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

Augusto De Sanctis [a,*], Michela Mariottini [b], Emiliano L. Fanello [b], Guillermo Blanco [c], Silvano E. Focardi [b], Cristiana Guerranti [b], Guido Perra [b]

[a] Abruzzo Institute of WWF Protected Areas, Nature Reserve "Sagittario Gorges", Via D'Annunzio 68, 65100 Pescara, Italy
[b] Department of Environmental Sciences, University of Siena, Siena, Italy
[c] Department of Evolutionary Ecology, National Museum of Natural History, CSIC, Madrid, Spain

Keywords:
Red-billed Chough
Biomonitoring
Polybrominated diphenyl ethers
Polychlorinated biphenyls
Polycyclic aromatic hydrocarbons
Hexachlorocyclohexane
Dichlorodiphenyltrichloroethane

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 dichloro-diphenyltrichloroethane (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.

V.

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 agro-ecosystems [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

* Corresponding author. Tel./fax: +39 85 4510236.
E-mail address: a.desanctis@wwf.it (A. De Sanctis).

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

Please cite this article as: A. De Sanctis, et al., Evaluating contamination in the Red-billed Chough Pyrrhocorax pyrrhocorax through non-invasive sampling, Microchem. J. (2012), http://dx.doi.org/10.1016/j.microc.2012.08.017

criterion) for each PAH compound was 0.5 ng/g. Procedural blanks and replicate samples were used as quality control procedures, and their reproducibility and recovery were high (>85%). For the Italian metapopulation we obtained 3 groups of 7 feathers and we presented data of all these three subsamples.

2.1.2. Analytical method for PBDEs

Pooled samples (~1 g), consisting of equal quantities of feathers from five to eight specimens, were extracted (Dionex mod. ASE 20 0 accelerated solvent extractor, Sunnyvale, USA), according to US-EPA (1996) method 3545A. Nineteen PBDEs were identified and quantified by GC/MS (ion trap mass detector: ThermoFinnigan, Trace™ GC 2000/GCQ Plus) with a RTX-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm; Resteck) using splitless injection mode and helium as carrier gas [19–21]. The limit of detection (LOD) of PBDEs, calculated as mean blank +3SD, was 0.6 pg μ/L = 20 pg/g wet mass. The accuracy and precision of the procedure were tested by analyzing Certified Reference Material WMF-01 (freeze-dried fish tissue), from Wellington Laboratories Inc. The results of two replicates agreed well with the certified values with an average error of 5%.

2.1.3. Analytical method for PCBs and organochlorine pesticides

Pooled samples (~1 g), consisting of equal quantities of feathers from five to eight specimens, were extracted (Dionex mod. ASE 20 0 accelerated solvent extractor, Sunnyvale, USA), according to US-EPA (1996) method 3545A. OC pesticides and PCB congeners were identified and quantified using a GC/MS (ion trap mass detector: ThermoFinnigan, Trace™ GC 2000/GCQ Plus) with a RTX-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm; Resteck) using splitless injection mode and helium as carrier gas [22]. A blank sample prepared by the same procedure used for the samples was included every five samples and results were blank corrected. Detection limits were 0.01 ng/g tissue. PCBs and OC pesticides were analyzed in Certified Reference Material CARP-2 provided by the National Research Council Canada (NRC). Results of two replicates were highly consistent with the certified values with an average error of 5%.

3. Results

3.1. Contaminants from combustion and/or industrial activities

3.1.1. Polycyclic aromatic hydrocarbons (PAHs)

PAHs were found only in Italian subsamples (Fig. 1) which contained 11 out of the 16 PAHs evaluated (Table 2). All the three subsamples showed similar levels of contamination but the presence of the different compounds was diversified. In the most contaminated Italian subsample, fluorene and fluoranthene accounted respectively for 46% and 54% of the total amount of PAH. In the subsample 1, the pyrene represented the 86% of the total amount of PAH while in the subsample 3 that showing the lowest level of contamination, seven compounds were found. Two to three rings PAH congeners accounted for 22% of the total amount of PAH in the three Italian subsamples, four rings PAH accounted for 66% and five to six rings PAH for 15%.

3.1.2. Polychlorinated biphenyls (PCBs)

PCBs were found only in two Spanish populations, Belinchóen and Getafe, near Madrid City, where we found very low values (1.3 and 5.1 ng/g w.w. respectively, Table 3). The 52 and the 138 congeners were responsible of this low level contamination, the latter presenting the highest value.

