

Trace element bioaccumulation in the plumage of common blackbirds (*Turdus merula* Linnaeus, 1758) from Agigea, Romania

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Abstract: The purpose of this research was to establish the baseline concentrations of thirteen trace elements (Cd, Co, Cr, Cu, Ni, Pb, Fe, Mg, Na, K, Sr, Ca, and Zn) in feathers of the common blackbird *Turdus merula* (n=9) at the Natura 2000 site "Marine Dunes from Agigea" (ROSCI0073). Both remiges and rectrices were pooled, and the quantification of trace elements was accomplished using two spectrometry analysis methods: high-resolution continuum source atomic absorption spectrometer with a graphite furnace (HR-CS GF-AAS) and flame atomic absorption spectrometer (FL-AAS). The concentration of Ca was the highest of the thirteen trace elements, registering at $209.9146 \pm 192.4098 \mu\text{g}\cdot\text{g}^{-1}$ w.w., while Cd had the lowest concentration levels in feathers, $0.0243 \pm 0.0158 \mu\text{g}\cdot\text{g}^{-1}$ w.w., respectively. However, the results obtained were significantly lower than those in the relevant literature. These findings suggest that the bioavailability of the analysed trace elements is minimal in our study area. Thus, it is imperative to conduct further research on the impact of these trace elements' accumulation and effects on avifauna in the Natura 2000 site "Marine Dunes from Agigea".

1. Introduction

Human activities have persistently introduced a wide range of pollutants into the environment (Meillère et al., 2016). Heavy metals represent recurring byproducts of industrial processes, with their emissions often leading to environmental contamination in surrounding areas (Costa et al., 2011). Due to their extensive use, and their persistence in the environment, certain heavy metals have been implicated in environmental pollution. The animals absorb metals from air, water, and food sources (Zaccaroni et al., 2011), bioconcentrating them across various trophic levels (Meillère et al., 2016). Consequently, there is a growing necessity to identify organisms, or specific anatomical components thereof, which can serve as effective biological indicators or monitors of specific pollutants (Weyers et al., 1988; Meillère et al., 2016).

The environmental repercussions of metals discharged from mining and smelting activity underscore their uptake by plants and subsequent integration into the biological tissues of various organisms, spanning herbivores, insects, earthworms, and molluscs. These organisms, functioning at lower trophic levels, subsequently serve as prey for species higher up the food chain (Durkalec et al., 2022). Birds, positioned at elevated trophic levels, assume significance as pivotal bioindicators of environmental contamination (Espín and Sánchez-Virosta, 2021) due to their capacity for pollutant accumulation and exhibit sensitivity to ecological alterations (Maznikova et al., 2024).

Metal exposure in avian species primarily occurs through dietary ingestion and inhalation, supplemented by geophagy as an alternative route for metal uptake. The absorption and accumulation of metals within avian populations are influenced by various factors, including the availability of metals, exposure routes, and intrinsic characteristics such as trophic position, feeding behaviours, age, and genetic diversity (Durkalec et al., 2022).

Measurements of blood parameters offer a more direct insight into the toxicological effects of chemicals on wildlife (Coeurdassier et al., 2012). Nevertheless, bird feathers have been extensively utilised in numerous biomonitoring endeavours and have demonstrated efficacy as indicators of heavy metal pollution.

Feather analysis is esteemed as an alternative, less intrusive method for assessing bird exposure to specific trace elements (Gil-Jiménez et al., 2020). However, it is essential to recognize that the detected concentration levels of elements in feathers correspond to the duration of exposure during the formation and growth of feathers. Furthermore, an additional layer of complexity arises from potential external contamination, such as dust or soil particles adhering to the feather surface, which may contain various elements. While this aspect may present challenges in assessing bioaccumulation in avian species, it can confer advantages in passive biomonitoring by reflecting the overall pollution levels within the bird's habitat (Durkalec et al., 2022).

The significance of certain avian species as bioindicators, particularly in the context of heavy metal monitoring, has been frequently underscored in the literature (Weyers et al., 1988; Meillère et al., 2016). Passerines predominantly reside within limited territories and exhibit non-migratory behaviour, confining their foraging activities to relatively small ranges. Consequently, contamination detected in passerine birds reflects localized pollution, rendering them valuable for identifying and monitoring local environmental degradation (Costa et al., 2011). Among insectivorous birds, the common blackbird (*Turdus merula* Linnaeus, 1758), a widespread species in the Western Palearctic, predominantly consumes insects and fruits during late summer to early winter, with earthworms comprising a significant portion of its annual diet. Blackbirds primarily feed on earthworms (56%) and insects (26%), while vegetation and other items (such as spiders, myriapods, or slugs) constitute 4% and 14%, respectively, of their total dietary intake (Fritsch et al., 2012). The selection of the blackbird was motivated by its ubiquitous presence and omnivorous feeding habits, allowing it to exploit a diverse array of habitats. Furthermore, previous research has demonstrated its capacity for accumulating contaminants such as trace metals (Fritsch et al., 2012).

Investigations into the bioavailability of trace elements in the "Marine Dunes from Agigea" Natura 2000 site (ROSCI0073), located on the western coast of the Black Sea, are limited compared to research conducted in other ecosystems. Hence, evaluating how passerine birds can indicate trace element pollution within the distinctive environmental setting of the "Marine Dunes from Agigea" is crucial.

The primary objectives of our study encompassed a dual focus: firstly, to meticulously assess the concentrations of various trace elements (including Cadmium (Cd), Nickel (Ni), Lead (Pb), Cobalt (Co), Chromium (Cr), Copper (Cu), Iron (Fe), Magnesium (Mg), Sodium (Na), Potassium (K), Strontium (Sr), Calcium (Ca), and Zinc (Zn)) present within the feathers of common blackbirds sourced from Agigea, Romania; and secondly, to conduct a comprehensive literature evaluation of the levels of exposure signified by these concentrations.

2. Materials and Methods

2.1. Study area

The sampling site, Agigea Bird Observatory, situated in Constanta County, a southern region of Romania, is a component of the Marine Biological Station "Prof. Dr. Ioan Borcea" Agigea (Figure 1). This location is recognized as a Natura 2000 site, specifically the "Marine Dunes from Agigea" (ROSCI0073). This site is of community importance and possesses two types of priority natural habitats: fixed coastal dunes with herbaceous vegetation (grey dunes) (2130) and subcontinental peri-Panonic scrub (40A0). Additionally, this protected area features a non-priority habitat, embryonic shifting dunes (white dunes) (2110) (Strugariu and Boc, 2022; Ghetu, 2012), according to the Natura 2000 classification.

