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Local Variability of Trace Element Concentration in Barn Swallow (*Hirundo rustica*) Nestlings from the Po Plain (Northern Italy)

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Abstract: Birds are commonly used as bioindicators, and their feathers are considered suitable tissues for assessing the presence of contaminants, such as trace elements, in the environment. In agroecosystems, trace elements' occurrence can be associated with both natural and anthropogenic processes, including vehicular traffic, traditional fertilizers, food feed additives for livestock, and the use of sewage sludge as fertilizer. Here, we evaluated the concentrations of twelve trace elements (Aluminium, Arsenic, Cadmium, Chromium, Copper, Iron, Mercury, Manganese, Nickel, Lead, Selenium, and Zinc) in the feathers of barn swallow (*Hirundo rustica*) nestlings. We then compared the concentrations of these elements between nestlings grown in areas amended or not amended with sewage sludge in 2019 and 2020 in a broad region of the Po Plain (Northern Italy). Multivariate analysis showed that the element content of the feathers significantly differed among years and areas, suggesting that the concentration of nestlings' feathers may indicate the local level of contamination. However, univariate analyses did not show clear spatial differences, possibly due to co-occurring sources of trace elements other than sewage sludge. These results suggest that barn swallow nestlings can be a reliable sentinel for the monitoring of local variation of the environmental occurrence of trace elements.

Keywords: agroecosystems; *Hirundo rustica*; sewage sludge; trace elements

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1. Introduction

Sentinel species, or biological indicators, are organisms that accumulate contaminants in their tissues and can thus be used to obtain a quali-quantitative profile of the contamination experienced by ecosystems in a given habitat or over a given period [1], by also providing useful indications for protecting human health [2]. Birds are often used as indicators of contamination due to their abundance, species richness [3,4], and ease of catching and handling [5]. They can also provide information both on their home range and migration routes [6–8] and, in the case of secondary consumers, can allow the assessment of contaminant transfer through the food web [9].

The application of birds in biomonitoring the occurrence, accumulation, and effects of contamination has increased over time [10], but researchers are still debating which tissue is most reliable, as accumulation rates vary according to the sample matrices, the focal element, the target bird species, and the timing of sampling [11]. The most accurate results may come from the integrated analyses of internal tissues such as the liver, brain, muscle, kidney, or in subcutaneous fat [12,13]. However, there are numerous counterindications to the use of these tissues, first and foremost related to the preservation and survival of individuals. Hence, researchers often focus on non-lethally collected samples, such as preen oil, blood, eggs, and feathers, as they offer several ethical and practical advantages [14]. In particular, bird feathers are considered among the most suitable tissues for ecotoxicological studies aiming at assessing the presence of persistent organic pollutants (POPs) and trace elements (reviewed in [15]), as well as emerging contaminants. Indeed, birds excrete trace elements into growing feathers, storing them in the calamus, rachis, and vane during moulting, when they are still connected to the animal bloodstream [5,6,16] and are therefore good descriptors of the average concentration of trace elements in the whole body [17,18]. Many studies also focused on small passerine species, which are usually more abundant and easier to catch and manipulate than larger species at the top of the food chain, such as raptors or piscivorous species [19], as biomonitors of trace elements by measuring the concentration of these elements in their feathers [20,21].

Among elements, trace metals and non-metals are non-biodegradable contaminants arising from natural and anthropogenic processes; some of them may have toxic effects even at low concentrations and may be bioaccumulated and biomagnified, inducing harmful effects on organisms across the trophic chain [22–25]. In agroecosystems, trace metals can occur through natural and anthropogenic processes, including erosion, hydrological processes and atmospheric deposition, industrial activities, and the use of sewage sludge as a fertilizer [26]. The current wastewater management practices strongly encourage the use of sewage sludge in agriculture (Directive 86/278/EEC). Indeed, after proper composting, the sludge can re-enter the agroecosystems as a soil improver or fertilizer due to its high content of organic matter, nitrogen, and phosphorus [27–32]. However, a growing concern exists about the effects of sludge application on terrestrial ecosystems and animal communities living in the spreading areas [33,34], because wastewater treatment plants (WWTPs) are not adequately designed to completely remove several contaminants and trace elements [35–37], which consequently may accumulate in the sludge, resulting in the transfer of organic and inorganic contaminants to the terrestrial environment [33,38,39]. Moreover, studies on the effects of sludge application in agriculture on vertebrate fauna are very scant [40] and this lack of awareness regarding the potential risks and benefits of their use as soil improvers contributes to the social amplification of the concerns about their use [34].

Here, we evaluated the bioaccumulation of twelve trace elements (Aluminium, Arsenic, Cadmium, Chromium, Copper, Iron, Mercury, Manganese, Nickel, Lead, Selenium, and Zinc) in the feathers of barn swallow (*Hirundo rustica*) nestlings. The study was performed in an area of the Po Plain (south-east of Milan, northern Italy) where, depending on both market choices and geological features of the soil, the use of sewage sludge as a soil amendment is either widespread or precluded. Similarly to other swallow species (e.g., the closely related tree swallow (*Tachycineta bicolor*) in the USA [41]), the barn swallow can be used for systematic biomonitoring of agroecosystems because of the following [9,42]: (1) it is widespread across the globe, allowing large-scale monitoring of the selected contaminants; (2) it occupies a high trophic level in the food web, being an insectivorous species; (3) during the breeding season, it is characterized by sedentary and synanthropic behaviour, foraging within 500 m of the breeding site [43]; and (4) it is a flagship species for the conservation of agroecosystems, with very well-known biology and ecology [43]. Nestlings, in particular, are ideal sentinels as they are fed with insects caught by parents in their foraging area around the nest site; therefore, the trace element signature in their feathers should reflect their environmental availability at a local spatial scale and in the period of feather growth.

