Arch. Environ. Contam. Toxicol. 39, 541–546 (2000)
DOI: 10.1007/s002440010138

ARCHIVES OF
Environmental
Contamination
and Toxicology

Can Excrement and Feathers of Nestling Songbirds Be Used as Biomonitors for Heavy Metal Pollution?

T. Dauwe,[1] L. Bervoets,[2] R. Blust,[2] R. Pinxten,[1] M. Eens[1]

[1] Department of Biology, University of Antwerp (U.I.A.), Universiteitsplein 1, B-2610 Wilrijk, Belgium
[2] Department of Biology, University of Antwerp (R.U.C.A.), Groenenborgerlaan 171, B-2020 Antwerp, Belgium

Received: 21 March 2000/Accepted: 9 July 2000

Abstract. Although birds have been frequently used as indicators of heavy metal pollution, few studies have examined pollutant levels in nestling passerines. In this paper we determined the levels of two essential (zinc and copper) and three nonessential heavy metals (lead, cadmium, and arsenic) in the excrement and feathers of great (*Parus major*) and blue tit (*Parus caeruleus*) nestlings at a polluted site (near a metallurgic factory) and a reference site (4 km farther east). The excrement of both great and blue tit nestlings contained significantly higher concentrations of arsenic, cadmium, copper, and lead at the polluted site. Zinc concentrations did not differ significantly between sites for both species. The feathers of great and blue tit nestlings accumulated significantly higher concentrations of lead at the polluted site than at the reference site. Zinc levels in the feathers of great tit nestlings were significantly higher at the reference site than at the polluted site. For all other elements considered, concentrations did not differ significantly between the two sites. There were no interspecific differences in metal levels between great and blue tits in both excrement and feathers. There was a significant positive correlation between the lead concentration in the excrement and feathers for both great and blue tits. We therefore conclude that excrement of great and blue tit nestlings can be used as a biomonitor for heavy metals (lead, cadmium, arsenic, and copper), whereas feathers appear only to be suitable as a biomonitor for lead pollution.

Heavy metal pollution poses a genuine threat to the quality of our environment. The need to determine the exposure to and the effects of pollutants, such as heavy metals, has led to the development of numerous biomonitoring programs. Assessing ecosystem health adequately by means of biomonitoring requires the selection of indicator species that are representative for the considered ecosystem (Burger and Gochfeld 1996). The accumulation of metals in indicator species might cause levels to increase with the age of the organism and with each succeeding step in the food chain (van Straalen and Ernst 1991). Therefore, there has been a tendency to evaluate pollutants in long-living species that are high on the food chain, such as birds (Burger 1993). Birds are exposed to heavy metals through air, water, and food. Once a metal has entered the body, it can be stored or accumulated, or it can be excreted (Burger 1993). Birds can rid their body of heavy metals through excrement or by depositing them in the uropygial gland, salt gland (Burger and Gochfeld 1985), and feathers (Burger 1993). Female birds can additionally excrete heavy metals through their eggs (Burger and Gochfeld 1993; Dauwe *et al*. 1999).

Bird feathers have been used intensively as an indicator tissue for metal exposure since the 1960s (Burger 1993). Metal levels in feathers reflect levels in the blood during the short period of feather growth when the feather is connected with blood vessels and metals are incorporated in the keratin structure. The specific incorporation of metals in feathers renders profiles inert and stable (Burger 1993) and may lead to higher concentrations than in internal tissues (Grue *et al*. 1986). Excrement has been used to a much lesser extent than internal organs or feathers in biomonitoring studies. Yet metal levels in excrement appear to be a sensitive indicator of heavy metals in the environment (Fitzner *et al*. 1995; Nyholm 1996; Spahn and Sherry 1999).

Although there are numerous studies that have used birds as biomonitors, most have focused on birds of prey (Denneman and Douben 1993; Esselink *et al*. 1995; Jager *et al*. 1996) and seabird species (Becker 1989; Burger *et al*. 1992). Because metals might accumulate with the age of the organism, the majority of studies have used adult birds. Nestling passerines have been used to a much lesser extent, although they are potentially good biomonitors for terrestrial point-source pollution (Burger and Gochfeld 1993; Burger 1996). Metal levels in nestling passerines may reflect local pollution levels because metal contamination will be obtained in a clearly defined time period and will originate from a limited parental foraging area (Furness 1993).

