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Birds' feathers – suitable samples for determination of environmental pollutants

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Abstract

The intensive development of industry and human population results in large amounts of different xenobiotic emitted into individual ecosystem components. As a consequence, monitoring of the level of pollution of particular elements of the environment by exotoxins has become a common interest. The determination of environmental changes by different types of biological indicators is called bioindication, which is used as one of the basic methods in the monitoring of environmental pollution. The following review paper contains comprehensive information about the use of bird feathers to assess the environmental contamination level. Types of contaminants (trace metals, microplastics, persistent organic pollutants) and analytical methods used for their determination are described in detail. In addition, the types of feathers used and the techniques for preparing them as samples for analysis are summarized.

Keywords: Birds' feathers; Trace metals; Microplastics; Persistent organic pollutants; Biomonitoring;

1. Introduction

Nowadays, human activity is responsible for introducing many xenobiotics into the environment. Persistent organic pollutants (POPs), toxic metals, and increasingly frequently mentioned micro-plastics are known to impose a serious potential risk to human and wildlife as they can be lingering, bioaccumulative and toxic [1]. POPs are present all over the world as a result of their wide-spread usage, long distance transport and persistence. Individual POPs have characteristic distribution patterns according to regional use patterns and their physico-chemical properties. Metals can enter the environment naturally and as a result of human actions. Prolonged exposure of biota results in the accumulation of toxic metals in their tissues (bioaccumulation) and an increase in the accumulation of

metals in each succeeding organism/link of the food chain (biomagnification). The environmental presence of toxic elements is a real threat to the quality and sustainability of ecosystems. The harmful effects of toxic metal contamination on organisms, including endocrine and nervous disorders, genetic mutation and certain physiological and behavioural abnormalities, are well known and widely described. In the last decade, a new type of plastic contaminant, described as microplastic, has attracted growing interest. Due to its size, microplastic can be consumed by both organisms from lower levels of the food chain (zooplankton, barnacles, fish) and organisms that occupy higher trophic levels, such as birds [2]. The classification of pollutants according to their legal "status" is presented in a schematic way in Figure 1.

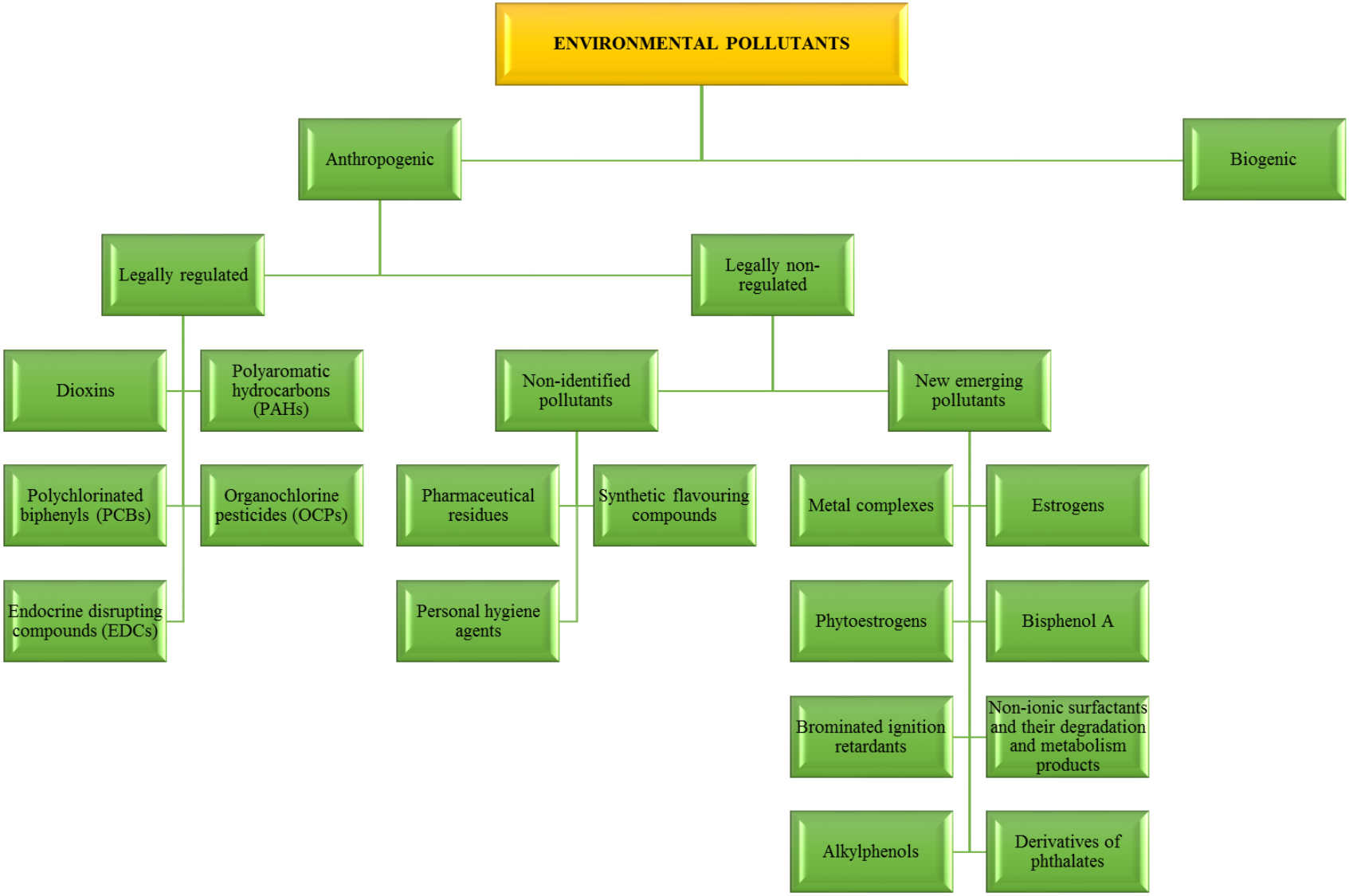

Figure 1: Classification of environmental pollutants.

The increasing amounts of hazardous chemicals released into the environment have affected the well-balanced nature of ecosystems and, as a result, the demand for environmental monitoring, assessment and remediation has never been greater. The need to identify exposure to and effects of contaminants has resulted in the creation of numerous biomonitoring schemes. A classification of biological methods for analysis and monitoring is presented in Figure 2. Proper assessment of the health status of ecosystems through biomonitoring requires the selection of indicator species that are representative of a given ecosystem[3]. Indicator organisms (or groups of organisms) can be classified according to their action and origin. A brief description of both types of indicators is presented in the Table 1.

Table 1: Classification of organisms by mode of action and origin.

CLASSIFICATION PARAMETER	NAME OF THE BIOMONITOR/ BIOINDICATOR	DESCRIPTION
MODE OF ACTION	Accumulative indicator	Organisms which accumulate one or more elements and/or compounds in the environment.
	Sensitive indicator	Organisms which show specific or non-specific alterations as a result of their exposure to a specified element, chemical compound or group of substances. Such changes may include: • Morphological changes; • Histological changes; • Changes in cell structure; • Changes in metabolic processes; • Behavioural changes • Changes in the structure of the population of organisms;
ORIGIN	Active bio-indicators/ biomonitors	Cultures are usually grown in a laboratory. They are used to study the accumulation of elements or compounds or specific or non-specific effects after exposure for a specific period of time, in a specific location (transplantation).
	Passive bio-indicators/ biomonitors	Organisms taken from their

	natural biotope and analysed to determine the accumulation of elements or compounds or specific or non-specific effects.

Organisms living in ecosystems differ in levels of contamination according to their diet, gender, age, size, or trophic level. Since the assessment of the pollution level of ecosystems by means of measuring the concentration of contaminants in all its components is problematic, time-consuming and labour-intensive, the use of indicator species which are exposed to environmental pollution and could best reflect the ecosystem's condition has become very common. Tissues and organs of living organisms that are exposed to contaminants both in the process of breathing and in the process of taking over the skin and with food can be a place of accumulation of various xenobiotics. An example is birds that certainly are exposed to various types of contamination. Birds can accumulate large amounts of xenobiotics due to their position in the food chain and sensitivity to environmental changes, which is why they are very often used as biomonitors of environmental pollution [4]. Of all bird species, predators are the most suitable for monitoring the contamination of terrestrial ecosystems as they are highly territorial and can be found worldwide. The best known indicator of exposure to pollutants is the level of contaminants in birds' internal tissue samples. However, nowadays, for both practical and ethical reasons, there is a growing interest in searching for samples alternative to internal tissues [4].

Analysing feathers is considered a non-destructive tool because feathers lost in the field or in nests can be collected without harming the animals [4]. The use of feathers as research material is becoming increasingly popular and offers many advantages, and the determination of impurities deposited in feathers of birds during their lifespan can provide valuable information on the quality of their environment. Both flight feathers (primary remiges and rectrices) and body feathers have been used in biomonitoring studies [5]. The most important milestones reported in the field of analytical and environmental chemistry are outlined in Figure 3. The advantages and disadvantages of the use of feathers as a tool for the biomonitoring of xenobiotics in the environment are graphically presented in Figure 4. The feathers may also provide a historical record of exposure at certain times of year on birds' annual cycle [6].

Contamination of feathers with xenobiotics may be endogenous or exogenous (Figure 5). When endogenous contamination predominates, the level of xenobiotics in the feathers reflects the level of contamination accumulated in the internal organs of a bird since the last moult cycle and the level of contamination of the food at the time of moulting.

