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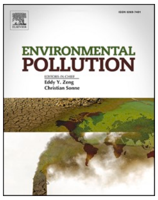

# Levels of persistent organic pollutants (POPs) and the role of anthropic subsidies in the diet of avian scavengers tracked by stable isotopes[☆]

J. Muñoz-Arnanz[a,*,1], A. Cortés-Avizanda[b,c,1,**], I. Donázar-Aramendía[d], E. Arrondo[c,e,f], O. Ceballos[g], P. Colomer-Vidal[a], B. Jiménez[a], J.A. Donázar[c]

[a] Department of Instrumental Analysis and Environmental Chemistry, Institute of Organic Chemistry (IQOG-CSIC), Juan de la Cierva 3, 28006, Madrid, Spain
[b] Department of Plant Biology and Ecology, Faculty of Biology, University of Seville, Av. Reina Mercedes 6, 41012, Seville, Spain
[c] Department of Conservation Biology, Estación Biológica de Doñana, CSIC, C/. Americo Vespucio 26, 41092, Seville, Spain
[d] Laboratorio de Biología Marina, Seville Aquarium R + D + I Biological Research Area, Department of Zoology, Faculty of Biology, University of Sevilla, 41012, Seville, Spain
[e] Centro de Investigación e Innovación Agroalimentaria y Agroambiental (CIAGRO-UMH), Miguel Hernández University, Elche, Spain
[f] Department of Zoology, University of Granada, Spain
[g] UGARRA, Avda. Carlos III 1, 31002, Pamplona, Navarre, Spain

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ABSTRACT

Persistent Organic Pollutants (POPs) have been identified as a significant factor driving declines in wildlife populations. These contaminants exhibit a dual tendency to biomagnify up the food chains and persist within tissues, rendering long-lived vertebrates, such as raptors, highly vulnerable to their adverse effects. We assessed the concentrations of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) in fledglings of two vulture species, the Egyptian vulture (*Neophron percnopterus*) and the griffon vulture (*Gyps fulvus*), coexisting in northern Spain. Vultures, currently facing a severe threat with a population decline exceeding 90%, represent one of the most critically endangered avian groups in the Old World. Despite this critical situation, there remains a scarcity of research examining the intricate relationship between contaminant levels and individual foraging behaviors. In parallel, we analyzed stable isotope levels ($\delta^{15}N$ and $\delta^{13}C$) in fledgling's feathers and prey hair to determine the association between individual dietary and contaminant burdens. Our findings revealed higher levels of PCBs in Egyptian vultures, while pesticide concentrations remained very similar between focal species. Furthermore, higher individual values of $\delta^{13}C$, indicating a diet based on intensive farming carcasses and landfills, were associated with higher levels of PCBs. While the levels of POPs found do not raise immediate alarm, the presence of individuals with unusually high values reveals the existence of accessible contamination sources in the environment for avian scavengers. The increasing reliance of these birds on intensive livestock farming and landfills, due to the decline of extensive livestock farming, necessitates long-term monitoring of potential contaminant effects on their populations.

## 1. Introduction

Persistent organic pollutants (POPs) are toxic chemicals that raise significant concern and are recognized as an important threat to environmental health. The potential detrimental effects of POPs on natural ecosystems have been widely studied. Due to the recalcitrant and lipophilic nature of POPs, they bioaccumulate, thus being able to cause a range of sublethal toxic effects that may lead to a decline in wildlife populations (Walker, 1990). In addition, these contaminants tend to bio-magnify through the trophic web. Consequently, species at higher trophic levels (e.g. raptors) have an elevated risk of harm when exposed to high concentrations of POPs (Bowerman et al., 1995; Gilbertson et al., 1991; Skaare et al., 2000). POPs are also known for their semi-volatile nature, which allows their transport over long distances in the

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[*] Corresponding author.
[**] Corresponding author.
*E-mail address:* juan.ma@iqog.csic.es (J. Muñoz-Arnanz).
[1] These authors contributed equally to this work.

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atmosphere, through the air, water, or some migratory species, before being deposited. Their persistence and mobility make it possible to detect them anywhere on the planet, including remote regions where they have never been used, such as the Arctic or Antarctic (AMAP, 2018; Casal et al., 2017; Mello et al., 2016; Morales et al., 2022; Roscales et al., 2019; Thomas et al., 1992).

Over the past decades, POPs have been introduced into the environment, causing very detrimental effects on ecosystems and wildlife populations (Brogan et al., 2017; Buck et al., 2020; Elliott et al., 1996; Krüger et al., 2022; Skaare et al., 2000; Walker, 1990). Due to the accumulation processes of these contaminants and their persistence in tissues (e.g. adipose tissue, liver, etc.), long-lived vertebrates are particularly susceptible to POP contamination (García-Fernández et al., 2008). Many of these chemical contaminants can disrupt both embryonic and postnatal development, and the ramifications of such interferences can be profound, often resulting in irreversible consequences. In some extreme cases, these disruptions can lead to premature death (Bustnes et al., 2015). However, it is important to note that in many instances, the adverse effects may not become apparent until the affected individual reaches adulthood. This delayed manifestation of negative consequences can include the loss of fertility, a distressingly common outcome documented in numerous studies (Assersohn et al., 2021; Kuo et al., 2022). Such fertility loss not only hinders the reproductive potential of affected individuals, but also poses a significant threat to the long-term survival of species (Elliott et al., 1996). The cumulative impact of fertility loss and premature mortality can seriously compromise the overall viability of endangered species (Bowerman et al., 1995; Elliott et al., 1996).