3.1.3. Polybrominated diphenyl ethers (PBDEs)

In all samples the level of contamination by PBDE compounds exceeded 100 pg/g (mean 1560 ± 3415 pg/g w.w., range 135–11,753 Fig. 2). The highest contamination was found in the Spanish locality of Rivas-Vaciamadrid, near Madrid city, in feathers collected in 1990. The other localities showed levels below 2000 pg/g w.w (Table 3). The contamination was due to six out of the nineteen PBDE congeners we measured (the congeners 7, 15, 47, 49, 66 and 99). Four samples presented only one congener, six samples two and two samples three. The congener 47 was found in all samples (range 135–5990 pg/g w.w.) while five samples coming from both the Spanish and Italian populations resulted positive for the congener 99 (range 90–4081 pg/g w.w.). Also the congener 15 was found in four samples from both countries (range 44–72 pg/g w.w.). Congeners 49 and 66 were found only in the Spanish samples while the congener 7 was found only in one Italian sample.

3.2. Agrochemicals

3.2.1. Hexachlorobenzene (HCB) and isomers and metabolites of dichlorodiphenyltrichloroethane (DDTs) and Hexachlorocyclohexane (HCHs)

Among pesticides, HCH isomers and HCB levels were always under the detectability limit. Six of the seven Spanish localities showed a low contamination by pp'DDE (mean 3.21 ± 3.34 ng/g w.w., range for positive samples 1.7–10.1 ng/g w.w.) The latter was the only metabolite/isomer detected within DDTs. The highest values were measured in El Espinar (Segovia) and Getafe (Table 3). The Italian subsamples were not polluted.

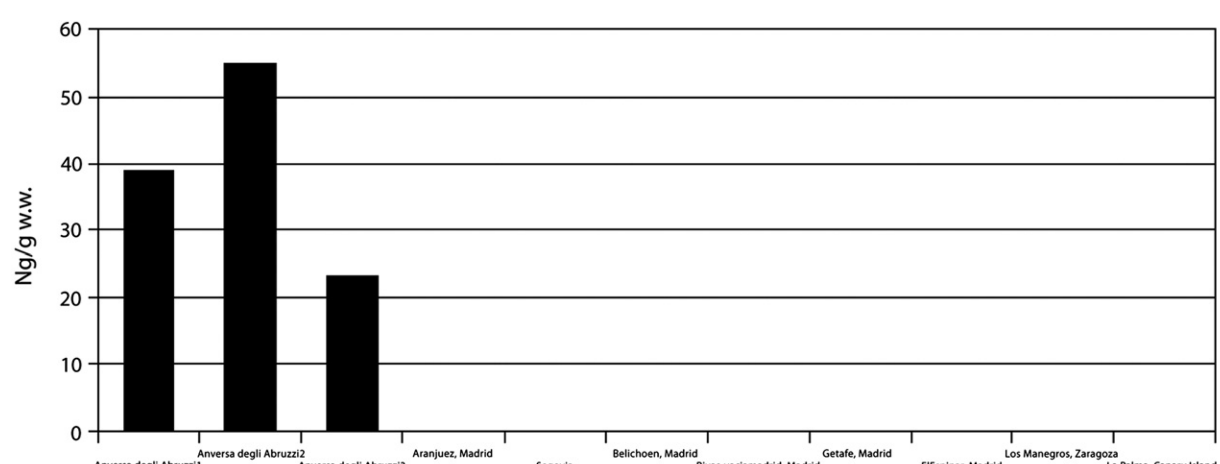

Fig 1. Values of PAHs expressed in ng/g w.w.

Please cite this article as: A. De Sanctis, et al., Evaluating contamination in the Red-billed Chough Pyrrhocorax pyrrhocorax through non-invasive sampling, Microchem. J. (2012), http://dx.doi.org/10.1016/j.microc.2012.08.017

Table 2
Values are expressed in ng/g per fresh weight. Abbreviations are: NaP (Naphthalene), Ace (Acenaphtylene), A (Acenaphthene), Fl (Fluorene), Phe (Phenanthrene), An (Anthracene), Flu (Fluoranthene), Py (Pyrene), BaA (Benz[a]antracene), Chry (Chrysene), BbF (Benz[b]fluoranthene), BkF (Benz[k]fluoranthene), BaP (Benz[a]pyrene), DBA (Dibenz[a,h]antracene), BghiP (Benzo[g,h,i]perylene) e IP (Indeno[1,2,3-cd]pyrene). Detection limit (LOD) was 0.5 ng/g.

