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RESEARCH ARTICLE

Retrospective biomonitoring of mercury and other elements in museum feathers of common kestrel *Falco tinnunculus* using instrumental neutron activation analysis (INAA)

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Abstract This study examines the potential to use instrumental neutron activation analysis (INAA) to explore temporal and geographical variation in exposure to heavy metals and other selected elements in common kestrel *Falco tinnunculus* using feathers from a natural history collection. The study gathered samples of two breast feathers from each of 16 adult male kestrel specimens from Naturalis Biodiversity Centre, collected in The Netherlands between 1901 and 2001. Feather samples were analysed for more than 50 elements, using INAA at the Reactor Institute Delft. Results (in mg/kg dw) were transformed into ratios of milligram of element per millimetre of feather length. The distribution of the mass fractions and ratios was plotted for each element against time and by geographical area. Observed mass fractions and/or ratios are discussed for selected elements (Hg, Cd, Zn, Pt, Pd, Se, Al, Rb, As, Sb, Cr, V, Cl, Br) known to have, at certain concentrations, adverse effects on raptors. Some samples show mass fractions of certain elements (Cr, Cd, Se, As) above levels known to have adverse effects. We conclude that the analysis of museum feathers using INAA provides reference values for concentrations of selected elements, including those of high societal concern such as Hg and Cd, against which to assess concentrations of these elements in feathers of present-day living raptor populations.

Keywords Raptor · Common kestrel · *Falco tinnunculus* · Feather · Museum specimen · Contaminant · Exposure · Metal · Element · INAA

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Introduction

Aim and objectives

The aim of this study was to use instrumental neutron activation analysis (INAA) to explore temporal and geographical variation in exposure to heavy metals and other selected elements in common kestrels *Falco tinnunculus* using feathers from a natural history collection. The study also aims to explore the potential offered by museum bird skin collections for retrospective biomonitoring of heavy metals and other elements using this analytical method.

The study was intended as a pilot with a view to initiating a larger project to examine temporal and geographic variation in exposure to heavy metals and other elements in raptor (and possibly other bird) species using museum specimens and to compare these with contaminant levels in present-day populations of the same species living in the same geographic regions.

The use of raptors as sentinels of environmental contamination

Birds can be useful sentinels of environmental contamination (e.g. Newton et al. 1993; Rattner 2009). Raptors (birds of prey, owls and scavengers) are especially suitable for monitoring persistent, bioaccumulative, toxic (PBT) chemicals (e.g. Sergio et al. 2005, 2006; Movalli et al. 2008a), because (a) they are typically relatively long-lived, apex predators; (b) they effectively integrate contaminant exposure over time (Furness 1993) and over relatively large spatial areas; (c) they are relatively easily studied and captured (especially nestlings) which facilitates collection of samples without sacrificing or harming animals (blood, feather, preen gland oil); and (d) populations can be relatively easily monitored and quantified. These are all criteria identified by the US National Research Council as requirements for sentinel species (NRC 1991). Raptors are also known to have measurable responses to PBT chemicals, ranging from residue accumulation to population decline. Indeed, the current ban under the Stockholm Convention on polychlorinated biphenyls (PCBs) and other PBT compounds that are potentially harmful to both people and wildlife has been partly based on exposure and effects data in raptors (Rattner 2009).

Raptor biomonitoring programmes in Europe

An overview of existing raptor contaminant monitoring activities in Europe has recently been published by Gómez-Ramírez et al. (2014). This found that, while there is a large number of biomonitoring programmes using raptors, only a few are established at a national scale, e.g. National Environment Monitoring Programme in Sweden (Helander et al. 2008), the Predatory Bird Monitoring Scheme (PBMS) in the UK (Walker et al. 2008), the Bird Monitoring Programme in Finland (Koskimies 1989) and the Monitoring Programme for Terrestrial Ecosystems (TOV) in Norway (Gjershaug et al. 2008). In other countries, such as Spain, Germany, Belgium, Italy and The Netherlands (Gómez-Ramírez et al. 2011; Jaspers et al. 2006; Kenntner et al. 2003; Movalli et al. 2008b; van den Brink et al. 2003), studies are typically limited in spatial extent and/or duration and are rarely repeated (García-Fernández et al. 2008). Overall, there appears to be widespread capability and expertise to use raptors to monitor the effectiveness of EU directives at a pan-European scale. However, existing national and subnational monitoring initiatives need to be reinforced and coordination at a pan-European scale improved (Movalli et al. 2008a). A paper on the selection of raptor samples for contaminant monitoring has recently been published by Espín et al. (2016a), and a sample monitoring protocol has been developed under the EURAPMON Research Networking Programme (Espín et al. 2016b).

The use of biological collections for retrospective studies

Biological collections in museums can contribute unique and invaluable insights into the study of pathogens, vectors of disease and environmental contaminants (Suarez and Tsutsui 2004). These collections represent a valuable repository of catalogued samples collected during periods when scientists were not aware about environmental contaminants. Those specimens, which can date back to the mid-1800s or even earlier, can be useful indicators of environmental contamination during a period of increasing industrialisation prior to the 1970s (Campbell and Drevnik 2015).

The preservation techniques used on museum skins is not always known and can contaminate samples. Washing does not always remove this external contamination. However, we used a washing method (Cortes Toro et al. 1993, see below) known to be effective for removal of external contamination by heavy metals and other elements, including inorganic Hg that was in the past commonly used to preserve museum skins (however, see discussion on As, below).

The use of museum feathers to establish reference values

Environmental contaminants are chemicals that exist at levels judged to be above those that would normally occur in any particular component of the environment. This raises the question of what is to be considered normal. With most man-made chemicals, such as pesticides, the situation is simple; any detectable level is abnormal as the compounds did not exist in the environment until released by man. On the other hand, chemicals such as metals, polycyclic aromatic hydrocarbons and methyl mercury are naturally occurring and were certainly or probably present in the environment before the appearance of human beings. The concentration of these chemicals varies from place to place and over time. This makes it difficult to judge their normal ranges of concentration (Walker et al. 2006).

One way to help inform these normal ranges, in birds, for naturally occurring chemicals is to conduct work on feathers from museum specimens with a view to establishing retrospective (historical) reference values. Feathers have long been known as a useful matrix for the study of a range of contaminants in birds, including mercury and other metals (e.g. Berg et al. 1966; Scanlon et al. 1980; Movalli 1993; Fasola et al. 1998), and more recently have been used to study organic pollutants such as DDT and metabolites (e.g. Dauwe et al. 2005; García-Fernández et al. 2013) and neonicotinoides (Taliansky-Chamudis et al. 2017). However, other raptor tissues, when available, may be better matrices for the study of many contaminants (Espín et al. 2016a).

Museum collections of raptor skins, at least for certain species, are relatively large, allowing for sufficient sample sizes to test for statistical significance of any differences in

contaminant levels observed over time and space. These skins offer certain other advantages in this respect; they are typically well documented, with data available on the species, sex, maturity, location and date of collection, allowing for interpretation of observed contaminant levels (Thompson et al. 1992). Specimens can date back to the mid-nineteenth century or even earlier and thus provide data on change in contaminant exposure during a period of increasing industrialisation through to the mid to late twentieth century (Campbell and Drevnik 2015).

Previous studies using museum raptor specimens

As early as the 1960s, Berg et al. (1966) analysed preserved bird specimens from the Swedish Museum of Natural History and showed that concentrations of accumulated mercury increased during the 1940s and 1950s. There are a few other examples of the use of museum collections for the retrospective analysis of trends in exposure to mercury (e.g. Dietz et al. 2006 for Greenland; Head et al. 2011 for the USA). However, we are not aware of other studies that, like ours, examine exposure to a wide range of metals and other elements using museum collections of raptor skins. This is also the first study reporting on the concentrations of contaminants in feathers of museum kestrels from the Netherlands.

Raptor specimens at Naturalis Biodiversity Centre

A number of natural history museums in Europe hold important collections of raptor skins dating from the last 150 years or so. Naturalis Biodiversity Centre is the result of a recent merger of the National Museum of Natural History of The Netherlands and the Zoological Museum of the University of Amsterdam and houses a large collection of bird skins. Such collections offer potential to investigate temporal and geographic variation in the exposure of raptors to contaminants such as Hg, Cd and certain POPs, which are amenable to study using feathers.

Relevance to EU chemical regulation and to environmental and human health

EU law on human and veterinary medicinal products (EC 2001, EC 2004a, EC 2004b), persistent organic pollutants (EC 2004c), industrial chemicals (EC 2006), plant protection products (PPPs) (EC 2009) and biocidal products (EU 2012) as well as aspects of the Water Framework Directive (WFD) (EC 2000), Marine Strategy Framework Directive (MSFD) (EC 2008) and Environmental Liability Directive (EC 2004d) aim to prevent and limit negative impacts of chemicals on human health and the environment. These laws are supplemented by global and regional conventions ratified by the EU such as the Helsinki Convention on the Baltic Sea (1992), OSPAR Convention on the NE Atlantic (EU 1998), Stockholm Convention on Persistent Organic Pollutants (EC 2004d) and Minamata Convention on Mercury (EU 2017).

A key issue with all such legislative instruments is to determine how effective they are. Measuring the numbers of registrations, authorisations and restrictions on chemicals only provides data on activities undertaken under the auspices of the EU directives. Such measures do not provide information on how *effective* the measures were in achieving mitigation targets; that requires monitoring of contaminants in selected environmental compartments.

Raptors can be particularly useful as sentinels of environmental contamination. The availability of reference values for selected contaminants, derived from museum specimens of a selected raptor species, can enable a better-informed assessment of modern-day values observed in the same species and thus a better-informed assessment of the effectiveness of regulatory controls on these contaminants.

When combined with modern-day monitoring of living raptor populations, historical reference values obtained from museum raptor skins can help to provide early warning of certain legacy and emerging contaminant threats and thereby reduce the costs to environmental and human health that may arise from such contamination (though other tissues such as liver and adipose tissues are more appropriate tissues to use for lipophilic compounds—see Espín et al. 2016a).

Materials and method

Considerations in selecting the study species

A number of issues should be taken in to account when selecting the species for study. These include

1. *Residential or migratory species and territorial fidelity.* Some raptor species (or one or the other sex of a species) are migratory and/or occupy very wide-ranging territories. In this case, studies can assess the overall exposure of individuals to contaminants, but it is difficult to make inferences about the geographical source of any contaminant detected. In other raptor species, the male and/or female typically reside in a restricted territory all year round, permitting deductions to be made both about overall exposure and about the geographical source of any contaminant detected.
2. *Number of specimens available in the museum collections.* A statistically valid number of specimens of the selected species (and sex) are required for each time period in order to explore temporal trends. If the intention is also to examine geographical patterns of contamination, then a statistically valid number is required both

for each time period and for each geographic area of interest. As museums tend to have higher numbers of specimens for relatively common and widespread species, it is such species for which a statistically valid number of specimens are most likely to be available.

3. *Likely sources of contamination and likelihood of exposure to specific contaminants through the food chain*. Certain raptor species feed predominantly on rodents, and therefore may be particularly exposed to contaminants that rodents are exposed to, such as fungicide seed dressings and rodenticides. Other raptor species feed predominantly on insect or seed-eating birds, and therefore may be particularly exposed to contaminants that these prey are exposed to, such as insecticides or fungicide seed dressings.

Selection of male kestrels as the species and sex for this study

Taking in to account the above considerations, we chose male kestrels *F. tinnunculus* as the species and sex for this study for the following reasons:

1. In general, the kestrel in The Netherlands is strongly territorial, depending on availability of food (voles and mice). If food is scarce, females in particular may leave the territory to seek better hunting grounds. Males need less biomass and probably have a greater need to defend their territory and will eat earthworms in addition to rodents (however, in a period of prolonged snow cover or frozen ground, males also may move from their territory). Moreover, females can excrete metals and other contaminants by egg laying resulting in greater variability in body burden (Lodenius and Solonen 2013).
2. The kestrel is a relatively common and widespread species in The Netherlands, both now and historically. The Naturalis Biodiversity Centre holds a relatively large collection of kestrel skins (over 600) dating from 1850 to 1980.
3. The kestrel in The Netherlands feeds predominantly on voles and mice (Goree et al. 1995), as elsewhere, e.g. Norway (Steen et al. 2011), Italy (Casagrande et al. 2008) and Finland (Valkama pers. comm.) but also can eat a lot of earthworms. This means that it is particularly exposed to contaminants that these rodents and worms may be exposed to, including fungicide seed dressings, herbicides, insecticides and elements such as mercury, lead and cadmium, from industrial, vehicular and other sources, deposited on vegetation and soil from the atmosphere.
4. The kestrel is widespread and common in much of Europe and has been relatively frequently studies by ecotoxicologists, providing a relatively large volume of data with which to compare historical contaminant levels.
5. Naturalis receives present-day specimens of dead kestrels, and kestrel nest sites (many use nest boxes in The Netherlands) are relatively accessible for sampling, offering opportunities in the future to collect present-day feather samples and compare present-day contaminant levels with historical levels.

