Arch. Environ. Contam. Toxicol. 28, 471–486 (1995)

ARCHIVES OF
Environmental
Contamination
and **Toxicology**

Biomonitoring Heavy Metals Using the Barn Owl *(Tyto Alba Guttata)*: Sources of Variation Especially Relating to Body Condition

H. Esselink[1], F. M. van der Geld[1,2], L. P. Jager[2], G. A. Posthuma-Trumpie[2], P. E. F. Zoun[2], A. J. Baars[2]*

[1] Zoological Laboratory & Science Shop Biology, University of Groningen, Postbus 14, 9750 AA Haren, The Netherlands
[2] DLO-Central Veterinary Institute, Department of Biochemistry and Toxicology, Postbus 65, 8200 AB Lelystad, The Netherlands

Received: 10 February 1994/Revised: 26 November 1994

Abstract. The feasibility of using the Barn Owl *(Tyto alba guttata)* to monitor environmental quality in the Netherlands was investigated, using Cd, Cu, Pb, Mn, and Fe as indicators for environmental contamination.

Throughout 1992, bird-watchers, volunteers, and officials submitted 53 birds. The age and geographical distribution of these birds formed a representative sample of the population. The following interrelationships were investigated: cause of death, nutrient reserve, age, time of death, place of death, body measurements, sex, condition, and heavy metal concentration in kidney, liver, and tibia.

Twenty-eight animals had died after collisions. Fifteen Barn Owls died of exhaustion. In total, twenty-four birds were exhausted, with coccidiosis or other parasitic gastrointestinal infections. The condition of the birds showed that as the birds' condition worsened, fat reserves were depleted before protein reserves. Significant linear relationships were found between decreasing protein reserves and decreasing dry weights of the liver, kidney, flight muscle and heart, but not of the tibia. An asymptotic, nonlinear relation was observed between dry organ weight and fat reserve. This suggested that fat reserves were only found when protein reserves exceeded 15% of the body mass at starvation. Concentrations of Cu and Fe in liver and kidney rose as protein reserves fell; the total content of Cu and Fe per organ, however, remained constant. The Mn concentration of these organs remained constant; Mn content increased with increasing organ sizes. Neither Cd nor Pb showed a clear relationship with parameters of body condition. The ratio between the organ content of Pb or Cd and the dry organ weight, however, revealed some birds from contaminated habitats.

The findings suggested that concentrations of environmental contaminants should be measured on a dry weight basis. Furthermore, depending on the pharmacokinetic characteristics of a contaminant, the total content of that contaminant per organ can be more informative than the concentration. In this one year sample of Barn Owls, no indications were found of toxic levels of Cd, Cu, Pb, Mn, or Fe in the Netherlands.

It is concluded that the Barn Owl is a suitable biomonitor. Furthermore, a network of volunteers can produce an informative sample of the Barn Owl population without interfering with the population.

Biomonitoring should give early warning of environmental deterioration or impending catastrophes. Heavy metals and pesticides are among the major ecotoxicants for which the earliest possible indications of rising levels, both at local and at more global scale, are needed. The ideal species to provide such data should 1) be widespread among habitats to allow for cross-habitat comparisons; 2) be territorial and nonmigratory, so that measured concentrations can be linked with a source area; 3) be near the top of a food chain so that various contaminants can be weighted with their biomagnification effects; 4) be so numerous that it is feasible to collect sufficiently large samples; 5) have sufficient body mass to allow low concentrations of toxicants to be measured accurately. Also, the sampled individuals should represent an entire population. The Barn Owl *(Tyto alba guttata)* fulfills these criteria and is therefore a candidate for biomonitoring.

In addition, the Barn Owl goes through regular cycles of great abundance of food and great scarcity of food. Thus, a dynamic interaction between levels of contaminants and generally good or poor body condition, with high or low reproduction can be integrated in the monitoring (Moriarty 1988). These fluctuations will confound the interpretation of data of a single year or area. In the end, however, these interactions should reveal information about the kinetics and dynamics of contaminants in animals and environment.

This feasibility study reports on the level of the heavy metals Pb, Cd, Cu, Fe, and Mn in liver, kidney, and tibia of Barn Owls *(Tyto alba guttata)* found dead in the Netherlands in 1992. These levels are related to data obtained by postmortem analysis (cause of death), ring recoveries (age and geographical

---

**Present address:* DLO-State Institute for Quality Control of Agricultural Products. Postbus 230, 6700 AE Wageningen, The Netherlands

Correspondence to: L. P. Jager

origin), and carcass analysis (fat and protein reserves, and other biological parameters).

Heavy Metals: Contamination and Biomagnification

Several regions in the Netherlands are contaminated with heavy metals. Sources and locations of pollution are zinc smelters in the south of the country (Budel), blast furnaces in the coastal area, metallurgical industries, waste dumps, and Rhine and Meuse sediments. High concentrations of heavy metals such as zinc, lead, cadmium, and copper can be found near these sources (Spierenburg *et al.* 1988; Di Giulio and Scanlon 1984). Other potentially important sources of heavy metal pollution are highways (Pb), shooting ranges (Pb; Bon and Boersema 1984), and intensive pig farming (Cu; Ernst 1991).

Because of biomagnification, animals at the end of a food chain, such as the Barn Owl, might carry high concentrations of heavy metals. Accumulation depends on the transfer of heavy metals in the food chain and thus on the composition of the diet at different trophic levels. Body load increases with age if intake exceeds metabolic rate and excretion (van Straalen 1988). After absorption, heavy metals accumulate in bone, kidney, and/or liver. Lead is mainly stored in calcareous tissues (Scheuhammer 1987; Cosson 1989). Metallothionein, a metal-binding protein of liver and kidney, preferentially binds zinc and cadmium, and possibly other heavy metals (Nordberg 1972; Vallee and Ulmer 1972; Osborn 1979; Osborn *et al.* 1979; Mayack *et al.* 1981; Aaseth and Norseth 1986).

Copper, manganese, and iron are essential elements that become toxic in high doses. Lead and cadmium have no known physiological role. At high doses, heavy metals are acutely lethally toxic. At low doses, they can cause sublethal toxic effects, such as slower reactions to stimuli or weight loss (Honda *et al.* 1990). Suppression of its immune system by lead or cadmium could make a host prone to infectious diseases (Locke *et al.* 1969; Cook *et al.* 1974; Koller *et al.* 1977; Custer *et al.* 1984; DeMent *et al.* 1986; Trust *et al.* 1990). The absorption of manganese, copper, and iron is normally regulated by homeostatic mechanisms (Friberg *et al.* 1986; Elinder 1986; Saric 1986). Toxic effects of heavy metals are also related to their bioavailability (Graveland 1990), and the organism's physiological status (Osborn 1979; Honda *et al.* 1986; Blomqvist *et al.* 1987; Krasowski and Doelman 1990). The toxicity of a heavy metal is also modulated by the availability of other heavy metals: the interactions between cadmium and copper, zinc or selenium are well known (Voogt *et al.* 1980; Goede and Voogt 1985). Lead interacts with calcium or phosphorus (Graveland 1990; Krasowski and Doelman 1990).

The Ecology of the Barn Owl in the Netherlands

The variation in body load of heavy metals in the Barn Owl can best be understood and interpreted in terms of the bird's ecology. The Barn Owl is a nocturnal raptor with a small food spectrum. In the Netherlands, 98% of its prey biomass are mice and shrews (*Microtidae, Soricidae* and *Muridae*). Only two species, Common Vole *(Microtus arvalis)* and Common Shrew *(Sorex araneus/coronatus)*, contribute to 62% of the weight of the total diet (de Bruijn 1979; de Jong 1980; van der Hut *et al.* 1992).

Vole population density cycles, with a period of about three or four years between very high (climax) and low (crash). The proportion of voles in the Barn Owls' diet reflects the vole population density. Consequently, the diet fluctuates strongly among years, among seasons (de Jong 1991a), and among regions (van der Hut *et al.* 1992). During climax years, the Barn Owl diet may consist of 70 to 100% voles. In crash years, the main source of food is the Common Shrew, which has about half the vole's body mass. This dietary fluctuation has an important impact on transfer of heavy metals to Barn Owls. The cadmium and lead concentrations in kidneys and liver were orders of magnitude higher in shrews than in voles (Ma 1989; Ma *et al.* 1991). In contaminated areas, tissue levels Cd and Pb found in shrews were hazardous to mammals (Ma *et al.* 1991).

Another result of this food spectrum is that the size of the Barn Owl population fluctuates with the population cycle in the common Vole. After a climax year, vole populations crash to a minimum. In response, the Barn Owl breeding population might decline to half that of the previous year (Schönfeld and Girbig 1975; Glutz von Blotzheim and Bauer 1980; Bunn *et al.* 1982; Shawyer 1987; van der Hut *et al.* 1992). Before 1963, the size of the Barn Owl population in the Netherlands was estimated to be about 3500 pairs in climax years and about 1800 pairs in crash years (Honer 1963). In the winter of 1962/63, very severe winter conditions coincided with a crash in vole population. In 1963, only a few dozen Barn Owl pairs started breeding. The Barn Owl population recovered very slowly to only 300 pairs by 1987 (Braaksma and de Bruijn 1976; de Jong 1983; SOVON 1987; de Jong 1989). In 1990, a climax year, the population reached 1130 pairs but crashed to 600 pairs in 1991 (de Jong 1991b, 1992).

Adult Barn Owls show year-round site fidelity. Their small hunting territories, about 3 km^2, are usually within 1 km from the nesting site (Bunn *et al.* 1982; Shawyer 1987). Only severe winter conditions and/or food shortage will force adult birds to disperse (Sauter 1956). Most of the young birds ringed as nestlings have been found dead near their birthplace, 50% within 20 km and 88% within 100 km (Dutch Ringing Scheme 1911–89). The Barn Owl has a high recovery rate and most birds are reported dead (±25%, personal communication R. Wassenaar). Furthermore, as at least 92% of the ringed birds are ringed as nestlings, accurate determination of age of these dead birds is possible (Anon. 1990).

Biomonitoring

Monitoring heavy metal concentrations in biota can reveal trends in time and space of the body burden of organisms (Reid and Hacker 1982). Biomonitoring is also used to explore the nature and extent of the biological impact of these metals (Ernst 1991). Heavy metal concentrations can be compared to background levels, and their bioavailability can be determined. Biomonitoring heavy metals can help when supervising and adjusting government action to correct this pollution (Canters and Snoo 1993). For legal and ethical reasons, often the animal species used to monitor contaminants at the end of the food chain in terrestrial ecosystems cannot be killed for this purpose. Therefore, this study used birds found dead. The body condition of such birds differs greatly, depending on season, gender (Hardy *et al.* 1981; Wijnandts 1984; Dijkstra *et al.* 1988), and cause of death (Piechocki 1960; Visser 1978). The possible

Table 1. Lower limits of determination of heavy metals in tissues on a dry weight basis. The average conversion factor from wet weight to dry weight was 3.75 for liver and 4.35 for kidney

Metal Tissue	Cadmium mg/kg	Copper mg/kg	Lead mg/kg	Manganese mg/kg	Iron mg/kg
Liver	0.07	1.5	0.64	0.45	1.9
Kidney	0.13	0.8	0.78	0.74	3.0
Tibia	0.03	0.6	1.10	0.37	5.6

impact of body condition on concentrations has never been considered when determining heavy metals in birds.

