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Evaluating contamination in the Red-billed Chough *Pyrrhocorax pyrrhocorax* through non-invasive sampling

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A B S T R A C T

Concentrations of 16 polycyclic aromatic hydrocarbons (PAHs), 19 polybrominated diphenyl ethers (PBDEs), 7 polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), isomers and metabolites of dichlorodiphenyltrichloroethane (DDTs) and Hexachlorocyclohexane (HCHs) were measured in the feathers of the Red-billed Chough *Pyrrhocorax pyrrhocorax*, a rare and protected corvid species inhabiting rural and mountainous areas. Feathers were collected in the wild from seven sites in the Iberian Peninsula, one in La Palma, Canary Islands, Spain and one site in Italy. HCH isomers and HCB resulted always under the detection limit. PAHs were found only in the Italian population (range 39.3–54.6 ng/g wet weight in the three subsamples). For PBDEs, the concentrations ranged from 135 to 11,753 pg/g (mean 1560 ± 3415 pg/g w.w.). Only one sample collected in the most urbanized Spanish site in 1990 exceeded 2000 pg/g. Twenty years later in the same site the concentration resulted very low. Six Spanish areas from the same meta-population, and one island population at La Palma showed a low contamination by *pp*'DDE (3.2 ± 3.3 ng/g w.w., range for positive samples 1.7–10.1 ng/g w.w.). PCBs were found only in two Spanish locations with the 52 and the 138 congeners. Overall the species showed low to intermediate levels of contamination in feathers probably due to its intermediate trophic position and/or to the integrity of its environment.

1. Introduction

The impacts of persistant toxic chemicals on wild animals are widely recognized, the consequences of exposure on animal health could be both acute and sublethal (reviewed by [1] for PAHs; by [2] for brominated flame retardants; by [3] for DDTs, HCB and HCS isomers). Birds have been recognized as suitable bioindicators of environmental health [4].

The Red-billed Chough *Pyrrhocorax pyrrhocorax* is a rare corvid species living mostly in rural and mountainous areas of Europe where has a scattered distribution, nesting from rocky coastal cliffs of west Ireland to high peaks of Pyrenees and the Alps, from Iberian sub-steppes to volcanic gorges of the Canary Islands. As a result, these fragmented populations could experience very different levels of contamination, and the study of the pollution is a prerequisite to address conservation efforts and also to scale the different conservation options. For example, information about the levels of contamination of persistent compound as DDT could be used to build specific extinction models for the different populations [5] to concentrate and to prioritize interventions on the most affected populations. This species is usually ecologically linked to low intensity agroecosystems [6]. In recent years some populations have undergone a severe decline possibly due to the abandonment or, on the opposite, intensification of farming activities promoting reduction of their feeding opportunities [6–8]. Besides its effects on prey availability, agricultural intensification can also expose birds to agrochemicals which is usually subtle due to the sublethal effect on the individuals [9].

Because of its synantropic nesting and roosting behavior, the Red-Billed Chough colonized several Spanish areas outside the former breeding range. They now breed and roosts on abandoned rural houses, historical buildings and other artefacts, and here could experience exposure to pollutant typically linked with industries, traffic and heating (anthropogenic activities).

As a consequence, the evaluation of the degree of exposure to the different contaminants should be part of the planning process to better address conservation efforts for each chough meta-population. Until now, studies comparing contamination levels between countries employed bird eggs, blood and/or other tissues with standard techniques but in some wild species it could be difficult to capture individuals or to sample nests from each population. Although in Spain hundreds of choughs were ringed each year thanks to its synantropy, in Italy less than ten individuals were captured in the last decade. The

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possibility to use non-invasive and inexpensive sampling techniques could represent a key factor to facilitate the monitoring of contamination in this species. Jaspers et al. [10] proposed to evaluate organic contamination in birds through feather analysis. Levels of organic pollutant in feathers were unaffected by external contamination [11] except with oil from the uropygial gland [12]. Feather analysis proved to be a very useful method not only to monitor contamination in adults [13,14] and nestlings [15] of different species but also to compare different populations of the same species at a regional scale [16]. Here we analyzed chough feathers coming from several Spanish and Italian populations to assess the presence of both agrochemicals and several environmental contaminants linked with urbanization and industrial development. We considered the brominated flame retardants polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), chlorinated pesticides such as hexachlorocyclohexane isomers (HCHs), hexachlorobenzene (HCB), isomers and metabolites of dichlorodiphenyltrichloroethane (DDTs) and polycyclic aromatic hydrocarbons (PAHs).

