Bird feathers as a non-invasive method for ecotoxicological monitoring; a rapid review

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Abstract

The decline in animal population with resulting risk of eventual disruption of ecosystem functioning has necessitated moves towards non-invasive sampling methods for ecotoxicological studies. These efforts have focused on developing sampling methodologies geared towards prioritizing conservation of biodiversity. Using a rapid review approach, 106 articles covering peer-reviewed studies, theses, and manuscripts under peer-review quantifying contaminants in feathers were examined. Meta-analysis of extracted information (n=91) revealed that most studies on feather contaminants originate from Europe (47.6 %) and Asia (32.9%), with a higher occurrence of such studies between 2015 and 2020. The most utilized feather-type across studies were body feathers (28.6%) and tail feathers (20%). Majority of the studies (60.5%) used feathers alone to estimate contaminant exposure and uptake; 14.8% used feathers and blood, while 12.3% used feathers and soft tissues (liver, kidney, muscle etc.). Inferences from the review reveal that feathers as a non-invasive sampling method provide advantages by having contaminant concentrations that are relatable with internal organs of birds, captures information on ambient contaminant concentrations based on exogenous contributions and application for historic studies. However, some available data indicate that selective uptake of some metals in organs of species could significantly reduce the number of contaminants stored in feathers and may limit its accuracy for biomonitoring. Furthermore, the different degrees of external deposition of persistent organic contaminants (PCBs, PBDEs, etc) onto the feather surface due to differential degradability/metabolization may limit the use of feathers to estimate exogenous effects. Nonetheless, while feathers have been used successfully as a non-invasive method for ecotoxicological monitoring of metals, metalloids and organic pollutants, knowledge on bird ontogeny and contaminant-specific trends in feathers could improve the accuracy of monitoring. Further efforts towards broadening the impact and advancement of this field method in future studies of avian research especially in developing countries is recommended.

Keywords: Non-invasive, non-destructive, feather contaminants, soft-tissues, birds

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Introduction

The rapid loss of diversity through habitat destruction, introduction of invasive species, and overexploitation coupled with climate change (Pimm *et al* 2014; Bellard *et al* 2012) has fuelled discussions among stakeholders on the likelihood of a 6th mass extinction (Barnosky *et al* 2011; Ceballos *et al* 2020). While about one in five vertebrate species is categorized as vulnerable, endangered or critically endangered by the IUCN's Red List (UNEP-WCMC 2018), a 60% decline in species biodiversity was also reported by the World Wildlife Fund (WWF 2018). Such declines in animal population and eventual disruption of ecosystem functioning and availability of services (Dirzo *et al* 2014), has necessitated monitoring and assessment of biodiversity loss through research methodologies geared towards prioritizing conservation of species biodiversity (Hoffmann *et al* 2010; Carroll *et al* 2018).

Various approaches to sampling have been adopted in the course of seeking and validating conservation strategies in wildlife research, (MacKay *et al* 2008; Garshelis and Noyce 2006). However adequate animal welfare through the adoption of protocols to minimize the pain and distress inflicted on research animals has become an issue of concern (Lund *et al* 2014).

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This in turn has expanded the application of the 3R (replace, reduce, refine) which inherently highlights the adoption of legal restrictions on animal use and guidelines on animal experiments in many countries (Sneddon *et al* 2017; Erkekoglu *et al* 2011). Although the 3R principles were originally proposed for laboratory animals, applications in wildlife research has been suggested, particularly through the use of non-invasive research methods, i.e. methods that do not affect the physical integrity of an animal or compromise its health and survival (Lefort *et al* 2022).

Following the recognition of birds as valuable sentinels of environmental quality, efforts to describe the movement of environmental contaminants through ecosystems have been made by quantification of contaminants in avian samples (Garcıa-Fernández 2014). Traditional methods for the collection of biological material used in contaminant quantification and other aspects of ecotoxicological research hitherto involve the capture and manipulation of individuals for drawing blood, scrapping tissue, or actual shooting of birds for soft tissue sampling (Taberlet *et al* 1999). However, these techniques have the potential to cause varying degrees of distress to individuals which may lead to lower survival rates in captured species like birds (Brown and Brown 2009; Owen 2011) and eventual adverse effects on their populations. As a result, specific efforts for non-invasive sampling of biological material to reduce harm to birds have increased the scope of samples to include eggshell swabbing or eggshell grinding (Martin-Galvez *et al* 2011; Egloff *et al* 2009), shed feathers (Horváth *et al* 2005; Johansson *et al* 2012; Miño and Del Lama 2009), faeces (Baumgardt *et al* 2013; Idaghdour *et al* 2003), buccal swabbing (Wellbrock *et al* 2012; Handel *et al* 2006; Yannic *et al* 2011) and feather plucking (Johansson *et al* 2012; Costantini *et al* 2008; Taberlet and Bouvet 1991).