This study focuses on sources of variation in heavy metal levels in Barn Owls, with emphasis on relationships between levels found and parameters of body condition. It is part of a collaborative research and surveillance project on raptor mortality in the Netherlands by the DLO-Central Veterinary Institute (Lelystad), the Netherlands Society for the Protection of Birds (Zeist), the Zoological Laboratory of the University of Groningen and the Ringing Scheme of the Netherlands Institute of Ecology (Heteren). The aim of this study was to determine whether biomonitoring is possible by analyzing Barn Owls found dead by volunteers of the Working Group on Wild Bird Mortality, and the general public.

Materials and Methods

Collection of Samples

Throughout the Netherlands, government officials and volunteers associated with the Working Group on Wild Bird Mortality send birds found dead to the Veterinary Institute when they suspect unnatural causes of death (Baars and Over 1989). On average, about 1000 wild birds are received for examination every year. For the present feasibility study, which covered the year 1992, participants were asked to send all raptors and owls found dead to the Veterinary Institute. The National Ringing Station also requested reporters of all ringing recoveries to send any birds found dead to the Veterinary Institute. This made it possible to obtain birds from throughout the country.

During postmortem examination at the Veterinary Institute, bird species and sex were determined, and certain standard measurements (fresh weight, tarsus length, and wing length) were taken. Many Barn Owls had been ringed as nestlings, so their exact age (± one month) was known. To establish the cause of death, macroscopic pathological findings were recorded, and radiological, histological, parasitological, bacteriological, virological, and/or toxicological examinations were done if deemed necessary (Lumeij *et al.* 1991). At dissection, the kidneys, left tibia, and left half of the liver were weighed, after being removed for heavy metal determination. These samples and the remaining cadavers were stored at −20°C until further analysis. Dissection, storage, and the materials used complied with the standard operating procedures of the diagnostic unit of the Veterinary Institute to prevent contamination of samples.

Preparation of Samples

Kidneys and Livers: After thawing, approximately 1 g of liver or kidney tissue was weighed in long-necked digestion tubes, dried overnight in an oven at 110°C, and then reweighed. The samples were digested in these tubes with pieces of carborundum using a mixture of H_2SO_4, $HClO_4$, and HNO_3 (2:1:10 v/v/v) according to van Beek *et al.* (1987). Because kidneys are inhomogeneous organs, whole kidneys were used. With large owls this required using more than one digestion tube. In these cases, the weighted mean value was used in further analysis.

Tibiae: The bones were boiled in deionized water for 20 min and dried overnight in an oven at 110°C, after any residuals of fat, muscle, and cartilage were removed. The dried material was ground, and 0.2 g of the bones was weighed accurately in long-necked digestion tubes. The bones were digested in a mixture of $HClO_4$ and HNO_3 (1:20 v/v) according to Hontelez *et al.* (1992).

Analysis of Samples

Amounts of lead, cadmium, copper, iron, and manganese were determined with a Perkin-Elmer Zeeman/3030 atomic absorption spectrometer with a HGA-600 graphite furnace as described by van Beek *et al.* (1987). Calibration curves for the metals were made from Titrisol® standard solutions of 1 mg of metal per ml (Merck). All reagents were of analytical grade (Merck). Sample solution concentrations were corrected for blank solutions. Limits of determination were defined as twice the standard deviation of blank controls and are summarized in Table 1. Samples from a stock of a dried and homogenized mixture of kidneys and livers of other animals were positive controls in each digestion run. The concentration of cadmium, lead, copper, iron, and manganese in this mixture was known. With each digestion run, three digestion tubes with reagent blanks were negative controls.

Carcass Analysis

After autopsy and dissection, the frozen cadavers were sent to the Zoological Laboratory. Measurements of total body length, length of wing, tail, sternum, and head were taken. The second half of the liver, the heart, and the breast muscles were removed and weighed. The carcass was plucked and weighed. The feathers were collected, dried at 75°C, and weighed. Liver, heart, breast muscles, and carcass were dried separately in an oven at 75°C until no further weight reduction occurred (dry weight). After being reweighed, the fat of the different parts was extracted in a Soxhlet-extractor by petroleum-ether until the extract remained colourless (approximately 1 day). The fat-free remains were dried again in an oven at 75°C until no further weight reduction occurred and weighed (fat-free dry weight). The fat content was determined by subtracting the fat-free dry weight from the gross dry weight. Subsequently, the heart, liver, breast muscles, and carcass were incinerated in an oven at 450–500°C. This took twelve hours for organs and twenty-four h for carcasses. Total protein weight was calculated by subtracting weight of ashes from fat-free dry weight.

The nutrient, fat, and protein reserves were estimated as described by Modderman *et al.* (in preparation). To calculate nutrient reserves, it was necessary to correct for the size of the individual bird (Piersma 1984). Data from nine starved Barn Owls were used as benchmark from which a relationship between body dimensions and dry weight could be derived. This relation, based on the assumption that those animals had exhausted their nutrient reserves, enabled us to calculate the minimal dry weight for all Barn Owls. According to Modderman *et* *al.* (in preparation), the product of head and wing length was the best estimation for minimal dry weight *(minimal dry weight (g) = 0.0046 * wing (mm) * head (mm) − 28.14;* $N = 9$ $R^2 = 0.90$ $p < 0.001$*)*. This relation was subsequently used to calculate structural mass (or mass at starvation) of all birds from their head and wing length. Structural mass is thus the calculated minimal mass of the Barn Owl based on its body dimensions. Because the protein reserves were obtained by subtracting the calculated minimal fat-free dry weight from the determined fat-free dry weight, their value can be negative. These birds actually had a lower fat-free dry weight than their calculated minimal fat-free dry weight. Biological variation in structural size of the starved animals used to construct the benchmark is expressed in the calculated protein reserves. All carcass weights were corrected for the organs removed.

Statistics

In earlier studies, metal levels showed a skewed distribution (Hontelez *et al.* 1992). Therefore, we opted for a nonparametric analysis and presentation of the data. Heavy metal concentrations were reported as median and range in mg per kg dry weight (Adrian and Stevens 1979; Scanlon 1982); fat and protein reserves were expressed as a percentage of the calculated structural mass. Differences between metal concentrations of male and female Barn Owls were tested using the Mann-Whitney rank sum test. Differences between metal concentrations in liver, kidney, and tibia were tested using the Wilcoxon's matched pairs signed rank test. The differences between weights and reserves were tested in the Mann-Whitney rank sum test. Probabilities of correlations were estimated using procedures and tables given by Diem (1982). When probability levels were below 5% ($p < 0.05$), the data sets were considered different.

Results

During 1992, fifty-three Barn Owls were sent to the Veterinary Institute. Twenty-five were male, twenty-one were female, and the gender of seven was not determined. Some birds were unsuitable for further analysis because their carcasses were incomplete or because of decay.

Primary cause of death of forty-five birds was established. Twenty-eight had suffered trauma, mainly due to road accidents. A sublethal dose of parathion was found in one, and at least seven were also emaciated. Seventeen birds showed signs of severe exhaustion only. For eight birds, no primary cause of death could be established. Besides primary cause of death, exhaustion was also noted as an additional symptom in road accident victims. With one exception, exhaustion coincided with the diagnosis of severe coccidiosis and/or other parasitic gastrointestinal infections.

The seasonal pattern of mortality (Figure 1) showed only the winter/early spring peak in mortality. The second and higher peak usually observed in late summer/early autumn was absent. No indications of any sex-linked distribution of mortality were observed. Of thirty-one Barn Owls, ages were known thanks to ringing recoveries. The age distribution (Figure 2) showed a large contribution of 2–3 years old ringed Barn Owls. The locations in which owls were found are represented in Figure 3.

Heavy Metals

Metal concentrations were determined in forty-three birds (Table 2). There were no significant differences in the metals measured between the sexes.

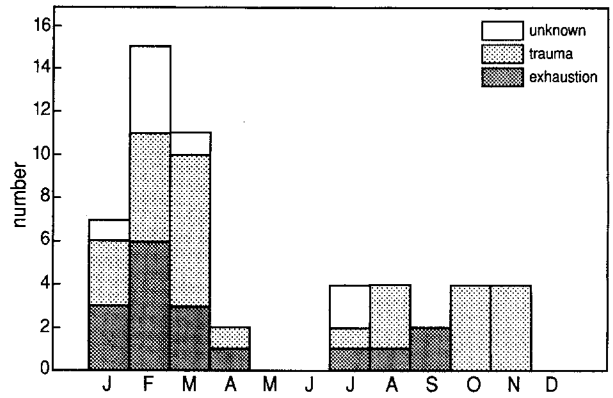

Fig. 1. Frequency distribution by month and cause of death of 53 Barn Owls sent to the DLO-Central Veterinary Institute in 1992

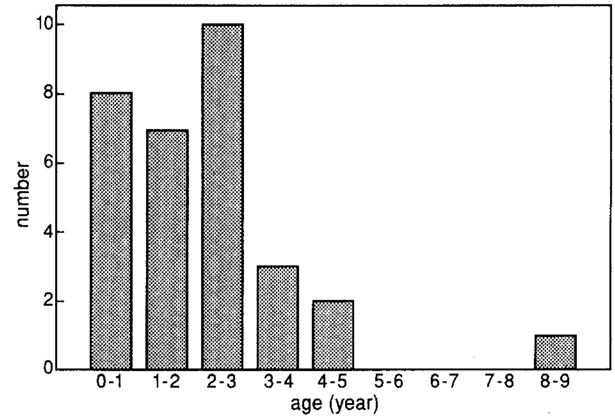

Fig. 2. Frequency distribution of age of 31 ringed Barn Owls sent to the DLO-Central Veterinary Institute in 1992

Cd levels were significantly higher in kidney than in liver; both levels exceeded Cd concentrations in tibia. The lowest Cu levels were found in tibia, and the highest were found in liver. Pb concentrations were highest in tibia and lowest in liver. The ratio between Pb concentration in liver and in kidney was variable. Mn concentrations found in liver were significantly higher than those found in kidney or tibia. Lowest Mn levels were found in tibia. Fe showed a distribution similar to Cu, but the large dispersion of Fe concentrations is notable.

We investigated possible correlations between kidney, liver, and tibia regarding both their concentration and their load of Cd, Cu, Pb, Mn, and Fe. In all cases, we had thirty-eight samples, therefore correlation coefficients between plus and minus 0.32 had probability levels below 5% and were not reported. The correlation coefficient for [Cd] in liver v. kidney was 0.73 and the correlation coefficient for Cd load between liver and kidney was 0.74. Lower coefficients were found for the correlations for [Cu], [Mn], [Pb], or [Fe] between liver and kidney ($R = 0.43$ for Cu and $R = 0.54$ for Pb). The loads of these metals in kidney v. liver had higher correlations ($R = 0.33$, 0.44, 0.47 and 0.37 for Cu, Mn, Pb, and Fe, respectively). For Cd, Cu, Mn, and Fe, no significant correlations were found between liver and tibia, or between kidney and tibia. Concentrations and loads of Pb in tibia varied with those of kidney and liver. R values were 0.39 and 0.37 for tibia v. kidney, and 0.57 and 0.62 for tibia v. liver.