2. Materials and Methods

2.1. Study Species

The barn swallow is a small migratory passerine bird whose breeding range encompasses the whole Holarctic region. Italian populations spend the non-breeding period mostly in west-African sub-Saharan countries [44,45]. This species breeds synanthropically in rural buildings and forages on aerial insects captured within 500 m of the breeding sites [43]. In the study area, the nestlings' diet is mainly represented by Formicidae (Hymenoptera, 23%), Aphodidae (Coleoptera, 22%), Tabanidae (Diptera, 21%), and Syrphidae (Diptera, 15%) [46]. In Italy, the breeding season lasts from early April until the end of August. During this period, females lay 1–3 clutches of 1–7 eggs, incubated by the female only for approximately 14 days; both parents feed the offspring until fledging at ca. 18–20 days.

2.2. Study Area and Field Methods

In 2019 and 2020, starting from 1st April, 17 barn swallow colonies located south-east of Milan (northern Italy), close to the Adda Sud Park, were visited every second week, and the content of nests was inspected to identify hatching date (see [43] for details on field methods). Due to COVID-19 pandemic-related restrictions, in 2020, we could not visit some of the colonies selected in 2019. Six groups of colonies were identified (Figure 1): two groups were colonies located in an area amended with sewage sludge which were sampled in either 2019 (hereafter, Sludge 19, N = 5 colonies, 1 of which with presence of livestock farming, 49 nestlings) or in 2020 (hereafter, Sludge 20, N = 5 colonies, 2 of which with presence of livestock farming, 61 nestlings); two groups were colonies in an area improved with manure or fertilizers and located close to the areas amended with sewage sludge which were sampled in either 2019 (hereafter, No-sludge 19C, N = 5 colonies, 4 of which with presence of livestock farming, 18 nestlings) or in 2020 (hereafter, No-sludge 20C, N = 1 colony, with presence of livestock farming, 14 nestlings); and finally, two groups were colonies located an area improved with manure or fertilizers but located far from the areas amended with sewage sludge which were sampled either in 2019 (hereafter, No-sludge 19F, N = 5 colonies, 4 of which with presence of livestock farming, 28 nestlings) or in 2020 (hereafter, No-sludge 20F, N = 3 colonies, 2 of which with presence of livestock farming, 21 nestlings). As barn swallows forage within a maximum distance of 500 m from the colony, and considering the large distance between the three considered groups of colonies, we are confident that the studied individuals did not move from a field amended with sewage sludge to a field amended with manure or fertilizer, nor vice versa. When nestlings were 15–18 days old, we plucked the fourth (counting outwards) right rectrix (R4) from 2 ($\pm$0.3 SD, range: 1–4) nestlings per brood. This age was selected to allow feathers to reach a minimum size for the analyses while reducing, at the same time, the risk of nestlings leaving the nest prematurely. The R4 feathers were stored in sealed plastic bags to avoid contamination until laboratory analyses.

2.3. Reagents and Apparatus

Ultrapure HNO_3, Trace-SELECT® (65% *w/w*), H_2O_2 (30% *w/w*), and certified multi-standard solution Merck VI for ICP-MS were purchased from Sigma-Aldrich (Milan, Italy), ultrapure water was from Carlo Erba Reagents s.r.l. (Cornaredo, Italy). A CEM Mars microwave oven (CEM s.r.l., Cologno al Serio, Italy) equipped with eight PTFE vessels (Xpress, 55 mL) and an XpressVapTM accessory was used for sample digestion and evaporation. Elements' measurement was performed by an inductively coupled plasma quadrupole mass spectrometer (ICP-MS) (Elan DRC-e, PerkinElmer, Shelton, CT, USA) equipped with a standard ICP torch, crossflow nebulizer, nickel sampler, skimmer cones, and dynamic reaction cell™ (DRC).