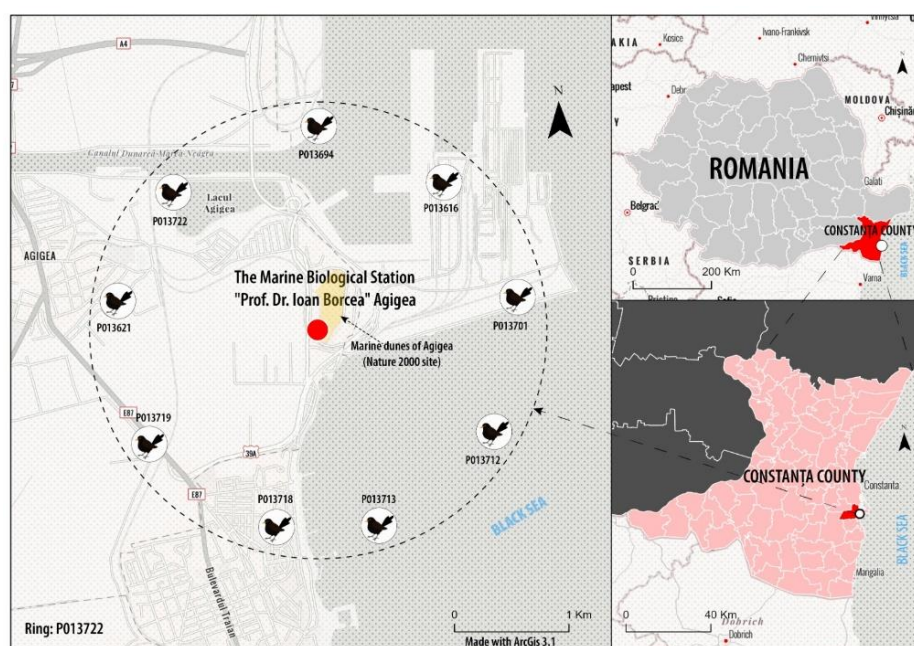


Figure 1. Location of the study area on the geographical map of Romania.

2.2. Sampling and sample analysis

For this study, we assisted in a ringing campaign targeting birds during the autumn migration season at the Agigea Bird Observatory in October 2019. Using mist nets, we captured several specimens of the common blackbird (*Turdus merula*) and collected nine individuals' flight and tail feathers. The remiges and rectrices were carefully chosen and sampled to avoid impediments to the individuals' long-term survival or flight. Notably, the common blackbird was among the top 10 most abundant species ringed during the 2019-2020 season (Baltag et al., 2021), ranking sixth in frequency. Additionally, the species occupied the fifth place in abundance during the 2021 and 2022 seasons (Baltag et al., 2022; Pintilioaei et al., 2023). The collected feather samples were transported to the laboratory in metal-free polyethylene zip bags, packed on ice, and stored in a freezer at a temperature of -21°C until analysis, following the established sampling protocol.

We employed sample preparation techniques that adhere to the protocols outlined in established scientific literature (Pilastro et al., 1993; Strungaru et al., 2017), taking into account variations in sample type and manufacturer recommendations for equipment. We thoroughly washed the feather samples twice, followed by a rinse with deionised water Milli-Q ($18.2 \text{ M}\Omega \cdot \text{cm}^{-2}$). Afterwards, we allowed them to air-dry at room temperature (22°C), cut them into smaller pieces, and weighed them to a precision of approximately 0.2-1.0 g.

The samples underwent treatment with nitric acid Suprapur® of 65% concentration (certified Merck Germany) and hydrogen peroxide EMSURE® ISO of 30% concentration (certified Merck Germany) in a 4:2 ratio, allowing for a 30-minute reaction time in decontaminated pressure vessels made of TFMTM-PTFE (DAP-60K type). A 3-step acid digestion program was employed for organic matrix samples using a Microwave Digestion System (Berghof SpeedWave MWS-2, Eningen, Germany). Following the cooling process, the liquid samples were adjusted to the mark with deionised water Milli-Q ($18.2 \text{ M}\Omega\cdot\text{cm}^{-2}$) in 50 mL clear polypropylene tubes and stored in the laboratory until trace element analysis.

We used two analytical methods to measure the amounts of different elements in our study. Firstly, we measured the concentrations of six metals (Cd, Co, Cr, Cu, Ni, and Pb) using a high-resolution continuum source atomic absorption spectrometer with a graphite furnace HR-CS GF-AAS (ContrAA 600-Analytik Jena, Germany) and Aspect CS 1.5.6.0 software. Afterwards, we used flame atomic absorption spectrometry (FL-AAS) with an air-acetylene flame spectrometer (GBC Avanta, Australia) and GBC Avanta ver. 2.02 software to determine signals from the other seven elements (Fe, Mg, Na, K, Sr, Ca, and Zn). We utilised specific wavelengths to detect the signals of each element. To calibrate the detection signals of both instruments for the analysed elements, we diluted a reference standard solution, the standard ICP multielement IV (Certipur®, $1000 \text{ mg}\cdot\text{L}^{-1}$), to a concentration of $5 \text{ }\mu\text{g}\cdot\text{L}^{-1}$ for detecting signals of Cd, Co, Cr, Cu, and Pb, a concentration of $10 \text{ }\mu\text{g}\cdot\text{L}^{-1}$ for detecting signals of Fe, Mg, Na, Sr, and Zn, a concentration of $100 \text{ }\mu\text{g}\cdot\text{L}^{-1}$ for detecting signals of Ca, and a concentration of $20 \text{ }\mu\text{g}\cdot\text{L}^{-1}$ for detecting signals of Ni and K. Calibration curves were created for each set of 20 liquid samples per analysed element to validate the precision of the working procedure. The calibration curve's coefficient R remained consistently above 0.98 for all elements analysed in the spectrometers. Both blank and spiked samples were included in every round of analysis to verify the purity of the chemicals. The quality of the method was assessed and confirmed using a certified reference material, specifically fish muscle (ERM-BB422), certified by the Research Center Institute for Reference Materials and Measurements. The recovery rates varied between 97 and 105%, indicating good outcomes. We determined the limit of detection (LOD) for each element analysed and acquired the data following elemental assays in $\mu\text{g}\cdot\text{g}^{-1}$ wet weight (w.w.).

2.3. Data analysis

Statistical analyses were performed with statistical analysis software GraphPad Prism ver. 6.04 for Windows. For all analysis, the significance level was set as $p < 0.05$. Samples yielding concentration values below the detection limits were treated as the value for the detection limit divided by the square root of two.

