**Biomonitoring of metals and metalloids with raptors from
Portugal and Spain: a review**

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BIOMONITORING OF METALS AND METALLOIDS WITH RAPTORS FROM PORTUGAL AND SPAIN: A REVIEW

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BIOMONITORING OF METALS AND METALLOIDS WITH RAPTORS FROM PORTUGAL AND SPAIN: A REVIEW

Abstract: The analysis of metals in different tissues of raptors has been an important tool for assessing metal pollution. Several studies using a range of sentinel raptor species have been carried out in Portugal and Spain since the 1980s in order to identify the adverse effects in the animals themselves and on their populations, to identify the contamination of the food-chain, to determine the levels of environmental contamination and to estimate human health risks.

The aim of this work is to provide synthesized information of the studies carried out in Portugal and Spain in the direct biomonitoring of metals and metalloids using raptors, through a systematic search of the published literature. The information is summarized taking into account specific issues such as monitored raptor species, sampling periods, monitored areas, type of samples, analytical techniques used in the determination of the metals and metalloids, the analysed metals and metalloids and overall analysis of the concentrations obtained.

There is a striking difference between the number of studies performed in Portugal and Spain, and most of them have been carried out in Spain. The Eagle owl, Black kite and Common buzzard were the species from which the greatest number of individuals has been analysed. Among the most analyzed biological samples, the blood and liver samples were used to measure the concentrations of all studied metals, while bone was mainly collected to evaluate chronic exposure to lead and feathers to evaluate exposure to mercury during feather growth. Atomic Absorption Spectrometry has been the most frequently performed technique to determine the majority of metals and arsenic. In general, the concentrations of metals detected in raptor samples from Portugal and Spain are low and insufficient to produce toxic side effects. Only lead, in certain cases, can be related with toxic side effects. However, it

25 should be given particular attention to mercury due to its high toxicity, its transport from an
26 aquatic environment to the adjacent terrestrial food web and because mercury toxicity
27 threshold have yet to be established for raptors.

28 This work confirms the need for further biomonitoring studies of metals with raptors,
29 especially in Portugal; the establishing of national programs in order to conduct long-term
30 studies; and creating a network between Portugal and Spain in order to study environmental
31 contamination using raptors.

32

33

34 **Keywords:** biomonitoring, environmental contamination, metals, raptors, Portugal,
35 Spain.

Draft

36 1. Introduction

37 Metals are natural constituents of all ecosystems that are present at low concentrations
38 in soils, plants and living organisms. Their distribution in the environment results from
39 natural processes and various anthropogenic activities. However, human activities
40 significantly increase their presence in the environment and in many ecosystems these
41 anthropogenic inputs exceed removals by natural biogeochemical cycles of metals
42 (Pacyna and Pacyna 2001; Florea and Busselberg 2006).

43 Some metals, such as copper (Cu), cobalt (Co), iron (Fe), molybdenum (Mo),
44 manganese (Mn), selenium (Se) and zinc (Zn) are considered important micronutrients
45 since they are required in relatively small amounts for many physiological processes in
46 living beings. If their supply is below physiological requirements it can result in
47 deficiency diseases, but in larger amounts they may be harmful and even toxic to
48 organisms. Other metals such as Cd, Pb and Hg are not necessary for any physiological
49 processes, they have toxic effects on living organisms and are classified as contaminants
50 or non-essential elements (Ernst et al. 1983; He et al. 2005; Florea and Busselberg 2006).
51 These are the most dangerous metals for both humans and animals, not only because of
52 their high toxicity but also due to their persistence and pronounced biological
53 accumulation and biomagnification in food chains (Burger 1995, 2008; García-Fernández
54 et al. 1996; Cristol et al. 2008). As is a metalloid or a semi-metal, and although there is
55 some uncertainty regarding its necessity for physiological processes, it is well known that
56 it has toxic effects on living beings (Uthus 1992; Eisler 1998; Shi et al. 2004; Hughes
57 2006). Unlike other toxic metals, As has a low rate of bioaccumulation and it is not
58 biomagnified in the food chain (Eisler 1998; Hughes 2006).

59 Environmental contamination by non-essential elements is a worldwide problem and
60 represents a serious threat to the stability of ecosystems and living organisms (Barbosa et
61 al. 2014). The need to determine the degree of exposure and the effects of contaminants
62 in both animals and humans has led to numerous biomonitoring studies. Direct
63 monitoring of air, soil, water and sediments can be useful to determine the degree of
64 contamination in a particular area; however, it does not provide a measure of
65 bioavailability and resultant uptake by biota or people. It is only through direct
66 biomonitoring (the analysis of contaminants in fluids and tissues of organisms) that the
67 actual exposure of organisms can be properly determined and related to the levels in the
68 physical environment (Beeby 2001; Gómez-Ramírez et al. 2014). In general, the
69 objectives of monitoring sentinel animals include data collection in order to: (1) estimate
70 human health risks; (2) identify contamination of the food-chain; (3) determine the levels
71 of environmental contamination; (4) identify adverse effects on animals; (5) examine
72 spatial or temporal trends in contamination levels (National Research Council (US) 1991;
73 Gómez-Ramírez et al. 2014).

74 Many of these studies are carried out with birds, which are particularly sensitive to
75 anthropogenic contamination (Anthony et al. 1993; Dauwe et al. 2000; Battaglia et al.
76 2005; Alleva et al. 2006; Burger and Gochfeld 2009; Naccari et al. 2009; Álvarez et al.
77 2013). Amongst birds, raptors (birds of prey, owls and scavengers) are considered
78 especially suitable for monitoring bioaccumulable and biomagnifiable pollutants, mainly
79 due to their position at the top of trophic chain (Furness 1993). They may also provide
80 information about the integration of contaminants that reach other species at the top of
81 trophic chain, such as humans (Furness 1993; Zolfaghari et al. 2007; Figueira et al.
82 2009). Other key characteristics make raptors good sentinels for environmental

contamination, namely their relatively large home range; relative ease with which individuals (particularly nestlings) can be captured and non-destructive samples (blood, feather, preen gland oil) can be collected; and relative ease with which populations can be quantified and monitored (Becker 2003; Gómez-Ramírez et al. 2014). Moreover, raptors are known to have measurable responses to persistent, bioaccumulative and toxic chemicals, ranging from residue accumulation to population decline (Newton et al. 1993; Mateo et al. 1997b; Berglund et al. 2007; Gangoso et al. 2009; Lee et al. 2012; Espín et al. 2014a).

Portugal and Spain are rich in bird life, holding breeding populations of 34 species of raptors from 56 species that breed regularly in Europe (Table 1). These countries are also home to important populations of several threatened species at an European level, such as Spanish Imperial eagle (*Aquila adalberti*), Red kite (*Milvus milvus*), Bonelli's eagle (*Hieraaetus fasciatus*), Egyptian vulture (*Neophron percnopterus*), Cinereous vulture (*Aegypius monachus*) and Bearded vulture (*Gypaetus barbatus*) (Burfield 2008). Moreover, Spain is home to more than 95% of all European avian scavengers and the world's entire Spanish imperial eagle population (Margalida 2012). Given the important ecological role (providing important ecosystems) of the raptor species, their high profile and popularity with the public health, the political commitment to halt biodiversity loss in Europe and the implications that toxic metals could have on their conservation, it is timely to review the studies directed towards the monitoring of toxic metals using raptors from Portugal and Spain as biomonitors.

The aim of this work is to provide synthesized information of the studies carried out in Portugal and Spain on the biomonitoring of metals and metalloids using raptors, through a systematic search of the published literature. Taking into consideration only the studies

107 that performed direct biomonitoring, the information is summarized taking in account the
108 following specific issues: monitored raptor species, sampling periods, monitored areas,
109 type of samples, the analytical techniques used in the determination of the metals, the
110 analysed metals and metalloids, and the concentrations obtained.

111

112 **1.1 Monitored raptors species**

113 Raptor species have been an important group of species used for monitoring purposes,
114 not only because many of them are excellent sentinels of environmental contamination
115 indicating specific environmental change, but also because they are valued natural
116 resources in their own right with an important ecological role (providing important
117 cultural and regulatory ecosystem), that may be adversely affected by toxicant exposure
118 (Becker 2003; Golden and Rattner 2003). Due to the conservation problems associated
119 with pollutant exposure and accumulation in some endangered raptor species in Europe
120 (BirdLife International, 2004), raptors in general, and endangered species in particular,
121 should be studied as a priority.

122 In table 1 are summarized the main characteristics (trophic position and the most
123 important prey, conservation status and migratory habits) of the raptor species breeding in
124 Portugal and Spain, specifying which ones were used for monitoring purposes of metals
125 and the total number of analysed individuals. The selection of the raptor species for
126 monitoring metals and metalloids has been mainly influenced by the availability of
127 samples, which in turn is conditioned by particular features of each species, such as
128 abundance and conservation status, geographical distribution, type of habitat and
129 migration habits. For example, the collection of samples from animals in their natural
130 environment can sometimes be difficult to accomplish in raptors from wooded or

131 mountainous areas, especially out of the breeding season, and the cost and inherent
132 difficulty in obtaining samples are also disadvantages (García-Fernández et al. 2008).
133 Collaboration with Wildlife Rehabilitation Centres (WRCs) can be useful for
134 environmental biomonitoring, mainly when it is difficult to collect samples from animals
135 in their natural environment, as it was in the sample collection of the Common buzzard
136 (*Buteo buteo*), one of the most commonly used species in biomonitoring studies in both
137 countries (García-Fernández et al. 1995, 1996, 1997; Mateo et al. 2003; Pérez-López et
138 al. 2008; Castro et al. 2011; Carneiro et al. 2014).

139 The type of diet has also been an important factor in the selection of the bird species,
140 since it influences the bioaccumulation of metals (García-Fernández et al. 1995; Palma et
141 al. 2005). García-Fernández et al. (1995) and Mateo et al. (2003) found the highest Pb
142 concentrations in tissues of bird species that usually feed on carrion, and the piscivorous
143 species are capable of accumulating higher concentrations of Hg in their tissues
144 (Thompson 1996; Evers et al. 2005). Most of the species frequently monitored in
145 Portugal and Spain are mixed feeders, often taking a mixture of mammalian, avian, fish
146 and, in some cases, invertebrate prey, which is considered by some authors a suitable
147 characteristic in biomonitoring studies of different contaminants (Battaglia et al. 2005).
148 However, diet variation may have major confounding effects in studies on biomonitoring
149 environmental contamination using birds of prey. Furthermore, due to the capacity of
150 some species to exploit a wide variety of food resources and consequently its diet
151 depends on the availability of optimal and suboptimal prey species, pollutant uptake and
152 accumulation can be highly variable among different territories and periods depending on
153 the diet composition (Mañosa et al. 2003; Ortiz-Santaliestra et al. 2015).

154 For this reason, some authors recommended the use of bird species with narrow and
155 inflexible diets in contamination studies, rather than generalist predators (Monteiro and
156 Furness 1995; Palma et al. 2005; Hollamby et al. 2006). However, true dietary specialists
157 are hard to find and constant diets across space and time are unwarranted.

158 The aim of the work is also a determining factor in the selection of the biomonitor bird
159 species. For example, species that have an unfavourable conservation status, such as the
160 Bearded vulture, Egyptian vulture and Spanish imperial eagle were used in research
161 studies that mainly aimed to assess the adverse effects of metals on their populations
162 (González and Hiraldo 1988; Hernández et al. 1988a; Donázar et al. 2002; Pain et al.
163 2005; Hernández and Margalida 2009; Rodriguez-Ramos et al. 2011).