Having been locally very abundant throughout the 19th and 20th centuries, vulture species are currently among the most threatened within the avian family in the Old Word. Historically, vultures' declines were caused by the negative effects of direct and indirect persecution having been estimated that in some cases more than 90% of the original population size has been lost at certain regions (Ogada et al., 2016; Safford et al., 2019). Currently, the effects of anthropization of the natural environment, particularly the pronounced changes in the farming systems, are gaining significance (Botha et al., 2017; Cortés-Avizanda et al., 2016; Cortés-Avizanda et al., 2015). Nowadays, in Europe, some vulture populations have been recovering thanks to conservation programs that have required important economic and human efforts (Safford et al., 2019). However, the scenario is unstable since many vulture populations, as also occurs in other areas of Asia and Africa, increasingly depend on resources provided by intensive farming and landfills with potential negative effects on individual health (Fernández-Gómez et al., 2022; Krüger et al., 2022; Plaza and Lambertucci, 2017; Tauler-Ametllller et al., 2019). In this sense, adverse effects have been detected linked to the exposure to legacy chemicals such as dichlorodiphenyltrichloroethane (DDT) and its metabolites, flame retardants, polycyclic aromatic hydrocarbons (PAHs), organophosphates, polychlorinated biphenyls (PCBs), and heavy metals such as lead (Arrondo et al., 2020; Krüger et al., 2022). However, there is very scarce research on the detailed relationship between levels of contaminants and the individual foraging behavior while birds exploit "risky" trophic sources such as the above-mentioned landfills, farms, etc. The scenario is complex because, in addition, the strategies deployed to search and exploit resources may differ between species composing a scavenger guild (Cortés-Avizanda et al., 2012), so their exposure risk and vulnerability to different POPs may also be asymmetric. To our knowledge, in our study area (see below) only (Ortiz-Santaliestra et al., 2019) addressed this question by measuring concentrations of various POPs in the blood of fledging Egyptian vultures (*Neophron percnopterus*). They found that individuals breeding close to urbanized areas had higher levels of some PCBs, but the birds' diets were not estimated. In this context, it is obvious that the effect of dependence on human resources on scavenger birds needs to be re-examined thoroughly at an individual level.

Here we examine the levels of PCBs and organochlorine pesticides (OCPs) in populations of Egyptian vultures (*Neophron percnopterus*) and griffon vultures (*Gyps fulvus*), that coexist in a Natural Park in northern Spain. We know that individuals of both species forage in highly humanized areas around the Park where they take advantage of both extensive and intensive livestock remains, as well as landfills (Cortés-Avizanda et al., 2015; Fernández-Gómez et al., 2022). We assessed the proportion of risky food sources in the diet of individuals using stable isotopes, δ$^{15}$N and, specifically, δ$^{13}$C, which indicates both the consumption of livestock raised under intensive conditions (such as pigs) and the utilization of landfills (Tauler-Ametller et al., 2018). Our main goals of this study were: i) to determine the presence and concentration of both groups of POPs in fledgling individuals of the two European vulture species; and ii) to examine the association of these pollutants with the scavenger's diet and trophic strategies as determined with analyses of stable isotopes. Our results will significantly enhance our understanding of the potential current impact of these contaminants on obligate avian scavengers; thereby, aiding in their conservation efforts.

## 2. Material and methods

### 2.1. Study area, focal populations and field procedures

The study was carried out in the Bardenas Reales de Navarra Natural Park (northern Spain, a zone close to 42,000 ha in the centre of the depression of the Ebro valley (Fig. 1). Altitudes range between 280 and 660 m a.s.l. large plains and small flat hills dominate the orography. The climate is continental Mediterranean with warm summers and cold dry winters. Barren oils, cereal cultures and pasturelands with Mediterranean pine woodlands, and scrubland in the hills of the southern part of the Natural Park, dominate the landscape. The surroundings of the park are heavily humanized, with a growing importance of intensive irrigated agriculture areas and a progressive abandonment of extensive livestock farming in favour of semi-intensive (sheep) or fully intensive (pigs) systems (see details in Cortés-Avizanda et al., 2009, 2015). The park holds important populations of cliff-nesting griffon and Egyptian vultures with 112 and 19 breeding pairs respectively in 2021.

In 2021, during labours of monitoring and ringing, we took blood

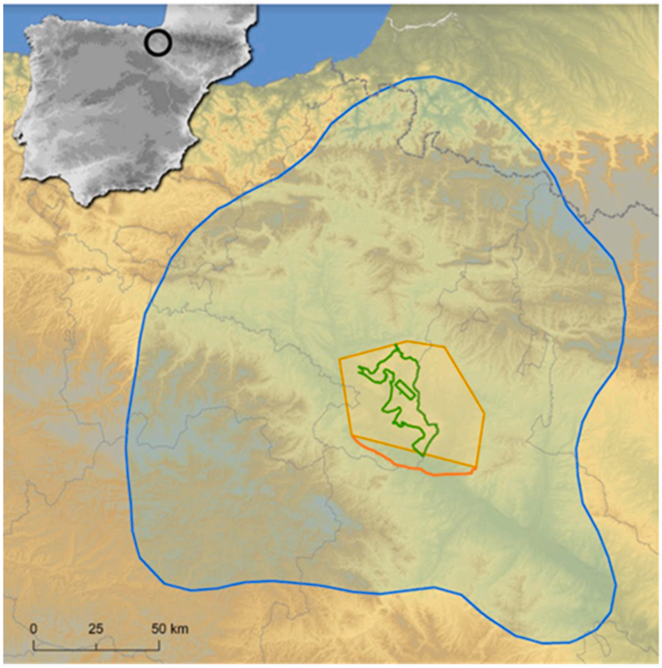

**Fig. 1.** Map of the study area.

samples (5 mL) from fledging Egyptian and griffon vultures. Samples were stored in heparin-lithium tubes and refrigerated during their transport to the laboratory. Blood was centrifuged at 3000 r.p.m. for 20 min to obtain plasma for POP analyses. Apart from this, two-three mantle feathers were picked up in each bird for stable isotope analyses. In total, we obtained respectively 14 and 15 individual samples for Egyptian and griffon vultures. In addition, and on the basis of previous knowledge, within the main areas where individuals of the two species forage regularly, we collected hair of the main mammal species consumed by the vultures (see below for details).