In the present study the metal levels in the excrement and feathers of nestling great (*Parus major*) and blue tits (*Parus caeruleus*) were examined and compared between a heavily polluted site (near a metallurgic factory) and a site 4 km east of the pollution source. Although both great and blue tits have

Correspondence to: T. Dauwe

been used frequently in ecological and behavioral investigations, it is only recently that they are being used in biomonitoring and ecotoxicological studies (Eeva and Lehikoinen 1995; Llacuna *et al*. 1995; Dauwe *et al*. 1999). Previous research with adult great and blue tits has revealed that they are very useful as biomonitors for heavy metal contamination and that they have the potential to become important indicators of terrestrial point-source pollution (Eens *et al*. 1999).

Materials and Methods

We collected excrement and feathers from great and blue tit nestlings at a polluted and a reference site both located in Antwerp (Belgium). The polluted site is bounded by a metallurgic factory, which is responsible for an extremely high local pollution with mainly arsenic, cadmium, copper, lead, and zinc (Dauwe *et al*. 1999; VMM 1999). Pollution originated mainly from dust from ore piles blown by the wind (Verbruggen 1993). Lead and cadmium deposition in the vicinity of the factory is the highest reported in the Flanders with lead and cadmium deposition being seven and eight times higher, respectively, than the guideline proposed by the World Health Organization (VMM 1999). At the polluted site there were 49 great tit and 42 blue tit nest boxes, all within 1 km of the pollution source. The reference site, the campus of the University of Antwerp, was situated 4 km east of the polluted site. At the reference site there were 43 great tit and 42 blue tit nest boxes.

Collection of Samples

As part of an intensive biomonitoring study, nest boxes at the polluted and reference site were checked daily during the breeding season of 1998. We were able to determine the date of hatching accurately for each nest. Fourteen days after the first egg hatched we collected the excrement and the outer left and right tail feathers of three randomly chosen nestlings.

Nyholm (1996) observed a lower variation in heavy metal concentration in the liver among nestling pied flycatchers (*Ficedula hypoleuca*) of the same clutch than among different clutches within the same locality. Assuming this is also the case for excrement and feathers of nestling great and blue tits, we pooled samples of three nestlings of the same nest to obtain sufficient sample to determine metal contamination. The outermost tail feathers were plucked and stored in envelopes. As a response to capture and handling, nestlings often defecate, and we caught excrement immediately in metal-free plastic containers. In total, we collected feathers from 18 great tit and 10 blue tit nests at the polluted and from 17 great tit and 7 blue tit nests at the reference site. At the polluted site we collected excrement from 10 great tit and 12 blue tit nests, whereas at the reference site we collected excrement of 8 great tit and 6 blue tit nests.

Preparation of Samples

Feathers were washed alternately with deionized water and acetone in the laboratory and were then put in 4-ml metal-free polypropylene vials. We determined the dry weight of the feathers after they were put for 24 h in an oven (60°C). Subsequently the feathers were digested in a 1:1 mixture of HNO_3 (70%) and H_2O_2 (30%). We completed the digestion with the microwave procedure described by Blust *et al*. (1988). After this, the samples were diluted by adding 4 ml deionized water and stored at −20°C until analysis. Excrement was directly collected in metal-free vials, and consequently external contamination is ignorable. We washed the samples briefly with deionized water, after which we followed the same digestion procedure as with nestling feathers.

Analysis of Samples

We measured arsenic, cadmium, copper, lead, and zinc levels in samples with an axial inductively coupled plasma-atomic emission spectrophotometer (ICP-AES) (Varian Liberty series II) (De Wit and Blust 1998). The concentrations are expressed in ppm ($\mu g\ g^{-1}$) based on dry weights. All specimens were analyzed in batches with certified reference material of the Community Bureau of Reference (*i.e*., mussel sample, CRM 278), blanks, and a standard calibration curve. Recovered concentrations of the certified samples were within 10% of the certified values, which is an acceptable margin (Gochfeld and Burger 1998). All samples were measured on the same day. We were not always able to measure all elements in each sample of feathers, which accounts for the differences in sample sizes.

Statistics

Statistical analysis of data was performed using SPSS v3.0 statistical software (SPSS 1986). Nonparametric Mann-Whitney U tests were applied to test for significant differences in metal concentrations between sites (polluted versus reference site) and between species (great versus blue tits). When carrying out multiple comparisons (*i.e*., when comparing metal levels between sites and between species), α was adjusted using a Bonferroni correction (Sokal and Rohlf 1981) to correct for the increased probability of type I errors. Therefore, we used a significance level of $\alpha = 0.025$ for all Mann-Whitney U tests. Spearman rank correlations were applied to examine relationships between feathers and excrement. The level of significance was set at $\alpha = 0.05$. Arithmetic means and standard errors are represented in the tables.