2. Materials and methods

2.1. Sampling areas and methods

In different years we collected flight and tail feathers of the species — rectrices and remiges primaries or secondaries — directly on the ground under the communal roosts. Feathers from all the collecting sites were first stored in paper envelopes at ambient temperature in laboratory shelves until 2010 and then mailed to the laboratory. There, they were stored in laboratory plastic bags for eight weeks before analysis. We collected feathers opportunistically during standard census of the species at their communal roosts. Feather sampling represents a routine work for field researchers censusing this rare and protected species often difficult to capture. In the latter 20 years, feathers revealed to be useful to obtain genetic material or for parasite studies, isotopic analysis and heavy metals monitoring. As a consequence, from 1990 we decided to collect and store feather samples during census from a variety of our study areas. In the present research we considered feathers coming from localities showing differences in environmental conditions from the same Iberian meta-population and from two other areas to cover a representative portion of the species geographic range. Moreover we choose feathers from a site sampled twice to evaluate diachronic evolution of contamination. We sampled the Canarian population (one locality at La Palma Island), the Iberian meta-population (seven localities) and the Italian Peninsula population (one locality). In Table 1 we showed the most salient characteristics of our sampling areas. For the Italian site for which we collected many feathers we prepared three sub-samples each composed of 7 feathers to evaluate also the variability of the contamination level in the same area. The roost sites where we collected feathers were used by dozens of individuals each night and, although we were prevented to discern between feathers coming from different individuals, the probability to sample the same individual could be considered as low.

Regarding potential problems with external contamination, pollutants reach the growing feathers via the blood accumulating in the feather structures. As a consequence, in the full-grown feathers, which lost their vascular connection, pollutant concentrations remain stable. This made the method useful especially in species difficult to capture or which nest is often impossible to visit to collect eggs. In fact, feathers could be collected in a wide range of conditions as cadavers of birds died due to traffic accident, natural causes or starvation [10] and, could be stored simply in paper envelope which could be easily send via mail. As mentioned above, Jaspers et al. [11] showed that also in these situations external contamination from the air was negligible. As a consequence, the use of feathers to monitor organic contamination in birds is spreading also through the sampling of museum specimen prepared dozens of years ago and kept at ambient temperature (see Discussion).

Feathers collected in the present study to further reduce the risk of external contamination by dust each feather was first cleaned before analysis with a laboratory brush and then washed with distilled water.

2.1.1. Analytical method for PAHs

PAHs were quantified for 16 compounds indicated by the US-EPA Clear Water Act as priority ones: NAP, naphthalene; ACP, acenaphthene; ACL, acenaphthylene; FL, fluorene; PHE, phenan-threne; AN, anthracene; PHL, fluoranthene; PYR, pyrene; B(a)A, benzo[a]anthracene; CRY, chrysene; B(b)F, benzo[b]fluoranthene; B(k)F, benzo[k,]fluoranthene; B(a)P, benzo[a]pyrene; IND, indeno [1,2,3-cd]pyrene; D(a,h)A, dibenzo [a,h]anthracene; B(g,h,i)P, ben-zo[ghi]perylene.

The analytical procedure for the extraction of feathers samples was similar to that described by Della Torre et al. [17] and Perra et al. [18]. About one gram (~1 g) of pooled samples (5–8 feathers) was extracted (Dionex mod. ASE 20 0 accelerated solvent extractor, Sunnyvale, USA) according to US-EPA (1996) method 3545A and quantified by high-performance liquid chromatography (HPLC) (Waters mod. 474 SFD and 996 PDA detectors, Milford, Massachusetts). The chromatographic separation was performed on a Supelcosil™ LC-PAH HPLC chromatographic column (250 × 4.6 mm i.d., particle size 5 lm, Supelco) with an acetonitrile:water gradient of 60:40 imposing a ramp to 100:0 within 30 min and hold 10 min, with a flow rate of 1.5 mL min. Quantitative analysis was done against a three-point linear calibration of PAH solution, obtained by dilution of the certified standard mixture TLC 16–PAH mix (Supelco). Satisfactory linearity was obtained, with values of the correlation coefficient R above 0.99. Detection limit, estimated as 3 s (IUPAC

Table 1
Physical data and environmental characteristics of the sampling sites. Categories as follows. *Urbanization level:* high: presence of towns in a 20 km radius; medium presence of sparse villages in a 20 km radius; low: presence of sparse rural houses; zero: remote area. Industrialization level: presence of relevant industrial sites in a 20 km radius (yes/not). *Presence of roads: high: presence of statal roads or highways in a 10 km radius; medium: presence of county roads in a 10 km radius; low: presence of local roads.*

Locality	Sampling date	Latitudine	Longitude	Elevation	Urbanization level	Industrialization level	Type of farming	Presence of roads
Sagittario Gorge Reserve, Anversa degli Abruzzi (AQ) — Italy	February 2010	41.9	13.8	600	medium	not	traditional	high
Aranjuez, Madrid, Spain	June and August 2009	40.0	3.5	550	high	not	intensive	medium
Segovia, Spain	Winter 2006	40.9	4.1	1000	high	yes	intensive	high
Belichóen, Madrid, Spain	September 2009	40.0	3.0	764	low	not	intensive	low
Getafe, Madrid, Spain	May and October 2009	40.3	3.6	575	high	yes	intensive	high
Rivas-vaciamadrid, Madrid, Spain	June 1990	40.3	3.5	572	high	yes	intensive	high
El Espinar, Segovia, Spain	November 2006	40.7	4.4	1272	low	not	traditional	low
Los Monegros, Zaragoza, Spain	Summer 2006	41.4	0.19	322	low	not	traditional	low
Barranco de los Cardos, La Palma, Canary Islands, Spain	October 2003	28.6	17.8	979	low	not	traditional	low