Table 2. Concentrations of heavy metals in kidney, liver, and tibia of Barn Owls (mg/kg dry weight) found dead in the Netherlands during 1992, expressed as medians for all birds and as ranges for males and females separately

Tissue	Metal	n#	Cd mg/kg	Cu mg/kg	Pb mg/kg	Mn mg/kg	Fe mg/kg
Kidney	median	42	1.09^a	14.5^a	0.94^a	6.7^a	785^a
	male-range	21	<0.13–11.3	5.2–28.3	<0.78–5.2	2.8–31.2	262–1593
	female-range	19	<0.13–8.8	10.9–23.0	<0.78–10.6	4.3–11.1	252–1610
Liver	median	41	0.55^b	29.2^b	$<0.64^b$	9.8^b	1466^b
	male-range	21	<0.07–2.7	12.6–105.3	<0.64–5.8	<0.45–16.7	47–7262
	female-range	19	<0.07–3.1	<1.50–102.1	<0.64–22.3	2.3–15.5	504–7274
Tibia	median	43	$<0.03^c$	1.8^c	1.54^c	2.6^c	45^c
	male-range	22	<0.03–0.21	<0.60–5.5	<1.10–10.7	1.0–4.8	12.5–896
	female-range	19	<0.03–0.28	<0.60–4.2	<1.10–11.7	0.60–6.7	7.6–143

Different suffixes in a column indicate significant differences ($p < 0.05$)

Two liver samples and one kidney sample were spilled during destruction and the sex of two birds was not determined

Table 3. Body mass and nutrient reserves of Barn Owls found dead in the Netherlands and sent to the DLO-Central Veterinary Institute in 1992. The ranges are given for both sexes. Nutrient reserves are % of calculated structural or starvation mass

	n #	Fresh weight g	Dry weight g	Fat-free dry weight g	Ash weight g	Fat reserve %	Protein reserve %
Median	43	255	70.13	62.95	13.50	11.72	12.64
Male-range	22	167–348*	41.13–99.24*	38.26–71.37*	9.76–14.86*	0.92–64.65	−1.23–33.48*
Female-range	19	218–365*	53.04–118.66*	51.99–83.51*	12.05–15.85*	1.38–88.24	−10.23–61.00*

Of two animals, sex was not determined

* Significant differences between sexes ($p < 0.05$)

Fig. 3. Locations in the Netherlands where dead Barn Owls were found, which were received by the DLO-Central Veterinary Institute in 1992. Note that one bird was found in Germany

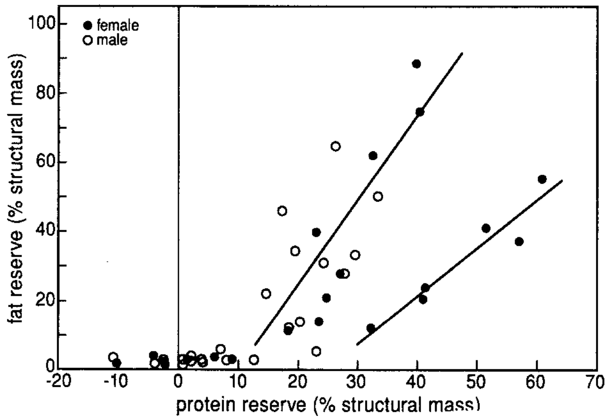

Fig. 4. Relation between fat and protein reserves of the Barn Owl (% of calculated structural mass). Different symbols are used to indicate sex of the animals found dead. In the birds with a fat reserve larger than 10%, two groups can be discerned. For the animals with a relatively high protein reserve (X) relative to their fat reserve (Y), a regression ($R^2 = 0.91$) of $Y = 1.4(\pm 0.2)*X - 34.3(\pm 5.2)$, ($n = 6$; ±SD) was calculated. Note that these females all died during February and March just before egg laying. For the other birds, a regression ($R^2 = 0.61$) of $Y = 2.4(\pm 0.5)*X - 24.1(\pm 15.0)$, ($n = 17$; ±SD) was calculated. For negative values, see M & M section

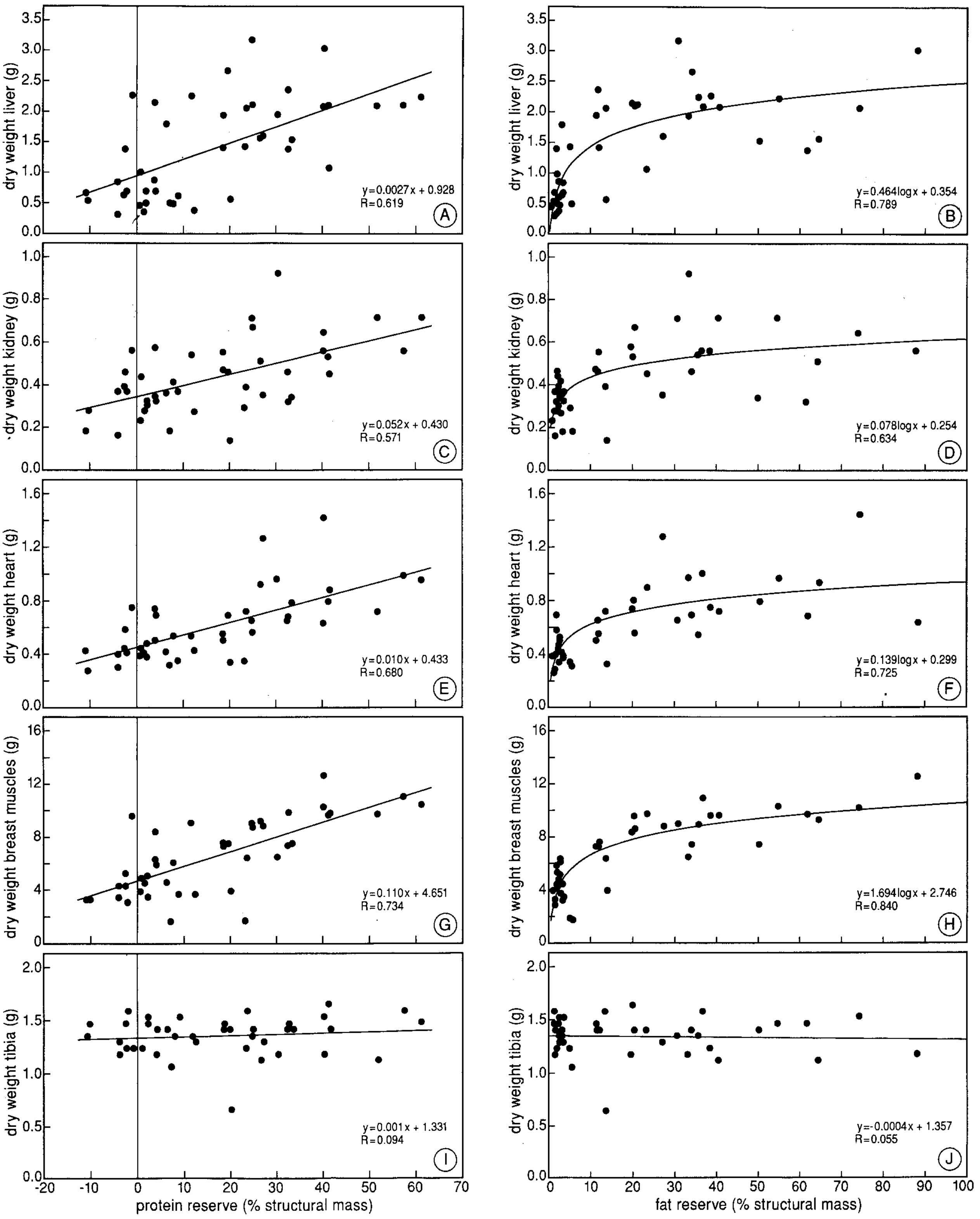

Fig. 5. Relation between tissue dry weight (g) and protein and fat reserves (% of calculated starvation dry weight) for liver (A & B), kidney (C & D), heart (E & F), breast muscles (G & H), and tibia (I & J) in the Barn Owls found dead and sent to the DLO-Central Veterinary Institute during 1992

In liver, significant correlations were found between [Cd] and [Mn] (R = 0.33), between [Cu] and [Mn] (R = 0.53), and between [Cu] and [Fe] (R = 0.48). The significant correlations between metal loads in liver were for Cd and Mn, 0.32, and for Cu and Mn, 0.53. Only between [Fe] and [Mn] was a significant correlation (0.32) found in the kidney. Correlations were found between Cu and Fe loads (0.47), between Cu and Mn loads (0.46), and between Fe and Mn loads (0.35). In tibia,

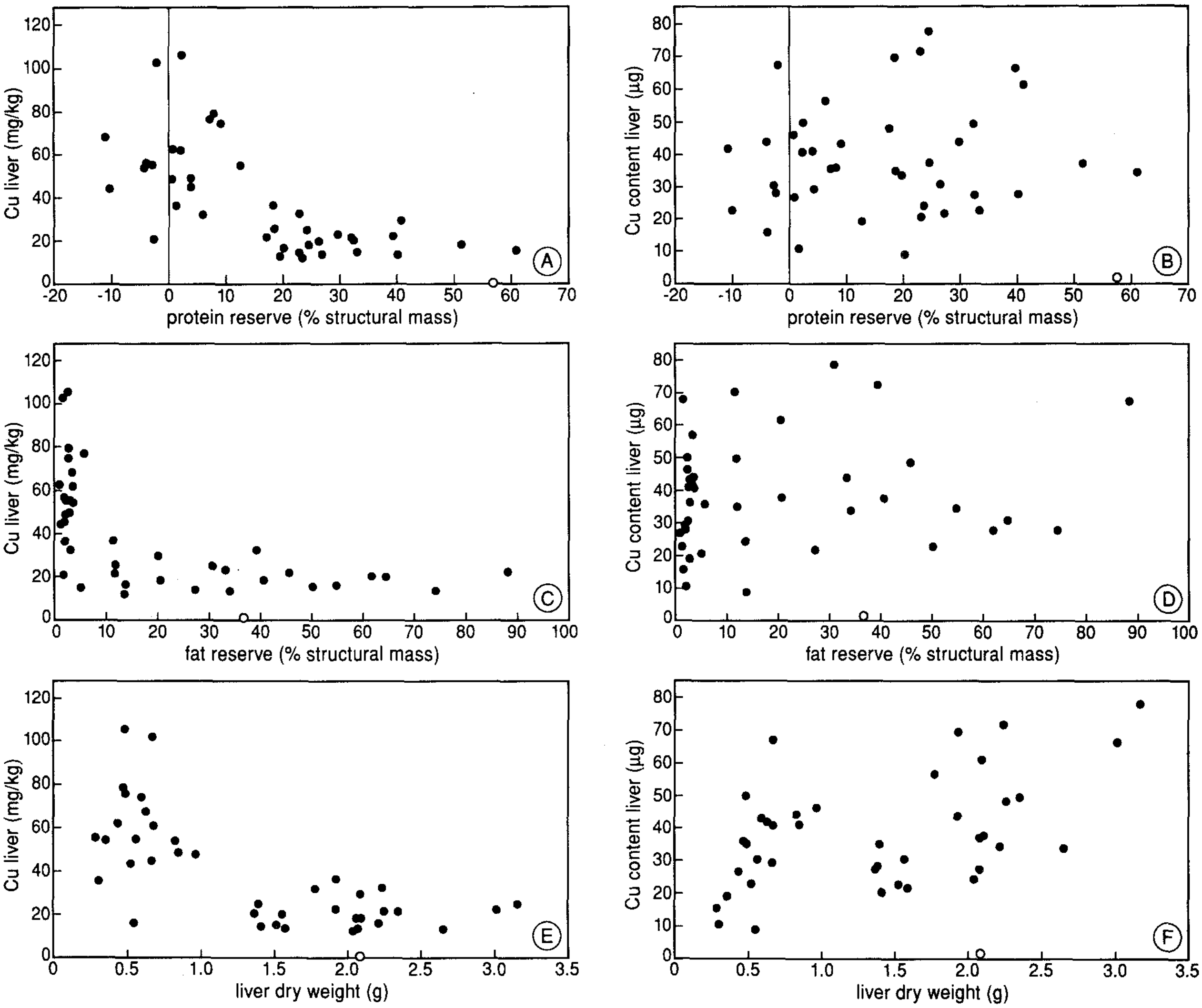

Fig. 6a. Relationships between Cu concentration (mg/kg dry weight) or Cu load (µg) of liver and protein reserve (% structural mass; A & B), fat reserve (% structural mass; C & D), and dry liver weight (g; E & F) of Barn Owls found dead in the Netherlands during 1992. Observations below the limit of determination are indicated by open circles