Selection of INAA as the analytical method for this study

In using museum feathers for ecotoxicological studies, the selection of the analytical method needs to take into account the range of contaminants for which feathers are a suitable matrix of study. Feathers are known to be a suitable matrix of study for mercury (Hg) and a range of other heavy metals and other elements, some of which (e.g. Hg) are bound in to the keratin during feather growth and others of which are deposited in the feather by the blood stream during feather growth but not structurally bound. For those elements that are firmly bound to the keratin (such as Hg), the concentration in the feather will be related to the exposure of the individual and the mass of the feather (mass-dependent contaminants), while for those elements that are not structurally bound, the concentration in the feather will be related to the exposure of the individual to the contaminant and the duration of feather growth as reflected by the feather length (time-dependent contaminants). For the former, concentrations are best expressed as milligrams per kilogram and for the latter, as milligrams per kilogram per millimetre length of feather (Bortolotti 2010).

For the current study, we selected INAA as the analytical method. INAA has proven to be a highly sensitive and versatile method for measuring trace elements in a wide variety of samples (Wainerdi and DuBeau 1963) at microgram levels (Corliss 1963). Almost 50 years ago, Devine and Peterle (1968) demonstrated the utility of neutron activation analysis in their investigations on populations of Canada geese *Branta canadensis*. It has been demonstrated that INAA can meet the highest metrological requirements (Greenberg et al. 2011).

INAA has several advantages. First, and critically when dealing with irreplaceable museum specimens, it requires only a very small sample size (we used two breast feathers from each specimen; there is no minimum mass for INAA). The removal of feathers from museum skins can have impact on the aesthetic quality and scientific value of these skins, and therefore, the number sampled from each skin must be kept as low as possible. INAA is moreover non-destructive, so the feather samples may be returned to the museum or used for other analyses. Second, INAA has very low limits of detection and has proven to be a highly sensitive and versatile method for measuring trace elements in a wide variety of samples at microgram levels (Parrish et al. 1983). Third, a single INAA

assay can provide concentration values for a large range of elements.

During INAA, the feathers are irradiated with neutrons. These neutrons interact with the nuclei of the atoms in the feathers and in doing so create radioisotopes. Gamma rays, with energies specific to each element, are emitted during the radioactive decay of the feather and are measured by a spectrometer. Concentrations of numerous elements are measured simultaneously (Bortolotti and Barlow 1986).

The Reactor Institute Delft (RID) at the University of Technology Delft offers several different assays which each detect a different range of elements. For the purposes of this study, we selected the assay which detects the following elements (listed in ascending order in the periodic table): Mg, Al, Si, Cl, K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Br, Rb, Sr, Zr, Mo, Pd, Ag, Cd, In, Sn, Sb, I, Cs, Ba, Hf, Ta, W, Re, Pt, Au, Hg, La, Ce, Nd, Sm, Eu, Tb, Dy, Yb, Lu, Th and U. This includes a number of elements of particular societal concern, including Hg and Cd.

Collection of museum feather samples

We sampled, in 2013, two breast feathers (total c. 10 mg) from each of 16 male kestrel skins ($n = 16$) dating from the period 1901–2001. Table 1 provides a list of feather samples with their Naturalis Biodiversity Centre specimen ID code, place and date of collection. These data were all available from the Naturalis Biodiversity Centre specimen labels.

The collection of each feather was performed following a protocol as follows. To avoid possible variations, only one operator collected and processed all samples, in a uniform manner. The operator wore plastic gloves. Individual feathers were isolated and pulled with clean, acid-rinsed, stainless steel forceps. Before taking the measurement of the length and weight of each feather, the operator removed the calamus of each feather[1] (with scissors) at the point where the first barbs of the vane join at the base of the feather; then, the length of each feather was measured (in mm) using a calliper, from the cut end (the base of the vane) to the tip. The feathers were measured flat on a clean table (when necessary placing the feathers under a sterile sheet of glass to flatten them). The feathers were then weighed (in mg) using an electronic balance.

Feathers were placed in labelled polyethylene vials, using forceps. Only one pair of forceps was used to collect the feathers and perform all handling operations. Feather samples were catalogued and stored at room temperature at Naturalis Biodiversity Centre until analysis. All the samples were transferred to TU Delft where the analyses were performed.

Cleaning and drying of breast feather samples

In order to remove external contamination from the surface of the feathers, a cleaning process was performed, as recommended for hair samples by the International Atomic Energy Agency (Cortes Toro et al. 1993), prior to analytical determination. The feathers were cleaned by adding ultrapure (up) water to the capsule and shaking for 5 min. This process was repeated three times, after which the capsules (with up water) were placed in an ultrasonic bath for 15 min. After cleaning, the feathers were dried in a desiccator. The drying process was followed by daily weighing of the capsules, until the mass was stable for 2 days.

Instrumental neutron activation analysis

The cleaned and dried samples were analysed using INAA at RID. The quality management system of this laboratory is accredited by the Dutch Council for Accreditation for compliance with the ISO/IEC 17025:2005 standard.

Specifically, the samples were irradiated during 2 min at a thermal neutron fluence rate of 1.5×10^{13} cm^{-2} s^{-1} and measured, after 1-min decay time, during 5 min at 1-cm distance from a 10% Ge detector. Then, they were irradiated during 5 h at a thermal neutron fluence rate of 4.6×10^{12} cm^{-2} s^{-1} and measured twice in a well-type Ge detector after 4 and 16 days of decay time, during 1 and 3 h, respectively. The acquired gamma-ray spectra were analysed using the Apollo software, resulting in a list of mass fractions with associated measurement uncertainties and detection limits of about 50 elements (Blaauw 1994). The entire analytical method has been validated (Bode and Blaauw 2012).

Counting of fault bars in remiges of the same birds

We analysed fault bars in remiges of the same birds (see Table 2). On each feather, 10 growth bars, centred on the point two thirds of the distance towards the tip of the feather, were measured (see Grubb 1989). This analysis was done by Yosef, who served as a blind test in respect to all other variables measured (e.g. Grubb and Yosef 1994). This measurement was done during a summer school in 2014 on the use of museum specimens for contaminant analysis in raptors.[2]

Growth bars (differently from fault bars) are regular cross-bands on feathers that denote 24-h periods of growth (Wood 1950; Grubb 1989). These bars are most commonly seen in the wing and tail feathers, as alternately dark and pale cross-

[1] The calamus is removed as this has a different growth rate and therefore could skew the concentration values per millimetre length of vane (Bortolotti 2010).

[2] http://www.eurapmon.net/sm6-naturalis-aug-2014

Table 1 List of samples, museum specimen ID, year, and location

Feather sample no.	Naturalis registration number	Year	Location	Latitude and longitude	Region
1a, 1b	RMNH.AVES.48827	1921	Noordwijk aan Zee	52° 14′ 23.86″ N, 4° 27′ 0.05″ E	S
2a, 2b	RMNH.AVES.24161	1956	Delft	52° 0′ 41.61″ N, 4° 21′ 25.66″ E	S
3a, 3b	RMNH.AVES.43127	1947	Ottersluis, Dordrecht	51° 47′ 31.08″ N, 4° 45′ 30.68″ E	I
4a, 4b	RMNH.AVES.86459	1995	Leiderdorp	52° 9′ 1.05″ N, 4° 31′ 41.64″ E	C
5a, 5b	RMNH.AVES.10550	1942	Scherpenisse	51° 32′ 43.95″ N, 4° 6′ 14.65″ E	I
6a, 6b	RMNH.AVES.192431	1905	Oegstgeest	52° 11′ 6.42″ N, 4° 28′ 29.12″ E	C
7a, 7b	ZMA.AVES. 36007	1981	Lisserbroek	52° 15′ 35.09″ N, 4° 34′ 15.96″ E	N
8a, 8b	RMNH.AVES.61829	1933	Voorburg	52° 4′ 31.46″ N, 4° 21′ 45.57″ E	S
9a, 9b	RMNH.AVES.13267	1947	Loosduinen	52° 19′ 7.29″ N, 4° 38′ 44.29″ E	N
10a, 10b	ZMA.AVES. 16757	1962	Motorway between Amsterdam and Rotterdam, some km S of Haarlemmermeer	52° 26′ 11.18″ N, 4° 49′ 11.47″ E	N
11a, 11b	RMNH.AVES.192428	1901	Leiden	52° 9′ 32.74″ N, 4° 29′ 50.09″ E	C
12a, 12b	ZMA.AVES. 36374	1977	Muiden	52° 19′ 39.10″ N, 5° 4′ 7.42″ E	I
13a, 13b	RMNH.AVES.194679	1910	Wassenaar	52° 8′ 25.84″ N, 4° 24′ 4.85″ E	S
14a, 14b	RMNH.AVES.18439	1951	Hazerswoude	52° 5′ 58.69″ N, 4° 35′ 0.08″ E	C
15a, 15b	RMNH.AVES.15775	1951	Wijde Aa	52° 10′ 53.03″ N, 4° 37′ 11.98″ E	C
16a, 16b	RMNH.AVES.103842	2001	Spaarndam	52° 24′ 42.65″ N, 4° 41′ 9.43″ E	N

bands, rarely in other tracts (Wood 1950). The difference in colour between the dark and light bars may be due to a more rapid development of the barbules during the day, hence more of them in a given space, and by a different deposition rate of lipochromic granules (Wood 1950). Since the first definition of ptilochronology (Grubb 1989), feather growth bar widths have become a standard index of nutritional condition in ornithological research (see Shawkey et al. 2003). The width of feather growth bars during each 24-h period at the time of moult depends on the amount of energy and nutrients invested by an individual into the regeneration process, which at the same time depends on the nutritional condition and quality of an individual (Grubb 1989, 2006). Feather growth as an index of nutritional condition is based on natural selection, which forces birds to regenerate lost feathers as rapidly as possible. Therefore, birds in better nutritional condition regenerate feathers much faster than individuals in poor condition (Grubb 1991; Grubb 2006). Thus, feather quality is a relatively accurate indicator of an individual's quality.

Fault bars are transparent bands in the feathers of birds produced under stressful and adverse conditions (i.e. Møller et al. 2009). Such bands are narrow malformations in feathers oriented almost perpendicular to the rachis where the feather vein and even the rachis may break. Breaks in the barbs and barbules result in small pieces of the feather vein being lost, while breaks in the rachis result in loss of the distal portion of the feather (e.g. Jovani and Rohwer 2016). Malnutrition was likely the first factor to be related to fault bars (Sebright 1826); a recent review however suggests that several environmental stressors may result in the development of fault bars in growing feathers (Jovani and Rower 2016).

Results

Data and data transformation

Annex 1a and 1b (spreadsheets <Annex 1a *Results & graphs – Mass-dependent 30Apr17.xlsx*> and <Annex 1b *Results & graphs – Time-dependent 30Apr17.xlsx*>) provide the data resulting from the INAA analysis, the transformation of the data and graphs. The following provides an explanation of each tab on the spreadsheet:

- The first tab 'INAA RESULTS' presents the INAA results (mg/kg).
- In Annex 1a, the second tab 'DATA mg/kg dw' presents the mass fractions for each element (mg/kg).
- In Annex 1b, the second tab 'RATIOS' calculates the ratios (mg/mm) of element mass per feather length, calculated as (element mass fraction (mg/kg) × feather mass (kg)) / (feather length (mm)). On the results and discussion below, other than for Hg (for which deposition in the feather is surely mass dependent as Hg binds to the keratin), we use mass of each element per millimetre of feather length (following Bortolotti 2010), taking in to account that deposition of many of the elements of interest in feathers may be time dependent (i.e. it relates to the length

of the time period during which the feather is growing and receiving an influx of blood carrying these elements) and not mass dependent (except for those elements that bind structurally to the keratin).
- The third tab 'Specimen AVGs' calculates the average ratio (mg/mm) for each bird, calculated as the average ratio from the two feathers sampled from each bird.
- The fourth tab 'Pivot GEO' calculates the average ratios (mg/mm) for each geographical area (see below for explanation of these areas).
- The fifth tab 'Distr TIME' presents graphs showing the distribution of specimen average ratios over time.
- The sixth tab 'Distr GEO' presents graphs showing the distribution of the specimen average ratios by geographic area (1 = north, 2 = centre, 3 = south, 4 = inner).