	Anversa degli Abruzzi, IT subsample 1	Anversa degli Abruzzi, IT subsample 2	Anversa degli Abruzzi, IT subsample 3
NaP	b0.5	b0.5	b0.5
Ace	0.9	b0.5	b0.5
A	b0.5	b0.5	b0.5
Fl	b0.5	25.3	b0.5
Phe	1.9	b0.5	b0.5
An	b0.5	b0.5	7.9
Flu	b0.5	29.2	b0.5
Py	34.8	b0.5	1.0
BaA	b0.5	b0.5	b0.5
Chry	b0.5	b0.5	0.6
BbF	b0.5	b0.5	5.1
BkF	b0.5	b0.5	1.1
BaP	b0.5	b0.5	0.8
DBA	b0.5	b0.5	b0.5
BghiP	b0.5	b0.5	b0.5
IP	1.6	b0.5	6.7
Σ16 PAH	39.2	54.5	23.2

4. Discussion

The use of feathers samples for biomonitoring was validated only recently for the organic compounds analyzed in the present study, except PAHs [13]. As a consequence, there are only a very few comparative data about the level of contamination experienced by different species of birds across Europe. Moreover, taking into account the most commonly used samples (blood, liver, muscles and eggs), data on heavy metals contamination were available for a wide range of countries, habitat and species but information on pesticides and industrial contaminants are usually restricted to raptors, tits and aquatic bird species [e.g. 23–26] or for birds living in areas with dramatic cases of pollution (e.g. oil spill accidents, [27,28]).

Moreover, to our knowledge, this is the first time that feathers of the Red-billed Chough have been analyzed for the presence of contaminants. As a consequence, the interpretation of results was somewhat difficult. Generally, it seemed that our populations did not suffer high levels of contamination from the toxical compounds here analyzed. Regarding pesticides (HCH, HCB and DDT-DDE) the observed values for the Red-billed Chough are usually lower than that showed in feathers by seven out of the eight aquatic and raptor species reported from Belgium [13] In our study two Spanish populations of the chough, El Espinar (Segovia) and Getafe (Madrid), showed DDT-DDE values slightly higher than those found in buzzards Buteo buteo feathers sampled in Belgium (10.1 and 10.3 ng/g versus 9.4 ng/g). If we also considered studies which searched pesticides in eggs or tissues, the levels of pesticide contamination should be considered low or moderate [e.g. 29,30] but usually levels of contamination in feathers are generally lower than in other tissues. Jaspers et al. [10] compared PCB, PBDE and DDT/DDE levels in feathers, muscle and liver of buzzard (Buteo buteo) simultaneously showing that the concentrations of such contaminants were, respectively, 8.4, 4.0 and 14.8 times higher in the muscle and 5.6, 2.0 and 6.9 times higher in the liver than in feathers. However, correction factor could be species-specific and should be used with caution. Unfortunately blood samples of the chough were difficult to obtain from all our sites. Moreover, levels of the pollutants in the blood are known to change in the same individual in the short term (e.g. for diet, body condition). Tissues as muscle or liver from died birds were not available and for ethical, logistic and conservation reasons we avoided to try to obtain eggs to analyze. So we were prevented to make comparisons of contamination level between different kinds of biological matter coming from the Red-billed Chough.

The toxical compounds linked with industrial emissions and with urbanization in land-use (e.g. traffic and heating) — PAHs, PCBs and PBDEs — showed quite contrasting results. The latter family of compounds were always above the detectability limit but at low or very low levels, except in the Spanish locality of Rivas-Vaciamadrid in feathers collected in 1990. There we measured contamination higher than the mean concentration of the other species studied so far (eight raptor/aquatic species[13] three raptors species [15]; two herons species [31]). Interestingly, Rivas-Vaciamadrid is one of the most anthropized of our sampling areas, being located near large infrastructures (the highway Madrid-Valencia), industries, and the largest rubbish dump of Spain associated to a solid-waste incinerator [32,33] and the city of Madrid at a distance of about 20 km. These high levels of PBDEs were found much reduced in feathers from chough inhabiting the same area (feathers collected in Getafe) about 20 years later. The high levels found at the 90s could have promoted the dramatic decline that this chough population experienced since them to nowadays in south-eastern Madrid [34, G. Blanco unpubl. data]. The BDE 47 and 99 congeners contributed for 89% of the total PBDEs we measured. In respect to the other congeners, they both have the highest biomagnification potential [35] and for this reason they are the most commonly congeners found in humans, despite their minor contribution to production [36].