correlations were found between [Cd] and [Mn] (0.37), between [Cu] and [Fe] (0.57), between [Cu] and [Pb] (0.40), between Cd and Mn loads (0.41), between Cu and Fe loads (0.48), and between Cu and Pb loads (0.45).

Carcass Analysis

Nutrient reserves of 43 Barn Owls were calculated (Table 3). Negative protein reserves calculated for individual birds reflect biological variation in the benchmark group (see M & M). Fresh, dry, and fat-free dry weights of the birds that died of exhaustion were significantly lower than those that died of trauma. A review of the ash weights showed that the two categories did not differ in size. As expected, significant differences in protein reserve and in fat reserve were observed: reserves of birds that died of exhaustion were very small, whereas nutrient reserves of trauma victims were generally much larger.

In total, significant differences were observed between sexes with regard to fresh, dry, fat-free dry, ash weights, and protein reserve (Table 3). Because carcasses used for these measurements either were from males, which had mostly died of exhaustion or from females, which had mostly died of trauma, at least part of these differences are probably artifacts. The findings relating to fresh, dry, fat-free weights and protein reserve can especially be attributed to an uneven distribution of sexes over death causes. Within a group of death cause, no significant differences were found in fresh, dry, fat-free dry, ash weights, and nutrient reserves between males and females. Although we found no difference between cause of death groups regarding the ash weights, this parameter showed sex differences in the total sample of Barn Owls. Also noteworthy is the lack of sex differences regarding the fat reserve in the whole sample.

If the fat reserve is larger than 10%, two linear relationships between fat and protein reserves can be distinguished (Figure 4). In males, and in females outside the prebreeding phase, this relationship differs sharply from a small group with relatively high protein reserves. This latter group consists only of females that died during February and March 1992, shortly before or during egg-laying. The data from emaciated birds (fat reserve < 10%) suggest a progressive reliance on protein as en-

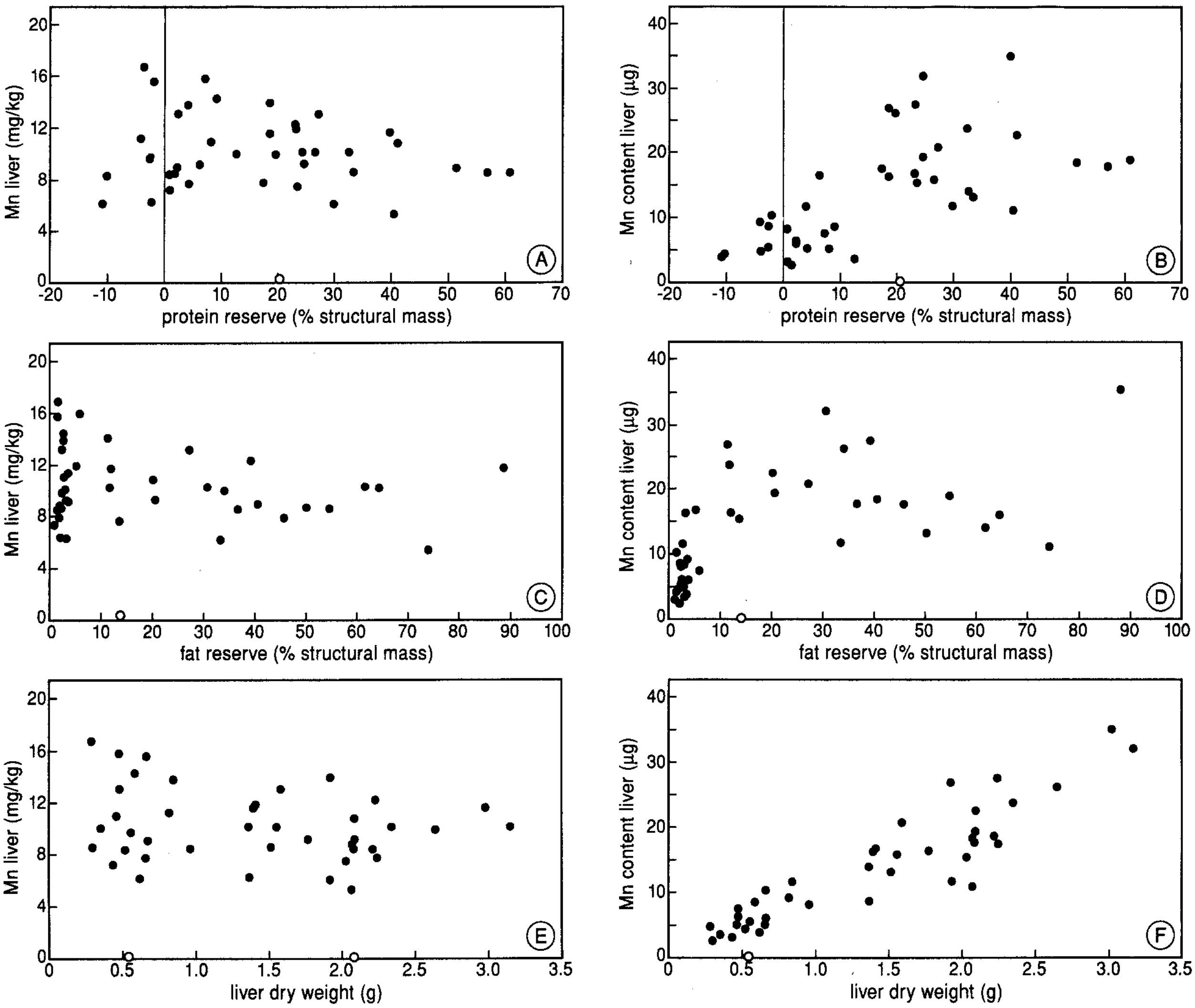

Fig. 6b. Relationships between Mn concentration (mg/kg dry weight) or Mn load (μg) of liver and protein reserve (% structural mass; A & B), fat reserve (% structural mass; C & D), and dry liver weight (g; E & F) of Barn Owls found dead in the Netherlands during 1992. Observations below the limit of determination are indicated by open circles

ergy store. A fat content of 2(1–6)% apparently represents 'structural fat' (Piersma *et al.* 1984) that cannot be mobilized.

Figure 5 shows the relationships between protein or fat reserves, and the dry weight of liver, kidney, breast muscles, heart, and tibia. The relations between protein reserve and dry weight of the soft tissues were linear. The relations between fat reserve and dry weight of the soft tissues were nonlinear but could be fitted to an exponential equation seemingly approaching a maximum. As expected, no relations were found between protein and fat reserves and dry weight of the tibia.

Heavy Metals and Body Condition

Heavy metal concentrations in tibia never showed a clear relationship with body condition. High concentrations of Cu were measured in the liver and/or kidneys of owls with low fat and protein reserves (Figure 6a). These organs, however, tend to become smaller as body condition worsens (Figure 5), and therefore metal concentrations could increase if metal load remains constant. To test this, we calculated the total amount of Cu in an organ and found it to be independent of body condition (Figure 6a). Similar results were obtained for Fe, in spite of the wide range of Fe concentrations and total organ load.

The Mn concentration in organs and tibiae did not vary with body condition. Correction for organ size (by calculating the total load of the organ) showed a relationship reflecting the size of the organ. This reveals that Mn concentrations remain constant with varying organ size (Figure 6b).

Neither concentrations nor loads of both Cd and Pb varied with body condition or organ size. The highest Pb concentrations, however, were found in livers, tibia, and kidneys of owls with low fat and protein reserves. If high Pb loads were encountered in tibia, the fat and protein reserves were low. However, when organ loads or concentrations of Cd or Pb are plotted against an indicator of body condition, the graph of organ load yields outliers (Figure 6c). All these outliers could be traced to collection locations in or very near areas contaminated with heavy metals. The reverse, however, was not observed.