3. Results

Throughout the ringing process, we meticulously documented a range of morphological and body condition indices for the subjects from whom we collected feathers for our study. These indices can be found in Table 1. Based on the moulting score, we determined that the ringed birds had yet to moult. Therefore, we can assume that the sampled feathers registered trace element concentrations throughout the growth process (Meillère et al., 2016). Weight, muscle, and fat scores generally suggested that most ringed individuals were physically healthy. Among the specimens, the majority were juveniles, with only one adult. Females accounted for 55.55% of the individuals, while males comprised 44.44%. Because of the uneven distribution of age ratios, incorporating this variable into analysing factors influencing trace element bioaccumulation was unsuitable. Without the age data, we conducted a Student t-test to explore sex-based differences in trace element concentration levels in common blackbird feathers. However, the observed differences between male and female birds were not statistically significant ($p > 0.05$).

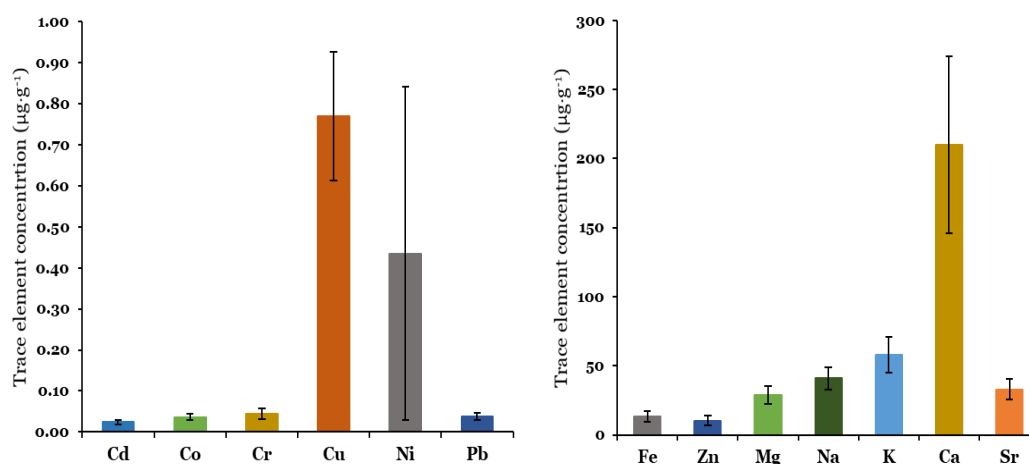
Table 1. Registered morphological and body condition indices of common blackbird individuals (n=9) ringed during autumn migration in October 2019, Agigea, Romania.

Ring	Age	Sex	Wing size (mm)	Tail size (mm)	Muscle score	Fat score	Moulting score	Weight (g)
P013694	JUV	M	128	104	1	2	0	98
P013616	JUV	F	125	94	2	0	0	74.8
P013701	AD	M	128	104	1	0	0	74.8
P013712	JUV	F	124	89	1	1	0	82.9
P013713	JUV	F	120	98	1	1	0	88.6
P013718	JUV	M	125	91	1	2	0	79.9
P013719	JUV	F	122	87	1	0	0	91.7
P013722	JUV	F	123	96	1	0	0	76.2
P013621	JUV	M	131	95	1	1	0	92.3

JUV – juvenile; AD – adult; M – male; F – female; muscle score: 1 – sternum easy to distinguish but not sharp, muscle neither depressed, sharp nor rounded; 2 – sternum still distinguishable and muscle slightly rounded; fat score: 0 – no visible fat, dark red; 1 – furcular region with a wide wedge of fat and a trace of fat on the abdomen, light red; 2 – furcular region completely covered but deeply concave and slips of fat on the abdomen, light yellow; moulting score: 0 – remaining old feathers

Assessing the trace element burden of specific bird species is crucial to understanding their health and well-being. However, it is essential to consider the migratory status of the species in question. Meillère and his team have pointed out that blackbirds can be sedentary or migratory. Interestingly, they observed that adult blackbirds residing in urban areas are more likely to be sedentary than migratory. Furthermore, they noted that dispersal is limited in male common blackbirds (Meillère et al., 2016). While it is known that juvenile blackbirds tend to stay around their natal territory for several weeks after fledging (Meillère et al., 2016), for our study, we could not determine whether the sampled birds were migratory. Therefore, we cannot exclude the possibility that they may have accumulated their trace element burden in a habitat other than where they were sampled.

The accumulation of trace element concentrations in feather samples from common blackbirds (*Turdus merula*) in Agigea, Romania, is represented in Figure 2 and it followed a grading order of $\text{Ca} > \text{K} > \text{Na} > \text{Sr} > \text{Mg} > \text{Fe} > \text{Zn} > \text{Cu} > \text{Ni} > \text{Cr} > \text{Pb} > \text{Co} > \text{Cd}$. Ca ($209.9146 \pm 192.4098 \mu\text{g}\cdot\text{g}^{-1}$ w.w.) exhibited the highest concentration values, while Cd ($0.0243 \pm 0.0158 \mu\text{g}\cdot\text{g}^{-1}$ w.w.) registered the lowest concentration values from the group of thirteen trace elements examined in blackbird feathers.

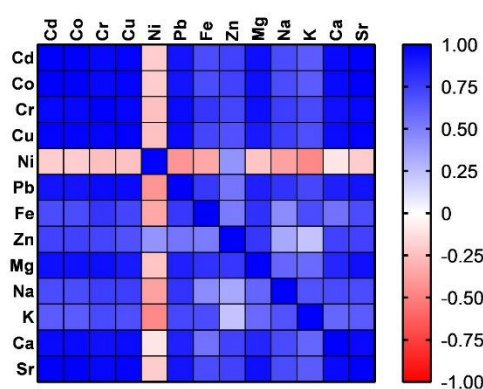
**Figure 2.** Variation of trace element concentrations in feather samples from common blackbird *Turdus merula* (n=9) from Agigea (Romania), which are expressed as mean ± standard error (SE) on $\mu\text{g}\cdot\text{g}^{-1}$ w.w. basis

In our study, we applied the Kolmogorov-Smirnov test to evaluate the normality of the data sets concerning trace metal concentrations in common blackbirds from Agigea, Romania. The results indicated that all values, except Ni, were normally distributed. Interestingly, an outlier value for the feathers of an adult male common blackbird affected the normality of the Ni dataset. We found that the descriptive statistics for concentration values did not reveal a high variability rate among the analysed trace elements, as outlined in Table 2. Our findings provide valuable insights into the distribution of trace metals in common blackbirds and highlight the need for further research in this area.