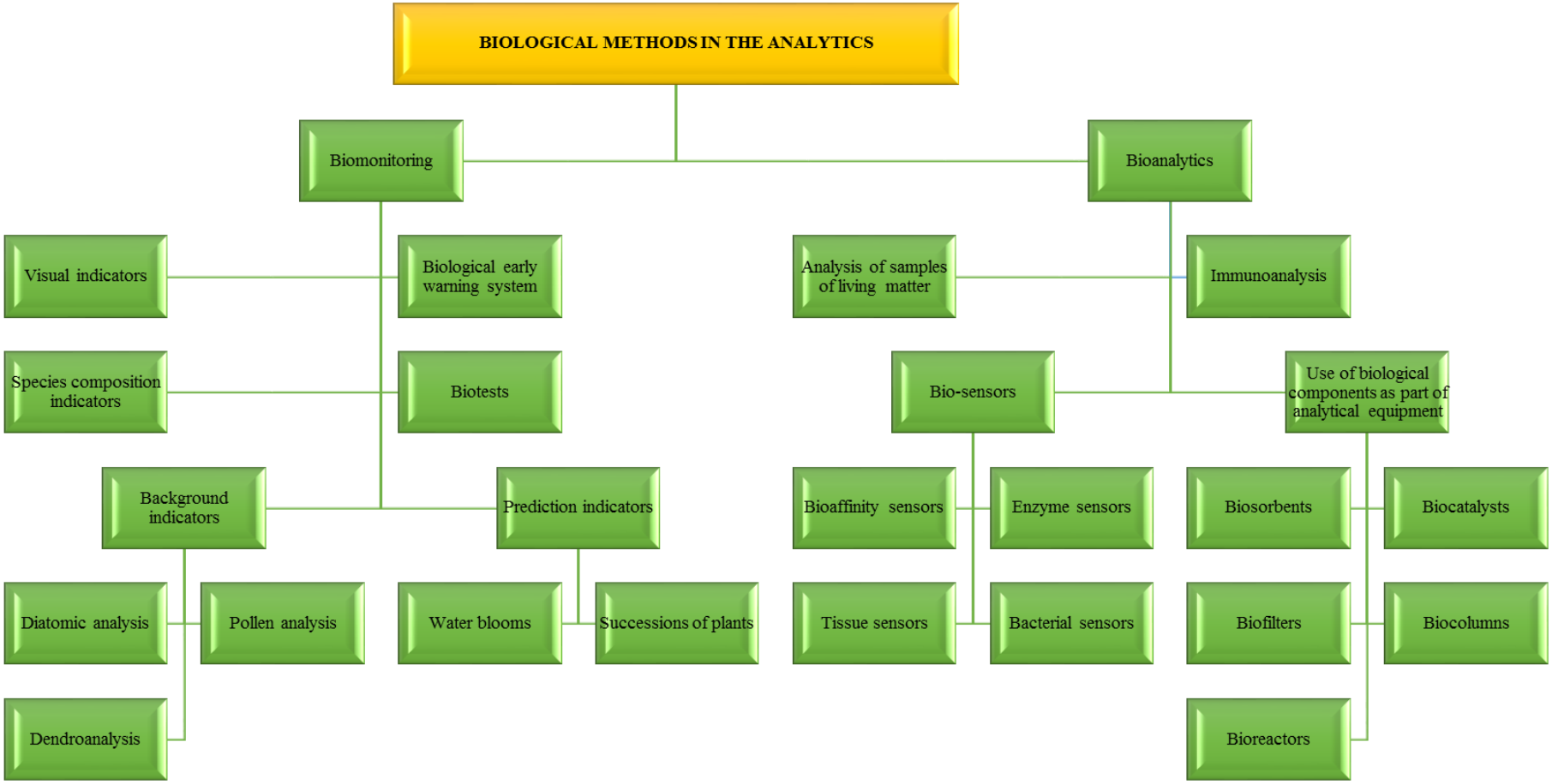

Figure 2: Biological methods in the analytics.

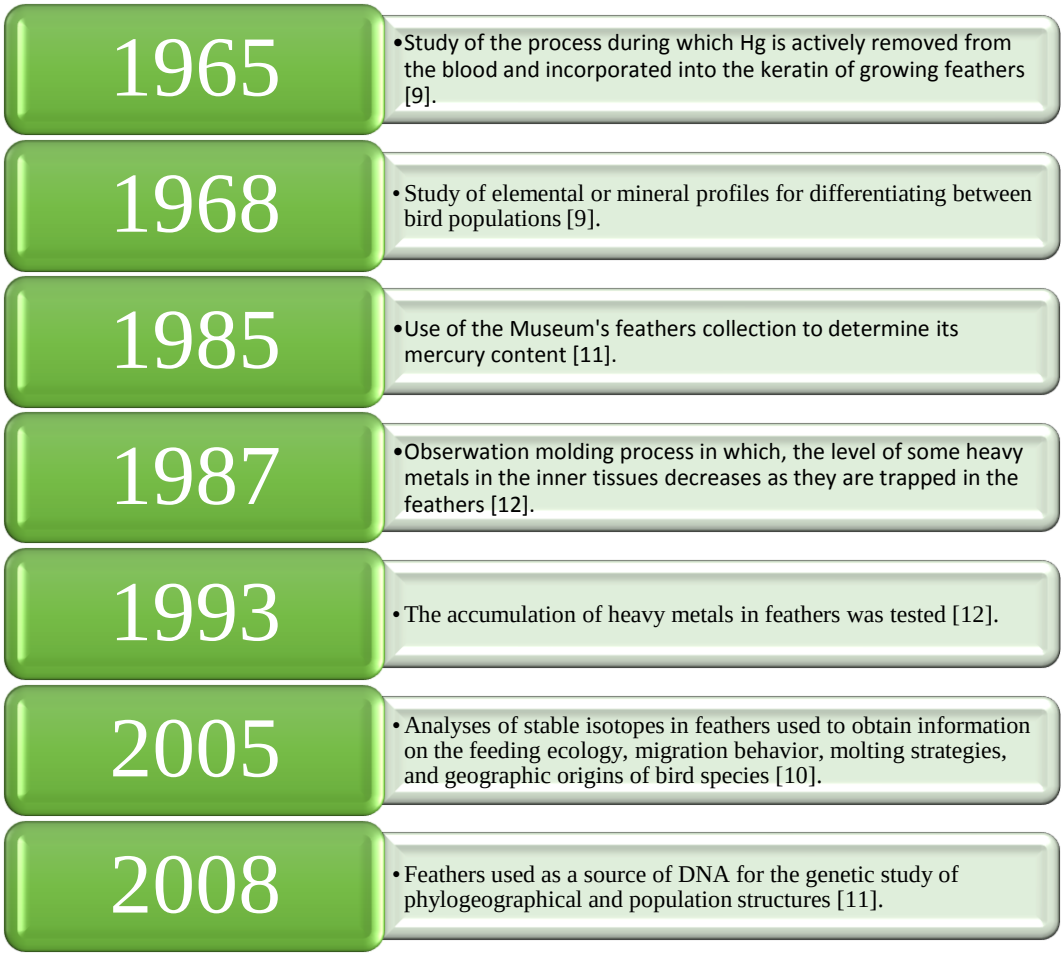

Figure 3: Information on the use of bird feathers in environmental analysis and monitoring over decades [7–10].

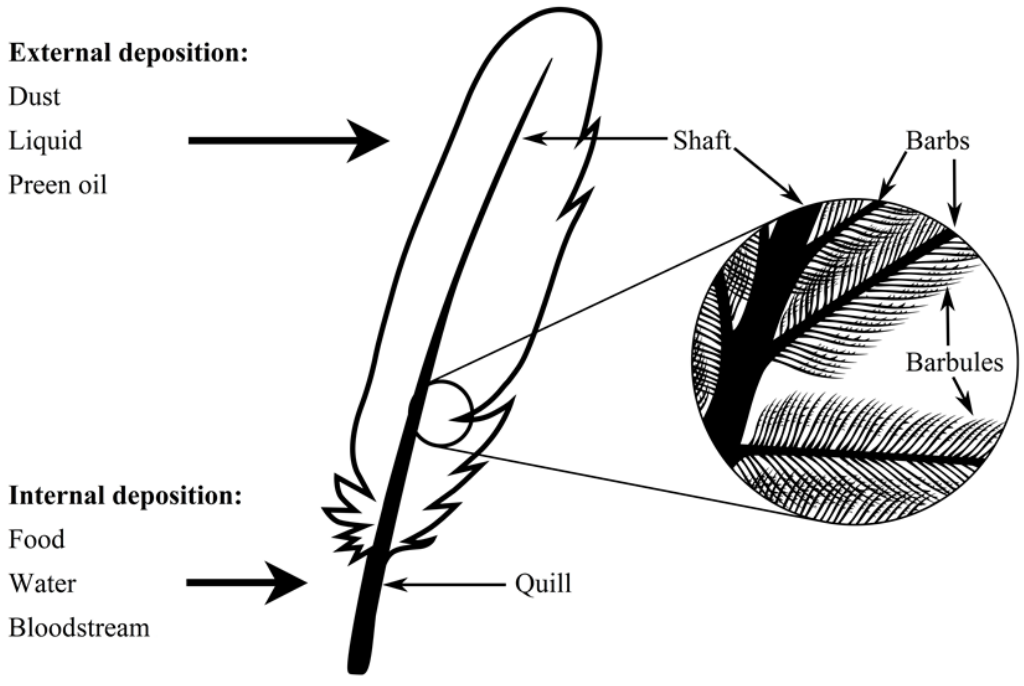

Figure 5: Schematic of the bird feather structure and possible contamination pathway.

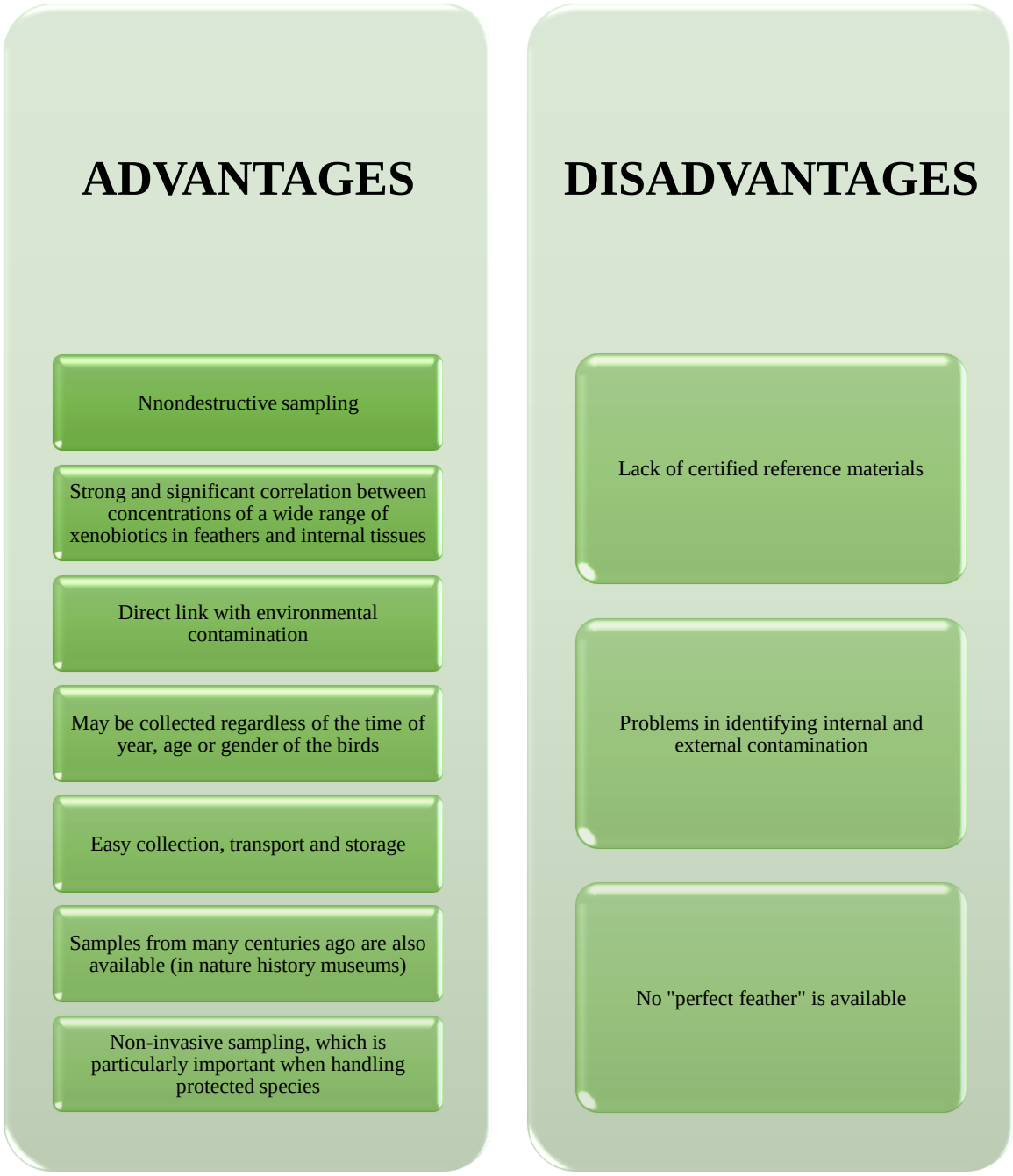

Figure 4: Advantages and disadvantages of using feathers in chemical analysis.