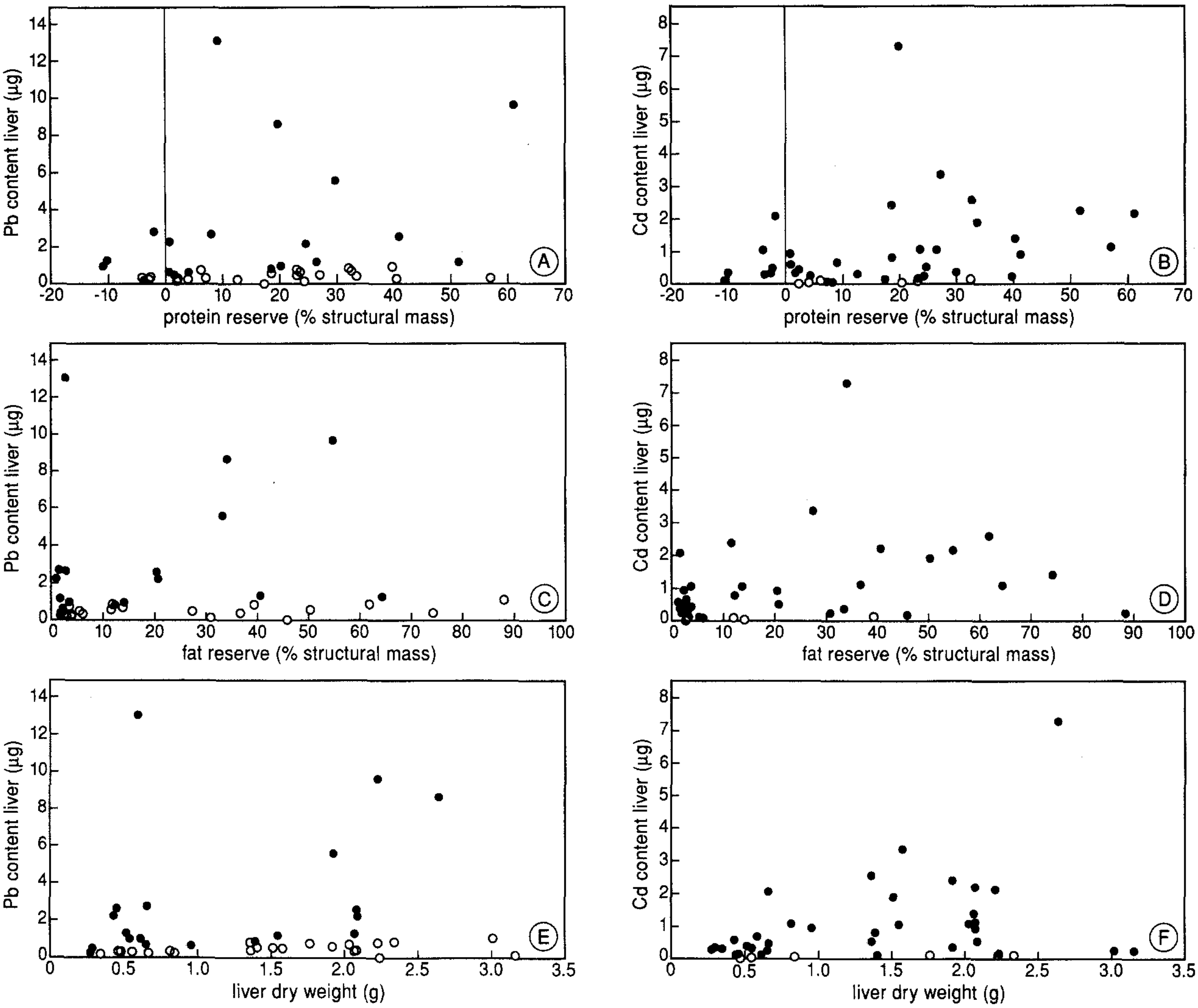

Fig. 6c. Relationships between Pb or Cd load (µg) of liver and protein reserve (% structural mass; A & B), fat reserve (% structural mass; C & D), and liver dry weight (g; E & F) of Barn Owls found dead in the Netherlands during 1992. Observations below the limit of determination are indicated by open circles

Heavy Metals and Age

Total Cd load in liver increased with the age of the bird (R = 0.69), as did Cd load in kidney (R = 0.73). Cu load in liver tended to decrease with age. No relationship was found between age and total Cu load in kidney. Cd and Cu loads in tibia were below the determination limits. No relationships between age and concentration or load of Mn, Fe, or Pb in kidney, liver, or tibia were observed.

Heavy Metals and Geographical Distribution

The number of birds investigated was too small to enable a conclusive comparison to be made of regions in the Netherlands. Nevertheless, corresponding with the results obtained by Hontelez *et al.* (1992), all birds with high loads (g) of both Pb and Cd had been found in areas contaminated with these metals. It is noteworthy that the single Barn Owl with high Pb but a low Cd load came from an area polluted by lead pellets from clay pigeon shooting. The distribution of birds with high Cu loads did not match the distribution of polluted industrial regions. A source of Cu emission is intensive pig farming because of the high concentrations of Cu in pig manure. A distribution map of Cu supplied by spreading pig manure, expressed as kg per hectare (Ernst 1991), did not match the distribution map of Cu levels found in Barn Owls. It is possible that the disparity of scale of these maps hinders detection of correlations. It might also result from recent restrictions of the use of Cu as a feed additive. Areas contaminated by Mn or Fe were not identified in the Netherlands.

Heavy Metals and Nutrient Reserves Throughout the Year 1992

Barn Owls found dead from January to March (the peak in Figure 1) showed higher Cd concentrations and loads in kidney and liver than birds found dead from July to November. Cd concentrations and loads in tibia increased during the first period and declined during the second period. Pb concentrations and loads in liver, kidney, and tibia were higher during the first period than during the second. Mn loads and concentrations in liver, kidney, and tibia remained fairly constant throughout the year. The same holds for Cu and Fe in liver and kidney. Cu and Fe loads and concentrations in tibia were higher in birds found during the January to March period than in birds found in July to November.

The dry weight of tibia remained constant throughout the year. Kidney, liver, heart, and breast muscle dry weights were variable during January to March in 1992, but increased during July to November. Fat and protein reserves (% structural mass) were highest in February and March.

Discussion

The volunteers of the Working Group on Wild Bird Mortality, the public, and government officials provided us with a representative sample of Barn Owls. The seasonal pattern of mortality (Figure 1) deviated somewhat from Barn Owl mortality patterns observed by others (Honer 1963; Braaksma and de Bruijn 1976; Hardy *et al.* 1981; Shawyer 1987; Newton *et al.* 1991; van den Tempel 1993). The sample showed only the winter/early spring peak in mortality, and not the higher peak usually observed in late summer/early autumn. The spring/summer period, with a low mortality rate, coincided with the breeding season. Age distribution (Figure 2) was similar to that of recovered ringed Barn Owls (Breaaksma and de Bruijn 1976). The age group 2–3 yeard old is large and refects that 1990 was a climax year for Barn Owls (de Jong 1991b). The geographical distribution of the locations in which owls were found (Figure 3) coincided reasonably well with the population distribution (Texeira 1979; SOVON 1987; de Jong 1989). From the comments received during this study and afterwards, we concluded that by slightly changing our approach to potential collaborators, we could easily have doubled the sample size.

Heavy Metals

Metal concentrations found in kidney, liver, and tibia for Cd, Cu, Pb, Mn, and Fe generally agree with those obtained by others in several bird species (Table 4). These data originate, however, from both uncontaminated and contaminated areas.

Denneman and Douben (1993) found Cd levels in both kidney and liver to be significantly lower at the control site than in Budel (Table 4). Cd levels at the control site match the bottom of our range. The highest Cd levels from the contaminated Budel site are similar to the upper part of our range. Levels that exceed 3 mg/kg dry weight in liver and in kidney would suggest hazardous environmental exposure to Cd (Scheuhammer 1987). Kidney levels of several birds in our sample were well above that threshold. The liver rapidly inactivates Cd with metallothionein; the kidney eliminates Cd more slowly. Therefore, a liver:kidney ratio > 1 indicates an acute ingestion of relatively high levels. Chronic absorption is reflected in a ratio < 1 (Scheuhammer 1987). The results match the latter case and imply a chronic burden with Cd, in some places at hazardous levels.

Other authors also found Cu levels in the liver to be the highest (Table 4). Some authors, however, reported higher concentrations in the kidneys than in the liver (Table 4). The lack of a significant difference between the polluted Budel site and the control site in Cu concentrations in Barn Owls (Denneman and Douben 1993), fits our observations.

Most of the literature data summarized in Table 4 also show bones as the tissue with the highest Pb concentration; however, the ratio between Pb concentration in liver and in kidney was variable. According to Scheuhammer (1987), Pb levels > 5 μg/g (dry weight) in bones of adult wild birds would suggest increased environmental exposure to Pb. In the present study of Barn Owls, most of the Pb concentrations in tibia, kidney, and liver (Table 2) resembled those reported from relatively uncontaminated areas. In contrast with Denneman and Douben (1993), our sample from contaminated areas had a few birds with high levels of Pb.

The finding that the concentrations and loads of Cu, Cd, Mn, Pb, and Fe in kidney and liver were largely intercorrelated was expected. Of the heavy metals studied, only Pb accumulates in bones, so we expected to find no relationships between liver and tibia, or between kidney and tibia for Cu, Cd, Mn, and Fe. Relationships were found between Pb in liver and tibia, and in kidney and tibia. Scheuhammer (1987) argues that the long-term exposure of birds to lead is reflected in Pb present in calcareous tissue. On the other hand, Pb in soft tissue reflects recent exposure of birds to lead. In a controlled experiment, Custer *et al.* (1984) found a better correlation between Pb in bones and Pb in liver than between Pb in bones and Pb in kidney. We also found higher correlations for Pb between liver and tibia than between kidney and tibia. These findings suggest that the kidneys form a distinct but connected compartment with pharmacokinetic characteristics different from those of tibia. The correlations between Pb in kidney and in liver suggest a closer pharmacokinetic tie. Of the soft organs, the kidney accumulates the highest levels of Pb. It seems therefore that kidney and tibia are indeed good indicators for recent and lifelong Pb exposure.

In liver and kidney, the heavy metals regulated homeostatically (Cu, Mn, and Fe) were linearly related. Unexpectedly, linear relationships were also found between Cu and Fe in tibia.

Heavy metal levels found in this study tentatively suggest that the Dutch Barn Owl population is not threatened by the current levels of heavy metal contamination in the Netherlands. Our sample is too restricted to allow a final evaluation.

Carcass Analysis

It is clear that in nutritionally stressed birds, fat reserves are depleted faster than protein reserves (Figure 4). Protein reserves decreased gradually, along with a steep decline of fat reserves. As organs mainly consist of protein, their size (as dry weight) showed a linear relationship with protein reserve (Figure 5). The observed parabolic relationship between organ size and fat reserves (Figure 5) reflects the use of fat as an energy source (when fat reserves were $> 10\%$), and the use of protein as an energy source (when fat reserves were $< 10\%$). Figures 4 and 5 imply that Barn Owls with fat reserves less than 10% of their structural mass are starving. Figure 4 clearly shows two distinct relationships between fat and protein reserves. The "high protein" group of birds, consisting exclusively of females found dead in February and March of 1992, deserves mention. Their organ sizes compared with their protein reserves are not

Table 4. Summary of 5 heavy metal concentrations (median and range) in 3 target tissues found in 12 bird species from various parts of the world