To demonstrate the exploration of possible geographic patterns, data were clustered a priori in groups as follows:

1. North (mostly north of 52° 15′ N) (samples 7, 9, 10, 16)
2. Centre (mostly between 52° 9′ N and 52° 15′ N) (samples 4, 6, 11, 14, 15)
3. South (close to coast, mostly south of 52° 9′ N) (samples 1, 2, 8, 13)
4. Inner (east of 4° 45′ E) (samples 3, 5, 12)

It should be noted that there is no particular rationale to these geographic groupings in relation to likely contaminant sources. They have been made simply to demonstrate how such geographical groupings can be made and analysed.

Table 2 Specimens in which rarest elements detected and those with high values of certain rare elements

Year	Specimen	Positive to				High values of			
		Zr	In	Nd	Lu	Ba	La	Ce	Ta
1901	11								
1905	6								
1910	13								
1921	1								
1933	8								
1947	3			1	1				
1947	9	1		1			1	1	
1951	14								
1951	15		1						
1952	5					1			
1955	4								
1956	2	1						1	1
1962	10								
1977	12								
1981	7								
2001	16								

Quantitative statistical analysis

Where the INAA results showed a high frequency (> 30%) of negative values (i.e. uncertainty largely overlapping with zero (Blaauw 2016)), the element in question was excluded from quantitative analysis. Such elements (Si, Cr, Ni, Rb, Sr, Zr, Mo, Pd, Ag, In, Ba, Nd, Tb, Lu, W, Re) were examined only for the presence/absence with the application of an exact Fisher *F* test (but no evident temporal or geographic pattern was found).

Moreover, for those elements (Au, Hf, Yb, Ta, Eu, Sm) where all INAA results had a maximum mass fraction < 0.10 mg/kg, such very low values did not result in any meaningful correlative analysis.

Consequently, we carried out quantitative statistical analyses for the remaining 29 elements (i.e. those with no or < 30% of, negative values): Mg, Al, Cl, K, Ca, Sc, Ti, V, Mn, Fe, Co, Cu, Zn, Ga, As, Se, Br, Cd, Sn, Sb, I, Cs, La, Ce, Dy, Ta, Pt, Hg, Th and U. For those elements, to consider only positive values of mass fraction, any negative values (ranging between − 0.03 and 0.00) were transformed to one tenth of the lowest positive value for the relative element.

Time patterns of elements' mass fractions were explored with linear and quadratic regression. Geographical patterns were explored with ANOVA. All regressions/differences were considered significant if $p < 0.05$. Statistical analyses were performed with the statistical packages SPSS and Prism.

Temporal and geographical pattern

The pattern of average time-dependent ratios (in mg/mm) for each specimen is almost independent of the year of collection. The only significant relations highlighted by the regression analysis were those showing a decrease of As milligrams per millimetre over time ($r = 0.503$, $p = 0.047$*) and an increase of Se milligrams per millimetre over time ($r = 0.576$, $p = 0.19$*) (Fig. 1a, b).

To look for significant patterns related to pollution events during the central period, a quadratic regression model was applied, but none of the elements showed a significant curve with such a maximum.

Notably, some of the rarest elements (Zr, In, Nd, Lu) were only detected in one to two specimens related to the middle period (1947–1962), altogether in four different birds (spec. 2, 3, 9, 15) (Table 2). Possibly due to the small size of the sample, none of these gave a significant result.

Several other rare elements (Ba, La, Ce, Ta) showed exceptionally high mass fraction values in the same period (1947–1952), mostly in the same samples (Table 2). For the same period, the highest values of cadmium (Cd) mass fractions

were detected (Fig. 2). Ce is a dopant in diesel oil (Costantini 2001) and has been measured also in nail clippings (Gomez Aracena et al. 2006). La is also often linked as an indicator for oil burning processes (e.g. Tribalis et al. 2016). The contamination in this period might reflect the aftermath of large-scale pollution during WWII.

The variance analysis highlighted a biased distribution for selenium Se ($F = 4.51$, $p = 0.24$, df 3 and 12), with most high values in bird samples collected in the 'north' area (Fig. 3a) and for mercury Hg ($F = 3.47$, $p = 0.05$, df 3 and 12), with lower values in the 'south' and 'inner' areas (Fig. 3b). However, as already noted, the geographical areas are arbitrary constructs, so this biased distribution should only be considered indicative.

Growth and fault bars

The average growth rate of each feather based on the growth bar measurement was 2.95 mm/day (± 2.8 SD), ranging from 2.27 to 3.42.

The number of fault bars was usually 0 (nine cases) or 1 (five cases), but two birds showed, respectively, five and six fault bars. The average number of fault bars was consequently 1/feather (± 1.83 SD).

The number of fault bars negatively correlated to the size of growth bars on the feathers ($r = -0.584$, $p < 0.01$), consistent with the fact that fault bars may be interpreted as a negative and growth bars a positive proxy of individual bird health status.

Analysis of variance did not evidence any temporal or geographical pattern in the growth bar size (respectively, $F = 0.662$, $p = 0.662$ ns, df 1 and 2 and $F = 0.897$, $p = 0.471$ ns, df 1 and 3). Application of the Kruskal-Wallis test to the fault bar counts did not reveal any temporal or geographical pattern (Kruskal-Wallis statistic, respectively, 3.79 with $p = 0.285$ ns, and 1.25 with $p = 0.535$ ns).

When compared with the measured element mass fractions, growth bar size showed a significant correlation only with the value of *K* (mg/kg, after log transformation $r = 0.61$, $p < 0.01$), all other elements ranging between $r = -0.45$ and $r = 0.42$ (ns). Such a result may possibly be explained by the role of *K* in proper growth in birds (e.g. NRC 1994).

The possible role of other elements on the residual variability of growth bar size was explored by submitting the data to a stepwise regression analysis. Using time-dependent data, a second element, Ca, was maintained in the model (stepwise regression model: $r = 0.685$, $F = 5.747$, $p < 0.05$; see Table 3). Feathers contain $CaCO_3$, and thus, Ca is needed to grow and maintain good feathers. Ca levels may depend both on Ca intake and Ca metabolism, the latter being affected by chemical pollutants such as DDT. Our data show no decrease of Ca level in the samples during the DDT period (1950s–1960s), so we may argue that growth bar size in these specimens is possibly explained only by nutritional status during the moulting period.

On the other hand, the two cases of high number of fault bars are related to single birds with exceptional mass fraction values of Ca (2663 mg/kg vs. a range of 432–1677 for the other 15 birds) or arsenic (460 mg/kg, residual range 7–316).

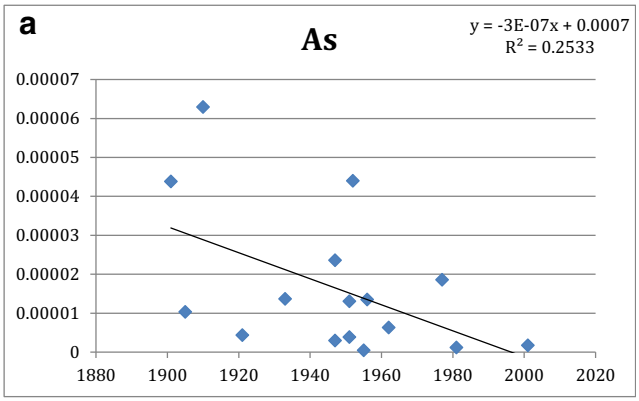
To understand the possible role of single elements in determining the appearance of a single fault bar, the milligram per kilogram values of the five birds showing one bar have been compared with the nine values of the birds showing no bars with Kolmogorov-Smirnov test for small samples. The only element showing a statistically significant difference is As ($Z = -2.2$, $p = 0.029$), but the effect is doubtful, as As mass fraction is significantly influenced by year (mg/kg = 5241.3–2.63*year; $r = 0.549$*). The Kolmogorov-Smirnov test performed on the residuals after the regression is not significant ($Z = -0.33$, $p = 0.797^{ns}$). Cu and Se approach the significance threshold (respectively, $Z = -1.93$, $p = 0.060$ and $Z = -1.80$, $p = 0.83$), suggesting a possible detectable difference with a bigger sample size.

A cumulative effect of combined elements has been tested by means of discriminant analysis, looking for the combination of elements able to separate birds carrying a fault bar from birds with unmarked feathers. A supervised analysis including only Cu and Se identifies a discriminant function correctly classifying 12 out of 14 birds (85.7%), with lower Cu and higher Se, or both, in the presence of a fault bar. An unsupervised analysis with a stepwise variable selection, considering all 29 selected elements but As, only included Ti and Se, correctly classifying 100% of the birds (Table 4, Fig. 4a, b).

While selenium (Se) is a metalloid trace element that birds need in small amounts for good health, high concentration of selenium in bird tissues may result in reproductive failure and adult mortality (Ohlendorf et al. 1987). Our results suggest that relatively higher values of selenium, as revealed by analysis of feathers, may influence the sensitivity of feather follicles to acute stress during feather growth (Jovani and Rohwer 2016), and possibly overall bird condition, either alone or in combination with other microelements. However, our values for Se do not exceed 2 mg/kg dw (3×10^{-7} mg/mm). This lies within the range of 1–4 mg/kg for the background concentration of Se in feathers (with 10% moisture content), according to Ohlendorf and Heinz (2009). An Se concentration of 5 mg/kg has been identified as a threshold warranting further study (USDI 1998).

Discussion

The uptake mechanisms for metals in feathers are inhalation of airborne particles, dry or wet deposition, contamination through direct contact with the environment (water, soil or vegetation), preening and ingestion of contaminated prey.



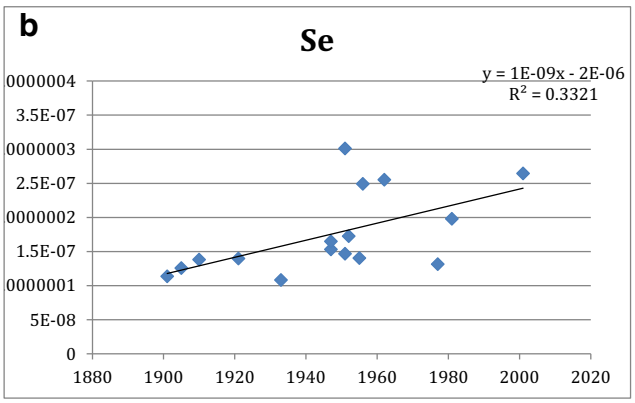


Fig. 1 **a** As, specimen average time-dependent ratios (mg/mm), 1901–2001. **b** Se, specimen average time-dependent ratios (mg/mm), 1901–2001

Most raptors ingest the feathers, furs and bones of their prey and discard excess material as pellets (Jensen et al. 2002). Metals can be lost from feathers through precipitation, bathing in water and rubbing (Goede and de Bruin 1986; Weyers et al. 1988).

Unlike many organic chemicals, metals cannot be easily metabolised into less toxic compounds. Metal pollution affects wildlife indirectly by altering habitat, food chains, community structure and between-species ecological interactions

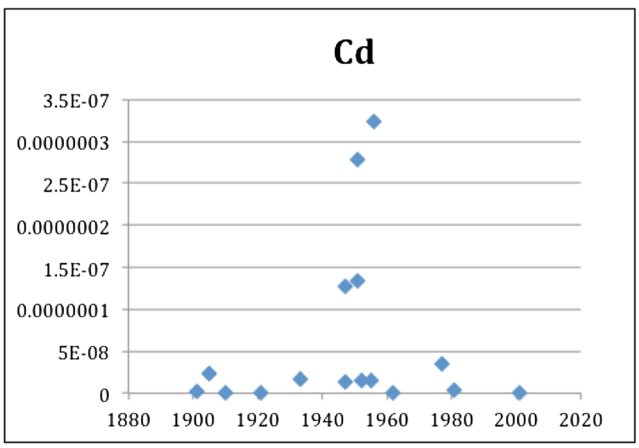


Fig. 2 Cd, specimen average time-dependent ratios (mg/mm), 1901–2001

(Koivula and Eeva 2010) but also with direct effects such as morphological or neurological changes, developmental instability during ontogeny and alteration in biochemical processes (e.g. changes in enzyme activities and free radical levels) (Scheuhammer 1987).

Metals are very reactive and thus toxic to many organisms when interfering with metabolism and important biochemical reactions (e.g. Scheuhammer 1987). The concentration of a given metal in the body depends on the level and duration of metal exposure. The toxicity can be related to the isotope forms of metals as well as the interactions with other xenobiotics (Gochfeld and Burger 1987; Koivula and Eeva 2010).

There is little information available about toxic threshold values of essential and trace elements in bird feathers (Theuerkauf et al. 2015).

