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The Use of Passerine Feathers to Evaluate Heavy Metal Pollution in Central Portugal

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Abstract In 2003, two sampling strategies were applied in order to evaluate metal contamination in passerine bird feathers. One strategy included the use of nest boxes and nestling great tits in a forest habitat (MU). The other strategy focused on the use of mist nets to capture adult blackbirds, robins and blackcaps in a fragmented non-forested habitat (EST). There was a significantly higher concentration of mercury in great tits (1.09 ± 0.40) sampled in MU and also in robins (3.44 ± 0.91) and blackcaps (1.62 ± 1.99) sampled in EST, in comparison to the reference site (0.66 ± 0.68; 2.04 ± 1.12; 0.32 ± 0.43; respectively). Concerning interspecific differences, robins accumulated significantly more metals than blackbirds or blackcaps.

Keywords Heavy metal pollution · Passerine bird feathers

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Heavy metals are recurrent waste products of industrial activities and their emission often results in the contamination of the surrounding environment (Eeva and Lehikoinen 2000). Bird feathers have been used intensively in numerous biomonitoring studies and they have been shown to be suitable indicators of heavy metal pollution (Dauwe et al. 2000; Janssens et al. 2002). Most studies were carried out on birds of prey (Jager et al. 1996) and on seabird species (Kim et al. 1996). However, most of these species are very mobile, feeding over a wide geographical area, making it difficult to determine where contaminants were acquired. On the other hand, passerines are mainly non-migratory residents, foraging along small ranges. This implies that the detected contamination refers to a limited area, making passerine birds suitable for identifying local pollution (Furness 1993; Chao et al. 2003). Although nestlings are also potential good monitors for terrestrial point-source pollution (Dauwe et al. 2000), they have been used to a much lesser extent. Metal concentrations in nestling feathers may reflect local pollution levels far better than those concentrations measured in adults because in nestling exposure occurs in a clearly defined time period and its source is limited to the parent's foraging area (Furness 1993).

Studies focusing on Mediterranean habitats are scarce in comparison to those focusing on other habitats (Costa et al. 2005). Therefore, it is necessary to assess the efficiency of passerine birds in monitoring heavy metal pollution in Mediterranean habitats.

In the present study, we analysed the feathers of nestling great tits (*Parus major*) and the feathers of three adult resident passerine bird species in two polluted areas (MU and EST) and in a reference site (MQ) located in a rural area. The three adult passerines correspond to blackbirds (*Turdus merula*), robins (*Erithacus rubecula*) and blackcaps (*Sylvia atricapilla*), which were chosen due to their availability in reasonable densities in all the areas. Because there were no forested areas along the polluted area EST (unsuitable for nest box studies), two sampling strategies were applied: one consisting on the use of nest boxes and nestling great tits in forest habitats (MQ vs MU) and the other consisting on the use of mist nets to capture passerine birds in fragmented non-forested habitats where the use of nest boxes is not feasible (MQ vs EST).

The main objective of this study was to evaluate the use of different passerine bird species as indicators of heavy metals according to different types of atmospheric pollution.

Materials and Methods

Feathers were obtained in two industrial areas identified as MU (National Pine Forest of Urso) and EST (Estarreja), and also in a forest area identified as MQ (National Pine Forest of Quiaios) where there is no direct or nearby influence of industrial effluents. The latter was used as the reference site when comparing levels of toxic elements in feathers collected in each study area.

The industrial area EST is a chemical compound located in the north of Estarreja city, 60 km to the north of the reference site. Agriculture fields and pine/eucalyptus stands dominate the area surrounding the compound. At this site, there are numerous factories that manufacture several products such as resins, fertilisers, chlorine (Cl_2) and caustic soda (NaOH). Mercury is used as a raw material in the productive process of chlorine and caustic soda (Ferreira 1993).

The industrial area MU is a pulp and paper mill located to the south of Figueira da Foz, 20 km to the south of the reference site. The area surrounding the factory is a maritime pine plantation dominated by *Pinus pinaster* interspersed by some *Pinus pinea* patches.