When exogenous contamination is prevalent, the content of feather contamination reaches a minimum shortly after moult completion and then increases when the bird is exposed to long-term xenobiotic pollution [11].

The overall aim of the present paper is to review a series of articles that focus on assessing the degree of xenobiotic contamination of organisms and ecosystems through the use of bird feathers as research material. This review is focused on discussion on the use of different types of bird feathers as an environmental biomonitoring tool. The use of several analytical techniques for the determination of a wide spectrum of xenobiotics has been reported. We estimate that birds' feathers are a very promising type of sample to be used as a source of information on the environmental condition.

2. Selection and preparation of the feathers

Since birds are sensitive to environmental changes and because of their specific position in the food chain, birds can accumulate high pollutant levels and thus they can be useful for the biomonitoring of environmental pollution. The best known source of indicators of the degree of birds' exposure to accumulative contaminants is their internal tissues. Nowadays, however, there is a trend and need to look for alternative samples to internal tissues due to ethical, practical, and conservational reasons. Among these alternatives feathers play an important role, because this kind of sample offers many advantages [4]. As that the collection of feathers in small numbers can be performed without causing permanent damage to the bird (since feathers lost in the field or in nests can be collected without having to directly handle individual birds), analysing feathers can be considered the ultimate non-destructive monitoring tool [4]. However, such aspects as collection time, moult period, feather type, external contamination on a feather's surface, gender, age, or the nutritional status of birds can influence the results and should be considered prior to final analysis [4]. In this section problems related with collection, selection and preparation of feather samples are discussed.

2.1. Collection of material

In general, responses of particular species of birds to human intrusions, the locations and accessibility of colonies are well known, thus, it is not difficult to design an action plan and determine the cost of a sampling programme. For biomonitoring, feathers are collected from birds at breeding or banding stations, wintering grounds and from museum specimens [12]. However, despite the fact that advanced analytical techniques exist which allow to gain a lot of analytical information on the pollutants in the environment, some difficulties for a single researcher and a team occur, the biggest of which is of the need to collect samples from hundreds or even thousands of individual birds from across the range of a

particular species [12]. Therefore, the primary rationale for systematic and organized feather collection is fostering studies at sampling intensity scales that are otherwise impossible to achieve.

One may think that systematic collection of feathers coming from migratory bird populations has a large research potential, however, such an endeavour would involve major logistical and financial hurdles [12].

In fact, collection of feathers requires significant regulatory coordination as well as oversight. Moreover, researchers might be granted permission to collect feathers from species that are not endangered as a part of their master banding permit [12].

There are specific principles guiding feather collection.. Firstly, collected feathers must be related to important, specific data including sampling date and locality. In addition, taxon identification and determination of age and sex must be made as precisely as possible. This way, data related to analyses of feathers would be analogous to data related to on-going national banding activities yielding many discoveries, including correctly identified taxa. The third principle is associated with reorganization of the limitations of such resources. For instance, it is not recommended to apply feather material, even of well identified taxa, in phylogenetic studies if approved samples are available or could be readily obtained.

Generally, researchers using feathers should carefully consider the costs as well as benefits of feathers versus other materials on a case-by-case basis, however it needs to be noted that there are clearly situations where broad-scale sampling of feathers and their analysis can provide valuable information that is otherwise unobtainable.

It also needs to be noted that consistent field sampling protocols are required to be adopted, as feathers constitute a complex issue and they are in fact not easy to collect. Thus, protocols of collection should be introduced to reduce the time and effort needed of banders while maximizing the new samples' utility [12].

2.2. Selection of material

All types of feathers can be used for pollutant analysis and other research including genetic studies. Selection of a type of feather depends on several issues, such as the moulting pattern of the species, preening behaviour and what is also important, on the study endpoint. In reported studies, such feathers as flight feathers, body feathers, and tail feathers have been used, but in some studies more than one feather type was used. Studies show that both plucked and moulted feathers can be analysed. Researchers prefer to use body feathers, rather than flight feathers, when feathers need to be plucked

(or cut close to the skin) from live birds. This is to avoid impairment of flight. But it is worth mentioning that plucking or cutting feathers may require a permit. However, feathers plucked from live birds were applied in about half as many research studies as feathers plucked from dead birds found in the field or from museum collections. Generally, moulted feathers are much easier to collect than plucked ones, however they have a drawback — information on age, sex and body condition of the bird from which the feather has originated and the time of moult, is usually lacking [4]. However, it is reported that application of moulted feathers appears to be similar to that of ones plucked from live birds.

2.3. Preparation of the samples

As feathers are a type of sample which is not homogeneous, and is exposed to various environmental factors and micro- and macro-pollution and dust, they need to be prepared properly prior to analysis. Firstly, feathers are typically washed with distilled water, then dried at room temperature, and cut into small pieces of approximately 1 mm [13]. In some studies, other solvents and washing agents such as water, surfactant solution, and acetone were investigated to remove external contamination [14]. However, in that case the washing solution is also collected after the washing for an appropriate analysis. In one study [15], different washing procedures were applied to compare their validity for removing external contamination from Common Magpie feathers. The results disclose a significant effect on the concentrations measured in feathers when washing with surfactant solution and acetone, compared to the control feathers. It was also observed that the concentrations in the washes were found highest for acetone and lowest for water, thus it was concluded that water is not suitable for removing preen oil secretions from feathers, while airborne particles and dust can be easily washed away with it. Washing with acetone or surfactant solution results in the leaching of some internal concentration. In the same study it was also indicated that preen oil is probably the main source of external contamination and that airborne contamination is probably of minor importance for organic compounds [15]. It needs to be mentioned that several washing steps are often performed to assure that all particles such as dust externally deposited on the surface of the feather are removed.

After basic sample preparation, a different type of extraction occurs, depending on the determined analytes and the choice of the final technique. The most popular technique is liquid-liquid extraction (LLE) performed at elevated temperatures (30-50 ^{o}C) [14,16]. Different mixtures of solvents are applied during LLE, depending on the analytes, the most popular, however, are: hexane:dichloromethane (analytes – organohalogenated compounds, e.g. polichlorinated biphenyls) [12,14,17], hexane:acetone (organochlorine pesticides) [12]. After extraction, the organic fractions are usually purified on a cartridge filled with acid silica and topped with anhydrous Na_2SO_4. The typical sample preparation procedure based on LLE is presented in Figure 4.

In addition to LLE, Soxhlet extraction is also applied [18]. After extraction, the organic fraction is purified on chromatography glass columns filled from the bottom with acidified silica and anhydrous sodium sulphate.

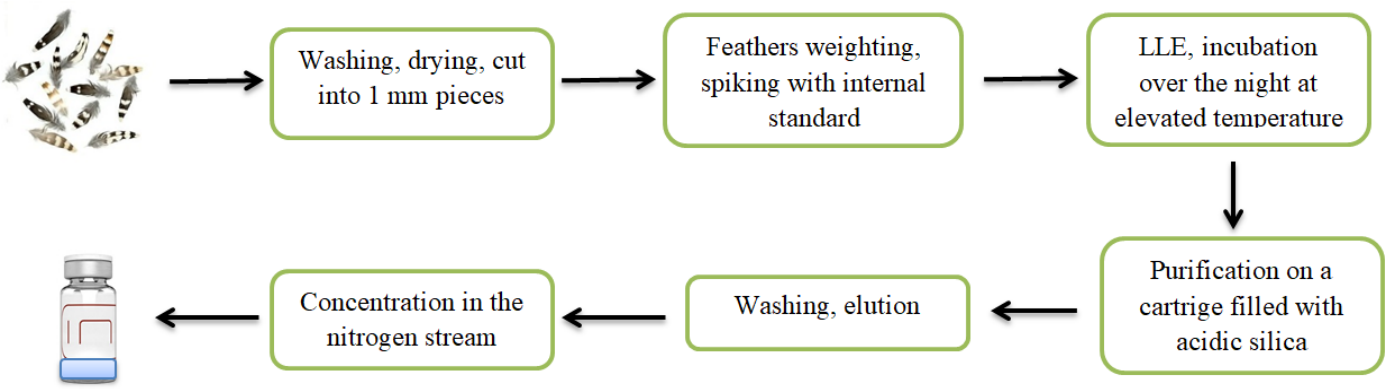

Figure 4. Schematic representation of feathers sample preparation based on LLE

3. Deposition rate of contaminants in feathers

Chemical analysis of feathers can be affected by a wide range of factors, therefore expressing the results as 'mass of contaminant per mass of feathers' is not the most accurate unit of measurement. The interpretation of the content of contaminations determined in bird feathers is affected for example by feather mass or its growth rate. Therefore, the solution to use the deposition rate (*DR*) factor as a unit of measurement in the test has been proposed [4]. Several options of calculation of this parameter are presented in Table 2.

Any results described in previous studies can be transformed by using the presented calculations, as it would be interesting to present results considering the mass of contaminants in relation to the mass of the feathers and their growth rate.

Table 2: Examples of the formulas for calculating the deposition rate factor [4,19,20].

DESCRIPTION	EQUATION
A pair of growth bars (dark and light) mark a 24-hour growth period. The formula may not be used for those species of birds where the growth bars are not clearly visible.	$DR = \frac{caontaminat\ mass\ [ng]}{pairs\ of\ measured\ growth\ bars\ [day]}$
The growth rate of feathers of different species of birds must be known (sometimes ecological studies are required). It should be taken into consideration that not all individuals of a given species have the same rate of feather growth.	$DR = \frac{contaminant\ mass\ [ng]}{feather\ length\ [nm]\ \times grow\ rate\ \left[\frac{mm\ feat\ her}{day}\right]}$

4. Feathers as a biomonitoring tool for detecting pollutants

4.1. Trace elements

Environmental pollution by toxic metals is a global problem and poses a serious threat to the quality of the environment. The need to identify levels of exposure and the effects of pollution has resulted in numerous biomonitoring studies. It is crucial to examine the baseline levels of trace elements in areas with relatively no contamination, to be treated as global reference values. Many of such tests are carried out on birds that are highly sensitive to anthropogenic pollution. Antarctica can be considered as one of the last impeccable environments. However, the results of the determination of toxic metals in feather samples of penguins living in that region show that some areas in Antarctica are not utterly pristine. The highest levels of elements such as lead (Pb), chromium (Cr) and zinc (Zn) were determined in feathers of birds inhabiting locations where many human activities such as plane and ship trips related to the tourism industry take place. Whereas concentrations of manganese (Mn), coper (Cu) or selenium (Se) are similar to those found in other parts of the world [21].