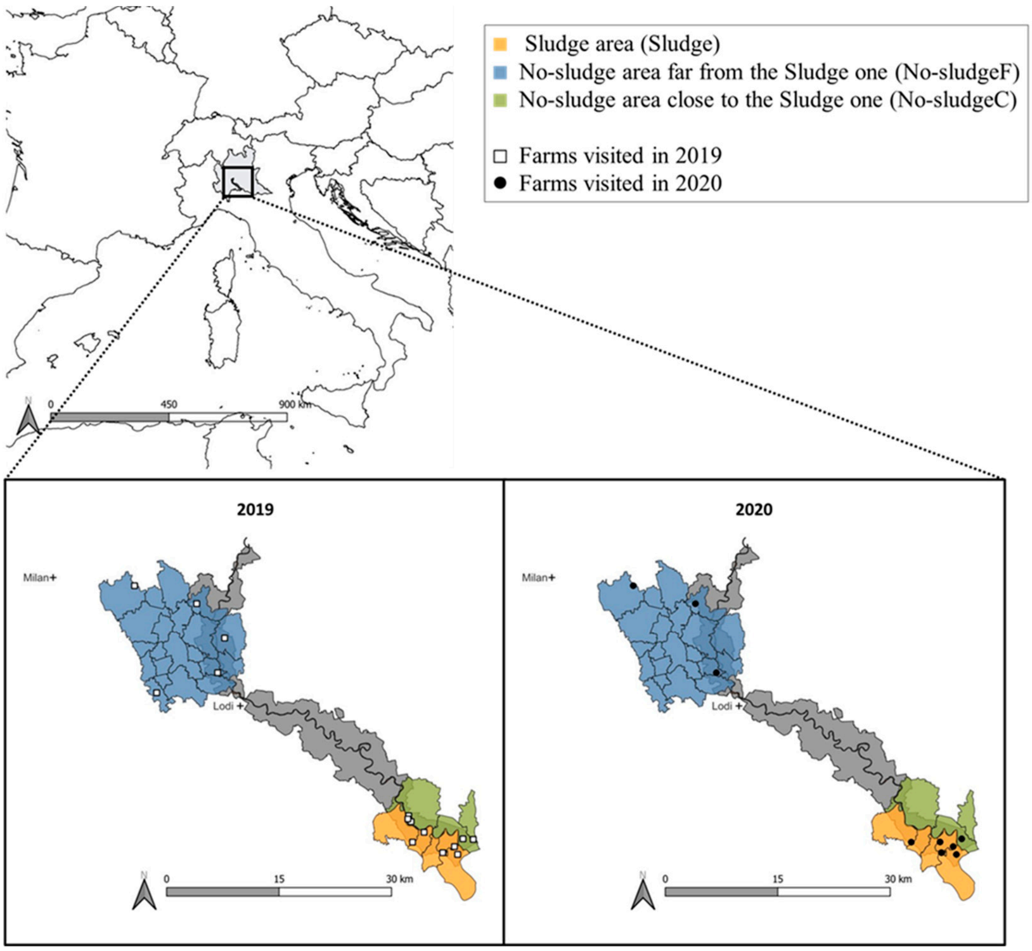

Figure 1. Map of the three sampling sites located south-east of Milan (northern Italy), close to the Adda Sud Park (dark grey area). Orange indicates the Sludge area (Sludge), blue the No-sludge area far from the Sludge one (No-sludge F), green the No-sludge area close to the Sludge one (No-sludge C). Open squares denote farms visited in 2019, while circles denote farms visited in 2020.

2.4. Analytical Procedure

The sample treatment and digestion were carried out as reported in [8]. Briefly, the feathers were washed with deionized water, then placed in an ultrasonic bath for 5 min with 1 M acetone solution to remove external bound metals and dirt particles, then rinsed in ultrapure water, and finally air-dried. Each feather was accurately weighed and digested (1600 W, 15 min, 200 °C) with a mixture of 5 mL of HNO_3 and 2 mL of H_2O_2. After cooling, the digested samples were evaporated to a small volume (approximately 0.5 mL) and diluted to 5 mL with ultrapure water in calibrated polypropylene tubes before analysis by dynamic reaction cell for inductively coupled plasma mass spectrometry (DRC-ICP-MS). The same procedure was used for reagent blanks. Three-point calibration curves were generated in the range of 5–500 µg/L. Method detection and quantification limits (MDLs and MQLs, respectively) were obtained from the instrumental detection and quantification limits (IDLs and IQLs, respectively) calculated using the residual standard deviation (Sy/x) of the linear regression parameters as $(3.3 \times Sy/x)$/slope and $(10 \times Sy/x)$/slope, respectively, and are referred to in the overall procedure (see Table S1 in [8]).

2.5. Statistical Analyses

Differences in trace element concentrations among the six groups of colonies were evaluated by both multivariate and univariate analyses. A total of 16 values (1 for As; 2 for Al; 1 for Cr; 4 for Cu; 1 for Hg; 1 for Mn; 2 for Pb; 3 for Cr; 1 for Fe) were considered outliers (i.e., they were larger than the mean value of the considered element ± 5 standard deviations) and subsequently removed from all the analyses.

To avoid biases due to differences in variances, we first calculated the standardized trace element concentrations of each feather by subtracting the mean value from the concentration of each element at each feather and dividing the result by the standard deviation. A redundancy analysis (RDA) was then performed to assess whether the patterns of trace element concentration (i.e., the standardized concentrations of all measured elements simultaneously) of feathers differed between the six groups of colonies. Missing values (i.e., outliers removed in the previous step of the analysis) were replaced using the MICE imputation procedure [47].

Differences in trace element concentrations among groups of colonies were further investigated by univariate linear mixed models, including farm identity as a random grouping factor. Post hoc tests were performed to test for pairwise differences between groups of colonies in both multivariate and univariate analyses. To avoid the inflation of type I errors, post hoc tests were always limited to comparisons between areas sampled in the same year (e.g., Sludge 19 vs No-sludge 19C; Sludge 19 vs No-sludge 19F; No-sludge 19C vs. No-sludge 19F) or between samples collected in different years but in the same area (e.g., Sludge 19 vs. Sludge 20). Overall, 9 pairwise comparisons were performed, and p-values were corrected with the Benjamini–Hochberg method.

Analyses were performed with the R statistical software version 3.6.2 [48] with the vegan [49], glmmTMB [50], and emmeans [51] packages.

3. Results and Discussion

Overall, 191 tail feathers from barn swallow nestlings were analysed. A total of 85 feathers were collected in areas where sewage sludge was not spread, while 106 were plucked from nestlings grown in areas amended with sewage sludge. Independently of the considered group, the most abundant elements measured in barn swallow feathers were Zn, Al, and Fe, with an average concentration (±SE) of 221 (±4), 95 (±5), and 78 (±2) µg/g, respectively. The concentration of the other elements was always lower than 11.6 (±0.4) µg/g, except for As and Cu, whose concentrations were below the MQLs (see [8]) in 18.5% and 1% of the samples, respectively. The trace element average concentrations decreased as follows: Zn > Al > Fe > Cu > Mn > Ni > Pb > Cr > Se > Hg > Cd > As. Mean values, standard errors, and minimum and maximum concentrations of the 12 trace elements are reported in Supplementary Table S1.

RDA showed that the trace element concentration of feathers significantly differed among the six groups of colonies ($F_{5,185}$ = 9.79, p = 0.001), with post hoc tests showing statistically significant pairwise differences among all groups of colonies ($F_{1,40} \geq 4.60$, $P_{FDR} < 0.01$ in all cases). These differences suggest that the trace element pattern of nestling feathers may reflect local environmental concentrations of trace elements. This is consistent with the results of a previous study carried out on adult barn swallows, where differences in trace element profiles between feathers grown in the African wintering grounds and in the Italian breeding ones were observed [8]. Moreover, the same study hypothesized the existence of two groups of individuals probably spending the non-breeding period in two different geographical areas based on the trace element profiles of their feathers grown in Africa [8], therefore suggesting that the feather's trace element profiles reflect the concentrations of trace elements in the areas where feathers are grown. The results of the present study further suggest that the nestlings' feathers may reflect the local environmental concentrations of trace elements at even smaller spatial and temporal scales because, before fledging, nestlings are fed by adults with insects caught within a few hundred meters of the nest for a few weeks. Indeed, it can be hypothesized that insects originating from or inhabiting contaminated agroecosystems may bioaccumulate contaminants over time, either through direct contamination or biomagnification, during both their larval and adult stages. Consequently, dietary exposure may be the primary source of nestlings' contamination, as barn swallow nests are mainly located inside rural buildings, protected from aerial deposition of trace elements. This pathway should be further investigated in future studies aimed to assess the bioaccumulation and biomagnification of trace elements in the food web of the agroecosystems.