	Metal organ		Cd kidney	Cd liver	Cu kidney	Cu liver	Cu bone	Pb kidney	Pb liver	Pb bone	Mn kidney	Mn liver	Mn bone	Fe kidney	Fe liver	Fe bone
Species		n	mg/kg DW	mg/kg DW	mg/kg DW	mg/kg DW	mg/kg DW	mg/kg DW	mg/kg DW	mg/kg DW	mg/kg DW	mg/kg DW	mg/kg DW	mg/kg DW	mg/kg DW	mg/kg DW
Barn Owl	Netherlands	42	1.09	0.55	14.5	29.2	1.8	0.94	<0.64	1.54	6.7	9.8	2.6	785	1466	45
	This study		<0.13–11.3	<0.07–3.1	5.2–28.3	<1.5–105.3	<0.6–5.5	<0.78–10.6	<0.64–22.3	<1.1–11.7	2.8–31.2	<0.5–16.7	0.6–6.7	252–1610	47–7274	7.6–896
	Netherlands	3	4.8	1.2	17	35		0.6	0.3							
	Budel[a]		0.5–12.8	0.2–3.1	13–19	18–59		0.4–0.8	0.2–0.3							
	Netherlands	3	0.5	0.2	16	13		0.7	0.2							
	Achterhoek[a]		0.4–0.6	0.1–0.9	14–17	8–106		0.4–0.9	0.1–0.2							
Buzzard	Netherlands[c]	35	2.1	0.6				0.9	0.7							
			0.2–3.7	0.1–4.6				0.2–3.7	0.1–10.9							
Eider	Netherlands[c]	26	15.3	6.5				0.7	0.7							
			4.5–43.5	1.8–17.7				0.3–2.6	0.1–4.9							
	Denmark[i]	42	38	13		333										
			3–134	1–44		25–2135										
Grey heron	Netherlands[c]	4	1.0	0.4				1.0	1.4							
			0.3–19.4	0.2–4.7				0.2–2.6	0.4–2.1							
	Germany	10	0.22	0.9				0.14	0.18							
	Schleswig-H[b]		0.17–0.27	0.11–0.7				0.10–0.17	0.10–0.27							
Common Tern	Canada	6	29.5	3.69	20.6	17.6	6.06	<10.0	<10.0	12.2						
	Hamilton[d]		11.2–53.9	1.28–5.29	18.2–23.8	12.9–21.6	5.15–8.14			9.70–15.9						
	USA	10	21.3	3.82	19.2	23.5	5.43	<10.0	<10.0	18.1						
	Massachusetts[d]		10.4–46.1	2.05–6.87	17.2–21.6	17.9–27.5	4.15–8.87			11.2–32.8						
Crested Tern	AUS.-N. Sol.[l]	10	5.09 ± 0.74	1.28 ± 0.23	1.29 ± 0.24	2.29 ± 0.16		0.23 ± 0.04	0.44 ± 0.04	0.43 ± 0.07	0.66 ± 0.02	1.08 ± 0.07		144 ± 8	280 ± 30	
	AUS.-Big I.[l]	10	2.45 ± 0.35	0.77 ± 0.08	1.04 ± 0.14	2.75 ± 0.43		0.16 ± 0.02	0.41 ± 0.02	0.42 ± 0.05	0.68 ± 0.03	1.07 ± 0.03		95 ± 6	153 ± 9	
Duck male	Poland	50		0.76 ± 0.06		1.5 ± 0.2			0.13 ± 0.02			4.2 ± 0.8			750 ± 110	
female	Gdansk Bay[j]	40		0.59 ± 0.10		1.3 ± 0.1			0.14 ± 0.03			4.9 ± 0.7			670 ± 130	
Feral Pigeons	UK-control	7	1.75	0.54 ± 0.05				4.34 ± 1.26	2.01 ± 0.29	5.73 ± 1.05						
	Chelsea	53	12.3 ± 2.05	2.45 ± 0.28				321.4 ± 45.3	21.6 ± 1.95	669 ± 45						
	Mortlake	15	1.52 ± 0.31	0.40 ± 0.07				48.7 ± 17.5	10.1 ± 2.36	282 ± 74						
	Heathrow[e]	15	50.7 ± 22.7	9.48 ± 3.15				9.87 ± 1.26	6.11 ± 1.09	106 ± 28						
Osprey	Eastern USA[f]	21		0.14		3.9			0.42						690–2800	
				<0.10–0.74		1.5–55			<0.10–4.6							
Mute Swan	Denmark[h]	99	23	12	34	2680		15	31							
Tit	Poland	9		0.32		9.4	3.3		2.9	5.0					1426	79
	Bialowieza[m]			0.35 ± 0.04		12.9 ± 2.1	7.5 ± 4.1		5.3 ± 2.4	8.3 ± 2.5					1720 ± 324	99 ± 28
	Poland	5		10.6		7.0	2.8		10.6	62.6					1464	286
	Dulowa Forest[m]			15.3 ± 4.4		11.5 ± 5.8	3.4 ± 1.1		11.5 ± 2.6	65.6 ± 8.8					1630 ± 403	299 ± 48
Cattle Egret	Korea[g]				2.80 ± 0.11	7.05 ± 2.96	0.79 ± 0.13				1.58 ± 0.15	3.47 ± 0.29	2.28 ± 0.19	122 ± 22.4	230 ± 39.8	50.2 ± 5.53
	USA-Texas[k]	9	1.08 ± 0.25	0.12 ± 0.02				0.94 ± 0.42	0.63 ± 0.13	7.93 ± 3.84	1.1 ± 0.87	1.8 ± 0.39	2.2 ± 0.39			

[a] Denneman and Douben, 1993 [b] Scharenberg 1989 (mean ± SD, wet weight) [c] Hontelez et al. 1992 [d] Connors et al. 1975 [e] Hutton and Goodman 1980 (mean ± SD) [f] Wiemeyer et al. 1987 [g] Honda et al. 1986 (mean ± SD, wet weight) [h] Elvestad et al. 1982 (mean values) [i] Karlog et al. 1983 (mean and range) [j] Szefer, 1983 (mean ± SD) [k] Hulse et al. 1980 (mean ± SD, wet weight) [l] Howarth et al. 1981 (mean ± SD, wet weight) [m] Sawicka-Kapusta et al. 1986 (median, mean ± SD)

atypical. Perhaps these birds were storing protein just before egg laying.

The fresh weights reported by Piechocki (in: Glutz von Blotzheim and Bauer 1980), although slightly higher, agree with our results. In birds that did not die of exhaustion, we calculated mean fat reserve (as % of fresh weight) of 6.2 for males and 6.3 for females. These values are similar to the fat reserves (as % of fresh weight) of 5.3 for males and 6.6 for females estimated by Piechocki (in: Glutz von Blotzheim and Bauer 1980). For Barn Owls that did not die of exhaustion, we calculated mean protein reserves (as % of fat-free dry weight) of 18 for males and 26 for females. Piechocki (in: Glutz von Blotzheim and Bauer, 1980) estimated comparable values named starvation resistance (22 for males and 25 for females). Female Barn Owls are reported to be somewhat larger than males (Piechocki in: Glutz von Blotzheim and Bauer 1980; Hardy *et al.* 1981). In our study, the significant difference in ash weight between sexes implies the same.

Heavy Metal Levels and Sources of Variation

We looked for different sources that could be responsible for variation in heavy metal levels found in the Barn Owl. These could include sex, age, body condition (and hence organ size), diet, and contamination of home range.

Reports on sex differences in Cd and Pb concentrations are variable (Cheney *et al.* 1981; Hutton 1981; Clausen *et al.* 1982; Karlog *et al.* 1983; Szefer 1983; Scheuhammer 1987). The difference in diet, weight increase of the female before egg laying, and the increased turnover of skeletal Ca needed for eggshell formation (Taylor 1970) might explain why a higher metal concentration was occasionally found in females. In our study, we found no differences between sexes, not even in the "high protein" group (prebreeding females) from Figure 4.

Except bones, changes in body condition lead to changes in the sizes of organs. This in turn might affect the metal concentrations or load in these organs. The results found in the present study and by Osborn (1979) suggest that the homeostatically regulated Cu, Fe, and Mn concentrations or loads in organs not only reflect the levels of these metals in the food, but also change with nutrient reserves and/or organ weight. Note the difference found between Cu (& Fe) on the one hand and Mn on the other hand (Figures 6a, 6b). In contrast to this physiologically-based regulation, Pb and Cd become virtually immobile after being taken up by the target organ. They are best expressed as an organ load. Relatively high concentrations found in the liver and kidneys of birds with very low protein and fat reserves might yield false positive signals in biomonitoring. Also, the lower concentrations encountered in owls with large reserves might give false negative signals. The higher concentrations and loads of Pb found in tibiae of birds in very poor condition is not easily explained. It might be that lifelong exposure to relatively high levels of Pb induced immunosuppression, which in turn leads to coccidiosis and then emaciation. In this pilot study, the collection locations of the outliers 'generated' by expressing our data as organ load were all in contaminated areas. For the moment, we suggest that both concentrations (g/kg dry weight) and loads (g) be determined in subsequent studies of heavy metals in animals. As noted in the Materials and Methods section, all measurements should be based on dry weight to prevent variation resulting from uncontrolled evaporation.

The kidneys and liver together can account for up to 75% of the total body load of Cd (Nordberg *et al.* 1985; Friberg *et al.* 1986). Furthermore, once bound to metallothionein, Cd has a very long biological half life (Friberg *et al.* 1986). Thus, an expected relationship between age and Cd load (but not necessarily concentration) in liver and kidney was indeed found. Cd levels increasing with age were also reported for several other bird species (Blomqvist *et al.* 1987; Honda *et al.* 1986; Hutton 1981; Cheney *et al.* 1981; Karlog *et al.* 1983). Results of Blomqvist *et al.* (1987) also suggest that Cu has an age-related concentration decrease. Surprisingly, we found no relation between age and Pb concentration in tibia. Calcareous tissue has a great affinity for Pb and, once bound, Pb is practically immobile. The skeleton accumulates about 90% of the total body load (Tsuchiya 1986; Stowe *et al.* 1973). Thus, Pb levels are reported to increase with age (Honda *et al.* 1986; Cheney *et al.* 1981). The important source of Pb for birds of prey would be the bones of their prey. Barn Owls do not digest such bones; they are vomited with hairs and feathers in pellets. This might be why we found no age-related bioaccumulation of Pb in tibiae.

Osborn (1979) investigated the seasonal changes in fat, protein, and metal load (Cd, Cu, Fe, and Zn) in the liver of Starling (*Sturnus vulgaris*.) He reported a sudden drop in the dry weight of liver just before moult, accompanied by a peak in Cd and Cu concentrations. However, if Cd and Cu were expressed as organ load, this peak was not observed. Our results for Cu confirm Osborn's observations, but no relationship was found for Cd.

Seasonal influences on heavy metals in Barn Owls are difficult to interpret. They reflect a combination of effects due to changes in diet and/or changes in physiology, related to reproduction and moult. As stated in the introduction, the diet might have an important impact on the transfer and accumulation of heavy metals in the Barn Owl apex of the food chain. Table 5 shows heavy metal concentrations observed in the Barn Owl (this study) and in their major food items (Ma 1989; Ma *et al.* 1991). The very large difference in organ metal load between voles and shrews reflects the difference in their average daily intake of Cd and Pb. Voles, being herbivores, feed mainly on grasses that hardly accumulate Cd or Pb. Shrews, being carnivores, feed mainly on earthworms, insects and their larvae, and spiders. Earthworms, the main food item of shrews, accumulate vast amounts of Cd and Pb (Denneman 1990; Ma *et al.* 1991). Note the differences in levels between polluted areas and control sites found in shrews and voles (Table 5). Another important fact is that Barn Owls need to catch at least two shrews to get the amount of food comparable to a vole. Clearly, a change in diet due to variation in vole densities between seasons and between years (see introduction) must have a major impact on the intake of heavy metals in Barn Owls. Only limited information is available about heavy metal levels in a few Barn Owls in relation to the heavy metal levels in their prey (Denneman and Hoven 1992; Denneman and Douben 1993). No time series on changes in diet related to intake of heavy metals and to levels of heavy metals in body compartments of the Barn Owl is currently available. With respect to our results, vole densities were high during 1992.