Regarding the two other families of compounds, on one hand PCB congeners appeared only in two Spanish population; in these cases

Table 3
Values of PBDEs, DDT-DDE and PCB expressed in ng/g w.w.. Only positive congeners are shown. The following congeners were searched but they resulted under the detectability threshold.

	Anversa 1	Anversa 2	Anversa 3	Aranjuez, Madrid	Segovia	Belichóen, Madrid	Rivas-vaciamadrid, Madrid	Getafe, Madrid	El Espinar, Segovia	Los Monegros, Zaragoza	La Palma Canary islands
PBDE-7	b20	39	b20	b20	b20	b20	b20	b20	b20	b20	b20
PBDE-15	44	b20	72	b20	54	58	b20	b20	b20	b20	b20
PBDE-47	167	256	154	240	346	376	5991	472	1647	255	135
PBDE-49	b20	b20	b20	b20	b20	b20	1681	b20	b20	b20	b20
PBDE-66	b20	b20	b20	b20	b20	80	b20	b20	b20	b20	b20
PBDE-99	541	b20	b20	b20	b20	b20	4081	91	249	126	b20
ΣPBDE	752	295	226	240	400	515	11,753	563	1896	381	135
pp-DDE	b0.1	b0.1	b0,1	3.4	3,9	3,9	b0.1	5.2	10.1	1.7	7.2
ΣDDT	b0.1	b0.1	b0.1	3.4	3.9	3.9	b0.1	5.2	10.1	1.7	7.2
PCB-52	b0.2	b0.2	b0.2	b0.2	b0.2	1.3	b0.2	b0.2	b0.2	b0.2	b0.2
PCB-138	b0.2	b0.2	b0.2	b0.2	b0.2	b0.2	b0.2	5.1	b0.2	b0.2	b0.2
ΣPCB	b0.2	b0.2	b0.2	b0.2	b0.2	1.3	b0.2	5.1	b0.2	b0.2	b0.2

PBDE: 3, 17, 28, 71, 77, 85, 100, 119, 126, 138, 153, 154 and 156.
PCB: 28, 101, 118, 153, 180.
DDT-DDE: op'-DDD, op'-DDE, op'-DDT, pp'-DDD, pp'-DDT

Please cite this article as: A. De Sanctis, et al., Evaluating contamination in the Red-billed Chough Pyrrhocorax pyrrhocorax through non-invasive sampling, Microchem. J. (2012), http://dx.doi.org/10.1016/j.microc.2012.08.017

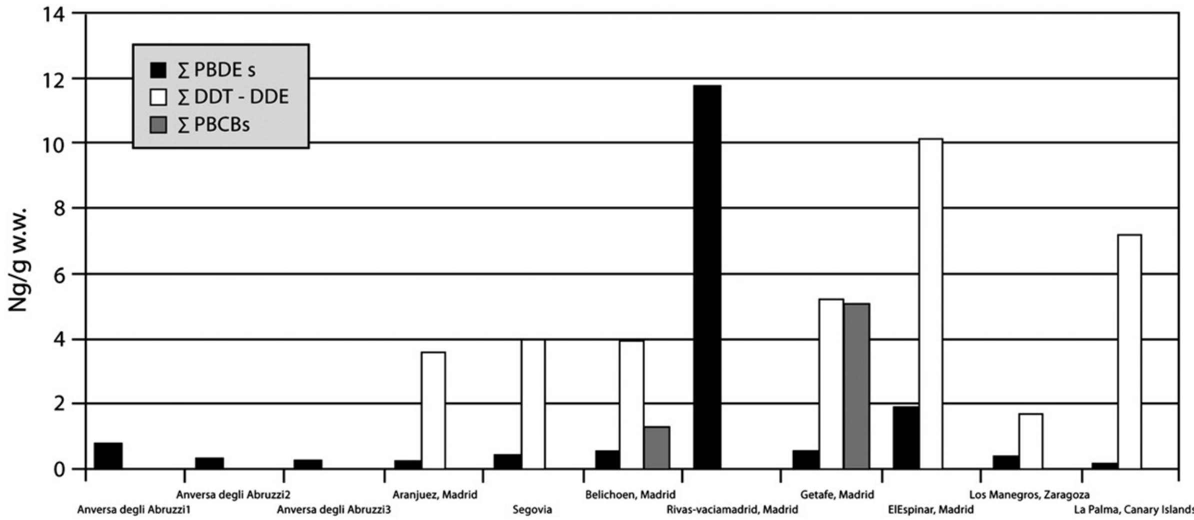

Fig. 2. Values of ΣPBDEs, ΣDDT-DDE, ΣPCBs. Values expressed in ng/g w.w.

they were found in low quantities as compared with other species (considering feathers and eggs and tissues: [14,30,37]).