Table 2. Descriptive statistics for trace element concentrations values registered in feather samples from common blackbird *Turdus merula* (n=9) from Agigea, Romania, which are expressed as mean, standard deviation (SD), minimum (min) and maximum (max) on $\mu\text{g}\cdot\text{g}^{-1}$ w.w. basis.

Trace element	Mean	SD	Min	Max
Cd	0.024	0.016	0.015	0.053
Co	0.036	0.024	0.022	0.078
Cr	0.045	0.038	0.016	0.112
Cu	0.770	0.471	0.401	1.674
Ni	0.435	1.217	0.001	3.680
Pb	0.039	0.025	0.010	0.083
Fe	13.400	11.134	2.827	35.122
Zn	10.343	10.307	1.386	29.427
Mg	28.526	19.386	16.459	71.938
Na	40.937	24.296	16.641	90.277
K	57.898	38.078	9.922	121.500
Ca	209.915	192.410	79.127	621.623
Sr	32.987	21.468	20.374	71.493

In the feather samples of common blackbirds from Agigea, Romania, we discovered several notable positive correlations among nearly all trace elements assessed. The majority of these connections were between essential elements (Co, Cr, Cu, Mg, Ca, Na) and non-essential elements such as Cd and Pb. However, there was one exception: Ni demonstrated only a weak positive correlation with Zn (as illustrated in Figure 3). These findings suggest that the bioaccessibility, bioavailability, and bioaccumulation of these trace elements in nature are intricate and influenced by the additive, antagonistic, or synergistic effects of their mixtures and combinations with other elements.



The colours correspond to the levels of correlation: with 1 indicating positive correlation (dark blue) and -1 indicating the negative correlation (dark red).

Figure 3. Correlation matrix heatmap (Pearson's correlation coefficient - r was used to compute the relevance and redundancy of the features) showing the relationship between trace element concentrations in feather samples of common blackbird *Turdus merula* (n=9) from Agigea (Romania).

4. Discussion

Feathers can contain contaminants from a variety of sources. Some elements are absorbed into the feather keratin during growth, while others may stick to the surface due to contact with contaminated soil, water, or preening gland secretions. Certain elements are known to accumulate more than others, while others do not accumulate at all and are solely from external contamination. This external contamination can make it challenging to interpret results, but it can also provide insight into the bird's environment. Cleaning feathers before analysis is necessary but may not entirely remove all contaminants (Durkalec et al., 2022).

Aloupi and his team thoroughly analysed feathers from Eurasian Wigeon (*Mareca penelope*) and Great Cormorant (*Phalacrocorax carbo*) using various cleaning procedures commonly suggested to minimise external contamination before analysis. After careful examination, they concluded that none of these methods can completely remove exogenous metallic deposits from the surface of feathers. For instance, when using one of the cleaning methods employed in their study, Milli-Q water, they achieved up to 87.7% removal for Cr (Aloupi et al., 2020). Our research utilised similar techniques with Milli-Q water, leading us to posit that the concentrations of the analysed elements may have arisen from either endogenous or exogenous sources.

Within the scientific literature, there is a lack of established threshold values for the thirteen elements we analysed in feather concentrations. As a result, we conducted a comparison of our findings with values documented in feathers from common blackbirds and related species in a variety of habitats and pollution gradients (Appendix 1).

Our analysis was based on directly comparing our w.w. feather concentrations with dry weight (d.w.) concentrations reported in the literature, as feathers have minimal moisture content (Albert et al., 2019).

It is well-established that Cd is a highly toxic metal ubiquitous in natural environments, existing in most soils, surface waters, and plants. In animals, the primary route of Cd exposure is through the consumption of contaminated food. Following ingestion, the metal is rapidly eliminated from the bloodstream and predominantly accumulates in the liver, where it is bound to metallothioneins - small cysteine-rich proteins induced transcriptionally by Cd. Subsequently, Cd-thionein is transported to the kidney for long-term storage. In light of the metal's exceptionally long biological half-life, Cd concentrations in the liver and kidney could be linked to chronic environmental exposure, as opposed to current exposure levels (Zaccaroni et al., 2011). Thus, lower Cd concentrations in feathers do not necessarily indicate a lack of metal accumulation in birds; rather, it could signify that the metal was primarily stored in their liver.

The concentration levels of Cd, $0.036 \pm 0.024 \mu\text{g} \cdot \text{g}^{-1}$ w.w., discovered in the washed and pooled feathers (flight and tail) of common blackbirds from a protected area in Agigea, Romania, were significantly lower than those found in the feathers of common blackbirds from control sites, where we expected only to find background levels. Our findings differed significantly from those of Costa et al. (2011), who discovered the lowest amounts of Cd ($0.12 \pm 0.08 \text{ mg} \cdot \text{kg}^{-1}$ d.w.) in the tail feathers of adult common blackbirds from Portugal's National Pine Forest of Quiaios, part of the protected area of Dunas de Mira (Natura 2000 PTCON055). Their values were almost four times higher than the values we recorded. Additionally, we observed a similar pattern in the feathers of the adult common myna (*Acridotheres tristis*), another species from Sturnidae, from a rural area in Pakistan, Baroghil Valley, Khyber-Pakhtunkhwa (Abbasi et al., 2015). These feathers had a high mean value of Cd of $0.35 \pm 0.20 \mu\text{g} \cdot \text{g}^{-1}$ d.w. Furthermore, our concentration values in the protected area were several times lower than those observed in contaminated areas or areas with varying pollution gradients. For instance, Weyers et al. (1988) documented the highest values in common blackbird flight feathers (washed $2.03 \pm 1.07 \text{ mg} \cdot \text{kg}^{-1}$ d.w. and unwashed $6.07 \pm 2.58 \text{ mg} \cdot \text{kg}^{-1}$ d.w.) in a polluted area from the Stolberg district (Germany).

In a study by Meillère et al. (2016), Co exhibited elevated levels in washed breast feathers from common blackbirds captured in an urban environment (Pointou-Charentes region) in France. The levels of contamination observed were $0.21 \pm 0.29 \mu\text{g} \cdot \text{g}^{-1}$ d.w. for male and female juveniles and $0.29 \pm 0.32 \mu\text{g} \cdot \text{g}^{-1}$ d.w. for male adults, respectively. These values were notably higher, by a factor of five and eight, than the levels of Co reported in our research, $0.036 \pm 0.024 \mu\text{g} \cdot \text{g}^{-1}$ w.w.