Results

Metal Levels in Excrement

All measured elements (arsenic, cadmium, copper, lead, and zinc) were detected in excrement of great and blue tit nestlings (Table 1). Both great and blue tit nestlings had significantly higher concentrations of arsenic, cadmium, copper, and lead in their excrement at the polluted site than at the reference site (Table 1). Zinc concentrations did not differ significantly between the two sites for both species (Table 1). In great and blue tits differences between the two sites were greatest for lead, being 35 and 22 times higher at the polluted site than at the reference site, respectively.

There were no significant differences between great and blue tits in the concentrations of arsenic, cadmium, copper, lead, and zinc in excrement (Table 1) both at the reference site (Mann-Whitney U tests, $p > 0.07$) and at the polluted site (Mann-Whitney U tests, $p > 0.1$).

Metal Levels in Feathers

In the feathers we detected all the considered elements (Table 2), except for cadmium in blue tits at the reference site, where

Table 1. Mean concentration (ppm dry weight ± SE) in the excrement of great and blue tit nestlings in a reference site and a polluted site

	Great Tit			Blue Tit		
	Reference Site	Polluted Site	p^a	Reference Site	Polluted Site	p^a
As	1.37 ± 0.59 (8)	16.03 ± 3.25 (10)	0.0007	1.28 ± 0.63 (6)	16.01 ± 4.13 (12)	0.002
Cd	5.72 ± 1.18 (8)	16.81 ± 3.58 (10)	0.021	3.11 ± 0.45 (6)	9.35 ± 1.16 (12)	0.002
Cu	36.16 ± 2.72 (8)	90.28 ± 18.29 (10)	0.001	37.50 ± 2.14 (6)	92.74 ± 10.95 (12)	0.0007
Pb	2.34 ± 0.727 (8)	80.40 ± 17.00 (10)	0.0005	5.54 ± 1.68 (6)	124.80 ± 32.90 (12)	0.0007
Zn	400.4 ± 42.9 (8)	429.4 ± 100.4 (10)	0.66	311.0 ± 16.7 (6)	317.4 ± 19.0 (12)	1.00

[a] p values from Mann-Whitney U tests applied to test for differences between the reference and the polluted site. To correct for the increased probability of type I error due to multiple testing (between species and between sites) a significance level of $\alpha = 0.025$ was used.

Table 2. Mean concentration (ppm dry weight ± SE) in the feathers of great and blue tit nestlings in a reference site and polluted site

	Great Tit			Blue Tit		
	Reference Site	Polluted Site	p^a	Reference Site	Polluted Site	p^a
As	2.55 ± 1.01 (7)	3.00 ± 1.05 (17)	0.80	6.44 ± 3.75 (2)	5.24 ± 1.83 (14)	0.51
Cd	0.053 ± 0.025 (10)	0.007 ± 0.004 (18)	0.12	ND[b] (7)	0.071 ± 0.057 (17)	0.35
Cu	5.78 ± 0.51 (10)	6.16 ± 0.45 (17)	0.58	4.90 ± 0.42 (7)	5.14 ± 0.78 (17)	0.60
Pb	0.51 ± 0.13 (10)	4.83 ± 1.08 (18)	0.00002	0.48 ± 0.15 (7)	3.68 ± 1.11 (17)	0.020
Zn	127.2 ± 12.4 (9)	97.9 ± 5.0 (17)	0.012	156.5 ± 16.7 (7)	119.0 ± 7.4 (15)	0.04

[a] p values from Mann-Whitney U tests applied to test for differences between the reference and the polluted site. To correct for the increased probability of type I errors due to multiple testing (between species and between sites) a significance level of $\alpha = 0.025$ was used.
[b] ND = concentration lower than the instrument detection limit.

levels were below detection limit. The feathers of great tit nestlings contained significantly higher concentrations of lead and lower concentrations of zinc at the polluted site than at the reference site. The concentrations of arsenic, cadmium, and copper did not differ significantly between the two sites (Table 2). Metal levels in the feathers of blue tit nestlings contained significantly higher concentrations of lead at the polluted site, whereas arsenic, cadmium, copper, and zinc levels did not differ significantly between the two sites (Table 2). Lead levels in the feathers of great and blue tit nestlings were nine and eight times higher at the polluted site than at the reference site, respectively.