Extensive research is also carried out into the determination of trace elements in feathers of birds living in close proximity to humans in cities. The concentration of toxic elements in the feathers of pigeons throughout the Parisian agglomeration has been tested. On the basis of the collected data, it was noted that the ratio of metal concentration in feathers to the concentration in the environment calculated using results from other studies was 2-90 times higher in the case of cadmium than in the case of other metals, which indicates a very high ecological significance of this element [22]. In other studies, determination of toxic metals in feathers of Canada geese breeding in the New Jersey Meadowlands was carried out. These extensive wetlands are located in the heavily urbanized estuary of the New York/New Jersey Harbor and have been contaminated by industrial, commercial and domestic wastewater run-off along the Hackensack River and the nearby waterways [23]. Other studies were based on determination of metal concentrations in the feathers of nestling great tits, all collected from four sites along a pollution gradient in Belgium. Obtained data suggest that metal contamination in feather samples increase significantly towards the pollution source [24]. Similar studies, where determined levels of toxic metals in the feathers of birds from site closest to the pollution were compared with levels of toxic elements in the feathers of birds from reference site, were carried out. At contaminated site arsenic (As), cadmium (Cd), cobalt (Co), Cu, mercury (Hg), nickel (Ni), Pb and Se levels were on average 2-40 times higher in comparison with a presumably non-contaminated reference site [25]. Studies of environmental degradation following environmental disasters, in which feathers were used as research material, have also been done. Studies highlighting the suitability of chick feathers of seabirds for assessing the impact of oil spills on trace elements contamination were extremely important for monitoring environmental pollution. During these tests contents of toxic metals in seabird feathers were measured in order to assess the temporal pattern of contaminant exposure following the *Prestige* oil spill in November 2002 [26]. Table 3 summarises,

for a broader comparison, the literature information on the average toxic metal content in feathers of various species from different biogeographical areas.

Among the environmental monitoring studies, there are also studies that focus only on the level of contamination of organisms and their habitats by a single metal, e.g. highly toxic Hg. Hg is uptaken by birds almost only with the food they consume, while other toxic metals (e.g. Cd, Pb) are exogenous and their levels depend on the exposure of the feathers to contamination. The mercury content is almost constant throughout a feather, unlike other metals [27]. Feathers are connected with the bloodstream by an artery, therefore during the process of forming feathers, Hg both from the diet and accumulated in the internal organs and released into the bloodstream, is transported to the keratin structure of the feathers. Accordingly, the constant concentration of Hg in the feathers represents the amount of Hg to which the birds were exposed during the formation of the feathers. Therefore, the process of forming feathers in birds, which takes place at a certain time of year, works as a detoxification system [27]. A similar process can be observed with other metals. Lead, for example, accumulates in feathers through active or passive diffusion from blood to feather follicles [28]. A number of studies have also been conducted regarding the cadmium content in feathers of birds of different ecological habits [29].

Taking into account the results of many studies on the determination of toxic metals in bird feathers, it can be concluded that feathers are useful for long-term monitoring of this contaminants in the environment and are also reliable for a better understanding of spatial and temporal trends.

4.1.1. Analytical methods for toxic metals in feathers.

Several methods have been used for the analysis of toxic metals in feathers. The predominant method used for the determination of Hg content is the cold vapor atomic absorption spectrometry (CVAA) method at a wavelength of 253.7 nm [30]. However, there are studies in which atomic fluorescence spectrophotometry (AFS) [31], was also used. All other metals were analysed with a graphite furnace atomic absorption spectrometer (GFAAS) [29], flame atomic absorption spectrometer (FAAS) [32], neutron activation analysis (NAA) [33], inductively coupled plasma mass spectrometer (ICP-MS) [34] and plasma atomic emission spectrometer (ICP-AES [35]. Precision and accuracy of the methods were mostly tested using certified reference materials. The determination of trace metals by spectroscopic techniques requires appropriate sample preparation. In the first step the feathers are washed vigorously in deionized water alternated with acetone [36] and air dried. The next step is digestion with an acid/ hydrogen peroxide mixture [36]. Sometimes this process can be assisted by microwaves [37]. At the final stage, the sample is diluted with water to the target volume[38].

Table 3: Literature data on the content of trace elements in birds feathers samples from different parts of the world (numerical values are given as originally stated in the cited literature).

Bird species	Study sites	Feather type	Unit	Element *												Sample pretretment	Analytical method	References
				Cd	Se	Co	Cr	As	La	Cs	Sc	Hg	Cu	Pb	Zn			
Laggar falcons (*Falco biarmicus jugger*)	Pakistan	Body feathers	(µg/ kg)	0.10 ± 0.05	2.76 ± 0.82	0.86 ± 0.30	1.98 ± 0.58	-	0.84 ± 0.52	0.20 ± 0.06	0.23 ± 0.10	3.09 ± 2.35	-	1.56 ± 1.12	107.40 ± 19.98	Mineralization in a CEM-MDS 81D microwave oven and carried to volume with 0.5% acqueous nitric acid solution.	AAS\NAA	[33]
Common eider (*Somateria mollissima*)	USA (Aleutian Islands, Alaska)	Breast feathers	(ng/ g)	79.8±3.96	878± 88.3	-	172±49. 9	138±18. 0	-	-	-	840±81. 5	-	993±13 2	-	Washing (acetone)\ digestion (nitric acid\ 30% hydrogen peroxide)\ dilution (deionized water)	CVAA\GFAA	[36]
Tufted puffin (Fratercula cirrhata)				80.3±12.9	6,600 ±344	-	1,820±2 30	136±25. 6	-	-	-	2,540±1 95	-	1,260± 339	-			
Pigeon guillemot (Cepphus columba)				31.0±6.02	3,350 ±259	-	1,670±4 02	157±25. 8	-	-	-	7110±6 57		1,280± 274	-			
Bald eagle (Haliaeetus leucocephalus)		Wing feathers		253±39.6	2,550 ±268	-	2,170±1 93	547±75. 8	-	-	-	4,910±7 72	--	4570±7 99	-			
White tern (Gygis alba)	Pacific Ocean (Midway Atoll)	Breast feathers	(ng/ g)	216±36.0	1290 ±85.0	-	1300±1 02	459±68. 7	-	-	-	1210±7 6.2	-	1380±6 93	-	Washing (acetone)\ digestion (nitric acid\ dilution (deionized water)	CVAA\GFAA	[38]
Bonin petrel (Pterodroma hypoleuca)				129±28.7	7850 ±213	-	1620±6 9.5	59.5±20 .6	-	-	-	19 700±1.0 80	-	1350±2 91	-			
Christmas shearwater (*Puffinus nativitatis*)				950±429	10 100± 1200	-	2350±4 85	360±20 0	-	-	-	939±10 7	-	2380±5 31	-			

Eurasian sparrowhawk (*Accipiter nisus*)	Belgium (Flanders)	Tail feathers	(µg/kg)	0.09±0.05	-	0.06±0.02	1.97±0.67	0.98±0.83	-	-	-	1.10±0.66	3.16±1.09	2.61±2.08	23.8±4.4	Washing (acetone\water)\ drying\digestion (nitric acid\ 30% hydrogen peroxide)\ dilution (deionized water)	ICP–MS	[39]
Little owls (*Athene noctua*)				0.05±0.02		0.22±0.08	0.50±0.16	0.08±0.03				0.32±0.08	8.98±1.38	3.99±1.95	35.7±2.0			
Little Egrets (Egretta garzetta)	China (Hong Kong)	-	(µg/g)	0.10 ± 0.04	-	-	0.9 ± 0.08	-	-	-	-	0.8 ± 0.3	13.0 ± 3.0	4.4 ± 1.2	83.9 ± 13.1	Washing (acetone\water)\ drying\ digestion (nitric acid 65% \ dilution (deionized water)	ICP-AES\ICP-MS\CVAAS	[40]
Night Herons (*Nycticorax nycticorax)*				0.04 ± 0.06	-	-	0.9 ± 0.3	-	-	-	-	1.7 ± 1.1	6.0 ± 1.9	0.7 ± 1.00	118.9 ± 12.4			
Antarctic prion (Pachyptila desolata)	UK (Bird Island, South Georgia)	-	(ng/g)	59 ± 110	7319 ±2192	605 ± 1295	-	411 ± 227	-	<LOD	<LOD	-	185292 ±644966	<LOD	113658 ± 364227	Oven-drying\ digestion (nitric acid\hydrogen peroxide)\ dilution (deionized water)	ICP-MS	[41]
Black-browed Albatross (T. melanophrys)	Argentina (Patagonian Shelf)	Primary feather	(µg/g)	0.33 ± 0.32	-	-	< LOD	-	-	-	-	-	4.86 ± 1.75	5.71 ± 5.67	102.76 ± 127.37	Washing (acetone\water)\ mineralization with 1:3 perchloric-nitric acid mixture	F-AAS	[32]
		Breast feathers		0.69 ± 0.67	-	-	<LOD	-	-	-	-	-	9.67 ± 3.22	3.17 ± 3.34	28.18 ± 67.37			
Flesh-footed Shearwater (Puffinus carneipes)	New Zeland (Kauwahaia Island)	Breast feathers	(ng/g)	71 ±41	-	104 ± 93	-	1229 ± 1454	-	-	-	7466 ±2360	14796 ±4330	347 ± 176	104291 ±31540	Washing (NaOH)\ digestion (nitric acid\hydrogen peroxide)\ dilution	ICP-MS	[42]

*Given are mean ± S.E. on a dry weight basis.

4.2. Persistent organic pollutants

Exposure to various types of organic pollutants is one of the main environmental hazards caused by human activity. Pollutants resistant to environmental degradation, and thus occurring in the environment for a long time, are called persistent organic pollutants (POPs). These impurities are transported long-range and they also have the ability to bioaccumulate, which may pose a threat to the environment and living organisms. These chemical compounds include mainly: polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polybrominated diphenyl ethers PBDEs or perfluorinated alkyl substances (PFASs).

In recent years, bird feathers have been increasingly used to monitor environmental pollution (Table 4). Figure 1 shows examples of POP chemicals, which have been determined in feathers.

4.2.1. PCBs

Polychlorinated biphenyls (PCB) are one of the main chemicals that contaminate bird feathers. PCBs are commonly found in various environmental elements, such as soils, sediments, air or fauna and flora. Their presence in a bird's life environment, and consequently in feathers, results from applications in the industry due to their stabilizing character and low flammability. Exposure of birds to PCBs causes a number of chronic effects, which mainly include dysfunction of the endocrine, nervous and reproductive systems [43]. Endocrine disruptions resulting from the birds' exposure to PCBs are one of the main causes of the reduction in the population of many bird species [44].

The content of polychlorinated biphenyls varies depending on the species of birds, which results from factors such as diet, migration or living environment. Research shows that feathers of carnivorous birds contain the largest amounts of polychlorinated biphenyls compared to birds with other eating habits, because of the biomagnification process [45]. A large accumulation has been recorded especially in the case of fish-eating birds, because fish accumulate highly lipophilic compounds from their surroundings, both through direct absorption from the water via gills and from the food intake. [46]. In the case of using migrating birds’ feathers as bioindicators for PCBs content in breeding areas, a better solution is to use a fledgling's feather [47]. The common bird species, whose feathers are used as an indicator of environmental pollution with polychlorinated biphenyls, is *Accipiter nisus*. Comparing the results of the determinations, differences in the content of PCBs in feathers of these birds depending on the region can be noticed - Iran (55 ng/g), Pakistan (3.56 ng/g), Belgium (160 ng/g) [48–50]. Feathers are therefore a good indicator of environmental pollution.

