

# Evaluation of the usefulness of bird feathers as a non-destructive biomonitoring tool for organic pollutants: A comparative and meta-analytical approach

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## Abstract:

In this study, we investigated whether bird feathers can be used as a non-destructive biomonitor for organic pollutants. We analysed the outermost tail feathers of 8 terrestrial and aquatic bird species from Belgium (8 species,  $n=108$ ) for polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (OCPs). Every compound class could be quantified in one single tail feather of the birds under study (sum PCBs ranging from 5.5 to 510 ng/g feather, sum PBDEs from 0.33 to 53 ng/g feather, sum DDTs from 1.5 to 730 ng/g feather), except for PBDEs in feathers of the common moorhen (*Gallinula chloropus*). Further, we calculated Pearson correlations between concentrations of organic pollutants in feathers and concentrations in corresponding muscle or liver tissue from the birds. Correlations were found significant in half of the cases of the terrestrial species, but were found not significant for the aquatic species, with the exception of a significant correlation of sum PCBs in the common moorhen. Only for the common buzzard (*Buteo buteo*) ( $n=43$ ) all correlations were found significant ( $0.32 < r < 0.77$ ). In order to cope for low statistical power, we performed a meta-analysis on all bird species together. This led to significant correlations between levels in feathers and corresponding levels in muscle or liver for all terrestrial birds ( $p < 0.05$  in all cases, effect size 0.59 ( $p,p'$ -DDE) to 0.71 ( $\sum$  PCB) for levels in feather and muscle). When correlations were recalculated excluding the birds that had died due to starvation, correlation coefficients for the terrestrial birds were found even higher (effect size up to 0.83 ( $\sum$  PCB)). These results have important implications for non-destructive and retrospective biomonitoring. Although our results suggest that exact concentrations in the body cannot be predicted using feathers, bird feathers can give a good estimate of contamination levels in a population and as such are a potential non-destructive biomonitoring tool for organic pollutants.

Outermost tail feathers and muscle tissue were also examined for nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ) stable isotope content in the different bird species. However,  $\delta^{15}\text{N}/\delta^{13}\text{C}$  signatures in feather or muscle were not significantly correlated with the corresponding levels of organic pollutants in these tissues. Various confounding factors (such as habitat, condition, age, sex) may have masked a relationship between  $\delta^{15}\text{N}$  values and organic pollutant levels in the birds under study.

## Keywords:

Feathers; Biomonitoring; Polybrominated diphenyl ethers; Polychlorinated biphenyls; Organochlorine pesticides; Meta-analysis; Stable isotopes

## 1. Introduction

Organohalogenated pollutants, such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and polybrominated diphenyl ethers (PBDEs), form an important group of environmental contaminants. They are widespread in the environment, have a high persistency, accumulate in biological tissues and biomagnify within food chains (Kunisue et al., 2003). While levels of PCBs and  $p,p'$ -dichlorodiphenyl-trichloroethane (DDT) are decreasing due to restrictions and legislation (Jones and De Voogt, 1999), levels of lower brominated PBDE congeners are still prominently present in the environment (Thomas et al., 2006), even though recent restrictions on the production and use of the penta- and octa-BDE mixtures have been declared in the European Union (Directive EEC, 2003). PBDEs are employed worldwide in consumer products (such as computers, TV casings and car interiors) as flame retardants (WHO, 1994). Due to bioaccumulation, high concentrations can be found in top predators, including predatory birds (Law et al., 2003; Lindberg et al., 2004; Jaspers et al., 2006a; Voorspoels et al., 2006).

Birds have extensively been used in the past as biomonitors of environmental contamination with persistent organic pollutants (Herzke et al., 2003; Lindberg et al., 2004; Walker et al., 2001). They are situated high on the food chain, thus accumulating high levels of organohalogenated pollutants, and they are sensitive to environmental changes (Furness, 1993). The amount of organohalogenated pollutants accumulating within the tissues of birds is related to their diet and corresponding trophic position (Borgå et al., 2004; Naso et al., 2003), but also to differences in accumulation among habitats and ecosystems (marine versus terrestrial).

Because practical and ethical reasons inhibit the sacrifice of free-living animals, methods for non-destructive biomonitoring have been developed (Furness, 1993). The use of hair, a keratinous tissue, has recently been evaluated as a method for the analysis of persistent organic pollutants (Zupancic-Kralj et al., 1981; Dauberschmidt and Wennig, 1998; Covaci et al., 2002; Altshul et al., 2004; D'Havé et al., 2005). Since feathers are composed of a keratinous matrix as well, they are potentially useful to study contamination with organic pollutants. In contrast to hair, which is continuously growing, feathers grow only for a certain period of time and are connected to the blood stream (and its circulating pollutants) only during this limited time period (Jaspers et al., 2004). While many biomonitoring studies on organic pollutants have previously focused on bird eggs, feathers have the advantage that they can be collected irrespective of season, age or sex. Moreover, since one feather can easily be removed from a living bird without causing severe damage, this technique could be valuable as a non-destructive biomonitoring tool for endangered species. Bird feathers have been used previously for monitoring heavy metals in numerous studies, but the use of feathers as monitors of organic pollutants has only recently been investigated (Dauwe et al., 2005; Jaspers et al., 2006b; Van den Steen et al., in press). The study of Dauwe et al. (2005) was performed on a small passerine bird (*Parus major*) and although concentrations of most PCBs and DDTs could be quantified, levels of other OCPs and PBDEs could not be measured in the feathers due to generally low levels in passerine species. In addition, given the large amount of feathers needed for analysis, feather sampling could not be considered non-destructive for passerine birds. Therefore, more recently, a study was performed on a predatory bird species, the common buzzard (*Buteo buteo*) with a high position on the food chain (Jaspers et al., 2006b). One tail feather of a buzzard was found to be sufficient to quantify concentrations of most PCBs, PBDEs and DDTs. Furthermore, significant correlations were found between levels of organic pollutants in feathers and muscle or liver tissue. Therefore, feathers of common buzzards, and probably other predatory birds, are useful as a nondestructive biomonitor for contamination with organic pollutants (Jaspers et al., 2006b). In the study of Van den Steen et al. (in press) European starlings (*Sturnus vulgaris*) were exposed to PCB 153 by silastic implants and levels were compared in feathers before and after (regrown feathers) the exposure experiment. The former study provided the first experimental evidence that feathers might be useful as a non-destructive biomonitoring tool for PCBs (Van den Steen et al., in press).