The concentrations of all measured elements—arsenic, cadmium, copper, lead, and zinc (Table 2)—did not differ significantly between great and blue tit nestlings at the polluted site (Mann-Whitney U tests, $p > 0.06$) and at the reference site (Mann-Whitney U tests, $p > 0.025$).

Excrement–Feather Comparison

We examined the correlations among metal levels in excrement and feathers for both great and blue tit nestlings. We collected from 18 great tit and 15 blue tit nests both excrement and feathers. There was a significant positive correlation between lead levels in feathers and in excrement for both great (Spearman rank correlation test, $r_{18} = +0.77$, $p < 0.0005$) and blue tits (Spearman rank correlation test, $r_{15} = +0.53$, $p < 0.05$; Figure 1). However, for blue tits the correlation was strongly influenced by a single point. When we removed this point the correlation was no longer significant (Spearman rank correlation test, $r_{14} = 0.43$, $p > 0.1$). For all other measured metals there were no significant correlations between the excrement and the feathers of nestlings (Spearman rank correlation test, $p > 0.1$).

Discussion

According to our expectations, metal levels in excrement of both tit species were significantly higher at the polluted site for all elements considered except zinc. Nyholm (1996) and Eeva (1996) pointed out that the concentration of essential and nonessential elements in excrement of passerines reflected the concentration of the food items that nestlings eat. Our results indicate that great and blue tit nestlings at the polluted site are

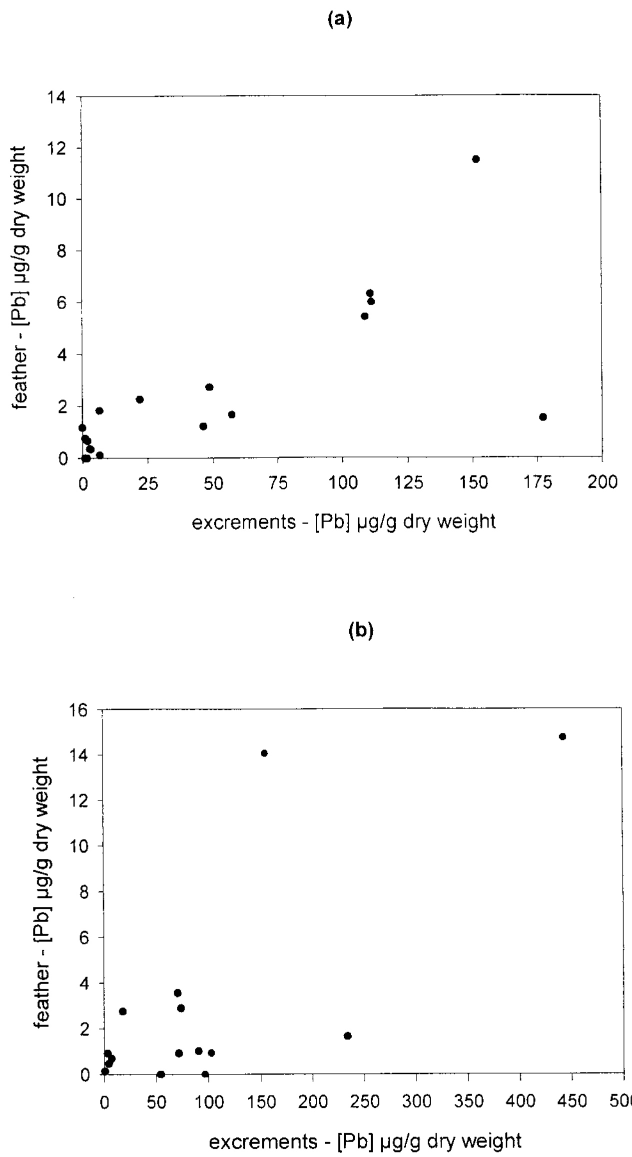

Fig. 1. Relationship between lead levels in the excrement and outer tail feathers of (a) great and (b) blue tit nestlings

confronted with higher arsenic, cadmium, copper, and lead concentrations in their food than at the reference site. Although the zinc deposition at the polluted site is higher than at the reference site (VMM 1999), zinc levels in excrement did not differ significantly between the two study sites. The transfer of essential elements, such as zinc, in the food web is modified by homeostatic mechanisms in organisms, which strive to keep levels physiologically adequate (Clarkson 1986). Thus, the absence of significantly higher levels of zinc at the polluted site indicates that the zinc contamination of the food items at the polluted site is similar to that of the reference site. These findings are consistent with the results of Nyholm (1995), who also found significant differences in the lead and cadmium levels of great tit excrement at different localities but found insignificant differences for zinc. However, further research is necessary to determine whether metal levels in excrement of great and blue tit nestlings reflect the metal concentrations in the food items (Spahn and Sherry 1999).