### 2.2. *Contaminant analysis*

#### 2.2.1. *Sample treatment*

Residue analysis was conducted using 500 μL of plasma for most samples (range: 40–500 μL). The samples underwent extraction and purification procedures as described in a previous study by Roscales et al. (2010), with an additional purification step involving a multilayer silica gel column. Initially, the samples were supplemented with a set of $^{13}$C-labeled standards of PCBs and organochlorine pesticides. After the extraction and purification processes, the final extracts were concentrated to ~1 mL under a gentle stream of nitrogen. Subsequently, the concentrated extracts were transferred to vials, further concentrated until almost completely dry, and finally reconstituted in a volume of 20 μL with $^{13}$C-PCB-70, -111, and −170 as injection standards for instrumental determination. Full information regarding the sample treatment procedure and standards used can be found in the Supplementary Material (SM).

#### 2.2.2. *Instrumental determination*

Targeted analysis was conducted on eighteen PCBs, including six indicator PCB (i-PCBs) congeners (# 28, 52, 101, 138, 153, 180), and twelve dioxin-like PCBs (DL-PCBs) encompassing four non-ortho congeners (# 77, 81, 126, 169) and eight mono-ortho congeners (# 105, 114, 118, 123, 156, 157, 167, 189). Additionally, eleven organochlorine pesticides (OCPs) were analyzed, namely PeCB, HCB, p,p'- and o,p'-isomers of DDT, DDE, DDD, and α-, β-, and γ-isomers of HCH.

PCBs were determined using gas chromatography coupled to high-resolution mass spectrometry (GC-HRMS) with a Trace GC Ultra gas chromatograph (Thermo Fisher Scientific, Milan, Italy) coupled to a high-resolution mass spectrometer (DFS, Thermo Fisher Scientific, Bremen, Germany) operating at a resolution of 10,000 (10% valley). OCPs were determined using GC coupled to low-resolution tandem mass spectrometry, employing a GC-MS/MS system (Agilent, Palo Alto, CA, USA) consisting of a 7010B QqQ spectrometer equipped with an electron impact (EI) high-efficiency source operating in the multiple reaction monitoring (MRM) mode, and coupled to a 7890B chromatograph equipped with a programmable temperature vaporization (PTV) inlet. The isotopic dilution technique was used for quantitation purposes for all target analyses. Detailed descriptions of all operating conditions can be found in (Muñoz-Arnanz et al., 2022) and in the SM.

#### 2.2.3. *QA/QC criteria*

One blank sample was processed for every five plasma samples, covering the entire analytical procedure. The correct identification and quantification of the target analytes was ensured following the criteria (a) similar GC retention times (±0.1 min) as those of standard compounds, (b) a ratio between the monitored ions or MRMs within ±15% of the theoretical values, and (c) a signal-to-noise ratio of 10 as the limit of quantification (LOQ). All analyte concentrations were corrected for recovery, and when necessary, for blank values. Calibration curves were checked daily. Further information regarding QA/QC, including recovery and LOD values is provided in the SM.

### 2.3. *Stable isotope analyses*

We analyzed stable isotopes of Nitrogen ($^{15}$N/$^{14}$N, expressed as δ$^{15}$N) and Carbon ($^{13}$C/$^{12}$C, δ$^{13}$C) in the collected hair and feather tissues. In the case of feathers, the barbs' tip was extracted over the entire length of the vane so that the isotope levels reflected the diet of the individual during the period of feather growth (approximately one month). Hair and feathers were cleaned with 2:1 chloroform: methanol, freeze-dried, powdered and 0.3–0.4 mg small quantity of each sample was packed into tin capsules. Isotopic analyses were performed at the Laboratory of Stable Isotopes of the Estación Biológica de Doñana, CSIC (www.ebd.csic.es/lie/index.html). For δ$^{13}$C and δ$^{15}$N determination, samples were combusted at 1020 °C using a continuous flow isotope-ratio mass spectrometry system (Thermo Electron) using a Flash HT Plus elemental analyser interfaced with a Delta V Advantage mass spectrometer. Stable isotope ratios were expressed in the standard δ-notation (‰) relative to Vienna Pee Dee Belemnite (δ$^{13}$C), atmospheric N2 (δ$^{15}$N) and Vienna Cañon Diablo Troilite (VCDT; δ 34S). Based on internal laboratory standards, measurement error was estimated as ± 0.1, ±0.2 and ±0.2‰ for δ$^{13}$C and δ$^{15}$N, respectively. These laboratory standards were previously calibrated with international standards supplied by the International Atomic Energy Agency (IAEA, Vienna).

For each vulture species, the composition of the diet was determined using the MixSIAR R-package (Stock et al., 2018). The model estimated the potential contribution of each isotopically distinct potential prey in the diet of the consumer based on the isotopic values of the consumer and its main potential prey. Based on previous knowledge about the diet of the two vulture species studied and the known foraging range areas for the populations of both species (see details in SM) we retained three categories for Egyptian vultures: i) pigs (*Sus scrofa domestica*), ii) sheep (*Ovis aries*), iii) wild rabbits (*Oryctolagus cuniculus*), and another three for griffon vultures: i) pig plus wild boar (*Sus scrofa*), ii) sheep plus wild rabbit, iii) other ungulates. It is of interest to note that as demonstrated by Tauler-Ametller et al. (2018), isotope signatures corresponding to domestic pigs would also indicate the consumption of trophic resources (butcher waste, human food remains) obtained in landfills. Because vultures eat meat, we standardized isotopic values between hair and muscle samples following the information found in Roth & Hobson (2000) so that we added quantities of −1.49 for δ$^{13}$C and +0.09 for δ$^{15}$N. Additionally, trophic enrichment factors (TEF) between diet to feather values were estimated for both species using SIDER (Healy et al., 2018). We obtained similar values for the two vultures (3.2 ± 1.3 ‰ for δ$^{15}$N and 1.3 ± 1.3‰ for δ$^{13}$C). The adequacy of the estimated TEF were evaluated using simulated mixing polygons (Smith et al., 2013). The MCMC parameters were set to 300,000 iterations with three Markov chains, a burn-in of 200,000 iterations, and a thinning ratio of 1:100. We assessed model convergence using Gelman-Rubin diagnostic (Gelman et al., 2013).