Since ecotoxicological information on birds is still inadequate and records are scanty with insufficient geographical representation (Hao *et al* 2021), increased investigations and research on wild bird populations and their ecosystems using feathers are anticipated. In addition, such non-invasive methods that are attractive for achieving a larger sample size should also be validated for more efficient use. Here, we conduct a rapid review to examine the use of feathers as non-invasive samples and highlight aspects of possible caution of this method for avian ecotoxicological research.

Materials and methods

This rapid review followed structure and methods recommended by Haby *et al* (2016) with little modifications.

Literature search

For this study, we first examined peer-reviewed publications without restriction on the year of publication. We searched web-based resources for bird studies from the earliest studies available reporting on outcomes of contaminant occurrence and quantification in feathers. Our search strategies included the following keywords: feather contaminant, bird contaminant and bird non-invasive. Database searches covered PubMed (www.ncbinlm.nih.gov/pubmed); Scopus (www.scopus.com); Web of science (www.webofscience.com); ScienceDirect (www.sciencedirect.com); Google Scholar (www.googlescholar.com); and African Journals Online (www.ajol.info). These databases were accessed through Google and Google Scholar websites.

Grey literature and manual search

In addition to peer-reviewed publications, unpublished studies undertaken by the authors (including undergraduate thesis, postgraduate thesis and manuscripts under peer-review) were captured in this rapid review.

Inclusion criteria for studies

All types of studies covering feather samples and contaminant profiling, or quantification were sought. Field, experimental, and historic studies using museum feathers to estimate contaminant uptake in birds were included in the study. Studies combining feathers and other tissues including soft tissues (liver, kidney, muscles) were also included in the review process. All available studies covering all types of birds and geographical areas/regions were included in this study.

Screening and selection of studies

Two review authors (AVC and AOA) conducted and screened literature according to the selection criteria. The full text of any potentially relevant papers was retrieved for closer examination. Each reviewer erred on the side of inclusion where there was any doubt about its inclusion to ensure no potentially relevant papers were missed.

The inclusion criteria were then applied against the full text version of the papers (where available) independently by two reviewers (AOA and AVC). Studies that considered contaminants in birds but did not quantify or profile contaminants in feathers were excluded in this review. Diverse views regarding eligibility of studies were resolved by discussion and consensus between the authors.

Data extraction

Information extracted from studies and reviewed included authors names, year of publication, study location/geographical region, sample type i.e., whether feathers alone were used for the study or other tissue types were sampled alongside feather type i.e., primaries, secondaries, down, tail or molted feathers; contaminant type; whether birds were exposed in the wild or under experimental conditions; direct mention of operational words ‘non-invasive’ ‘non-destructive’ in study justifications. In the absence of these operational words, we also searched for and considered phrases, sentences or similar words that also point to the use of feathers as a 'non-invasive' 'non-destructive' method. Data extraction was performed by one reviewer (AVC) and checked by a second reviewer (AOA). Divergent opinions were resolved through discussion and consensus.

Data analysis

Findings from the included publications were visualized (using tables, descriptive charts) and synthesized into a narrative summary.

Results and Discussion

Literature search

A total of 106 potentially relevant full-text papers were screened (Table 1). Subsequently, 85 published articles, one manuscript under peer-review plus five undergraduate and postgraduate theses (n=91) fulfilled the eligibility criteria and were included. Four of the included papers were review articles.

Publication assessment

The articles considered were published between 1984 and 2022, and majority of the articles were published between 2015 and 2022, while the least number of articles on contaminants in feathers occurred before 2005 (Figure 1). We observed that 47.6% of the studies were published in Europe, 32.9% in Asia, 12.2% in North America and 3.7% in South America and Africa (Figure 2). This relative occurrence of bird contaminant studies confirms earlier reports of the continuing lack of ornithological research capacity in most of West Africa (Cresswell 2018) and probably the rest of Africa. Aside the characteristic high bird diversity of West Africa, this subcontinent is a crucial non-breeding area for over one-third of European breeding species (Cresswell 2018). As such, the relatively lower percentage of bird studies for Africa reported in this review inherently communicates a research deficit and limited capacity prerequisite for targeted bird conservation.

From publications assessed, 60.5% of studies used feathers alone to estimate contaminant exposure and uptake; 14.8% used feathers and blood, 12.3% used feathers and soft tissues (liver, kidney, muscle etc.) while 12.2% used feathers in combination with egg, soft tissues, excrement and preen oil respectively (Figure 3). The most utilized feather-type across studies was the body feathers (28.6%), followed by tail feathers (20%), breast feathers (14.3%), and primaries (12.9%) with other feather types accounting for a cumulative 24.4% (Figure 4). Metals were the most prominent contaminants profiled in birds' feathers (73.2%) compared to organics (26.8%) (including PBDEs, organochlorines, PCBs etc.) (Figure 5).

