Belg. J. Zool. - Volume 129 (1999) - issue 2 - pages 439-447 - Brussels 1999

Received: 24 December 1998

ARE EGGSHELLS AND EGG CONTENTS OF GREAT AND BLUE TITS SUITABLE AS INDICATORS OF HEAVY METAL POLLUTION?

TOM DAUWE [1], LIEVEN BERVOETS [2], RONNY BLUST [2], RIANNE PINXTEN [1] AND MARCEL 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

e-mail: eens@uia.ua.ac.be

Abstract. We examined whether eggs of the Great Tit (*Parus major*) could be used as indicators for lead, arsenic, cadmium, copper and zinc pollution. We collected eggs from two sites with different pollution levels, and measured heavy metal levels in the egg content and eggshell separately. At the polluted site, situated near a metallurgic factory in Hoboken (Belgium), eggshells contained significantly higher concentrations of arsenic, cadmium and lead than did eggshells at the reference site on the campus of the University of Antwerp. Egg contents also contained significantly higher concentrations of lead at the polluted site than at the reference site. Both at the polluted and reference site egg contents contained higher concentrations of zinc and copper than did the eggshell. The eggshell contained higher arsenic and lead concentrations than did the egg content at the polluted site but not at the reference site. There was a clear discrepancy between essential and non-essential elements. Copper and zinc, two essential elements, were highest in the egg content, while arsenic and lead were higher in the eggshell. Moreover, the concentrations of essential elements did not differ significantly between the two sites. We also collected eggs from Blue Tits (*Parus caeruleus*) at the polluted site, and compared metal levels between the eggshell and egg content. Differences between the shell and content were similar to those for the Great Tit. At the polluted site we found no significant differences in metal levels between the two species. Our study indicates that Great and Blue Tits sequester non-essential heavy metals in their eggs, especially in the eggshell. Therefore the eggshell is suitable as an indicator for heavy metal pollution.

Key words: Heavy metals, bioindicators, eggs, Great Tit, Blue Tit, *Parus major*, *Parus caeruleus*.

INTRODUCTION

Terrestrial birds are exposed to heavy metals through air, water and their food. Once a metal has entered the body it can be stored or accumulated, or it can be excreted (BURGER, 1993). The accumulation of metals in organs causes levels to increase with age of the organism and with each succeeding step in the food chain (VAN STRAALEN & ERNST, 1991). Concentrations in long-lived carnivores can reach high levels, and may reach toxic or even lethal levels. Birds can rid the body of heavy metals through the faeces or by depositing them in the uropygial gland, salt gland (BURGER & GOCHFELD, 1985) and feathers (BURGER, 1993). Females can also eliminate heavy metals by sequestering them in their eggs, which may jeopardise the developing embryo. Although some studies failed to detect elevated levels in the eggs of experimentally dosed females (PATTEE, 1984), others have shown elevated levels of lead and cadmium (MAEDGEN *et al.*, 1982).

Assessing ecosystem health is a daunting task, requiring the selection of indicator species that are representative of the system (BURGER & GOCHFELD, 1996). Because many contaminants can bioaccumulate, there has been a tendency to evaluate toxins in species that are high in the foodchain (BURGER & GIBBONS, 1998). Small passerines such as the Great Tit (*Parus major* L.) and the Blue Tit (*Parus caeruleus* L.) may be useful bioindicators. They are primarily insectivorous during the breeding season, and high in the foodchain. They live in many different habitats and often in large densities. They are territorial in the breeding season, and non-migratory in many populations (CRAMP & PERRINS, 1993). They nest in holes, and use nestboxes, so they are easy to study. These characteristics make them very suitable for monitoring point source contamination (EENS *et al.*, 1999).

Biomonitoring requires effective determination of the load of pollutants in the indicator species. Most studies have used internal organs, but demand for non-invasive monitoring techniques has led to the introduction of feathers, faeces and eggs as bioindicators (BURGER, 1993). Eggs are suitable as bioindicators because 1) they come from a specific fragment of the population; 2) they are only formed in a specific period; 3) they have a consistent composition; 4) they are easily sampled and 5) the removal of one egg from a nest has a minor effect on population parameters (FURNESS, 1993). Although eggs have distinct advantages as bioindicators they have scarcely been used to determine heavy metal pollution. Furthermore, most studies have focused on seabirds and waterfowl, while songbirds have received little attention (MORERA *et al.*, 1997; NYHOLM, 1998). NYHOLM (1998) recently stressed that there is almost no information on the transfer of heavy metals into eggs of passerine birds.