Differences in the concentrations of each element among feathers collected in the six groups of colonies were further investigated with univariate analyses. Figure 2 shows wide fluctuations in trace element concentrations among areas and years of study. In 2019, feathers from areas amended with sewage sludge had significantly higher Ni and Se concentrations as compared to those from the closest No-sludge area, while Zn, Se, Cd, and Ni were higher as compared to those of the furthest No-sludge area. Conversely, in 2020, feathers from the Sludge area had lower concentrations of Al, Mn, Pb, and As as compared to those of the closest No-sludge area and lower concentrations of Cu, Mn, and As as compared to the furthest No-sludge area ones. A significant increase within the study area between 2019 and 2020 was found for Mn in all areas; Cu and Pb in both No-sludge areas; Hg in Sludge and No-sludge F areas; Al in the No-sludge C area; Fe in the Sludge area; and Se and As in the No-sludge F area. Conversely, Cd, As, and Se decreased significantly from 2019 to 2020 in the Sludge area only. The lack of consistency in trace element concentrations between years and areas can be attributed to a number of processes with both anthropogenic and natural origin. For instance, Cd and Zn are emitted from tire wear, and substantial amounts of Cd and Zn are added to pig feed to increase productivity and are subsequently disposed in agroecosystems. In addition, human activities such as air pollution fallout and standard agricultural practices can contribute to the variability in the metal content of soils. However, it is worth noting that the area under study is a plain flat, north of the Po River, which is characterized by intensive, standardized agriculture based on maize and winter cereal production. This, together with the presence of atmospheric stagnation caused by thermal inversion, ensures that the primary sources of metal contamination of soil remain consistent and have an equivalent impact on the different agricultural environments. The lithological texture of the soils can also contribute to the variability of metal concentrations in the soils. The plains to the north of the Po River, formed by the erosion of alpine relief, consist mainly of sands and gravels with a lower capacity to retain metal ions compared to the clay plains south of the river [52], and indeed a certain uniformity in the surface concentrations of metal ions in the soils of the lowlands lying north the Po River has been reported by [52,53]. On the basis of this information, it can be concluded that sludge disposal is one of the most important carriers of trace elements, including toxic metals, in agroecosystems and it can represent the main source of variability of their concentration in the study areas. Unfortunately, it was not possible to analyse the composition of the sewage sludge spread in the study areas. Therefore, the present work represents a first exploration of the potential contamination of agroecosystems by sewage sludge. However, we are aware that certain trace elements may enter the study area from alternative sources, such as vehicular traffic, conventional fertilizers, animal feed additives, or soil amendments other than sewage sludge. For future investigations, it is essential to prioritize a more detailed investigation of the pathways involved in the accumulation of trace elements in barn swallows. In addition, assessment of the potential adverse effects associated with the accumulation of such elements should be a focus of these forthcoming studies.

