

## The Use of Bird Feathers for the Monitoring of Cadmium Pollution

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**Abstract.** The cadmium contamination mechanism in bird feathers was investigated using starlings fed with diets containing 10 and 50 ppm Cd for five months. The experiment started about two months before the beginning of the annual complete feather molt and lasted until most of the birds completed the molt of the primaries. Concentrations of Cd in liver, kidney, and uropygial gland were highly correlated, but uropygial gland concentration was about 100 times lower. Cadmium was found both in old and new feathers, in a dose-related manner. Old feathers showed higher metal concentrations than new ones and primaries higher than secondaries. Feather Cd concentration correlated with Cd concentration in liver, kidney, and the uropygial gland. The use of bird feathers are, therefore, a reliable method for monitoring cadmium pollution, but differences between feather type and age must be considered to correctly interpret data collected in the field.

Certain bird species are important as biomonitors for environmental heavy metal pollution (Howarth *et al.* 1981; 1982; Appelquist *et al.* 1984; Frank 1986). In particular, birds foraging on animals accumulate higher metal levels than those feeding on plants (Lindberg and Odsjö 1983; Gochfeld and Burger 1987) and they are particularly exposed to metal toxicity. In the last 25 years, many studies dealt with the use of bird feathers as a tissue suitable for the monitoring of heavy metal pollution (Berg *et al.* 1966; Lindberg and Odsjö 1983). As Burger and Gochfeld (1991) pointed out, analysis of feathers offers several advantages: feathers can be easily collected and stored during routine ringing activity; birds can be released unharmed; even endangered species can be repeatedly sampled in large numbers; finally, museum bird skins give the opportunity for a historical approach (Westmark *et al.* 1975; Appelquist *et al.* 1985).

Contamination of the feather by metals can be endogenous or exogenous (Goede and de Bruin 1984). When endogenous contamination prevails, the metal level in the feathers reflects the level of the metal accumulated in liver and kidney since the last molt cycle and the food contamination level at the moment of the molt. Feather metal levels remain constant until the next molt cycle, as it has been demonstrated for mercury (Furness *et al.* 1986; Braune and Gaskin 1987). When exogenous contamination prevails, feather metal content reaches its minimum soon after molt completion, and it increases afterwards if the

bird is exposed to extended metal pollution. Goede and de Bruin (1984, 1985), found that the feathers of waders feeding in a heavily polluted lagoon became readily contaminated by selenium, arsenic, and lead. Since direct metal uptake from the environment to the feathers was not observed, they concluded that the feathers became contaminated by the secretion products of uropygial glands during the preening activity.

No conclusive data are available for cadmium on the route of feather contamination. Several studies have been carried out on the feather cadmium content in species with different ecological habits (Osborn *et al.* 1979; Tataruch and Lidauer 1984; Kooiker 1986). Weyers *et al.* (1988), studying the blackbird (*Turdus merula*) feathers from an area with a strong fallout of heavy metals (in particular lead, zinc, and cadmium), concluded that the metal content in feathers resulted from a direct environmental contamination due to airborne pollution. Unfortunately, uropygial glands were not analyzed and so it is not possible to ascertain whether there was a correlation between the concentrations of these metals in these two tissues. However, high levels of cadmium have been found in feathers of bird species living in areas with no air pollution (Cheng *et al.* 1984; Burger and Gochfeld 1991). There is evidence, therefore, that cadmium can accumulate on bird feathers, but the mechanisms regulating this process are still unclear. The aim of this paper is to investigate, under controlled conditions, the mechanism of internal feather contamination by cadmium, and consequently to assess if and to what extent feathers can be used to monitor cadmium in the environment. We experimental fed a group of starlings (*Sturnus vulgaris*) food containing cadmium for 5 months, starting two months before the beginning of the molt and spanning its entire length.