### 2.4. *Analyses of relationships between contaminants and diet*

Linear models (LM) were carried out considering the summary values of PCBs and DDTs as response variables. Since the distribution of values in both variables was not normal, we proceeded to consider two groups of values, above and below the median in each case (binomial error). Then, for both, PCBs and DDTs, linear models were adjusted with the response variables i) "species" (Egyptian/griffon) ii) δ$^{13}$C level, iii) δ$^{15}$N. We also fitted the interactions between species and both isotope levels. LMs were fitted using lm4 package (Bates et al., 2015). Model comparison was made following Burnham & Anderson (2002), so we considered models with DeltaAICc <2.0 to be supported by the data. All analyses were performed using R Statistical Software version v4.0.3 (R Core Team, 2021).

## 3. Results

### 3.1. Contaminants

The concentrations of ∑PCBs and ∑OCPs were comparable in both species of vultures (Egyptian vulture (EV, n = 14) and griffon vulture (GV, n = 15)) (Table 1). For PCBs, a higher amount was found in EVs (median: 0.564 pg/μL, range: 0.062–1.03 pg/μL) relative to that of GVs (median: 0.282 pg/μL, range: 0.059–16.1 pg/μL). However, for pesticides, the concentrations were very similar between Egyptian vultures (median: 0.349 pg/μL, range: 0.131–2.13 pg/μL) and griffons (median: 0.350 pg/μL, range: 0.116–52.1 pg/μL). It should be noted that the ranges of pollutants were always broader in griffons due to the existence of two specimens with particularly elevated concentrations.

Regarding PCBs, all congeners were detected in at least 80% of samples (Table 1), with the exception of PCB 189 (<13%) and the non-ortho congeners PCB-81 (0%), −126 (7% of GV), and −169 (0%). No striking differences were observed in the congener profiles between both species (Fig. 2), although some disparate abundances were detected among the predominant congeners, with a greater contribution of higher chlorinated congeners (PCB-138, -153, and −180), but also PCB-52 in GVs, relative to that in EVs. Conversely, greater contributions of PCB-101, -105, and −118 were registered in Egyptian vultures.

As for OCPs, the relative abundance of each pesticide family conspicuously varied between both species (Fig. 3). Thus, the OCP burden in GVs was dominated by the presence of DDTs (average of 92.4%), followed by HCHs (4.8%) and chlorobenzenes (2.8%). In EVs, DDTs accounted for 45.6% of the total burden, followed by HCHs (41.4%) and chlorobenzenes (13.0%).

In both species, *p,p'*-DDE and *p,p'*-DDT accounted for ≈95% and ≈1%, respectively, of the total DDT content, which can be read as a clear indication of the aged contents of this pesticide. Pertaining HCHs, the α-isomer was predominant over the β-isomer (≈67% and ≈82% in GVs and EVs, respectively). The ɣ-isomer could not be determined due to a pervasive chromatographic interference in all samples, making it not possible the calculation of isomer ratios aimed to decipher the HCH origin (technical *vs.* lindane usage).

### 3.2. Relationship between contaminants and stable isotopes

Considering the values corresponding to the summation of PCBs in each individual, equivalent models were obtained indicating the effect of δ$^{13}$C levels and species. The first model was slightly higher in weight and smaller AICc (Table 2), and showed that higher values of $^{13}$C were associated with higher levels of PCBs whereas the alternative model revealed higher values for Egyptian vultures (Table 3). Our MixSiar models showed that higher values of $^{13}$C were associated with diets relying mainly on domestic pigs. This prey category was more predominant in the diet of Egyptian vultures (Fig. 4, Table S3.2).

The modeling of the sum of DDTs showed that the null model outfitted the rest of models (Table 2). It was followed by the model accounting for the variable “Species”, but this parameter overlapped with zero (result not shown).

## 4. Discussion

The impact of contaminants on the conservation of threatened species, particularly birds of prey, has been one of the conservation priorities since the mid-20th century, and as a result, highly stringent regulations have achieved significant success (Sumasgutner et al., 2021). Our results demonstrate that the levels of PCBs and OCPs in two European scavenger bird species, each with a distinct trophic strategy within the guild of obligate scavengers, are remarkably low and lower than those previously documented for these species in the same study areas in previous decades (Gómara et al., 2004). On the other hand, δ$^{13}$C isotope levels are valuable for detecting significant variations between primary producers employing different photosynthetic pathways (C3 *vs.* C4) (O'Leary et al., 1992) as it remains relatively stable during trophic

**Table 1**
Concentrations (pg/μL) of PCBs and OCPs in plasma samples of Egyptian and griffon vulture fledgings in Bardenas Reales Natural Park (Navarra, northern Spain).