Table 5. Concentrations of lead and cadmium in kidney and liver, as indicator of heavy metals in the food chain of the Barn Owl in the Netherlands

	Species	Barn Owl		Shrews				Voles			
				polluted area		control site		polluted area		control site	
Metal organ		mg/kg DW	n	mg/kg DW	n	mg/kg DW	n	mg/kg DW	n	mg/kg DW	n
Pb kidney	median	0.94	42	269*	12	18.2*	10	15.8*	17	5.9*	16
	range	<0.78–10.6		49.0–1267		3.7–104		5.3–56.3		1.7–49.6	
Pb liver	median	<0.64	41	15.9*	12	2.2*	10	5.1*	17	2.8*	16
	range	<0.64–22.3		5.9–34.5		1.0–7.3		2.9–27.3		1.0–11.2	
Cd kidney	median	1.09	42	151**	32	29**	33	2.0**	51	0.19**	43
	range	<0.13–11.3		15–406		3.0–75		0.48–16		0.01–1.3	
Cd liver	median	0.55	41	200**	31	26**	33	3.8**	52	0.12**	44
	range	<0.07–3.1		16–541		2.1–66		0.07–9.8		0.06–0.23	

* From Ma (1989)
** From Ma *et al.* (1991)

Biomonitoring

The degree of heavy metal contamination in the Netherlands varies from unpolluted to relatively severely polluted areas. It is therefore important to continue biomonitoring to trace changes in the environment. Biomonitoring can show temporal changes, and the spatial distribution of the environmental load of micro-contamination. Barn Owls with higher levels of Cd and Pb expressed as organ load were found in contaminated areas.

Ongoing analyses of the Barn Owl and other bird species, such as those investigated by Hontelez *et al.* (1992), may provide an estimate of the extent and nature of accumulation of heavy metals at the end of food chains in different parts of the Netherlands. From our results, it is clear that biomonitoring heavy metals at the end of a food chain entails the understanding of different sources of variation found in concentrations and loads in target organs. This is why it is necessary to understand the behaviour of the individual metals and to know the ecology of the species involved.

Diet composition of the Barn Owl is not complicated. Because of its diet, Pb intoxication by consuming hunter or poacher killed or crippled prey is unlikely. For the same reason, however, rodenticides and crop protection agents are likely to affect Barn Owls. Switching from vole-dominated to a more shrew-dominated diet must have a major impact on the transfer of heavy metals, especially in polluted areas. A time series spanning several years is not yet available. In cooperation with the Barn Owl Working Group, between years, between seasons, and geographical variation in diet composition could be determined by analyzing pellets from breeding sites. Mice censuses, with emphasis on voles, are currently being taken in various areas in the Netherlands. Thus, samples can be collected for determination of heavy metal loads in different mice species of different geographical origin. Only after a long time series can the characteristics of the Barn Owl be assessed regarding biomagnification and bioaccumulation of heavy metals or other ecocontaminants. The findings for Pb illustrate the influence of bioavailability. An intriguing question is how the Barn Owl copes with a large intake of heavy metals (Table 5) when nutrient reserves are low.

In biomonitoring, the sample taken from the population should be representative. This study was conducted exclusively on Barn Owls found dead; therefore, the sample might be biased by comparison with the living population. Most of the birds sent in had a road accident as primary cause of death. There are no indications that a disproportional part of the road kills of owls and birds of prey are in poor condition or diseased (Smith *et al.* 1987; Newton *et al.* 1991; this study). Over the past thirty years, road kills constitute 40% of the dead Barn Owl ring recoveries (R. Wassenaar, personal communication), so road kills probably form a reasonable sample of the existent population. Our study shows that even birds with exhaustion (disease) as the primary cause of death form a usable sample to study contaminants of which the organ load (g) is an important parameter.

Based on this assessment of the value of the Barn Owl as a biomonitor, we recommend biomonitoring for at least a decade, a time span long enough to include two vole cycles. The Barn Owl is on the Dutch list of protected species, and a Species Recovery Plan is being set up (Osieck 1986; van der Hut *et al.* 1992). Continued biomonitoring with this bird could usefully be incorporated in the plan to rehabilitate the Barn Owl. It would also be interesting to investigate rodenticide levels in the Barn Owl in the Netherlands. Determination of heavy metals to moult feathers could enlarge the Barn Owl sample (see also Denneman and Douben, 1993). For biomonitoring, it would be worthwhile to continue measuring Cd, Cu, and Pb and to extend the scope to other heavy metals such as Hg, Al, and Zn.

Acknowledgments. The authors thank Lieske van der Klip for her help with the autopsies. GertJan de Graaf and Aura Widjaja of the DLO-Central Veterinary Institute optimized the heavy metal analysis. Wil Bax, Leonard Holleman, Christa Maätita, Irma Roksnoer, and Rinse Wassenaar of the Ringing Station (Netherlands Institute of Ecology) conscientiously channelled all reported carcasses of ringed raptors to DLO-Central Veterinary Institute and provided us with the recovery data. Nelly Eck, Willem van Hal, Barbara van Oosten, and Jacob Hogendorf of the Laboratory of Plant Ecology (University of Groningen) and our colleagues of the Zoological Laboratory bravely endured the smell of the carcasses throughout the year. The students Frank Rijnierse, Yvo Muller, Roel Modderman, Gerwin Koopmans, Rolf Ahlers, Inge Palm, Rogier van Oosten, and Karen de Boer helped with collecting the data. Gerrit Hartman and Jouke Franke of the Department of Biology (University of Groningen) supplied us with the additional equipment needed. We gratefully acknowledge the help of those who took the pains to send the carcasses found to the Veterinary Institute. Dick Visser created the figures. Finally, we thank Arie van Noordwijk, Wim Denneman, Nani Stam, Jelte van Andel, Rudi Drent, Maurien Butter, and Joy Burrough for their useful comments on the manuscript. This study was supported by a seed grant from the University of Groningen to H.E.

References

Aaseth J, Norseth T (1986) Copper In: Friberg L, Nordberg GF, Vouk V (eds) Handbook on the toxicology of metals, II, 2nd edition. Elsevier, Amsterdam, pp 233–254

Adrian WJ, Stevens ML (1979) Wet versus dry weight for heavy metal toxicity determinations in duck liver. J Wildlife Diseases 15:125–126

Anonymous (1990) In volle vlucht. Het ringwerk in Nederland; analyse en beleid. [Bird banding in the Netherlands: Analysis and policy.] Royal Dutch Academy of Sciences, Heteren/Amsterdam, pp 31

Baars AJ, Over H eds (1989) Wild bird mortality in the Netherlands, 1975–1989. DLO-Central Veterinary Institute, Lelystad and Netherlands Society for Protection of Birds, Zeist

Blomqvist S, Frank A, Petersson LR (1987) Metals in liver and kidney tissues of autumn-migrating dunlin *Calidris alphina* and curlew sandpiper *Calidris ferruginea* staging at the Baltic Sea. Marine Ecology Progress Series 35:1–13

Braaksma S, de Bruijn O (1976) De kerkuilstand in Nederland. [New data on Barn Owls *Tyto alba* in the Netherlands.] (with English summary) Limosa 49:135–187

Bunn DS, Warburton AB, Wilson RDS (1982) The Barn Owl. T and AD Poyser, Calton

Canters KJ, de Snoo GR (1993) Chemical threat to birds and mammals in the Netherlands. Review Environ Contam Toxicol 130:1–29

Cheney MA, Hacker CS, Schroder GD (1981) Bioaccumulation of lead and cadmium in the Louisiana Heron *(Hydranassa tricolor)* and the Cattle Egret *(Bubulcus ibis)*. Ecotoxicol Environ Safety 5:211–224

Clausen B, Elvestad K, Karlog O (1982) Lead burden in Mute Swans from Denmark. Nordic Veterinary Medicine 34:83–91

Connors PG, Anderli VC, Risebrough RW, Gilbertson M, Hays H (1975) Investigations of heavy metals in Common Tern populations. Canadian Field-Naturalist 89:157–162

Cook JA, Marconi EA, Di Lunzio NR (1974) Lead, cadmium, endotoxin interaction: Effect on mortality and hepatic function. Toxicol Applied Pharmacol 28:292–302

Cosson RP (1989) Relationships between heavy metal and metallothionein-like protein levels in the liver and kidney of two birds: The Greater Flamingo and the Little Egret. Comparative Biochem Physiol 94C:243–248

Custer TW, Franson JC, Pattee OH (1984) Tissue lead distribution and hematological effects in American Kestrels (*Falco sparverius* L.) fed biologically incorporated lead. J Wildlife Diseases 20:39–43

de Bruijn O (1979) Voedseloecologie van de Kerkuil *Tyto alba* in Nederland. [Food ecology of the Barn Owl in the Netherlands.] (with English summary) Limosa 52:91–154

de Jong J (1980) De Kerkuil in Friesland. [Barn Owl in the province of Friesland.] (with English summary) Limosa 53:142–144

de Jong J (1983) De Kerkuil. [The Barn Owl] Kosmos, Utrecht

de Jong J (1989) Aantal broedparen Kerkuil neemt toe. [Number of breeding pairs of the Barn Owl is increasing.] Vogels 52(9):182–183

de Jong J (1991a) Protection et recherches sur la consommation alimentaire et le bilan énergétique chez la Chouette Effraie, *Tyto alba*. [Protection and study of diet and energy balance of the Barn Owl] In: Juillard M, Bassin P, Baudvin H, Génot JC, Ravussin PA, Rebetez C (eds) Rapaces nocturnes; Actes du 30^{e} Colloque interrégional d'ornithologie Porrentruy (Suisse), novembre 1990. Nos Oiseaux: Société romande pour l'étude et al protection des oiseaux, Prangins, pp 109–122

de Jong J (1991b) Aantal broedparen van de Kerkuil boven de duizend. [More than one thousand breeding pairs of the Barn Owl] Bull. Society for the Protection of Birds *(Nieuwsbrief Kerkuil 1991)* 4(3):3

de Jong J (1992) Bull. Society for the Protection of Birds *(Nieuwsbrief Kerkuil 1992)* 5(2):3–12

de Voogt P, van Hattum B, Feenstra JF, Copius Peereboom JW (1980) Exposure and health effects of Cadmium. Toxicol Environ Reviews 3:89–109

DeMent SH, Chisolm JJ, Barber JC, Strandberg JD (1986) Lead exposure in an "urban" Peregrine Falcon and its avian prey. J Wildlife Diseases 22:238–244

Denneman WD (1990) A comparison of the diet of two *Sorex araneus* populations under different heavy metal stress. Acta Theriologica 35:25–38

Denneman WD, Hoven M (1992) Ecosystem transfer of Cadmium and effects of the central food chain of Insectivora. In: Teller A, Mathy P, Jeffers JNR Responses of forest ecosystems to environmental changes. Elsevier, NY, pp 612–615

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

Diem K (1982) *Wissenschaftliche Tabellen Geigy* Teilband Statistik, 8.Auflage, Basel.

