats (*Rattus rattus* L.) and Algerian mice (*Mus*  
407 *spretus*) living close to abandoned mine areas in the Iberian Peninsula, our results are also  
408 low (Pereira et al., 2006). Considering that the sampling area is unpolluted, low As  
409 concentrations are therefore to be expected. For the European badger, the results were  
410 within the range of those observed in previous studies (<1.2 mg kg<sup>-1</sup> dw in liver and  
411 kidney) (Table 2). In Spain, there has been limited research on this particular PTE in  
412 terrestrial wildlife. Notably, a study conducted by Millán et al. (2008) in badgers from  
413 the Doñana National Park did not detect this element. We propose that this discrepancy  
414 can be attributed to the use of a less precise method compared to the one used in our  
415 current research, however, differences in environmental factors should not be excluded  
416 either. As far as we know, no data about the levels of As in wild terrestrial mammals from  
417 our study area are available. Few studies have been mainly carried out in livestock and  
418 domestic animals, and our results were higher than those obtained by López-Alonso et al.  
419 (2007) in dogs from urban and rural areas of Spain (aprox. 0.039 and 0.06 mg kg<sup>-1</sup> dw).  
420 We hypothesised that the main reason for this difference may be that some of the badgers  
421 come from farming areas, where As has been widely used as a pesticide and fertiliser.  
422 The concentration of As in badgers samples could be related to behavioural habits, as  
423 they spend a large part of their time in their burrows, and may even be exposed to As-  
424 polluted soils as a consequence of spreading pesticides in adjacent sites.

425 Based on the results obtained and the average concentration of the elements quantified,  
426 these data can provide valuable information for wildlife management in the region.  
427 Elevated concentrations of Zn and Hg in these animals suggest the current problem of the  
428 health status of local ecosystems in NW Spain due to possible contamination. This could  
429 alert wildlife authorities and environmental managers to the need to investigate and  
430 address specific sources of contamination that could affect not only wildlife, but also  
431 human health, water and soil quality. Thus, these results indicate a significant concern in  
432 terms of environmental quality in northwest Spain. Zn and Hg are PTEs that can have  
433 detrimental effects on aquatic and terrestrial ecosystems. The accumulation of these  
434 metals in wild mammal tissues indicates a possible entry of contaminants into the local  
435 food chain. This could have implications for biodiversity, as chronic exposure to metals  
436 can affect the health and reproduction of local species. In addition, these data can be an  
437 early warning signal to identify specific geographic areas that may require more stringent  
438 environmental management. Finally, in terms of environmental risk assessment, the  
439 detection of elevated Hg concentrations in hedgehogs raises questions about exposure of  
440 other organisms in the region. This could lead to further investigations into the sources  
441 and routes of mercury exposure, as well as more detailed risk assessments to evaluate  
442 potential health and environmental impacts.

### 443 3.2. Correlation study

444 We observed a ‘very strong’ and ‘strong’ correlation between Hg stored in the liver and  
445 kidney of both species ( $r=0.8050$ ,  $p<0.0001$  for hedgehogs;  $r=0.7699$ ,  $p<0.0001$  for  
446 badger). Moreover, similar strong correlation between these organs was also observed for  
447 Pb in hedgehogs and badgers ( $r=0.6463$ ,  $p<0.0001$ ;  $r=0.6030$ ,  $p<0.005$ ) (Fig. 2).  
448 However, only in hedgehog, a positive very strong correlation between the levels of both  
449 organs was found for Cd ( $r=0.8786$ ,  $p<0.0001$ ), strong for As ( $r=0.6963$ ,  $p<0.0001$ ) and  
450 moderate for Zn ( $r=0.5594$ ,  $p<0.0005$ ). This association could be attributed to the high  
451 reabsorption rate and blood transfer, as well as similar detoxification dynamics between  
452 the liver and kidney (Boening, 2000; Cooke, 2011; Ma, 2011). These correlations have  
453 been observed in previous studies carried out in hedgehogs and badgers, and even other  
454 wild mammals (Petrović et al., 2014; Kalisińska et al., 2021; Dahmardeh Behrooz et al.,  
455 2022).