### Materials and Methods

#### Animals and Treatment

Thirty-six adult starling were randomly assigned to three treatment groups; group A (n = 14) and B (n = 14) were fed on a CdCl<sub>2</sub> supplemented diet with a nominal concentration of 10 and 50 µg/g, respectively; final concentrations in the food were found by analysis to be 10.27 µg/g (SE = 0.47, n = 14) and 55.23 (ES = 1.63, n = 23), respectively, on a dry weight basis. Group C (n = 8), with no cadmium in the diet, was used as the control. Sexes were equally distributed in treatment and control groups. All animals were individually housed,

and water and food were provided ad libitum. The starling has a complete feather molt once a year, at the end of the breeding season (Feare 1984).

Cadmium treatment started at the beginning of April. Feather status was checked every 2–3 days, and molt scores (0 = old feather, 5 = full grown new feather, 1–4 = intermediate stages) of primaries and secondaries were recorded according to Ginn and Melville (1983). The length of the cadmium exposure for the old feathers was expressed as the time (weeks) elapsed since the beginning of the treatment to the collection date for each feather. A different procedure was followed for the feathers which grew during the experiment. Because new primaries and new secondaries take several days to grow to their final length, the distal part of each feather was exposed to Cd for a longer time than the proximal part. A figure expressing the length of the exposure was therefore estimated as the time elapsed since the date when new feathers were a half of their final length (score = 3) to the date of collection, thus approximating the average time of exposure of these feathers. Eight birds (three from group A, three from group B, and two from group C) were sacrificed by decapitation 9 weeks after the beginning of the experiment when the molt started, eight were sacrificed after 14 weeks three from group A, three from group B, and two from group C) and the rest were sacrificed after 22 weeks when most of the birds had completed the molt.

#### Tissue Preparation and Analyses

At sacrifice, three inner primaries and three outer secondaries were collected, wearing surgical gloves and using stainless steel scissors. Feathers were stored in labelled plastics bags at c.  $-20^{\circ}\text{C}$ . Livers, kidneys and uropygial glands were removed and stored at  $-80^{\circ}\text{C}$ . Before metal analysis, groups of three feathers (secondaries or primaries) were vigorously shaken with deionized water for 10 min ( $4\times$ ), dried at  $60^{\circ}\text{C}$  for 1h and weighed. Approximately 0.5 g wet tissue of kidney and liver, or the whole organ in the case of the uropygial gland, was lyophilized and weighed.

All samples were digested in 2 ml of concentrated  $\text{HNO}_3$  ARISTAR in pressurized teflon vessels at  $160^{\circ}\text{C}$  for 3 h. After cooling at room temperature, sample solutions were quantitatively transferred to volumetric flasks and diluted to 10 ml with deionized water.

Analysis was performed by atomic absorption spectrophotometry (Perkin Elmer 4000) with deuterium background correction. Standards in the range of 0–1  $\mu\text{g/g}$  were prepared daily from a stock solution of 1,000  $\mu\text{g/g}$  Cd in 0.1N  $\text{HNO}_3$  solution. One blank per 8 samples was run with the same procedure. All the laboratory material equipment was previously cleaned in a solution of concentrated  $\text{HNO}_3$  and  $\text{HCl}$  (in a 1:3 proportion) for 24 h, and rinsed in deionized water ( $4\times$ ).

#### Statistical Analysis

Data were analyzed by analysis of variance, followed by Tukey multiple range test to determine significant differences between means. Correlation analysis was used to test the association between two variables (Sokal and Rohlf 1981). All means are presented  $\pm$  SE unless otherwise noted; statistical significance was accepted at  $P \leq 0.05$ , and tests were two tailed.