At the other hand, PAH compounds resulted above the levels of detectability only in the Italian population, which was sampled inside a Natural Reserve area. The average concentration of ΣPAHs in Italian samples was within the range of values described also for eggs of the Red-billed Choughs in Britain [30 ng/g, 38]. Unfortunately, in the literature there were no studies comparing PAH values in feathers and eggs in the same species and correction factors were not available. As suggested before, levels of pollutants in feathers are generally lower than in the eggs and our values could represent an indication of higher exposure to PAH in our Italian population. Our results should be interpreted with cautions given the novel approach to evaluate PAHs used in this study. In fact, our samples were collected in different years and those collected in Italy were the most recent ones. We are not aware of degradation process for the different PAH congeners in feather structures. Behrooz [37] measured for the first time organic compounds levels in feathers of a museum collection of many bird species stuffed several years before but did not analyzed PAHs. In our study we mostly found tetra and pentacyclic congeners. Generally, an increase in the size of a PAH molecule results in an increase in electrochemical stability [39] which in turn contribute to the persistence of PAHs in the environment [40]. As BDE 47 and BDE 99, the PAHs we found in the Italian site as fluoranthene have a high potential for biomagnification and are known to exert toxic effects and possess mutagenic or carcinogenic properties [41]. Tetra and pentacyclic PAHs present less volatile properties and usually derives from local sources. Two possible but not mutually exclusive causes could explain the exposure of the Red-Billed Chough Italian population to PAHs. The roost site used year-round by the individuals of the colony and also their nest-sites are on a cliff crossed by a county road. The roost site is at a distance of 50 m by this infrastructure which is heavily used in the touristic season. Some nests directly overlooked the road at a distance of 10–20 m. Moreover, the rocky gorge in this point is very narrow and it is possible that traffic emissions did not disperse over a wider area. Fluoranthene and pyrene are known to be the major contributors from anthropogenic combustion sources [42]. Perico et al. [43] measuring in humans metabolites of pyrene excreted with urine, linked the exposure to pyrene to the traffic intensity. The second explanation for exposure to PAHs could refer to emissions from forest burning [44]. The interior of the Abruzzo region was affected in the 2007 by large forest fires just at 15–20 km from the collecting sites (about 10,000 hectares in the July-August 2007). As suggested by Vidal et al. [45], the large amount of poisonous emissions from fires could entail the exposure of birds to large amount of PAHs. In the case of the chough, the species forage over wide areas which are distant up to 15–35 km from the roosting and nesting site. In this reserve biomonitoring through measures on PAHs in tits eggs (De Sanctis unpublished data) confirmed the exposure of wildlife to these pollutants despite the environment surrounding the sampling site was considered in good state.

The observed level of toxic compounds accumulation seemed to agree with the ecological role the Red-billed Chough, which occupies a lower trophic level than raptors and aquatic birds such as gulls and herons, preying mainly on vertebrates. The chough mostly feeds on soil invertebrates, seeds and fruits (for Spain [46], for the Central Appennines, Italy [47]) so in this species the consequences of the biomagnification process should be less severe. Zhang et al. [28] reported a positive association between trophic level and contamination and Behrooz et al. [37] showed that the highest levels of DDTs and PCBs were found in the carnivorous species compared to herbivorous ones. It is also possible that exposure to contaminants is overall less than that experienced in other areas due to the remoteness of most of our sampling sites with scarce sources of pollution, as demonstrated for tits living in areas with different degree of urbanization [24].

5. Conclusions

Our study indicated that the sampled Red-billed Chough populations show low to intermediate levels of contamination in feathers. These could be linked both to the trophic position of the species and/or to the integrity, at least by a contamination point of view, of the environment surrounding its breeding and wintering sites. At the same time we demonstrated that in some particular cases birds could accumulate specific toxic compounds. Although we could only tentatively advance some explanations to identify exposure routes for the different groups of molecules, at the same time we provide important baseline information that can be used for future research.

The Red-billed Chough is protected under the EU "Birds" Directive 147/2009/CEE, which requires the establishment of Special Protection Areas (SPA) devoted to the conservation of this species. After gazetting, SPA required the drafting and implementation of conservation plans with site-specific measures such as limitations of the use of pesticides and other contaminants. As a consequence, the evaluation of the degree of exposure to contaminants should be part of the planning process to better address conservation efforts for each meta-population. The possibility to use non-invasive and very expeditious sampling

Please cite this article as: A. De Sanctis, et al., Evaluating contamination in the Red-billed Chough Pyrrhocorax pyrrhocorax through non-invasive sampling, Microchem. J. (2012), http://dx.doi.org/10.1016/j.microc.2012.08.017

techniques could represent a key factor to facilitate the monitoring of contamination in this species due to the difficulties to capture the individuals from each population.