Different bird species belong to different ecosystems and food chains, occupying different trophic positions within these food chains. To define these species characteristics, stable isotope ratios of carbon ( $^{13}\text{C}/^{12}\text{C}$ ) and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) can be used as integrators and tracers of ecological processes (Robinson, 2001). Carbon isotopic ratio may define an isotopic signature for the different primary producers of an ecosystem, allowing, for example, the distinction between marine and terrestrial food chains. On the other hand, nitrogen isotopic ratio offers the possibility of estimating the trophic level of organisms, because the  $\delta^{15}\text{N}$  value of animal tissues is generally positively related with trophic position (Hobson and Welsh, 1992). However, this  $\delta^{15}\text{N}$  enrichment is variable; it is often diet-related and varies among different food chains (Mac Cutchan et al., 2003; Vanderklift and Ponsard, 2003). The  $\delta^{15}\text{N}$  signature of primary producers of a food chain is dependent of inorganic nitrogen sources, and is therefore also potentially useful for distinction between terrestrial and marine food sources. Previously, stable isotopes analysis of feathers has been reported to allow insights into the feeding ecology of marine and terrestrial birds (Smith et al., 2003).

In this study we report levels of organohalogenated pollutants (PCBs, OCPs and PBDEs) in a single tail feather of eight bird species (both terrestrial and aquatic) collected in Flanders, Belgium. We examine correlations between levels of these organic pollutants in feathers with those in corresponding muscle and liver tissue (data previously reported by Jaspers et al., 2006a; Voorspoels et al., 2006) of these different bird species. As these levels are often related to the diet, we use stable isotopes as trophic tracers by measurements of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in feathers and muscles.

## 2. Materials and methods

### 2.1. Collection of samples

Between October 2003 and June 2004, 108 carrions of 8 different bird species (9 grey herons-*Ardea cinerea* (aquatic), 12 herring gulls-*Larus argentatus* (aquatic), 11 common moorhens-*Gallinula chloropus* (aquatic), 9 barn owls-*Tyto alba* (terrestrial), 10 long-eared owls-*Asio otus* (terrestrial), 43 common buzzards-*B. buteo* (terrestrial), 3 kestrels-*Falco tinnunculus* (terrestrial) and 11 sparrowhawks-*Accipiter nisus* (terrestrial)) were collected in collaboration with Wildlife Rescue Centres (WRCs) in Flanders (Vogel-bescherming Vlaanderen vzw, Belgium, <http://www.vogelbescherming.be>). Fellow workers of the WRCs of Oostende, Opglabbeek, Merelbeke, Malderen and Herenthout carried out the sampling of the birds, with the permission of the Flemish Department of Environment and Infrastructure. The birds were found dead or had died shortly after collection. Frequent causes of death included traffic accidents, natural causes and starvation. No birds were killed for the purpose of this study. We collected the outermost tail feathers from the birds and stored them in paper envelopes until further analysis, which is described below. One feather was used for analysis of organic pollutants, the other was used to determine the stable isotope ratios (see below). Liver and pectoral muscle were excised and stored at -20 °C until sample preparation. The results of the internal tissue analysis are reported elsewhere (Jaspers et al., 2006a; Voorspoels et al., 2006).

### 2.2. Analysis of organic pollutants

In all samples, 7 PBDE congeners (28, 47, 99, 100, 153, 154, and 183), brominated biphenyl (BB) 153, 25 PCB congeners (18, 28, 52, 74, 95, 99, 101, 105, 110, 118, 128, 132, 138, 149, 153, 156, 167, 170, 177, 180, 183, 187, 194, 196 and 199), hexachlorobenzene (HCB), chlordanes (cz's-chlordane (CC), *trans*-chlordane (TC), *trans*-nonachlor and oxychlordane, expressed here as CHLs), hexachlorocyclohexanes ( $\alpha$ -,  $\beta$ -,  $\gamma$ -HCHs) and *p,p'*-DDT and metabolites (*p,p'*-DDE and *p,p'*-DDD), expressed here as DDTs, were analysed.

Left outermost tail feathers were washed with distilled water, dried at room temperature and cut in pieces of ~ 1 mm. Depending on the species, 100 to 500 mg of the feathers was weighed and incubated overnight at 40 °C with hydrochloric acid (4 N) and a mixture of hexane and dichloromethane (4:1, v:v). After liquid extraction, clean-up was performed on acidified silica, as described by Dauwe et al. (2005).

For PBDEs and OCPs, analysis was done using a gas chromatograph equipped with a HT-8 capillary column (25 m x 0.22 mm x 0.25  $\mu$ m), linked to a mass spectrometer (GC/MS) operated in electron capture negative ionisation (ECNI) mode. PCBs were analysed using a GC/MS equipped with a DB-1 capillary column (30 m x 0.25 mm x 0.25  $\mu$ m), operated in electron ionisation (EI) mode. Limits of quantification (LOQ) were calculated as three times the standard deviation of the analyte values in procedural blanks. For analytes that were not detected in procedural blanks, LOQs were calculated for a signal-to-noise ratio equal to 10. LOQs for the analysed compounds ranged between 0.1 and 0.4 ng/g feather.

### 2.3. Stable isotope measurements

Right outermost tail feathers were washed with a mix of chloroform/ methanol (2:1, v:v) and dried at 50 °C for 48 h. Afterwards, the feathers were cut into small pieces and ground with a micro grinder. Muscle samples were also dried at 50 °C (48 h) and subsequently ground into a homogenous powder. Nitrogen and carbon stable isotopes ratios were measured on a V. G. Optima (VG Instrument, UK) IR-MS coupled to an N-C-S elemental analyser (Carlo Erba, Italy). Stable isotope ratios were expressed as a delta ( $\delta$ ) in ‰ according to:

$$\delta X = \left( \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right) \times 1000,$$

where  $X$  is  $^{15}\text{N}$  or  $^{13}\text{C}$  and  $R$  is the corresponding ratio  $^{15}\text{N}/^{14}\text{N}$  or  $^{13}\text{C}/^{12}\text{C}$  of the sample or of a standard. International standards were vPDB (Vienna Peedee Belemnite) standard and atmospheric  $\text{N}_2$  for carbon and nitrogen, respectively. Reference materials were IAEA-N1 ( $\delta^{15}\text{N} = +0.4 \pm 0.2\text{‰}$ ) and IAEA-CH-6 (sucrose) ( $\delta^{13}\text{C} = -10.4 \pm 0.2\text{‰}$ ). Experimental precision (based on the standard deviation of replicates of an internal standard) was 0.3 and 0.4‰ for carbon and nitrogen, respectively.