The objective of this study was to determine to what extent Great and Blue Tits sequester heavy metals in their eggs. BURGER (1994) showed that in marine birds there is a discrepancy between the egg content and the eggshell in heavy metal concentration (see also MORERA *et al.*, 1997). Therefore we also analysed the egg content and the eggshell separately. For Great Tits we collected eggs from two sites: at a heavily polluted site in Hoboken close to a metallurgic factory, and at the campus of the University of Antwerp (UIA) about four kilometres to the east of the polluted site. The latter study area is considered as a reference site in this study. Environmental pollution by trace metals is important in the vicinity of the smelter of Hoboken (VAN GRIEKEN, 1996). Dust deposition is the major source of pollution, and affects the immediate surroundings of the factory (VERBRUGGEN, 1994).

MATERIAL AND METHODS

In this study we examined metal levels in eggs coming from a polluted and a reference site. The polluted site at Hoboken was bounded on the west by a metallurgic factory. This nageoires dorsale et anale et le complexe hémaxanal de type 3 sont des traits qui peuvent se manifester dans de multiples familles de téléostéens mais qui se retrouvent chez tous ou chez beaucoup de Tselfatiiformes (obs. pers.). Cela renforce donc encore l'idée que *Zanclites xenurus* appartient bien à cet ordre.

Zanclites **et les autres genres de Tselfatiiformes**

Zanclites JORDAN, 1924 se distingue de tous les autres genres connus de Tselfatiiformes par au moins onze caractères particuliers :

(1) Le supraorbitaire s'allonge fortement et s'articule non seulement avec le frontal mais aussi avec le ptérotique dont il éloigne le dermosphénotique. Chez les autres Tselfatiiformes, le supraorbitaire est plus court et ne longe que le frontal, permettant ainsi au dermosphénotique de s'articuler avec le ptérotique (obs. pers.).

(2) la tête de de l'ectoptérygoïde se renfle et s'accole contre un processus osseux de la face externe de l'entoptérygoïde, formant ainsi un puissant contrefort contre lequel s'appuyait, sans doute, un gros palatin. Une telle structure n'existe pas chez les autres Tselfatiiformes (obs. pers.).

(3) L'ectoptérygoïde prend la forme d'une épaisse baguette osseuse édentée. L'ectoptérygoïde montre la forme plus classique d'un os plat, relativement étroit, effilé à ses deux extrémités et presque toujours denticulé chez les autres membres de l'ordre (obs. pers.).

(4) Le carré est réduit, de très faible hauteur et exhibe un condyle articulaire étiré. Cet os offre un développement normal chez les autres Tselfatiiformes (obs. pers.).

(5) Le préoperculaire montre une branche ventrale très allongée et une branche dorsale courte. Chez les autres genres de Tselfatiiformes, les deux branches du préoperculaire sont subégales ou bien la branche dorsale se révèle plus longue que la branche ventrale (obs. pers.).

(6) la *dilatator fossa* est estompée sur la surface du ptérotique et simplement délimitée vers le haut par un processus osseux. Chez les autres Tselfatiiformes, la *dilatator fossa* est étroite et peu profonde mais clairement marquée néanmoins (obs. pers.).

(7) les hémapophyses sont complètement ou partiellement soudées aux corps vertébraux abdominaux. Chez les autres Tselfatiiformes, les hémapophyses de la région abdominales sont autogènes (obs. pers.). Chez *Tselfatia*, néanmoins, on observe aussi une certaine tendance à la fusion des arcs avec les centres vertébraux correspondants (TAVERNE, sous presse a).

(8) les ptérygophores anaux forment des expansions aliformes qui s'imbriquent les unes dans les autres. Il n'y a pas d'élargissement distal de l'axonoste en plateau comme chez les autres Tselfatiiformes (obs. pers.).