| | Egyptian vulture (n = 14) | | | Griffon vulture (n = 15) | | |
|---|---|---|---|---|---|---|
| | Minimum | Maximum | Median | Minimum | Maximum | Median |
| PCB28 | 0.012 | 0.059 | 0.028 | <LOQ | 0.566 | 0.017 |
| PCB52 | <LOQ | 0.195 | 0.088 | 0.020 | 3.07 | 0.072 |
| PCB77 | <LOQ | 0.007 | 0.002 | <LOQ | 0.020 | 0.003 |
| PCB81 | <LOQ | <LOQ | 0 | <LOQ | <LOQ | 0 |
| PCB101 | <LOQ | 0.284 | 0.134 | 0.014 | 2.32 | 0.070 |
| PCB105 | 0.005 | 0.096 | 0.044 | 0.003 | 0.647 | 0.022 |
| PCB114 | <LOQ | 0.010 | 0.003 | <LOQ | 0.029 | 0.002 |
| PCB118 | <LOQ | 0.275 | 0.142 | 0.004 | 1.85 | 0.060 |
| PCB123 | 0.001 | 0.017 | 0.004 | <LOQ | 0.030 | 0.001 |
| PCB126 | <LOQ | <LOQ | 0 | <LOQ | 0.002 | 0 |
| PCB138 | 0.007 | 0.052 | 0.026 | 0.002 | 2.68 | 0.011 |
| PCB153 | 0.009 | 0.124 | 0.034 | <LOQ | 2.56 | 0.011 |
| PCB156 | <LOQ | 0.013 | 0.008 | 0.001 | 0.157 | 0.002 |
| PCB157 | <LOQ | 0.004 | 0.002 | <LOQ | 0.080 | 0.001 |
| PCB167 | <LOQ | 0.019 | 0.002 | <LOQ | 0.218 | 0.001 |
| PCB169 | <LOQ | <LOQ | 0 | <LOQ | <LOQ | 0 |
| PCB180 | 0.006 | 0.161 | 0.012 | 0.002 | 1.83 | 0.003 |
| PCB189 | <LOQ | 0.010 | 0 | <LOQ | 0.103 | 0 |
| **ΣPCBs** | **0.062** | **1.03** | **0.564** | **0.059** | **16.1** | **0.282** |
| PeCB | <LOQ | 0.018 | 0.013 | <LOQ | 0.034 | 0.008 |
| HCB | 0.024 | 0.075 | 0.052 | 0.024 | 0.246 | 0.092 |
| α-HCH | <LOQ | 1.66 | 0 | <LOQ | 1.00 | 0.011 |
| β-HCH | <LOQ | 0.295 | 0 | <LOQ | 0.413 | 0.008 |
| o,p'-DDE | <LOQ | 0.017 | 0.001 | <LOQ | 0.936 | 0 |
| p,p'-DDE | 0.068 | 1.02 | 0.132 | 0.025 | 49.4 | 0.076 |
| o,p'-DDD | <LOQ | 0.007 | 0.002 | <LOQ | 0.213 | 0.002 |
| p,p'-DDD | <LOQ | 0.006 | 0 | <LOQ | 0.206 | 0.004 |
| o,p'-DDT | <LOQ | 0.009 | 0.003 | <LOQ | 0.393 | 0.006 |
| p,p'-DDT | <LOQ | 0.007 | 0.003 | <LOQ | 0.326 | 0.009 |
| ΣDDTs | 0.078 | 1.04 | 0.136 | 0.028 | 51.4 | 0.100 |

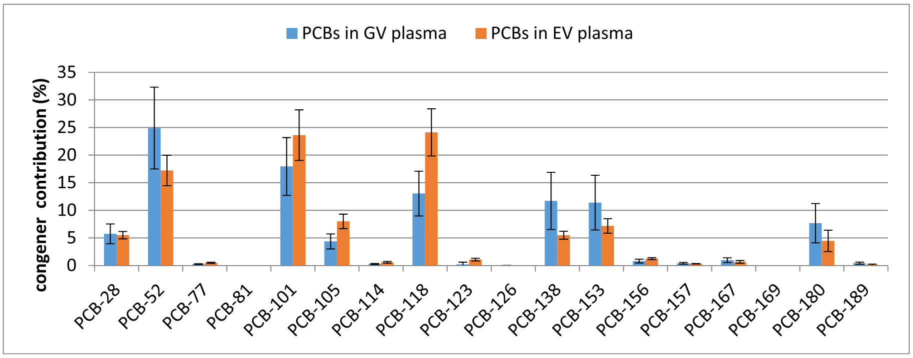

**Fig. 2.** Average PCB congener profile in plasma of fledging of griffons (GV) and Egyptian vultures (EV). Error bars represent standard errors (SE).

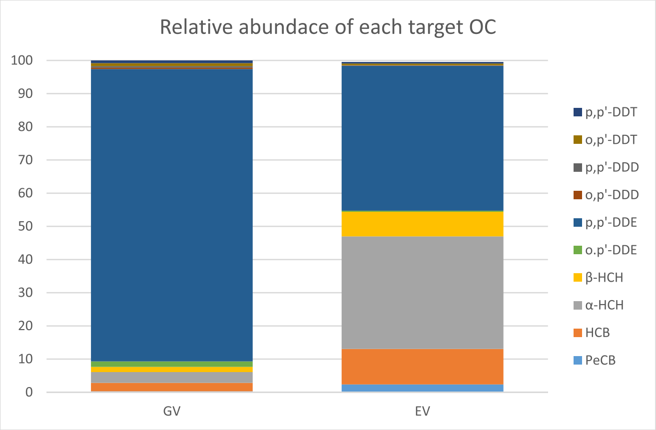

**Fig. 3.** Average contribution of OCPs in plasma of fledglings of griffons (GV) and Egyptian vultures (EV). Error bars represent standard errors (SE).

transfers, showing minimal change (about 1% ± 1SD) (Tieszen et al., 1983). It is recognized that examination of $\delta^{13}$C levels can allow to evaluate the dependence of individuals from human derivate food resources. Specifically, higher $\delta^{13}$C levels may be indicative of a diet comprising carcasses of livestock maintained with feed-stuffs based on C4 plants and reared in intensive, as well as human waste found in landfills (Tauler-Ametller et al., 2018; Duclos et al., 2020; Gámez et al., 2022; Vedel et al., 2022). Simultaneously, the examination of stable isotope values revealed that a diet based on carcasses of intensive farming and landfills is associated with higher levels of PCBs, as found for the endangered Egyptian vultures. Thus, these analysis techniques prove to be a valuable tool for identifying the exposure of avian scavenger species to sources of POP contamination.