An extremely important element in assessing environmental pollution based on the content of PCBs in feathers is choosing the right type of feathers. The most appropriate type for biomonitoring the environment are feathers from a bird's body surface [51]. However, it cannot be unambiguously

determined whether feathers of resident or migratory birds are a better indicator for PCBs. Everything depends on the purpose of the research being conducted. It is then extremely important to trace all stages of a bird's life in order to take into account factors resulting from its migration which can influence the outcome. Resident birds are good indicators of local contamination and migratory birds may be useful in research on a broader scale. Studies have shown that using hatchling feathers to assess the actual state of the environment is a better approach than using adult birds' feathers. It is related to the still existing connection of the feather with the blood of the hatchling and elimination of physiological and ecological variables when studying nestlings, in contrast to adults [52].

To determine the content of polychlorinated biphenyls in bird feathers, in most cases the gas chromatography technique coupled with mass spectrometry is used. The second detector utilized for this purpose is the electron capture detector (ECD), because the feathers are samples with a complex matrix composition. High content of compounds other than the targeted one and the same retention time of PCBs leads to coelution, which falsifies the results obtained with the use of the GC-ECD technique.

4.2.2. OCPs

Organochlorine pesticides (OCPs) are a structurally heterogeneous class of semivolatile organic compounds. They have properties similar to other persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs). After being released to the environment, they are resistant to decomposition processes, be it physical, biological, chemical and photochemical, and thus remain in the environment. In addition, these compounds can accumulate in the food chain due to their lipophilic bioaccumulative and biomagnification properties [53]. The most commonly studied persistent chloroorganic pesticides are dichlorodiphenyltrichloroethane (DDT) and its metabolites, hexachlorobenzene (HCB), β-hexachlorocyclohexane (β-HCH) and endosulfan. Exposure to OCPs has resulted in a variety of toxic and harmful health effects, including cancer, immune and reproductive disorders in living organisms, including humans and wild fauna and flora [53]. The influence of OCPs on the survival and reproduction of birds has also been documented. These compounds have caused the thinning of eggshells, impaired development and impairment of the nervous system of the hatchlings [54].

The content of this group of pollutants depends mainly on the range of a given species. Although prohibitions on the use of OCPs have been implemented in developed countries, many of these compounds may still be used in developing countries in America and South Asia [55]. Based on the research, much higher concentrations of OCPs in bird feathers from Patagonia have been documented (6.49±5.95 μg g-1) [56] compared to results obtained for birds living in Spain (870.48±614.48 ng/g) [57] or Ireland (17.94±2.19 ng/g) [58]. Therefore, feathers can be a biomonitor to determine the

environmental contamination of given regions of the world. In view of the long half-life of chloroorganic pesticides, these compounds can be subject to long-distance atmospheric transport, therefore the use of OCPs can cause contamination not only in the place of use but also in other parts of the world, even in virgin areas such as the Arctic and Antarctica. Penguin feathers can be a good tool for biomonitoring OCPs, both because it is a resident species and because their diet can give a clear picture of the available fixed amount of pesticides in endemic fauna. In addition, the method of collecting samples is completely non-invasive and does not adversely affect penguin colonies [59]. Feathers were also used to reflect the changes in their environment, namely to depict the differences in the levels of OCPs in urban and rural areas. On the basis of the conducted research, it was shown that the OCP concentrations in magpie (*Pica pica*) feathers living in rural areas were significantly higher compared to the industrialized/urbanized areas. Feathers can, therefore, reflect regional variations in pollution levels, which increases their applicability as a non-destructive tool for monitoring organic pollutants. [60]. The content of OCPs in bird feathers also depends on their species, and more specifically on the movement pattern or diet. Birds of prey are particularly sensitive to environmental changes. In addition, due to their position in the food chain, they accumulate high levels of pollution. For this reason, species of carnivore birds are most often used for environmental research [50,61,62]. OCPs in Mongolian plover (*Charadrius mongolus*) feathers have been shown to reflect OCPs in the environment, so the feathers can be considered as appropriate tools to assess the toxicological and environmental risks [61]. Another application is the use of feathers of various species of birds as a tool to determine the progress of remedial actions in various regions of the world, e.g. in the area of the Caspian Sea [45]. It should also be noted that feathers from museum collections can also be used for studying the contents of OCPs in feathers, which allows comparing the past state of the environment with the current one [46,50].

For the determination of OCPs in feather samples, a gas chromatography technique coupled with an electron capture detector or mass spectrometry is mainly used. The results obtained using these techniques are comparable, and the limits of quantification are in each case about 0.1 ng/g of feathers.

4.2.3. PBDEs

Polybrominated diphenyl ethers (PBDEs) are compounds that strongly affect the natural environment. They are so harmful that they have been banned in many developed countries, such as Canada, Japan, or the EU member states. In many countries, PBDEs are still used in plastics, synthetic textiles, electronic goods, building materials and polyurethane foams. Due to the high potential of bioconcentration and biomagnification they easily penetrate the air, soil, water or sediment in production, disposal, and recycling processes [63]. Despite legal restrictions, the concentration of PDBEs is still high in bird feathers from many European countries [49,52]. Exposure to PBDEs has been shown to cause many toxicological effects (reproductive/developmental effects in laboratory

animals and wildlife). Because birds are widespread and sensitive to environmental changes, and occupy the highest position in the food chain, they are commonly used to monitor PBDE levels [64]. The amount of PBDEs accumulating in the tissues of birds is related to their diet and position in the trophic chain, but also to the differences in accumulation between various habitats and ecosystems (water/land)[49]. According to literature reports on PBDE, it has been suggested that birds living in terrestrial environments are more exposed to higher brominated BDEs than aquatic species [65]. Many studies have been carried out on the content of PBDEs in bird feathers. It was stated that levels of sum PBDEs were 10 to 40 times lower than levels of sum PCBs in feather samples. The researchers found that the contents of PBDEs can be determined using a single feather of a predatory bird, sampled in a non-destructive way. That is why feathers of predatory birds seem more suitable as a tool for assessing environmental levels of organic pollutants than feathers of small, herbivorous birds [49]. The conducted research confirmed that feathers of predatory birds, especially their fledglings, to be a good non-destructive biomonitoring strategy for assessing exposure to PBDEs [52]. In the case of Lack kite (*Milvus migrans*) and Spotted owlet (*Athene brama*) it was noted that also in the case of PBDEs, nestling or growing feathers may be better predictors of the state of the environment civilization's great solution. The gas chromatography technique is used to determine compounds from the PBDE group. In most cases, it is used in combination with electron capture negative ion mass spectrometry.

4.2.4. PFASs

Perfluorinated substances (PFASs) belong to a group of chemical compounds characterized by a completely fluorinated hydrophobic linear carbon chain combined with a hydrophilic functional group. They are widely used for both industrial and consumer purposes because of their physicochemical properties, such as thermal stability, surface-active properties, and water or oil resistance [66]. These compounds are also resistant to various types of degradation, which is why they are persistent in the environment and have the potential to bioaccumulate. In addition, they are very toxic; it has been documented that PFASs are associated with neuro-, immune- and hepatotoxicity [67]. PFOA and PFOS are the most common substances among PFASs, however their production and use are currently banned. Despite this, they are still present in the environment, which is why it is extremely important to find tools to monitor these pollutants.

Birds, due to their position in the food chain and susceptibility to the collection and biomagnification of pollution from both aquatic and terrestrial ecosystems, can be a good reflection of the state of the environment. Especially feathers obtained from predatory birds can be good indicators of environmental pollution. Prey birds are often protected species, which is why it is so important to look for non-destructive biomonitoring techniques. The use of feathers to determine the content of PFASs has many advantages: sampling does not have a negative impact on the bird's body; moulted feathers can be collected even from a nest. Moreover, the samples do not require special storage and transport

conditions. Samples of feathers and livers taken from barn owls (*Tyto alba*) were tested. Based on the obtained results, it was found that the content of PFOS in feathers was caused by internal impurities. Most likely they were introduced into the body along with food, while the PFOA content in feathers was caused by external impurities, i.e. wet or dry deposition from atmospheric air thus, the feathers can probably be used as a passive probe for PFOAs [68]. The concentrations of PFHxS and PFOS were also compared in various bird species with different diets. It has been proven that feathers of carnivore birds are characterized by higher concentrations of PFASs. This confirms the thesis that in the case of PFAS content, the birds' diet is the most important factor [69]. In addition, the selection of the individual birds for research is also very important. It has been documented that territorial and non-migratory species are most important for assessing the local state of environmental pollution. The best solution, however, is to use feathers from the fledglings, as they are only exposed to the environment in which they were hatched, and in addition, are fed pray caught near the nest. Fledgling feathers can be non-destructive tools to assess the state of the environment in the area of their existence [70].

For the determination of PFASs, the high-performance liquid chromatography technique coupled with mass spectrometry is mainly used. This technique is characterized by high sensitivity and resolution, which makes it possible to obtain a low limit of detection, even below 0.04 ng/g of feathers [71].

4.2.5. OPEs

Organophosphate esters (OPEs) are chemical substances used as flame retardants, antifoams and plasticizers. They are constituents of many plastic products, electronic equipment, furniture, building materials, varnishes and hydraulic fluids. OPEs are therefore additional substances in polymeric materials, i.e. they are not strongly associated with them, so they can easily get from the products into the environment due to abrasion, oxidation or leaching [72]. These substances are characterized by persistence, bioaccumulation ability, toxic (mutagenic, carcinogenic or neurotoxic) properties [73].

In recent years, feathers have become common samples for environmental measurements, as the method of collecting is non-invasive and non-destructive. In order to determine OPEs in the environment, however, the feathers were used in very few studies. In one of them, OPE concentrations in the kittiwake (*Rissa tridactyla*) feathers and blood plasma from Svalbard were compared. Based on the conducted research, it was found that feathers obtained from adult birds are not useful for testing OPEs pollutants, and in the future, the focus should be on nestling studies [47]. The level of organic contaminants in two types of feathers from cinereous vulture (*Aegypius monachus*) nestlings was also investigated. In addition to the studies of the usually used contour feathers, it was decided to carry out experiments also on the down feathers. Based on the results obtained, it was found that nestling feathers are an extremely important tool for the determination of POPs in the environment. In addition, down feathers are also useful and can be used as OPE biomonitors, and with such samples higher

detection rates and higher concentrations of OPEs can be obtained compared with contour feathers [74].

The use of feathers as OPE biomonitors is a new analytical issue, and for this reason many studies are needed to explain the deposition of these substances in feathers.

4.2.6. PAHs

Polycyclic aromatic hydrocarbons (PAHs) are highly toxic, carcinogenic and mutagenic. Due to their bioaccumulation in marine sediments, they produce toxic effects in organisms at the lowest level of marine food chain (benthos) [75]. Polycyclic aromatic hydrocarbons get into the environment mainly from various industrial undertakings. However, they can also come from incomplete combustion of fossil fuels [58]. It has been proven that feathers could predict the pollution of PAHs. Aquatic birds' feathers have greater potential for the bioindication of PAHs, because water birds diminished their body burdens by placing contaminants into growing feathers [76]. The use of bird feathers as a biomonitoring tool for PAHs is a new approach. It elucidated the pollution status of PAHs in feathers of Little Egret using HPLC- DAD-FLD technique [76]. The European storm petrels' feathers were also tested for PAHs and their profiles in breast feathers [58]. Gas chromatography and mass spectrometer run in EI mode were used for the analysis.