## 2.4. Statistical analysis

All statistical analyses were performed using Statistica for Windows (Statsoft 1997, Tulsa, OK, USA), GraphPad InStat® version 3.06 for Windows (GraphPad Software Inc., San Diego, CA, USA) and Comprehensive Meta-Analysis version 1.025 (Biostat® 1998, New Jersey, USA). Samples with levels below the LOQ were assigned a value of  $p \times \text{LOQ}$ , with ' $p$ ' as the proportion of measurements with levels above the LOQ (Voorspoels et al., 2002). Compounds with over 50% of the measurements below the LOQ were excluded from statistical analysis (i.e. CBs 18, 28, 52, 74, 105, 132, 156 and 167,  $\alpha$ -HCH, TC, CC, BB 153 and  $p,p'$ -DDD). Data were not normally distributed (Shapiro-Wilks test,  $p > 0.05$ ) and were therefore log-transformed ( $\log_{10}(x+1)$ ) to meet normal distribution requirements. The level of significance was set at  $\alpha = 0.05$  throughout this study.

Parametric Pearson correlations were calculated between log-transformed concentrations of organic pollutants in feathers and in liver or muscle tissue, and also between log-transformed levels of organic pollutants and  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  values in feathers and muscle, respectively. Significant differences among correlation coefficients were investigated by performing a test for equality of two dependent correlations, as described by Steiger (1980). For each species, we presented the significant results after applying Bonferroni correction as additional information to the tables. However, these results need to be interpreted with caution seeing the fact that by reducing the type I error (Bonferroni correction), the chance of making a type II error is increased. This means that applying the Bonferroni correction could reduce the possibility of detecting significant results. For more discussion on this topic, we refer to Moran (2003); Nakagawa (2004) and Garamszegi (2006).

A meta-analysis was performed to investigate the overall relationship between levels of organic pollutants in feathers and levels in muscle or liver. While the number of publications using meta-analysis has increased dramatically in social and medical sciences, and also recently in evolutionary biology (Arnqvist and Wooster, 1995; Garamszegi and Eens, 2004), this method has up until now received little attention in (eco)toxicological research. Metaanalysis is the statistical procedure for combining data from multiple studies. When the treatment effect (or effect size) is consistent from one study to the next, meta-analysis can be used to identify this common effect. When the effect varies from one study to the next, meta-analysis may be used to identify the reason for the variation (Borenstein and Rothstein, 1999). Because of the limitations of a single study it has been recognized that data from multiple studies must be synthesized to yield more definitive results. Prior to the introduction of meta-analysis, this synthesis took the form of descriptive reviews, which were rather subjective and impossible to carry out for more than a few studies involved. Meta-analysis, by contrast, applies objective formulas to summarize data of any number of studies, by examining the generality of a relationship, taking sample size into account (Hedges and Olkin, 1985; Cooper and Hedges, 1994). The current meta-analysis was conducted to provide estimates of the general relationship between levels of organic pollutants in feathers and internal tissues of birds. It was based on the calculated Pearson correlation coefficients of the different bird species (Borenstein and Rothstein, 1999). By combining the different bird species into a meta-analysis, problems with low statistical power could be avoided. Analysis was performed together for all birds and separately for the aquatic and terrestrial species (analysis of variance for correlation, fixed effects,  $z$ -transformed; Borenstein and Rothstein, 1999). Then, we repeated this meta-analysis using the Pearson correlations based on the data excluding the birds killed by starvation. Tests for assumption of homogeneity within classes were not significant, except for CB 101 between feather and muscle tissue within the aquatic species. Deviation from homogeneity between classes (aquatic or terrestrial) was only significant for CB 101 and CB 149 between feather and muscle.

The concentration results for 43 common buzzards, previously reported in the study of Jaspers et al. (2006b), were also incorporated in this study in order to compare with other bird species and to include them in the meta-analysis. The data obtained for kestrels were not included in any of the statistical analysis since the sample size was too small (only 3 individuals).

## 3. Results and discussion

### 3.1. Levels and profiles in feathers

#### 3.1.1. Levels

Most organohalogenated pollutants could be measured in feathers of the bird species under study, except for the common moorhen that had non-detectable levels for PBDEs, CHLs and HCB (Table 1). PBDEs were quantified in all samples (except the common moorhen) with mean levels  $\pm$  95% confidence interval (CI) ranging from  $2.2 \pm 0.59$  to  $11 \pm 6.5$  ng/g feather. In general, mean levels of sum PBDEs were 10 to 40 times lower than levels of sum PCBs in feather samples. Sparrowhawks showed high levels of all analysed organic pollutants with mean

levels of sum DDTs (mean 230 ng/g feather) far above levels in the other species. Herring gulls showed highest PCBs levels (mean 220 ng/g), while OCPs levels in feathers were rather low in comparison to other predatory bird species (mean sum DDTs, HCHs, CHLs and HCB were 12, 1.1, 0.46 ng/g feather and not detected, respectively).