Previous studies have also reported detectable levels of lead, cadmium, and zinc in excrement of great tit nestlings (Nyholm 1995), little blue heron (*Egretta caerulea*) chicks (Spahn and Sherry 1999), and great blue heron (*Ardea herodius*) chicks (Fitzner *et al.* 1995). The zinc levels in excrement in our study are comparable with those reported by Nyholm (1995), who found zinc levels up to 525 ppm in the excrement of great tit nestlings. Lead and cadmium levels on the other hand were markedly lower in other studies: the highest lead and cadmium levels described by Nyholm (1995) are, respectively, 24 ppm and 9.4 ppm. On the basis of trophic-level considerations it can be expected that levels in insectivorous passerines are lower than in piscivorous birds, such as herons (Burger *et al.* 1999). Lead and cadmium concentrations reported by Fitzner *et al.* (1995) were, respectively, 6 ppm and 0.5 ppm, whereas Spahn and Sherry (1999) found 1.59 ppm and 0.11 ppm of lead and cadmium, respectively, in guano. Lead and cadmium levels at the polluted site in our study were markedly higher, which suggests a high contamination of lead and cadmium at the polluted site.

For most metals (except arsenic) levels in the feathers were considerably lower than levels in excrement (see Tables 1 and 2). This is consistent with the results of Spahn and Sherry (1999), who found lower concentrations of lead and cadmium in the feathers of nestling herons than in guano. The outermost tail feathers of both great and blue tit nestlings contained significantly elevated levels of lead at the polluted site. Moreover, zinc levels in feathers were significantly higher at the reference site for great tit nestlings. Arsenic, cadmium, and copper levels did not differ significantly between the two sites. This was surprising to us because we have found elevated cadmium, copper, lead, and zinc levels in adult great and blue tit feathers at the polluted site compared to the reference site (Dauwe *et al.* unpublished). Our results may suggest that nestlings at the polluted site have not accumulated higher metal concentrations of copper, cadmium, and arsenic than at the reference site. Homeostatic mechanisms (*e.g.*, excretion through excrement) may still control adequately metal levels in tissues at this early stage. However, Nyholm (1995) showed that the liver of nestling pied flycatchers had significantly elevated levels of lead, arsenic, and cadmium at a polluted site. It seems unlikely that nestling great and blue tits in our study did not accumulate higher concentrations of metals considering the differences in metal contamination of excrement between the two sites. We suggest that arsenic, cadmium, and copper levels in nestling feathers may not adequately reflect the body burden of the nestlings. Furthermore, metal accumulation in feathers of nestling great and blue tits may be suppressed. Honda *et al.* (1986) found that young great egrets (*Egretta alba*) sequester a lower percentage of their body burden in feathers than adults do. Additionally, Burger and Gochfeld (1992) found significantly lower levels of lead and mercury in partially formed feathers than in fully grown feathers. Because not fully grown feathers still have an active blood supply, Burger and Gochfeld (1992) suggested that relatively low levels of metals in the blood may lower metal concentrations in partially formed feathers. Moreover, feathers of great and blue tit nestlings consist mainly of shaft, which contains lower metal levels than the vane (Goede and de Bruin 1984). Therefore the outermost tail feathers of 15-day-old great and blue tit nestlings cannot be used as a biomonitor for arsenic, cadmium, and copper.

Although there were few significantly elevated concentrations in the feathers at the polluted site, lead levels in the feathers of great and blue tit nestlings were significantly higher. Moreover, we found a significant positive correlation between lead levels in the feathers and excrement in both tit species, suggesting that nestling feathers are potentially good monitors for lead pollution. Although our results may suggest that lead accumulates better in feathers than other elements, studies of Burger (1993) and Burger and Gochfeld (1993) have shown that this is not the case. Presumably the high contamination with lead at the polluted site (Eylenbosch *et al*. 1984; VMM 1999) causes the difference in lead content in the feathers between the two sites, whereas this may not be the case for the other elements. The extreme differences in the lead levels in the excrement between the two sites indicate that lead is the most important pollutant at the polluted site and that tits are probably exposed to extreme concentrations in their food. Moreover, these findings are consistent with an earlier study in which we also found extreme differences in lead contamination in the eggshell and egg content of great and blue tits between the two sites (Dauwe *et al*. 1999). For instance, lead levels in the eggshells of great tits were 40 times higher at the polluted site than at the reference site. Moreover, in great tit eggs, only lead levels were significantly elevated at the polluted site. Further research, including study sites with intermediate contamination levels, is necessary to investigate whether feather analysis is a good predictor of lead burden in nestlings.