For some legacy contaminants such as Hg, a known neurotoxin, there is already a global concern as shown by the recent Minamata Convention on Mercury, a global treaty to protect human health and the environment from the adverse effects of this metal. However, other metals and elements are already, or may prove to be, of societal concern. This study aims to provide data also on these other elements.

Discussion of observed mass fractions of selected elements potentially toxic to raptors

The small number of samples (n = 16) for this study means that we have only three to seven samples per time period and only three to five samples per geographic area. This allows us only to look for some broad, obvious pattern. We make some observations here on values observed for some selected elements of potential toxicity to raptors. Some of these elements (e.g. Hg, Cd) produce chronic effects on biota and are considered hormone disruptors and immunosuppressive agents, as well as causing adverse effects on the nervous and reproductive systems.

Mercury

Our Hg values range from 0.4 to 4.5 mg/kg dw. According to Eisler (1987), Hg concentrations of 5–40 mg/kg in bird feathers are associated with adverse effects, including impaired reproduction (Lodenius and Solonen 2013). Burger and Gochfeld (2000b) similarly considered Hg levels in feathers greater than 5 mg/kg to be associated with adverse reproductive effects in birds. Concentrations of 15 mg/kg Hg may be required for adverse effects in some predatory birds (Spry and Wiener 1991; Wiener and Spry 1996; Burger and Gochfeld 2009). Feather Hg concentrations of 10–15 mg/kg are thought to be harmful to bald eagles *Haliaeetus leucocephalus* (Cristol et al. 2012). However, Bowerman et al. (1994) instead affirmed that Hg (max. 66 mg/kg in feathers) did not affect reproduction of bald eagle in the

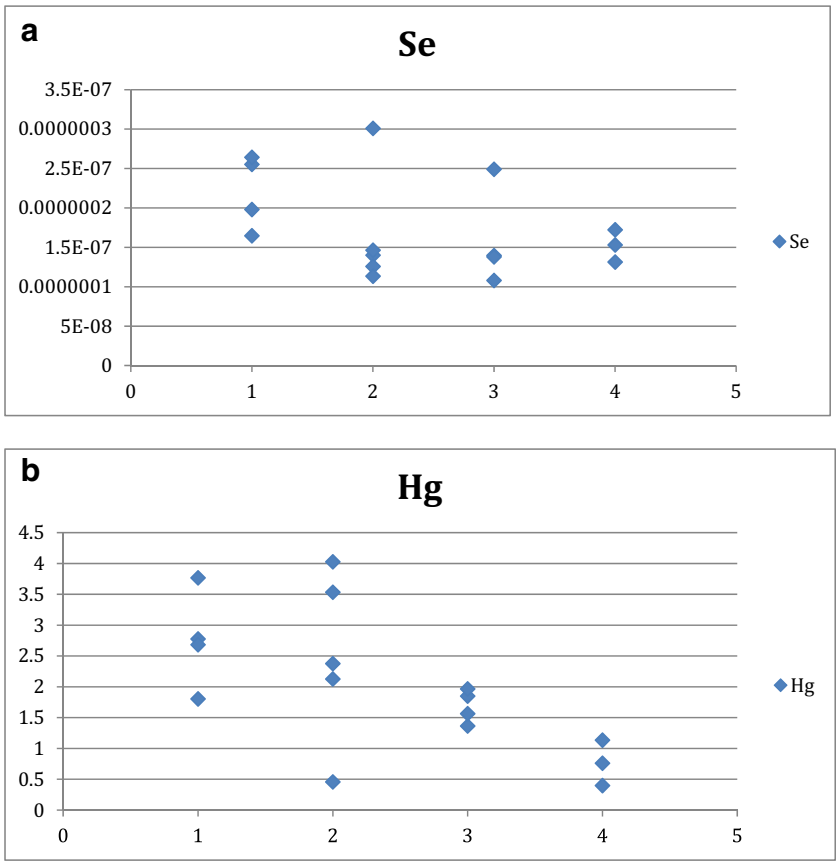


Fig. 3 **a** Geographical distribution of Se, specimen average time-dependent ratios (mg/mm) (1 = north, 2 = centre, 3 = south, 4 = inner). **b** Geographical distribution of Hg, specimen average mass-dependent values (mg/kg dw) (1 = north, 2 = south, 3 = centre, 4 = inner)

Table 3 Stepwise regression analysis applied to all elements (time-dependent data)

Model summary						
Model	*R*	*R*-squared	Adjusted *R*-squared		Std. error of the estimate	
1	0.524	0.275	0.223		0.328	
2	0.685	0.469	0.388		0.292	
ANOVA						
Model		Sum of squares	df	Mean square	*F*	Sig.
1	Regression	0.573	1	0.573	5.310	0.037
	Residual	1.510	14	0.108		
	Total	2.083	15			
2	Regression	0.978	2	0.489	5.747	0.016
	Residual	1.106	13	0.085		
	Total	2.083	15			
Coefficients						
Model		Unstandardised coefficients (*B*)	Std. error	Standardised coefficients (β)	*t*	Sig.
1	Constant	2.580	0.179		14.380	0.000
	K	22,701.6	9851.6	0.524	2.304	0.037
2	Constant	2.773	0.182	15.221	0.000	
	K	27,007.0	8967.3	0.624	3.012	0.010
	Ca	− 1758.7	806.3	− 0.452	− 2.181	0.048

The procedure stopped at the second step, only including K and Ca as explanatory variables for growth bars width as a dependent variable. Step 1 predictors: constant and *K* mass fraction. Step 2 predictors: constant, *K* mass fraction and Ca mass fraction

Table 4 Discriminant analysis results separating birds with feathers not carrying or carrying fault bars

Supervised model (Cu and Se)					
Eigenvalue	% Of variance	Cumulative %	Canonical correlation		
0.600	100	100	0.612		
Standardised canonical discriminant function coefficients					
Se 0.679					
Cu − 0.535					
Unsupervised model					
Eigenvalue	% Of variance	Cumulative %	Canonical correlation		
2.000	100	100	0.817		
Standardised canonical discriminant function coefficients					
Se 1.574					
Ti − 1.410					
Unsupervised model statistics					
Step	Variables entered	Wilks' lambda	df 1	df 2	df 3
1	Se	0.690	1	1	12
2	Ti	0.333	2	1	12
Step	Variables entered	Exact *F*	df 1	df 2	Sig.
1	Se	5.387	1	12	0.039
2	Ti	11.002	2	11	0.002

A supervised model including Se and Cu correctly classified 12 out of 14 birds (85.7%). An unsupervised model selected Se and Ti as explanatory variables, correctly classifying 100% of the birds

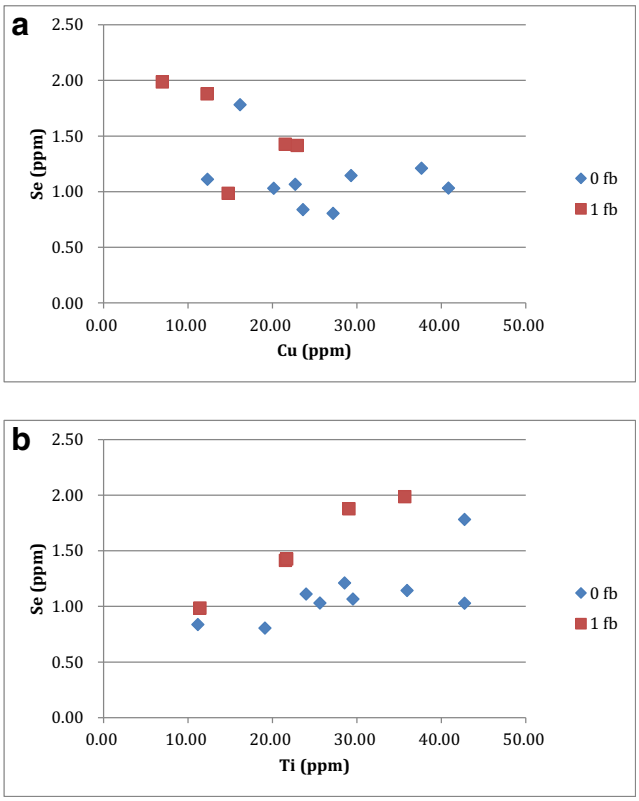


Fig. 4 **a** Se in relation to Cu concentrations (ppm) and number of fault bars. **b** Se in relation to Ti concentrations (ppm) and number of fault bars

Great Lakes region in North America. Our Hg values are below those associated with adverse effects.

Feathers are an excellent resource for retrospective analysis of Hg (Head et al. 2011). Hg is incorporated into the feather at the time of formation where it remains stably bound to keratin and is thus physiologically unavailable (Appelquist et al. 1984). More than 70% of a bird's total body burden of Hg can be found in the feathers, but this percentage can vary across species and with the degree of exposure (Braune and Gaskin 1987; Honda et al. 1986). More than 90% of total feather Hg exists as methyl-Hg (MeHg), the form that is bioaccumulative and bioavailable in aquatic food chains (Thompson and Furness 1989).

Feather Hg concentrations reflect an integration of Hg found in the blood at the time of feather formation (due to recent dietary intake) and Hg in the muscle (accumulated over a longer time period) (Evers et al. 2005). Levels of Hg and other contaminants in bird feathers are strongly correlated to those in bird muscle or liver (e.g. Thompson et al. 1991; Bearhop et al. 2000) suggesting that the analysis of feathers is a viable alternative to estimate the overall contamination status of the birds.

Feathers are an effective and commonly used indicator of overall MeHg body burden. There is a direct relationship between concentration of MeHg in feathers and body burden of MeHg during the time of feather growth. However, feather type (e.g. flight vs. body) and species' moult patterns affect this relationship (Bond et al. 2015). Once grown, feathers are inert, and the Hg bound within the feather is stable (Appelquist et al. 1984). It is therefore possible to examine temporal trends in feather MeHg (and therefore MeHg body burden) using dated museum specimens, allowing retrospective analyses (Bond et al. 2015).

All feathers could possibly be used to screen at high contamination sites, but when specific information on internal tissue concentrations is required, body feathers are probably the most appropriate. Body contour feathers have been shown to be most representative of total body MeHg burden (e.g. Doi and Fukuyama 1983; Furness 1996; Movalli 2000; Vo et al. 2011).

Inorganic Hg (IHg) compounds (usually $HgCl_2$) were used up to the 1930s to preserve museum bird skins, and this can affect the observed valued of Hg using INAA (which does not distinguish organic from IHg). Head et al. (2011) analysed feather samples in museums in the USA and found no feather sample after 1936 had evidence of IHg contamination, but several prior to 1936 did show evidence of contamination. They concluded that one may assume that all Hg found after the 1930s is organic. Hogstad et al (2003) affirmed that MeHg is the only appropriate measure of a birds' Hg body burden when analysing museum specimens with unknown preservation histories.

However, Strekopytov et al. (2017) note that mercury treatments were used in the USA up to the 1980s. They examined Hg in kestrel feathers from four specimens (collected between 1872 and 1954) from the Natural History Museum in London and observed highly elevated values (4409 to 26,960 ppm). They conclude that any value over 1000 ppm is likely to relate to museum preservative treatment.

IHg preservation does not interfere with the selective measurement of MeHg in feathers. While INAA measures only total Hg, it is non-destructive and the proportions of IHg and MeHg can be calculated through subsequent GC-MS analysis. In any case, our samples did not show unusually high levels of Hg for those samples dating from before 1940. It seems safe to assume that the Hg observed is therefore organic and not derived from preservation methods. It is probable that Hg was not used in preservation of these museum samples (van der Mije, pers. comm.). Mashroofeh et al. (2015), analysing Hg content of feathers from Iranian museums, assumed 85–90% of Hg in feathers to be MeHg and therefore analysed for total Hg.

MeHg concentrations in biota have increased due to anthropogenic Hg in many regions. Museum feathers from an endangered seabird from the North Pacific (the black-footed albatross *Phoebastria nigripes*) showed a sharp increase in MeHg concentrations after 1940 (Vo et al. 2011). Dietz et al. (2009) analysed museum samples from marine food webs in the Arctic and observed that Hg concentrations in marine mammals and birds of prey began to rise in the mid to late nineteenth century and accelerated in the twentieth century. The authors attributed > 90% of this rise to anthropogenic Hg sources (Sunderland and Selin 2013).