(9) le cinquième hypural est réduit et ne touche pas la petite vertèbre terminale urale I et II. Le cinquième hypural des autres Tselfatiformes est bien développé et s'articule sur le dessus de la petite vertèbre terminale (obs. pers.).

(10) L'arc neural de la petite vertèbre préurale I est soudé à cette dernière. Cet arc est autogène chez les autres Tselfatiiformes (obs. pers.).

(11) Le supratemporal et le posttemporal sont articulés. Ce n'est pas le cas chez les autres Tselfatiiformes (obs. pers.).

site is characterised by an extremely high and localised pollution, mainly with arsenic, cadmium, copper, lead and zinc, caused by emissions and dust from ore-piles, blown up by wind. In October 1997, we added 10 Blue Tit and 21 Great Tit nestboxes to the 32 Blue Tit and 28 Great Tit nestboxes that were already present at this site. The reference site, the campus of the University of Antwerp, was situated about four kilometres east from the polluted site. At the reference site there were 43 Great Tit nestboxes, available since 1995.

We collected eggs from nests that were abandoned (either spontaneously or due to human disturbance), and non-viable eggs from Great and Blue Tit nests in Hoboken and the UIA, from April till June 1998. Since BLUS (1982) illustrated that one egg of a nest is representative for the entire clutch, we collected only one egg of a clutch (but see MORERA *et al.*, 1997; FURNESS, 1993). We collected nine Great Tit eggs at the polluted site and five at the reference site. We also collected 12 Blue Tit eggs at the polluted site. The eggs were stored in the refrigerator until analysis. All eggs were analysed in the same week.

In the laboratory, eggs were opened round the airchamber with a stainless steel dissection needle. The content of the egg was transferred to an acid-washed polypropylene, 10 ml vial. The inside and outside of the eggshells were washed vigorously in deionised water in the laboratory to remove loosely adherent internal and external contamination (see BURGER, 1994). We assumed that by this procedure most of the aerial contamination from heavy metals was removed from the outside of the eggshells. The eggshell was then put in a separate acid-washed polypropylene, 10 ml vial. To determine dry weight, the samples were, before weighing, put in an oven (60°C) for 24 hours. After we determined the dry weight, we added a 1:1 mixture of HNO_3 (70%) and H_2O_2 (30%) to the egg content and eggshell. We completed the destruction with the microwave procedure described by BLUST *et al.* (1988). After microwave destruction the samples were diluted with deionised water and stored at -20°C until analysis.

We measured cadmium, copper, arsenic, lead and zinc with an axial Inductively Coupled Plasma – Atomic Emission Spectrophotometer (ICP-AES) (Varian Liberty series II). The concentrations are expressed in ppm based on dry weight. All measurements were performed on the same day. All specimens were analysed in batches, along 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 & BURGER, 1998)

We used SPSS statistical software to perform the statistical analyses. According to the Kolmogorov-Smirnov goodness-of-fit test all data sets had normal distributions. We used student's t-tests to compare metal levels among sites and species, and paired t-tests to compare metal levels between the egg content and eggshell.

RESULTS

Intersite comparison

The metal levels in the egg content of Great Tits differed significantly between the polluted and reference site only for lead. The lead concentration in the egg content in Hoboken was more than 15 times higher than levels found at the campus. For all other metals considered, we could not find any significant differences (Table 1). However, we should emphasise that there was a considerable difference in the mean concentrations of arsenic and cadmium between the two sites, being respectively two and 16 times higher at the polluted site in Hoboken.

TABLE 1

Mean concentrations of heavy metals (± SE) in the egg content and eggshell from Great and Blue Tits in the polluted (Hoboken) and reference (campus of the University of Antwerp) sites. Concentrations are expressed in ppm (µg g-1). Parametric student's t-tests were used to test for significant differences (P < 0.05) in Great Tits between sites, and in Hoboken between species. P-values are given in brackets.