Zinc levels in the outer tail feathers, contrary to our expectations, were lower at the polluted site than at the reference site. The zinc concentrations in the excrement of the nestlings indicate that at the polluted site the concentration of zinc in the food items is comparable to that of the reference site. We therefore expected that zinc levels in the feathers would not be significantly different between the two sites. However, physiological alterations caused by pollution may have an influence on the uptake and the accumulation of zinc. Exposure to high levels of nonessential elements, such as lead or cadmium, increases the levels of zinc-binding proteins, such as protoporphyrin in the blood (Blus *et al*. 1995; Heinz *et al*. 1999) and metallothionein in the kidney (Elliott and Scheuhammer 1997). Di Guilio and Scanlon (1985) have shown that elevated levels of cadmium can significantly increase zinc concentrations in the kidney, probably due to metallothionein induction. These alterations may result in lowered levels of zinc accessible for accumulation in the feathers. Although it is clearly indicated that lead has a role in the metabolism of zinc, it remains to be demonstrated whether it has an impact on the accumulation of zinc in feathers.

Previous studies, which focused mainly on seabirds (Burger and Gochfeld 1993, 1995; Burger 1996) and herons (Fasola *et al*. 1998; Spahn and Sherry 1999), have reported detectable levels of lead and cadmium in feathers of nestling birds. Burger (1993) reported lead and cadmium levels in feathers of nestling Franklin's gulls (*Larus pipixcan*) of 0.8 and 0.21 ppm, respectively, while feathers of roseate tern (*Sterna dougallii*) and black skimmer (*Rynchops niger*) chicks accumulated up to 0.5 and 4.1 ppm respectively (Burger and Gochfeld 1993). Little blue heron chicks sequestered on average 1.01 ppm lead and 0.47 ppm cadmium in their feathers (Spahn and Sherry 1999). Fasola *et al*. (1998) detected lead levels in feathers of heron chicks up to 4.5 ppm and cadmium levels up to 0.64 ppm. The cadmium concentrations reported in our study are considerably lower than those reported in other studies. Lead concentrations, on the other hand, were comparable or even higher in our study.

There were no significant interspecific differences in metal concentrations between the two tit species. The diet of great and blue tit nestlings is very much alike. They are fed solely insects with a dominance of Lepidoptera larvae (Cramp and Perrins 1993). A study by Eens *et al*. (1999) found interspecific differences in lead, copper, and zinc levels in feathers of adult great and blue tits, with significantly higher levels in the latter species. Differences in feeding ecology and metabolic rate between the two species are probably responsible for differences in contamination. However, these small differences in the exposure to heavy metals caused by differences in ecology or metabolic rate may not yet be revealed in nestlings. Only essential elements tended to differ between the two species, suggesting that physiological differences may have a more important role than differences in metal exposure.

In conclusion, our data suggest that sampling excrement of nestling great and blue tits is a powerful means to assess the presence of certain food chain contaminants. Therefore, excrement can be a useful biomonitor for heavy metal pollution. Feathers of nestling great and blue tits on the other hand, appear only to be suitable for monitoring lead pollution. However, further research is necessary to determine whether heavy metal levels in excrement and feathers reflect adequately internal tissue levels or food chain exposure levels. Great and blue tits, which are important model species in behavioral and ecological research, have several characteristics that make them suitable as biomonitors for point-source contamination. They are ubiquitous and abundant, which permits sampling from almost any wooded area across much of Europe. Both species readily nest in man-made nest boxes, so breeding populations of both species can be rapidly established and easily monitored. Although in most cases adult birds are monitored, the use of nestling passerines has several advantages (Furness 1993). Nestlings are restricted to the nest, and they will be fed with food items collected close to the nest site (Cramp and Perrins 1993). Therefore, the heavy metal contamination will originate from a restricted area around the nest. Moreover, contamination will be accumulated in the short period of nestling growth. Nestling great and blue tits can easily be monitored, and they are relatively insensitive to disturbances at the nest. This allows the setup of a network of volunteers that can monitor great and blue tit nestlings with no interference to the population. Furthermore, the nestlings may be the stage of development at which toxicological effects are particularly evident (Furness 1993). Therefore, there are few, if any, drawbacks to using great and blue tit nestlings as a sentinel for metal pollution.