An area located in the National Pine Forest of Quiaios (MQ), Figueira da Foz, with presumably very limited pollution, was chosen as a reference site. MQ is a maritime pine forest included in the protected area of Dunas de Mira (Natura 2000 PTCON055). The area is a 70–80 year old man-made plantation dominated by *Pinus pinaster* interspersed by some *Pinus pinea* patches. Agriculture fields are present in the edges of the area.

In MQ and MU nest boxes (n = 108) were equally distributed along three quadrangular 4 ha sampling plots. The distance between boxes was 40 m. Nest boxes were checked regularly to gather data on several breeding parameters (Costa et al. 2005). When great tit nestlings were 15 (±1) days of age, the two outermost tail feathers were collected from 164 nestlings (MQ – 92, MU – 72) from 32 nests (MQ – 19, MU – 13).

In EST (on the 29th of May) and in MQ (on the 9th and the 14th of May) 47 adult blackbirds, robins and blackcaps were caught in mist nets. The captured birds were measured, ringed and the two outermost tail feathers were collected.

In the case of adult birds, each feather sample corresponded to an individual bird, whereas samples of nestling tits corresponded to a pool of feathers collected from three nestling birds of the same nest (Dauwe et al. 2000) thus ensuring a sufficient amount of feathers for heavy metal analysis.

Feathers were stored in sterile, metal-free plastic ependorf tubes and maintained at −20°C until analysis. To obtain the feather's dry weight, feathers were left in an oven (60°C) till constant weight. About 0.2 g of feathers of each sample was rinsed in tap water and deionised water to eliminate adsorbed external contamination (Burger 1993). Each sample was treated with nitric acid overnight (15 mL). The wet-ash procedure was continued with heating in a water bath, followed by the addition of hydrogen peroxide (2 mL). This treatment was repeated till full digestion. The filtrate was diluted with double distilled water up to a volume of 25 mL. Finally, the digested material was analysed for heavy metals using a combination of analytical techniques: ICP-AES (inductively coupled plasma – atomic emission spectrometry) was used to estimate Cu, Zn, Cd, Ni and Pb (EPA procedures: EPA 6010B); total Hg was estimated using a cold vapor atomic absorption (CVAA) technique (EPA procedures: EPA 7471A). Concentrations are expressed in mg kg^{-1} based on dry weight values. All samples were analyzed in batches with certified reference material (mussel sample- CRM 278, Community Bureau of Reference) and blanks. Recovered concentrations of the certified samples were within 10% of the certified values, which is considered an acceptable margin (Gochfeld and Burger 1998). The limit of detection was 1 mg kg^{-1} for Cu, Zn, Ni, Pb and 0.1 mg kg^{-1} for Cd and Hg.

Statistical analyses were performed with SAS statistical software 9.2 (SAS Institute 2003).

Differences in heavy metal values were compared between study areas using the t-test for unequal variances, after checking for normal distribution. Values that did not present a normal distribution were $\log_{10}$-transformed to normalize distributions. In adult birds, due to the small number of samples above the analytical detection limits, confidence limits were generated for sample estimates by the bootstrap technique – 1,000 repetitions (Quinn and Keough 2002). Analysis of variance (ANOVA) was used to compare metal concentrations between species for each study area (followed by Tukey's post-hoc test). Samples yielding concentration values below the detection limits were removed from the analysis. For all analysis, the significance level was set at $p < 0.05$.

Results and Discussion

One objective of our study was to compare metal concentrations in the feathers of nestling great tits between two forest habitats exposed to different levels of pollution (an industrial area-MU and a forest area-MQ). The industrial site presented significantly higher values of mercury (Table 1). The concentrations of nickel, copper and zinc presented no significant differences between areas and they were even slightly higher in the forest area (MQ) (Table 1). In other studies reporting concentrations of heavy metals in nestling birds (Dauwe et al. 2000; Janssens et al. 2002), the concentrations of copper and zinc were also not significantly different between reference and polluted sites. These authors suggest that great tit nestling feathers cannot be used as biomonitors for zinc and copper because they may not reflect adequately the nestling body burden. According to Dauwe et al. (2000) homeostatic mechanisms (e. g., excretion through the excrements) may still adequately control metal levels in tissues at this early stage of maturity.