164 Most of the raptor species breeding in Portugal and Spain have been used at some
165 stage for monitoring metals. However, in many cases, the number of analysed specimens
166 was very low, being fewer than 20 (Cinereous vulture, Golden eagle *Aquila chrysaetos*,
167 Short-toed eagle *Circaetus gallicus*, Montagu's harrier *Circus pygargus*, Hobby *Falco*
168 *subbuteo*, Peregrine falcon *Falco peregrinus*, Long-eared owl *Asio otus* and Scops owl
169 *Otus scops*). Amongst the monitored raptors, Eagle owl (*Bubo bubo*) (n=979), Black kite
170 (*Milvus migrans*) (n=428) and Common buzzard (n=428) are the species from which the
171 greatest number of individuals has been analysed. The Spanish imperial eagle is found
172 only in the Iberian Peninsula and it is considered one of the most threatened birds of prey
173 in Europe (González et al. 2008). Several studies have analysed the risk of metals for the
174 conservation of this species in a relatively large number of individuals (n=257). In
175 Portugal, only the raptor species Common buzzard, Griffon vulture (*Gyps fulvus*), Eagle
176 owl, Bonelli's eagle (*Hieraaetus fasciatus*), Little owl (*Athene noctua*), Tawny owl (*Strix*
177 *aluco*) and Barn owl (*Tyto alba*) were used for monitoring purposes, wherein were

178 sampled less than 20 individuals in the last three referred species (Palma et al. 2005;
179 Figueira et al. 2009; Lourenço et al. 2011; Carneiro et al. 2014, 2015). Eagle owl and
180 Common buzzard gather relevant features, such as favourable conservation status, wide
181 geographical distribution, non-migratory habits, and high territoriality and diversified
182 diet, which make them suitable biomonitors. Although the Black kite is a migratory bird,
183 its other life history traits make it also a suitable species for contaminants monitoring.
184 They often breed in habitats that are highly affected by agricultural, industrial and urban
185 pollution. Therefore, they were important indicators of environmental contamination in
186 an environmental disaster (Aznalcóllar mining accident) and in polluted areas (Benito et
187 al. 1999; Blanco et al. 2003; Baos et al. 2006a, 2006b; Álvarez et al. 2013). Furthermore,
188 some studies have shown that Black kites are susceptible to metal toxicity and
189 contamination has been suspected as an important factor that contributes to the decline of
190 their populations. In general, the least sampled species for metal-monitoring purposes are
191 those with an unfavourable conservation status and/or with migratory habits.

192 In future studies, other raptor species such as the Eurasian sparrowhawk (*Accipiter*
193 *nisus*), Common kestrel (*Falco tinnunculus*), Little owl, Tawny owl and Barn owl can be
194 used more frequently for biomonitoring purposes of metals, since they have the
195 characteristics essential to be suitable biomonitors, namely their relative high abundance
196 that can easily provide a large number of samples for environmental biomonitoring.
197 Future efforts should also be focused on the study of those species that have
198 demonstrated their usefulness in matters of conservation, such as the Black-winged kite
199 (*Elanus caeruleus*), Bonelli's eagle, Cinereous vulture, Egyptian vulture, Golden eagle,
200 Red kite and Osprey (*Pandion haliaetus*).

201

202 1.2. Sampling periods and monitored areas

203 In table 2 are listed the sampling periods and the bird species studied by each
204 monitored area of Portugal and Spain. There is a striking difference between Portugal and
205 Spain regarding the studies performed on the biomonitoring of metals using raptors. To
206 our knowledge, to date only six of these studies have been performed in Portugal.
207 Although these studies have enclosed several areas of the country, there is still a lack of
208 information, especially regarding the centre and the southern coast of the country. In
209 Spain, despite several studies being carried out, there are still many areas (mainly in the
210 North and West) that have not been monitored.

211 Amongst the most sampled bird species, the Common buzzard stands out as having
212 been monitored over practically the entire Iberian Peninsula, while the Eagle owl and the
213 Black kite were mostly sampled in Murcia, south-eastern Spain and in Doñana National
214 Park, south-western Spain, respectively. Concerning the analysed metals, unlike Hg that
215 was mostly analysed in Portugal and south-west Spain, Pb was monitored over a larger
216 number of geographic areas.

217 The monitored area is often closely related to the purpose of each study and the
218 location of specific sources of metals, such as environmental disasters, industry,
219 agriculture, hunting activities or urbanization. For example, in the south-west of Spain,
220 some studies were carried out after an environmental disaster (Aznalcóllar toxic spill) in
221 order to evaluate its effects on bird populations (Benito et al. 1999; Baos et al. 2006a,
222 2006b). The selection of the area to monitor is also related with the distribution of the
223 species intended to be used as monitors and its consequent conservation status. For
224 example, research with threatened species like the Spanish imperial eagle has been made
225 in central Spain and Doñana National Park, south-western Spain. Before the

226 reintroduction project of the Bearded vulture in the Southern Iberian Mountains (LIFE04
227 NAT/ES/000056), the Iberian population was only distributed in the Pyrenees, and thus
228 research studies on this species were limited to this geographical area (Hernández and
229 Margalida 2009).

230 Furthermore, the geographic location of the research groups also influences the
231 monitored areas. The research groups who have taken on the monitoring of raptors as a
232 major branch of their research are located in Spain, namely in Doñana (south-west),
233 Madrid (central), Barcelona (north-east) and Murcia (south-east). For this reason, central,
234 north-eastern and southern Spain are the areas in which a greatest number of studies have
235 been performed. The research groups in central and south-western Spain have focused to
236 a high degree on two species with an unfavourable conservation status (Black kite and
237 Spanish Imperial eagle) and the studies carried out in the south-east and north-east Spain
238 have covered a greater variety of species with a more favourable conservation status. In
239 Portugal, because there are not any research groups primarily directed towards
240 monitoring environmental contaminants with raptors, only a few isolated studies have
241 been performed.

242 The earliest monitoring studies started in 1980s in central and south-west Spain,
243 mainly focused on the Spanish Imperial eagle species (Hernández et al. 1986, 1988a;
244 González and Hiraldo 1988). It was during the period between 1997 and 2005 that the
245 majority of investigations were performed and therefore a greater number of species and
246 areas were evaluated. In recent years there has been a decrease in investigations directed
247 to the biomonitoring of toxic metals with raptors in Spain, which perhaps is strongly
248 related with a reduction in funding for research and development by the Spanish
249 government in 2011 (Margalida 2012). In Portugal such studies have been scarce. The

250 first study was published in 2005 with the Bonelli's eagle (Palma et al. 2005), after which
251 only three studies were published with new results about the metal levels in raptors
252 (Lourenço et al. 2011; Carneiro et al. 2014, 2015).

253 The research carried out in both countries has been sporadic in terms of both time
254 and location, and using different type of samples and species (e.g. Hernández et al. 1986,
255 1988b; González et al. 1984; Negro et al. 1993; Mateo et al. 1999; Donazar et al. 2002;
256 Figueira et al. 2009; Hernández and Margalida 2009; Rodríguez-Ramos et al. 2011).
257 Furthermore some of the studies have focused on isolated events or potential sources,
258 such as the Aznalcóllar toxic spill, solid-waste incinerators and mining areas (Benito et
259 al. 1999; Blanco et al. 2003; Baos et al. 2006a, 2006b; Gómez-Ramírez et al. 2010). As
260 such, the available information does not allow making a temporal and spatial analysis
261 regarding metal concentrations. Nonetheless, studies in the South-east Spain (Murcia)
262 with the Common Buzzard and Eagle owl showed that Pb exposure has decreased in the
263 last 20 years (García-Fenández et al. 1995, 1997; Martínez-López et al. 2004; Gómez-
264 Ramírez et al. 2010; Espín et al. 2014b), which could be explained by the closure of
265 mines in 1991 and the ban on leaded petrol in 2001 (García-Fenández et al. 2005; Espín
266 et al. 2014b). Mateo et al. (2007) also found a decrease in the number of Pb shot in the
267 pellets of the Spanish Imperial Eagle from South Spain over the course of time, which
268 may be due to the ban of waterfowl hunting. However, in Portugal, a recent study with
269 the Common Buzzard showed higher blood Pb concentrations than those found in the
270 studies performed in South-east Spain and previously mentioned. On the other hand,
271 Common buzzards sampled in North-west Spain (Galicia) presented higher hepatic
272 concentrations of As, Cd, Hg and Pb than those sampled in Portugal and South-east Spain
273 (García-Fernández et al. 1995, 1997; Hermoso de Mendoza et al. 2006; Pérez-López et al.

2008; Castro et al. 2011; Carneiro et al. 2014). The studies carried out in South-west of Spain after the Aznalcóllar toxic spill, revealed a decreasing trend over time during the period between 1999 and 2002 in the levels of As and Pb in Black kites from the affected area (Benito et al. 1999; Baos et al. 2006a, 2006b). Nonetheless, Black kites sampled in Madrid in 2001, presented blood Pb levels higher than those reported for birds sampled in the area affected by the toxic spill regardless the year (Blanco et al. 2003).

280

281 1.3. Type of samples

282 The 44 biomonitoring studies with raptors carried out in Portugal and Spain have
283 measured metals in a variety of different biological samples. In table 3 are listed the
284 metals and metalloids measured in each raptor species from Portugal and Spain *per*
285 sample type and the total number (n) of the different analysed samples for each species.

286 Choosing the type of samples to be used depends directly on the ultimate aim of the
287 study and, in a great degree, on the kinetics of the metals to be analysed. Furthermore, the
288 number of available samples is conditioned by several issues such as ethics, legal issues,
289 census, and human and financial resources (García-Fernández et al. 2008). For example,
290 the conservation status of a species is a conditioning factor in selecting the sample type,
291 so samples that can be collected from live birds in a non-destructive way are preferred.

292 Blood has been the preferred sample in most biomonitoring studies performed in
293 Portugal and Spain, in order to measure the concentrations of all studied elements. Blood
294 samples provide the most direct and non-lethal assessment of dietary exposure, primarily
295 reflecting recent exposure and to a lesser extent recently metabolized contaminants
296 (Evers et al. 2005; Shlosberg et al. 2011; Álvarez et al. 2013). Moreover, blood levels
297 strongly correlate with concentrations in other internal tissues and blood samples can be

298 easily and repeatedly obtained from the same individual if required (García-Fernández et
299 al. 1996; Burger and Gochfeld 1997; Kahle and Becker 1999; Kenow et al. 2007).

300 Non-invasive samples such as eggs and feathers have also been collected in some
301 species. In the initial periods of biomonitoring with raptors in Spain, eggs were frequently
302 collected to evaluate a variety of different metals in twelve raptor species (González et al.
303 1984; Hernández et al. 1986, 1988a, 1988b; González and Hiraldo 1988; Negro et al.
304 1993). Perhaps because eggs are not indicative of exposure in all individuals of the
305 population, such as males and juvenile birds, and it is difficult to obtain a sufficient
306 number of samples, they have not often been collected in more recent studies. However,
307 they are a homogenous sample that can be directly related with reproductive effects,
308 which is particularly important in the case of birds with an unfavourable conservation
309 status (Hernández et al. 1988a; Negro et al. 1993; Hargreaves et al. 2010; Gómez-
310 Ramírez et al. 2014).