Acknowledgments. We thank Dimitri van Pelt, Els Van Duyse, and Sylvie Hurtrez-Boussès for help with the fieldwork. This study was supported by the Fund for Scientific Research Flanders (FWO). Ronny Blust and Marcel Eens are research associates of the FWO; Lieven Bervoets is supported by a FWO postdoctoral fellowship and Rianne Pinxten by FWO project G.0075.98. This study was also supported by FWO project G.0397.00 and by the Research Council of the University of Antwerp (NOI-BOF UA-2000 project).

References

Becker PH (1989) Seabirds as monitor organisms of contaminants along the German North Sea shore. Helgoländer Meeresuntersuchungen 43:394–403

Blus LJ, Henny CJ, Hoffman DJ, Grove RA (1995) Accumulation in and effects of lead and cadmium on waterfowl and passerines in Northern Idaho. Environ Pollut 89:311–318

Blust R, van der Linden A, Verheyen E, Decleir W (1988) Evaluation of microwave heating digestion and graphite furnace atomic absorption spectrometry with continuum source background correction for the determination of Fe, Cu and Cd in brine shrimp. J Anal At Spectrom 3:387–393

Burger J (1993) Metals in avian feathers: bioindicators of environmental pollution. Rev Environ Toxicol 5:203–311

Burger J (1996) Heavy metal and selenium levels in feathers of Franklin's gulls in interior North America. Auk 113:399–407

Burger J, Gochfeld M (1985) Comparison of nine heavy metals in salt gland and liver of great scaup (*Aythya marila*), black duck (*Anas rubripes*) and mallard (*Anas platyrhynchos*). Comp Biochem Physiol 81:287–292

Burger J, Gochfeld M (1992) Trace element distribution in growing feathers: additional excretion in feather sheats. Arch Environ Contam Toxicol 23:105–108

Burger J, Gochfeld M (1993) Lead and cadmium accumulation in eggs and fledgling seabirds in the New York bight. Environ Toxicol Chem 12:261–267

Burger J, Gochfeld M (1995) Biomonitoring of heavy metals in the Pacific basin using avian feathers. Environ Toxicol Chem 14: 1233–1239

Burger J, Gochfeld M (1996) Ecological and human health risk assessment: a comparison. In: Di Guilio RT, Monosson E (eds) Interconnections between human and ecosystem health. Chapman and Hall, London, pp 127–148

Burger J, Schreiber EAR, Gochfeld M (1992) Lead, cadmium, selenium and mercury in seabird feathers from the tropical mid-Pacific. Environ Toxicol Chem 11:815–822

Burger J, Woolfenden GE, Gochfeld M (1999) Metal concentrations in the eggs of endangered Florida scrub-jays from central Florida. Arch Environ Contam Toxicol 37:385–388

Clarkson TW (1986) Effects-general principles underlying the toxic action of metals. In: Friberg L, Nordberg GF, Vouk VB (eds) Handbook on the toxicity of metals. Elsevier, pp 128–148

Cramp S, Perrins CM (1993) Handbook of the birds of Europe, the Middle East and North Africa. The birds of the western Palearctic, vol. VII, Flycatchers to Shrikes. Oxford University Press, London

Dauwe T, Bervoets L, Blust R, Pinxten R, Eens M (1999) Are eggshells and egg contents of great and blue tit suitable as indicators of heavy metal pollution? Belg J Zool 129:439–447

De Wit M, Blust R (1998) Determination of metals in saline and biological matrices by axial inductively coupled plasma atomic emission spectrometry using microconcentric nebulization. J Anal Atomic Spectrom 13:515–520

Denneman WD, Douben PET (1993) Trace metals in primary feathers of the barn owl (*Tyto alba guttatus*) in The Netherlands. Environ Pollut 82:301–310

Di Guilio RT, Scanlon PF (1985) Effect of cadmium ingestion and food restriction on energy metabolism and tissue metal concentrations in mallard ducks (*Anas platyrhynchos*). Environ Res 37:433–444