Acknowledgements

This study was funded by the Natural Reserve and WWF Oasis "Sagittario Gorge", Anversa degli Abruzzi (AQ, Italy) municipality.

We thank Paola Laiolo who gave very useful suggestions on a early version of the paper and Chiara Rizzi for her support in editing the figures.

References

[1] P.H. Albers, Birds and polycyclic aromatic hydrocarbons, Avian Poult. Biol. Rev. 17 (2006) 125–140.
[2] P.O. Darnerud, Toxic effects of brominated flame retardants in man and in wildlife, Environ. Int. 29 (2003) 841–853.
[3] A. Mitra, C. Chatterjee, F.N. Mandal, Synthetic chemical pesticides and their effect on birds, Res. J. Environ. Toxicol. 5 (2011) 81–96.
[4] J. Burger, M. Gochfeld, in: Marine Birds as Sentinels of Environmental Pollution EcoHealth, 1, 2004, pp. 263–274.
[5] M. Nakamaru, Y. Iwasa, J. Nakanishi, Extinction risk to bird populations caused by DDT exposure, Chemosphere 53 (2003) 377–387.
[6] G. Blanco, J.L. Tella, I. Torre, Traditional farming and key foraging habitats for chough Pyrrhocorax pyrrhocorax conservation in a Spanish pseudosteppe landscape, J. Appl. Ecol. 35 (1998) 232–239.
[7] A. Robertson, A.M. Jarvis, K.R. Day, Habitat selection and foraging behavior of breeding choughs Pyrrhocorax pyrrhocorax L. in county Donegal, Proc. R. Ir. Acad. 95 (1995) 69–74.
[8] C. Kerbiriou, F. Gourmelon, F. Jiguet, L.E. Viol, E. Isabell, F. Bioret, R. Julliard, Linking territory quality and reproductive success in the Red-billed Chough Pyrrhocorax pyrrhocorax: implications for conservation management of an endangered population, Ibis 148 (2006) 352–364.
[9] D.M. Fry, Reproductive effects in birds exposed to pesticides and industrial chemicals, Environ. Health Perspect. 103 (Suppl. 7) (October 1995) 165–171.
[10] V.L.B. Jaspers, S. Voorspoels, A. Covaci, M. Eens, Can predatory bird feathers be used as a non-destructive biomonitoring tool of organic pollutants? Biol. Lett. 2 (2006) 283–285.
[11] V.L.B. Jaspers, A. Covaci, E. Steen Van Den, M. Eens, Is external contamination with organic pollutants important for concentrations measured in bird feathers? Environ. Int. 33 (2007) 766–772.
[12] V.L.B. Jaspers, A. Covaci, P. Deleu, H. Neels, M. Eens, Preen oil as the main source of external contamination with organic pollutants onto feathers of the common magpie (Pica pica), Environ. Int. 34 (2008) 741–748.
[13] V.L.B. Jaspers, S. Voorspoels, A. Covaci, G. Lepoint, M. Eens, Evaluation of the usefulness of bird feathers as a non-destructive biomonitoring tool for organic pollutants: a comparative and meta-analytical approach, Environ. Int. 33 (2007) 328–337 (Erratum in: Environ Int. 33(2007): 714–5).
[14] F. Rajaei, A.E. Sari, N. Bahramifar, M. Savabieasfahani, M. Ghasempouri, Persistent organic pollutants in muscle and feather of ten avian species from Māzandarān Province of Iran, on the Coast of the Caspian Sea, Bull. Environ. Contam. Toxicol. 87 (2011) 678–683.
[15] I. Eulaers, A. Covaci, D. Herzke, M. Eens, C. Sonne, T. Moum, L. Schnug, S.A. Hanssen, T.V. Johnsen, J.O. Bustnes, V.L.B. Jaspers, A first evaluation of the usefulness of feathers of nestling predatory birds for non-destructive biomonitoring of persistent organic pollutants, Environ. Int. 37 (2011) 622–630.
[16] V.L.B. Jaspers, A. Covaci, P. Deleu, M. Eens, Concentrations in bird feathers reflect regional contamination with organic pollutants, Sci. Total Environ. 407 (2009) 1447–1451.