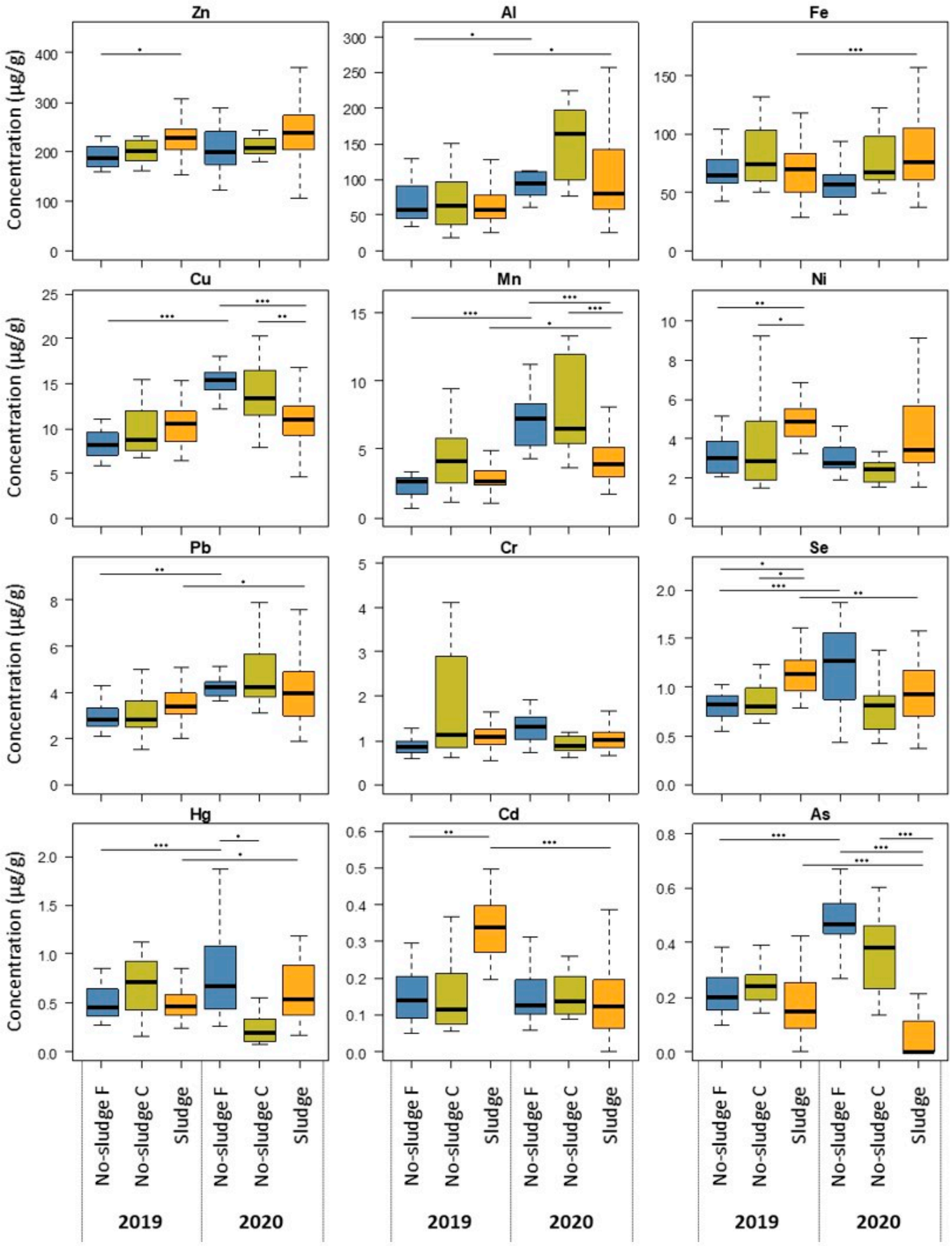

Figure 2. Trace element concentrations in nestling feathers grown in the three considered areas in 2019 and 2020. Orange box plots represent feathers grown in the Sludge area (Sludge), blue box plots represent feathers grown in the No-sludge area far from the area amended with sewage sludge (No-sludge F), green box plots represent feathers grown in the No-sludge area close to the area amended with sewage sludge (No-sludge C). Statistically significant differences between groups of colonies are indicated by lines with asterisks (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$). Contaminants are presented in decreasing mean concentration order. Full details of post hoc tests are provided in Supplementary Table S2.

Among the considered elements, particular attention should be given to toxic metals known to cause sub-lethal effects even at low concentrations, such as Cd, Pb, and Hg [54]. Cd has carcinogenic, teratogenic, and possibly mutagenic effects on the biota; it is known to modify Ca uptake, with adverse effects on skeletal integrity and egg production [54]. Besides the physiological effects, sub-lethal doses of Cd are also suggested to induce changes in anti-predator and reproductive behaviour, negatively affecting individual survival and fitness [55]. Lead poisoning is among the major causes of death in wild animals, largely caused by the ingestion of lead pellets used for shooting [56]. However, Pb particles can also dissolve in soil water, becoming bioavailable and entering the trophic chain. Sub-lethal effects of Pb on birds include pathologies associated with reproductive and immune functions, impaired thermoregulation and feeding behaviour, and lower nestling survival [54]. Finally, Hg is one of the most studied toxic metals in birds, highly toxic even at small concentrations. Its adverse effects include neurological, endocrine, and immune disruption with consequences on development, neurocognitive function, and reproduction [57–60]. To understand the potential risks to bird populations, it is pivotal to define the concentrations above which the considered trace metal induces sub-lethal behavioural or reproductive effects. While different studies defined the threshold values in internal tissues such as the liver or kidney, a lower number of studies set a threshold for feather concentrations, and, in addition, most of them have been conducted on seabirds. For Cd, the sub-lethal and behavioural effects occurred at a concentration that, in feathers, ranges from 0.1 to 2 μg/g, depending on the seabird species considered, while for Pb and Hg, sub-lethal effects emerge at concentrations lower than 4 and 5 μg/g, respectively [54]. Irrespective of the considered group, more than 75% of individuals in our study showed a concentration of Cd exceeding the adverse effect concentration of 0.1 μg/g. In addition, concentrations above the threshold values were reached by 38% and 47% of nestlings for Pb and Hg, respectively. Considering these results, we might speculate that the exposure to Cd, Pb, and Hg can result in the onset of sub-lethal effects in barn swallow nestlings, or in further stages of its life cycle. However, it is important to bear in mind that the feather threshold of sub-lethal concentrations for the elements in question have been found in seabird species with different ecological and physiological characteristics and metabolic pathways compared to our model species, while no studies are currently available for terrestrial species. In addition, as Cd, Hg, and Pb bioaccumulate in the tissues, we might hypothesize that the concentrations of those elements would further increase during the individual's life above the concentrations measured in our analyses carried out on feathers, potentially impacting population dynamics via their sub-lethal effects on reproduction.

4. Conclusions

The results of our study suggest that barn swallow nestlings may serve as valuable local sentinels for monitoring potential trace element contamination. However, more evidence is needed to establish standardized protocols for their use as biomonitors. In fact, the variation in trace element concentrations within the same region over different years is typically greater than the differences observed between different regions. This weakens the predictive value of feather analyses in indicating contamination levels within a particular region. Furthermore, the relationship between the trace element concentrations found in nestling feathers and the application of sewage sludge as a soil amendment remains unclear. This could be due to the influence of various other human-related or ecological factors that may affect the observed patterns of trace elements in feathers. Therefore, further research is needed to investigate the transfer of sludge-derived trace elements through the food chain and to assess their impacts on the fitness traits of barn swallows. In conclusion, while our study highlights the potential of barn swallow nestlings as indicators of trace element contamination, further research is required to establish standardized protocols and gain a deeper understanding of the ecological implications and effects on barn swallow fitness.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/environments10080145/s1, Table S1: Mean, standard error (SE), minimum (Min) and maximum (Max) concentrations of the 12 trace elements measured in feathers collected in the six groups of colonies; Table S2: Result of the linear mixed model of differences in trace element concentrations among groups of colonies.