According to Sevillano-Caño et al. (2020), Cr, specifically Cr (IV), is considered a heavy metal with a well-defined toxicological role. However, we mainly encounter this trace element in foods as Cr (III), and its supplementation could significantly improve carbohydrate and lipid metabolism in certain stress situations, such as type 2 diabetes (Sevillano-Caño et al., 2020). Further research is necessary to determine the toxicological or essential nature of the Cr levels detected in birds' feathers.

In an urban environment in the Pointou-Charentes region, France, Cr concentrations in the breast feathers of common blackbirds were $6.23 \pm 11.85 \mu\text{g} \cdot \text{g}^{-1}$ d.w. for male and female juveniles and $6.17 \pm 9.61 \mu\text{g} \cdot \text{g}^{-1}$ d.w. for male adults, respectively (Meillère et al., 2016). These values were significantly higher than the Cr reported in our research ($0.045 \pm 0.038 \mu\text{g} \cdot \text{g}^{-1}$ w.w.). Concentrations ranging from 0.30 to $0.89 \mu\text{g} \cdot \text{g}^{-1}$ d.w. were found in a mixture of feathers from common blackbirds collected in a reference site, St. Jaume de Frontanya, and a polluted area, Cercs, in Spain (Llacuna et al., 1995). Cr concentrations as high as $1.11 \pm 0.70 \text{mg} \cdot \text{kg}^{-1}$ d.w. were found in pooled feathers of adult common myna (*Acridotheres tristis*) in an urban environment, Soan Valley, in Pakistan. In the same study, Cr levels dropped almost twice as much and reached $0.60 \pm 0.34 \text{mg} \cdot \text{kg}^{-1}$ d.w. at a site that was thought to be "unpolluted" because it was in the Baroghil Valley, Khyber-Pakhtunkhwa, 250 km away from a town (Abbasi et al., 2015).

Elevated levels of Cu were found in feathers of common blackbirds from a polluted site in Cercs, Spain, $23.26 \pm 33.72 \mu\text{g} \cdot \text{g}^{-1}$ d.w., respectively (Llacuna et al., 1995). Surprisingly, adult common blackbirds from a protected area in Portugal, the National Pine Forest of Quiaios, also registered relatively high values of Cu, $11.00 \pm 4.88 \text{mg} \cdot \text{kg}^{-1}$ d.w. (Costa et al., 2011). These concentrations were several times higher than those we found in Agigea, Romania - $0.770 \pm 0.401 \mu\text{g} \cdot \text{g}^{-1}$ w.w.

Regarding Ni concentrations in common blackbird feathers from Agigea, Romania, we detected mean levels of $0.435 \pm 1.217 \mu\text{g} \cdot \text{g}^{-1}$ w.w., which were similar to values registered in a reference site in Spain, St. Jaume de Frontanya - $0.50 \pm 0.64 \mu\text{g} \cdot \text{g}^{-1}$ d.w. (Llacuna et al., 1995). Surprisingly, Cr mean levels of $0.78 \pm 0.30 \text{mg} \cdot \text{kg}^{-1}$ d.w., which were slightly higher than ours, were registered in tail feathers from adult common blackbirds captured in a protected area from Portugal, the National Pine Forest of Quiaios (Costa et al., 2011). However, the highest Cr mean values ($7.95 \pm 10.32 \mu\text{g} \cdot \text{g}^{-1}$ d.w.) were detected by Meillère et al. (2016) in breast feathers of male adult common blackbirds from an urban area in the Pointou-Charentes region, France.

Pb is an inorganic pollutant that can adversely affect various body systems in birds, leading to issues with haematological, neurological, immunological, reproductive, and behavioural functions (Durkalec et al., 2022). Even low levels of Pb exposure can result in oxidative stress, decreased δ -aminolevulinic acid dehydratase levels in the blood, vitamin and essential element depletion, cognitive and behavioural disruptions, and increased susceptibility to pathogens. In birds, sub-lethal Pb exposure may cause endocrine disruption, affecting hormone levels related to breeding and stress responses, including corticosterone and testosterone, which are critical in balancing survival and reproduction (Fritsch et al., 2019). Feathers may reflect a bird's dietary exposure to Pb (Durkalec et al., 2022). Unfortunately, we could not find any literature with benchmarks directly linking Pb concentrations in feathers to bird health effects (Fritsch et al., 2019).

Pb concentrations in this study were not comparable to those in other studies with similar designs. Pb levels were usually around seventeen to twenty-four orders of magnitude higher (Meillère et al., 2016; Scheifler et al., 2006; Abbasi et al. 2015). The closest values were reported in breast feathers of male and female juvenile common blackbirds in France - $0.66 \pm 0.64 \mu\text{g} \cdot \text{g}^{-1}$ d.w. (Meillère et al., 2016). Fritsch and his

research team (2012) from France detected mean Pb levels in flight feathers from adult and juvenile common blackbirds along a pollution gradient that ranged between 1.59 and 32.8 $\mu\text{g}\cdot\text{g}^{-1}$ d.w. However, these were not the highest Pb concentrations registered for common blackbirds. Weyers et al. (1988) detected Pb values of 60.5 ± 34.6 $\text{mg}\cdot\text{kg}^{-1}$ d.w in washed flight feathers of juvenile common blackbirds in a polluted area from Stolberg district (Germany). In the United States, similar Sturnidae species, American robin (*Turdus migratorius*) and starling (*Sturnus vulgaris*) registered the highest values for Pb concentrations in a mixture of washed feathers from an urban area in Illinois, 79.7 and 225.1 $\mu\text{g}\cdot\text{g}^{-1}$ d.w., respectively (Getz et al., 1977). Researchers found in flight feathers from another comparable species, clay-coloured thrush (*Turdus grayi*), Pb levels of 4.19 $\mu\text{g}\cdot\text{g}^{-1}$ d.w. in a rural area from Mexico and 3.1 $\mu\text{g}\cdot\text{g}^{-1}$ d.w. in an urban environment from Merida, Yucatan (Nava-Diaz et al., 2015).