4.2.7. Phenols

In recent years, compounds from the group of phenols in the samples of feathers of European herring gulls have been determined. The content of bisphenol A (BPA), 4-tert-octylphenol (OP), and 4-nonylphenol (NP) was examined. These compounds got to the feathers from the feeding area during the moulting of birds. Due to their ability to disrupt homeostasis, embryonic development and reproduction, these compounds are a dangerous factor in the habitat of birds. The main sources of these compounds are: thermal paper receipts, bike helmets, police shields, reading glasses, circuit boards, flat screen televisions and smart phones, incubators, nebulisers, implants, artificial joints, internal electronics (BPA), car tires, paints and varnishes (OP), detergents, cleaners, industrial applications, but also in cosmetics (NO) [77]. A significant amount of these compounds penetrates into waters and sediments, which are bioaccumulated by plankton and fish. In the case of endocrine active phenols, their content also increases with increasing trophic levels. For this reason predatory water birds are the most exposed to phenols, not only through food, but also because of the high content of these compounds in their living environment. Polyphenols are particularly susceptible to endocrine as they are gulls, because in addition to eating fish, they also find food in landfills or in garbage containers, which is why they swallow fragments of plastic or foil. In addition, gulls feed on the entrails of fish gutted by fishermen on the seashore, which further increases the exposure of gulls to such compounds as BPA, OP, or NP [77]. These compounds eventually accumulate in bird feathers. The studies also showed that the age of a given bird influences the level of EDCs. Higher mean BPA

contents were measured in the feathers of mature birds, while the reverse dependence was obtained in the case of NP.

The content of endorphin active phenol compounds in bird feather samples is determined using liquid chromatography. Also in this case, it is necessary to perform an earlier extraction using organic solvents such as methanol and ammonium acetate. However, the quantities of solvents consumed are small. Enrichment of analytes is carried out using the SPE technique.

In conclusion, bird feathers are an extremely useful tool for monitoring environmental pollution. As can be seen in Fig. 2, feathers of both aquatic and terrestrial birds are used for research. The selection of birds depends on the research purpose. On the basis of resident bird feather analysis, it is possible to assess the condition of the environment in a given region, while using feathers of migratory birds it is possible to determine the exposure of organisms living in regions less-developed in industrial terms, such as India, where birds often overwinter. If the feathers are a matrix for the analysis of environmental contaminates, the bird species from which the feathers are obtained should be chosen properly. The most appropriate option is birds of prey. As it can be seen in the graph (Fig. 2), research on birds of prey (carnivore and scavenger) constitutes 67% of all research.

4.3. Microplastics

Plastics are mostly made of synthetic polymers including polyethylene or polystyrene, which are derived from polymerisation of monomers extracted from oil or gas. They are considered civilization's great solution, becoming the predominant material of modern life. Since the introduction of plastics into industry in the 1950s, the accumulation of plastic waste has become an emerging global environmental issue. Worldwide plastic production increased from 1.5 million tonnes in 1950 to 335 million tonnes in 2016. Europe is the second largest, after Asia, producer of plastic at global level with 60 million tonnes manufactured in 2016, corresponding to 19% of the total production. It was estimated that up to 80% of the waste discarded in landfills, or left in the open dumps or in the natural environment is plastic [78].

In recent years, there has been increasing environmental concern about microplastics (MPs), defined as the particles with a size smaller than 5 mm [78]. However, it was also suggested to consider fragments lower than 10 mm [79], 2-6 mm [80], 2 mm [81] and 1 mm [82]. Depending on their origin source, they can be divided into two groups: primary and secondary microplastics. Primary MPs, engineered to be small, are used as exfoliants for personal care products [83], in sand-blasting media or in synthetic fibers for the production of clothes [84]. They enter the environment directly as micron-sized granules during manufacture, transportation or use. More abundant in the natural environment are secondary microplastics, derived from the breakdown of larger plastic pieces as a result of

different conditions such as temperature changes, physical abrasion or mechanical action [85]. During these processes plastic debris becomes smaller and finally appears in the microscopic size.

Table 4: State of the art of feathers' application in the POPs determination and quantification

MATRIX			SAMPLING			SAMPLE PREPARATION			DETERMINATION				Ref.
BIRDS TYPE	SPECIES	STUDY AREA	FEATHERS TYPE	COLLECTION	STORAGE	PRE-PREPARATION STEP	EXTRACTION	PURIFYING	ANALYTE	TECHNIQUE	RESULTS	LOD/LOQ	
terrestrial, resident, scavenger	• Turkey vulture (Cathartes aura) • American black vulture (Coragyps atratus) • Southern crested caracaras (Polyborus plancus)	Argentina	primary wing feathers	fresh-molted feathers	• plastic bag • room temperature	• washing with tap water, distilled water and Milli-Q water • drying at room temperature • cutting into pieces	• incubation with HCl and hexane:acetone (2:1, v/v) at 37 °C overnight • extraction with hexane:acetone (3:1, v/v)	• filtration using anhydrous sodium sulfate • fractionation via Florisil column chromatography	OCPs	GC-ECD	• ΣOCP: 0.35-26.5 μg/g ww • ΣDDT: nd-11.7 μg/g ww • ΣHCH: nd–6.53 μg/g ww • ΣHeptachlor: nd-18.2 μg/g ww • ΣDrin: nd-8.89 μg/g ww • ΣEndosulfan: nd-10.7 μg/g ww	LOD: 0.03-0.41 ng/g ww	[56]
aquatic, migrant, carnivore	• Razorbill (Alca torda)	East of Spain	primary wing feathers	feathers from dead birds	-	• washing with tap water, distilled water and Milli-Q water • drying at room temperature • cutting into pieces	• incubation with HCl and hexane:acetone (2:1, v/v) at 37 °C overnight • extraction with hexane:acetone (3:1, v/v)	• filtration using anhydrous sodium sulfate • fractionation via Florisil column chromatography	OCPs	GC-ECD	• ΣOCP: nd-2104.05 ng/g dw • ΣDDT: nd-1596.24 ng/g dw • ΣHCH: nd-683.56 ng/g dw • ΣHeptachlor: nd-624.98 ng/g dw • ΣDrin: nd-281.31 ng/g dw • ΣEndosulfan: nd-500.02 ng/g dw • ΣCyclodien: nd-1103-22 ng/g dw	LOD: 0.03-0.14 ng/g dw	[57]
terrestial/aquatic, resident, carnivore	• Gentoo penguins (Pygoscelis papua) • Chinstrap penguin (Pygoscelis antarctica)	Western Antarctica	moulting feathers	-	-	-	diethyl ether:n-hexane (1:1, v/v)	filtration	OCPs	GC	• ΣDDT: 1.922–3.011 μg/g • ΣHCH: 0.02–0.658 μg/g		[59]
terrestrial, resident, carnivore	Western burrowing owls (Athene cunicularia hypugea)	California	body feathers from breast, sides and back	feathers from birds captured on nests	in acetone-rinsed, prelabeled glass jars placed on ice	homogenization	extraction with 5% ethanol in ethyl acetate	cleaning up with automated gel permutation chromatography	OCPs	GC-FPD GC-ECD GC-MS	DDE: 0.05–1.02 μg/g		[86]
• aquatic, partial migrant, carnivore • aquatic, resident, planktivore • aquatic, resident, planktivore • aquatic, resident, carnivore • aquatic, resident, planktivore	• Northern fulmar (Fulmarus glacialis) • Crested auklet (Aethia cristatella) • Auklet-crumb (Aethia pusilla) • Pacific gull (Larus schistisagus) • Gray petrel (Oceanodroma furcate)	Russia	-	-	−20 °C	homogenization	extraction with hexane	-	OCPs	GC-EI-MS	• ΣOCP: 29-8289 ng/g lipid • ΣDDT: 975–1978 ng/g lipid		[87]
• aquatic, migrant, herbivore • aquatic, migrant, herbivore • aquatic, migrant, omnivore (mostly herbivore) • aquatic, migrant, omnivore (mostly herbivore) • aquatic, migrant, carnivore • aquatic, migrant, carnivore • aquatic, migrant, omnivore • terrestrial, resident, omnivore • terrestrial, migrant, omnivore (mostly herbivore) • aquatic, migrant, carnivore • aquatic, migrant, omnivore (mostly herbivore) • aquatic, migrant, carnivore	• Greylag goose (Anser anser) • Bean goose (Anser fabalis) • Mallard duck (Anas platyrhynchos) • Garganey (Anas querquedula) • White stork (Ciconia ciconia) • Black headed gull (Larus ridibundus) • Herring gull (Larus argentatus) • Ring-necked pheasant (Phasianus colchicus) • Common wood pigeon (Columba palumbus) • Common tern (Sterna hirundo) • Mute swan (Cygnus olor) • Ruff (Philomachus pugnax)	Romania	quill	feathers from birds captured on nests	sealed in paper envelopes	grinding in a pestle	• extraction with petroleum ether for 15 min with shaking • left overnight and decantation • filtration on anhydrous sodium sulfate • repeated 3 times	• cleaning on fluorisyl column	OCPs	GC-ECD	• ΣDDT: 26.5-43.6 ng/g ww • ΣHCH: 3.4-11.3 ng/g ww		[88]
• aquatic, migrant, carnivore	Little Egret (Egratta Garzetta)	China	body feathers	feathers from birds (nestlings) captured on nests	• envelopes • room temperature	• washing with distilled water and acetonitrile • drying • cutting into pieces	extraction with HNO_3 (69%) at room temperature for 48 h	filtration under vacuum through glass fibre filter	OCPs PAHs	GC-μECD HPLC- DAD-FLD	• ΣOCP: 21.74-25.84 ng/g dw • ΣPAH: 25.66-70.44 ng/g dw		[76]
aquatic, migrant, carnivore	• European storm petrel • Hydrobates pelagicus	Ireland	breast feathers	feathers from birds captured on nests	at room temperature	• washing with distilled water • soaking for 20 min • drying in folded tissue paper for 2 h	extraction with 37% HCl and n-hexane:acetone (2:1,v/v) at 37 °C for 15 h	-	PAHs PCBs PBDEs OCPs	GC-EI-MS	• ΣPCB: 27.26±1.52 ng/g ww • ΣOCP: 17.94±2.19 ng/g ww • ΣPBDE: 4.59±0.42 ng/g ww • ΣPAH: 38.96±3.59 ng/g ww		[58]
aquatic, migrant, carnivore	• Cattle egret (Bubulcus ibis) • Little egret (Egretta garzetta)	Pakistan	breast feathers	-	paper envelopes	• washing with tap water and MiliQ water • drying at room temperature overnight	• Soxlet extraction with DCM for 16 h	fractionation via chromatography glass columns with acidified silica	PBDEs	GC-SIM-MS	ΣPBDE: 0.34-1.3 ng/g dw	LOD: 0.1-0.3 ng/g dw	[63]
• terrestrial/aquatic, migrant, omnivore • terrestrial/aquatic migrant, carnivore	• Lack kite (Milvus migrans) • Spotted owlet (Athene brama)	Pakistan	tail feathers	feathers from birds captured on nets	−20 °C	• washing with distilled water • drying at room temperature overnight • cutting into pieces	• incubation with HCl and hexane:dichloromethane (4:1, v/v) at 45 °C overnight	• filtration using SPE cartridges with acidified silica with anhydrous sodium sulfate • fractionation on SPE silica cartridge	PBDEs OCPs	GC-ECNI-MS	• ΣPCB: 18-332 ng/g dw • ΣOCP: 88-4567 ng/g dw • ΣDDT: 4.37-73.24 ng/g dw • HCB: 0.06-0.48 ng/g dw • TN: 0.01-0.11 ng/g dw • ΣPBDE: 0.30-7.88 ng/g dw	LOQ: 0.01-0.05 ng/g dw	[48]