Eens M, Pinxten R, Verheyen RF, Blust R, Bervoets L (1999) Great and blue tits as indicators of heavy metal contamination in terrestrial ecosystems. Ecotox Environ Safety 44:81–85

Eeva T (1996) Direct and indirect effects of air pollution on two hole-nesting bird species. Annales Universitatis Tukuensis, Turku

Eeva T, Lehikoinen E (1995) Egg shell quality, clutch size and hatching success of the great tit (*Parus major*) and the pied flycatcher (*Ficedula hypoleuca*) in an air pollution gradient. Oecologia 102:312–323

Elliott JE, Scheuhammer AM (1997) Heavy metal and metallothionein concentrations in seabirds from the Pacific coast of Canada. Mar Pollut Bull 34:794–801

Esselink H, van der Geld FM, Jager LP, Posthuma-Trupie GA, Zoun PEF, Baars AJ (1995) Biomonitoring heavy metals using the barn owl (*Tyto alba guttata*): sources of variation especially relating to body condition. Arch Environ Contam Toxicol 28:471–486

Eylenbosch WJ, van Sprundel MP, Clara RR (1984) Lead pollution in Antwerpen, Belgium. Ann Acad Med Singapore 13:224–230

Fasola M, Movalli PA, Gandini C (1998) Heavy metal, organochlorine pesticide, and PCB residues in eggs and feathers of herons breeding in northern Italy. Arch Environ Contam Toxicol 34:87–93

Fitzner RE, Gray RH, Hindts WT (1995) Heavy metal concentrations in great blue heron fecal castings in Washington state: a technique for monitoring regional and global trends in environmental contaminants. Bull Environ Contam Toxicol 55:398–403

Furness RW (1993) Birds as monitors of pollutants. In: Furness RW, Greenwood JJD (eds) Birds as monitors of environmental change. Chapman and Hall, London, p 86

Gochfeld M, Burger J (1998) Temporal trends in metal levels in eggs of the endangered roseate tern (*Sterna dougallii*) in New York. Environ Res 77:36–42

Goede AA, de Bruin M (1984) The use of bird feather parts as a monitor for metal pollution. Environ Pollut 37A:287–309

Grue CE, Hoffman DJ, Beyer WN, Franson LP (1986) Lead concentrations and reproductive success in European starling *Sturnus vulgaris* nesting within highway roadside verges. Environ Pollut 42A:157–182

Heinz GH, Hoffman DJ, Sileo L, Audet DJ, Lecaptain LJ (1999) Toxicity of lead-contaminated sediment to mallards. Arch Environ Contam Toxicol 36:323–333

Honda K, Min BL, Tatsukawa R (1986) Distribution of heavy metals and their age-related changes in the eastern great white egret, *Egretta alba modesta*, in Korea. Arch Environ Contam Toxicol 15:185–197

Jager LP, Rijnierse FVJ, Esselink H, Baars AJ (1996) Biomonitoring with the buzzard *Buteo buteo* in The Netherlands: heavy metals and sources of variation. J Orn 137:295–318

Llacuna S, Gorriz A, Sanperra C, Nadal J (1995) Metal accumulation in three species of passerine birds (*Emberiza cia*, *Parus major*, and *Turdus merula*) subjected to air pollution from a coal-fired power plant. Arch Environ Contam Toxicol 28:298–303

Nyholm NEI (1995) Effects of environmental pollution on breeding populations of birds in southern Poland. Water Air Soil Poll 85:829–834

Nyholm NEI (1996) Measurements of heavy metals in terrestrial birds and mammals. Proc Symp Development of Environmental Technology in the Barents Region, Kemi, April 18–19 1995, pp 79–98

Sokal RR, Rohlf FJ (1981) Biometry. WH Freeman, New York

Spahn SA, Sherry TW (1999) Cadmium and lead exposure associated with reduced growth rates, poorer fledging success of little blue heron chicks (*Egretta caerulea*) in South Louisiana wetlands. Arch Environ Contam Toxicol 37:377–384

SPSS (1986) SPSS reference guide. Chicago, IL

van Straalen NM, Ernst E (1991) Metal biomagnification may endanger species in critical pathways. Oikos 62:255–256

Verbruggen A (1993) Report on the environment and nature in Flanders. Learning to change. Flemish Environment Agency, Vlaamse Milieumaatschappij

VMM (1999) Studie van de luchverontreiniging in de omgeving van Union Minière vestiging Hoboken, Jaarrapport 1998. Vlaamse Milieumaatschappij, Erembodegem 1999