456 The interaction among the metals for each tissue was also assessed. A significant very  
457 strong correlation between Cd and Hg was observed in hedgehogs for liver ( $r=0.8023$ ,  
458  $p<0.0001$ ) and strong correlation in kidney samples ( $r=0.7932$ ,  $p<0.0001$ ) (Fig. 2). In  
459 liver samples, a positive moderate correlation between As and Cd for hedgehogs  
460 ( $r=0.5104$ ,  $p<0.01$ ) and strong for badgers ( $r=0.6434$ ,  $p<0.05$ ) was observed, as well as  
461 for As and Hg for badgers ( $r=0.6014$ ,  $p<0.05$ ). These results suggest that the liver (which  
462 plays a role in metal detoxification) is related to these correlations. The correlation  
463 between Cd and Hg has not been shown in hedgehogs before. However, this association  
464 is in agreement with that reported in previous biomonitoring studies. For example, these  
465 results were reported in white-toothed shrew or red fox from Spain (Sánchez-Chardi et  
466 al., 2007b; Millán et al., 2008) and in river otter (*Lutra lutra*) from Hungary (Lanszki et  
467 al., 2009). In our study, a positive moderate correlation was also observed between  
468 hepatic Zn and Pb in hedgehogs ( $r=0.5973$ ,  $p<0.0001$ ). According to a previous study,  
469 the bioaccumulation and toxicity of heavy metals depend on the interaction between  
470 essential and nonessential metals (Neila et al., 2017). Similar to our results, positive  
471 correlations have been found between hepatic Zn and Pb levels in mammals (Millán et  
472 al., 2008; Lanszki et al., 2009). These correlations, however, seem to differ between  
473 species and tissues, making it essential to obtain a better understanding of the relationship  
474 among trace elements, their toxicokinetics and metabolism.

475 Principal Component Analyse (PCA) was used to explore the existence of behaviour  
476 patterns in the samples and the possible correlations between variables, considering the  
477 biological factors assessed. Fig. 3 shows the plot of scores and loading of PCA obtained  
478 from the PTEs concentrations of the samples, being specified by species (Fig. 3A) and  
479 age (Fig. 3B). PCA provided the explanation of 55% of the data variance accumulated.  
480 Two principal components were extracted, the first component (PC1), which explains  
481 35.74% of total variation, stands the strong correlation between the levels of Hg in liver  
482 and kidney. The PC1 charge was negative for all metals, mainly Hg and Cd which were  
483 the variable with the highest negative loading. For other metals, a similar trend has been  
484 observed, with the exception of Zn in the kidney, which has a positive loading. This trend  
485 in the accumulation of Hg, as abovementioned, is due to the fact that both organs are  
486 involved in Hg detoxification, and from a biological point of view, both display a similar  
487 distribution pattern (Kalisińska, 2019). The second principal component (PC2), which  
488 explains 19.26% of the variation, reflects the correlation of As and Pb in both organs,

489 being the variables with the highest negative loadings for Pb and Zn, and the highest  
490 positive loading for As; Hg has no influence.

491 In the first PCA (Fig. 3A), there was a tendency to cluster the specifications by species,  
492 since the overlap between hedgehogs and badgers (circled) is the smallest of all. The  
493 Kruskal-Wallis test results or the species factor were consistent with these findings, since  
494 statistically significant differences were observed for PTEs ( $p<0.05$ ). Therefore, the  
495 differences may be due to the fact that they are two completely different species, and in  
496 each species the pattern of accumulation of PTEs will be different. Badgers tend to follow  
497 the pattern established by Zn and Pb, while hedgehogs are attributed to Cd and Hg. The  
498 hypotheses we put forward are that these trends may be generally influenced by dietary  
499 habits. Behaviour could be another parameter, as hedgehogs are insectivorous linked to  
500 (sub)urban areas being affected to a greater extend by exposure to Cd and Hg (D'Havé et  
501 al., 2006). Another parameter is the physiological function, for example hedgehogs are  
502 hibernators, and during this period, the stress caused by PTEs body burden may be crucial  
503 (Rautio et al., 2010). Finally, it is interesting to note the behaviour of hepatic Zn, which  
504 increases when it decreases in the kidney and vice versa, in spite this did not result in a  
505 significant negative correlation. Badgers tended to follow this trend possibly because  
506 omnivores accumulate Zn first in the liver followed by the kidney, and also because one  
507 of the places where Zn homeostasis is maintained is in the hepatic tissue (Kosik-Bogacka  
508 & Lanocha-Arendarzyk, 2019).