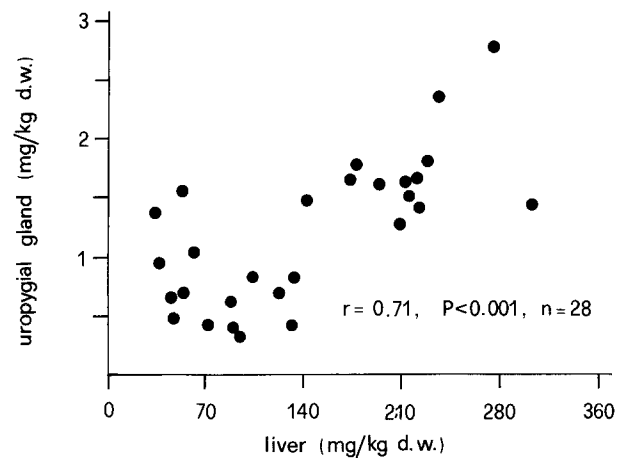
#### Results

The birds in the experiment started their annual complete primary molt at the end of May (median day = 28/5, SD = 8.85,

**Table 1.** Cadmium concentration in liver and kidney in the three treatment groups (10 ppm and 50 ppm of dietary Cd and controls)

Group	Liver			Kidney		
	Mean <sup>a</sup>	SE	n	Mean <sup>a</sup>	SE	n
A	75.71	9.21	14	116.03	12.21	14
B	208.49	12.90	14	308.93	21.10	14
C	2.29	0.60	8	5.87	0.99	8

<sup>a</sup>  $\mu\text{g/g}$ , dry weight



**Fig. 1.** Cadmium concentration in the liver and the uropygial gland of the starling fed diets with 10 and 50 ppm Cd. Six birds (3 + 3) were sacrificed after 9 weeks of exposure to the metal, at the beginning of the annual complete molt, six (3 + 3) after 14 weeks, and sixteen (8 + 8) after 22 weeks, when most of the individuals had completed the molt. Controls, whose uropygial gland Cd concentrations were below the detection limit of the instrument used, have been excluded

$n = 30$ ). Length of the molt was approximately 100 days (median = 101, SD = 11.4,  $n = 19$ ). No significant differences in molt timing were found as a function of age, sex and treatment. Starlings from groups A and B accumulated cadmium in liver and kidneys at a high level; a small amount of this metal was also found in the organs of the birds of group C (Table 1). Cadmium was also found in the uropygial gland of the treated birds but not in the controls. Concentration of cadmium in uropygial gland was lower than in liver, and the two values were positively correlated (Figure 1), as well as cadmium concentration of kidney ( $r = 0.64$ ,  $P < 0.001$ ,  $n = 28$ ).

Starlings accumulated cadmium both in the old and new feathers, in a dose-related manner (Table 2;  $F = 24.8$ ,  $P < 0.001$ ,  $n = 96$ ). Cadmium content of the feathers from control birds was below the detection limit of the instrument used ( $n = 14$ ). Within the treatment groups, the old feathers had more cadmium than the new ones and primaries accumulated more than the secondaries ( $F = 19.3$ ,  $P < 0.001$ ,  $n = 45$ , and  $F = 46.4$ ,  $P < 0.001$ ,  $n = 51$  for group A and B respectively). These differences did not depend on the length of the exposure time: the ratios between cadmium contents and time of exposure (weeks) still show the same differences ( $F = 3.95$ ,  $P = 0.01$ , and  $F = 34.0$ ,  $P < 0.001$ , for group A and B respectively, Table 3).

Feather cadmium concentrations were correlated with Cd concentration in the uropygial gland, liver and kidney (Table

**Table 2.** Feather Cd concentration in old and new primaries and secondaries in the group A (10 ppm Cd in the diet) and B (50 ppm Cd in the diet). Old feathers are those present on the wing when the exposure to cadmium started; new feathers are those grown during the experiment. Cadmium content of the feathers from control birds was below the detection limit of the instrument used. [Different superscripts within columns indicate significant difference between the means ( $P < 0.05$ )]