Interestingly, our results show that, in general, the concentrations for all target pollutants in both vulture species were very low, with median values for ∑PCBs and ∑OCPs in the range of sub-ppb, and in clear contrast with concentrations reported in other areas of the world such as India (mean ∑PCBs: 226 and 253 pg/μL, mean ∑DDTs: 8.8 and 35.9 pg/μL, mean ∑HCHs: 76.6 and 86.9 pg/μL) for Egyptian vultures and congeneric *Gyps* species, respectively (Dhananjayan et al., 2011). Higher values were also observed in griffon vultures of Greece (mean ∑PCBs: 10.64 ng/g, mean ∑DDTs: ca. 4.7 ng/g and mean ∑HCHs: ca. 4.23 ng/g (Goutner et al., 2011). Gómara et al. (2004) reported pollutants found in Egyptian vulture adults and chicks' samples of 1999–2001, Spain, including samples of our study area. They found concentrations about one order of magnitude higher. Specifically, 4.12 pg/μL for ∑DDTs and 22 pg/μL for ∑PCBs (both values corresponding to geometric mean). The comparison among studies can be strongly influenced by diverse factors such as the variable age of the sampled animals (nestlings, immature, and mature), period of sampling, and body condition, among others (Bustnes et al., 2013), but we are confident that the determined concentrations reflected current environmental concentrations in their foraging areas. In summary, the reduced values found in our study seem to indicate a clear decrease in the environmental concentrations of all these POPs. This reduction was expected in response to regulations that have banned the production and use of both PCBs and DDT in Spain for several decades now, and globally since the enforcement of the Stockholm Convention in 2004 (UNEP, 2021). Even though this reduction in environmental concentrations of legacy POPs is not generalized across geographical areas and ecosystems, in fact, it has recently been described in distinct bird species such as seabirds from South Korea (Jang et al., 2022), the northeast Pacific (Elliott et al., 2023), or from a pan-geographic study encompassing West Greenland, Norway, and central Sweden (Sun et al., 2020). In Spain, decline trends in OCPs have been reported, for instance, in Eurasian Eagle-owls (*Bubo bubo*) from the southeast of the country (2003–2007, Gómez-Ramírez et al., 2019), and in up to seven different bird species inhabiting Doñana National Park in southwestern Spain (1999–2021, Peris et al., 2023).

Focusing on the last decade, interestingly, the concentrations reported by Ortiz-Santaliestra et al. (2019) in the plasma of fledgling Egyptian vultures from northwestern Spain, and sampled in 2012–2016, were notably lower than those detected in our study area. Among the common target pollutants for both studies, these authors only detected *p,p'*-DDE in 22.4% of the samples, with a geometric mean of 0.017 pg/μL. Additionally, PCB-180 was the only PCB congener determined above its limit of detection, with a geometric mean of 0.084 pg/μL. These differences cannot be attributed to differences in the age of the fledglings in both studies since they were all sampled at the time of ringing, in the final phase of growth, when they were between 45 and 60 days old (see above references). The observed differences can be attributed to the fact that Egyptian vultures in our study area exploits resources in very humanized regions, exposing them to potential contamination sources. In contrast, the vultures studied by Ortiz-Santaliestra et al. (2019) were located in the foothills of the Pyrenees, where they seem to rely more on trophic resources associated with extensive livestock farming. However, they also forage in garbage dumps when these are in proximity to their territories (Cerecedo-Iglesias et al., 2023).

Remarkably, our findings on concentrations of PCBs, which are higher than those of DDTs for both vulture species, aligns with that described for Egyptian vultures studied by Gómara et al. (2004). It, however, contradicts what has traditionally been described for many raptor species in Spain where typically, *p,p'*-DDE show the highest concentrations, followed by PCBs (García-Fernández et al., 2008). These findings can be elucidated by the circumstance that, as we stated above, our studied populations forage within the Ebro Valley, an area highly altered in terms of human population density, industrial development, and the prevalence of landfills frequently exploited by scavengers (as detailed below). Indeed, previous research has demonstrated that birds of prey that hunt in regions with a dense network of power lines and other electric infrastructures reveal elevated levels of PCBs

**Table 2**
Competing models resulting of the fitting of values of Total PCBs (above) and Total DDTs (below) in relation to the values of stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) and species (Spp). The interaction between both isotopes and species were also fitted. Top-ranked models (within 2 delta AICc) are shown in bold and italics.

Total PCBs

| Model | Intercept | $\delta^{13}C$ | Spp | $\delta^{15}N$ | $\delta^{13}C$:Spp | $\delta^{15}N$:Spp | df | logLik | AICc | delta | weight |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 21.65 | 0.9411 | | | | | 2 | −17.58 | 39.6 | 0 | 0.313 |
| 3 | 0.9163 | | + | | | | 2 | −17.923 | 40.3 | 0.69 | 0.222 |
| 6 | 20.03 | 0.897 | | 0.06627 | | | 3 | −17.568 | 42.1 | 2.47 | 0.091 |
| 4 | 22.35 | 0.9729 | + | | | | 3 | −17.579 | 42.1 | 2.5 | 0.09 |
| 1 | 0.06899 | | | | | | 1 | −20.084 | 42.3 | 2.7 | 0.081 |
| 7 | 0.4205 | | + | 0.05003 | | | 3 | −17.917 | 42.8 | 3.17 | 0.064 |
| 5 | −4.173 | | | 0.4602 | | | 2 | −19.193 | 42.8 | 3.23 | 0.062 |
| 12 | 18.95 | 0.8187 | + | | + | | 4 | −17.536 | 44.7 | 5.12 | 0.024 |
| 8 | 21.88 | 0.9869 | + | 0.07876 | | | 4 | −17.564 | 44.8 | 5.17 | 0.024 |
| 23 | −1.585 | | + | 0.2531 | | + | 4 | −17.851 | 45.4 | 5.75 | 0.018 |
| 24 | 20.44 | 1.036 | + | 0.3342 | | + | 5 | −17.466 | 47.5 | 7.92 | 0.006 |
| 16 | 18.54 | 0.8343 | + | 0.07579 | + | | 5 | −17.522 | 47.7 | 8.03 | 0.006 |
| 32 | 17.37 | 0.8919 | + | 0.3226 | + | + | 6 | −17.429 | 50.7 | 11.06 | 0.001 |