terrestrial/aquatic, migrant, carnivore	• Spur-winged lapwing (Vanellus spinosus)	Turkey	tail feathers	feathers from birds captured on nests	4 °C	• incubation with distilled water at 40 °C for an hour • drying at 40 °C • cutting into pieces	• incubation with HCl and hexane:dichloromethane (4:1, v/v) at 40 °C overnight • extraction with hexane:dichloromethane (4:1, v/v)	• filtration using SPE cartridges with acidified silica with anhydrous sodium sulfate	PCB OCPs	GC-MS	• ΣDDT: 14-1040 ng/g dw • ΣHCH: 5-98 ng/g dw • ΣCHL: 21-149 ng/g dw • Dicofol: 4-227 ng/g dw	• LOD: 0.03±0.01 ng/g dw • LOQ: 0.06±0.025 ng/g dw	[54]
aquatic, migrant, carnivore	White-tailed eagles (Haliaeetus albicilla)	Greenland	pectoral body, primary and tail feathers	feathers from dead birds	polyethylene plastic sealed bags	• washing with water or acetone • cutting into pieces	• incubation with HCl and hexane:dichloromethane (4:1, v/v) 45 °C overnight	filtration using SPE cartridges with acidified silica	PCBs	GC-ECNI-MS	• ΣPCB: 2.3-4200 ng/g dw • DDE: 1.5-2740 ng/g dw • ΣHCH: 0.31-51 ng/g dw • ΣCHL: 0.61-670 ng/g dw • HCB: 0.19-18 ng/g dw • ΣPBDE:0.08-27 ng/g dw	LOQ: 0.1-1 ng/g dw	[62]
• terrestial, migrant, carnivore • terrestial, migrant, carnivore • terrestial, migrant, carnivore • terrestial, migrant,carnivore • terrestial, migrant, carnivore • terrestial, migrant, carnivore • terrestial, resident, carnivore • terrestial, migrant, carnivore • terrestial/aquatic, partial migrant, carnivore • terrestial, residen, carnivore/herbivore • terrestial, resident, herbivore • terrestial, resident, herbivore • terrestial, migrant, carnivore • terrestrial, resident, scavenger • terrestrial, resident, omnivore • terrestrial, migrant, carnivore • terrestrial, resident, carnivore/herbivore • terrestrial, migrant, carnivore	• Greater spotted eagle (Aquila clanga) • Sparrowhawk (Accipiter nisus) • Kestrel (Falco tinnunculus) • Merlin (Falco columbarius) • Hobby (Falco subbuteo) • Peregrine Falcon (Falco peregrinus) • Little owl (Athene noctua) • European scops owl (Otus scops) • Short-eared owl (Asio flammeus) • Chukar (Alectoris chukar) • Black francolin (Francolinus francolinus) • Collared dove (Streptopelia decaocta) • Hoopoe (Upupa epops) • Raven (Corvus corax) • Magpie (Pica pica) • European roller (Coracias garrulus) • Houbara bustard (Chlamydotis undulata) • Common cuckoo (Cuculus canorus)	South-West of Iran	tail feathers	feathers from museum collections	• paper envelopes • room temperature	• washing with tap water and distilled water • drying at room temperature • cutting into pieces	• incubation with HCl and hexane:dichloromethane (4:1, v/v) at 40 °C overnight • extraction with hexane:dichloromethane (4:1, v/v)	• filtration using SPE cartridges with acidified silica with anhydrous sodium sulfate	PCBs OCPs	GC-ECD	• ΣPCB: <LOQ-182 ng/g dw • ΣDDT: 1-295 ng/g dw • ΣHCH: 9-212 ng/g dw • HCB: <LOQ-92 ng/g dw	LOQ: 0.2-0.5 ng/g dw	[50]
• aquatic, migrant, omnivore • aquatic, migrant, carnivore/herbivore • aquatic, migrant, omnivore • aquatic, migrant, carnivore • aquatic, migrant, carnivore • aquatic, migrant, carnivore • aquatic, migrant, carnivore • aquatic, migrant, carnivore • aquatic, migrant, carnivore	• Mallard (Anas platyrhynchos) • Common teal Teal (Anas crecca) • Pintail (Anas acuta) • Common gull (Larus canus) • Little gull (Hydrocoloeus minutus) • Black-headed gull (Chroicocephalus ridibundus) • Little grebe (Tachybaptus ruficollis) • Black-necked grebe (Podiceps nigricollis) • Great crested grebe (Podiceps cristatus) • Great cormorant (Phalacrocorax carbo)	Iran	-	feathers from injured or dead birds	-	• washing with distilled water • drying at room temperature • cutting into pieces	• incubation with HCl and hexane:dichloromethane (4:1, v/v) at 40 °C overnight	filtration using SPE cartridges with acidified silica with anhydrous sodium sulfate	PCBs OCPs	GC-ECD	• ΣPCB: 4.6-355.7 ng/g ww • ΣDDT: 5.2-439.5 ng/g ww • ΣHCH: <LOQ-140.7 ng/g ww • HCB: <LOQ-689 ng/g ww	LOQ: 0.1-0.8 ng/g ww	[45]
• aquatic, resident, carnivore • terrestrial/aquatic, resident, carnivore • aquatic, migrant, carnivore • aquatic, resident, carnivore • aquatic, migrant, omnivore • aquatic, migrant, carnivore • terrestrial, migrant, carnivore • terrestrial, resident, herbivore • aquatic, migrant, carnivore • aquatic, migrant, carnivore • aquatic, migrant, carnivore/herbivore • aquatic, migrant, omnivore • aquatic, migrant, herbivore • aquatic, migrant, herbivore • aquatic, migrant, omnivore	• Pied kingfisher (Ceryle rudis) • White-breasted kingfisher (Halcyon smyrnensis) • Night hero(Nycticorax nycticorax) • Little bittern (Ixobrychus mintus) • Coot (Fulica atra) • Cattle egret (Bubulcus ibibs) • Bittern (Botaurus stellaris) • Purple galllinule (Prophyrio porphyrio) • Slender-billed gull (Larus genei) • White pelican (Pelecanus onocrotalus) • Teal (Anas crecca) • Mallard (Anas platyhychos) • Wigeon (Anas penelope) • Shoveler (Anas clypeata) • Ferruginous duck (Aythya nyroca)	Iran	tail feathers	feathers from museum collections	• paper envelopes • room temperature	• washing with tap water and distilled water •drying at room temperature • cutting into pieces	• incubation with HCl and hexane:dichloromethane (4:1, v/v) 40 °C overnight • extraction with hexane:dichloromethane (4:1, v/v)	filtration using SPE cartridges with acidified silica with anhydrous sodium sulfate	PCBs OCPs	GC-ECD	• ΣPCB: <LOQ-151 ng/g dw • ΣDDT: 2-112 ng/g dw • ΣHCH: <LOQ-95 ng/g dw • HCB: <LOQ-95 ng/g dw	LOQ: 0.2-0.5 ng/g dw	[46]
terrestiral, resident/migrant, carnivore	Mongolian plover (Charadrius mongolus)	India	body feathers	feathers from birds captured on nests	⩾20°C	• washing with detergent free from OCs	• Soxlet extraction with diethyl ether:hexane (3:1, v/v) for 7 h	fractionation via Florisil column chromatography	PCBs OCPs	GC-ECD	• ΣPCB: <20 ng/g ww • ΣDDT: 26-34 ng/g ww • ΣHCH: 4.4-17 ng/g ww • ΣCHL: <0.2 ng/g ww • HCB: 0.3 ng/g ww		[61]