Feather	Group A			Group B		
	Mean <sup>a</sup>	SE	n	Mean <sup>a</sup>	SE	n
Old primaries	2.09 <sup>a</sup>	0.37	10	7.41 <sup>a</sup>	1.07	9
Old secondaries	0.65 <sup>b</sup>	0.13	8	2.24 <sup>b</sup>	0.26	13
New primaries	0.96 <sup>b</sup>	0.19	18	1.88 <sup>c</sup>	0.34	15
New secondaries	0.24 <sup>b</sup>	0.03	11	0.84 <sup>c</sup>	0.11	12

<sup>a</sup> ( $\mu\text{g/g}$ , dry weight)

**Table 3.** Ratios between cadmium feather concentration and weeks of exposure in the group A (10 ppm Cd in the diet) and B (50 ppm Cd in the diet). [Different superscripts within columns indicate significant difference between the means ( $P < 0.05$ )]

Feather	Group A			Group B		
	Mean <sup>a</sup>	SE	n	Mean <sup>a</sup>	SE	n
Old primaries	0.22 <sup>a</sup>	0.43	10	0.78 <sup>a</sup>	0.12	9
Old secondaries	0.10 <sup>ab</sup>	0.02	8	0.33 <sup>b</sup>	0.05	13
New primaries	0.22 <sup>ab</sup>	0.07	18	0.19 <sup>c</sup>	0.05	15
New secondaries	0.03 <sup>b</sup>	0.00	11	0.12 <sup>c</sup>	0.06	12

<sup>a</sup> ( $\mu\text{g/g/weeks}$ )

**Table 4.** Correlation coefficients ( $r$ ) and probabilities ( $P$ ) between cadmium concentration in the feathers and in uropygial gland, liver, and kidney of the starlings from group A (10 ppm Cd in the diet) and from group B (50 ppm Cd in the diet). Sample sizes are given in brackets

		OS <sup>a</sup>	OP <sup>b</sup>	NS <sup>c</sup>	NP <sup>d</sup>
		(n = 9)	(n = 17)	(n = 7)	(n = 19)
Uropygial gland	r	0.639	0.672	0.762	0.633
	P	n.s.	0.003	0.047	0.004
Liver	r	0.708	0.652	0.781	0.599
	P	0.033	0.005	0.038	0.007
Kidney	r	0.843	0.706	0.608	0.672
	P	0.004	0.002	n.s.	0.002

<sup>a</sup> OS = old secondaries

<sup>b</sup> OP = old primaries

<sup>c</sup> NS = new secondaries

<sup>d</sup> NP = new primaries

4). Still significant was the correlation calculated on the pooled data, disregarding age (old or new) and type (primaries or secondaries) of the feathers, but a lower coefficient was obtained ( $r = 0.38$ ,  $P < 0.001$ ,  $n = 96$ ).

## Discussion

The presence of cadmium in the diet of starlings resulted in cadmium accumulation in the feathers. The observed concen-

trations in feathers ranged between 0.3% and 3% of the levels of the metal in the liver. It is therefore confirmed that feathers can accumulate cadmium, as already reported for some free-living seabirds (Osborn *et al.* 1979; Howarth *et al.* 1981, 1982; Cheng *et al.* 1984; Honda *et al.* 1986; Burger and Gochfeld 1991; Gochfeld *et al.* 1991) and passerines (Tataruch and Lidauer 1984; Kooiker 1986; Weyers *et al.* 1988).