Di Giulio RT, Scanlon PF (1984) Heavy metals in tissues of waterfowl from the Chesapeake Bay, USA. Environmental Pollution Series A 35:29–48

Dijkstra C, Daan S, Meijer T, Cavé AJ, Foppen RPB (1988) Daily and seasonal variations in body mass of the Kestrel in relation to food availability and reproduction. In Dijkstra C: Reproductive tactics in the Kestrel *Falco tinnunculus,* and study in evolutionary biology (PhD thesis, University of Groningen). Van Denderen B.V., Groningen pp 15–39

Elinder CG (1986) Iron In: Friberg L, Nordberg GF, Vouk V (eds) Handbook on the toxicology of metals, II, 2nd edition. Elsevier, Amsterdam, pp 276–297

Elvestad K, Karlog O, Clausen B (1982) Heavy metals (copper, cadmium, lead, mercury) in Mute Swans from Denmark. Nordic Veterinary Medicine 34:92–97

Ernst WHO (1991) Zware metalen, biomonitoring. [Heavy metals, biomonitoring.] In: Straalen NM van, Verkleij JAC (eds) Leerboek Oecotoxicologie [Textbook Ecotoxicology.] VU Uitgeverij Amsterdam pp 15 & 321

Friberg L, Kjellström T, Nordberg GF (1986) Cadmium. In: Friberg L, Nordberg GF, Vouk V (eds) Handbook on the toxicology of metals, II, 2nd edition. Elsevier, Amsterdam, pp 130–184

Glutz von Blotzheim UN, Bauer KM (1980) Handbuch der Vögel Mitteleuropas. Akademische Verlagsgesellschaft, Wiesbaden pp 234–276

Goede AA, de Voogt P (1985) Lead and cadmium in waders from the Dutch Wadden Sea. Environmental Pollution Series A 37:311–322

Graveland J (1990) Effects of acid precipitation on reproduction in birds. Experientia 46:962–970

Hardy AR, Hirons GJM, Stanley PI (1981) The relationship of body weight, fat deposits and moult to the reproductive cycles in wild Tawny Owls and Barn Owls. In: Cooper JE, Greenwood AG (eds) Recent advances in the study of raptor diseases. Chiron Publications, Keighley, pp 159–163

Honda K, Lee DP, Tatsukawa R (1990) Lead poisoning in swans in Japan. Environ Pollut 65:209–218

Honda K, Min BY, Tatsukawa R (1986) Distribution of heavy metals and their age-related changes in the Eastern Great White Egret, *Egretta alba modesta,* in Korea. Archives Environ Contam Toxicol 15:185–197

Honer MR (1963) Observations on the Barn Owl *(Tyto alba guttata)* in the Netherlands in relation to its ecology and population fluctuations. Ardea 51:158–195

Hontelez LCMP, van den Dungen HM, Baars AJ (1992) Lead and cadmium in birds in the Netherlands: A preliminary survey. Archives Environ Contam Toxicol 23:453–456

Howarth DM, Hulbert AJ, Horning D (1981) A comparative study of heavy metal accumulation in tissues of the Crested Tern, *Sterna Bergii,* breeding near industrialized and non-industrialized areas. Aust Wildl Res 8:665–672

Hulse M, Mahony JS, Schroder GD, Hacker CS, Pier SM (1980) Environmentally acquired lead, cadmium, and manganese in the Cattle Egret, *Bulbulcus ibis,* and the Laughing Gull, *Larcus atricilla.* Archives Environ Contam Toxicol 9:65–78

Hutton M (1981) Accumulation of heavy metals and selenium in three seabird species from the United Kingdom. Environmental Pollution Series A 26:129–145

Hutton M, Goodman GT (1980) Metal contamination of feral pigeons *Columbia livia* from the London area; Part 1: Tissue accumulation of lead, cadmium and zinc. Environmental Pollution Series A 22:207–217

Karlog O, Elvestad K, Clausen B (1983) Heavy metals (cadmium, copper, lead and mercury) in Common Eiders *(Somateria mollissima)* from Denmark. Nordic Veterinary Medicine 35:448–451

Koller LD, Brauner JA (19797) Decreased β-lymphocyte response after exposure to lead and cadmium. Toxicol Applied Pharmacol 42:621–624

Krasowski M, Doelman P (1990) Lood in milieu en voeding in Nederland. [Lead in the environment and in the food of the Netherlands.] Ministry of Housing, Physical Planning and Environment, The Hague

Lee DP, Honda K, Tatsukawa R, Won PO (1989) Distribution and residue level of mercury, cadmium and lead in Korean birds. Bulletin Environ Contam Toxicol 43:550–555

Locke LN, Bagley GE, Frickie DN, Young LT (1969) Lead poisoning and aspergillosis in an Andean Condor. JAVMA 155:1052–1056

Lumeij JT, Westerhof I, Smit T, Spierenburg TJ (1991) Diagnosis and treatment of poisoning in raptors from the Netherlands: Clinical case reports and review of 2,750 postmortem cases, 1975–1988. In: Raptor Biomedicine, Eds Redig PT, Cooper JE, Remple JD, Hunter DB, Hahn T, University of Minnesota Press, Minneapolis, pp 233–238

Ma WC (1989) Effect of soil pollution with metallic lead pellets on lead bioaccumulation and organ/body weight alterations in small mammals. Arch Environ Contam Toxicol 18:617–622

Ma WC, Denneman WD, Faber JH (1991) Hazardous exposure of ground-living small mammals to cadmium and lead in contaminated terrestrial ecosystems. Arch Environ Contam Toxicol 20:266–270

Mayack LA, Bush PB, Fletcher OJ, Page RK, Fendley TT (1981) Tissue residues of dietary cadmium in Wood Ducks. Arch Environ Contam Toxicol 10:637–645

Moriarty F (1988) Ecotoxicology, The study of pollutants in ecosystems. second edition. Academic Press, London pp 203

Newton I, Wyllie I, Asher A (1991) Mortality causes in British Barn Owls *Tyto alba,* with a discussion of aldrin-dieldrin poisoning. Ibis 133:162–169

Nordberg GF (1972) Cadmium metabolism and toxicity. Experimental studies on mice. Environ Physical Biochem 2:7–36

Nordberg GF, Kjellström T, Nordberg M (1985) Chapter 6 In: *Cadmium and Health. A toxicological and epidemiological appraisal.* Friberg L, Elinder CG, Kjelström T, Nordberg GF (eds) CRC Press, Boca Raton, FL

Osborn D (1979) Seasonal changes in the fat, protein and metal content of the liver of the Starling *Sturnus vulgaris.* Environ Pollut 19:145–155

Osborn D, Harris MP, Nicholson JK (1979) Comparative tissue distribution of mercury, cadmium and zinc in three species of pelagic seabirds. Comparative Biochem Physiol 64C:61–67

Osieck ER (1986) Bedreigde en karakteristieke vogels in Nederland. [Threatened and characteristic birds in the Netherlands.] (with English summary) Netherlands Society for Protection of Birds, Zeist

Piechocki R (1960) Über die Winterverluste der Schleiereule (Tyto alba). Vogelwarte 20:274–280

Piersma T (1984) Estimating energy reserves of Great Crested Grebes *Podiceps cristatus* on the basis of body dimensions. Ardea 72:119–126

Piersma T, Davidson N, Evans P (1984) Estimation of the protein reserves of waders: The use and misuse of standard muscle volume. Wader Study Group Bulletin 42:19–22

Reid M, Hacker CS (1982) Spatial and temporal variation in lead and cadmium in the Laughing Gull, *Larus atricilla.* Marine Pollut Bull 13:387–389

Saric M (1986) Manganese In: Friberg L, Nordberg GF, Vouk V Handbook on the toxicology of metals, II, 2nd edition. Elsevier, Amsterdam, pp 354–386

Sawicka-Kapusta K, Kozlowski J, Sokolowska T (1986) Heavy metals in Tits from polluted forests in Southern Polland. Environmental Pollution series A 42:297–310

Sauter U (1956) Beitrage zur Okologie der Schleiereule (Tyto alba) nach den Ringfunden. Vogelwarte 18:109–151

Scanlon PF (1982) Wet and dry weight relationships of Mallard *(Anas platyrhynchos)* tissues. Bulletin Environ Contam Toxicol 29:615–617

Scharenberg W (1989) Schwermetalle in Organen und Federn von Graureihern *(Ardea cinera)* und Kormoranen *(Phalacrocorax carbo).* J Ornithol 130:25–33

Scheuhammer AM (1987) The chronic toxicity of aluminum, cadmium, mercury, and lead in birds: A review. Environ Pollut 46:263–295

Schönfeld M, Girbig G (1975) Beiträge zur Brutbiologie der Schleiereule, *Tyto alba,* unter besonderer Berücksichtigung der Abhängigkeit von der Feldmausdichte. Hercynia 12:257–319

Shawyer CR (1987) The Barn Owl in the British isles: Its past, present and future. The Hawk Trust, London

Smit T, Bakhuizen T, Jonkers DA (1987) Doodsoorzaken van Torenvalken *Falco tinnunculus* in Nederland. [Causes of Kestrel death in the Netherlands.] (with English summary) Limosa 60:175–178

SOVON *Cooperating Organizations of Bird Research in the Netherlands* (1987) Atlas van de Nederlandse vogels. [Atlas of Dutch birds.] (with English legends), Arnhem pp 316–317

Spierenburg TJ, de Graaf GJ, Baars AJ, Brus DHJ, Tielen MJM, Arts BJ (1988) Cadmium, zinc, lead, and cooper in livers and kidneys of cattle in the neighborhood of zinc refineries. Environ Monitoring Assess 11:107–114

Stowe HD, Goyer RA, Krigman MM, Wilson M, Cates M (1973) Experimental oral lead toxicity in young dogs. Clinical and morphologic effects. Archives Pathol 95:106–116

Szefer P (1983) Investigations of trace metals in Long-tailed Duck (*Clangula hyemalis* L.) from the Gdansk Bay. Science Total Environ 29:269–276

Taylor TG (1970) The role of the skeleton in eggshell formation. Ann Biol Anim Biochem Biophys 26:273–283

Texeira RM (1979) Atlas van de Nederlandse Broedvogels. [Atlas of breeding birds in the Netherlands.] De Lange van Leer BV, Deventer, pp 194–195

Trust KA, Miller MW, Ringelman JK, Orme IM (1990) Effects of ingested lead on antibody production in Mallards *(Anas platyrhynchos).* J Wildlife Diseases 26:316–322

Tsuchiya K (1986) Lead In: Handbook on the toxicology of metals, Volume II, Friberg L, Nordberg GF, Vouk VB (eds) Elsevier, Amsterdam, pp 298–353

Vallee BL, Ulmer DD (1972) Biochemical effects of mercury, cadmium, and lead. Annual Review Biochem 785:91–128

van Beek H, Greefkes HCA, Baars AJ (1987) Determination of copper, iron, manganese, lead and cadmium in automatically wet-digested animal tissue by graphite-furnace atomic-absorption spectrometry with Zeeman background correction. Talanta 34:580–582

van Bon J, Boersema JJ (1984) De belasting van de Nederlandse bodem door metallisch lood. [The loading of metallic lead in Dutch soil.] Landschap 4:282–289

van der Hut RMG, de Jong J, Osieck ER (1992) Biologie en bescherming van de kerkuil *Tyto alba*: Aanzet tot het beschermingsplan. [Biology and conservation of the Barn Owl *Tyto alba*: Towards a conservation plan.] (with English summary) Technical Report #7, Netherlands Society for Protection of Birds, Zeist

van Straalen NM (1988) Ecotoxicologische theorievorming over opname, effecten en doorgifte van stoffen in dierpopulaties. [Development of an ecotoxicological theory on uptake, effects and transfer of pollutants in animal populations.] Milieu 2:40–45

van den Tempel MW (1993) Vogelslachtoffers door het wegverkeer [Bird victims of road traffic.] (with English summary). Technical Report #11, Netherlands Society for the Protection of Birds, Zeist pp 51–72

Wiemeyer SN, Schmeling SK, Anderson A (1987) Environmental pollutant and necropsy data for Ospreys from the eastern United States, 1975–1982. J Wildlife Diseases 23:279–291

Wijnandts H (1984) Ecological energetics of the Long-eared Owl *Asio otus*. Ardea 72:1–92