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References

1. Holt, E.A.; Miller, S.W. Bioindicators: Using Organisms to Measure Environmental Impacts | Learn Science at Scitable. *Nat. Educ. Knowl.* **2011**, *3*, 8.
2. Conti, M.E. Environmental Biological Monitoring. *Biol. Monit.* **2008**, *30*, 1–23. [CrossRef]
3. Golden, N.H.; Rattner, B.A. Ranking Terrestrial Vertebrate Species for Utility in Biomonitoring and Vulnerability to Environmental Contaminants. *Rev. Environ. Contam. Toxicol.* **2003**, *176*, 67–136. [CrossRef] [PubMed]
4. Smith, P.N.; Cobb, G.P.; Godard-Codding, C.; Hoff, D.; McMurry, S.T.; Rainwater, T.R.; Reynolds, K.D. Contaminant Exposure in Terrestrial Vertebrates. *Environ. Pollut.* **2007**, *150*, 41–64. [CrossRef] [PubMed]
5. Denneman, W.D.; Douben, P.E.T. Trace Metals in Primary Feathers of the Barn Owl (*Tyto alba guttatus*) in The Netherlands. *Environ. Pollut.* **1993**, *82*, 301–310. [CrossRef]
6. Bortolotti, G.R. Flaws and Pitfalls in the Chemical Analysis of Feathers: Bad News–Good News for Avian Chemoecology and Toxicology. *Ecol. Appl.* **2010**, *20*, 1766–1774. [CrossRef]
7. Borghesi, F.; Migani, F.; Andreotti, A.; Baccetti, N.; Bianchi, N.; Birke, M.; Dinelli, E. Metals and Trace Elements in Feathers: A Geochemical Approach to Avoid Misinterpretation of Analytical Responses. *Sci. Total Environ.* **2016**, *544*, 476–494. [CrossRef]
8. Parolini, M.; Sturini, M.; Maraschi, F.; Profumo, A.; Costanzo, A.; Caprioli, M.; Rubolini, D.; Ambrosini, R.; Canova, L. Trace Elements Fingerprint of Feathers Differs between Breeding and Non-Breeding Areas in an Afro-Palearctic Migratory Bird, the Barn Swallow (*Hirundo rustica*). *Environ. Sci. Pollut. Res.* **2021**, *28*, 15828–15837. [CrossRef]
9. Hollamby, S.; Afema-Azikuru, J.; Waigo, S.; Cameron, K.; Rae Gandolf, A.; Norris, A.; Sikarskie, J.G. Suggested Guidelines for Use of Avian Species as Biomonitors. *Environ. Monit. Assess.* **2006**, *118*, 13–20. [CrossRef]
10. Barton, M.G.; Henderson, I.; Border, J.A.; Siriwardena, G. A Review of the Impacts of Air Pollution on Terrestrial Birds. *Sci. Total Environ.* **2023**, *873*, 162136. [CrossRef]
11. Jaspers, V.L.B.; Covaci, A.; Herzke, D.; Eulaers, I.; Eens, M. Bird Feathers as a Biomonitor for Environmental Pollutants: Prospects and Pitfalls. *TrAC Trends Anal. Chem.* **2019**, *118*, 223–226. [CrossRef]
12. Gebbink, W.A.; Letcher, R.J. Comparative Tissue and Body Compartment Accumulation and Maternal Transfer to Eggs of Perfluoroalkyl Sulfonates and Carboxylates in Great Lakes Herring Gulls. *Environ. Pollut.* **2012**, *162*, 40–47. [CrossRef]
13. Sagerup, K.; Savinov, V.; Savinova, T.; Kuklin, V.; Muir, D.C.G.; Gabrielsen, G.W. Persistent Organic Pollutants, Heavy Metals and Parasites in the Glaucous Gull (*Larus hyperboreus*) on Spitsbergen. *Environ. Pollut.* **2009**, *157*, 2282–2290. [CrossRef]
14. Dauwe, T.; Bervoets, L.; Pinxten, R.; Blust, R.; Eens, M. Variation of Heavy Metals within and among Feathers of Birds of Prey: Effects of Molt and External Contamination. *Environ. Pollut.* **2003**, *124*, 429–436. [CrossRef] [PubMed]
15. Rutkowska, M.; Płotka-Wasylka, J.; Lubinska-Szczygeł, M.; Różańska, A.; Możejko-Ciesielska, J.; Namieśnik, J. Birds' Feathers – Suitable Samples for Determination of Environmental Pollutants. *TrAC Trends Anal. Chem.* **2018**, *109*, 97–115. [CrossRef]
16. Hahn, E.; Hahn, K.; Stoeppler, M. Bird Feathers as Bioindicators in Areas of the German Environmental Specimen Bank—Bioaccumulation of Mercury in Food Chains and Exogenous Deposition of Atmospheric Pollution with Lead and Cadmium. *Sci. Total Environ.* **1993**, *139–140*, 259–270. [CrossRef] [PubMed]
17. Costa, R.A.; Eeva, T.; Eira, C.; Vaqueiro, J.; Vingada, J.V. Assessing Heavy Metal Pollution Using Great Tits (*Parus major*): Feathers and Excrements from Nestlings and Adults. *Environ. Monit. Assess.* **2013**, *185*, 5339–5344. [CrossRef]
18. Peterson, S.H.; Ackerman, J.T.; Toney, M.; Herzog, M.P. Mercury Concentrations Vary Within and Among Individual Bird Feathers: A Critical Evaluation and Guidelines for Feather Use in Mercury Monitoring Programs. *Environ. Toxicol. Chem.* **2019**, *38*, 1164–1187. [CrossRef]
19. Burger, M.; Gochfeld, J. Age Differences in Metals in the Blood of Herring (*Larus argentatus*) and Franklin's (*Larus pipixcan*) Gulls. *Arch. Environ. Contam. Toxicol.* **1997**, *33*, 436–440. [CrossRef] [PubMed]
20. Llacuna, S.; Gorriz, A.; Sanpera, C.; Nadal, J. Metal Accumulation in Three Species of Passerine Birds (*Emberiza cia*, *Parus major*, and *Turdus merula*) Subjected to Air Pollution from a Coal-Fired Power Plant. *Arch. Environ. Contam. Toxicol.* **1995**, *28*, 298–303. [CrossRef]
21. Eens, M.; Pinxten, R.; Verheyen, R.F.; Blust, R.; Bervoets, L. Great and Blue Tits as Indicators of Heavy Metal Contamination in Terrestrial Ecosystems. *Ecotoxicol. Environ. Saf.* **1999**, *44*, 81–85. [CrossRef]