311 In recent years the usefulness of feathers as a biomarker of metals exposure has been
312 investigated, and in Portugal and Spain they have been frequently used in recent studies
313 to evaluate mainly Hg and Pb exposure (Martínez-López et al. 2004; Pain et al. 2005;
314 Palma et al. 2005; Ortego et al. 2006; Figueira et al. 2009; Cardiel et al. 2011; Castro et
315 al. 2011; Lourenço et al. 2011; Rodríguez-Ramos et al. 2011; Debén et al. 2012; Martínez
316 et al. 2012; Espín et al. 2014c). The use of feathers has several advantages over other
317 tissues for the measurement of metals contamination in birds. Feathers are easy to collect
318 non-invasively, from both dead and living birds, or can be easily found in nests, which is
319 particularly appropriate for rare and declining species; and since they do not require
320 refrigeration, feathers can be easily stored for decades which makes them useful for long-
321 term studies (Movalli 2000; Adout et al. 2007; Lodenius and Solonen 2013). Moreover,

the specific incorporation of metals in feathers renders profiles inert and stable and may lead to higher concentrations than in internal tissues, particularly for Hg, so those concentrations in feathers can be more easily measured (Furness et al. 1986; Dauwe et al. 2000; Burger et al. 2008; Burger and Gochfeld 2009). What is more, some studies indicate that it is possible to correlate metal levels in feathers with concentrations in blood and internal tissues (Dauwe et al. 2002; Martínez-López et al. 2004, 2005). However, there are some factors that influence the deposition of metals into feathers, such as variation between feathers, variation between different parts of a feather, moulting patterns and changes of diet during moulting, which make the interpretation of metal levels difficult (Dauwe et al. 2003; Jaspers et al. 2004; Debén et al. 2012; Martínez et al. 2012). With the exception of Hg, alongside endogenous deposition, external contamination (atmospheric deposition or during preening) may have an important impact on the metal concentrations found in feathers, which can also make the interpretation of the results difficult (Dauwe et al. 2002, 2003; Jaspers et al. 2007; Lodenius and Solonen 2013). In order to avoid the problem of external contamination, Cardiel et al. 2011 recommends the analysis of metals in the feather rachis. Except the barbs of new or actively growing feathers, barbs are more prone to surface contamination. The authors also recommend the routine use of aluminium (Al) analysis in studies regarding metal analysis in feathers, in order to improve the interpretation of those data.

Different non-invasive methods, such as the collection of regurgitated pellets, have also been carried out and used to evaluate Pb exposure (Mateo et al. 1999, 2001, 2007). Like blood samples, they can give information on the exposure frequency, seasonality and the food items associated with metal exposure, however these methods can

345 sometimes be difficult to accomplish in raptors from wooded or mountainous areas,
346 especially out of the breeding season (Mateo et al. 1999).

347 Despite the difficulties inherent to the collection of internal tissues, they have also
348 been collected for monitoring metal exposure and assessing their risk on bird populations
349 from Portugal and Spain. Most metals are stored in internal tissues such as the liver,
350 kidneys and bones, which have a tendency to accumulate them over time. Consequently
351 these tissues contain higher concentrations of some metals than those that are generally
352 found in the blood and other tissues of the birds (García-Fernández et al. 1996, 1997; Ek
353 et al. 2004; Naccari et al. 2009). The concentrations of metals in internal tissues represent
354 the levels of metals in the diet, which in turn reflect the degree of contamination of the
355 ecosystem (Monteiro et al. 1998; Castro et al. 2011). Collaboration with WRCs can
356 provide a sufficiently large number of such samples. However, the interpretation of
357 results requires great care, since the cause of death or illness may interfere with the
358 kinetics of certain compounds. In Spain, most studies were preferentially performed using
359 liver and bone samples followed by the kidney. The liver is the first target of most
360 elements and consequently it can be a good indicator of recent exposure (Braune and
361 Gaskin 1987; Scheuhammer 1987; Battaglia et al. 2005), for this reason it was used to
362 measure the concentrations of all studied metals in many bird species. Bone was the
363 preferred sample for evaluating Pb exposure because it is a long-term repository for this
364 metal, containing approximately 90% of the total body burden in mammals and birds and
365 reflecting life-time exposure (Scheuhammer 1987; García-Fernández et al. 1997; Mateo
366 et al. 2003; Gangoso et al. 2009). The kidney has been mainly analysed to measure Cd
367 concentrations, since this organ accumulates about one half of the Cd total body burden
368 and its Cd content is extremely stable. Therefore the kidney is considered an adequate

369 biomarker of chronic exposure to Cd (Scheuhammer 1987; García-Fernández et al. 1996).
370 Other internal tissues, such as the brain, are not frequently used for monitoring metals
371 due to its low rate of accumulation there (García-Fernández et al. 1996, 1997).

372

373 **1.4. Analytical techniques used in the determination of metals and metalloids**

374 The analytical techniques commonly used for metals detection in biological samples
375 of raptors from Portugal and Spain have been atomic spectroscopy methods such as
376 Atomic Absorption Spectrometry (AAS), the voltammetric stripping technique Anodic
377 Stripping Voltammetry (ASV), Inductively Coupled Plasma-Atomic Emission
378 Spectrometry (ICP-AES) and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)
379 (Table 4).

380 The element(s) to be analysed, the sensitivity of the technique to the respective
381 element(s) and the associated costs were the main factors which influenced the selection
382 of the detection method. Except for Hg determination, Graphite Furnace (GF) - AAS and
383 ASV have been the most frequently performed techniques to determine most of the
384 metals, due to their relative cost and the sensitivity of the techniques. For determination
385 of Hg, Cold Vapour (CV) - AAS method has always been the preferred technique, while
386 the most recent investigations used a single-purpose atomic absorption spectrometer
387 (direct Hg analyser) by applying additional steps in the CV-AAS procedure. ICP-MS is
388 also very sensitive and it has recently been applied to various biological matrices.

389 The major drawback of AAS techniques (flame, graphite furnace, hydride generation
390 and cold vapour) is the impossibility of performing simultaneous multi-elemental
391 analysis. The ASV technique, modified by García-Fenández et al. (1995), has shown to
392 be very useful, mainly in the measurement of blood Cd concentrations, as it has detection

393 limits that were equivalent or lower than those of the ICP-MS (García-Fernández et al.
394 1995, 1996; Espín et al. 2014a). The major drawbacks are the difficulties associated with
395 handling, storage and disposal of its Hg based electrodes, difficulties which make its
396 application undesirable. In fact, environmental regulations and health considerations
397 restrict its continuous application due to the high toxicity of Hg (Stankovic et al. 2011).

398 ICP-MS technique is a very powerful tool for the determination of metals at ultra-trace
399 levels in different matrices, allowing simultaneous multi-elemental analysis with high
400 sensitivity. ICP-MS combines the simultaneous multi-element capabilities of ICP-OES
401 techniques with exceptional detection limits, equivalent or lower than those of GF-AAS
402 (Thomas 2001; Cornelis and Nordberg 2007). However, its major drawbacks - high
403 purchase and running costs and highly skilled laboratory operator required - are the main
404 reasons for its infrequent use in biomonitoring studies with raptors in the Iberian
405 Peninsula. Although the purchase cost of an ICP-MS device is high, it has a high level of
406 productivity and becomes comparatively economical when many samples and/or
407 elements need to be determined.

408 A number of methodological issues complicate the literature concerning the levels and
409 effects of metals on organisms, populations, and ecosystems and influence our ability to
410 compare tissue levels. These mainly include changes in technology over the years, the use
411 of wet weight (w.w.) vs. dry weight (d.w.), statistical calculation (for example, use of
412 arithmetic mean and geometric mean) and the non-standardization of the quantification
413 limits calculation. Changes in technology mainly influence detection levels, which in turn
414 affects the number of samples with detectable levels.

415

416

417 1.5. Metals and metalloids studied and concentrations obtained

418 According to the results found in the literature, in the table 5.1 and 5.2 are listed the
419 ranges of the means of the metals and metalloid concentrations found in blood and tissues
420 of the studied raptor species from Portugal and Spain.

421 Both essential and non-essential metals have been analysed, but the essential metals
422 have received the least interest. Cu and Zn were the essential metals most frequently
423 analysed; Cu has been analysed mainly in eggs and Zn in liver and also in the eggs of
424 different raptor species. Se was only evaluated in the blood of three raptors species from
425 the south-west of Spain. In general, the concentrations of Zn, Cu and Se in the bird
426 species studied were within the healthy range of physiological levels in several bird
427 species, including several raptor species (Blanco et al. 2003; Baos et al. 2006a, 2006b;
428 Pérez-López et al. 2008; Álvarez et al. 2013; Espín et al. 2014a, 2014b).

429 Regarding the non-essential elements, Pb and Cd have been the most studied, while
430 Hg and As have received the least interest.

431 Pb is one of the most toxic metals and has historically been shown to affect wildlife
432 (Fisher et al. 2006; Guitart et al. 2010; Bedrosian et al. 2012). In the Iberian Peninsula,
433 contamination deriving from hunting activities has increased in relative importance in
434 recent decades and consequently it has received special concern, particularly when the
435 birds have scavenger feeding habits and an unfavourable conservation status. Numerous
436 authors have presented the Pb risk to raptors in Spain, including predators and scavengers
437 (Mateo et al. 2001; Donázar et al. 2002; García-Fernández et al. 2005b; Pérez-López et
438 al. 2008; Hernández and Margalida 2009). It has been a limiting factor for the
439 conservation of some endangered species, as the Pyrenean bearded vulture (Hernández
440 and Margalida 2009, Mateo et al. 2015), Egyptian vulture (Donázar et al. 2002; Gangoso

et al. 2009) and Spanish imperial eagle (Pain et al. 2005; Rodriguez-Ramos et al. 2011). Pb shot in regurgitated pellets of raptors has been documented in the Red kite (Mateo et al. 2001, 2007), Marsh harrier (Mateo et al. 1999, 2007), Spanish imperial eagle (Mateo et al. 2001, 2007) and Peregrine falcon (Mateo et al. 2007). Pb poisoning has been described in the Golden eagle (Cerradelo et al. 1992), Spanish Imperial eagle (Hernández 1995), Eagle owl, Red kite (Mateo et al. 2003) and in four species of vultures: Griffon vulture (Mateo et al. 1997a), Cinereous vulture (Hernández and Margalida 2008), Bearded vulture (Hernández and Margalida 2009) and Egyptian vulture (Rodriguez-Ramos et al. 2009). Elevated concentrations of Pb have been detected in blood of Griffon vultures both in Portugal and Spain (García-Fernández et al. 2005b; Espín et al. 2014a; Carneiro et al. 2015). Besides acute toxicity, it has also been observed subtle detrimental effects of Pb in some species, namely reduction on bone mineralization in Egyptian vulture (Gangoso et al. 2009); biochemical changes such as decreased on δ -aminolevulinic acid dehydratase (δ -ALAD) activity, an enzyme participating in the synthesis of haeme group in the erythrocyte, in Booted eagle (*Hieraaetus pennatus*), Common buzzard, Northern goshawk and Eagle owl (Martínez-López et al. 2004; Gómez-Ramírez et al. 2010); and oxidative damage in Griffon vultures (Espín et al. 2014).