Hg is probably the metal of most concern for adverse biological effects (Walker et al. 2000), and some recent studies have documented increasing levels of Hg in biota, especially in the Arctic (Mallory et al. 2015). In Sweden, rather constant levels of Hg have been found in birds of prey during the period 1840–1940. After the introduction of alkyl Hg compounds for seed dressing, the concentrations increased significantly amounting to at least 10–20 times the previous level and decreased sharply after the Hg ban in 1966 (Berg et al. 1966; Westermark et al. 1975; Johnels et al. 1979; Wallin 1984). In Finland, Hg levels in feathers of Eurasian sparrowhawks *Accipiter nisus* increased sharply in the 1960s and decreased thereafter slowly. In the Eurasian kestrel, Hg levels were high already in the 1950s but afterwards showed a similar decrease to that in the Eurasian sparrowhawk (Lodenius and Solonen 2013). In Central Norway, Bustnes et al. (2013) observed no temporal changes in Hg levels in tawny owl *Strix aluco* tail feathers ($n = 633$) collected annually between 1986 and 2005.

Published results show rather variable concentrations of Hg in feathers for different species of birds of prey (see Table 1 in the review of Lodenius and Solonen 2013). In terrestrial food chains, Hg concentrations are often lower and Denneman and Douben (1993) concluded that barn owls from a polluted area in the Netherlands are not adversely affected by heavy metals (Lodenius and Solonen 2013).

Selenium

Our samples show an increase in Se values over time, with values reaching almost 2 mg/kg dw (3.01×10^{-7} mg/mm) in some feathers, levels associated with sublethal adverse effects in raptors. Burger and Gochfeld (2009) reported that for Se, feather levels of 3.8 to 26 mg/kg (depending upon species) result in mortality (converted after Burger 1993) and of 1.8 mg/kg result in sublethal adverse effects (after Heinz 1996). Our samples also show that feathers with higher levels of both Se and Ti, or with higher levels of Se and lower levels of Cu, are more likely to exhibit a fault bar, representing metabolic stress.

Ansara-Ross and Wepener (2013) reported selenium levels in owl breast feathers lower than those reported in feathers from the laggar falcon (0.95–5.2 mg/kg dw) (Movalli 2000) and bald eagles (0.8–3.2 mg/kg dw) (Bowerman et al. 1994).

The interrelationship of Hg and Se is complex, as each is known to ameliorate or decrease the toxic effect of the other (Falnoga et al. 2006; Komsta-Szumska et al. 1983). Se binds and inactivates Hg (Fenstad et al. 2017). For this reason, some authors have suggested that the molar ratio of Se to Hg may be an indicator of the potential protective effect of Se on Hg, with a ratio somewhere above 1:1 being protective (Ralston 2008, 2009; Ralston et al. 2008 Burger et al. 2013). While Se acts as an antagonist to methyl Hg, there are indications that the amounts of Se in birds often are insufficient to bind all body Hg (Odsjö et al. 2004; Lodenius and Solonen 2013). In our samples, we found no correlation between Hg mass fractions and Se ratios ($r = 0.245$, $p = 0.360$ ns).

Cadmium

Cd values for some feathers exceed 1 mg/kg dw (3×10^{-7} mg/mm). Such levels may be associated with adverse effects (Burger and Gochfeld 2009). The highest levels of Cd were observed for the central time period 1947–1956 (Fig. 2).

Cd is not a nutritionally essential element for animals (Furness 1996). In addition to being toxic above certain levels, Cd may induce deficiencies of essential elements through competition at active sites in biologically important molecules (Walker et al. 1996). Cd causes sublethal behavioural effects at lower concentrations than lead and mercury (Eisler 1985), but feather levels known to cause adverse effects in the birds themselves have not been determined from laboratory studies. However, conversion factors developed from Burger (1993) suggest that feather levels that are associated with adverse effects would range from 0.1 mg/kg (shearwaters) to 2 mg/kg (terns) (Burger and Gochfeld 2009). In birds, chronic

exposure to Cd reduces reproductive success, e.g. by reducing Ca intake and egg production as well as increased susceptibility to stresses, diseases and oxidative and histopathological damage (Koivula and Eeva 2010).

Jager et al. (1996) analysed buzzards found dead in the Netherlands and found that most non-urban parts of the country were moderately contaminated with Cd, Cu and Pb; the north-south gradient in aerosol deposition and soil content of heavy metals (especially Cd and Pb) was reflected in the common buzzard *Buteo buteo*. Hontelez et al. (1992) reported that several regions of The Netherlands near zinc smelters or dumping grounds were polluted by Cd, Zn, Pb and Cu and analysis of soil, invertebrates and small mammals had shown a considerable contamination of Cd and Pb in particular areas. We found a highly significant correlation between Cd and Zn levels ($r = 0.754$, $p = 0.0007$) suggesting that these two elements may have come from zinc smelters.

Zinc

We found Zn levels in the range 122 to 423 mg/kg dw (1.76 to 5.83×10^{-5} mg/mm). Ansara-Ross and Wepener (2013) found Zn levels in breast feathers of the African grass owl *Tyto capensis* and barn owl *T. alba* in South Africa in the range 16.9–103 and 6.11–34.1 mg/kg dw, respectively. Movalli (2000) found Zn in the range 70–151 mg/kg in feathers of the laggar falcon *Falco biarmicus jugger* in Pakistan.

Zn is a trace element essential for proper body function (Movalli 2000) and is thought also to provide protection against the renal toxicity of Cd (e.g. Hutton 1981). Most animal species are tolerant of excessive intake of Zn (Goede 1985).

Platinum and palladium

We found levels of Pt and Pd ranging from zero to 0.65 and 1.7 mg/kg dw (5.86×10^{-8} and 1.31×10^{-7} mg/mm), respectively, reaching similar levels at the upper end of this range to those found by Jensen et al. (2002) in Sweden. Jensen et al. (2002) found concentrations ranged from 0.3 to 1.8 mg/kg for Pt in three raptor species in Sweden and concluded that there is evidence for increasing platinum group element (PGE) concentrations from 1917 to 1999 in two of the species studied, the peregrine falcon *Falco peregrinus* and Eurasian sparrowhawk, reflecting the introduction of automobile catalyst exhaust systems. They also affirmed that PGE contamination of feathers is predominantly external in the form of nanometre-sized particles externally attached to the feather.

Until recently, it was believed that PGEs are relatively inert, but it has now been shown that these metals undergo environmental transformations into more reactive species (Lustig et al. 1996, 1997, 1998; Rauch and Morrison 2000). The toxicity of Pt has been investigated in detail (Lindell 1997), but less is known about Pd and Rh. Recently, Pd has been increasingly favoured over Pt as the catalytic metal for oxidation in automobile catalysts (e.g. Palacios et al. 2000a, 2000b). This is a concern because it has been shown that Pd is more mobile in the environment than either Pt or Rh (e.g. Jarvis et al. 2001; Moldovan et al. 2001).

Aluminium

We found indications for Al levels in the range of 100 to 800 mg/kg dw (6.42×10^{-6} to 1.06×10^{-4} mg/mm). However, the related NAA signal for can only be selectively be assigned to Al if also the amounts of Si and P have been measured. The latter (P) cannot be done by INAA, and the Si amount is also difficult to measure in feathers. However, both interferences are only relevant for very high mass fraction ratios of P vs. Al and Si vs. Al. It has been assumed that for our study, the interferences can, for a first estimate, be neglected.

Ansara-Ross and Wepener (2013) found Al concentrations in South African owl feathers similar to *T. alba* and *A. noctua* from Belgium (13.7–49.5 mg/kg dw) (Dauwe et al. 2003). Bustnes et al. (2013) analysed different elements in tail feathers (only the lower shaft of the feather was used) of tawny owls in Central Norway from 1986 to 2005. In their samples, Al showed decreasing levels until the mid-1990s, but a considerable increase afterwards (concentrations up to 146.1 mg/kg dry weight). They found this pattern of changes in Al concentrations difficult to interpret. Two strong drivers for increasing Al concentrations in Norwegian soil have been identified: sea salt deposition on land and high production of dissolved organic carbon (Lange et al. 2006). Events with high deposition of sea salt in soil in Norway are related to weather patterns such as storms, and possibly precipitation and snow melt may lead to changes in the soil pH which again may lead to release of Al (Hindar et al. 2004; Lange et al. 2006). It is thus possible that variation in weather may have increased the availability of Al to voles and thence to owls since the mid-1990s.

Al has a ubiquitous natural distribution and use in cooking ware, cans, medications, food processing and water purification ensure considerable human exposure. It is generally held that Al does not have a biological function. A potential role for Al in the pathogenesis of Alzheimer's disease has been postulated (Markesbery et al. 1981).

INAA is a sensitive method that requires no sample dissolution, chemical processing or separations. Hence, the potential for reagent and laboratory contamination is minimal compared to other analytical techniques used for determining trace element content. This is especially important when dealing with an element such as Al, which is abundant in most laboratory ware and in common dust. V is a good indicator for oil burning, and both Al and V are common components of dust

(Bortolotti and Barlow 1985), so it is important to use an effective method of laundering to remove external depositions of Al and V from feathers. Nuclear reaction interferences do exist, but with this technique, they are precisely evaluated by independent determination of the interfering elements in the same sample (Markesbery et al. 1981).

According to some authors, for some elements such as Al and Rb, increased pH in the soil, for example, through changes in precipitation will increase the release to the food chain (Nyholm and Tyler 2000; Steinnes 2009; Bustnes et al. 2013).

Increased atmospheric deposition of sulphuric and nitrogenous compounds has been demonstrated in The Netherlands (e.g. Berendse and Aerts 1987; Roelofs et al. 1996), which alters the chemical status of the soil by increasing nitrogen availability, inducing soil acidification and mobilising toxic metal ions (e.g. Houdijk et al. 1993; De Graaf et al. 1998; Roem and Berendse 2000).

Rubidium

We found Rb levels from 0 to 1.975 mg/kg dw (6.79×10^{-8} to 1.21×10^{-7} mg/mm). This compares with values of 0.018 to 1.24 mg/kg in tawny owl 1986–2005 in the area surrounding Trondheim, Norway (Bustnes et al. 2013).

Rb is a metal that occurs naturally in soil and with high mobility thus readily available for plant uptake (Nygård et al. 2012). In Norway in the same species, the same authors found that Rb increased by 61% over the 20-year period, and as Nyholm and Tyler (2000) found that low pH in forest soil increases uptake of Rb in plants, which is then propagated through the food web, these authors affirmed that both increased Al and Rb in tawny owl feathers may be an indication of lower pH values in the soil in the owl habitats.

Arsenic

Our samples show relatively high values for As for samples for the period from 1900 to 1930 (14.4 to 596.9 mg/mm dw, 4.35×10^{-6} to 6.3×10^{-5} mg/mm), which may presumably be attributed to the use of arsenic in the early decades of the twentieth century to treat and preserve museum specimens. Indeed, Desjardins (2016) using an XRF analyser found high levels of As in a range of mounted specimens and skins of various taxa in the collection of Naturalis (of 69 specimens examines, 33 had As levels over 1000 ppm). It is possible that relatively high levels of Ag and Au in these same samples are due to the presence of impurities in the arsenic compounds used to treat and preserve these feathers.

As, especially in its inorganic forms, can cause the death of an individual, produce sublethal effects and affect reproduction on birds. It accumulates mostly in body tissues such as liver and kidney, in particular in top predators (e.g. barn owl, sparrowhawk, kestrel) (Koivula and Eeva 2010).

Differences between species in accumulation of toxic elements have been explained by diet (Bezel et al. 1994). Erry et al. (1999) suggested that As burdens can vary between raptor species according to variation in their diet and variation in the ability to metabolise and excrete As.

Antimony

Our levels are in the range 0 to 11.06 mg/kg dw (0 to 8.55×10^{-7} mg/mm). Ansara-Ross and Wepener (2013) found Sb levels in breast feathers of African grass owl and barn owl in South Africa in the range 0–0.724 and 0–1.29 mg/kg dw, respectively. In The Netherlands, Denneman and Douben (1993) found a level of 22.1 mg/kg dw in barn owl feathers from a contaminated site and a level of 0.34 mg/kg dw for a relatively uncontaminated site.

Chromium

Cr values for 14 of the 32 feathers are above 3 mg/kg dw (5×10^{-7} mg/mm) with a highest value 20.65 mg/kg dw (2.3×10^{-6} mg/mm). Levels above 2.8 mg/kg may be associated with adverse effects (Burger and Gochfeld 2000a). High amounts of Cr in a diet have been shown to cause altered growth patterns and reduction in survival, e.g. in the offspring of American black duck *Anas rubripes* (Eisler 2000).

Vanadium

Our values for V were in the range of 0.086 to 3.3 mg/kg dw (2.1×10^{-8} to 3.87×10^{-7} mg/mm). Bond and Lavers (2011) found vanadium levels of 0.21–0.92 mg/kg in breast feathers of flesh-footed shearwaters *Puffinus carneipes*. Ansara-Ross and Wepener (2013) found 0.14 mg/kg dw in breast feathers of South African owls.