	Arsenic	Cadmium	Lead	Copper	Zinc
INTERSITE COMPARISON					
Egg content					
UIA	0.22 ± 0.14	0.05 ± 0.01	0.13 ± 0.05	4.8 ± 0.8	69 ± 13
Hoboken	0.45 ± 0.25	0.8 ± 0.6	2.0 ± 0.4	5.5 ± 0.7	62 ± 3
t-test	NS	NS	4.95 (0.001)	NS	NS
Eggshell					
UIA	1.2 ± 0.6	0.08 ± 0.02	0.37 ± 0.16	1.72 ± 0.23	19 ± 6
Hoboken	4.2 ± 0.8	0.31 ± 0.08	15 ± 4	3.2 ± 0.5	28 ± 5
t-test	2.6 (0.03)	2.9 (0.02)	3.6 (0.007)	NS	NS
INTERSPECIFIC COMPARISON					
Egg content					
Blue Tit	0.33 ± 0.13	0.17 ± 0.14	2.2 ± 0.6	5.4 ± 0.7	60 ± 8
Great Tit	0.45 ± 0.25	0.8 ± 0.6	2.0 ± 0.4	5.5 ± 0.7	62 ± 3
t-test	NS	NS	NS	NS	NS
Eggshell					
Blue Tit	3.7 ± 1.2	0.15 ± 0.02	7.4 ± 1.1	2.8 ± 1.1	32 ± 8
Great Tit	4.2 ± 0.8	0.31 ± 0.08	15 ± 4	3.2 ± 0.5	28 ± 5
t-test	NS	NS	NS	NS	NS

When comparing metal levels in eggshells of Great Tits between the two sites, we found significantly higher concentrations of arsenic, cadmium and lead at the polluted site. Lead levels in Hoboken were 40 times higher than at the reference site. Arsenic and cadmium levels at the polluted site were almost four times higher than at the reference site. The essential elements, zinc and copper, did not differ significantly between the two sites (Table 1) but the mean concentrations were respectively 50 and almost 100% higher at the polluted site in Hoboken.

Comparison between eggshell and egg content

At the reference site we found a significantly higher concentration of zinc and copper in the egg content than in the eggshell of Great Tits. The other elements were higher in the eggshell but not significantly so (Fig. 1). At the polluted site we also found a significantly higher concentration of zinc and copper in the egg content, but the eggshell also contained a significantly higher arsenic and lead concentration (Fig. 2).

We also compared metal levels in the egg content and the eggshell of Blue Tits from Hoboken. Arsenic and lead concentrations were significantly higher in the eggshell. The egg content had a significantly higher copper concentration, while for zinc a similar trend was observed (Fig. 3).

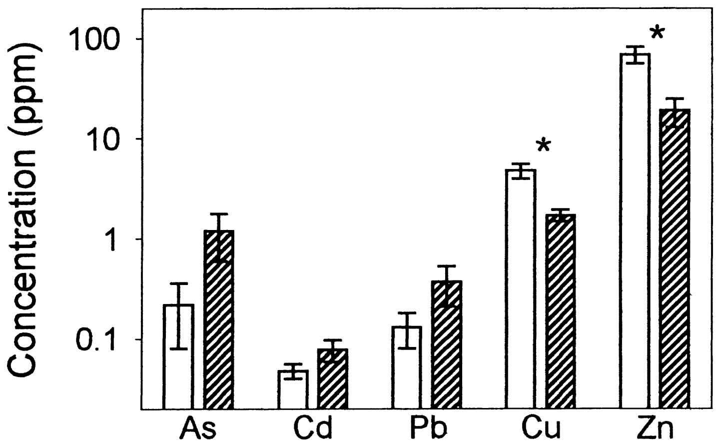

Fig. 1. – The mean concentration (± SE) of heavy metals in the egg content (open bars) and eggshell (striped bars) of Great Tits from the reference site. An asterisk denotes a significant difference between the egg content and the eggshell (P < 0.05) in a paired t-test. The concentration axis has a logarithmic scale.