Cd, as well as Pb, is a persistent and ubiquitous metal that can cause several deleterious effects in living beings (Burger 2008). In general, in the studies carried out in Portugal and Spain, it has been the element with the lowest concentrations in all types of analysed samples (Table 5.1, 5.2) and the blood Cd concentrations are often below the detection limits of commonly used techniques (García-Fernández et al. 1995; Carneiro et al. 2014, 2015, Ortiz-Santaliestra et al. 2015). The highest blood Cd concentrations were

obtained for the Black kite feeding in the area affected by a toxic spill (Doñana National Park, south-west Spain) (Benito et al. 1999) and for the Northern goshawk from a non-polluted Mediterranean forest (Murcia, south-eastern Spain) (Martinez-López et al. 2005), but the researchers considered that concentrations were generally low. Regarding the concentrations in internal tissues, the highest concentrations were observed in the liver of the Tawny owl and Long-eared owl from Galicia (north-west Spain) (Pérez-López et al. 2008). Scheuhammer (1987) suggested that Cd levels above 3 µg/g d.w. in liver and above 8 µg/g d.w. in kidney might indicate increased environmental exposure. According to these thresholds, it is possible that there is an increased environmental exposure to Cd mainly in north-west Spain. Renal and hepatic Cd concentrations described in raptors from Portugal and Spain are lower than the suggested threshold tissue concentrations, above which Cd acute intoxication might be expected (40 µg/g w.w. in liver and 100 µg/g w.w. in kidney) (Furness 1996) (Table 5.1). The Cd concentrations in eggs are not believed to have caused adverse effects on reproductive success or shell thickness (González et al. 1984; Hernández et al. 1986, 1988a, 1988b; González and Hiraldo 1988). However, Cd exposure, even at low levels, seems to be associated with genotoxic effects and immunological stress in Black kites (Blanco et al. 2004; Baos et al. 2006b).

Comparatively with Pb and Cd, few studies have evaluated the exposure to Hg in raptors from Portugal and Spain. However, Hg has been a contaminant of concern because it accumulates in the tissues of wildlife species and can adversely affect their reproduction, especially in higher trophic level species (Wolfe et al. 1998; Scheuhammer et al. 2007; Tartu et al. 2013). Although Hg biomagnification in predatory birds is generally greater in aquatic than in terrestrial food webs, some studies have found large

489 accumulations of Hg in birds at the top of terrestrial trophic chains (Palma et al. 2005;
490 Figueira et al. 2009; Lourenço et al. 2011). Furthermore, the relatively high blood Hg
491 concentrations observed in the Peregrine falcon (south-west of Spain) and in the
492 Common buzzard (Portugal) (Alvárez et al. 2013; Carneiro et al. 2014) support the
493 hypothesis of the transport of aquatic Hg into the adjacent terrestrial food web, proposed
494 by Cristol et al. (2008). Although all mean blood-Hg levels detected in the studies
495 performed in Portugal and Spain were lower than the threshold that is reported as high
496 risk for avian piscivores (i.e. Common loon, *Gavia immer*) of 300 µg/dl (Evers et al.
497 2008), the sensitivity of the species to toxic elements varies considerably and toxicity
498 threshold have not yet been established for raptors. According to the Hg threshold level
499 of 3 µg/dl associated with alterations in antioxidant enzymes' activity (Espín et al. 2014a,
500 2014b), many birds could end up being susceptible to suffering sub-lethal effects related
501 with oxidative stress. Regarding Hg levels in feathers and eggs, Palma et al. (2005) found
502 a strong relationship between them, with feather levels of 4.1 µg/g w.w. corresponding to
503 eggs containing the benchmark of 1.0 µg/g w.w, which is the lowest concentration
504 associated with deformities of particularly sensitive embryos, though it is unlikely to
505 affect more than a small percentage of eggs (Heinz and Hoffman 2003). Therefore, it is
506 unlikely that Hg contamination can negatively affect the reproduction of the studied bird
507 populations. The initial research studies (1980s) carried out with eggs in the south-west
508 and central Spain, reached this same conclusion (González et al. 1984; Hernández et al.
509 1986, 1988a; González and Hiraldo 1988). However, sub-lethal effects are likely to occur
510 at concentrations far lower than those required to produce more pronounced pathological
511 effects (Scheuhammer 1987; Thompson 1996).

512 As was the least studied toxic element in raptors from Portugal and Spain perhaps
513 because, despite being a toxic metalloid, it has a low rate of bioaccumulation and it is not
514 biomagnified in the food chain (Eisler 1998; Hughes 2006). The As concentrations
515 obtained in the studied species could be considered as indicative of low and background
516 amounts, of no ecotoxicological concern (Blanco et al. 2003; Baos et al. 2006a, 2006b;
517 Pérez-López et al. 2008; Carneiro et al. 2014).

518

519 **2. Conclusion**

520 In conclusion, the analysis of toxic metals in different tissues of raptors has been an
521 important tool for assessing environmental contamination by these elements in Portugal
522 and Spain. Several studies using a range of sentinel raptor species have been carried out
523 since the 1980s. However, there is a striking difference between the number of studies
524 performed in Portugal and Spain, having been scarce in Portugal.

525 The Eagle owl, Black kite and Common buzzard were the species from which the
526 greatest number of individuals have been analysed. Due to conservation problems, the
527 Spanish Imperial eagle was also the subject of several biomonitoring studies. The study
528 of other raptor species that have demonstrated their usefulness in matters of conservation
529 and environmental risk assessment is recommended.

530 The studies performed have measured the metal concentrations in a variety of different
531 biological samples employing different analytical techniques. Blood was the type of
532 sample collected in the greatest number, followed by feathers, liver and bone. Feathers
533 have been frequently collected in recent studies to evaluate mainly Hg and Pb exposure.
534 Blood and liver were used to measure the concentrations of all studied metals in many
535 bird species, while bone was mainly collected to evaluate chronic Pb exposure.

536 Regarding analytical techniques, AAS has been the most frequently performed technique
537 to determine most of the metals and metalloids, mainly due to its relatively low cost and
538 good sensitivity. The high cost of ICP-MS has limited its application. However this
539 technique allows simultaneous multi-elemental analysis with higher sensitivity and it has
540 a high productivity, becoming comparatively economical when many samples and/or
541 elements need to be determined.

542 In general, the concentrations of metals detected in raptor samples from Portugal and
543 Spain are generally low and of an insufficient level to produce toxic side effects. Only Pb,
544 in certain cases, can be related to toxic side effects and it has been a limiting factor in the
545 conservation of some endangered species, such as the Pyrenean bearded vulture, Egyptian
546 vulture and Spanish imperial eagle. Although in Spain, the Griffon vulture has a positive
547 population trend, in Portugal Pb exposure could be a risk for the conservation of this
548 endangered species. Even though Pb has received the greatest level of concern among
549 researchers, more efforts are necessary to assess its risk to the conservation of other
550 endangered species and to human health. Regarding Hg, the transport of the aquatic into
551 the adjacent terrestrial food web, the lack of information concerning its exposure in many
552 raptor species and the need to establish the toxicity threshold Hg for raptors, support the
553 need for further biomonitoring studies of this metal.

554 The research carried out in Portugal and Spain has been sporadic in terms of both time
555 and space. Financial support has been the main limiting factor in implementing
556 biomonitoring studies with raptors on a large spatial and temporal scale. However, given
557 the toxicity of the metals and As, it is advisable to establish at a national scale, both in
558 Portugal and Spain, biomonitoring programmes using raptors in order to conduct long-
559 term studies that would link the survival of birds to their contaminant burdens. A network

560 needs to be developed between the two countries and between toxicologists, veterinarians
561 and ornithologists studying raptor populations to encompass analysis of samples collected
562 in WRCs and by ornithologists. Coordination between existing biomonitoring schemes in
563 other European countries and expansion of monitoring into other European countries is
564 also needed.

565

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1090 **Table 1.** Main characteristics (trophic position, diet, conservation status and migratory habits) of the raptor species breeding in Portugal and
 1091 Spain, specifying which ones were used for monitoring purposes of metals and the total number of analysed individuals.

Common name	Scientific name	Trophic position (Most important prey)	Conservation status PTSP		Migratory habits	Total number of analysed individuals	References
Accipitridae Family							
Northern goshawk	Accipiter gentilis	Bird and mammal predator (Red-legged partridge, Domestic pigeon, European rabbit) (Mañosa 1994; Petronillo and Vingada 2002; Catry et al. 2010)	VU	LC	Resident	117	Hernández et al. 1986; Mateo et al. 2003; Martínez-López et al. 2004, 2005; Hermoso de Mendoza et al. 2006; Pérez-López et al. 2008; Castro et al. 2011; Debén et al. 2012; Martínez et al. 2012
Eurasian sparrowhawk	Accipiter nisus	Bird predator (Passeriformes) (Mañosa and Oro 1991; Catry et al. 2010)	LC	LC/VU	Resident	22	García-Fernández et al. 1995, 1996; Hermoso de Mendoza et al. 2006; Pérez-López et al. 2008
Cinereous vulture	Aegypius monachus	Carrion consumer (Livestock carcasses) (Catry et al. 2010; Moreno-Opo et al. 2010)	CR	VU	Resident	18	Hernández et al. 1986; Hermoso de Mendoza et al. 2006; Cardiel et al. 2011
Spanish Imperial eagle	Aquila adalberti (heliaca)	Bird and mammal predator (European rabbit) (Sánchez et al. 2008; Catry et al. 2010)	CR	EN	Resident	257	González et al. 1984; Hernández et al. 1986, 1988a; González and Hiraldo 1988; Mateo et al. 2003; Pain et al. 2005; Rodríguez-Ramos et al. 2011
Golden eagle	Aquila chrysaetos	Bird and mammal predator/Carrion consumer (European rabbit) (Catry et al. 2010; Sánchez-Zapata et al. 2010)	EN	NT	Resident	8	Hernández et al. 1988b; Cerradelo et al. 1992; García-Fernández et al. 1996; Mateo et al. 2003
Common buzzard	Buteo buteo	Bird and mammal predator (Small mammals) (Mañosa and Cordero 1992; Tapia et al. 2007; Catry et al. 2010)	LC	LC	Resident	428	Hernández et al. 1988b; García-Fernández et al. 1995, 1996, 1997; Mateo et al. 2003; Martínez-López et al. 2004, 2005; Hermoso de Mendoza et al. 2006; Pérez-López et al. 2008; Castro et al. 2011; Martínez et al. 2012; Carneiro et al. 2014

1092 **Table 1.** (Continued) Main characteristics (trophic position, diet, conservation status and migratory habits) of the raptor species breeding in
1093 Portugal and Spain, specifying which ones were used for monitoring purposes of metals and the total number of analysed individuals.

Common name	Scientific name	Trophic position (Most important prey)	Conservation status		Migratory habits	Total number of analysed individuals	References
			PT	SP			
Accipitridae Family							
Short-toed Eagle	<i>Circetus gallicus</i>	Predator (Reptiles: snakes and lizards) (Gil and Pleguezuelos 2001; Catry et al. 2010)	NT	LC	Reproductive/ Migratory	3	García-Fernández et al. 1996
Marsh harrier	<i>Circus aeruginosus</i>	Bird and mammal predator (Small mammals and birds) (Catry et al. 2010; Cardador et al. 2012)	VU	LC	Resident/ Visitor	79	Hernández et al. 1986; Mateo et al. 1999; Álvarez et al. 2013
Hen harrier	<i>Circus cyaneus</i>	Bird and mammal predator (Lagomorphs and birds)(García and Arroyo 2005; Catry et al. 2010)	CR/ VU	LC	Resident/ Visitor	-	-
Montagu´s harrier	<i>Circus pygargus</i>	Predator (Lagomorphs, birds, insects) (Arroyo 1997; Catry et al. 2010)	EN	VU	Reproductive Migratory	10	García-Fernández et al. 1996; Hermoso de Mendoza et al. 2006
Black-winged kite	<i>Elanus caeruleus</i>	Predator (Small mammals, birds, reptiles, insects)(Catry et al. 2010)	NT	NT	Resident	-	-
Bearded vulture	<i>Gypaetus barbatus</i>	Carrion consumer (Bones of wild and domestic ungulates) (Margalida et al. 2005, 2007; Hernández and Margalida 2009)	RE	EN	Resident	136	Hernández and Margalida 2009; Mateo et al. 2015
Griffon vulture	<i>Gyps fulvus</i>	Carrion consumer (Wild and domestic ungulates) (Donázar 1993; Catry et al. 2010; Donázar et al. 2010; Moreno-Opo et al. 2015)	NT	LC	Resident	245	García-Fernández et al. 1995, 2005b; Mateo et al. 2003; Cardiel et al. 2011; Espín et al. 2014a; Carneiro et al. 2015
Bonelli´s eagle	<i>Hieraetus fasciatus</i>	Bird and mammal predator (Rabbits, pigeons, partridges) (Moleón et al. 2007; Catry et al. 2010)	EN	EN	Resident	154	García-Fernández et al. 1995, 1996; Mateo et al. 2003; Palma et al. 2005; Figueira et al. 2009; Ortiz-Santaliestra et al. 2015
Booted eagle	<i>Hieraetus pennatus</i>	Bird and mammal predator (Songbirds) (García Dios 2006; Catry et al. 2010)	NT	LC	Reproductive Migratory	144	González et al. 1984; Hernández et al. 1988b; García-Fernández et al. 1995, 1996; Mateo et al. 2003; Martínez-López et al. 2004, 2005

1094 **Table 1.** (Continued) Main characteristics (trophic position, diet, conservation status and migratory habits) of the raptor species breeding in
 1095 Portugal and Spain, specifying which ones were used for monitoring purposes of metals and the total number of analysed individuals.