[17] C. Della Torre, I. Corsi, F. Nardi, G. Perra, M.P. Tomasino, S.E. Focardi, Transcriptional and post-transcriptional response of drug-metabolizing enzymes to PAHs contamination in red mullet (Mullus barbatus, Linnaeus, 1758): a field study, Mar. Environ. Res. 70 (2010) 95–101.
[18] G. Perra, K. Pozo, C. Guerranti, D. Lazzeri, V. Volpi, S. Consolini, S. Focardi, levels and spatial distribution of polycyclic aromatic hydrocarbons (PAHs) in superficial sediment from fifteen Italian Marine protected Areas (MPA), Mar. Pollut. Bull. 62 (2011) 874–877.
[19] M. Mariottini, I. Corsi, C. Della Torre, T. Caruso, A. Bianchini, I. Nesi, S. Focardi, Biomonitoring of polybrominated diphenyl ether (PBDE) pollution: a field study, Comp. Biochem. Physiol. C Toxicol. Pharmacol. 148 (2008) 80–86.
[20] C. Guerranti, S.E. Focardi, Differences in POP levels between conventional and omega-3 fatty acid-enriched milk and dairy products, ISRN Toxicology, 2011. article ID 541694, 7 pages.
[21] C. Guerranti, M. Palmieri, S.E. Focardi Mariottini, Persistent organic pollutants in human milk from central Italy: levels and time trends, ISRN Toxicology, 2011. article ID 107514, 6 pages.
[22] I. Corsi, M. Mariottini, A. Badesso, T. Caruso, N. Borghesi, S. Bonacci, A. Iacocca, S. Focardi, Contamination and sub-lethal toxicological effects of persistent organic pollutants in the European eel (Anguilla anguilla) in the Orbetello lagoon (Tuscany, Italy), Hydrobiologia 550 (2005) 1–13.
[23] P. Wegner, G. Kleinstauber, F. Baum, F. Schilling, Long-term investigation of the degree of exposure of German peregrine falcons (Falco peregrinus) to damaging chemicals from the environment, J. Org. 146 (2011) 34–54.
[24] E. Steen Van den, R. Pinxten, V.L.B. Jaspers, A. Covaci, E. Barba, C. Carere, M. Cichoń, A. Dubiec, T. Eeva, P. Heeb, B. Kempenaers, J.T. Lifjeld, T. Lubjuhn, R. Mänd, B. Massa, J.A. Nilsson, A.C. Norte, M. Orell, P. Podzemny, J.J. Sanz, J.C. Senar, J.J. Soler, A. Sorace, J. Török, M.E. Visser, W. Winkel, M. Eens, Brominated flame retardants and organochlorines in the European Environment using great tit eggs as a biomonitoring tool, Environ. Int. 35 (2009) 310–317.
[25] M.G. Pereira, L.A. Walker, J. Wright, J. Best, F. Richard, F. Shore, Polycyclic Aromatic Hydrocarbons (PAHs) in eggs from Gannets, Golden Eagle and Merlins, Organohalogen Compd. 70 (2008) 166–169.
[26] F. Rajaei, N. Bahramifar, A.E. Sari, M. Ghasempouri, PCBs and Organochlorine Pesticides in ducks of Fereydoon-kenar Wildilife Refuge in Iran, Bull. Environ. Contam. Toxicol. 84 (2010) 577–581.
[27] C. Pérez, A. Velando, I. Munilla, M. Lòpez-Alonso, D. Oro, Monitoring polycyclic aromatic hydrocarbon pollution in the marine environment after the Prestige oil spill by means of seabird blood analysis, Environ. Sci. Technol. 42 (2008) 707–713.
[28] X. Zhang, X. Luo, J. Liu, Y. Luo, S. Chen, Polychlorinated biphenyls and organochlorinated pesticides in birds from a contaminated region in South China: association with trophic level, tissue distribution and risk assessment, Environ. Sci. Pollut. Res. 18 (2011) 556–565.
[29] M.G. Pereira, L.A. Walker, J. Wright, J. Best, R.F. Shore, Concentrations of polycyclic aromatic hydrocarbons (PAHs) in the eggs of predatory birds in Britain, Environ. Sci. Technol. 43 (2009) 9010–9015.
[30] P. Movalli, M. Lo Valvo, G. Pereira, D. Osborn, Organochlorine pesticides and polychlorinated biphenyl congeners in lanner Falco biarmicus feldeggii Schlegel Chicks and Lanner Prey in Sicily, Italy, Ambio 37 (2008) 445–451.