Total DDTs

| Model | Intercept | $\delta^{13}C$ | Spp | $\delta^{15}N$ | $\delta^{13}C$:Spp | $\delta^{15}N$:Spp | df | logLik | AICc | delta | weight |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.06899 | | | | | | 1 | −20.084 | 42.3 | 0 | 0.366 |
| 3 | 0.2877 | | + | | | | 2 | −19.925 | 44.3 | 1.99 | 0.135 |
| 5 | −0.5012 | | | 0.06185 | | | 2 | −20.067 | 44.6 | 2.28 | 0.117 |
| 2 | 1.542 | 0.06433 | | | | | 2 | −20.07 | 44.6 | 2.29 | 0.117 |
| 4 | −23.78 | −1.095 | + | | | | 3 | −19.434 | 45.8 | 3.51 | 0.063 |
| 7 | 1.095 | | + | −0.08138 | | | 3 | −19.906 | 46.8 | 4.46 | 0.039 |
| 23 | 10.16 | | + | −0.9899 | | + | 4 | −18.602 | 46.9 | 4.55 | 0.038 |
| 12 | −40.46 | −1.856 | + | | + | | 4 | −18.604 | 46.9 | 4.56 | 0.037 |
| 6 | 0.463 | 0.03558 | | 0.04564 | | | 3 | −20.064 | 47.1 | 4.77 | 0.034 |
| 24 | −19 | −1.407 | + | −1.165 | | + | 5 | −17.873 | 48.4 | 6.04 | 0.018 |
| 8 | −23.18 | −1.121 | + | −0.1175 | | | 4 | −19.395 | 48.5 | 6.14 | 0.017 |
| 32 | −38.98 | −2.399 | + | −1.341 | + | + | 6 | −16.821 | 49.5 | 7.14 | 0.01 |
| 16 | −39.88 | −1.89 | + | −0.1338 | + | | 5 | −18.556 | 49.7 | 7.4 | 0.009 |

**Table 3**
Estimates and standard errors resulting from the two top ranked models fitting the individual levels of Total PCBs to the values of stable isotopes and species (Table 2). Confidence intervals (7.5–92.5%) of the estimates are shown.

Model 2

| | Estimate | Std. error | 7.5% | 92.5% |
|---|---|---|---|---|
| (Intercept) | −21.6526 | 10.3863 | 7.4694 | 37.7100 |
| $\delta^{13}C$ | 0.9411 | 0.4515 | 0.3234 | 1.6376 |
| $R^2 = 0.1586$ | | | | |

Model 3

| | Estimate | Std. error | 7.5% | 92.5% |
|---|---|---|---|---|
| (Intercept) | 0.9163 | 0.5916 | 0.1037 | 1.8345 |
| Species (griffon) | −1.6094 | 0.8062 | −2.8233 | −0.4843 |
| $R^2 = 0.1384$ | | | | |

(Garcia-Heras et al., 2018; Gioia et al., 2014).

Our results using stable isotopes provide strong support for the idea that when birds feed in areas heavily influenced by human activities or consume common food sources from these areas, their contaminant levels are higher. Statistical models precisely show that higher levels of $\delta^{13}C$, which characterize a diet based on intensive farming carcasses (pigs) and landfills (Tauler-Ametller et al., 2018), are associated with higher levels of PCBs. Materials frequently found in landfills, such as plastics and e-waste, are recognized sources of PCBs (Robinson, 2009; Teuten et al., 2009). An alternative model to $\delta^{13}C$ also indicates that the Egyptian vulture is more exposed to PCBs (considering the sum of all compounds examined). Indeed, diet models show that the diet of both avian scavengers, although based globally on the same food resources, is proportionally different because the Egyptian vulture's diet includes a higher proportion of the pig/landfill category. In fact, the association of Egyptian vultures with human waste is well-known worldwide (Ortiz-Santaliestra et al., 2019).

The relative PCB abundance profiles were very similar in the Egyptian vulture and the griffon vulture, with indicator PCBs (PCB-28,-52,-101,-118,-138,-153, and −180) accounting for most of the PCB content in both species (Fig. 2). This was expected, as these congeners are typically found in environmental samples and have been commonly described in different tissues, including plasma, in birds of prey of different ages and from different geographical areas (e.g. kestrel eggs from the Canary Islands (Buck et al., 2020), vultures' plasma from India (Dhananjayan et al., 2011), feathers of Spanish cinereous vulture nestlings (Monclús et al., 2018) or plasma of Canadian Cooper's hawks (Brogan et al., 2017). It is worth noting, however, that the higher chlorinated congeners (PCB-138, -153, and −180), which usually exhibit the highest rates of bioaccumulation and biomagnification, due to their heightened resistance to (bio)degradation and higher lipophilicity (Drouillard et al., 2001), were not the major contributors in our samples. Instead, lower-chlorinated congeners (tetra- and penta-chlorinated), such as PCB-52, -101, and −118, accounted for the highest contribution to the total PCB burden. Interestingly, there was found also a high proportion of PCB-105, which was also reported by Gómara et al. (2004). No point sources of PCBs are expected to have affected the sampled animals, given the low concentrations detected along with the strict regulations on the production and use of them. In consequence, it is plausible that this higher contribution of lower chlorinated PCBs can respond to the preferential long-range transport and subsequent accumulation of these congeners in this northern area.

In contrast to PCBs, the profiles of OCPs exhibited significant differences between the two species (Fig. 3), which was unexpected. For griffon vultures, the primary OCP contributor was p,p'-DDE, accounting for approximately 88% of the burden. Conversely, in Egyptian vultures, two specific pollutants were prominent: *p,p'*-DDE at around 44% and α-HCH at approximately 34%. The underlying reasons for this phenomenon remain unknown. One hypothesis is that variations in the metabolism and subsequent bioaccumulation of contaminants can differ between the two species. For instance, Griffon vultures (*Gyps* spp) are known to have an extremely acidic digestive pH (values of