Common name	Scientific name	Trophic position (Most important prey)	Conservation status		Migratory habits	Total number of analysed individuals	References
			PT	SP			
Accipitridae Family							
Black kite	<i>Milvus migrans</i>	Predator/Carrion consumer (European rabbits, birds, fish, carrion) (Viñuela and Veiga 1992; Catry et al. 2010)	LC	LC	Reproductive Migratory	428	González et al. 1984; Hernández et al. 1986, 1988b; García-Fernández et al. 1996; Benito et al. 1999; Blanco et al. 2003; Mateo et al. 2003; Baos et al. 2006a, 2006b; Cardiel et al. 2011; Álvarez et al. 2013
Red kite	<i>Milvus milvus</i>	Predator/Carrion consumer (Small mammals, birds, fish, carrion) (García et al. 1998; Catry et al. 2010)	CR	EN	Resident/ Visitor	37	González et al. 1984; Mateo et al. 2003; Hermoso de Mendoza et al. 2006; Cardiel et al. 2011
Egyptian vulture	<i>Neophron percnopterus</i>	Carrion consumer (Carcasses of mammals and birds, garbage dumps) (Donázar et al. 2002; Hidalgo et al. 2005; Margalida et al. 2012)	EN	EN	Reproductive Migratory	234	Donázar et al. 2002; Gangoso et al. 2009
Honey buzzard	<i>Pernis apivorus</i>	Invertebrate predator (Larvae of hymenopterans) (Catry et al. 2010)	VU	LC	Reproductive Migratory	-	-
Pandionidae family							
Osprey	<i>Pandion haliaetus</i>	Fish predator (Fish) (Catry et al. 2010; Siverio et al. 2011)	CR	EN	Resident/ Visitor	-	-
Falconidae family							
Eleonora's falcon	<i>Falco eleonarae</i>	Predator (Insects) (Madroño et al. 2004)	RE	NT	Reproductive migratory	-	-
Lesser kestrel	<i>Falco naumanni</i>	Predator (Insects) (Madroño et al. 2004; Catry et al. 2010; Pérez-Granados 2010)	VU	VU	Reproductive Migratory	41	Negro et al. 1993
Hobby	<i>Falco subbuteo</i>	Bird and invertebrate predator (Small birds and insects) (Madroño et al. 2004; Catry et al. 2010)	VU	NT	Reproductive Migratory	7	González et al. 1984

1096 **Table 1.** (Continued) Main characteristics (trophic position, diet, conservation status and migratory habits) of the raptor species breeding in
1097 Portugal and Spain, specifying which ones were used for monitoring purposes of metals and the total number of analysed individuals.

Common name	Scientific name	Trophic position (Most important prey)	Conservation status		Migratory habits	Total number of individuals analysed	References
			PT	SP			
Falconidae family							
Barbary falcon	<i>Falco pelegrinoides</i>	Bird predator (Domestic pigeon) (Madroño et al. 2004)	I	EN	Resident	-	-
Peregrine falcon	<i>Falco peregrinus</i>	Bird predator (Domestic pigeon) (López-López et al. 2009)	VU	LC	Resident	18	Hernández et al. 1988b; Mateo et al. 2003; Álvarez et al. 2013
Common kestrel	<i>Falco tinnunculus</i>	Predator (Insects, rodents, birds) (Gil-Delgado et al. 1995; Catry et al. 2010)	LC	LC	Resident	94	García-Fenández et al. 1995,1996, 1997, 2005a; Hermoso de Mendoza et al. 2006; Pérez-López et al. 2008; Lourenço et al. 2011
Strigidae family							
Tengmalm's owl	<i>Aegolius funereus</i>	Mammal predator (Voles) (Korpimaki 1988)	-	NT	Resident	-	-
Short-eared owl	<i>Asio flammeus</i>	Bird and mammal predator ¹ (Small mammals, birds, insects) (Madroño et al. 2004; Catry et al. 2010)	EN	NT	Visitor/ Reproductive	-	-
Long-eared owl	<i>Asio otus</i>	Mammal predator (Small mammals) (Delibes et al. 1984; Catry et al. 2010)	DD	LC	Resident	5	García-Fernández et al. 1996; Pérez-López et al. 2008
Little owl	<i>Athene noctua</i>	Predator (Insects, small mammals) (Delibes et al. 1984; Tomé et al. 2008; Catry et al. 2010)	LC	LC	Resident	52	García-Fernández et al. 1995, 1996, 1997; Pérez-López et al. 2008; Lourenço et al. 2011
Eagle owl	<i>Bubo bubo</i>	Bird and mammal predator (European rabbit) (Catry et al. 2010; Gómez-Ramírez et al. 2010)	NT	LC	Resident	979	García-Fenández et al. 1995, 1996, 1997; Mateo et al. 2003; Ortego et al. 2006; Gómez-Ramírez et al. 2010; Lourenço et al. 2011; Espín et al. 2014b, 2014c
Scops owl	<i>Otus scops</i>	Predator (Insects) (Catry et al. 2010)	DD	LC	Reproductive Migratory	1	García-Fernández et al. 1996

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1099 **Table 1.** (Continued) Main characteristics (trophic position, diet, conservation status and migratory habits) of the raptor species breeding in
 1100 Portugal and Spain, specifying which ones were used for monitoring purposes of metals and the total number of analysed individuals.

Common name	Scientific name	Trophic position (Most important prey)	Conservation status		Migratory habits	Total number of individuals analysed	References
			PT	SP			
Strigidae family							
Tawny owl	<i>Strix aluco</i>	Predator (Invertebrates, rodents) (Villarán Adánez 2000; Catry et al. 2010)	LC	LC	Resident	74	Pérez-López et al. 2008; Castro et al. 2011; Lourenço et al. 2011; Debén et al. 2012
Tytonidae family							
Barn owl	<i>Tyto alba</i>	Predator (Rodents)(Gigirey et al. 2004; Catry et al. 2010)	LC	LC	Resident	33	García-Fernández et al. 1996; Pérez-López et al. 2008; Lourenço et al. 2011

1101 PT (Portugal), SP (Spain), I (Indeterminate), DD (Data deficient), LC (Least concern), NT (Near threatened), VU (Vulnerable), EN (Endangered), CR (Critically
 1102 endangered), RE (Regionally extinct)

1104 **Table 2.** Sampling periods and bird species studied by each monitored area of Portugal and Spain.

Areas	Sampling Periods	Species (n)
North-west, Spain	1997-2005 ^(Castro et al. 2011)	Common buzzard (n=146), Tawny owl (n=71), Northern goshawk (n=81), Barn owl (n=16), Eurasian sparrowhawk (n=16), Bonelli's eagle (n=11), Common kestrel (n=6), Long-eared owl (n=4), Little owl (n=3)
	1999-2005 ^(Hermoso de Mendoza et al. 2006)	
	2001-2009 ^(Martínez et al. 2012)	
	2010-2011 ^(Ortiz-Santaliestra et al. 2015)	
North-east, Spain	UP* ^(Pérez-López et al. 2008; Debén et al. 2012)	Bearded vulture (n=136), Bonelli's eagle (n=85)†, Common buzzard (n=53)†, Griffon vulture (n=52)†, Marsh harrier (n=47), Eagle owl (n=21)†, Northern goshawk (n=9)†, Black kite (n=8)†, Red kite (n=6)†, Booted eagle (n=5)†, Egyptian vulture (n=10), Peregrine falcon (n=4)†, Golden eagle (n=3)†, Spanish Imperial eagle (n=1)†
	1990-1991 ^(Cerradillo et al. 1992)	
	1992-1995 ^(Mateo et al. 1999)	
	1998-2001 ^{(Mateo et al. 2003)†}	
	2002 ^(Gangoso et al. 2009)	
	2004-2013 ^(Mateo et al. 2015)	
	2008 ^(Hernández and Margalida 2009)	
West, Spain	2010-2011 ^(Ortiz-Santaliestra et al. 2015)	Montagu's harrier (n=8), Cinereous vulture (n=6), Griffon vulture (n=5), Red kite (n=5), Common buzzard (n=3)
	2010-2012 ^(Carneiro et al. 2015)	
Centre, Spain	1979-1984 ^(González and Hiraldo 1988)	Spanish Imperial eagle (n=127)†, Black kite (n=91)†, Common buzzard (n=56)†, Eagle owl (n=53)†, Egyptian vulture (n=19), Marsh harrier (n=17), Northern goshawk (n=11)†, Booted eagle (n=9)†, Peregrine falcon (n=8)†, Red kite (n=6)†, Golden eagle (n=4)†, Bonelli's eagle (n=3)†, Griffon vulture (n=2)†
	1982-1985 ^(Hernández et al. 1986)	
	1985-1986 ^(Hernández et al. 1988b)	
	1986-1987 ^(Hernández et al. 1988a)	
	1997-2008 ^(Rodríguez-Ramos et al. 2011)	
	1998-2001 ^{(Mateo et al. 2003)†}	
	2002-2003 ^(Ortego et al. 2006)	
	2003-2004 ^(Gangoso et al. 2009)	
East, Spain	2001 ^(Blanco et al. 2003)	Griffon vulture (n=66)
	2011 ^(Espin et al. 2014a)	
South, Spain	1980-1999 ^(Pain et al. 2005)	Spanish Imperial eagle (n=75), Lesser kestrel (n=41), Bonelli's Eagle (n=30)
	1988-1991 ^(Negro et al. 1993)	
	2010-2011 ^(Ortiz-Santaliestra et al. 2015)	
South-west, Spain	1980-1983 ^(González et al. 1984)	Black kite (n=318), Spanish Imperial eagle (n=54), Eagle owl (n=30)†, Marsh harrier (n=15), Red kite (n=10), Booted eagle (n=7), Hobby (n=7), Little owl (n=7)†, Barn owl (n=6)†, Peregrine falcon (n=6), Egyptian vulture (n=3), Common buzzard (n=2), Tawny owl (n=1)†
	1982-1985 ^(Hernández et al. 1986)	
	1985-1986 ^(Hernández et al. 1988b)	
	1986-1987 ^(Hernández et al. 1988a)	
	1999 ^(Benito et al. 1999)	
	1999-2000 ^(Alvárez et al. 2013)	
	1999, 2001-2003 ^(Baos et al. 2006a)	
	1999-2002 ^(Baos et al. 2006b)	
	2003 ^(Gangoso et al. 2009)	
South-east, Spain	2003-2007 ^{(Lourenço et al. 2011)†}	Eagle owl (n=844), Booted eagle (n=123), Common kestrel (n=87), Common buzzard (n=43), Little owl (n=34), Griffon vulture (n=29), Northern goshawk (n=16), Eurasian sparrowhawk (n=6), Barn owl (n=4), Bonelli's eagle (n=4), Short-toed eagle (n=3), Black kite (n=2), Montagu's harrier (n=2), Golden eagle (n=1), Long-eared owl (n=1), Scops owl (n=1)
	1993 ^(García-Fernández et al. 1995, 1996)	
	1994 ^(García-Fernández et al. 1997)	
	1995-1997, 2001 ^(García-Fernández et al. 2005a)	
	1999-2000 ^(Martínez-López et al. 2004, 2005)	
	2003 ^(García-Fernández et al. 2005b)	
	2003-2007 ^(Gómez-Ramírez et al. 2010)	
	2006-2012 ^(Espin et al. 2014c)	
Balearic Islands, Spain	2011-2012 ^(Espin et al. 2014b)	
Balearic Islands, Spain	1982-1985 ^(Hernández et al. 1986)	Cinereous vulture (n=9)
Canary Islands, Spain	1998-2001 ^(Donazar et al. 2002)	Egyptian vulture (n=191)
	1999-2005 ^(Gangoso et al. 2009)	
Portugal	1992-2001 ^(Palma et al. 2005; Figueira et al. 2009)	Common buzzard (n=125), Griffon vulture (n=71), Eagle owl (n=31)*, Bonelli's eagle (n=21), Little owl (n=8)*, Barn owl (n=7)*, Tawny owl (n=2)*, Common kestrel (n=1)
	2003-2007 ^{(Lourenço et al. 2011)†}	
	2007-2012 ^(Carneiro et al. 2014, 2015)	