All articles were examined for occurrences of 'non-invasive' 'non-destructive' terms as descriptor of feather sampling methods and justification for use in contamination-related studies in birds. Of the articles examined, 47.5% described bird feathers as 'non-destructive' method to justify its use for contaminant

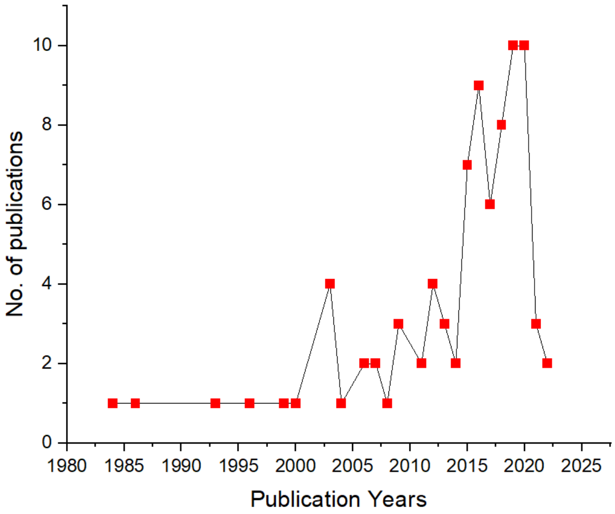

Figure 1. Number of contaminants in bird feather studies across the years

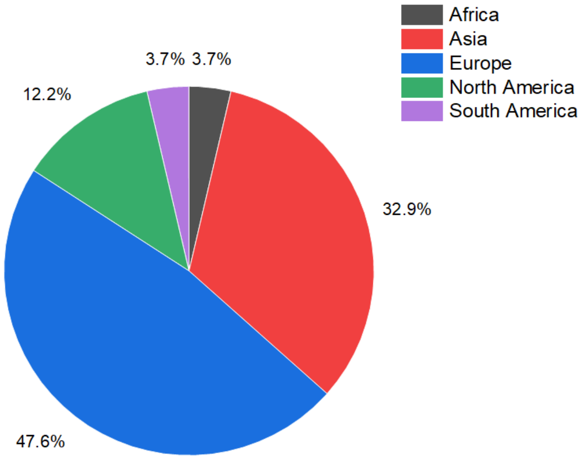

Figure 2. Occurrence of contaminant in feather studies by continent from literature search

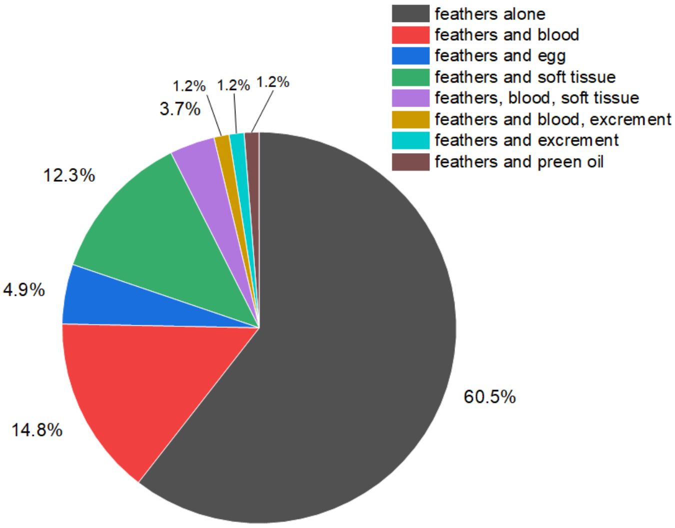

Figure 3. Overview of studies with either bird feathers alone or combined with other types of tissues

evaluation in birds. Other terms used include ‘non-invasive (30.5%) and non-lethal (6.8%). Few articles, 5.1% used both ‘non-invasive’ ‘non-destructive’ to describe feather sampling methods for bird studies and other terminologies ranging from non-harming to non-health affecting were used to describe a cumulative 10.2% of the studies (Figure 6).

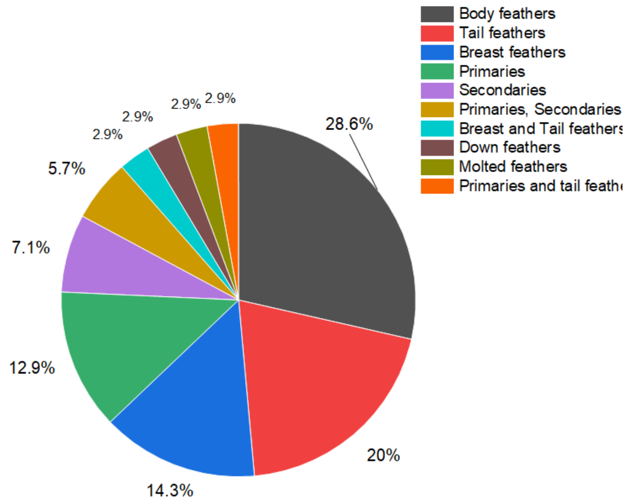

Figure 4. Bird feather studies that used single feathers or combined feather types