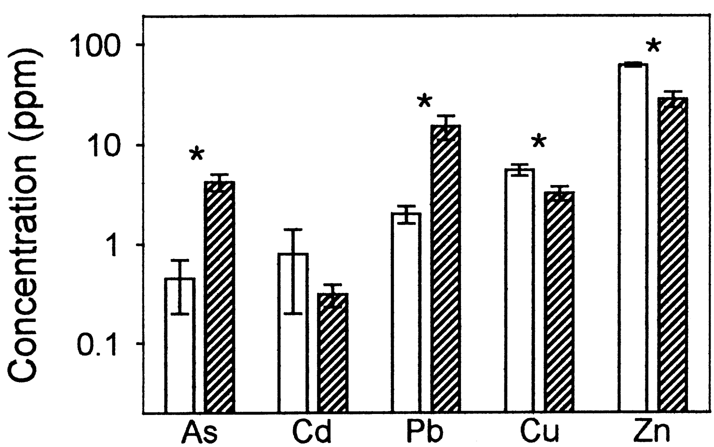

Fig. 2. – The mean concentration (± SE) of heavy metals in the egg content (open bars) and eggshell (striped bars) of Great Tits from the polluted site. An asterisk denotes a significant difference between the egg content and the eggshell (P < 0.05) in a paired t-test. The concentration axis has a logarithmic scale.

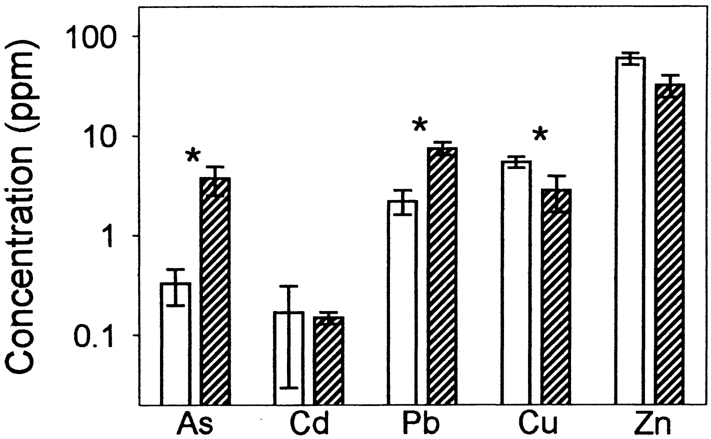

Fig. 3. – The mean concentration (± SE) of heavy metals in the egg content (open bars) and eggshell (striped bars) of Blue Tits from the polluted site. An asterisk denotes a significant difference between the egg content and the eggshell ($P < 0.05$) in a paired t-test. The concentration axis has a logarithmic scale.

Interspecific comparison

At the polluted site where both Great and Blue Tit eggs were collected, we found no significant differences between the two species in metal levels, neither in the eggshell nor in the egg content (Table 1). The mean concentrations in both species were generally similar, except for cadmium (both eggshell and egg content) and for lead (eggshell only). For these metals, concentrations were higher in Blue Tits than in Great Tits.

DISCUSSION

Our study showed marked differences in metal levels in eggshells and egg contents between the polluted and the reference site. Although some studies have failed to detect elevated levels of trace metals in experimentally dosed birds (PATTEE, 1984), our study clearly showed that Great and Blue Tits do sequester heavy metals in their eggs. Especially for lead levels, there were enormous differences between the two sites. We anticipated that lead levels would differ the most because the area round the metallurgic factory is known for its extreme lead pollution (EYLENBOSCH *et al.*, 1984). However, it is still remarkable that the differences were so extreme between the study sites, which are only four kilometres apart. Most studies that have used eggs as a bioindicator, measured organochlorines and mercury because they cause eggshell thinning (KOSTER *et al.*, 1993; OHLENDORF & HARRISON, 1986). Only a few studies have examined other heavy metals (mostly lead and cadmium) in eggs (GOCHFELD & BURGER, 1998), and even less have studied the eggshell and egg content separately (BURGER, 1993; MORERA *et al.*, 1997; NYHOLM, 1998; REID & HACKER, 1982). The levels of lead, cadmium and zinc found at the polluted site in our study are among the highest reported in the literature. Most previous studies have used marine or piscivorous birds as indicator species (OHLENDORF & HARRISON, 1986; FASOLA *et al.*, 1998). We found only one study that examined metal levels in eggs from Great and Blue Tits. KOTH (1983) measured zinc and lead levels in whole eggs of Great and Blue Tits in urban areas. For both species, levels at the polluted site in our study were markedly higher than those detected in KOTH's study.