The lowest levels of Zn concentrations (92 ± 22 $\text{mg}\cdot\text{kg}^{-1}$ d.w.) were detected in flight feathers of nestling common blackbirds captured in a polluted area from Stolberg, Aachen district (Germany) and the highest values (188 ± 47 $\text{mg}\cdot\text{kg}^{-1}$ d.w.) were recorded in the same area in remiges of juvenile common blackbirds (Weyers et al., 1988). These Zn concentrations were significantly higher than those found in remiges and rectrices from juvenile common blackbirds in Agigea, Romania (10.343 ± 10.307 $\mu\text{g}\cdot\text{g}^{-1}$ w.w.). Upon reviewing the available literature, we found that the Zn concentration value closest to our own was obtained from feathers of the adult common myna (*Acridotheres tristis*) in a reference site located in a rural area of Pakistan, specifically the Baroghil Valley in Khyber-Pakhtunkhwa. The recorded Zn concentration in this sample was found to be 60.83 ± 5.95 $\mu\text{g}\cdot\text{g}^{-1}$ d.w., which was six times higher than our own measured value (Abbasi et al., 2015).

The breast feathers of adult male common blackbirds in an urban region of Western France (Pointou-Charentes) had the highest mean concentrations of Fe, at 217 ± 218 $\mu\text{g}\cdot\text{g}^{-1}$ d.w. Male and female juvenile common blackbirds had Fe levels of 91 ± 68 $\mu\text{g}\cdot\text{g}^{-1}$ d.w., according to Meillère et al. (2016). Abbasi et al. (2015) found similar Fe concentrations (112.01 ± 11.16 $\mu\text{g}\cdot\text{g}^{-1}$ d.w.) in pooled feathers of the adult common myna (*Acridotheres tristis*) captured in an urban environment, Soan Valley, in Pakistan. Feather samples collected from individuals in a rural area of Pakistan, specifically the Baroghil Valley in Khyber-Pakhtunkhwa, registered Fe values of 88.85 ± 5.19 $\mu\text{g}\cdot\text{g}^{-1}$ d.w. These concentrations were several times higher than those found in feathers of common blackbirds from Agigea, Romania (13.400 ± 11.134 $\mu\text{g}\cdot\text{g}^{-1}$ w.w.).

5. Conclusions

This report presents a pioneering analysis of the levels of thirteen trace element in feathers of common blackbird, an abundant species across its distribution range. The results of the study indicate that relatively low levels of the thirteen trace elements accumulated in our sampled feathers compared to values reported in previous studies. This finding confirms the bioavailability of trace elements in the study area while also suggesting that the area around the Natura 2000 site - known as "Marine Dunes from Agigea" - is not heavily polluted by these elements, thus not causing any behavioural or internal problems for the breeding population of *Turdus merula*. Moreover, the results suggest that the analysis of feathers is an effective means of assessing the level of trace elements in the entire animal, regardless of the means of entry (inhalation/ingestion), thus obviating the need to kill it. However, further studies are necessary to estimate the environmental loading of these trace elements in the study site and to evaluate the risk they pose to exposed organisms.

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Appendix 1. Trace element concentrations in different types of feather samples of common blackbird and related species from worldwide similar studies (values are expressed as they are mentioned in the literature)

Country	Study area	N	Breast feathers	Flight feathers	Tail feathers	Pooled feathers	Studied by
Cd							
France	Noyelles-Godault, Nord - Pas-de-Calais ₁	12 ^{TM,W,i}	NA	0.186±0.118	NA	NA	Fritsch et al. (2012) ^{q,r,s}
	Noyelles-Godault, Nord - Pas-de-Calais ₂	19 ^{TM,W,i}	NA	0.193±0.156	NA	NA	
	Noyelles-Godault, Nord - Pas-de-Calais ₃	8 ^{TM,W,i}	NA	0.298±0.147	NA	NA	
	Noyelles-Godault, Nord - Pas-de-Calais ₄	12 ^{TM,W,i}	NA	0.561±0.191	NA	NA	
	Noyelles-Godault, Nord - Pas-de-Calais ₅	16 ^{TM,W,i}	NA	0.469±0.323	NA	NA	
	Noyelles-Godault, Nord - Pas-de-Calais ₆	22 ^{TM,W,i}	NA	0.699±0.503	NA	NA	
	Noyelles-Godault, Nord - Pas-de-Calais ₇	11 ^{TM,W,i}	NA	0.745±0.444	NA	NA	
	Noyelles-Godault, Nord - Pas-de-Calais ₈	26 ^{TM,W,i}	NA	2.18±3.04	NA	NA	
	Noyelles-Godault, Nord - Pas-de-Calais ₉	10 ^{TM,W,i}	NA	1.65±0.965	NA	NA	
	Pointou-Charentes region, Western France ₁₀	6 ^{TM,W,j,m} 25 ^{TM,W,k}	0.103±0.108 0.127±0.125	NA NA	NA NA	NA NA	Meillère et al. (2016) ^{q,r,s}
Germany	Stolberg ₁₁	5 ^{TM,U,a,l}	NA	0.76±0.53	NA	NA	Weyers et al. (1988) ^{t,r,s}
		8 ^{TM,W,b,l}	NA	0.60±0.33	NA	NA	
		5 ^{TM,W,c,j}	NA	0.70±0.33	NA	NA	
		7 ^{TM,W,d,j}	NA	2.03±1.07	NA	NA	
		5 ^{TM,U,d,j}	NA	6.07±2.58	NA	NA	
Pakistan	Baroghil valley, Khyber-Pakhtunkhwa ₁₅	9 ^{AT,W,k}	NA	NA	NA	0.35±0.20	Abbasi et al. (2015) ^{q,r,s}
	Soan valley, Punjab province ₁₀	9 ^{AT,W,k}	NA	NA	NA	0.78±0.25	Abbasi et al. (2015) ^{q,r,s}
Poland	Żeromski Park, Szczecin ₁₀	115 ^{TM,W,e,m}	NA	0.21±0.13	0.22±0.15	NA	Fritsch et al. (2019) ^{q,r,s}
	Cemetery Park, Szczecin ₁₀	20 ^{TM,W,m}	NA	NA	0.26±0.17	NA	
	Żeromski Park, Szczecin ₁₀	63 ^{TM,W,f,o}	NA	0.18±0.09	0.24±0.18	NA	
	Cemetery Park, Szczecin ₁₀	9 ^{TM,W,o}	NA	NA	0.22±0.10	NA	
	Żeromski Park, Szczecin ₁₀	51 ^{TM,W,g,n}	NA	0.23±0.15	0.20±0.11	NA	
	Cemetery Park, Szczecin ₁₀	11 ^{TM,W,n}	NA	NA	0.29±0.21	NA	
Portugal	National Pine Forest of Quiaios ₁₂	11 ^{TM,W,k}	NA	NA	0.12±0.08	NA	Costa et al. (2011) ^{t,r,s}
	Estarreja ₁₃	11 ^{TM,W,k}	NA	NA	0.38±0.20	NA	
Spain	Cercs, Northeast Spain ₁₁	8 ^{TM,W,h}	NA	NA	NA	0.23±0.27	Llacuna et al. (1995) ^{q,r,s}
	St. Jaume de Frontanya ₁₄	4 ^{TM,W,h}	NA	NA	NA	0.31±0.61	
Romania	Agiea, Constanta ₁	9 ^{TM, W, i, m}	NA	NA	NA	0.024±0.016	Present study (2024) ^{q, u, s}
Co							
France	Pointou-Charentes region, Western France ₁₀	11 ^{TM,W,j,m}	0.21±0.29	NA	NA	NA	Meillère et al. (2016) ^{q,r,s}
		27 ^{TM,W,k,n}	0.29±0.32	NA	NA	NA	
Romania	Agiea, Constanta ₁	9 ^{TM, W, i, m}	NA	NA	NA	0.036±0.024	Present study (2024) ^{q, u, s}
Cr							
France	Pointou-Charentes region, Western France ₁₀	12 ^{TM,W,j,m}	6.23±11.85	NA	NA	NA	Meillère et al. (2016) ^{q,r,s}
		30 ^{TM,W,k,n}	6.17±9.61	NA	NA	NA	