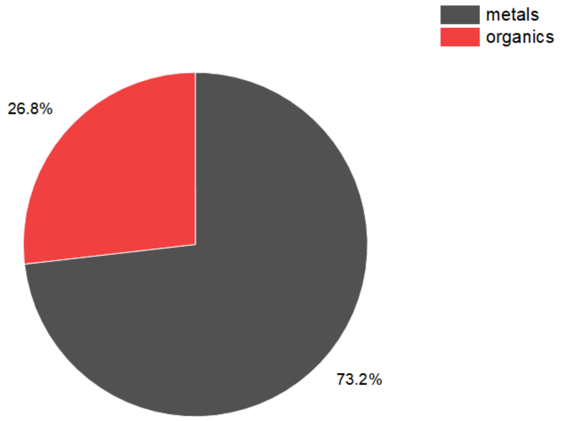

Figure 5. Bird feather studies with either metals or organic pollutants

Advantages of bird feathers for contaminant estimation

As skin derivatives, bird feathers can accumulate metals (Wang *et al* 2017), and they may outperform internal tissue samples as non-destructive bioindicators of metals because collecting feather samples causes minimal harm to birds (Berglund 2018; Van Aswegen *et al* 2019). The storage and transportation conditions required for feathers are also simpler because they are non-perishable compared to other abiotic and biotic materials.

Feathers and blood are the most targeted tissues to quantify trace element concentrations in birds mainly because they can be easily and non-destructively sampled on a large number of live individuals (Burger and Gochfeld 2004). More importantly, the proportion of the body burden stored in the feathers is relatively constant for some elements, particularly mercury (Hg) (Monteiro and Furness 1995; Burge 1993).

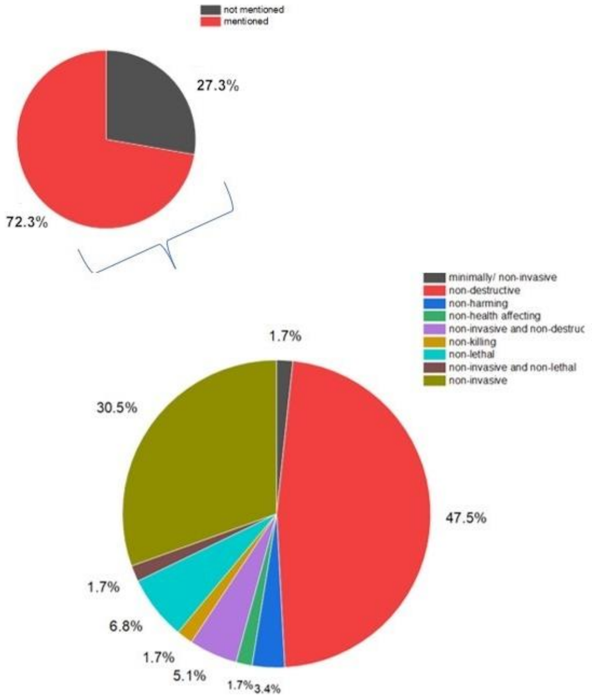

Figure 6. Bird feather studies with mentions of ‘non-invasive’ descriptors for feather sampling

One major feature of ecotoxicological importance that the use of feathers presents, is the ability to estimate contaminant uptake in bird internal organs through estimates from feathers. Yao *et al* (2021) in their study on metal(loid)s V, Mn, Co, Zn, and As in feathers and internal tissues including heart, liver, kidneys, muscles and bones demonstrated that the contents of some elements in feathers were positively correlated with those in internal tissues. For example, Co, As, and Cd in the heart, V and Co in the kidneys, Cd in the liver, Pb in bones, and As in muscles. Although challenges have been documented for metal contaminants like total mercury (THg), where feather contaminants concentration can be highly variable within an individual bird (Bond and Diamond 2008), possible applications of adult feathers in mercury monitoring has been recommended for non-migratory/resident bird species with extremely small home ranges (or other ecology) where THg concentrations in feathers are highly correlated to levels in internal tissues (Ackerman *et al* 2012). Feather samples have also proven to be samples of last resort when more invasive sampling methods need to be avoided (such as for endangered species), or when using museum specimens to examine long-term temporal trends, because no other tissue is available (Bond *et al* 2015; Monteiro and Furness 1997).

Burger *et al* (2014) among their study objectives sought to determine the relationship of metal levels among tissues, including mercury and selenium in muscle, liver, brain, fat and breast feathers. Although there were significant differences among tissues for all metals, correlations among metals in tissues were varied, with mercury levels being positively correlated for muscle and brain, and for liver and breast-feathers. Findings from Pilastro *et al* (1993) on cadmium exposures to birds revealed that feather Cd concentrations correlated with Cd concentrations in liver, kidney and uropygial gland. They however opined that the higher concentrations observed in primaries compared to secondaries could be attributed to greater abundance of primaries compared to secondaries. The same explanation was also applied to higher Cd levels in old feathers compared to new feathers. They further explained that disparities in soft tissue (liver and kidney) contaminant concentrations and feather concentrations could be attributed to data on birds of different ages living in the same habitat.