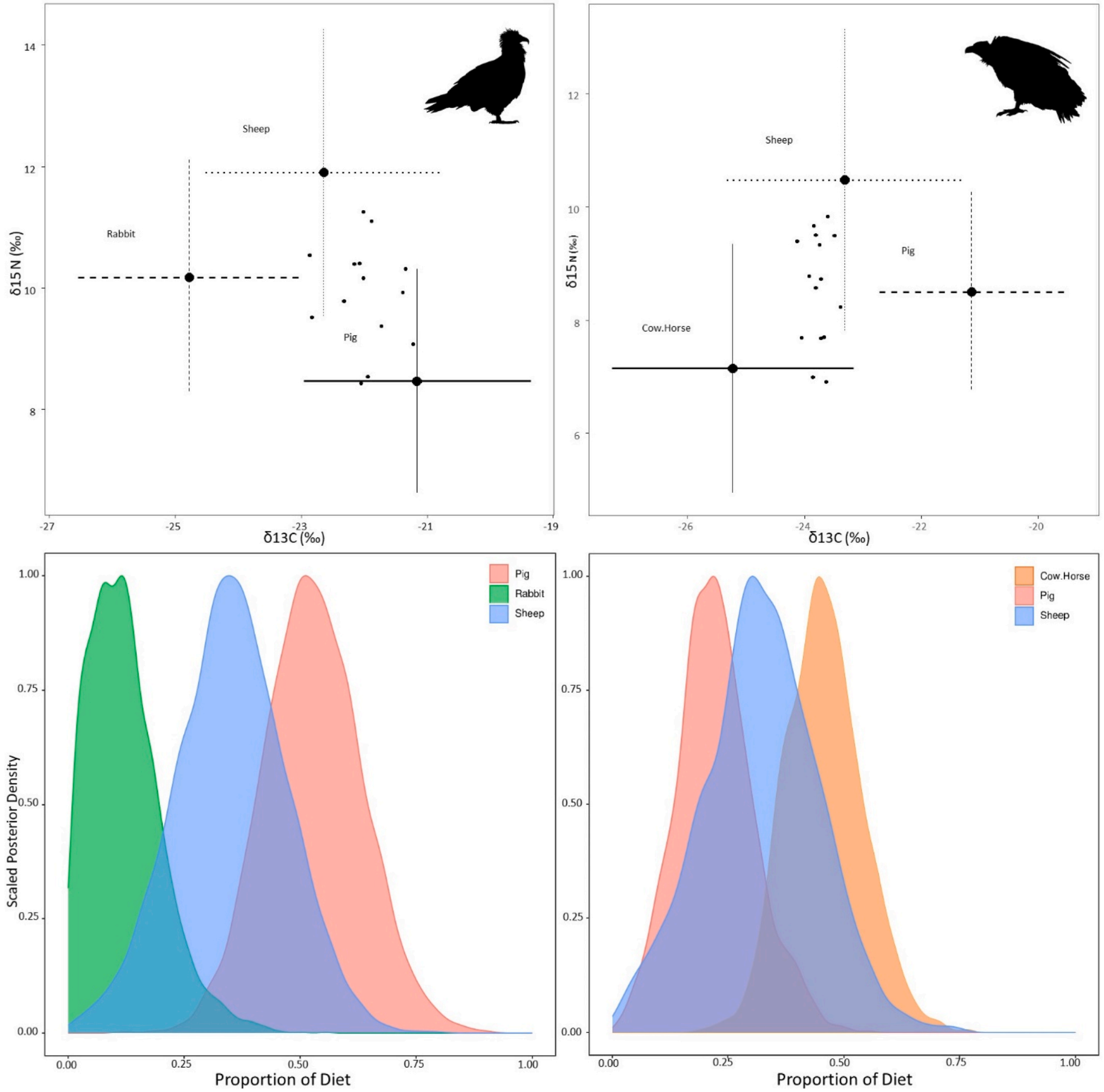

**Fig. 4.** Above: Isoplots representing the values of $\delta^{13}$C and $\delta^{15}$N in prey items (Black crosses) and individuals (dots) for the two studied vulture species. Below: Diet composition based on the SIAR model. Left: Egyptian vulture; Right: griffon vulture.

approximately 1), which suggests efficient degradation of less persistent compounds (Houston and Cooper, 1975). While the digestive pH of Egyptian vultures is not well-documented, it could be less acidic due to their diet's resemblance to that of hunting birds of prey (Houston and Cooper, 1975). Furthermore, Egyptian vultures frequently consume carrion from small animals (birds, reptiles, amphibians) found in irrigated areas within the study region (Cortés-Avizanda et al., 2015), potentially increasing their exposure to contaminants such as α-HCH (Mirás-Avalos et al., 2023; Vega et al., 2016). Nonetheless, these hypotheses warrant comprehensive exploration in future research.

## 5. Conclusion

The low concentrations of PCBs and OCPs found in avian scavengers in northern Spain indicate that, as has already been demonstrated in other species and study systems, the implementation of restrictive regulations on these contaminants, in effect at least since 2004, has been effective. With such low concentrations, negative effects on the fitness of the target species are not expected (Gómara et al., 2008). However, it is advisable to maintain surveillance in this regard because certain individuals, in our case the Griffon vultures, have shown remarkably high levels of organochlorine pesticides. This distribution of values with extreme cases of high levels is a pattern that also appears in other studies on contaminants in birds of prey (Gómara et al., 2004; Negro et al., 1993) and reveals that under certain circumstances, some individuals may be unusually exposed to sources of contamination.

Finally, our study demonstrates the utility of stable isotopes as a tool for determining the potential exposure of the populations of avian scavengers to sources of contamination, particularly PCBs. This can be of particular value in a scenario where the food sources that scavenging birds worldwide depend on are rapidly changing due to the decline of extensive livestock, which is being replaced by intensive farming, and the increasing dependence of these birds on landfills (Blanco et al., 2019; Cerecedo-Iglesias et al., 2023; Cortés-Avizanda et al., 2016; Plaza et al., 2019). Future conservation plans should continue to monitor the levels of POPs concerning the diet of individuals in populations of conservation concern to be able to detect in time possible sources of contaminants that may emerge and undermine the success of management measures.

## CRediT authorship contribution statement

**J. Muñoz-Arnanz:** Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing, Methodology. **A. Cortés-Avizanda:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **I. Donázar-Aramendía:** Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **E. Arrondo:** Methodology, Writing – original draft, Writing – review & editing. **O. Ceballos:** Methodology, Writing – review & editing, Writing – original draft. **P. Colomer-Vidal:** Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **B. Jiménez:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Supervision. **J.A. Donázar:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – original draft, Writing – review & editing.

## Declaration of competing interest

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

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2023.123188.

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