**Table 1:** Mean concentrations  $\pm$ 95% confidence interval (CI) of different organohalogenated compounds (ng/g) in outermost tail feathers of different bird species from Belgium (n=108)

Compound	Grey heron	Common moorhen	Herring gull	Barn owl	Long eared owl	Common buzzard	Sparrowhawk	Kestrel
N	9	11	12	9	10	43	11	3
Sum PCBs	91 $\pm$ 80	25 $\pm$ 14	220 $\pm$ 61	140 $\pm$ 73	120 $\pm$ 100	40 $\pm$ 11	160 $\pm$ 66	57 $\pm$ 70
Sum PBDEs	7.7 $\pm$ 9.5	n.d.	11 $\pm$ 6.5	3.8 $\pm$ 1.0	6.3 $\pm$ 4.0	2.2 $\pm$ 0.59	10 $\pm$ 8.7	4.4 $\pm$ 1.0
Sum DDTs	21 $\pm$ 10	12 $\pm$ 3.5	12 $\pm$ 4.0	48 $\pm$ 35	110 $\pm$ 140	9.4 $\pm$ 3.4	230 $\pm$ 88	110 $\pm$ 120
Sum HCHs	3.5 $\pm$ 1.5	2.4 $\pm$ 1.2	1.1 $\pm$ 0.25	5.3 $\pm$ 2.3	2.5 $\pm$ 1.7	1.9 $\pm$ 0.47	4.6 $\pm$ 1.5	1.2 $\pm$ 0.68
Sum CHLs	0.32 $\pm$ 0.28	n.d.	0.46 $\pm$ 0.23	2.5 $\pm$ 1.6	0.63 $\pm$ 0.44	2.6 $\pm$ 3.2	1.5 $\pm$ 0.54	2.1 $\pm$ 3.5
HCB	5.0 $\pm$ 4.6	n.d.	n.d.	1.1 $\pm$ 0.21	4.9 $\pm$ 3.3	0.70 $\pm$ 0.27	1.0 $\pm$ 0.20	2.9 $\pm$ 0.78

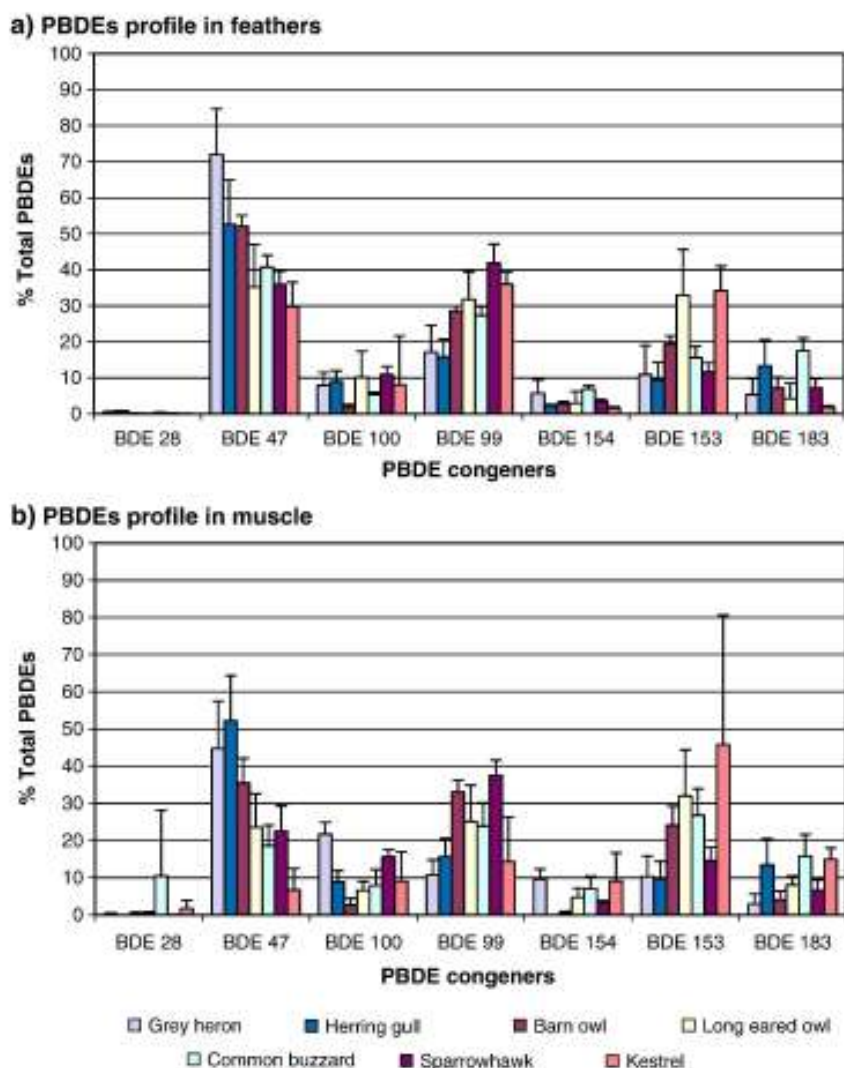
n.d., non detectable.

Because common moorhens feed on a diet with varying proportions of animal and plant material (Snow and Perrins, 1998), they had lower intake of organic pollutants and therefore showed lower accumulation compared to predatory birds. On the other hand, the diet of sparrowhawks consists almost entirely of small birds (up to 98%). The other terrestrial birds analysed in the present study mainly feed on small mammals and to a lesser extent small birds (Snow and Perrins, 1998). Small bird species feeding on seeds treated with seed dressings can accumulate pesticides, which could result in higher concentrations of pesticides in the predatory birds that preyed on them (Ratcliffe, 1993). This could explain the relatively high levels of organic contamination in sparrowhawks and the differences in accumulation compared to the other terrestrial predatory birds. The different accumulation profiles of herring gulls can possibly be explained by its very opportunistic feeding habits, consuming not only fish, but a variety of items, even garbage (Snow and Perrins, 1998). Furthermore, birds feeding mainly in aquatic environments are expected to show lower OCPs levels, than birds feeding in terrestrial environments where pesticides are commonly applied.