The suitability of feathers in estimating persistent organic contaminants (POPs) has been reported. Yin *et al* (2018) reported that higher brominated congeners, e.g., BDE-209, -153, -207 and -196 showed comparable dominance in both feather and muscle. Furthermore, the significantly correlated concentrations of lower brominated congeners in feather with those in muscle ($p<0.05$), suggests that feathers could efficiently reflect low brominated BDEs in the internal tissue of birds of prey. This suitability of feather samples was further confirmed by Jaspers *et al* (2006) who examined if there is a correlation between levels of organic pollutants in liver and muscle and levels in the corresponding feathers as a non-destructive biomonitor of organic pollutants. They demonstrated that polychlorinated biphenyls (PCBs), Dichlorodiphenyl trichloroethane (DDT) and polybrominated diphenyl ethers (PBDEs) are measurable in one single tail feather of common buzzards (*Buteo buteo*) and that levels in this feather and internal tissues were significantly related to each other.

Meyer *et al* (2009) investigated the perfluorinated compounds (PFC) exposure of five different bird species (Grey heron, Herring gull, Eurasian sparrowhawk, Eurasian magpie, and Eurasian collared dove) from the same geographic region in Belgium, using both feathers and liver tissue. Overall, there was a significant positive correlation (Pearson, $R=0.622$, $p<0.01$) between the feather and liver perfluorooctane sulfonate (PFOS) levels, indicating that feathers could be an alternative bioindicator for PFOS exposure in birds. Authors, however, did not observe significant correlations between the PFOS levels in the feathers and livers of the individual species.

Another advantage of the use of feathers is seen in its ability to reflect ambient environmental concentrations of certain contaminants. Tasneem *et al* (2020) in their study comparing contaminant concentrations in feathers and soft tissues reported concentration trends of metals and As within tissues in the order of tail feathers > pectoral muscles > blood. This indicates that feathers could be advantageous in capturing the combined effect of exogenous and endogenous contamination (Jaspers *et al* 2019) particularly for toxic metalloids that readily bioaccumulates in living tissues (Sánchez-Virosta *et al* 2015). This usefulness of feathers in discerning habitat exogenous contaminations and exposures was also confirmed by reports of Yang *et al* (2018) and Jaspers *et al* (2013). Kunisue *et al* (2003) were of the opinion that feathers of avian species are useful bioindicators to elucidate contamination status of organochlorines in breeding grounds, stopover sites and wintering grounds, because resident birds directly reflect the specific local pollution status of sampling area, and migratory birds reflect not only the pollution status of sampling area but also those of their migratory routes. Adeogun *et al* (2022) (under review) demonstrated that feather concentrations of metals and OCPs in birds (Bronze mannikin, Blue-spotted wood dove, Yellow-throated long claw, Senegal kingfisher and African jacana) were strongly correlated with the trophic tendencies and habitat preferences of each bird within lake wetlands.

Some authors (Jaspers *et al* 2013; García-Fernández *et al* 2013) have demonstrated that feathers could accurately reflect PFOS exposure in a population, but not necessarily of each individual. The moulting season and pattern of the birds should also be taken into account (Burger 1993). Feathers could be an indication of PFC levels during the feather growth period, when the feathers are connected to the bloodstream (Meyer *et al* 2009). Furthermore, the correlation between feather and liver PFOS levels suggests that feathers could be an alternative to measuring birds' exposure to PFOS. Jaspers *et al* (2013) in studies using correlation coefficients to estimate the strength of the linear relationship between congener levels in feathers and levels in soft tissues, showed that PFOS levels in tail feathers and liver were highly correlated, but not muscle tissues. This may be due to the chemical properties of PFASs, which are mainly bound to proteins in the blood, thus reducing the suitability of muscle tissue for estimating such contaminants.

All review publications on bird feather examined for this report also allude to the usefulness of feathers for monitoring environmental pollution both due to its non-invasive sample collection protocol and its high correlation between the levels of contaminants in the environment and those in the feathers (Rutkowska *et al* 2018; Abbasi *et al* 2016a; Jaspers *et al* 2019). Other review reports considered the usefulness of feather samples for discerning temporal and spatial patterns of xenobiotic occurrence and ecotoxic effects in endangered or threatened species without endangering populations (Rutkowska *et al* 2018).

Limitations of feathers for contaminant estimation

For some contaminants, feathers have low priority as a preferred tissue for sampling. In particular, feather total mercury (THg) concentrations are highly variable within an individual bird (Bond and Diamond 2008; Cristol *et al* 2012; Furness *et al* 1986) and poorly correlated with THg concentrations in internal tissues (Eagles-Smith *et al* 2008; Evers *et al* 1998). Concentrations in internal tissues have been documented to represent a more likely risk of current methylmercury toxicity. Furthermore, THg concentrations in feathers represent THg concentrations in blood at the time of feather growth, which is a combination of the bird's body burden of mercury, via redistribution of mercury among internal tissues during moult, and recent mercury acquired through diet (Braune and Gaskin 1987; Furness *et al* 1986). Not only is the timing of feather moult often unknown, but moult may represent a time when internal mercury concentrations are rapidly changing due to mercury transfer to feathers (Ackerman *et al* 2011; Condon and Cristol 2009) and the often-associated nutritional stress. Yao *et al* (2021) also corroborated this limitation of feather-sampling for estimating exposure to some essential elements (Cr, Mn, Cu and Zn).