22. Roig, N.; Sierra, J.; Martí, E.; Nadal, M.; Schuhmacher, M.; Domingo, J.L. Long-Term Amendment of Spanish Soils with Sewage Sludge: Effects on Soil Functioning. *Agric. Ecosyst. Environ.* **2012**, *158*, 41–48. [CrossRef]
23. Manzetti, S.; van der Spoel, D. Impact of Sludge Deposition on Biodiversity. *Ecotoxicology* **2015**, *24*, 1799–1814. [CrossRef] [PubMed]
24. Zhang, Y.; Gu, A.Z.; Cen, T.; Li, X.; He, M.; Li, D.; Chen, J. Sub-Inhibitory Concentrations of Heavy Metals Facilitate the Horizontal Transfer of Plasmid-Mediated Antibiotic Resistance Genes in Water Environment. *Environ. Pollut.* **2018**, *237*, 74–82. [CrossRef] [PubMed]
25. Xu, Y.; Seshadri, B.; Bolan, N.; Sarkar, B.; Ok, Y.S.; Zhang, W.; Rumpel, C.; Sparks, D.; Farrell, M.; Hall, T.; et al. Microbial Functional Diversity and Carbon Use Feedback in Soils as Affected by Heavy Metals. *Environ. Int.* **2019**, *125*, 478–488. [CrossRef]
26. Masindi, V.; Muedi, K.L. Environmental Contamination by Heavy Metals. In *Heavy Metals*; Saleh, H.E.-D.M., Aglan, R.F., Eds.; IntechOpen: london, UK, 2018; pp. 115–132.
27. Donatello, S.; Cheeseman, C.R. Recycling and Recovery Routes for Incinerated Sewage Sludge Ash (ISSA): A Review. *Waste Manag.* **2013**, *33*, 2328–2340. [CrossRef] [PubMed]
28. Kirchmann, H.; Börjesson, G.; Kätterer, T.; Cohen, Y. From Agricultural Use of Sewage Sludge to Nutrient Extraction: A Soil Science Outlook. *Ambio* **2017**, *46*, 143–154. [CrossRef] [PubMed]
29. Grobelak, A.; Grosser, A.; Kacprzak, M.; Kamizela, T. Sewage Sludge Processing and Management in Small and Medium-Sized Municipal Wastewater Treatment Plant-New Technical Solution. *J. Environ. Manag.* **2019**, *234*, 90–96. [CrossRef]
30. Grobelak, A.; Czerwińska, K.; Murtaś, A. General Considerations on Sludge Disposal, Industrial and Municipal Sludge. In *Industrial and Municipal Sludge*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 135–153.
31. Rorat, A.; Courtois, P.; Vandenbulcke, F.; Lemiere, S. Sanitary and Environmental Aspects of Sewage Sludge Management. In *Industrial and Municipal Sludge*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 155–180.
32. Bandini, F.; Taskin, E.; Vaccari, F.; Soldano, M.; Piccinini, S.; Frache, A.; Remelli, S.; Menta, C.; Sandro Cocconcelli, P.; Puglisi, E. Anaerobic Digestion and Aerobic Composting of Rigid Biopolymers in Bio-Waste Treatment: Fate and Effects on the Final Compost. *Bioresour. Technol.* **2022**, *351*, 126934. [CrossRef]
33. Wear, S.L.; Acuña, V.; McDonald, R.; Font, C. Sewage Pollution, Declining Ecosystem Health, and Cross-Sector Collaboration. *Biol. Conserv.* **2021**, *255*, 109010. [CrossRef]
34. Ekane, N.; Barquet, K.; Rosemarin, A. Resources and Risks: Perceptions on the Application of Sewage Sludge on Agricultural Land in Sweden, a Case Study. *Front. Sustain. Food Syst.* **2021**, *5*, 647780. [CrossRef]
35. Deblonde, T.; Cossu-Leguille, C.; Hartemann, P. Emerging Pollutants in Wastewater: A Review of the Literature. *Int. J. Hyg. Environ. Health* **2011**, *214*, 442–448. [CrossRef]
36. Cantinho, P.; Matos, M.; Trancoso, M.A.; dos Santos, M.M.C. Behaviour and Fate of Metals in Urban Wastewater Treatment Plants: A Review. *Int. J. Environ. Sci. Technol.* **2016**, *13*, 359–386. [CrossRef]
37. Magni, S.; Binelli, A.; Pittura, L.; Avio, C.G.; Della Torre, C.; Parenti, C.C.; Gorbi, S.; Regoli, F. The Fate of Microplastics in an Italian Wastewater Treatment Plant. *Sci. Total Environ.* **2019**, *652*, 602–610. [CrossRef]
38. Plumlee, M.H.; Gurr, C.J.; Reinhard, M. Recycled Water for Stream Flow Augmentation: Benefits, Challenges, and the Presence of Wastewater-Derived Organic Compounds. *Sci. Total Environ.* **2012**, *438*, 541–548. [CrossRef]
39. Tang, J.; Tang, H.; Sima, W.; Qiu, B.; Zou, D.; Liang, R.; Dong, J.; Liang, C.; Wan, S.; Li, Z.; et al. Physicochemical Properties and Heavy Metal Pollution Characteristics and Correlation Analysis of Sludge Landfill. *Int. J. Environ. Anal. Chem.* **2021**, 1–10. [CrossRef]
40. Washburn, B.E.; Begier, M.J. Wildlife Responses to Long-Term Application of Biosolids to Grasslands in North Carolina. *Rangel. Ecol. Manag.* **2011**, *64*, 131–138. [CrossRef]
41. Lovett, R.A. How Birds Are Used to Monitor Pollution. *Nature* **2012**. [CrossRef]
42. Szép, T.; Hobson, K.A.; Vallner, J.; Piper, S.E.; Kovács, B.; Szabó, D.Z.; Møller, A.P. Comparison of Trace Element and Stable Isotope Approaches to the Study of Migratory Connectivity: An Example Using Two Hirundine Species Breeding in Europe and Wintering in Africa. *J. Ornithol.* **2009**, *150*, 621–636. [CrossRef]
43. Ambrosini, R.; Bolzern, A.M.; Canova, L.; Arieni, S.; Møller, A.P.; Saino, N. The Distribution and Colony Size of Barn Swallows in Relation to Agricultural Land Use. *J. Appl. Ecol.* **2002**, *39*, 524–534. [CrossRef]
44. Liechti, F.; Scandolara, C.; Rubolini, D.; Ambrosini, R.; Korner-Nievergelt, F.; Hahn, S.; Lardelli, R.; Romano, M.; Caprioli, M.; Romano, A.; et al. Timing of Migration and Residence Areas during the Non-Breeding Period of Barn Swallows *Hirundo rustica* in Relation to Sex and Population. *J. Avian Biol.* **2015**, *46*, 254–265. [CrossRef]
45. Pancerasa, M.; Ambrosini, R.; Romano, A.; Rubolini, D.; Winkler, D.W.; Casagrandi, R. Across the Deserts and Sea: Inter-Individual Variation in Migration Routes of South-Central European Barn Swallows (*Hirundo rustica*). *Mov. Ecol.* **2022**, *10*, 51. [CrossRef] [PubMed]
46. Grigolo, C.P.; Sicurella, B.; Musitelli, F.; Romano, A.; Caprioli, M.; Rubolini, D.; Ambrosini, R.; Gobbi, M. Diet Heteogeneity and Antioxidant Defence in Barn Swallow *Hirundo rustica* Nestlings. *Avocetta* **2019**, *43*, 127–137.
47. van Buuren, S.; Groothuis-Oudshoorn, K. Mice: Multivariate Imputation by Chained Equations in R. *J. Stat. Softw.* **2011**, *45*, 1–67. [CrossRef]
48. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2016.