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1106 **Table 2.** (Continued) Sampling periods and bird species studied by each monitored area of
1107 Portugal and Spain.

1108
1109 *UP (unknown period), †The author does not provide the number of species collected in each area and thus an
1110 estimate (mean) was performed.

1111 **NOTE:** The author and their collaborators, Cardiel et al. 2011 does not specify the sampling area and period for the
1112 species Griffon vulture (n=20), Cinereous vulture (n=3), Black kite (n=9) and Red kite (n=10).

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1113 **Table 3.** The metals and metalloids measured in each raptor species from Portugal and Spain *per* sample type and the total number (n) of the
1114 different samples analysed for each species.

	Blood (n=2087)	Liver (n=594)	Kidney (n=318)	Brain (n=193)	Bone (n=593)	Feathers (n=884)	Eggs (n=268)
Northern goshawk	Cd ^(Martínez-López et al. 2005) Pb ^(Martínez-López et al. 2004) (n=6)	As ^(Hermoso de Mendoza et al. 2006; Pérez-López et al. 2008) Cd ^(Hermoso de Mendoza et al. 2006; Pérez-López et al. 2008; Castro et al. 2011) Hg ^(Castro et al. 2011) Pb ^(Hermoso de Mendoza et al. 2006; Pérez-López et al. 2008; Castro et al. 2011) Zn ^(Hermoso de Mendoza et al. 2006; Pérez-López et al. 2008) (n=22)	Cd ^(Castro et al. 2011) Hg ^(Castro et al. 2011) Pb ^(Castro et al. 2011) (n=16)		As ^(Mateo et al. 2003) Pb ^(Mateo et al. 2003) (n=18)	Cd ^(Martínez-López et al. 2005; Castro et al. 2011) Hg ^(Castro et al. 2011; Martínez et al. 2012) Pb ^(Martínez-López et al. 2004; Castro et al. 2011; Debén et al. 2012) (n=36)	Cd ^(Hernández et al. 1986) Cu ^(Hernández et al. 1986) Hg ^(Hernández et al. 1986) Pb ^(Hernández et al. 1986) Zn ^(Hernández et al. 1986) (n=2)
Eurasian sparrowhawk	Cd ^(García-Fernández et al. 1996) (n=1)	As ^(Hermoso de Mendoza et al. 2006; Pérez-López et al. 2008) Cd ^(García-Fernández et al. 1995, 1996; Hermoso de Mendoza et al. 2006; Pérez-López et al. 2008) Pb ^(García-Fernández et al. 1995; Hermoso de Mendoza et al. 2006; Pérez-López et al. 2008) Zn ^(Hermoso de Mendoza et al. 2006; Pérez-López et al. 2008) (n=25)	Cd ^(García-Fernández et al. 1995, 1996) Pb ^(García-Fernández et al. 1995) (n=9)	Cd ^(García-Fernández et al. 1995, 1996) Pb ^(García-Fernández et al. 1995) (n=9)	Cd ^(García-Fernández et al. 1995, 1996) Pb ^(García-Fernández et al. 1995) (n=9)		
Cinereous vulture		As ^(Hermoso de Mendoza et al. 2006) Cd ^(Hermoso de Mendoza et al. 2006) Pb ^(Hermoso de Mendoza et al. 2006) Zn ^(Hermoso de Mendoza et al. 2006) (n=6)			Pb ^(Cardiel et al. 2011) (n=3)	Al ^(Cardiel et al. 2011) Pb ^(Cardiel et al. 2011) (n=3)	Cd ^(Hernández et al. 1986) Cu ^(Hernández et al. 1986) Hg ^(Hernández et al. 1986) Pb ^(Hernández et al. 1986) Zn ^(Hernández et al. 1986) (n=9)

1115 **Table 3.** (Continued) The metals and metalloids measured in each raptor species from Portugal and Spain *per* sample type and the total number
 1116 (n) of the different samples analysed for each species.

	Blood (n=2087)	Liver (n=594)	Kidney (n=318)	Brain (n=193)	Bone (n=593)	Feathers (n=884)	Eggs (n=268)
Cinereous vulture		As ^(Hermoso de Mendoza et al. 2006) Cd ^(Hermoso de Mendoza et al. 2006) Pb ^(Hermoso de Mendoza et al. 2006) Zn ^(Hermoso de Mendoza et al. 2006) (n=6)			Pb ^(Cardiel et al. 2011) (n=3)	Al ^(Cardiel et al. 2011) Pb ^(Cardiel et al. 2011) (n=3)	Cd ^(Hernández et al. 1986) Cu ^(Hernández et al. 1986) Hg ^(Hernández et al. 1986) Pb ^(Hernández et al. 1986) Zn ^(Hernández et al. 1986) (n=9)
Spanish Imperial eagle		Pb ^(Rodríguez-Ramos et al. 2011) (n=15)			As ^(Mateo et al. 2003) Pb ^(Mateo et al. 2003; Pain et al. 2005; Rodríguez-Ramos et al. 2011) (n=90)	Pb ^(Pain et al. 2005; Rodríguez-Ramos et al. 2011) (n=195)	Cd ^(González et al. 1984; Hernández et al. 1986, 1988a; González and Hiraldo 1988) Cu ^(González et al. 1984; Hernández et al. 1986, 1988a; González and Hiraldo 1988) Hg ^(González et al. 1984; Hernández et al. 1986; González and Hiraldo 1988) Pb ^(González et al. 1984; Hernández et al. 1986, 1988a; González and Hiraldo 1988) Zn ^(González et al. 1984; Hernández et al. 1986, 1988a; González and Hiraldo 1988) (n=95)
Golden eagle	Cd ^(García-Fernández et al. 1996) (n=1)	Cd ^(García-Fernández et al. 1996) Pb ^(Cerradelo et al. 1992) (n=4)	Cd ^(García-Fernández et al. 1996) Pb ^(Cerradelo et al. 1992) (n=4)	Cd ^(García-Fernández et al. 1996) (n=1)	As ^(Mateo et al. 2003) Cd ^(García-Fernández et al. 1996) Pb ^(Mateo et al. 2003) (n=4)		Cd ^(Hernández et al. 1988b) Cu ^(Hernández et al. 1988b) Hg ^(Hernández et al. 1988b) Pb ^(Hernández et al. 1988b) Zn ^(Hernández et al. 1988b) (n=3)

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1118 **Table 3.** (Continued) The metals and metalloids measured in each raptor species from Portugal and Spain *per* sample type and the total number
1119 (n) of the different samples analysed for each species.

	Blood (n=2087)	Liver (n=594)	Kidney (n=318)	Brain (n=193)	Bone (n=593)	Feathers (n=884)	Eggs (n=268)
Common buzzard		As ^(Hermoso de Mendoza et al. 2006; Pérez-López et al. 2008; Carneiro et al. 2014)					
		Cd ^(García-Fernández et al. 1995, 1996; Hermoso de Mendoza et al. 2006; Pérez-López et al. 2008; Castro et al. 2011; Carneiro et al. 2014)	As ^(Carneiro et al. 2014)		As ^(Mateo et al. 2003)	Cd ^(Martínez-López et al. 2005; Castro et al. 2011)	Cd ^(Hernández et al. 1988b)
	As ^(Carneiro et al. 2014)		Cd ^(García-Fenández et al. 1995, 1996; Castro et al. 2011; Carneiro et al. 2014)	Cd ^(García-Fernández et al. 1995, 1996)	Cd ^(García-Fernández et al. 1995, 1996)	Hg ^(Castro et al. 2011; Martínez et al. 2012)	Cu ^(Hernández et al. 1988b)
	Cd ^(García-Fernández et al. 1995, 1996; Martínez-López et al. 2005; Carneiro et al. 2014)	Hg ^(Castro et al. 2011; Carneiro et al. 2014)	Hg ^(Castro et al. 2011; Carneiro et al. 2014)	Pb ^(García-Fernández et al. 1995, 1997)	Pb ^(García-Fernández et al. 1995, 1997; Mateo et al. 2003)	Pb ^(Martínez-López et al. 2004; Castro et al. 2011)	Hg ^(Hernández et al. 1988b)
Short-toed eagle	Pb ^(García-Fernández et al. 1995, 1997; Martínez-López et al. 2004; Carneiro et al. 2014)	Pb ^(García-Fernández et al. 1995, 1997; Hermoso de Mendoza et al. 2006; Pérez-López et al. 2008; Castro et al. 2011; Carneiro et al. 2014)	Pb ^(García-Fernández et al. 1995, 1997; Castro et al. 2011)	(n=17)	(n=120)	(n=56)	Pb ^(Hernández et al. 1988b)
	(n=113)	Zn ^(Hermoso de Mendoza et al. 2006; Pérez-López et al. 2008)	(n=91)				Zn ^(Hernández et al. 1988b)
		(n=191)					(n=4)
	Cd ^(García-Fernández et al. 1996)	Cd ^(García-Fernández et al. 1996)	Cd ^(García-Fernández et al. 1996)	Cd ^(García-Fernández et al. 1996)	Cd ^(García-Fernández et al. 1996)		
Marsh harrier	(n=2)	(n=3)	(n=3)	(n=3)	(n=2)		
							Cd ^(Hernández et al. 1986)
	Hg ^(Alvárez et al. 2013)						Cu ^(Hernández et al. 1986)
	Pb ^(Mateo et al. 1999)						Hg ^(Hernández et al. 1986)
	Se ^(Alvárez et al. 2013)						Pb ^(Hernández et al. 1986)
	(n=52)						Zn ^(Hernández et al. 1986)
							(n=17)

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1122 **Table 3.** (Continued) The metals and metalloids measured in each raptor species from Portugal and Spain *per* sample type and the total number

1123 (n) of the different samples analysed for each species.