### 509 3.3. Age and sex influence

510 The age of an organism often influences the accumulation of PTEs like Cd. In our study,  
511 consistent with expectations, adult hedgehogs showed more significant Cd accumulation  
512 in their kidneys compared to their younger counterparts, with mean concentrations being  
513 6.99 and 2.45 mg kg<sup>-1</sup> dw; respectively ( $p<0.05$ ) (Fig. 4). This age-related accumulation  
514 can be attributed to the prolonged exposure coupled with metals' extended biological  
515 half-life (Cooke, 2011). Another contributing factor is the detoxifying nature of nephritic  
516 tissue. Specifically, to neutralize Cd's toxic effects, the body Cd-MT (metallothioneins)  
517 complexes (Sánchez-Chardi et al., 2007a). While our results demonstrate this pattern in  
518 hedgehogs, similar tendencies, albeit not statistically significant, were also observed in  
519 badgers, particularly concerning As accumulation. Previous studies corroborate our  
520 findings, having reported hepatic and renal Cd accumulations in adult hedgehogs. Similar  
521 patterns were also observed for other metals like Hg, Pb, and As (Rautio et al., 2010;  
522 Dahmardeh Behrooz et al., 2022; Jota Baptista et al., 2023). However, while the PTE  
523 concentrations detected in this study are relatively low, understanding their significance  
524 would require insight into the concentrations in the subsequent trophic level. In the  
525 absence of such data, drawing a conclusive level of concern remains challenging.  
526 Nonetheless, our findings highlight potential exposure risks to apex predators that  
527 primarily feed on older animals, hinting at bioaccumulation effects up the food chain.

528 Contrary trends were observed in the levels of Zn in hedgehogs and, generally, in badgers  
529 where the young tended to accumulate relatively more PTEs than adults. It has been  
530 suggested that young animals are more susceptible to Zn accumulation than older animals  
531 due to their high uptake of food for energy requirements and their high absorption rate  
532 (Kosik-Bogacka & Lanocha-Arendarzyk, 2019). Similar findings have been reported for

533 hedgehogs from Finland, in which the Zn concentration decreased with age (Rautio et al.,  
534 2010). However, in badgers, Van den Brink & Ma (1998) found an age-influence in the  
535 oldest from the Netherlands, which had accumulated significantly high levels of Cd, Zn  
536 and Pb. Renal Pb accumulation in adult badgers has also been recognised (Ma, 2011),  
537 although this was not observed in our study. The fact that young badgers have  
538 accumulated more Pb than adults may be due to the fact that Pb is an analogue of calcium  
539 which is rapidly exchanged, being higher in juveniles when compared to adults  
540 (Skerfving and Bergdahl, 2015). When the age factor was evaluated, PCA showed an  
541 overlap between young and adults (Fig. 3B). This plot determines that the factor does not  
542 have an influence and is therefore not acting on the set of variables to. However, it seems  
543 that young animals tended to show a marked trend for Hg, Cd and As.

544 Data about gender-related metal accumulation in European hedgehog and Eurasian  
545 badger are scarce. Even though gender has a physiological function in metal  
546 accumulation, as abovementioned in the introduction section, little is known about its  
547 effects as a consequence of differences in hormonal and reproductive state, gene  
548 expression and metabolic rate between males and females (Burger et al., 2007). In the  
549 present study, we did not observe statistically significant differences in either species  
550 ( $p>0.05$ ) (Supplementary materials; Fig. 1S). In addition, PCA do not shows a significant  
551 trend on PTEs concentrations when the sex factor was considered, being data almost  
552 overlap (Supplementary materials; Fig. 2S). Thus, inter-population variation caused by  
553 differences in exposure and uptake of elements may also have influenced this discordant  
554 result. Generally, as reflected in the figures, we observed a slight trend of PTEs  
555 accumulation in females compared to males. Moreover, females tended to show a marked  
556 trend for Hg and Cd, while Pb and Zn were for males. Some authors claim that these  
557 differences are attributed to reduced dispersal rate when rearing pups, leaving them closer  
558 to areas contaminated by point sources or differences between feeding habits (Dibbern et  
559 al., 2021; Sánchez et al., 2022). These trends are consistent with a recent study developed  
560 by Jota Baptista et al. (2023), where significant differences in Pb concentration in the  
561 liver of hedgehogs from Portugal were observed; however, the opposite of those showed  
562 by Dahmardeh Behrooz et al. (2022) in Brant hedgehogs, whose Hg levels in the kidney  
563 were higher in males when compared to females; in another study, no difference between  
564 males and females was observed (Rautio et al., 2010). Nonetheless, the results obtained  
565 in the present study are in accordance with those carried out in insectivorous small  
566 mammals such as European mole, and even in mammal that are higher up the trophic  
567 chain (Komarnicki et al., 2000; Scheirs et al., 2006; López-Alonso et al., 2007; Millán et  
568 al., 2008).