Little is known about the toxicity of V in birds, but histopathological changes in the intestine, kidney, liver and heart have been detected in mallards and Canada geese. Blood chemistry changes, intestinal haemorrhage, indications of hepatic oxidative stress, liver lesions and kidney congestion have been shown in chronic feeding trials. Even low levels of V can diminish egg production and cause alteration in metabolic rates (laying hens and mallards) (Koivula and Eeva 2010).

Chlorine and bromine

Haskins et al. (2013) using INAA found significant Cl and Br concentrations in feather samples from a turkey vulture *Cathartes aura* in Canada, being 1600–7200 and 11–80 mg/kg, respectively (Haskins et al. 2013). Our levels were

comparable to these levels, in the range of 500 to 2760 mg/kg dw (1.5×10^{-5} to 3×10^{-4} mg/mm) and 3.6–160 mg/kg dw (5.14×10^{-7} to 1.73×10^{-5} mg/mm) for Cl and Br, respectively.

INAA is a convenient and quick method and indeed one of the very few analytical methods currently available for measuring the total amounts of Cl, Br and I. This characteristic has been used to assess simultaneously the amounts of extractable organochloronated (EOCl), extractable organobrominated (EOBr) and extractable organoiodinated compounds (EOI) in one extraction (e.g. Xu et al. 2011).

Conclusions

This is the first study in Europe to use museum feather samples to analyse such an extensive range of elements, including many elements for which there are no or few published data. The study demonstrates the potential to exploit the raptor collection at Naturalis Biodiversity Centre and other similar collections worldwide to explore temporal and geographic variation in levels of selected elements, including some important contaminants such as Hg and Cd. It has provided for the first time data that can contribute towards the development of reference values for contaminants in a raptor species in the Netherlands. It has demonstrated, inter alia, that

- *Mass fraction values (mg/kg) and ratios (mg/mm) can be obtained for selected elements* (including elements of high societal concern, such as Hg, Cd and Cs) *using INAA with very small samples* (two breast feathers), such that sampling does not harm the aesthetic or research value of the sampled specimens.
- *INAA analysis can detect very low mass fractions of elements* in one to two breast feathers and can provide values for a *wide range of elements in a single assay.*
- Data from this study suggest that there are mass fractions and ratios of some elements (e.g. Cr, Cd, Se) for certain dates and locations that are *at levels likely to have had adverse effects on kestrel health and reproduction*.
- *It would be possible, given a larger, statistically valid sample size, to examine temporal patterns* for the mass fraction values (mg/kg) and ratios (mg/mm) of selected elements in a given geographical area (e.g. the whole of Netherlands, or selected regions within The Netherlands).
- *The analysis of museum feather specimens using INAA can provide reference values for mass fractions (mg/kg) and/or ratios (mg/mm) of selected elements* (including elements of high societal concern such as Hg and Cd), against which to assess the mass fractions of these same elements in present-day living raptor populations.
- *Such contaminant studies are best suited to common, widely distributed raptor species* which meet the following criteria: species (and sex) that is resident and with high territorial fidelity (as opposed to migratory or vagrant) such that any contaminants found may reasonably be assumed to reflect exposure in the location from which the specimen was originally collected; relatively common and widely distributed species with a large number of specimens in the museum collection, allowing for statistically robust sampling for selected time periods and/or selected geographical areas; likely dietary exposure to a range of contaminants of societal concern; species that is widespread in Europe and relatively frequently studied, providing data with which to compare results; possibility of sampling present-day populations to compare present-day with historical contaminant levels.

In conclusion, this study has shown that INAA, using very small feather samples from museum specimens, delivers data on element mass fractions (mg/kg) and ratios (mg/mm) for a wide range of elements in a single assay, allowing the testing of various hypotheses related to patterns of contamination in time and space, and providing reference values against which to assess contaminant levels in present-day living raptor populations. This can have great relevance for the monitoring of elements of high societal concern, including mercury and cadmium.

Enhanced collaboration between ecotoxicologists and museums (and, indeed, environmental specimen banks and other collections) on contaminants in birds offers an important new direction for research using these collections (Movalli et al. in press). With planning, use of birds (and indeed other higher taxa) as biomonitors can be coupled with standard museum processing and preservation to simultaneously achieve very different scientific gains (Rocque and Winker 2005). Increasingly refined analytical abilities will continue to enhance the usefulness of museum specimens for contaminant studies as new techniques such as INAA reduce the amount of sample required for analyses.

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References

Ansara-Ross TM, Wepener V (2013) The use of feathers in monitoring bioaccumulation of metals and metalloids in the South African endangered African grass-owl (*Tyto capensis*). Ecotoxicology 22: 1072–1083

Appelquist HS, Asbirk S, Drabaek I (1984) Mercury monitoring—mercury stability in bird feathers. Mar Pollut Bull 15(1):22–24

Bearhop S, Waldron S, Thompson D, Furness R (2000) Bioamplification of mercury in Great Skua *Catharacta skua* chicks: the influence of

trophic status as determined by stable isotope signatures of blood and feathers. Mar Poll Bull 40(92):181–185

Berendse F, Aerts R (1987) Competition between *Erica tetralix* L. and *Molinia caerulea* L. Moench as affected by the availability of nutrients. Acta Oecol-Oec Plant 5:3–14

Berg W, Johnels A, Sjöstrand B, Westermark T (1966) Mercury content in feathers of Swedish birds from the past 100 years. Oikos 17:71–83

Bezel VS, Bolshakov VN, Vorobeichik EL (1994) Populyatsionnayaekotoksikologiya' (Population Ecotoxicology). Nauka, Moscow

Blaauw M (1994) The holistic analysis of gamma-ray spectra in instrumental neutron activation analysis. Nucl Instrum Methods A353: 269–271

Blaauw M (2016) Detection limits should be a thing of the past in gamma-ray spectrometry in general as well as in neutron activation analysis. J Radioanal Nucl Ch 309(1):39–43

Bode P, Blaauw M (2012) Performance and robustness of a multi-user, multi-spectrometer system for INAA. J Radioanal Nucl Ch 291: 299–305

Bond AL, Hobson KA, Branfireun BA (2015) Rapidly increasing methyl mercury in endangered ivory gull (*Pagophila eburnea*) feathers over a 130 year period. Proc R Soc B 282(1805):20150032

Bond AL, Lavers JL (2011) Trace element concentrations in feathers of flesh-footed shearwaters *(Puffinus carneipes*) from across their breeding range. Arch Environ Con Tox 61:318–326

Bortolotti GR (2010) Flaws and pitfalls in the chemical analysis of feathers: bad news–good news for avian chemoecology and toxicology. Ecol Appl 20(6):1766–1774

Bortolotti GR, Barlow JC (1985) Neutron activation analysis of bald eagle feathers: analytical precision and sources of sampling variation. Can J Zool 63:2707–2718

Bortolotti GR, Barlow JC (1986) Potential use of feather chemistry as an indicator of relative growth. Wilson Bull 98(4):516–525

Bortolotti GR, Marchant TA, Blas J, German T (2008) Corticosterone in feathers is a long-term, integrated measure of avian stress physiology. Funct Ecol 22(3):494–500

Bourgeon SE, Leat HK, Magnusdottir E, Fisk AT, Furness RW, Strom H, Hanssen SA, Petersen AE, Olafsdottir K, Borga K, Gabrielsen GW, Bustnes JO (2012) Individual variation in biomarkers of health: influence of persistent organic pollutants in Great skuas (*Stercorarius skua*) breeding at different geographical locations. Environ Res 118:31–39

Bowerman W, Evans E, Giesy J, Postupalsky S (1994) Using feathers to assess risk of mercury and selenium to bald eagle reproduction in the Great-Lakes region. Arch Environ Con Tox 27:294–298

Braune BM, Gaskin DE (1987) Mercury levels in Bonaparte's gulls (*Larus philadelphia*) during autumn molt in the Quoddy region, New Brunswick, Canada. Arch Environ Con Tox 16:539–549

Bryan AL, Brant HA, Jagoe CH, Romanek CS, Brisbin IL (2012) Mercury contamination in nestling wading birds relative to diet in the southeastern United States: a stable isotope analysis. Arch Environ Con Tox 63:14

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

Burger J, Gochfeld M (2000a) Metals levels in feathers of 12 species of seabirds from Midway Atoll in the northern Pacific Ocean. Sci Total Environ 257:37–52

Burger J, Gochfeld M (2000b) Metals in albatross feathers from midway atoll: influence of species, age, and nest location. Environ Res 82: 207–221

Burger J, Gochfeld M (2009) Comparison of arsenic, cadmium, chromium, lead, manganese, mercury and selenium in feathers in bald eagle (*Haliaeetus leucocephalus*), and comparison with common eider (*Somateria mollissima*), glaucous-winged gull (*Larus glaucescens*), pigeon guillemot (*Cepphus columba*), and tufted puffin (*Fratercula cirrhata*) from the Aleutian Chain of Alaska. Environ Monit Assess 152:357–367

Burger J, Jehl JR, Gochfeld M (2013) Selenium:mercury molar ratio in eared grebes (*Podiceps nigricollis*) as a possible biomarker of exposure. Ecol Indic 34:60–68

Bustnes JO, Bårdsen BJ, Bangjord G, Lierhagen S, Yoccoz N (2013) Temporal trends (1986–2005) of essential and non-essential elements in a terrestrial raptor in northern Europe. Sci Total Environ 2013:458–460

Campbell LM, Drevnik PE (2015) Use of catalogued long-term biological collections and samples for determining changes in contaminant exposure to organisms. In: Blais JM, Rosen MR, Smol JP (eds) Environmental contaminants. Using natural archives to track sources and long-term trends of pollution, Springer, Netherlands, pp 431–459

Casagrande S, Nieder L, Di Minin E, La Fata I, Csermely D (2008) Habitat utilization and prey selection of the kestrel *Falco tinnunculus* in relation to small mammal abundance. Ital J Zool 75(4):401–409

Clarkson TW (1994) The toxicity of mercury and its compounds. In: Watras CJ, Huckabee JW (eds) Mercury pollution: integration and synthesis. Lewis Publishers, Boca Raton, pp 631–641

Corliss WR (1963) Neutron activation analyses. U.S. Atomic Energy Comm. Div. Tech. Info, Oak Ridge, Tennessee

Cortes Toro E, De Goeij J, Bacso J, Cheng YD, Kinova L, Matsubara J et al (1993) The significance of hair mineral analysis as a means for assessing internal body burdens of environmental pollutants: results from an IAEA Co-ordinated Research Programme. J Radioanal Nucl Ch 167(2):413–421

Costantini MG (2001) Evaluation of human health risk from cerium added to diesel fuel. Health Effects Institute 9:64

Cristol DA, Mojica EK, Varian-Ramos CW, Watts BD (2012) Molted feathers indicate low mercury in bald eagles of the Chesapeake Bay, USA. Ecol Indic 18:20–24

Cuvin-Aralar MLA, Furness RW (1991) Mercury and selenium interaction: a review. Ecotox. Environ Safe 21:348–364

Dauwe T, Bervoets L, Pinxten R, Blust R, Eens M (2003) Variation of heavy metals within and among feathers of birds of prey: effects of moult and external contamination. Environ Pollut 124:429–436

Dauwe T, Jaspers V, Covaci A, Schepens P, Eens M (2005) Feathers as a nondestructive biomonitor for persistent organic pollutants. Environ Tox Chem 24(2):442–449

De Graaf MCC, Bobbink R, Roelofs JGM, Verbeek PJM (1998) Differential effects of ammonium and nitrate on three heathland species. Plant Ecol 135:185–196

Desjardins RB (2016) Arsenic and pre-1970s museum specimens: using a hand-held XRF analyzer to determine the prevalence of arsenic at Naturalis Biodiversity Center. Collection Forum 2016 30(1):7–14

Devine T, Peterle TK (1968) Possible differentiation of natal areas of North American waterfowl by neutron activation analysis. J Wildlife Man 32(2):274–279

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

Dietz R, Riget FF, Boertmann D, Sonne C, Olsen MT, Fjeldsa J, Falk K, Kirkegaard M, Egevang C, Asmund G, Wille F, Moller S (2006) Time trends of mercury in feathers of West Greenland birds of prey during 1851-2003. Environ Sci Technol 40(19):5911–5916

Dietz R, Outridge PM, Hobson KA (2009) Anthropogenic contributions to mercury levels in present-day Arctic animals—a review. Sci Total Environ 407:6120–6131

Doi R, Fukuyama Y (1983) Metal content in feathers of wild and zoo-kept birds from Hokkaido, 1976-1978. Bull Environ Con Tox 31:1–8

EC (1992) Convention on the Protection of the Marine Environment of the Baltic Sea Area, 1992. OJ L 73, 16.3.1994, p. 20–45