	Blood (n=2087)	Liver (n=594)	Kidney (n=318)	Brain (n=193)	Bone (n=593)	Feathers (n=884)	Eggs (n=268)
Montagu's harrier	Cd ^(García-Fernández et al. 1996) (n=1)	As ^(Hermoso de Mendoza et al. 2006) Cd ^(García-Fernández et al. 1996; Hermoso de Mendoza et al. 2006) Pb ^(Hermoso de Mendoza et al. 2006) Zn ^(Hermoso de Mendoza et al. 2006) (n=9)	Cd ^(García-Fernández et al. 1996) (n=1)	Cd ^(García-Fernández et al. 1996) (n=1)	Cd ^(García-Fernández et al. 1996) (n=1)		
Bearded vulture	Pb ^(Hernández and Margalida 2009; Mateo et al. 2015) (n=101)	Pb ^(Hernández and Margalida 2009; Mateo et al. 2015) (n=30)			Pb ^(Hernández and Margalida 2009; Mateo et al. 2015) (n=43)		
Griffon vulture	Cd ^(García-Fernández et al. 1995; Espín et al. 2014a; Carneiro et al. 2015) Cu ^(Espín et al. 2014a) Hg ^(Espín et al. 2014a; Carneiro et al. 2015) Pb ^(García-Fernández et al. 1995, 2005b; Espín et al. 2014a; Carneiro et al. 2015) Zn ^(Espín et al. 2014a) (n=216)	As ^(Hermoso de Mendoza et al. 2006) Cd ^(Hermoso de Mendoza et al. 2006) Pb ^(Hermoso de Mendoza et al. 2006) Zn ^(Hermoso de Mendoza et al. 2006) (n=5)			As ^(Mateo et al. 2003) Pb ^(Mateo et al. 2003; Cardiel et al. 2011) (n=24)	Al ^(Cardiel et al. 2011) Pb ^(Cardiel et al. 2011) (n=20)	
Bonelli's eagle	As ^(Ortiz-Santaliestra et al. 2015) Cd ^(García-Fernández et al. 1995, 1996; Ortiz-Santaliestra et al. 2015) Cu ^(Ortiz-Santaliestra et al. 2015) Hg ^(Ortiz-Santaliestra et al. 2015) Pb ^(García-Fernández et al. 1995; Ortiz-Santaliestra et al. 2015) Zn ^(Ortiz-Santaliestra et al. 2015) (n=127)				As ^(Mateo et al. 2003) Pb ^(Mateo et al. 2003) (n=6)	Hg ^(Palma et al. 2005; Figueira et al. 2009) (n=21)	

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1125 **Table 3.** (Continued) The metals and metalloids measured in each raptor species from Portugal and Spain *per* sample type and the total number
1126 (n) of the different samples analysed for each species.

	Blood (n=2087)	Liver (n=594)	Kidney (n=318)	Brain (n=193)	Bone (n=593)	Feathers (n=884)	Eggs (n=268)
Booted eagle	Cd (García-Fernández et al. 1995, 1996; Martínez-López et al. 2005) Pb (García-Fernández et al. 1995; Martínez-López et al. 2004) (n=58)	Cd (García-Fernández et al. 1995, 1996) Pb (García-Fernández et al. 1995) (n=8)	Cd (García-Fernández et al. 1995, 1996) Pb (García-Fernández et al. 1995) (n=7)	Cd (García-Fernández et al. 1995, 1996) Pb (García-Fernández et al. 1995) (n=7)	As (Mateo et al. 2003) Cd (García-Fernández et al. 1996) Pb (Mateo et al. 2003) (n=17)	Cd (Martínez-López et al. 2005) Pb (Martínez-López et al. 2004) (n=60)	Cd (González et al. 1984; Hernández et al. 1988b) Cu (González et al. 1984; Hernández et al. 1988b) Hg (González et al. 1984; Hernández et al. 1988b) Pb (González et al. 1984; Hernández et al. 1988b) Zn (González et al. 1984; Hernández et al. 1988b) (n=7)
Black kite	As (Benito et al. 1999; Blanco et al. 2003; Baos et al. 2006a, 2006b) Cd (García-Fernández et al. 1996; Benito et al. 1999; Blanco et al. 2003; Baos et al. 2006a, 2006b) Co (Benito et al. 1999) Cu (Benito et al. 1999; Blanco et al. 2003; Baos et al. 2006a, 2006b) Hg (Alvárez et al. 2013) Pb (Benito et al. 1999; Blanco et al. 2003; Baos et al. 2006a, 2006b) Se (Alvárez et al. 2013) Sb (Benito et al. 1999) Zn (Benito et al. 1999; Blanco et al. 2003; Baos et al. 2006a, 2006b) Tl (Benito et al. 1999) (n=334)	Cd (García-Fernández et al. 1996) (n=2)	Cd (García-Fernández et al. 1996) (n=2)	Cd (García-Fernández et al. 1996) (n=2)	As (Mateo et al. 2003) Cd (García-Fernández et al. 1996) Pb (Mateo et al. 2003; Cardiel et al. 2011) (n=31)	Al (Cardiel et al. 2011) Pb (Cardiel et al. 2011) (n=9)	As (Blanco et al. 2003) Cd (González et al. 1984; Hernández et al. 1986, 1988b; Blanco et al. 2003) Cu (González et al. 1984; Hernández et al. 1986, 1988b; Blanco et al. 2003) Hg (González et al. 1984; Hernández et al. 1986, 1988b) Pb (González et al. 1984; Hernández et al. 1986, 1988b; Blanco et al. 2003) Zn (González et al. 1984; Hernández et al. 1986, 1988b; Blanco et al. 2003) (n=70)

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1128 **Table 3.** (Continued) The metals and metalloids measured in each raptor species from Portugal and Spain *per* sample type and the total number
 1129 (n) of the different samples analysed for each species.

	Blood (n=2087)	Liver (n=594)	Kidney (n=318)	Brain (n=193)	Bone (n=593)	Feathers (n=884)	Eggs (n=268)
Red Kite		As ^(Hermoso de Mendoza et al. 2006) Cd ^(Hermoso de Mendoza et al. 2006) Pb ^(Hermoso de Mendoza et al. 2006) Zn ^(Hermoso de Mendoza et al. 2006) (n=5)			As ^(Mateo et al. 2003) Pb ^(Mateo et al. 2003; Cardiel et al. 2011) (n=22)	Al ^(Cardiel et al. 2011) Pb ^(Cardiel et al. 2011) (n=10)	Cd ^(González et al. 1984) Cu ^(González et al. 1984) Hg ^(González et al. 1984) Pb ^(González et al. 1984) Zn ^(González et al. 1984) (n=10)
Egyptian vulture	As ^(Donázar et al. 2002) Cd ^(Donázar et al. 2002) Cu ^(Donázar et al. 2002) Pb ^(Donázar et al. 2002; Gangoso et al. 2009) Zn ^(Donázar et al. 2002) (n=195)				Pb ^(Gangoso et al. 2009) (n=39)		
Lesser kestrel							Cd ^(Negro et al. 1993) Cu ^(Negro et al. 1993) Hg ^(Negro et al. 1993) Pb ^(Negro et al. 1993) Zn ^(Negro et al. 1993) (n=41)
Hobby							Cd ^(González et al. 1984) Cu ^(González et al. 1984) Hg ^(González et al. 1984) Pb ^(González et al. 1984) Zn ^(González et al. 1984) (n=7)

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1131 **Table 3.** (Continued) The metals and metalloids measured in each raptor species from Portugal and Spain *per* sample type and the total number
1132 (n) of the different samples analysed for each species.

	Blood (n=2087)	Liver (n=594)	Kidney (n=318)	Brain (n=193)	Bone (n=593)	Feathers (n=884)	Eggs (n=268)
Peregrine falcon	Hg ^(Alvárez et al. 2013) Se ^(Alvárez et al. 2013) (n=6)				As ^(Mateo et al. 2003) Pb ^(Mateo et al. 2003) (n=9)		Cd ^(Hernández et al. 1988b) Cu ^(Hernández et al. 1988b) Hg ^(Hernández et al. 1988b) Pb ^(Hernández et al. 1988b) Zn ^(Hernández et al. 1988b) (n=3)
Common kestrel	Cd ^(García-Fernández et al. 1995, 1996) Pb ^(García-Fernández et al. 1995, 1997) (n=26)	As ^(Hermoso de Mendoza et al. 2006; Pérez-López et al. 2008) Cd ^(García-Fernández et al. 1995, 1996; Hermoso de Mendoza et al. 2006; Pérez-López et al. 2008) Pb ^(García-Fenández et al. 1995, 1997, 2005a; Hermoso de Mendoza et al. 2006; Pérez-López et al. 2008) Zn ^(Hermoso de Mendoza et al. 2006; Pérez-López et al. 2008) (n=93)	Cd ^(García-Fenández et al. 1995, 1996) Pb ^(García-Fernández et al. 1995, 1997, 2005a) (n=85)	Cd ^(García-Fernández et al. 1995, 1996) Pb ^(García-Fernández et al. 1995, 1997, 2005a) (n=87)	Cd ^(García-Fenández et al. 1995, 1996) Pb ^(García-Fenández et al. 1995, 1997, 2005a) (n=72)	Hg ^(Lourenço et al. 2011) (n=1)	
Little owl	Cd ^(García-Fernández et al. 1995, 1996) Pb ^(García-Fernández et al. 1995, 1997) (n=11)	As ^(Pérez-López et al. 2008) Cd ^(García-Fenández et al. 1995, 1996; Pérez-López et al. 2008) Pb ^(García-Fenández et al. 1995, 1997; Pérez-López et al. 2008) Zn ^(Pérez-López et al. 2008) (n=64)	Cd ^(García-Fernández et al. 1995, 1996) Pb ^(García-Fernández et al. 1995, 1997) (n=34)	Cd ^(García-Fenández et al. 1995, 1996) Pb ^(García-Fenández et al. 1995, 1997) (n=34)	Cd ^(García-Fenández et al. 1995, 1996) Pb ^(García-Fenández et al. 1995, 1997) (n=19)	Hg ^(Lourenço et al. 2011) (n=15)	
Long-eared owl		As ^(Pérez-López et al. 2008) Cd ^(García-Fernández et al. 1996; Pérez-López et al. 2008) Pb ^(Pérez-López et al. 2008) Zn ^(Pérez-López et al. 2008) (n=6)	Cd ^(García-Fernández et al. 1996) (n=1)	Cd ^(García-Fenández et al. 1996) (n=1)	Cd ^(García-Fenández et al. 1996) (n=1)		

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1134 **Table 3.** (Continued) The metals and metalloids measured in each raptor species from Portugal and Spain *per* sample type and the total number

1135 (n) of the different samples analysed for each species.