569 In the present study, male badgers showed a slight tendency to accumulate As and Cd,  
570 while females accumulated Zn and Pb, but this was not statistically different ( $p>0.05$ ).  
571 Low levels in females may be attributed to the transfer of PTEs to the foetus during  
572 gestation and lactation (Shore et al., 2011). In contrast to these results, Cd accumulation  
573 in female badgers has been reported (Van den Brink & Ma, 1998; Millán et al., 2008).  
574 Thus, there is no clear evidence about the relationship between gender and metal  
575 accumulation, suggesting that the accumulation can differ among species, seasons or  
576 reproductive status. However, these results in badgers are not consistent with that of other  
577 mesocarnivores, such as the American mink (*Neovison vison*) (Mayak, 2012). Sex-related  
578 differences in metal accumulation have also been identified in other biomonitoring

579 investigations, with females exhibiting higher metal concentrations compared to males in  
580 terrestrial wild mammals (Hernández-Moreno et al., 2013). Some researchers have  
581 attributed this phenomenon to differences in the efficiency of metallothionein (MT)  
582 synthesis in females (Cooke, 2011). While the influence of sex on metal accumulation  
583 has been explored to a limited extent, it underscores the need to elucidate the  
584 toxicokinetics of individual inorganic elements in males and females across different  
585 species. This consideration should be incorporated into future biomonitoring studies  
586 (Neila et al., 2017).

#### 587 **4. Conclusions**

588 Our research provides current levels of potentially toxic elements (PTEs) in wildlife  
589 terrestrial ecosystems in NW Spain. This is the first contribution to report baseline Hg  
590 levels in the European hedgehog in the Iberian Peninsula, finding these concentrations  
591 above the threshold value for serious health effects, and nephritic reference levels in  
592 terrestrial wild mammals in our study area were also determined for the first time. This  
593 research also showed an age-related in Cd accumulation and this physiological factor  
594 should, therefore, be considered in the assessment of PTEs body burdens. However, sex  
595 had no effect, so it is necessary to improve our knowledge on this factor. In addition, our  
596 results should be included in a database with baseline values for PTEs bioaccumulation  
597 in European insectivores and mesocarnivores. The data obtained in this ecotoxicological  
598 study have significant potential applications in wildlife management, environmental  
599 quality improvement and environmental risk assessment in the northwest of the Iberian  
600 Peninsula. Moreover, our results underline the importance of proper environmental  
601 management and the need for further research to determine the exact sources of this  
602 contamination. In summary, this study highlights the importance of addressing and  
603 mitigating PTEs contamination in terrestrial ecosystems in NW Spain. This knowledge is  
604 fundamental for the conservation of biodiversity and the protection of environmental  
605 quality in this region. It is therefore necessary to monitor and understand the presence of  
606 PTEs in local ecosystems, and to stress the need for appropriate conservation and  
607 environmental management measures to protect both wildlife and the natural  
608 environment.



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- 861

862 **Statements & Declarations**

863 **Ethical Approval**

864 We have read and have abided by the statement of ethical standards for manuscripts  
865 submitted to *Environmental Science and Pollution Research*.

866 **Consent to Participate**

867 All authors have approved the manuscript and agree with the content.

868 **Consent to Publish**

869 All authors have approved the version to be published and gave consent to submit the  
870 manuscript.

871 **Authorship contribution**

872 As PhD student who participated to the whole study, mainly focusing on organization of  
873 data collection, as well as formal analysis and interpretation of results, and drafting of the  
874 article were performed by Javier García-Muñoz. Statistical study, as well as the critical  
875 revision of the article were performed by Nunzio Antonio Cacciola, Federico Plazzi,  
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888 **Availability of data and materials**

889 All data and materials will be made available upon request.

890

891 **Artwork**

892 GraphPad Prism (9.02) and R (R Core Foundation, 2023) was used to create the artwork.

893

894 **Figure Captions**

895 **Fig. 1.** Distribution of the sampling areas for European hedgehog (●) and badger (●) in  
896 Galicia, northwestern Spain. ☒ represents the main human settlement.

897 **Fig. 2.** Correlation matrix of the variables analysed in the liver and kidney of European  
898 hedgehog (A) and European badger (B). Blank square means no statistically significant  
899 correlation. Blue circles are positive correlation. Red circles are negative correlation. The  
900 size of the circle marks the correlation coefficient. \*\*\*( $p < 0.001$ ).

901 **Fig. 3.** Biplot of PC1 versus PC2 with loadings of Zn, Hg, Cd, Pb and As measured in  
902 liver and kidney of 39 samples of European hedgehog and 20 samples of European  
903 Badger. The colours represent the (A) species (red: hedgehogs, blue: badgers), and (B)  
904 age (red: adults, blue: young). The total variance explained by the first two principal axes  
905 is 55%.

906 **Fig. 4.** Distribution of Zn, Hg, Cd, Pb and As ( $\text{mg kg}^{-1} \text{ dw}$ ) in the liver and kidneys for  
907 both species, European hedgehog and Eurasian badger, according to age. Box plots  
908 represent median values and 25–75 percentile. Significance levels were \* $p < 0.05$ .