Besides down feathers of chicks, sampling juvenile birds for contaminant monitoring purposes is not advised, because THg concentrations, for example, in internal tissues (including blood) change rapidly as chicks age due to mass dilution and mercury transfer into growing feathers (Kenow *et al* 2007; Ackerman *et al* 2011) and, therefore, are difficult to interpret.

Fromant *et al* (2016) estimated metal levels in feather and soft tissue in the sea bird and reported that feather trace element concentrations, particularly Hg, were not significantly correlated to levels in other tissues. They attributed this discrepancy to a temporal mismatch between concentrations in metabolically active (soft tissues) versus inactive tissues (feathers). Some studies have inferred that the low metabolic and storage feature common to feathers and muscles will likely make their metal contaminant concentrations more relatable compared to the highly metabolic situation and turnover rate of liver tissues (Osičková *et al* 2014). It all leads back to the fundamental understanding that, once the feather is formed, the blood supply atrophies, with no further element being deposited. Thus, while feather Hg concentrations remain unchanged since their last moult, Hg concentrations in the other tissues progressively increased through dietary intake (bioaccumulation). Thus, feather Hg concentration reflects Hg levels of internal organs at the time of the previous moult, but not at the time of sampling.

The ability of some metals to be selectively taken up by certain tissues could also affect the ability of feather contaminant concentrations to be representative of soft-tissue concentrations. Fromant *et al* (2016) further attributed feather-soft-tissue disparity to the selective uptake of Cd and Pb in kidney and bone where they are firmly bound to organic and inorganic compounds, respectively, thus only enter feathers in trace amounts (Stewart *et al* 1994; Walsh 2018). This is consistent with previous studies elsewhere that not all trace metals exhibit positive relationships between the contents in feathers and those in internal tissues (Tsipoura *et al* 2008; Mikoni *et al* 2017). Thus cautious use of feather concentrations to predict soft tissue burdens for these two non-essential elements is necessary (Nam *et al* 2005).

Evidence from studies attributing lower correlation coefficients observed for PBDEs and DDTs in comparison to PCBs to different degrees of external deposition onto the feather surface or by different metabolization rates (Jaspers *et al* 2006) may have limiting implications on the use of feathers for esimating ambient environmental concentrations of organic contaminants. As such, the use of feathers for this purpose may be more applicable to metals (Burger 1993) compared to organics, which are less influenced by exogenous contamination (Dauwe *et al* 2005).

Reports by Espín *et al* (2016) indicate that feathers as a biomonitoring tool may provide the best opportunities for widescale studies, however its reliability should be tested on a wider range of compounds. This is consistent with reports by Jaspers *et al* (2019) who emphasized the existence of knowledge gaps on feather useage that require systematic and experimental information to concretize the basis of their suitability for other types of pollutants.

Conclusion

The lack of correlations between some feather contaminants and concentration of same in soft tissues does not indicate that feathers are not good indicators of internal contamination, but rather that the temporal integration of contaminants into feathers must be carefully considered. Further studies investigating feather and internal contaminant concentrations during moult are highly needed to understand the mechanism of excretion, in particular for POPs. These findings about metals in feathers and soft tissues possibly imply that contaminant-specific effects and feather-type specific effects be considered as a guide in its use as a non-invasive sampling strategy. Efforts geared towards taking cognisance of bird species biology (moult-patterns, age-composition of the population), ecology (movements, diet, migration pattern) and physiology could improve the use of feathers as a non-invasive method. Furthermore, promoting the use of feathers as a 3R approach to monitoring environmental contaminants could further the course of reduced biodiversity loss and sustainable use of terrestrial ecosystems as specified in SDG 15 of the 2030 Agenda for sustainable development.

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Table 1: Reviewed publications and extracted information