49. Oksanen, J.; Blanchet, F.G.; Friendly, M.; Kindt, R.; Legendre, P.; McGlinn, D.; Minchin, P.R.; O'Hara, R.B.; Simpson, G.L.; Solymos, P.; et al. Vegan: An Introduction to Ordination. *Management* **2019**, *1*, 1–10.
50. Brooks, M.; Bolker, B.; Kristensen, K.; Maechler, M. Package 'GlmmTMB'. 2022. Available online: https://cran.r-project.org/web/packages/glmmTMB/glmmTMB.pdf (accessed on 1 April 2023).
51. Lenth, R.; Singmann, H.; Love, J.; Buerkner, P.; Herve, M. Emmeans: Estimated Marginal Means, Aka Least-Squares Means. R Package Version. 2018. Available online: https://CRAN.R-project.org/package=emmeans (accessed on 1 April 2023).
52. Mantovani, I. Cartografia Geochimica della Pianura Padana Centrale a Ovest di Mantova. Master's Thesis, University of Bologna, Bologna, Italy, 2014.
53. Amorosi, A.; Sammartino, I. Influence of sediment provenance on background values of potentially toxic metals from near-surface sediments of Po coastal plain (Italy). *Int. J. Earth Sci.* **2007**, *96*, 389–396. [CrossRef]
54. Burger, J.; Gochfeld, M. Metal Levels in Feathers of 12 Species of Seabirds from Midway Atoll in the Northern Pacific Ocean. *Sci. Total Environ.* **2000**, *257*, 37–52. [CrossRef]
55. Pedersen, H.C.; Saether, M. Effects of Cadmium on Parental Behaviour in Free-Living Willow Ptarmigan hens. *Ecotoxicology* **1999**, *8*, 1–7. [CrossRef]
56. Pokras, M.A.; Kneeland, M.R. Lead Poisoning: Using Transdisciplinary Approaches to Solve an Ancient Problem. *Ecohealth* **2008**, *5*, 379–385. [CrossRef]
57. Heinz, G.H.; Hoffman, D.J.; Klimstra, J.D.; Stebbins, K.R.; Kondrad, S.L.; Erwin, C.A. Species Differences in the Sensitivity of Avian Embryos to Methylmercury. *Arch. Environ. Contam. Toxicol.* **2009**, *56*, 129–138. [CrossRef]
58. Tan, S.W.; Meiller, J.C.; Mahaffey, K.R. The Endocrine Effects of Mercury in Humans and Wildlife. *Crit. Rev. Toxicol.* **2009**, *39*, 228–269. [CrossRef] [PubMed]
59. Tartu, S.; Goutte, A.; Bustamante, P.; Angelier, F.; Moe, B.; Clément-Chastel, C.; Bech, C.; Gabrielsen, G.W.; Bustnes, J.O.; Chastel, O. To Breed or Not to Breed: Endocrine Response to Mercury Contamination by an Arctic Seabird. *Biol. Lett.* **2013**, *9*, 20130317. [CrossRef] [PubMed]
60. Goutte, A.; Barbraud, C.; Meillère, A.; Carravieri, A.; Bustamante, P.; Labadie, P.; Budzinski, H.; Delord, K.; Cherel, Y.; Weimerskirch, H.; et al. Demographic Consequences of Heavy Metals and Persistent Organic Pollutants in a Vulnerable Long-Lived Bird, the Wandering Albatross. *Proc. R. Soc. B Biol. Sci.* **2014**, *281*, 20133313. [CrossRef] [PubMed]