EC (2000) Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. OJ L327, 22.12.2000, p. 1–73

EC (2001) Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use. OJ L 311, 28/11/2004

EC (2008) Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive). OJ L 164, 25.6.2008, p. 19–40

EC (2009). Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market. OJ L 309, 24.11.2009, p. 1–50

Eisler R (1985) Cadmium hazards to fish, wildlife and invertebrates: a synoptic review. Technical report (No. PB-86-116779/XAB). Patuxent Wildlife Research Center, Laurel, MD (USA)

Eisler R (1987) Mercury hazards to fish, wildlife, and invertebrates: a synoptic review (No. PB-87-179115/XAB; BIOLOGICAL-85 (1.10)). Patuxent Wildlife Research Center, Laurel, MD (USA)

Eisler R (2000) Handbook of chemical risk assessment: health hazards to humans, plants, and animals, three volume set (Vol. 1). CRC press

Erry BV, Macnair MR, Meharg AA, Shore RF, Newton I (1999) Arsenic residues in predatory birds from an area of Britain with naturally and anthropogenically elevated arsenic levels. Env Poll 106:91–95

Espín S, García-Fernández AJ, Herzke D, Shore RF, van Hattum B, Martínez-López E, Couerdassier M, Eulaers I, Frisch C, Gómez-Ramírez P, Jasper V, Krone O, Duke G, Helander B, Mateo R, Movalli P, Sonne C, Van den Brink NW (2016a) Tracking pan-continental trends in environmental contamination using sentinel raptors—what types of samples should we use? Ecotoxicology 25(4):777–801

Espín S, García-Fernández AJ, Herzke D, Shore RF, van Hattum B, Martínez-López E, Couerdassier M, Eulaers I, Frisch C, Gómez-Ramírez P, Jasper V, Krone O, Duke G, Helander B, Mateo R, Movalli P, Sonne C, Van den Brink NW (2016b) Summary: sampling and contaminant monitoring protocol for raptors (available in 9 languages) Available at: http://www.eurapmon.net/results/contaminant-monitoring-protocol

EU (1998) Council Decision 98/249/EC of 7 October 1997 on the conclusion of the Convention for the protection of the marine environment of the North-East Atlantic (Paris Convention). OJ L 104, 3.4.1998

EU (2012) Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products. OJ L 167, 27.6.2012, p. 1–123

EU (2017) Council Decision (EU) 2017/939 of 11 May 2017 on the conclusion on behalf of the European Union of the Minamata Convention on Mercury. OJ L 142, 2.6.2017, p. 4–39

Eulaers I, Covaci A, Herzke D, Eens M, Sonne C, Moum T, Schnug L, Hanssen SA, Johnsen TV, Bustnes JO, Jaspers VLB (2011) A first evaluation of the usefulness of feathers of nestling predatory birds for non-destructive biomonitoring of persistent organic pollutants. Environ Int 37(3):622–630

Evers DC, Burgess NM, Champoux L, Hoskins B, Major A, Goodale WM, Taylor RJ, Poppenga R, Daigle T (2005) Patterns and interpretation of mercury exposure in freshwater avian communities in northeastern North America. Ecotoxicology 14:193–221

European Commission (2004a) Directive 2004/28/EC of the European Parliament and of the Council of 31 March 2004 amending Directive 2001/82/EC on the Community code relating to veterinary medicinal products. OJ L 136, 30.4.2004, p. 58–84

European Commission (2004b) Regulation (EC) no 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency. OJ L 136, 30.4.2004, p. 1–33

European Commission (2004c) Regulation (EC) no. 850/2004 of the European Parliament and of the Council of 29 April 2004 on Persistent Organic Pollutants and Amending Directive 79/117/EC http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri¼CELEX:32004R0850:EN:NOT

European Commission (2004d) Regulation (EC) No 850/2004 of the European Parliament and of the Council of 29 April 2004 on persistent organic pollutants and amending Directive 79/117/EEC. OJ L 158, 30.4.2004, p. 7–49

European Commission (2006) Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the registration, evaluation, authorisation and restriction of chemicals (REACH) http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri¼CELEX:32006R1907:EN:NOT

European Commission (2007) Community implementation plan for the Stockholm Convention on Persistent Organic Pollutants. Staff Working Document SEC(2007)341 http://ec.europa.eu/environment/pops/pdf/sec_2007_341.pdf

European Commission (2012) Regulation (EC) no. 528/2012 of the European Parliament and of the Council concerning the making available on the market and use of biocidal products. http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32012R0528

Falnoga I, Tusek-Znidaric M, Stegnar P (2006) The influence of long-term mercury exposure on selenium availability in tissues: an evaluation of data. Biometals 19:283–294

Fasola M, Movalli PA, Gandini C (1998) Heavy metal, organochlorine pesticide, and PCB residues in eggs and feathers of herons breeding in northern Italy. Arch Environ Con Tox 34(1):87–93

Fenstad AA, Bustnes JO, Lierhagen S, Gabrielsen KM, Öst M, Jaatinen K et al (2017) Blood and feather concentrations of toxic elements in a Baltic and an Arctic seabird population. Mar Pollut Bull 114(2):1152–1158

Furness RW (1993) Birds as monitors of pollutants. In: Furness RW, Greenwood JJD (eds) Birds as monitors of environmental change. Chapman and Hall, London, pp 86–143

Furness RW (1996) Cadmium in birds. In: Nelson Beyer W, Heinz GH, Redmon-Norwood AW (eds) Environmental Contaminants in Wildlife – Interpreting Tissue Concentrations. CRC Press, Boca Raton, FL, pp 389–404

Furness RW, Mulhead SJ, Woodburn M (1986) Using bird feathers to measure mercury in the environment: relationships between mercury content and moult. Mar Pollut Bull 1:27–30

García-Fernández AJ, Calvo JF, Martínez-López E, Maria-Mojica P, Martínez JE (2008) Raptor ecotoxicology in Spain: a review on persistent environmental contaminants. Ambio 37(6):432–439

García-Fernández AJ, Espín S, Martínez-López E (2013) Feathers as a biomonitoring tool of polyhalogenated compounds: a review. Environ Sci Technol 47(7):3028–3043

Gjershaug JO, Kalas JA, Nygård T, Herzke D, Folkestad AO (2008) Monitoring of raptors and their contamination levels in Norway. Ambio 37(6):420–424

Gochfeld M, Burger J (1987) Heavy metal concentrations in the liver of three duck species: influences of species and sex. Environ Pollut 45:1–15

Goede AA (1985) Mercury, selenium, arsenic and zinc in waders from the Dutch Wadden Sea. Environ Pollut Ser A 37:287–309

Goede AA, de Bruin M (1986) The use of feathers for indicating heavy metal pollution. Environ Monit Assess 7:249–256

Gomez-Aracena J, Riemersma RA, Gutiérrez-Bedmar M, Bode P, Kark JD, Garcia-Rodríguez A et al (2006) Toenail cerium levels and risk of a first acute myocardial infarction: the EURAMIC and heavy metals study. Chemosphere 64(1):112–120

Gómez-Ramírez P, Martínez-López E, María-Mojica P, León-Ortega M, García-Fernández A (2011) Blood lead levels and δ-ALAD inhibition in nestlings of Eurasian eagle owl (*Bubo bubo*) to assess lead exposure associated to an abandoned mining area. Ecotoxicology 20:131–138

Gómez-Ramírez P, Shore RF, van den Brink NW, van Hattum B, Bustnes JO, Duke G, Fritsch C, García-Fernández AJ, Helander BO, Jaspers V, Krone O, Martínez-López E, Mateo R, Movalli P, Sonne C (2014) An overview of existing raptor contaminant monitoring activities in Europe. Environ Int 67:12–21

Greenberg R, Bode P, De Nadai Fernandes E (2011) Neutron activation analysis: a primary method of measurement. Spectrchim Acta B 66: 193–241

Grubb TC (1989) Ptilochronology: feather growth bars as indicators of nutritional status. Auk 106(2):314–320

Grubb TC (1991) A deficient diet narrows growth bars on induced feathers. Auk 108(3):725–727

Grubb TC (2006) Ptilochronology: feather time and the biology of birds (No.15). Oxford University Press

Grubb TC, Yosef R (1994) Habitat-specific nutritional condition in loggerhead shrikes (*Lanius ludovicianus*): evidence from Ptilochronology. Auk 111(3):756–759

Haskins SD, Kelly DG, Weir RD (2013) Trace element analysis of turkey vulture (*Cathartes aura*) feathers. J Radioanal Nucl Chem 295: 1331–1339

Head JA, DeBofsky A, Hinshaw J, Basu N (2011) Retrospective analysis of mercury content in feathers of birds collected from the state of Michigan (1895-2007). Ecotoxicology 20(7):1636–1643. https://doi.org/10.1007/s10646-011-0738-6

Heinz GH (1996) Selenium in birds. In: Beyer WM, Heinz GH, Redmon-Norwood RW (eds) Environmental contaminants in wildlife: interpreting tissue concentrations. Lewis Publishers, Boca Raton, SETAC Sp P, pp 447–458

Helander B, Bignert A, Asplund L (2008) Using raptors as environmental sentinels: monitoring the white-tailed sea eagle *Haliaeetus albicilla* in Sweden. Ambio 37(6):425–431

Henny CJ, Blus LJ, Hoffman DJ, Sileo L, Audet DJ, Snyder MR (2000) Field evaluation of lead effects on Canada geese and mallards in the Coeur d'Alene River Basin, Idaho. Arch Environ Con Tox 39:97–112

Henny CJ, Hill EF, Hoffman DJ, Spalding MG, Grove RA (2002) Ninetheenth century mercury: hazard to wading birds and cormorants of the Carson River, Nevada. Ecotoxicology 11:213–231

Hindar A, Tørseth K, Henriksen A, Orsolini Y (2004) The significance of the North Atlantic oscillation (NAO) for sea-salt episodes and acidification-related effects in Norwegian rivers. Environ Sci Technol 38:26–33

Hogstad O, Nygård T, Gatzschmann P, Lierhagen S, Thingstad PG (2003) Bird skins in museum collections: are they suitable as indicators of environmental metal load after conservation procedures? Environ Mon Assess 87(1):47–56

Honda K, Min DY, Tatsukawa R (1986) Organ and tissue distribution of heavy metals, and age related changes in the eastern great white egret *Egretta alba modesta* in Korea. Arch Environ Con Tox 15: 185–197

Hontelez L, Van den Dungen H, Baars A (1992) Lead and cadmium in birds in the Netherlands: a preliminary survey. Arch Environ Contam Toxicol 23(4):453–456

Houdijk ALFM, Verbeek PJM, Van Dijk HFG, Roelofs JGM (1993) Distribution and decline of endangered herbaceous heathland species in relation to the chemical composition of the soil. Plant Soil 148:137–143

Hutton M (1981) Accumulation of heavy metals and selenium in three seabird species from the United Kingdom. Environ Pollut A 26(2): 129–145

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

Jarvis KE, Parry SJ, Piper JM (2001) Temporal and spatial studies of automobile catalyst-derived platinum, rhodium, and palladium and selected vehicle-derived trace elements in the environment. Environ Sci Technol 35:1031–1036

Jaspers VLB, Voorspoels S, Covaci A, Eens M (2006) Can predatory bird feathers be used as a non-destructive biomonitoring tool of organic pollutants? Biol Lett 2(2):283–285

Jensen KH, Rauch S, Morrison GM, Lindberg P (2002) Platinum group elements in the feathers of raptors and their prey. Arch Environ Con Tox 4:338–347

Johnels A, Tyler G, Westermark T (1979) A history of mercury levels in Swedish fauna. Ambio 8:160–168

Jovani R, Rohwer S (2016) Fault bars in bird feathers: mechanisms, and ecological and evolutionary causes and consequences. Biol Rev Camb Philos Soc. https://doi.org/10.1111/brv.12273

Kenntner N, Krone O, Altenkamp R, Tataruch F (2003) Environmental contaminants in liver and kidney of free-ranging northern goshawks (*Accipiter gentilis*) from three regions of Germany. Arch Environ Con Tox 45(1):128–135

Koivula MJ, Eeva T (2010) Metal-related oxidative stress in birds. Environ Poll 158:2359–2370

Komsta-Szumska E, Reuhl K, Miller D (1983) The effect of methylmercury on the distribution and excretion of selenium by the guinea pig. Arch Toxicol 54(4):303–310

Koskimies P (1989) Birds as a tool in environmental monitoring. Ann Zool Fenn 26:153–166

Lange H, Solberg S, Clarke (2006) Aluminium dynamics in forest soil waters in Norway. Sci Total Environ 367:942–957