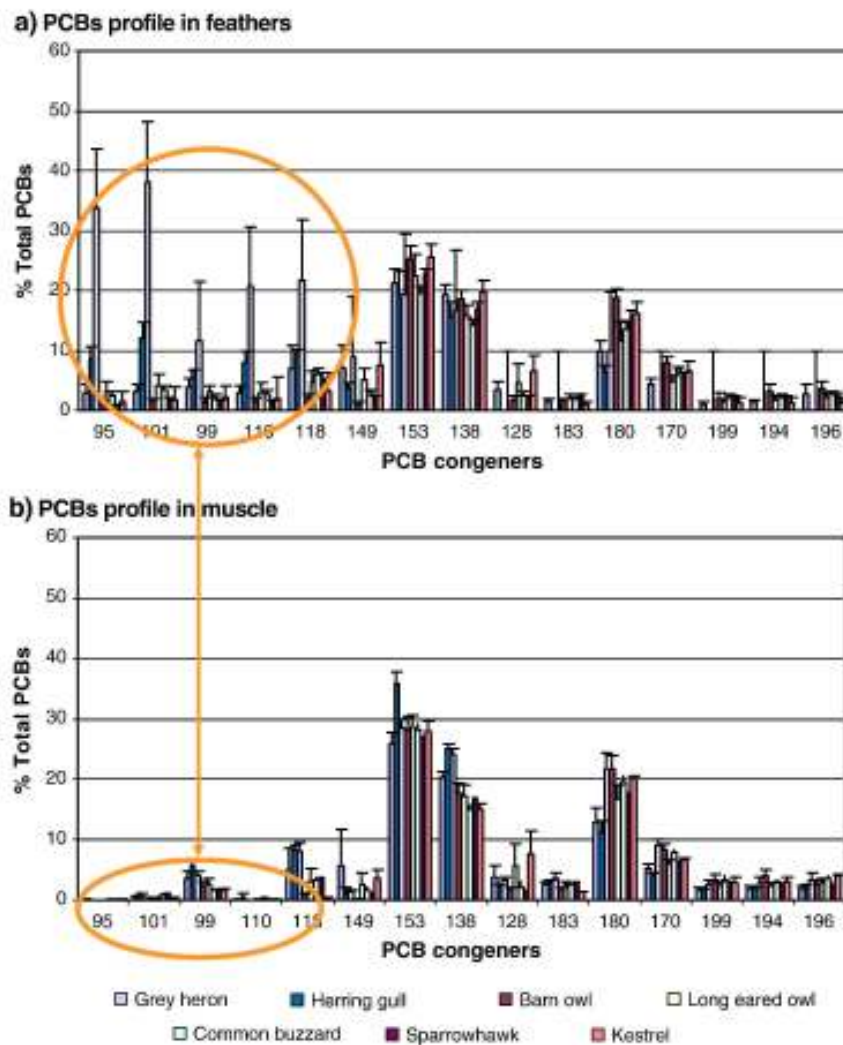
Dauwe et al. (2005) have reported concentrations of organic pollutants in feathers of a small passerine bird, the great tit. PBDEs, HCB and CHLs could not be detected in feathers of great tits, similar to the results for the common moorhen in the present study. Sum PCBs concentrations were similar (median 50 ng/g, range 28-87 ng/g) to those in the common moorhen (mean $\pm$ CI: 25 $\pm$ 14 ng/g, range 3.5-81 ng/g), while sum DDT concentrations were rather low (median 4.1 ng/g, range 2.3-11 ng/g), even lower than concentrations reported in the common moorhen (mean $\pm$ CI: 12 $\pm$ 3.5 ng/g, range 6.6-26 ng/g). Overall, concentrations measured in the birds of this study (not including the common moorhen) were higher than concentrations measured by Dauwe et al. (2005). Moreover, all organohalogenated pollutants (even PBDEs, HCB and CHLs) could be quantified in one single feather of the predatory birds under study. Therefore feathers of predatory birds seem more suitable as an assessment tool for environmental levels of organic pollutants, than feathers of small passerine birds or common moorhens.

Data on the corresponding concentrations in the tissues of the studied birds are published elsewhere (Jaspers et al., 2006a; Voorspoels et al., 2006). Concentrations measured in the feathers were 100 to 1000 times lower than levels reported in liver and muscle tissue of these birds. Levels reported in liver and muscle tissue of kestrels were low compared to the other terrestrial bird species. However, levels measured in the feathers of kestrels were in the same range as levels in the feathers of the other predatory birds (Table 1). Moreover, levels of DDTs measured in the tissues of kestrels were low (median sum DDTs 2700 and 4500 ng/g lipid in liver and muscle, respectively), while levels in feathers (mean sum DDTs 110 ng/g) were relatively high compared to the other species. The limited number of samples (3 kestrels) was, however, prohibiting achievement of clear-cut conclusions.

**Fig. 1:** Percentage contribution of selected PBDE congeners to the total PBDE load in feathers (a) and in muscle tissue (b) of different aquatic and terrestrial birds from Belgium. Error bars represent 2 SE.



**Fig. 2:** Percentage contribution of selected PCB congeners to the total PCB load in feathers (a) and in muscle tissue (b) of different aquatic and terrestrial birds from Belgium. Error bars represent 2 SE.



### 3.1.2. Profiles

Since no significant differences in profiles have previously been found between liver and muscle tissue (Jaspers et al., 2006a; Voorspoels et al., 2006), results below are only discussed with profiles in muscle tissue.

A different PBDE profile could be observed between feathers and muscle tissue in the birds under study (Fig. 1). BDE 47 was the most prominent congener in feathers of most bird species, while this congener had a lesser contribution in muscle tissue, for which BDE 99 and 153 were more important in most bird species. BDE 47 had the highest contribution in the aquatic species (i.e. heron and gull), in feathers as well as in muscles, while the contribution of BDE 99 and 153 increased towards the terrestrial species (Fig. 1). Concentrations of BDE 183 in heron were low, but concentrations in herring gull were similar to levels measured in terrestrial birds of prey.

It has been suggested for PBDEs that birds feeding in terrestrial environments are more exposed to the higher brominated BDE congeners than aquatic species (Law et al., 2003; Jaspers et al., 2006a). Moreover it is assumed that the octa-mix may be more widespread in terrestrial than in aquatic environments (Law et al., 2003). This could be different for the herring gull, since this omnivorous bird is feeding on aquatic prey as well as food found on the shore (Snow and Perrins, 1998), which could explain the deviating profile found for the herring gull in this study.

The PCB profile also differed between feathers and muscle tissue (Fig. 2). Less persistent chlorinated PCB congeners (i.e. CB 95, 101 and 110) had a relatively higher contribution in feathers than in muscle tissue. Contributions of these congeners were particularly high in feathers of the common moorhen. Earlier research has

also indicated that the percentage of lower chlorinated congeners (tetra- and penta-CB congeners), including less persistent congeners, measured in feathers is higher than in internal tissues (Dauwe et al., 2005).