[31] R.N. Malik, C. Moeckel, K.C. Jones, D. Hughes, Polybrominated diphenyl ethers (PBDEs) in feathers of colonial water-bird species from Pakistan, Environ. Pollut. 159 (2011) 2044–3055.
[32] G. Blanco, O. Frías, B. Jimenez, G. Gómez, Factors affecting variability and potential uptake routes of heavy metals in black kites exposed to emissions from a solid-waste incinerator, Environ. Toxicol. Chem. 22 (2003) 2711–2718.
[33] G. Blanco, B. Jimenez, O. Frías, J. Millan, J.A. Dávila, Contamination with non-essential metals from a solid-waste incinerator correlate with nutritional and immunological stress in prefledging black kites (Milvus migrans), Environ. Res. 94 (2004) 94–101.
[34] G. Blanco, Chova Piquirroja, (Pyrrhocorax pyrrhocorax) in: Atlas de las Aves Invernantes de la Comunidad de Madrid (1999–2001), Seo/Birdlife, Madrid, Spain, 2002, pp. 280–281.
[35] B.C. Kelly, M.G. Ikonomou, J.D. Blair, A.E. Morin, F.A.P.C. Gobas, Food web-specific biomagnification of persistent organic pollutants, Science 317 (5835) (2007) 236–239.
[36] N. Wu, T. Herrmann, O. Paepke, J. Tickner, R. Hale, E. Harvey, M. La Guardia, M.D. Mclean, T. Webster, Human exposure to PBDEs: associations of PBDE body burdens with food consumption and house dust concentrations, Environ. Sci. Technol. 41 (2007) 1584–1589.
[37] R.D. Behrooz, A. Esmaili-sari, S.M. Ghasempouri, N. Bahramifar, A. Covaci, Organochlorine pesticide and polychlorinated biphenyl residues in feathers of birds from different trophic levels of South-West Iran, Environ. Int. 35 (2009) 285–290.
[38] R.F. Shore, J. Wright, J.A. Horne, T.H. Sparks, Polyciclic Aromatic Hydrocarbons (PAH) residues in the eggs of coastal-nesting birds from Britain, Mar. Pollut. Bull. 38 (1999) 509–513.
[39] R.G. Harvey, Polycyclic aromatic hydrocarbons, Wiley-VCH, New York, N.Y., 1997.
[40] R.J. Wilcock, G.A. Corban, G.L. Northcott, A.L. Wilkins, A.G. Langdon, Persistence of polycyclic aromatic compounds of different molecular size and water solubility in surficial sediment of an intertidal sandflat, Environ. Toxicol. Chem. 15 (1996) 670–676.
[41] W.H. Clements, J.T. Oris, T.E. Wissing, Accumulation and food chain transfer of fluoranthene and benzo[a]pyrene in Chironomus riparius and Lepomis macrochirus, Arch. Environ. Contam. Toxicol. 26 (1994) 261–266.
[42] S. Sporstøl, N. Gjøs, R.G. Lichtenthaler, K.O. Gustavsen, K. Urdal, F. Oreld, Source identification of aromatic hydrocarbons in sediments using GC/MS, Environ. Sci. Technol. 17 (1983) 282–286.
[43] A. Perico, M. Gottardi, V. Boddi, P. Bavazzano, E. Lanciotti, Assessment of exposure to polycyclic aromatic hydrocarbons in police in Florence, Italy, through personal air sampling and biological monitoring of the urinary metabolite 1-hydroxypyrene, Arch. Environ. Health 56 (2001) 506–512.
[44] E.J. Kim, S.D. Choi, Y.S. Chang, Levels and patterns of polycyclic aromatic hydrocarbons (PAHs) in soils after forest fires in South Korea, Environ. Sci. Pollut. Res. Int. 18 (2011) 1508–1517.
[45] M. Vidal, J. Domìnguez, A. Luiz, Spatial and temporal patterns of polycyclic aromatic hydrocarbons (PAHs) in eggs of a coastal bird from northwestern Iberia after a major oil spill, Sci. Total Environ. 409 (2011) 2668–2673.
[46] J.J. Soler, M. Soler, Diet of the Red-Billed Chough Pyrrhocorax pyrrhocorax in south-east Spain, Bird Study 40 (1993) 216–222.
[47] R. Caldoni, A. De Sanctis, Some data on the diet of the Red-billed Chough Pyrrhocorax pyrrhocorax in the WWF reserve of the Sagittario Gorges (Abruzzo, Central Apennines), Alula 15 (2009) 147–150.