Country	Study area	N	Breast feathers	Flight feathers	Tail feathers	Pooled feathers	Studied by
Pakistan	Baroghil valley, Khyber-Pakhtunkhwa ¹⁵	9 _{AT,W,k}	NA	NA	NA	0.60±0.34	Abbasi et al. (2015) _{q,r,s}
	Soan valley, Punjab province ¹⁰	9 _{AT,W,k}	NA	NA	NA	1.11±0.70	
Spain	Cercs, Northeast Spain ¹¹	8 _{TM,W,h}	NA	NA	NA	0.89±0.83	Llacuna et al. (1995) _{q,r,s}
	St. Jaume de Frontanya ¹⁴	4 _{TM,W,h}	NA	NA	NA	0.30±0.48	Llacuna et al. (1995) _{q,r,s}
Romania	Agigea, Constanta ¹	9 _{TM, W, i, m}	NA	NA	NA	0.045±0.038	Present study (2024) _{q, u, s}
Cu							
France	Pointou-Charentes region, Western France ¹⁰	13 _{TM,W,j,m}	9.17±1.08	NA	NA	NA	Meillère et al. (2016) _{q,r,s}
		31 _{TM,W,k,n}	10.10±3.38	NA	NA	NA	
Pakistan	Baroghil valley, Khyber-Pakhtunkhwa ¹⁵	9 _{AT,W,k}	NA	NA	NA	1.04±0.72	Abbasi et al. (2015) _{q,r,s}
	Soan valley, Punjab province ¹⁰	9 _{AT,W,k}	NA	NA	NA	2.19±0.81	
Portugal	National Pine Forest of Quiaios ¹²	11 _{TM,W,k}	NA	NA	11.00±4.88	NA	Costa et al. (2011) _{t,r,s}
	Estarreja ¹³	11 _{TM,W,k}	NA	NA	10.43±2.99	NA	
Spain	Cercs, Northeast Spain ¹¹	8 _{TM,W,h}	NA	NA	NA	23.26±33.72	Llacuna et al. (1995) _{q,r,s}
	St. Jaume de Frontanya ¹⁴	4 _{TM,W,h}	NA	NA	NA	11.38±5.50	
Romania	Agigea, Constanta ¹	9 _{TM, W, i, m}	NA	NA	NA	0.770±0.401	Present study (2024) _{q, u, s}
Ni							
France	Pointou-Charentes region, Western France ¹⁰	13 _{TM,W,j,m}	6.86±10.93	NA	NA	NA	Meillère et al. (2016) _{q,r,s}
		31 _{TM,W,k,n}	7.95±10.32	NA	NA	NA	
Pakistan	Baroghil valley, Khyber-Pakhtunkhwa ¹⁵	9 _{AT,W,k}	NA	NA	NA	1.73±0.56	Abbasi et al. (2015) _{q,r,s}
	Soan valley, Punjab province ¹⁰	9 _{AT,W,k}	NA	NA	NA	1.88±0.51	
Portugal	National Pine Forest of Quiaios ¹²	11 _{TM,W,k}	NA	NA	0.78±0.30	NA	Costa et al. (2011) _{t,r,s}
	Estarreja ^{13z}	11 _{TM,W,k}	NA	NA	2.15±1.20	NA	
Spain	Cercs, Northeast Spain ¹¹	8 _{TM,W,h}	NA	NA	NA	0.82±0.57	Llacuna et al. (1995) _{q,r,s}
	St. Jaume de Frontanya ¹⁴	4 _{TM,W,h}	NA	NA	NA	0.50±0.64	
Romania	Agigea, Constanta ¹	9 _{TM, W, i, m}	NA	NA	NA	0.435±1.217	Present study (2024) _{q, u, s}
Pb							
France	Besançon ¹⁰	11 _{TM,U,p}	3.15±1.77	NA	7.75±4.50	NA	Scheifler et al. (2006) _{q,r,s}
	Vernierfontaine ¹⁵	12 _{TM,U,p}	1.37±1.07	NA	1.82±1.14	NA	
	Besançon ¹⁰	11 _{TM,W,p}	NA	NA	4.89±3.18	NA	
	Vernierfontaine ¹⁵	12 _{TM,W,p}	NA	NA	0.73±0.65	NA	
	Noyelles-Godault, Nord - Pas-de-Calais ¹	12 _{TM,W,i}	NA	4.77±6.65	NA	NA	Fritsch et al. (2012) _{q,r,s}
	Noyelles-Godault, Nord - Pas-de-Calais ²	19 _{TM,W,i}	NA	1.59±0.964	NA	NA	
	Noyelles-Godault, Nord - Pas-de-Calais ³	8 _{TM,W,i}	NA	4.88±2.06	NA	NA	
	Noyelles-Godault, Nord - Pas-de-Calais ⁴	12 _{TM,W,i}	NA	6.37±1.60	NA	NA	
	Noyelles-Godault, Nord - Pas-de-Calais ⁵	16 _{TM,W,i}	NA	9.05±7.05	NA	NA	
	Noyelles-Godault, Nord - Pas-de-Calais ⁶	22 _{TM,W,i}	NA	18.0±15.0	NA	NA	
	Noyelles-Godault, Nord - Pas-de-Calais ⁷	11 _{TM,W,i}	NA	15.2±9.39	NA	NA	

