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Bird feathers as a biomonitor for environmental pollutants: Prospects and pitfalls

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ABSTRACT

Due to increasing amounts of hazardous chemicals released into the environment, there is a high demand for developing easy and non-destructive biomonitoring tools. In a recent paper published in Trends in Analytical Chemistry, Rutkowska et al. (2018) concluded that feathers are a good matrix for biomonitoring of environmental pollutants in birds. In this commentary, we discuss the general conclusion of this paper. We provide several examples for which this statement is not correct, and we emphasize that only for legacy persistent organic pollutants (POPs) and mercury there is currently enough scientific evidence to use feathers as a reliable biomonitor, given that appropriate sampling designs and QA/QC protocols are taken into account. Furthermore, we discuss different pretreatment (e.g. feather washing) and analytical protocols along with specific QA/QC to be considered. In summary, this commentary provides an overview of the prospects and pitfalls when using feathers as a biomonitor for environmental pollutants.

1. Introduction

In a paper recently published in Trends in Analytical Chemistry, Rutkowska et al. [1] have presented an extensive literature review on the use of bird feathers to assess contamination levels of various environmental pollutants. The discussed environmental pollutants range from trace metals and legislated persistent organic pollutants to emerging organic pollutants (such as per- and polyfluoralkylated substances (PFASs), organophosphate esters (OPEs) and phenols) and microplastics.

Although we acknowledge the value of such comprehensive review, we have reservations about the main conclusion presented by the authors that "Feathers can be successfully used as environmental pollution monitors". We will provide several examples for which this statement is not correct (as will be detailed further below), and underline the lack of knowledge of emerging contaminants in particular. In addition, we wish to make important clarifications regarding preparation, analysis, and restrictions on the use of feathers for environmental pollutant analysis. Our main message is that scientists aiming to employ feathers in future research should be well aware of the prospects and pitfalls when using feathers as biomonitors for environmental pollution.

2. Discussion

2.1. Suitability of feathers for different types of pollutants

In the conclusion section, Rutkowska et al. [1] state that "Feathers can be successfully used as environmental pollution monitors because the proportion of contaminants in the body to the level present in the feathers is relatively constant for each xenobiotic and there is a high correlation between the levels of contaminants in the food of seabirds and those in feathers". This may indeed be the case for legislated persistent organic pollutants (POPs), in particular polychlorinated biphenyls and *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE), as indicated by several published articles (reviewed by García-Fernández et al. [2]). However, this statement is not true for many metals that show external

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contamination on the feather surface [3–6], and has recently been questioned for emerging compounds, such as PFASs and novel flame retardants [7]. Indeed, for many pollutants the correlations between the concentrations in feathers and internal tissues are not known (Table 1), and there may be differences among feather types. There are three different routes of how pollutants can be transferred to feathers; i) during feather growth through the connection to the blood circulation of the bird, ii) during preening via preen oil and iii) via contact with the environment (air, dust and water). Different chemical groups will be transferred via different routes according to their physical-chemical properties, with POP-like lipophilic chemicals mostly being found in the preen oil and polar chemicals such as PFASs and some OPEs being transferred during the growth of the feathers and the contact with the environment. This means that for some chemicals, concentrations measured in feathers may not be related to internal tissue concentrations but rather originate from external sources.

There is, thus, a great need for further studies to examine associations between concentrations in different feather types and internal tissues for different classes of contaminants, and to investigate the importance and extent of external contamination. In addition, concentrations in feathers and internal tissues may not always be correlated when there is a time difference between the formation of the feathers and the sampling of the feathers. Depending on the research questions, it is generally preferred to sample nestling feathers which are recently grown or still connected to the blood circulation rather than feathers from adult birds [3,8]. For example, adult migrating bird species may grow their feathers on the overwintering grounds, which subsequently may not show associations to the concentrations in the body of birds on the breeding grounds [e.g. 9]. The collection of museum samples may also present some challenges, such as the preservation, degradation of some contaminants over time, different types of feathers available, contamination during storage (e.g. OPEs) or through the use of disinfectants (e.g. arsenic [6]) and the lack of metadata on the sampled individuals [8,10]. We refer to Table 1 for an overview of the general suitability of feathers for different pollutants, and the knowledge gaps that still exist.