The separate analyses of the egg content and eggshell showed a marked difference in the accumulation of heavy metals in both samples. Moreover, our results suggested that there was also a discrepancy between essential and non-essential elements. In both the eggshell and the egg content, mean metal levels were higher at the polluted site, but the differences between the two study sites were greater in the eggshell, which resulted in significantly higher concentrations not only of lead but also of cadmium and arsenic. The mean lead, cadmium and arsenic levels were also higher in the eggshell than in the egg content. The eggshell is mainly composed of calcium, and SCHEUHAMMER (1987) found that trace metals such as cadmium and lead may interact with the metabolic pathway of calcium. Consequently, they may be incorporated more easily in the eggshell. The mean zinc and copper concentrations were, contrary to the non-essential elements, higher in the egg content. This was predictable, because zinc and copper are embedded in the quaternary structure of some proteins, and the egg content has a higher protein concentration than the eggshell. For the considered essential elements, zinc and copper, there were marked differences in the metal levels in the eggshell between the polluted and the reference site. In the egg content however, they did not differ markedly between the two sites, indicating that zinc and copper concentrations are homeostatically controlled in the egg content.

We did not find any significant differences in metal levels between the Great and Blue Tit eggs, although the mean concentrations of cadmium (both in the eggshell and in the egg content) and lead (only in the eggshell) were higher in Blue Tits. At the polluted site, we have found significantly higher concentrations of lead in the outer tail feathers of Blue Tits compared with Great Tits. In general, all heavy metals were higher in the feathers of Blue Tits (unpublished data). BURGER & GOCHFELD (1991) showed that metal levels in females were correlated with metal levels in their eggs, so we anticipated that there would be interspecific differences. However, the differences between the two species in metal levels in the outer tail feathers were not reflected in the metal levels in the eggs. Since this might be due to the small sample size in the present study, further research is necessary in this respect.

In conclusion, our data suggest that eggshells from Great and Blue Tits are suitable as bioindicators. Both essential and non-essential elements are accumulated in the eggshell. Although the egg content is less suitable as a bioindicator, because zinc and copper levels appear to be homeostatically controlled, the higher levels of non-essential elements found in our study indicate the extent to which a developing embryo may be exposed. Quite clearly, the elevated exposure of Great and Blue Tit females to lead at the polluted site caused increased amounts of lead in their eggs. Further work is, therefore, necessary to examine whether embryos or nestlings at the polluted site show an increased mortality rate. Finally, we must emphasise that our sample size was very limited, and that further research on the accumulation of heavy metals in the eggs of Great and Blue Tits is, therefore, necessary.

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): Lieven Bervoets is supported by a Postdoctoral Fellowship, while Ronny Blust and Marcel Eens are Research Associates of the FWO. This study was also supported by the Research Council of the University of Antwerp (Rianne Pinxten) and by FWO-project G.0075.98 (Rianne Pinxten).

REFERENCES

BLUS, L.J. (1982) – Further interpretation of the relation of organochlorine residues in brown pelican eggs to reproductive success. *Environ. Pollut.*, **28**: 15-33.

BLUST, R., A. VAN DER LINDEN, E. VERHEYEN & W. DECLEIR (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. (1994) – Heavy metals in avian eggshells: another excretion method. *J. Toxicol. Environ. Health*, **41**: 207-220.

BURGER, J. & J.W. GIBBONS (1998) – Trace elements in egg contents and egg shells of Slider Turtles (*Trachemys scripta*) from the Savannah River Site. Arch. Environ. Contam. *Toxicol.*, **34**: 382-386.

BURGER, J. & M. GOCHFELD (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. & M. GOCHFELD (1991) – Cadmium and lead in Common Terns (Aves: *Sterna hirundo*): relationship between levels in parents and eggs. *Environ. Monit. Assess.*, **16**: 253-258.

BURGER, J. & M. GOCHFELD (1996) – Ecological and human health risk assessment: a comparison. In*: Interconnections between human and ecosystem health*. DI GUILIO, R.T. & E. MONOSSON (Ed.). Chapman and Hall, London: 127-148.