Country	Study area	N	Breast feathers	Flight feathers	Tail feathers	Pooled feathers	Studied by
	Noyelles-Godault, Nord - Pas-de-Calais ⁸	26 _{TM,W,i}	NA	68.9±54.8	NA	NA	Meillère et al. (2016) _{q,r,s}
	Noyelles-Godault, Nord - Pas-de-Calais ⁹	10 _{TM,W,i}	NA	32.8±20.1	NA	NA	
	Pointou-Charentes region, Western France ¹⁰	13 _{TM,W,j,m}	0.66±0.64	NA	NA	NA	
		30 _{TM,W,k,n}	1.00±0.76	NA	NA	NA	
Germany	Stolberg ¹¹	5 _{TM,U,a,l}	NA	8.5±2.2	NA	NA	Weyers et al. (1988) _{t,r,s}
		8 _{TM,W,b,l}	NA	14.3±8.3	NA	NA	
		5 _{TM,W,c,j}	NA	24.8±8.6	NA	NA	Weyers et al. (1988) _{t,r,s}
		7 _{TM,W,d,j}	NA	60.5±34.6	NA	NA	
		5 _{TM,U,d,j}	NA	450±253	NA	NA	
Pakistan	Baroghil valley, Khyber-Pakhtunkhwa ¹⁵	9 _{AT,W,k}	NA	NA	NA	0.95±0.50	Abbasi et al. (2015) _{q,r,s}
	Soan valley, Punjab province ¹⁰	9 _{AT,W,k}	NA	NA	NA	2.15±0.06	
Poland	Żeromski Park, Szczecin ¹⁰	115 _{TM,W,e,m}	NA	3.8±2.0	6.7±3.8	NA	Fritsch et al. (2019) _{q,r,s}
	Cemetery Park, Szczecin ¹⁰	20 _{TM,W,m}	NA	NA	4.0±2.4	NA	
	Żeromski Park, Szczecin ¹⁰	63 _{TM,W,f,o}	NA	3.2±1.8	6.8±4.1	NA	
	Cemetery Park, Szczecin ¹⁰	9 _{TM,W,o}	NA	NA	4.1±3.4	NA	
	Żeromski Park, Szczecin ¹⁰	51 _{TM,W,g,n}	NA	4.3±2.1	6.6±3.6	NA	
	Cemetery Park, Szczecin ¹⁰	11 _{TM,W,n}	NA	NA	3.9±1.4	NA	
Portugal	National Pine Forest of Quiaios ¹²	11 _{TM,W,k}	NA	NA	2.35±0.77	NA	Costa et al. (2011) _{t,r,s}
	Estarreja ¹³	11 _{TM,W,k}	NA	NA	4.89±3.22	NA	
Spain	Cercs, Northeast Spain ¹¹	8 _{TM,W,h}	NA	NA	NA	2.48±2.34	Llacuna et al. (1995) _{q,r,s}
	St. Jaume de Frontanya ¹⁴	4 _{TM,W,h}	NA	NA	NA	5.25±5.23	
Mexico	Merida, Yucatan ¹⁰	14 _{TG,W}	NA	3.1	NA	NA	Nava-Diaz et al. (2015) _{q,r,v}
	Merida, Yucatan ¹⁵	26 _{TG,W}	NA	4.19	NA	NA	
United States	Champaign-Urbana, Illinois ¹⁰	10 _{TMi,W,h}	NA	NA	NA	79.7	Getz et al. (1977) _{q,r,w}
	Champaign-Urbana, Illinois ¹⁰	13 _{SV,W,h}	NA	NA	NA	225.1	
	Champaign-Urbana, Illinois ¹⁵	10 _{SV,W,h}	NA	NA	NA	25.3	
Romania	Agigea, Constanta ¹	9 _{TM, W, i, m}	NA	NA	NA	0.039±0.025	Present study (2024) _{q, u, s}
Zn							
France	Pointou-Charentes region, Western France ¹⁰	13 _{TM,W,j,m}	159±13	NA	NA	NA	Meillère et al. (2016) _{q,r,s}
		31 _{TM,W,k,n}	172±42	NA	NA	NA	
Germany	Stolberg ¹¹	5 _{TM,U,a,l}	NA	107±46	NA	NA	Weyers et al. (1988) _{t,r,s}
		8 _{TM,W,b,l}	NA	92±22	NA	NA	
		5 _{TM,W,c,j}	NA	132±70	NA	NA	
		7 _{TM,W,d,j}	NA	188±47	NA	NA	
		5 _{TM,U,d,j}	NA	574±135	NA	NA	
Pakistan	Baroghil valley, Khyber-Pakhtunkhwa ¹⁵	9 _{AT,W,k}	NA	NA	NA	60.83±5.95	Abbasi et al. (2015) _{q,r,s}
	Soan valley, Punjab province ¹⁰	9 _{AT,W,k}	NA	NA	NA	91.05±12.34	
Portugal	National Pine Forest of Quiaios ¹²	11 _{TM,W,k}	NA	NA	109.78±10.16	NA	Costa et al. (2011) _{t,r,s}
	Estarreja ¹³	11 _{TM,W,k}	NA	NA	140±36.84	NA	
Spain	Cercs, Northeast Spain ¹¹	8 _{TM,W,h}	NA	NA	NA	155.40±76.62	Llacuna et al. (1995) _{q,r,s}
	St. Jaume de Frontanya ¹⁴	4 _{TM,W,h}	NA	NA	NA	131.90±69.70	
Romania	Agigea, Constanta ¹	9 _{TM, W, i, m}	NA	NA	NA	10.343±10.307	Present study (2024) _{q, u, s}

Country	Study area	N	Breast feathers	Flight feathers	Tail feathers	Pooled feathers	Studied by
<i>Fe</i>							
France	Pointou-Charentes region, Western France ¹⁰	13	91±68	NA	NA	NA	Meillère et al. (2016) _{q,r,s}
		_{TM,W,j,m} 31	217±218	NA	NA	NA	
Pakistan	Baroghil valley, Khyber-Pakhtunkhwa ¹⁵	9	NA	NA	NA	88.85±5.19	Abbasi et al. (2015) _{q,r,s}
	Soan valley, Punjab province ¹⁰	_{AT,W,k} 9	NA	NA	NA	112.01±11.16	
Romania	Agigea, Constanta ¹	9 _{TM, W, i, m}	NA	NA	NA	13.400±11.134	Present study (2024) _{q, u, s}



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