The different PBDE and PCB profiles in feathers and muscle might possibly be explained by external contamination of organohalogenated compounds on the feather surface. Although the importance of external contamination has been extensively studied in the context of heavy metal monitoring via feathers (Burger, 1993; Dauwe et al., 2002; Jaspers et al., 2004), external contamination onto the feather surface should also be considered when measuring concentrations of organic pollutants. Lower brominated PBDE/chlorinated PCB congeners are assumed to contribute more to external contamination from the air than higher brominated/chlorinated congeners (Vorhees et al., 1997), similar to what is observed by Dauwe et al. (2005) for PCBs in feathers. The presence of low chlorinated PCB congeners in feathers of common moorhens might indicate a potential source of external contamination for these birds. However, the presence of the less persistent congeners can also be explained by their presence in the blood at the time of feather formation. This results in relatively higher concentrations of chlorinated less persistent congeners in feathers in comparison to liver and muscle tissue, in which preferably persistent congeners accumulate (Altshul et al., 2004). Additionally, aquatic birds intensively preen their feathers with oil from the uropygial gland to render feathers waterproof. This uropygial oil may also be considered an additional source of external contamination (Pilastro et al., 1993; Van den Brink, 1997; Dauwe et al., 2002), or it may perhaps be possible that this uropygial oil acts as an adhesive for external contamination originating from the air. Clearly, further experimental work is needed to elucidate the importance and possible routes of external contamination of organic pollutants onto feathers.

**Table 2 :** Pearson correlation coefficients calculated between  $\log_{10}$  levels of organohalogenated pollutants in feathers (ng/g) and in muscle/liver tissue (ng/g ww) of different bird species from Belgium

Species	Tissue	N	CB 99	CB 101	CB 149	CB 153	CB 180	Sum PCBs	Sum BDEs	<i>p,p'</i> -DDE
Grey heron	Muscle	9	0.27	-0.07	0.56	0.51	0.58	0.49	0.43	0.20
	Liver	9	0.12	-0.13	0.42	0.18	0.24	0.21	0.12	0.17
Herring gull	Muscle	12	0.23	0.02	0.23	0.48	0.41	0.21	0.50	0.43
	Liver	-	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Common moorhen	Muscle	11	0.47	n.d.	0.03	0.91**	0.92**	0.89**	n.d.	0.68*
	Liver	-	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Common buzzard	Muscle	43	0.71**	0.50**	0.66**	0.75**	0.77**	0.76**	0.73**	0.50**
	Liver	43	0.41**	0.36*	0.53**	0.63**	0.63**	0.60**	0.43**	0.32*
Long eared owl	Muscle	10	0.63	-0.08	0.90**	0.74*	0.69*	0.75*	0.54	0.62*
	Liver	10	0.42	-0.19	0.81**	0.69*	0.74*	0.71*	0.29	0.59
Barn owl	Muscle	9	0.53	-0.05	0.59	0.60	0.63	0.57	0.75*	0.86**
	Liver	9	0.41	-0.13	0.49	0.46	0.47	0.41	0.66	0.68*
Sparrowhawk	Muscle	11	0.45	0.42	0.43	0.48	0.43	0.42	0.63*	0.68*
	Liver	11	0.30	0.43	0.36	0.38	0.45	0.38	0.60	0.33

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\* significant after Bonferroni correction, n.a., not analysed; n.d., non-detectable; CB *x*: persistent PCB congener.

### 3.2. Correlations between feathers and tissues

#### 3.2.1. Differences among species

Correlations between concentrations in feathers and liver or muscle tissue of the different bird species are listed in Table 2. For sum PCBs and sum PBDEs, correlations between feather and muscle were significant in three of the seven bird species, while for *p,p'*-DDE significant correlations were established in five of the seven bird species (Table 2). When correlations were recalculated excluding the birds that had died due to starvation, correlation coefficients for the terrestrial birds were in most cases found higher (Table 3). These results indicate the importance of controlling for the condition of the birds. Similar correlation coefficients as those in the present study have been reported for heavy metals between levels in feathers and internal tissues (V ranging from 0.51 to 0.84 between feathers and liver, and from 0.31 to 0.74 between feathers and muscle; Burger, 1993). Moreover, D'Have et al. (2005) have recently evaluated the use of hair of hedgehogs (*Erinaceus europaeus*) as a non-destructive biomonitoring tool for PBDEs. These authors observed positive relationships between hair and internal tissues that, after removal of two outliers in their data set, were in the lower range of correlations found in our study ( $0.43 < r < 0.53$ ,  $n = 40$ ; D'Have et al., 2005).



A high variability could be seen among species in both correlation coefficients and significance (Tables 2 and 3). Only for the buzzard ( $n = 43$ ) most correlations were found significant. For the aquatic species grey heron ( $n = 9$ ) and herring gull ( $n = 12$ ), correlation coefficients were found low and not significant. However, for the common moorhen ( $n = 11$ ), also an aquatic bird species, a high and significant correlation was found for levels of sum PCBs between feather and muscle, in addition to a significant correlation in  $p,p'$ -DDE levels (Table 2). This discrepancy might be explained by variation in diet: herring gulls are very opportunistic feeders, changing their diet in accordance to available items (also garbage), while common moorhens feed on a more controlled diet of insects and plant material (Snow and Perrins, 1998). Therefore concentrations in the feathers of common moorhens originate from a similar diet than concentrations found in the tissues of these birds, whereas for the herring gull the diet at the time of feather formation could be different from the current diet.

**Table 3:** Pearson correlation coefficients calculated between  $\log_{10}$  levels of organohalogenated pollutants in feathers (ng/g) and in muscle/liver tissue (ng/g ww) of different bird species from Belgium, excluding birds killed by starvation

Species	Tissue	N	CB 99	CB 101	CB 149	CB 153	CB 180	Sum PCBs	Sum BDEs	$p,p'$ -DDE
Herring gull	Muscle	8	0.18	-0.37	-0.27	0.54	0.52	0.11	0.43	0.51
Common moorhen	Muscle	10	0.49	n.d.	0.05	0.90**	0.90**	0.87**	n.d.	0.22
Common buzzard	Muscle	26	0.71**	0.47*	0.72**	0.85**	0.86**	0.85**	0.76**	0.51**
	Liver	26	0.46*	0.26	0.69**	0.67**	0.67**	0.64**	0.44*	0.40*
Barn owl	Muscle	5	0.13	-0.47	0.48	0.60	0.86	0.74	0.66	0.92*
	Liver	5	-0.12	-0.37	0.57	0.61	0.83	0.73	0.59	0.83
Sparrowhawk	Muscle	5	0.81	0.78	0.74	0.60	0.52	0.62	0.86	0.77
	Liver	5	0.75	0.79	0.71	0.60	0.60	0.60	0.94*	0.44

For heron and long-eared owl, sample sizes were too small when excluding starved birds and therefore these species were not included in the table below. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* significant after Bonferroni correction, n.a., not analysed; n.d., non-detectable; CB<sub>x</sub>: persistent PCB congener.