CRAMP, S. & C.M. PERRINS (1993) – *Handbook of the birds of Europe, the Middle East and North Africa. The birds of the Western Palearctic*. Volume VII Flycatchers to Shrikes. Oxford University Press, London.

EENS, M., R. PINXTEN, R.F. VERHEYEN, R. BLUST & L. BERVOETS (1999) – Great and blue tits as indicators of heavy metal contamination in terrestrial ecosystems. *Ecotox. Environ. Safety*. In press.

EYLENBOSCH, W.J., M.P. VAN SPRUNGEL & R.R. CLARA (1984) – Lead pollution in Antwerp, Belgium. *Ann. Acad. Med. Singapore*, **13**: 224-230.

FASOLA, M., P.A. MOVALLI & C. GANDINI (1998) – Heavy metals, organochlorine pesticide, and PCB residues in eggs and feathers of herons breeding in northern Italy. *Arch. Environ. Contam. Toxicol.*, **34**: 87-93.

FURNESS, R.W. (1993) – Birds as monitors of pollutants. In: *Birds as monitors of environmental change.* FURNESS, R.W. & J.J.D. GREENWOOD (Eds.). Chapman and Hall, London: 86-143.

GOCHFELD, M. & J. BURGER (1998) – Temporal trends in metal levels in eggs of the endangered Roseate Tern (*Sterna dougallii*) in New York. *Environ. Research*, **77**: 36-42.

KOSTER, M.D., D.P. RYCKMAN, D.V.C. WESELOH & J. STRUGER (1996) – Mercury levels in Great Lakes Herring Gull (*Larus argentatus*) eggs. *Environ. Pollut.*, **93**: 261-270.

KOTH, T. (1983) – Zum eisen- blei- und zinkgehalt in eiern von Kohlmeisen (*Parus major*), Blaumeisen (*Parus caeruleus*) und Feldsperling (*Passer montanus*). *Luscinia*, **45**: 23-61.

MAEDGEN, J.L., C.S. HACKER, G.D. SCHRODER & F.W. WEIR (1982) – Bioaccumulation of lead and cadmium in the Royal Tern and Sandwich Tern. *Arch. Environ. Contam. Toxicol.*, **11**: 99-102.

MORERA, M., C. SANPERA, S. CRESPO, L. JOVER & X. RUIZ (1997) – Inter- and intraclutch variability in heavy metals and selenium levels in Audouin's Gull eggs from the Ebro Delta, Spain. *Arch. Environ. Contam. Toxicol.*, **33**: 71-75.

NYHOLM, N.E.I. (1998) – Influence of heavy metal exposure during different phases of the ontogeny on the development of Pied Flycatcher, *Ficedula hypoleuca*, in natural populations. *Arch. Environ. Contam. Toxicol.*, **35**: 632-637.

OHLENDORF, H.M. & C.S. HARRISON (1986) – Mercury, selenium, cadmium and organochlorines in eggs of three Hawaiian seabird species. *Environ. Pollut.* Ser. B, **11**: 169-191.

PATTEE, O.H. (1984) - Eggshell thickness and reproduction in American Kestrels exposed to chronic dietary lead. *Arch. Environ. Contam. Toxicol.*, **13**: 29-34.

REID, M. & C.S. HACKER (1982) – Spatial and temporal variation in lead and cadmium in the Laughing Gull (*Larus atricilla*). *Mar. Pollut. Bull.*, **13**: 387-389.

SCHEUHAMMER, A.M. (1987) – The chronic toxicity of aluminium, cadmium, mercury and lead in birds: a review. *Environ. Pollut.*, **46**: 263-295.

VERBRUGGEN, A. (1994) – *Leren om te keren: milieu- en natuurrapport Vlaanderen*. Garant, Leuven, 823 pp.

VAN GRIEKEN, R. (1996) – Milieu- en natuurrapport Vlaanderen 1996. Wetenschappelijk rapport III.3. Verspreiding van zware metalen.

VAN STRAALEN, N.M. & E. ERNST (1991) – Metal biomagnification may endanger species in critical pathways. *Oikos*, **62**: 255-256.