S/N	Reference	Year	Study location	Sample type	Feather type	Contaminant type	Wild /experimental	direct mention of non-invasive-destructive method	
1	Monclús *et al* (2022)	2022	Europe	feathers	Moulted	organics	historic	yes	minimally/ non-invasive
2	Strekopytov *et al* (2017)	2017	Europe	feathers	Primaries	metals	historic	yes	non-destructive
3	Sani *et al* (2020)	2019	Africa	feathers	Body	metals	wild	yes	non-destructive
4	Malik and Zeb (2009)	2009	Asia	feathers	Body	metals	wild	yes	non-destructive
5	Dolan *et al* (2017)	2017	Europe	feathers and blood	Body	metals	wild	yes	non-destructive
6	Burger (1996)	1996	North America	feathers	Breast	metals	wild	yes	non-destructive
7	Abdullah *et al* (2015)	2015	Asia	feathers and egg	Breast	metals	wild	yes	non-destructive
8	Yao *et al* (2021)	2021	Asia	feathers and soft tissue	Breast	metals	wild	yes	non-destructive
9	Scheifler *et al* (2006)	2006	Europe	feathers and blood	Breast and Tail	metals	wild	yes	non-destructive
10	Muralidharan (2018)	2018	Asia	feathers	Down	metals	wild	yes	non-destructive
11	Panda *et al* (2020)	2020	Asia	feathers	NS	metals	wild	yes	non-destructive
12	Grúz *et al* (2019)	2019	Europe	feathers	Primaries, Secondary	metals	wild	yes	non-destructive
13	He *et al* (2020)	2020	Asia	feathers	Secondaries	metals	wild	yes	non-destructive
14	Markowski *et al* (2013)	2013	Europe	feathers	Secondaries	metals	wild	yes	non-destructive
15	Bada and Omotoriogun (2019)	2019	Africa	feathers	Tail	metals	wild	yes	non-destructive
16	Gushit *et al* (2016)	2016	Africa	feathers	Tail	metals	wild	yes	non-destructive
17	Veerle *et al* (2004)	2004	Europe	Feathers	Tail	metals	wild	yes	non-destructive
18	Aver *et al* (2020)	2019	South America	feathers	Body	organics	wild	yes	non-destructive
19	Briels *et al* (2019)	2019	Europe	feathers and blood	Body	organics	wild	yes	non-destructive
20	Eulaers *et al* (2011)	2011	Europe	feathers and blood	Body	organics	wild	yes	non-destructive
21	Zhao *et al* (2019)	2019	Asia	feathers and egg	Body	organics	wild	yes	non-destructive
22	Randulff *et al* (2022)	2022	Europe	feathers preen oil	Body	organics	wild	yes	non-destructive
23	Abbasi *et al* (2016a)	2016	Asia	feathers	Primaries, Secondary, Tail	organics	wild	yes	non-destructive

S/N	Reference	Year	Study location	Sample type	Feather type	Contaminant type	Wild /experimental	direct mention of non-invasive-destructive method	
24	Jaspers *et al* (2007)	2007	Europe	feathers	Tail	organics	wild	yes	non-destructive
25	Jaspers *et al* (2009)	2009	Europe	feathers	Tail	organics	wild	yes	non-destructive
26	Jaspers *et al* (2013)	2013	Europe	feathers and soft tissue	Tail	organics	wild	yes	non-destructive
27	Abbasi *et al* (2017a)	2017	Asia	feathers and blood	Tail	organics	wild	yes	non-destructive
28	Jaspers *et al* (2006)	2006	Europe	Feathers and soft tissue	Tail	organics	wild	yes	non-destructive
29	Fromant *et al* (2016)	2016	Europe	feathers, blood, soft tissue	Body	metals	wild	yes***	non-destructive
30	Pilastro *et al* (1993)	1993	Europe	feathers and soft tissue	Primaries, Secondary	metals	experimental	yes	non-harming
31	Durmuş (2018)	2018	Asia	feathers	NS	metals	wild	yes	non-harming
32	Squadrone *et al* (2016)	2016	Europe	Feathers	Body	metals	wild	yes	non-health affecting
33	Fernando *et al* (2020)	2020	Asia	feathers	Body	metals	wild	yes	non-invasive
34	Pedro *et al*. (2015)	2015	North America	feathers	Body	metals	wild	yes	non-invasive
35	Iemmi *et al* (2021)	2021	Europe	feathers and blood	Body	metals	wild	yes	non-invasive
36	Abbasi *et al* (2015a)	2015	Asia	feathers	Body, Tail, Primaries	metals	wild	yes	non-invasive
37	Kushwaha (2016)	2016	Asia	feathers	Moulted	metals	wild	yes	non-invasive
38	Di Marzio *et al* (2018)	2018	Europe	feathers	Primaries	metals	wild	yes	non-invasive
39	Martínez *et al* (2012)	2012	Europe	feathers	Primaries	metals	wild	yes	non-invasive
40	Debén *et al* (2012)	2012	Europe	feathers	Primaries, Secondary	metals	wild	yes	non-invasive
41	Dauwe *et al* (2003)	2003	Europe	feathers	Primaries, Tail	metals	wild	yes	non-invasive
42	Frantz *et al* (2012)	2012	Europe	feathers	Secondaries	metals	wild	yes	non-invasive
43	Zolfaghari *et al* (2007)	2007	Asia	feathers	Tail	metals	wild	yes	non-invasive
44	Adrogué *et al* (2019)	2019	South America	feathers	Body	organics	wild	yes	non-invasive
45	Gómez-Ramírez *et al* (2017)	2017	Europe	feathers and blood	Body	organics	wild	yes	non-invasive
46	Abbasi *et al* (2017b)	2017	Asia	feathers	Body, Tail, primaries, secondaries	organics	wild	yes	non-invasive
47	Yin *et al* (2018)	2018	Asia	Feathers and soft tissue	Primaries	organics	wild	yes	non-invasive
48	Meyer *et al* (2009)	2009	Europe	feathers and soft tissue	Tail	organics	wild	yes	non-invasive
49	Movalli (2000)	2000	Asia	feathers	Breast	metals	wild	yes	non-invasive and non-destructive
50	Pandiyan *et al* (2020)	2020	Asia	feathers	Breast	metals	wild	yes	non-invasive and non-destructive
51	Monclús *et al* (2018)	2018	Europe	feathers	Down and Body	organics	wild	yes	non-invasive and non-destructive
52	Martínez-López *et al* (2015)	2015	South America	feathers	Primaries	organics	wild	yes	non-invasive and non-lethal
53	Burger *et al* (2008)	2008	Europe	Feathers and egg	Down	metals	wild	yes	non-invasively
54	Goede and De Bruin (1986)	1986	Europe	feathers	Primaries, Vane, Shaft	metals	wild	yes	non-killing
55	Mikoni *et al* (2017)	2017	North America	feathers and soft tissue	Body	metals	wild	yes	minimal sampling invasiveness
56	Low *et al* (2020)	2020	North America	feathers	Primaries	metals	wild	yes	non-lethal
57	Lane *et al* (2020)	2020	North America	feathers and blood	Primaries, Tail	metals	wild	yes	non-lethal
58	DeSorbo *et al* (2020)	2020	North America	feathers and blood	Breast	metals	wild	yes	non-lethally
59	Squadrone *et al* (2018)	2018	Europe	feathers	Body	metals	experimental	no	
60	Ackerman *et al* (2016)	2016	North America	feathers, blood, soft tissue	Primaries, down	metal	historic	no	
61	Bustnes *et al* (2013)	2013	Europe	feathers	Tail	metals	historic	no	