### 3.2.2. General patterns (using meta-analysis)

Table 4 presents the results of meta-analyses performed with all bird species combined and for the aquatic and terrestrial species separately. Results are given when all birds are included in the meta-analysis (Table 4A) and when the starved birds are excluded from the metaanalysis (Table 4B). Almost all correlations were found significant, with the exception of some correlations for the aquatic species (mostly with liver). Correlation coefficients for the terrestrial species ranged from 0.38 to 0.72 between feather and muscle and from 0.27 to 0.61 between feather and liver (Table 4A). Again, when the starved birds were excluded from the meta-analysis, correlation coefficients for the terrestrial species improved ( $0.44 < r < 0.85$  between feather and muscle;  $0.27 < r < 0.68$  between feather and liver), except for the correlations coefficients of  $p,p'$ -DDE, which remained unchanged. Our results indicate that sample sizes of most separate species were too small to obtain significant correlations and that by increasing the sample size (in this case by combining them in a meta-analysis) significant (or more significant) correlations with internal tissues could be established.

For each species, we also calculated Pearson correlations for three persistent PCB congeners (penta CB 99, hexa CB 153 and hepta CB 180) and two less persistent PCB congeners (penta CB 101, hexa CB 149) to investigate whether correlations differed according to the degree of chlorination and persistency (Tables 2-4). Correlation coefficients were found lowest for the less persistent congener CB 101 (Tables 2-4). This was confirmed by tests for the equality of two dependent correlations (Steiger, 1980). The correlations between feather and muscle were significantly lower for CB 101 than for CB 153 or CB 180, and correlations between feather and liver were also found significantly lower for CB 101 than for CB 149, CB 153 or CB 180. Similar to the present study, earlier research has indicated that the percentage of lower chlorinated congeners (tetra- and penta-PCB congeners), including less persistent congeners, measured in feathers is higher than in internal tissues (Dauwe et al., 2005). Since lower chlorinated congeners are more readily biodegradable, they do not accumulate to the same extent in internal tissues, as do higher chlorinated congeners. Therefore, it seems reasonable that correlations are higher for the persistent PCB congeners (e.g. CB 153 and CB 180), as these congeners accumulate in a more comparable way into feathers and tissues than the lower chlorinated congeners do. In addition to differences in transport and metabolism, external contamination with lower chlorinated PCB congeners onto the feather surface cannot be excluded (see above).

**Table 4:** Results of the meta-analysis performed on Pearson correlation coefficients of  $\log_{10}$  concentrations between feathers (ng/g) and muscle or liver tissue (ng/g ww) of different bird species from Belgium

Meta-analysis	Tissue	N	CB 99	CB 101	CB 149	CB 153	CB 180	Sum PCBs	Sum BDEs	p,p'-DDE
<i>A) Including all birds</i>										
Aquatic	Muscle	32	0.33	-0.38*	0.26	0.71**	0.71**	0.62**	0.47*	0.48**
	Liver	9	0.12	-0.13	0.42	0.18	0.24	0.21	0.12	0.17
Terrestrial	Muscle	73	0.66**	0.38**	0.67**	0.71**	0.72**	0.71**	0.70**	0.59**
	Liver	73	0.40**	0.27*	0.55**	0.59**	0.61**	0.57**	0.47**	0.40**
Combined	Muscle	105	0.58**	0.18	0.58**	0.71**	0.72**	0.69**	0.66**	0.56**
	Liver	82	0.37**	0.23*	0.54**	0.56**	0.58**	0.55**	0.44**	0.38**
<i>B) Excluding, birds which had died by starvation</i>										
Aquatic	Muscle	22	0.34	-0.66**	-0.11	0.77**	0.73**	0.65**	0.36	0.36
	Liver	4	0.20	-0.27	-0.23	0.17	0.15	0.05	-0.22	0.57
Terrestrial	Muscle	36	0.69**	0.44**	0.71**	0.83**	0.85**	0.83**	0.76**	0.59**
	Liver	36	0.45**	0.27	0.68**	0.66**	0.68**	0.64**	0.52**	0.45**
Combined	Muscle	58	0.60**	0.07	0.51**	0.81**	0.82**	0.79**	0.71**	0.52**
	Liver	40	0.44**	0.25	0.66**	0.65**	0.67**	0.63**	0.50**	0.45**

The significant negative correlations for CB101 in aquatic birds may be due to type I errors, i.e. spurious correlations found by chance as a result of many comparisons carried out at the same time. \* $p < 0.05$ , \*\* $p < 0.01$ ; n.a., not analysed; n.d., non-detectable, CB.x: persistent PCB congener.

In general, higher correlation coefficients were found between feathers and muscle samples than between feathers and liver samples in all species (Tables 2-4). Assuming that concentrations in feathers reflect the circulating concentrations in the body at the time of their formation, feathers may not reflect recent changes in contamination. Since the turnover rate in liver (a highly metabolically active tissue; Voet and Voet, 1995) is higher than in muscle, concentrations in liver probably reflect more recent exposure, which may explain the lower correlation coefficients found between feather and liver samples. Future research should investigate the relationship between concentrations in newly grown feathers and concentrations in blood sampled during feather formation.

Although, at present, our results cannot explain all variation observed between levels in feathers and internal tissues, bird feathers can provide an overall assessment of contamination levels. Given that feathers can be easily preserved and that bird collections in museums and private collections date from the late 1700s, feathers may be useful for retrospective biomonitoring of organohalogenated pollutants, as successfully done for heavy metals (Burger, 1993), and to study regional and temporal trends of contamination with organohalogens (Rocque, 2005). While additional studies need to investigate the influence of external contamination and other confounding factors, feathers seem to represent a promising non-destructive biomonitoring tool for organic pollutants in (predatory) birds.