In addition, the specific moulting sequence can be of concern [11], which is highly species dependent, with some species moulting their sets of flight feathers over several years [12]. Therefore, the sampling of body feathers can be an alternative, as these feathers moult more regularly and evenly throughout the year and have been shown to correlate well with internal body concentrations of POPs [13,14] and mercury [15]. However, extensive variation between body feathers for lead in Northern goshawk (*Accipiter gentilis*) and tawny owl (*Strix aluco*) has been recorded [16], and therefore the use of body feathers may not be suitable for all compounds and species. Recently, down feathers have also been studied as an alternative biomonitor [17]. It is also possible to collect moulted feathers from adults at the nest, but then the issue of external contamination is of higher concern than when sampling nestlings. If one is interested in biomonitoring the internal exposure of birds of prey for trace metals, it is important to analyse freshly moulted feathers or to have procedures to eliminate external contamination [e.g. [18]], which is not easy to achieve and deserves further (experimental) study. On the other hand, feathers could potentially be used as a passive monitor for environmental concentrations of metals and elements [5,18] and other emerging pollutants [e.g. [19,20]], thus not reflecting the actual concentrations of contaminants in the bird, but rather a snapshot of the pollutant load in the environment of the bird. This could be the case for microplastics as well, although Reynolds and Ryan [21] indicated that their sampling protocol (i.e. using feather brushings and faecal samples from seven duck species) likely underestimated the occurrence of micro-plastics in South-African wetlands. This has been misinterpreted by Rutkowska et al. [1] as Reynolds and Ryan [21] did not specifically advocate for the use of feathers as a biomonitor for microplastics.

Lastly, the specific habits of the bird species, from which the feathers are obtained, are of crucial importance for the study design. Some bird species migrate long distances, and therefore may in general not be suitable to collect feathers from at the breeding grounds if they were grown on the wintering grounds. This has been misinterpreted by Rutkowska et al. [1] in section 4.2.5 on OPEs. The reason for which Svendsen et al. [9] recommended collecting feathers from nestlings and not adults, is not a general statement for OPEs, but rather a general consideration when using migratory birds, irrespective of the nature of pollution investigated. In that sense, knowledge of the bird species' biology is of high importance when planning a biomonitoring study [3,22]. For instance, biomonitoring of the local terrestrial environment cannot be solely done using raptor species, due to their relatively large foraging areas. Instead passerine bird species, with smaller home ranges, are more appropriate to successfully monitor local contamination for example around point sources [6,23]. However, it should be noted that only a limited amount of feathers can be collected from small birds without harming them. Therefore, the

Table 1
Current information regarding the suitability of feathers for biomonitoring of different types of pollutants in birds. If not enough information is available to draw any conclusions, this is indicated with a question mark ("?").

	Reflecting internal concentrations	External contamination	Suitability as a biomonitor	Remarks	Selected references
Metals and elements	For Hg and some other metals	Important issue for some metals	Depending on the metal	External contamination is dependent on the location. Different elements may be important in sediment or air	[3–6,8,16,18,25,33]
PCBs	Strong	Minor issue	Yes	External contamination mostly from preen oil (internal exposure)	[2,27]
DDE	Strong	Minor issue	Yes	External contamination mostly from preen oil (internal exposure)	[2,27]
PBDEs	Strong	Potential issue	Yes	External contamination mostly from preen oil (internal exposure)	[2,27]
PFASs	Weak – Strong	Potential issue for some compounds	?	Good correlations found for some compounds, but not for others. External contamination should be further investigated	[7,19,34]
OPEs	No	Important issue?	?	High detection in feathers and low detection in blood could be due to either external contamination or high metabolism	[7,20]
PAHs	?	?	?	Only few studies to date	[35]
Bisphenols and Alkylphenols	?	?	?	Only 1 study to date	[36]
Neonicotinoids	?	?	?	Only 1 study to date	[37]
Microplastics	?	Yes	?	Only 1 study to date (analysing external contamination using feather brushings)	[21]
Pharmaceuticals	?	?	?	Only 1 study to date (on fluoxetine)	[38]

required amount of feather material, which is likely to depend largely on the pollutant studied as well as on the pollutant concentrations, should be checked in the literature [e.g. [6, 23]] or with the respective analytical lab.

2.2. Collection and storage of feathers

Although the second part of the conclusion, "it is easy to collect feathers non-invasively and it is possible to store test material for many years" [1] is largely correct, it is important to know what is meant by non-invasively collecting feathers. If the authors are only referring to the sampling of moulted feathers around the nest, this is indeed very easy to perform. However, this could have several drawbacks regarding the suitability of the collected material. One does not know the exact individual from which the moulted feathers originated, and the moulted feather could have been laying there for weeks or months, and is thus prone to external contamination or degradation. It is important to take this into account when washing the feathers (see next section) and when interpreting the results. Another non-invasive sampling method would be cutting the feathers from living birds (instead of plucking). In contrast to collecting flight feathers, the collection of body feathers from living birds generally allows for better standardization and minimal harm to the birds [13,14,24]. In respect to storage, also here a strict quality assurance/quality control (QA/QC) protocol needs to be followed to prevent contamination and protect the integrity of the feathers. Therefore, no opportunistic selection of stored feathers can be recommended for contaminant analysis, if the applicable QA/QC protocol was not followed. Once collected, feathers can be stored for many years, but they need to be stored under controlled conditions, protected from potential sources of external contamination and degradation, such as UV radiation and moisture. Dry feathers can be stored either in paper envelopes or alumina foil, further sealed in polypropylene (PP) zip-lock bags at room temperature, or dirty and wet feathers can be stored in the freezer. However, analyses of certain pollutants which can leach from PP zip-lock bags such as softeners, UV stabilizers and other additives as well as microplastics may not be possible for these samples. More information about the collection, transport, storage and pre-treatment of feathers can be found in the sampling and contaminant monitoring protocol for raptors [24].