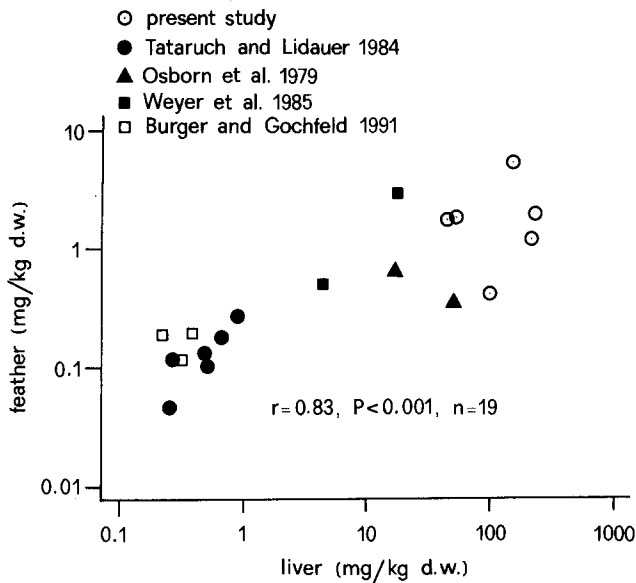
Both old and new feathers accumulated Cd during the experiment; we conclude that external deposition is the main mechanism of feather contamination. Otherwise, we should have observed minimal or absent contamination in the old feathers and higher levels in the new ones. External contamination could depend on two different mechanisms: (1) direct uptake of the metal caused by a contact with contaminated food; or (2) intestinal absorption of the metal from the food, and excretion through the uropygial gland, which is the only dermal gland in birds (Jacob and Ziswiler 1982). In the first case, the expected cadmium concentration in the feathers of individuals from the group B (50 ppm Cd in the diet) should be about as high as five times the concentration observed in the feathers of the group A (10 ppm in the diet). Instead, observed cadmium concentrations in the group B were about 2.5 times the observed concentration in the group A (Table 2). This is roughly the same ratio we found between the cadmium concentration in the liver and the kidney of the two groups (Table 1). Therefore, we suggest that direct, external contamination from the food to the feathers does not seem to be the main mechanism of contamination. Moreover, cadmium concentrations of liver, kidney, uropygial gland and feathers are significantly correlated (Table 4). The data support the hypothesis that the cadmium bioaccumulated from the diet is partially excreted through the uropygial gland; thus, contaminating the feathers. In the case of birds living in marine habitats, it is possible that also the salt glands play a role in the excretion of cadmium (Howarth *et al.* 1981, 1982), as it has been demonstrated for lead (Buggiani and Rindi 1980).

There is a positive, significant correlation between Cd levels in feathers and liver when pooling the few available data from literature on Cd concentration in feathers and liver in birds (Osborn *et al.* 1979; Tataruch and Lidauer 1984; Weyers *et al.* 1985; Burger and Gochfeld 1991) and data from the present study (Figure 2). This correlation suggests that feathers can be a measure of the exposure to cadmium (*i.e.*, of the dietary cadmium uptake) in very different environmental conditions.

Observed differences in cadmium concentration between type and age of the starling feathers are not easy to interpret. Given that primaries are usually more worn than secondaries, and that old feathers are more worn than new feathers (Svensson 1984), a possible explanation could be that the different wearing status of the feathers has an influence on their permeability to the excretions from the uropygial gland.

These differences between type of feather could also explain why in field studies, no clear association has been found between Cd concentrations of liver and feathers (Stock *et al.* 1989). It is also possible that cadmium concentration in the uropygial gland, and consequently in the feathers, may depend on the actual metal intake, while the levels in liver and kidney are basically related to the exposure time (Friberg *et al.* 1986). Thus, if data from birds of different ages living in the same habitat are analyzed together, correlations between cadmium concentration in the liver and in the feathers could be blurred.

The use of bird feathers for monitoring cadmium pollution is apparently a reliable method. However, the biology (molt pat-



**Fig. 2.** Cadmium concentration in the liver and in the feathers of different bird species. Points are the mean cadmium concentration values for homogeneous groups from the cited studies (*i.e.* species, age, period, area); data from the present experiment are the mean concentration in the feathers and the liver of the birds from groups A and B after 9, 14, and 22 weeks from the beginning of treatment, regardless the type and age of the feather

tern, age composition of the population), the ecology (movements, diet, migration pattern) and the physiology of the birds should be well known. Otherwise, the results obtained may be liable to misinterpretation.

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