### 3.3. Stable isotopes in feathers and tissues

The relationship between carbon or nitrogen stable isotope values ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) and the corresponding PCBs levels in the tissues of the different bird species is shown in Fig. 3. No significant correlations were found, for any of the analysed organohalogenated pollutants, neither in feathers nor in muscle. Our data show larger differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values among aquatic than among terrestrial species (Fig. 3).

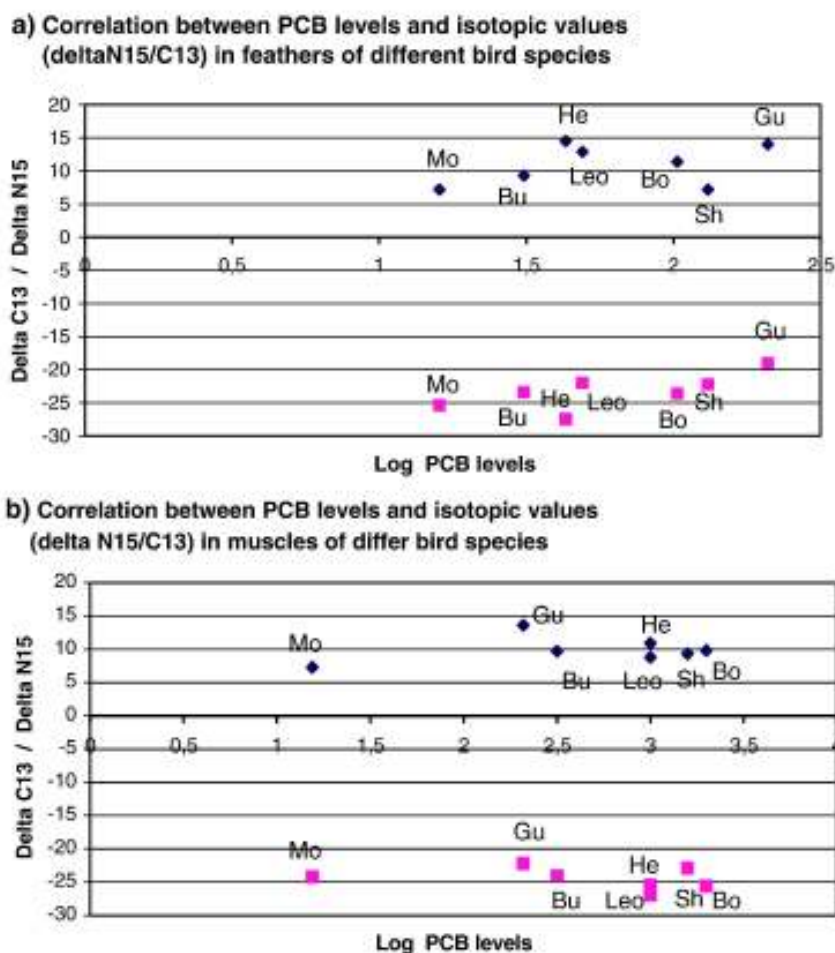
Zimmermann et al. (1997) have suggested that PCB patterns are influenced more by individual dietary factors than by interspecific differences in metabolic capacity. However, migratory patterns, proximity to sources and breakdown by metabolic processes in the bird can possibly lead to different accumulation profiles as well (Hobbs et al., 2002). Various confounding factors (such as habitat, condition, age, sex) may have masked a possible relationship between  $\delta^{15}\text{N}$  values and organic pollutant levels in the birds of the present study. However, there could be other reasons, for example that the differences in trophic position among species were too small to define them correctly or that the studied birds were belonging to different food chains, making comparisons difficult. In comparison, Thompson et al. (1998) found no relationship between isotope values and mercury contamination in feathers of seabirds. Mercury concentrations in body feathers were markedly different between northern fulmars (*Fulmaris glacialis*) and great skuas (*Catharacta skua*), reflecting differences in diet, but there was no corresponding difference in trophic status as measured through nitrogen stable isotope signatures. Most other studies have usually been performed on aquatic food webs (Borga et al., 2004; Ruus et al., 2002), which are possibly easier to link to  $\delta^{15}\text{N}$  values, given that diets are generally more constant within aquatic

environments.

Even within species, no significant correlation was found between  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values and levels of organic pollutants. This could be due to the fact that birds were collected randomly from locations all over Flanders. Therefore, although the birds might have fed on similar prey, contamination load in prey might differ among sites (Morrissey et al., 2004).

No significant correlations were found between levels of  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  in feathers and muscles of individual bird species, except for  $\delta^{15}\text{N}$  values in the common moorhen ( $r = 0.9$ ) and  $\delta^{13}\text{C}$  values in the barn owl ( $r = 0.91$ ). The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in feathers originate from the isotopic signatures of the blood during their formation. Differences in feeding habits over time could result in different isotopic values in feathers and muscle. An additional confounding factor could be the unknown moulting status of individual birds. As mentioned above, common moorhens feed on a controlled diet of insects and plant material (Snow and Perrins, 1998), explaining the correlation found between stable isotopic signatures in muscle and feathers. In conclusion, it seems important that isotopic measurements should be compared with contaminant levels analysed in the same tissue, as isotopic measurements in different tissues are not necessarily correlated.

**Fig. 3:** Correlations between  $\log_{10}$  PCB levels (ng/g ww) and  $\delta^{15}\text{N}$  (◆) and  $\delta^{13}\text{C}$  (■) values in feather (a) and muscle tissue (b) of different bird species from Belgium. Abbreviations: He: grey heron, Bo: barn owl, Leo: long-eared owl, Bu: common buzzard, Sh: sparrowhawk, Mo: common moorhen, Gu: herring gull.



#### 4. Conclusions

Concentrations of most organohalogenated pollutants (i.e. PCBs, DDTs, PBDEs, HCHs, CHLs and HCB) could be quantified in one single tail feather of different aquatic and terrestrial bird species. Significant correlations were found between levels of most organic pollutants in feathers and muscle or liver tissue of terrestrial predatory birds. Although it may not be possible to predict exact concentrations in internal tissues by analysing organic pollutants in bird feathers, feathers could be useful as a non-destructive biomonitoring tool for the

assessment of overall contamination levels with organic pollutants.

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