	Blood (n=2087)	Liver (n=594)	Kidney (n=318)	Brain (n=193)	Bone (n=593)	Feathers (n=884)	Eggs (n=268)
Eagle Owl	Cd ^(García-Fenández et al. 1995, 1996; Espín et al. 2014b) Cu ^(Espín et al. 2014b) Hg ^(Espín et al. 2014b, 2014c) Pb ^(García-Fenández et al. 1995, 1997; Gómez-Ramírez et al. 2010; Espín et al. 2014b) Zn ^(Espín et al. 2014b) (n=834)	Cd ^(García-Fenández et al. 1995, 1996) Pb ^(García-Fenández et al. 1995, 1997) (n=26)	Cd ^(García-Fenández et al. 1995, 1996) Pb ^(García-Fenández et al. 1995, 1997) (n=26)	Cd ^(García-Fenández et al. 1995, 1996) Pb ^(García-Fenández et al. 1995, 1997) (n=26)	As ^(Mateo et al. 2003) Cd ^(García-Fenández et al. 1995, 1996) Pb ^(García-Fenández et al. 1995, 1997; Mateo et al. 2003) (n=58)	Hg ^(Ortego et al. 2006; Lourenço et al. 2011; Espín et al. 2014c) (n=322)	
Scops Owl		Cd ^(García-Fenández et al. 1996) (n=1)	Cd ^(García-Fenández et al. 1996) (n=1)	Cd ^(García-Fenández et al. 1996) (n=1)	Cd ^(García-Fenández et al. 1996) (n=1)		
Tawny Owl		As ^(Pérez-López et al. 2008) Cd ^(Pérez-López et al. 2008; Castro et al. 2011) Hg ^(Castro et al. 2011) Pb ^(Pérez-López et al. 2008; Castro et al. 2011) Zn ^(Pérez-López et al. 2008) (n=51)	Cd ^(Castro et al. 2011) Hg ^(Castro et al. 2011) Pb ^(Castro et al. 2011) (n=34)			Cd ^(Castro et al. 2011) Hg ^(Castro et al. 2011; Lourenço et al. 2011) Pb ^(Castro et al. 2011; Debén et al. 2012) (n=54)	
Barn owl	Cd ^(García-Fenández et al. 1996) (n=1)	As ^(Guitart et al. 1994; Pérez-López et al. 2008) Cd ^(Guitart et al. 1994; García-Fenández et al. 1996; Pérez-López et al. 2008) Pb ^(Guitart et al. 1994; Pérez-López et al. 2008) Se ^(Guitart et al. 1994) Zn ^(Pérez-López et al. 2008) (n=25)	Cd ^(García-Fenández et al. 1996) (n=4)	Cd ^(García-Fenández et al. 1996) (n=4)	Cd ^(García-Fenández et al. 1996) (n=3)	Hg ^(Lourenço et al. 2011) (n=13)	

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1138 **Table 4.** Analytical techniques used in the determination of metals in biological samples of
1139 the raptors from Portugal and Spain.

Analytical technique	Elements
Atomic Absorption Spectrometry (AAS)	
<ul style="list-style-type: none">Flame	Al (Cardiel et al. 2011) , Zn (González et al. 1984; Hernández et al. 1986, 1988a, 1988b; Benito et al. 1999; Donázar et al. 2002; Blanco et al. 2003; Baos et al. 2006a, 2006b; Ortiz-Santaliestra et al. 2015) , Cu (González et al. 1984; Hernández et al. 1986, 1988a, 1988b; Donázar et al. 2002; Blanco et al. 2003; Ortiz-Santaliestra et al. 2015) , Hg (Ortiz-Santaliestra et al. 2015)*, Pb (González et al. 1984; Hernández et al. 1986, 1988a, 1988b; Hernández and Margalida 2009) , Cd (González et al. 1984; Hernández et al. 1986, 1988a, 1988b)
<ul style="list-style-type: none">Graphite furnace	As (Benito et al. 1999; Donázar et al. 2002; Blanco et al. 2003; Baos et al. 2006a, 2006b; Ortiz-Santaliestra et al. 2015) , Cd (Benito et al. 1999; Donázar et al. 2002; Blanco et al. 2003; Baos et al. 2006a, 2006b; Castro et al. 2011; Ortiz-Santaliestra et al. 2015) , Co (Benito et al. 1999) , Cu (Benito et al. 1999; Blanco et al. 2003; Baos et al. 2006a, 2006b) , Pb (Benito et al. 1999; Mateo et al. 1999; Donázar et al. 2002; Blanco et al. 2003; Mateo et al. 2003; Pain et al. 2005; Baos et al. 2006a, 2006b; Gangoso et al. 2009; Hernández and Margalida 2009; Cardiel et al. 2011; Castro et al. 2011; Rodríguez-Ramos et al. 2011; Debén et al. 2012; Mateo et al. 2015; Ortiz-Santaliestra et al. 2015) , Sb (Benito et al. 1999) , Tl (Benito et al. 1999)
<ul style="list-style-type: none">Hydride generation	As (Mateo et al. 2003)
<ul style="list-style-type: none">Cold vapour	Hg (González et al. 1984; Hernández et al. 1986; Palma et al. 2005; Ortego et al. 2006; Figueira et al. 2009; Castro et al. 2011; Lourenço et al. 2011; Martínez et al. 2012; Álvarez et al. 2013; Espín et al. 2014a, 2014b, 2014c)
Inductively Coupled Plasma (ICP)	
<ul style="list-style-type: none">Atomic/Optical Emission Spectrometry (AES)/(OES)	Pb (Mateo et al. 1999; Gangoso et al. 2009)
<ul style="list-style-type: none">Mass Spectrometry (MS)	As (Pérez-López et al. 2008; Carneiro et al. 2014) , Cd (Pérez-López et al. 2008; Carneiro et al. 2014, 2015) , Hg (Carneiro et al. 2014, 2015) , Pb (Pérez-López et al. 2008; Carneiro et al. 2014, 2015) , Se (Alvarez et al. 2013) , Zn (Pérez-López et al. 2008)
Anodic Stripping Voltammetry (ASV)	Cd (García-Fenández et al. 1995, 1996; Martínez-López et al. 2005; Espín et al. 2014a, 2014b) , Cu (Espín et al. 2014a, 2014b) , Pb (García-Fenández et al. 1995, 1997, 2005a, 2005b; Martínez-Lopez et al. 2004; Gómez-Ramírez et al. 2010; Espín et al. 2014a, 2014b) , Zn (Espín et al. 2014a, 2014b)

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1141 * Mercury/Híbrid system coupled to the AAS

1142 **Table 5.1.** Range of mean concentrations of the studied metals and metalloids in blood, liver and kidney samples of the studied raptors species
 1143 from the Iberian Peninsula.

	Blood (µg/dl)	Liver (µg/g d.w.)	Kidney (µg/g d.w.)
As	0.8 (Black kite) ^(Benito et al. 1999) _ 26.24 (Black kite) ^(Baos et al. 2006b)	0.10 (Common buzzard) ^(Carneiro et al. 2014) _ 6.88 (Tawny Owl) ^(Pérez-López et al. 2008)	0.18 (Common buzzard) ^(Carneiro et al. 2014)
Cd	n.d. (Griffon vulture, Bonelli's eagle) ^(Carneiro et al. 2015; Ortiz-Santaliestra et al. 2015) _ 0.68 (Black kite) ^(Benito et al. 1999)	0.05 (Booted eagle) ^(García-Fenández et al. 1995) _ 5.52 (Tawny Owl) ^(Pérez-López et al. 2008)	0.13 (Booted eagle) ^(García-Fenández et al. 1995) _ 1.81 (Common kestrel) ^(García-Fenández et al. 1995)
Hg	n.d. (Griffon vulture) ^(Carneiro et al. 2015) _ 20.94 (Common buzzard) ^(Carneiro et al. 2014) , 56.7* (Peregrine falcon) ^(Alvárez et al. 2013)	1.39 (Common buzzard) ^(Carneiro et al. 2014)	2.09 (Common buzzard) ^(Carneiro et al. 2014)
Pb	2.45* (Bearded vulture) ^(Hernandez and Margalida 2009) , 2.78 (Black kite) ^(Baos et al. 2006b) _ 43.07 (Griffon vulture) ^(García-Fenández et al. 2005b)	0.39 (Common buzzard) ^(García-Fenández et al. 1995) _ 6.64 (Common kestrel) ^(Pérez-López et al. 2008)	0.63 (Common buzzard) ^(García-Fenández et al. 1995) _ 1.65 (Eurasian sparrowhawk, Eagle owl) ^(García-Fenández et al. 1995, 1997)
Cu	n.d. (Bonelli's eagle) ^(Ortiz-Santaliestra et al. 2015) _ 36.86 (Black kite) ^(Baos et al. 2006b)	Not measured	Not measured
Zn	330 (Black kite) ^(Benito et al. 1999) _ 528 (Black kite) ^(Baos et al. 2006b)	134.24 (Griffon vulture) ^(Hermoso de Mendoza et al. 2006) _ 360.3 (Tawny Owl) ^(Pérez-López et al. 2008)	Not measured
Se	53,8*, 43,9* (Peregrine falcon) ^(Alvárez et al. 2013)	Not measured	Not measured
Al	Not measured	Not measured	Not measured

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1145 **d.w.** (dry weight), **w.w.** (wet weight), **n.d.** (not detected), ***GM** (Geometric mean)

1146 **Table 5.2** Range of mean concentrations of the studied metals and metalloids in brain, bone, feathers and eggs samples of the studied raptors
1147 from the Iberian Peninsula.

	Brain (µg/g w.w.)	Bone (µg/g d.w.)	Feathers (µg/g d.w.)	Eggs (µg/g w.w.)
As	Not measured	†	Not measured	0.01 (Black kite) ^(Blanco et al. 2003) – 0.02 (Black kite) ^(Blanco et al. 2003)
Cd	0.003 (Booted eagle) ^(García-Fenández et al. 1995) – 0.02 (Common kestrel) ^(García-Fenández et al. 1995)	0.01 (Common buzzard) ^(García-Fenández et al. 1995) – 0.125 (Eurasian sparrowhawk) ^(García-Fenández et al. 1995)	0.02 (Northern goshawk, Booted eagle) ^(Martínez-López et al. 2005) – 0.03 (Common buzzard) ^(Martínez-López et al. 2005)	0.006 (Black kite) ^(Blanco et al. 2003) – 0.18 (Black kite) ^(González et al. 1984)
Hg	Not measured	Not measured	0.09 (Eagle owl) ^(Ortego et al. 2006) – 2.43 (Bonelli’s eagle) ^(Palma et al. 2005; Figueira et al. 2009)	0.03* (Black kite) ^{Hernandez et al. 1988} , 0.19 (Booted eagle) – 0.45 (Black kite) ^(González et al. 1984)
Pb	0.02 (Common buzzard) ^(García-Fenández et al. 1995, 1997) – 0.14 (Common buzzard) ^(García-Fenández et al. 1995)	0.46 (Bearded vulture) ^(Mateo et al. 2015) – 46.35 (Eagle owl) ^(García-Fernández et al. 1995)	0.033 (Spanish Imperial eagle) ^(Rodríguez-Ramos et al. 2011) – 1.01 (Common buzzard) ^(Martínez-Lopez et al. 2004)	0.05 (Black kite) ^(Blanco et al. 2003) – 0.82 (Spanish Imperial eagle) ^(González and Hiraldo 1988) , 1.40* (Golden eagle) ^(Hernandez et al. 1988)
Cu	Not measured	Not measured	Not measured	0.65 (Black kite) ^(Blanco et al. 2003) – 2.07 (Hobby) ^(González et al. 1984)
Zn	Not measured	Not measured	Not measured	10.2 (Spanish Imperial eagle) – 14.79 (Black kite) ^(González et al. 1984)
Se	Not measured	Not measured	Not measured	Not measured
Al	Not measured	Not measured	n.d. in the rachis (Griffon vulture, Cinereous vulture, Black kite, Red kite) ^(Cardiel et al. 2011) – 1144 in the barbs (Griffon vulture) ^(Cardiel et al. 2011)	Not measured

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1149 **d.w.** (dry weight), **w.w.** (wet weight), **n.d.** (not detected), ***GM** (Geometric mean), **† Median**

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