• aquatic, migrant, scavenger • terrestrial, resident, carnivore • terrestrial, partial migrant, carnivore • terrestrial, resident, carnivore • terrestrial, resident, carnivore • terrestrial, resident, carnivore • terrestrial, resident, carnivore • aquatic, migrant, carnivore • aquatic, migrant, carnivore	• Black kite (Milvus migrans) • Eurasian sparrowhawk (Accipiter nisus), • Common kestrel (Falco tinnunculus), • Red-necked falcon (Falco chicquera) • Indian vulture (Gyps indicus) • White-rumped vulture (Gyps bangalensis), • Spotted owlet (Athene brama) • Little owl (Athene noctua) • Great cormorant (Phalacrocorax carbo) • Grey heron (Ardea cinerea).	Pakistan	tail, body, primary and secondary feathers	-	• zipped plastic bags • −20 °C	• washing with deionized water • covering with standard laboratory paper • drying in ambient temperature • cutting into pieces	• incubation in HCl and hexane:dichloromethane (4:1, v:v) at 45 °C overnight • extraction with hexane:dichloromethane (4:1, v:v)	-	PCBs OCPs	GC-ECNI-MS	• ΣPCB: 0.03–16 ng/g dw • ΣDDT: 0.11–2163 ng/g dw • HCB: 0.02–34 ng/g dw • TN: 0.01–0.13 ng/g dw		[51]
terrestrial, resident, omnivore	• Magpie (Pica pica)	Belgium	tail feathers	feathers from captured birds	• paper envelopes • room temperature	• drying at room temperature • cutting into pieces	• incubation with HCl and hexane:dichloromethane (4:1, v/v) at 40 °C overnight	filtration using SPE cartridges with acidified silica	PCBs PBDEs OCPs	GC-ECNI-MS	• ΣPCB: 2.92-236 ng/g dw • ΣDDT: 1.68-151 ng/g dw • ΣPBDE: 0.14-1.81 ng/g dw	LOQ: 0.10- 0.50 ng/g dw	[60]
• aquatic, migrant, carnivore • aquatic, migrant, omnivore • aquatic, migrant, omnivore, • terrestrial, migrat, carnivore • terrestrial, migrant, carnivore • terrestrial, partial migrant, scavenger • terrestrial, migrant, carnivore • terrestrial, resident, carnivore	• Grey herons (Ardea cinerea) • Herring gulls (Larus argentatus) • Common moorhens (Gallinula chloropus) • Barn owls (Tyto alba) • Long-eared owls (Asio otus) • Common buzzards (B. buteo) • Kestrels (Falco tinnunculus) • Sparrowhawks (Accipiter nisus)	Belgium	tail feathers	feathers from dead birds	paper envelopes	• washing with distilled water • drying at room temperature • cutting into pieces	• incubation with HCl and hexane:dichloromethane (4:1, v/v) at 40 °C overnight	filtration using SPE cartridges with acidified silica	PCBs PBDEs OCPs	GC-ECNI-MS	• ΣPCB: 5.5-510 ng/g dw • ΣDDT: 1.5-730 ng/g dw • ΣPBDE: 0.33-53 ng/g dw	LOQ: 0.1-0.4 ng/g dw	[49]
• terrestrial, migrant, carnivore • terrestrial, migrant, carnivore • terrestrial, migrant, carnivore	• Northern goshawks (Accipiter gentilis) • White-tailed eagles (Haliaeetus albicilla) • Golden eagles (Aquila chrysaetos)	Norway	chest and back feathers	feathers from birds (nestlings) captured on nests	• paper envelopes • room temperature	• washing with distilled water • drying at room temperature overnight • cutting into pieces	• incubation with HCl and hexane:dichloromethane (4:1, v/v) 45 °C overnight	filtration using SPE cartridges with acidified silica	PCBs PBDEs OCPs	GC-ECNI-MS	• ΣPCB: 6,78-140 ng/g dw • DDE: 3.15-145 ng/g dw • ΣHCH: 0.107-1.37 ng/g dw • HCB: 0.161-3.50 ng/g dw • ΣPBDE: 0.538-7.56 ng/g dw	LOQ: 0.10- 0.50 ng/g dw	[52]
aquatic, migrant, carnivore	Black-legged kittiwakes (Rissa tridactyla)	Norway	body feathers from back, head and wings	feathers from birds captured on nests	-	• washing with Milli-Q water • drying • cutting into pieces	extraction with cyclohexane/acetone (3:1, v:v) with sonication for 15 min	-	PCBs PBDEs OCPs OPEs	GC-ESI-QqQ-MS	• ΣPOP for Blomstrandhalvøya: 72.9±8.63 ng/g ww • ΣPOP for Krykkjefjellet: 29.6±1.67 ng/g ww		[47]
terrestrial, partial migrant, scavenger	Cinereous vulture (Aegypius monachus)	Spain	down, contour feathers	feathers from birds (nestlings) captured on nests	• paper envelopes • room temperature	• washing with distilled water • drying at room temperature, • cutting into pieces	• incubation in HCl and hexane:dichloromethane (4:1, v:v) at 45 °C • extraction with hexane:dichloromethane (4:1, v:v)	• cleaning on on Florisil® cartridges • cleaning on acidified silica	PCBs PBDEs OCPs OPEs	GC-ECNI-MS	• ΣPCB: 0.32-6.16 ng/g dw • ΣDDT: 0.09-6.10 ng/g dw • ΣHCH: 0.06- 3.79 ng/g dw • HCB: 0.04-0.69 ng/g dw • ΣOPEs: 7.63-72.32 ng/g dw • ΣPBDE: 0.06-1.35 ng/g dw	LOQs: • HCB, HCHs, PCBs: 0.005 ng/g dw • DDTs: 0.1 ng/g dw • PDBES: 0.003 ng/g dw • OPEs: 1.0 ng/g dw	[74]
terrestrial, partial migrant, carnivore	Sparrowhawk (Accipiter nisus)	Tibet	wings feathers	-	-	• washing with ultrapure water • freezing • cutting into pieces	• acid digestion coupled with organic solvent extraction • alkaline digestion coupled with organic solvent extraction • ion pair extraction • organic solvent extraction • mixed with acid, alkaline or pure solvent at 37 °C for 16 h • reextraction with fresh MTBE	-	PFASs	HPLC–ESI–MS/MS	• ΣPFCA: 1.80 3.33 ng/g dw • ΣPFSA: 3.60-11.84 ng/g dw	• LOD: 0.024-0.039 ng/g dw • LOQ: 0.08-0.13 ng/g dw	[71]
terrestrial, resident, carnivore	Barn owls (Tyto alba)	Belgium	tail feathers	feathers from dead birds	• low density polyethylene zip lock bags • room temperature	• washing with distilled water • drying at room temperature • washing with hexane in ultrasonic bath for 10 min	• extraction with methanol in an ultrasonic bath 3 times for 10 min • extraction with HCL in methanol 3 times for 10 min	cleaning up with ENVI-carb and glacial acetic acid	PFASs	HPLC-QTOFMS	• PFOA: <14.1-670 ng/g ww • PFHxS: <1.9-8.1 ng/g ww • PFOS: <2.2-56.6 ng/g ww	LOD: 2.2-14.1 ng/g ww	[68]
terrestrial, resident, carnivore	White-tailed eagle (Haliaeetus albicilla)	Norway	body feathers from chest and back	feathers from birds (nestlings) captured on nests	• plastic bags • room temperature	• washing with ultrapure water, • drying at room temperature, • cutting into pieces, • washing with hexane in an ultrasonic bath for 10 min	extraction with HCl with sonication for 10 min, 3 times	cleaning up with ENVI-carb and glacial acetic acid	PFASs	UHPLC-MS/MS	ΣPFAS: 4.54–25.61 ng/g		[70]

• aquatic, migrant, carnivore • aquatic, migrant, omnivore • terrestrial, resident, carnivore • terrestrial, resident, omnivore • terrestial, resident, herbivore	• Grey Heron (Ardea cinerea) • Herring Gull (Larus argentatus) • Eurasian Sparrowhawk (Accipiter nisus) • Eurasian Magpie (Pica pica) • Eurasian Collared Dove (Streptopelia decaocta)	Belgium	tail feathers	feathers from dead birds	-	• washing with acetonitrile • digestion in nitric acid at room temperature for 48 hours • adding sodium hydroxide • filtration through glass fibre filter	extraction using SPE Oasis HLB Plus cartridges	-	PFASs	HPLC-MS/MS	• PFHxS: <LOQ-33 ng/g dw • PFOS: 27-310 ng/g dw		[69]
aquatic, migrant, carnivore	Herring Gull (Larus argentatus)	Poland	covert feathers	feathers from dead birds	synthetic materials	• washing with acetone • drying	extraction with methanol, ammonium acetate and chloric acid (VII) with sonication at 20 °C for 10 min	purification on Oasis HLB glass cartridges	Phenols	LC-FLD	• BPA: 29.3–512.4 ng/g dw • OP: 28.4–563.6 ng/g dw • NP: 4.9–151.3 ng/g dw	LOQs: • BPA: 2.0 ng/g dw • OP: 0.5 ng/g dw • NP: 0.5 ng/g dw	[77]

dw – dry weigh, ww – wet weigh

Microplastics have gained much attention in recent years as an ubiquitous pollutant posing a threat to wildlife and humans. So far, detection of MPs in the environment is economically unjustified and there is a lack of their effective removal mechanisms. MPs appear to be a top environmental problem as they have spread globally to even the most remote environments. Recent studies have shown that MPs are widespread in sediments [89], oceans [90], air and arctic sea ice [91]. Ingestion of microplastics has been reported for various organisms including fish [92], turtles [93], crustaceans [94].

In recent years, birds were used as useful indicators for environmental plastic pollution because they usually breed in colonies, making them available for study purposes. In addition, their large habitats let researchers gather appropriate samples from certain locations. A lot of research has been focused on the examination of stomach content of different bird species as a good strategy for monitoring the accumulation of MPs. It was reported that freshwater birds have similar MP concentrations to marine ones. According to the data, 56% of seabirds [95] and 55% of freshwater habitants were affected by microplastics [96] predicting that MPs will have an impact on 99% of birds species via ingestion or entanglement by 2050 [97]. It is important to highlight that microplastics are metabolically active in the digestive tract of birds. Furthermore, sample collection from stomach seems to be too invasive, making it questionable when taking into account rare or endangered species. Therefore, birds' feathers seems to be well suited to serve as an indicator for plastic contamination in the environment as easily collected materials without any harmful effect on living birds. To date, only one study on the presence of microplastics in feathers was published. Recently, Reynolds and Ryan (2018) analysed 408 feathers collected from live African duck species including Cape Teal *Anas capensis*, Red-billed Teal, Yellow-billed Duck, White-faced Duck and Egyptian Goose. No significant differences were observed between the examined bird species in the abundance of microplastic fibres in the feathers. To deeply explore MP accumulation in freshwater ecosystem, the authors also took fresh faecal samples. The data clearly confirmed that the concentration of microplastics was higher for the feathers compared to faecal specimens, suggesting that a larger portion of microplastics had been ingested than the results indicated. The above-mentioned data confirmed that using feathers the amount of the microplastics in the natural environment could be determined more accurately.

Of particular concern is the fact that microplastics may also lead to the increased exposure of contaminants. They consist of toxic substances that are used in plastic manufacture but also they can absorb a number of airborne pollutants such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT) and trace elements [98]. The relationship between the concentration of microplastics ingested and the concentration of trace elements in feathers were confirmed. In Flesh-footed Shearwater fledgings' breast feathers, high amounts of chromium and silver were confirmed to be related with the mass of ingested MPs [99]. The potential for the presence of microplastics in the digestive tract has been also revealed in Laysan Albatros (*Phoebastria*

immutabilis) and Bonin Petrel (*Pterodroma hypoleuca*) fledgings from Midway Atoll, North Pacific Ocean. A high number of ingested plastic microparticles were correlated with the concentration of trace elements such as iron, manganese, rubidium, strontium and lead in Bonin Petrel breast feathers and chlorine in Laysan Albatross breast feathers. This proves that toxic chemical contaminants could be transferred by microplastics to wildlife and their level is linked to the concentration of microplastics consumed.

The negative impact of microplastics on the environment has become a global concern. Due to mass plastic production and its persistance in the natural environment, it could be predicted that the contamination with microplastics is likely to increase in the future. First of all, the consumption of single-use products derived from synthetic polymers should be cut. There is a need to design waste management strategies and effective and sufficient legislation, raise society's awareness of this global problem generally caused by using certain products on a daily basis. The removal of all microplastics is impossible and would not even be effective. Stakeholders should be aware that microplastics will become smaller and more toxic over time. Researchers should propose some solutions by improving methods to estimate the level of microplastics, understanding the consequences of their plague, and prevent their negative effect on wildlife and humans. These methods should not have a harmful effect on any living organism. Life without polymers seems to be unimaginable, therefore a lot of efforts should be put to develop an alternative to traditional plastics. One reasonable option seems to be the use of biodegradable plastics of natural origin. Nowadays applied biodegradable polymers are composed of synthetic additives with partial decomposition ability, leaving behind an abundance of synthetic, persistent polymers that will release into the environment. A good choice could be polyhydroxyalkanoates, biopolyesters produced by microorganisms, which are fully biodegradable into non-toxic products. Further effective research should be conducted to introduce them into the high-end markets as high value added biopolymers that may be used in many applications.

5. Final conclusion

Many pollutants affect living organisms all over the world and many of them are not easily degraded, making them very persistent. There is currently a great need to obtain data on the same species in a broad range of geographical regions and for comparative data on many various species representing different trophic levels from the same area. Each approach can provide valuable information. The birds are visible, ubiquitous and intensively studied, and in many cases seem to be more sensitive to environmental pollution than other vertebrates. Feathers can be successfully used as environmental pollution monitors because of the fact that 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 the feathers. In addition, it is easy to

collect feathers non-invasively and it is possible to store test material for many years. Therefore, feathers are particularly useful for creating temporal and spatial patterns without affecting populations and for evaluating xenobiotic contamination in the endangered or threatened species and provide information on incorporation paths and ecotoxic effects.

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