The levels of cadmium and lead were below the detection limits in the majority of the samples of the industrial area (MU) thus drastically reducing the number of samples. Even though nickel and lead were the only heavy metals emitted by the paper and pulp facilities in 2002 (EPER 2009), in MU no traces of lead were detected. Furthermore, the present results show a significant higher concentration of mercury in MU when comparing to MQ (Table 2). Because of its persistence and bioaccumulation potential, mercury is regarded as a global pollutant (EPER 2009). The high presence of mercury in MU could be a result of a past long-term emission, when environmental concerns were not considered a priority and regulations for this kind of emissions were lacking. One should also consider possible current emissions from factories which still release mercury during their production processes. Either way, mercury constitutes a matter of great concern and should be regularly monitored considering its well-known toxicity.

Table 1 Comparison between heavy metal levels (mg kg^{-1}) determined in feathers of nestling great tits in MQ and MU using a *t* test (mean ± standard deviation)

	n	MQ	n	MU	*t*	*p*
Cu	17	7.15 ± 2.22	13	7.08 ± 1.43	0.11	0.92
Zn	18	105.24 ± 25.67	13	98.37 ± 13.71	0.33	0.75
Hg	17	0.66 ± 0.68	13	1.09 ± 0.40	−3.17	**0.006**
Cd	10	0.17 ± 0.11	1	0.20	–	–
Ni	12	2.44 ± 1.97	2	1.24 ± 0.18	2.16	0.06
Pb	5	2.23 ± 1.18	0	–	–	–

In what concerns adult birds, mercury was also found in samples collected in the chemical compound area (EST). In 1988, the amount of mercury in liquid effluents was 8.5 mg/l/year (Tavares 1995) and according to EPER (2009) in 2002 gas emissions amounted to 7.61 kg/year. As expected, EST presented the highest levels of mercury in the three bird species (although not significantly higher in blackbird) (Table 2).

Cadmium was also significantly higher in EST in all species (Table 2). Although cadmium is not generally available to incorporate into feathers, in this study cadmium was consistently detected in the three species implying a very high level of cadmium in the surrounding habitats. The agriculture-dominated landscape and the presence of a fertilizer factory in EST may account for such high cadmium levels considering that cadmium may originate from anthropogenic activities such as the use of phosphate fertilisers (EPER 2009).

Lead and zinc were also significantly higher in EST (Table 2) in the case of robins. A Similar result was found for the blackcap, although, significant differences were detected for zinc values only. Considering their well-known hazardous effect on the environment, these results emphasise the need to regularly monitor areas presenting industrial usages similar to those located in EST.

Differences in metal levels between species and areas may be produced by different diets, feeding strategies and metabolic rates. Considering that blackbirds feed mostly on earthworms, this species was expected to accumulate more metals (Llacuna et al. 1995). However, results showed that robins accumulated more metals presenting differences between areas (Table 2). Robins eat mainly insects and feed on the ground. They are relatively smaller and present a higher metabolic rate (Root 1990; Eens et al. 1999), which might account for the higher accumulation of metals when comparing different species (Table 3). Blackcaps are also insectivorous and smaller. However, they feed mainly on trees thus reducing their exposure to metals when comparing with species that feed on the ground. The present results highlight that robins may constitute an efficient species to be used in studies dealing with heavy metal monitorization (Table 3).