S/N	Reference	Year	Study location	Sample type	Feather type	Contaminant type	Wild /experimental	direct mention of non-invasive-destructive method	
62	Solonen *et al* (1999)	1999	Europe	feathers	Body	metals	wild	no	
63	Boncompagni *et al* (2003)	2003	Asia	Feathers and egg	Body	metals	wild	no	
64	Abbasi *et al* (2015b)	2015	Asia	feathers	body, primaries	metals	wild	no	
65	Norouzi *et al* (2012)	2012	Asia	feathers	Breast	metals	wild	no	
66	Bourbour *et al* (2019)	2019	North America	feathers	Breast	metals	wild	no	
67	Burger *et al* (2014)	2014	North America	feathers and soft tissue	Breast	metals	wild	no	
68	Aziz *et al* (2021)	2021	Asia	feathers	Breast and Tail	metals	wild	no	
69	Badry *et al* (2019)	2019	Europe	feathers	Moulted, Body	metals	wild	no	
70	Yang *et al* (2018)	2018	Asia	feathers and soft tissue	NS	metals	wild	no	
71	Goede and De Bruin (1984)	1984	Europe	feathers	Primaries	metals	wild	no	
72	Adams *et al* (2020)	2020	North America	feathers and blood	Primaries	metals	wild	no	
73	Theuerkauf *et al* (2015)	2015	Europe	feathers and shaft	Primaries, Secondary	metals	wild	no	
74	Chatelain *et al* (2014)	2014	Europe	feathers	Secondaries	metals	wild	no	
75	Su *et al* (2020)	2020	Asia	feathers and blood	Secondaries	metals	wild	no	
76	Dauwe *et al* (2000)	2000	Europe	feathers and excrement	Tail	metals	wild	no	
77	Tasneem *et al* (2020)	2020	Asia	feathers, blood, soft tissue	Tail	metals	wild	no	
78	Binkowski and Sawicka-Kapusta (2015)	2015	Europe	feathers and blood, excrement	Primaries	metals	wild	no	
79	Eulaers *et al* (2014)	2014	Europe	feathers and blood	Body	organics	wild	no	
80	Malik *et al* (2011)	2011	Asia	feathers	Breast	organics	wild	no	
81	Kunisue *et al* (2003)	2003	Asia	feathers and soft tissue	NS	organics	wild	no	
82	Espín *et al* (2016)	2016	Review	Review	Review	Review	Review	yes	non-invasive
83	Abbasi *et al* (2016b)	2016	Review	Review	Review	Review	Review	yes	non-invasive
84	Jaspers *et al* (2019)	2019	Review	Review	Review	Review	Review	yes	non-invasive
85	Rutkowska *et al* (2018)	2018	Review	Review	Review	Review	Review	yes	non-invasive
86	Emasoga (2019)	UP	Africa	feathers	Moulting	metals	wild	yes	non-invasive
87	Oluwakotanmi (2019)	UP	Africa	feathers	Primaries	metals	wild		non-invasive
88	Egware (2019)	UP	Africa	feathers	Primaries	metals and organics	wild	yes	non-invasive
89	Okali (2019)	UP	Africa	feathers	Primaries	metals	wild	Yes	non-invasive
90	Fadahunsi (2019)	UP	Africa	feathers	Primaries	metals and Organics	wild	Yes	non-invasive
91	Adeogun *et al* (2022)	UPR	Africa	feathers	Body, Primaries	Metals and Organics	wild	yes	non-invasive

NS= Not specified, UP =unpublished, UPR = under peer review

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