2.3. Preparation and extraction of feathers for different types of pollutants

Section 2 in the paper of Rutkowska et al. [1] describes the preparation of the feathers. Although this section provides a very general description of the analysis of feathers for traditional POPs, no specification has been made regarding different pollutants and their specific preparation. This section may therefore be confusing for the reader since POPs, metals, PFASs, emerging flame retardants and other classes of pollutants each require different amounts of sample for analysis and undergo different preparation and extraction procedures. It is therefore important that relevant literature is consulted before preparing the feathers (e.g. Ref. [18] for metals [13], for POPs [7], for PFASs and [20] for current-use flame retardants). It is also unfortunate that the milestones in Figure 3 are not reflecting the development of new extraction methods for other pollutants than metals.

Regarding the preparation of feathers for metal analysis, the washing step is very important as many metals are present in high concentrations on the external surface of the feathers [5,18,25]. This is of concern when considering feathers as a potential biomonitor, and this discussion is missing in the section 4.1 of Rutkowska et al. [1]. In addition, it is very important to use non-metallic tools and metal-free liquids when washing and handling the feathers before performing trace elemental analysis. It should also be noted that washing procedures to eliminate external contamination have been extensively investigated only for metals [3,26] and POPs [27]. Still, we are currently lacking standardised washing procedures for metals and POPs and different authors have used different methods [2,25,27,28]. Current recommendations can be found in the sampling and contaminant monitoring protocol for raptors [24]. Extensive knowledge on washing procedures for other legacy and emerging contaminants in feathers is currently not available.

Variability due to external contamination can be reduced by restricting analysis to the shaft or rachis as external contamination tends to be greater for the vane than the shaft and the shaft is more easily and effectively cleaned [8]. Indeed, the rachis has been used in several studies for the monitoring of Pb exposure from ammunition sources in birds of prey [4,29,30]. However, García- Seoane et al. [31] reported high intraspecific variability and analytical difficulties due to the very low levels of arsenic, cadmium and lead in the rachis, which may limit the suitability of the rachis when concentrations are low. Yet, a new methodological approach has recently been suggested to limit and correct for external contamination with metals and elements on the feather surface, including analysing only the rachis of the feather, using specific washing procedures and the use of a reference element to correct for external contamination from sediment [18]. However, it should be noted that currently no reference materials are available specific for feathers. In addition, the use of field and analytical method blanks or passive air samplers may contribute to control for potential external contamination during both field sampling, storage and laboratory preparation.

Regarding the preparation of feathers for POPs and PFASs, the fine cutting or milling of the feathers is crucial for effective extraction of the contaminants. However, before performing washing and/or cutting of feathers, it is important to record the weight and the length of the feathers [32]. As indicated in section 3 of Rutkowska et al. [1], the deposition rate of contaminants in feathers can potentially be calculated, but this has in practice only rarely been done. Although this has been proposed previously [2], its application is limited to flight feathers with clear growth bars that correspond to daily growth.

Lastly, all kind of micro-plastic analyses in feathers needs to be carried out under particle-controlled conditions and follow strict QA/QC protocols including field and analytical method blanks and specific preparation of all materials and chemicals in contact with the feathers.

3. Conclusions

In conclusion, we do not support the general statement by Rutkowska et al. [1] that feathers can successfully be used as monitors for any environmental contaminant. Feathers can be useful as a biomonitor for POPs, mercury and several other metals under the conditions that appropriate sampling designs and pre-treatment of samples along with QA/QC protocols during storage, preparation and analysis are taken into account. Still, even for mercury a recent study has indicated that still several knowledge gaps existed related to variation within feather parts, among feather types and between feathers of the same type [33]. Quite clearly, more research is needed to investigate whether feathers can be useful to monitor the internal concentrations of other classes of contaminants. We hope to have clarified the specific considerations that need to be taken into account before investigating pollutants in feathers. The most important aspects relate to the choice of the bird species and the possibility of external contamination on the feathers, which in turn depends on the compounds

under study. The latter should inform the required collection, storage and pre-treatment strategies of the samples. In addition, the study species and the type of feathers collected need to be well considered in advance. Feathers are not a general solution for pollution biomonitoring: the specific bird species, the type of feather, the type of pollution and potential external contamination are very important to consider for a successful biomonitoring strategy. If the recommendations listed above are taken into account, feathers can be used as a good biomonitoring tool for legacy POPs and selected metals. However, there are many knowledge gaps remaining that need to be systematically and experimentally addressed in order to conclude on the suitability of feathers for other types of pollutants.

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