In conclusion, this study suggests that the use of great tit nestlings' feathers is a useful method to evaluate the presence of heavy metals, such as mercury, in the forest environment. Furthermore, great tits are ubiquitous and abundant, and sometimes the only forest passerine species available in reasonable densities in polluted areas. They readily nest in manmade nest boxes and so breeding populations can easily be monitored. Great tit nestlings are also easily monitored because they are relatively insensitive to nest disturbance (Janssens et al. 2002). Another advantage of using nestlings is the fact that they are restricted to their

Table 2 Comparison between heavy metal levels (mg kg^{-1}) determined in feathers of adult blackbird (n = 11), robin (n = 14) and blackcap (n = 22) in MQ and EST using a t-test (mean ± standard deviation) and 95% confidence limits (in brackets)

	MQ	EST	*t*	*p*
Blackbird				
Cd	0.12 ± 0.08 (0.03–0.18)	0.38 ± 0.20 (0.16–0.5)	3.05	**0.02**
Cu	11.00 ± 4.88 (8.09–14.13)	10.43 ± 2.99 (8.81–12.64)	0.27	0.79
Ni	0.78 ± 0.30 (0.5–1.06)	2.15 ± 1.20 (1.10–2.81)	2.74	**0.04**
Pb	2.35 ± 0.77 (1.52–3.15)	4.89 ± 3.22 (2.92–7.10)	2.04	0.09
Zn	109.78 ± 10.16 (102.03–121.59)	140.10 ± 36.84 (120.46–166.88)	2.11	0.08
Hg	0.50 ± 0.27 (0.26–0.73)	1.05 ± 0.95 (0.47–1.81)	1.46	0.19
Robin				
Cd	0.11 ± 0.13 (0.03–0.18)	0.37 ± 0.29 (0.06–0.65)	2.36	**0.04**
Cu	17.06 ± 6.29 (13.53–21.18)	13.66 ± 4.89 (8.76–18.56)	1.29	0.21
Ni	1.13 ± 0.90 (0.60–1.73)	1.02 ± 0.48 (0.5–1.38)	0.33	0.75
Pb	2.04 ± 1.12 (1.41–2.67)	4.14 ± 2.07 (2.48–6.60)	2.59	**0.03**
Zn	113.57 ± 19.50 (102.84–125.14)	135.69 ± 21.59 (108.54–150.18)	2.25	**0.04**
Hg	2.04 ± 1.12 (1.52–3.75)	3.44 ± 0.91 (2.5–4.06)	2.64	**0.02**
Blackcap				
Cd	0.20 ± 0.15 (0.11–0.29)	0.36 ± 0.27 (0.24–0.51)	2.14	**0.046**
Cu	15.04 ± 7.50 (10.95–20.22)	13.74 ± 5.28 (11.15–16.64)	0.45	0.66
Pb	3.05 ± 1.22 (2.30–3.84)	3.57 ± 2.15 (2.48–4.64)	0.73	0.47
Zn	87.83 ± 14.36 (79.26–96.59)	121.05 ± 27.69 (107.09–136.44)	3.67	**0.002**
Hg	0.32 ± 0.43 (0.01–0.29)	1.62 ± 1.99 (0.65–2.71)	2.27	**0.04**

Table 3 Interspecific metal accumulation differences between the three bird species studied, blackbird (n = 11), robin (n = 14) and blackcap (n = 22), from an ANOVA analysis

	MQ				EST			
	F	*df*	*p*	Bird	F	*df*	*p*	Bird
Cu	2.88	2.26	0.08		1.39	2.27	0.27	
Zn	8.50	2.26	**0.002**	Blackcap[b]	1.49	2.27	0.25	
Hg	5.61	2.26	**0.01**	Robin[a]	3.67	2.27	**0.04**	Robin[a]
Cd	1.51	2.26	0.24		0.42	2.26	0.66	
Ni	0.24	2.25	0.78		22.79	2.26	**<0.0001**	Blackbird[a]
Pb	2.30	2.26	0.12		0.70	2.27	0.51	

[a] Passerine bird that showed significantly higher metal concentration than the two other studied species

[b] Passerine bird that showed significantly lower metal concentration than the two other studied species

nest and their diet items are collected on the immediate vicinity of the nest box. Therefore the heavy metal contamination refers to a restricted area around the nest (Furness 1993). This study also suggests that the use of robin feathers is a reliable method to evaluate the presence of heavy metals in fragmented agriculture habitats where the use of nest boxes is not possible.

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