Lodenius M, Solonen T (2013) The use of feathers of birds of prey as indicators of metal pollution. Ecotoxicology 22:1319–1334

Lustig S, Zang S, Beck W, Schramel P (1998) Dissolution of metallic platinum as water soluble species by naturally occurring complexing agents. Mikrochim Acta 129:189–194

Lustig S, Zang S, Michalke B, Beck W, Schramel P (1997) Influence of micro-organisms on the dissolution of metallic platinum emitted by automobile catalytic converters. Environ Sci Pollut Res 4:141–145

Lustig S, Zang S, Michalke B, Schramel P, Beck W (1996) Transformation behaviour of different platinum compounds in a clay-like humic soil: speciation investigations. Sci Total Environ 188:195–204

Mallory ML, Braune BM, Provencher JF, Callaghan DB, Gilchrist HG, Edmonds ST, Allard K, O'Driscoll NJ (2015) Mercury concentrations in feathers of marine birds in Arctic Canada. Mar Poll Bull 98: 308–313

Markandya A, Taylor T, Long A, Murty MN, Murty S, Dhavala K (2008) Counting the cost of vulture decline—an appraisal of the human health and other benefits of vultures in India. Ecol Econ 67:194–204

Markesbery WR, Ehmann WD, Hossain TIM, Alauddin M, Goodin DT (1981) Instrumental neutron activation analysis of brain aluminium in Alzheimer disease and ageing. Ann Neurol 10:511–516

Mashroofeh A, Bakhtiari AR, Ghobeishavi A, Ahmadpour M, Asadi A, Ahmadpour M et al (2015) Mercury levels in avian feathers from different trophic levels of eight families collected from the northern region of Iran. Environ Monit Assess 187(5):275

Møller AP, Erritzøe J, Nielsen JT (2009) Frequency of fault bars in feathers of birds and susceptibility to predation. Biol J Linn Soc 97:334–345

Moldovan M, Rauch S, Gómez M, Palacios MA, Morrison GM (2001) Bioaccumulation of Pt, Pd and Rh from urban particulates and sediments by the freshwater isopod *Asellus aquaticus*. Wat Res 35: 4175–4183

Movalli P (1993) Il contenuto di metalli e di pesticidi organo-clorurati nell'avifauna nidificante come potenziale parametro di valutazione ecotossicologica. Ph.D. thesis. University of Milan, Italy. (Italian)

Movalli P (2000) Heavy metals and other residues in feathers of lagger falcon *Falco biarmicus jugger* from six districts in Pakistan. Environ Pollut 109:267–275

Movalli P, Duke G, Osborn D (2008a) Introduction to monitoring for and with raptors. Ambio 37(6):395–396

Movalli P, Lo Valvo M, Pereira MG, Osborn D (2008b) Organochlorine pesticides and polychlorinated biphenyl congeners in lanner *Falco biarmicus feldeggii* Schlegel chicks and lanner prey in Sicily, Italy. Ambio 37(6):445–451

Movalli P, Dekker R, Koschorreck J, Treu G (in press) Bringing together raptor collections in Europe for support of contaminant research and monitoring in relation to EU and regional chemical regulations. **Environ Sci and Poll Res**

Newton I, Wyllie I, Asher A (1993) Long-term trends in organochlorine and mercury residues in some predatory birds in Britain. Environ Pollut 79:143–151

Nygård T, Steinnes E, Royset O (2012) Distribution of 32 elements in organic surface soils: contributions from atmospheric transport of pollutants and natural sources. Water Air Soil Pollut 223:699–713

Nyholm NEI, Tyler G (2000) Rubidium content of plants, fungi and animals closely reflects potassium and acidity conditions of forest soil. For Ecol Manag 134:89–96

NRC (1991) Animals as sentinels of environmental health hazards. Committee on Animals as Monitors of Environmental Hazards. Washington, DC: National Academy Press

NRC (1994) Nutrient requirements of poultry. Edition, Ninth Revised

Odsjö T, Roos A, Johnels A (2004) The tail feathers of osprey nestlings (*Pandion haliaetus L.*) as indicators of change in mercury load in the environment of southern Sweden (1969–1998): a case study with a note on the simultaneous intake of selenium. Ambio 33:133–137

Ohlendorf HM, Hothem RL, Aldrich TW, Krynitsky AJ (1987) Selenium contamination of the grasslands, a major California waterfowl area. Sci Total Environ 66:169–183

Ohlendorf HM, Heinz GH (2009) Selenium in Birds. In: Beyer WN, Meador JP (eds) Environmental Contaminants in Biota: Interpreting Tissue Concentrations, Second edn. Taylor & Francis, Boca Raton, FL

Palacios MA, Gómez MM, Moldovan M, Morrison G, Rauch S, McLeod C, Li R, Laserna J, Lucena P, Caroli S, Alimonti A, Petrucci F, Bocca B, Schramel P, Lustig S, Zischka M, Wass U, Stenbom B, Luna M, Saenz JC, Santamaria J, Torrents JM (2000a) Platinum-group elements: quantification in exhaust fumes and surface modifications of the catalytic surface. Sci Total Environ 257:1–15

Palacios MA, Moldovan M, Gómez MM (2000b) The automobile catalyst as an important source of PGE in the environment. In: Alt F, Zereini F (eds) Anthropogenic platinum-group-element emissions and their impact on man and environment. Springer Verlag, Berlin

Parrish JM, David T, Rogers DT, Prescott F (1983) Identification of natal locales of peregrine falcons (*Falco peregrinus*) by trace-element analysis of feathers. Auk 100(3):560–567

Ralston NVC (2008) Selenium health benefit values as seafood safety criteria. EcoHealth 5:442–455

Ralston NVC (2009) Introduction to 2nd issue on special topic: selenium and mercury as interactive environmental indicators. Environ Bioindicat 4(4):286–290

Ralston NVC, Ralston CR, Blackwell JL III, Raymond LJ (2008) Dietary and tissue selenium in relation to methylmercury toxicity. Neurotoxicology 29:802–811

Rattner BA (2009) History of wildlife toxicology. Ecotoxicology 18:773–783

Rauch S, Morrison GM (2000) Routes for bioaccumulation and transformation of platinum in the urban environment. In: Alt F, Zereini F (eds) Anthropogenic platinum-group-element emissions and their impact on man and environment. Springer Verlag, Berlin

Rocque DA, Winker K (2005) Use of bird collections in contaminant and stable-isotope studies. Auk 122(3):990–994

Roelofs JGM, Bobbink R, Brouwer E, De Graaf MCC (1996) Restoration ecology of aquatic and terrestrial vegetation on non-calcareous sandy soils in The Netherlands. Acta Bot Neerl 45:517–541

Roem WJ, Berendse F (2000) Soil acidity and nutrient supply ratio as possible factors determining changes in plant species diversity in grassland and heathland communities. Biol Conserv 92:151–161

Scanlon PF, Oderwald RG, Dietrick TJ, Coggin JL (1980) Heavy metal concentrations in feathers of ruffed grouse shot by Virginia hunters. B Environ Contam Tox 25(1):947–949

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

Scheuhammer AM, Atchison CM, Wong AHK, Evers DC (1998) Mercury exposure in breeding common loons (*Gavia immer*) in Central Ontario, Canada. Environ Toxicol Chem 17(2):191–196

Sebright JS (1826) Observations upon hawking. Printed for J, Harding, London, U.K.

Sergio F, Newton I, Marchesi L (2005) Top predators and biodiversity. Nature 436:192

Sergio F, Newton I, Marchesi L, Pedrini P (2006) Ecologically justified charisma: preservation of top predators delivers biodiversity conservation. J Appl Ecol 43:1049–1055

Shawkey MD, Beck ML, Hill GE (2003) Use of a gel documentation system to measure feather growth bars. Journal of Field Ornithology 74(2):125–128

Spalding M, Frederick PC, McGill HC, Bouton SN, McDowell LR (2000) Methylmercury accumulation in tissues and its effects on growth and appetite in captive great egrets. J Wildlife Dis 36(3): 411–422

Spry DJ, Wiener JG (1991) Metal bioavailability and toxicity to fish in low-alkalinity lakes: a critical review. Environ Pollut 71:243–304

Steen R, Løw LM, Sonerud GA, Selås V, Slagsvold T (2011) Prey delivery rates as estimates of prey consumption by Eurasian kestrel *Falco tinnunculus* nestlings. Ardea 99(1):1–8

Steinnes E (2009) Soils and geomedicine. Environ Geochem Health 31(5):523–535

Strekopytov S, Brownscombe W, Lapinee C, Sykes D, Spratt J, Jeffries TE, Jones CG (2017) Arsenic and mercury in bird feathers: identification and quantification of inorganic pesticide residues in natural history collections using multiple analytical and imaging techniques. Microchem J 130:301–309

Suarez AV, Tsutzui ND (2004) The value of museum collections for research and society. Bioscience 54(1):66–74

Sunderland EM, Selin NE (2013) Future trends in environmental mercury concentrations: implications for prevention strategies. Environ Health 12(1):1

Taliansky-Chamudis A, Gómez-Ramírez P, León-Ortega M, García-Fernández AJ (2017) Validation of a QuECheRS method for analysis of neonicotinoids in small volumes of blood and assessment of exposure in Eurasian eagle owl (Bubo bubo) nestlings. Sci Total Environ 595:93–100

Theuerkauf J, Haneda T, Sato NJ, Ueda K, Kuehn R, Gula R, Watanabe I (2015) Naturally high heavy metal concentrations in feathers of the flightless kagu *Rhynochetos jubatus*. Ibis 157:177–180

Thompson DR, Bearhop S, Speakman J, Furness R (1998) Feathers as a means of monitoring mercury in seabirds: insights from stable isotope analysis. Environ Pollut 101:193–200

Thompson DR, Furness RW (1989) Comparision of the levels of total and organic mercury in seabird feathers. Mar Pollut Bull 20:577–579

Thompson DR, Furness RW, Walsh PM (1992) Historical changes in mercury concentrations in the marine ecosystem of the north and north-east Atlantic Ocean as indicated by seabird feathers. J Appl Ecol 29(1):79–84

Thompson DR, Hamer KC, Furness RW (1991) Mercury accumulation in great skuas *Catharacta skua* of known age and sex and its effects upon breeding and survival. J Appl Ecol 28:672–684

Tribalis A, Panagiotou GD, Bourikas K, Sygellou L, Kennou S, Ladas S et al (2016) Ni catalysts supported on modified alumina for diesel steam reforming. Catalysts 6(1):11

U.S. Department of the Interior (USDI) (1998) Guidelines for interpretation of the biological effects of selected constituents in biota, water, and sediment. National Irrigation Water Quality Program Information Report No. 3. USDI, Denver, CO

Van den Brink NW, Groen NM, De Jonge J, Bosveld ATC (2003) Ecotoxicological suitability of floodplain habitats in The Netherlands for the little owl *(Athene noctua vidalli*). Environ Pollut 122(1):127–134

Vo AE, Bank MS, Shine JP, Edwards SV (2011) Temporal increase in organic mercury in an endangered pelagic seabird assessed by century-old museum specimens. PNAS 108(18):7466–7471

Wainerdi RE, DuBeau NP (1963) Nuclear activation analysis. Science 139(3559):1027–1033

Walker CH, Hopkin SP, Sibly RM, Peakall DB (1996) Principles of ecotoxicology. Taylor & Francis, London

Walker CH, Hopkin SP, Sibly RM, Peakall DB (2000) Principles of Ecotoxicology, 2nd edn. Taylor & Francis, London

Walker CH, Hopkin SP, Sibly RM, Peakall DB (2006) Principles of ecotoxicology, 3rd edn. Francis, Taylor and

Walker L, Shore RF, Turk A, Gloria Pereira M, Best J (2008) The predatory bird monitoring scheme: identifying chemical risks to top predators in Britain. Ambio 37(6):466–471

Wallin K (1984) Decrease and recovery patterns of some raptors in relation to the introduction and ban of alkyl-mercury and DDT in Sweden. Ambio 13:263–265

Westermark T, Odsjo T, Johnels AG (1975) Mercury content of bird feathers before and after Swedish ban on alkilmercury in agriculture. Ambio 4:87–92

Wiener JG, Spry DJ (1996) Toxicological significance of mercury in freshwater fish. In: Beyer WN, Heinz GH, Redmon-Norwood AW (eds) Environmental contaminants in wildlife: interpreting tissue concentrations. SETAC SP P, Lewis, Boca Raton, FL, pp 297–339

Wood HB (1950) Growth bars in feathers. Auk 67:486–491

Xu D, Ma L, Chen Y, Chai Z, Xu G, Li S (2011) Hybrid NAA for study of organic halogens in precipitation in Beijing, China. Proc in Radiochem 1:323–326