

Synchrotron radiation analysis in the study of pollution in the ring-billed gull (*Larus delawarensis*): a novel application of the technique

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Synchrotron radiation analysis is a powerful tool for identifying pollutants. Here, we explore the distribution of elements in the flight feathers of ring-billed gulls, *Larus delawarensis*, to identify potential pollutants and their distribution throughout the feather. Our analysis using Synchrotron Radiation Analysis and Environmental Scanning Electron Microscopy has identified a variety of metals that are integral parts of the feather structure including: calcium (Ca), strontium (Sr), zinc (Zn) and iron (Fe). The darker portions of the flight feather were enriched with Zn and Fe, which supports the suggestion that feather melanins are efficient ligands of some metals found in the environment, sequestering potentially harmful particles away from the body. However, trace lead (Pb) acquired from the regional pollution was detected only in females, and it was distributed across both the melanized and non-melanized portions of the feather suggesting that only some metals are efficiently sequestered by melanins. Overall this study highlights a potential method for identifying pollutants using bird feathers, which has broader implications on environmental sensing as well as avian and human health. Copyright © 2013 John Wiley & Sons, Ltd.

Introduction

Environmental pollution by heavy metals and persistent organic compounds represents one of the most important problems facing modern society. Biomonitoring of pollutants in appropriate indicator species can provide an effective means of assessing the distribution and potential impact of those pollutants in the environment. Effective biomonitoring requires choosing both suitable indicator species and efficient analytical techniques. In this study, two techniques were employed: Synchrotron Radiation Analysis (SRA) and Environmental Scanning Electron Microscopy (E-SEM). The results are indicative of the relative strength of two distinct analyses.

Synchrotron Radiation Analysis uses an intense X-ray beam to excite X-ray Fluorescence (SXRF) from very small volumes of material under ambient conditions with little or no sample preparation.^[1] In principle, SXRF may be used to identify specific elements at concentrations as low as parts per billion and may provide quantitative results when suitable standards are available. In addition, the resulting X-ray spectra (X-ray Absorption Near Edge Structure, XANES, and Extended Absorption Fine Structure, EXAFS) may be used to probe the chemistry for specific atoms (oxidation state, coordination number and nearest neighbour distance). SRA thus provides an attractive biomonitoring tool given the low detection limits and potential for chemical information. SRA also avoids the problems associated with high vacuum techniques and is a non-destructive method that may be useful with rare samples and samples, which might be subjected to further analysis using multiple analytical methods. This technique has been used recently to study trace metals as biomarkers in the fossil record.^[2]

In this study, SXRF is evaluated as a possible analytical technique for estimating pollution levels using feathers collected from ring-billed gulls, *Larus delawarensis*, from Western Lake Ontario in Ontario, Canada. Here, we include E-SEM as a complementary technique to provide both morphological information and useful electron induced X-ray fluorescence spectra.^[3]

The presence of contaminants in bird feathers represents a possible indicator for both metal and organic pollutants.^[4–6] Some birds often travel long distances to feed and/or during migration, thus establishing the locations of pollution sources. This behaviour has the advantage of integrating data from large areas, providing evidence of the extent of pollution. In contrast, local nesting colonies and stable resident populations may be

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used to identify specific localized contamination sources. Collection of feathers is a minimally stressful means of obtaining samples from live birds, and, in many cases, lost feathers can be easily obtained without disturbing or handling the bird making this method ideal for examining both threatened and endangered species. Our study provides a first attempt to combine SRA and E-SEM to bird feathers, and highlights the utility of this combination of instruments.

Methods

Sample collection

Feathers were collected from ring-billed gulls at a nesting colony in Windermere Basin in Hamilton, Ontario, Canada (43°15'49.30" N, 79°46'54.83" W) as part of a behavioural study.^[7] The study site is located in an urban industrial area near two integrated steel mills and is known to be contaminated by both airborne and ingestible mutagenic pollutants, including heavy metals.^[7–10] An adult male (band no. 69422853) and an adult female (band no. 69422829) were captured in May 2007 using circular walk-in wire mesh traps placed on their respective nests several days prior to egg hatching. At the time of capture, the birds were banded and measured, and the fourth primary feather was collected from each bird. The proximal part of these feathers is pale gray, whereas the distal part is black. Thus, the black part of the feather should contain a higher concentration of melanin.

Synchrotron radiation analysis

The male and female feathers were prepared for SRA by lightly wiping each feather with Triton X-100 (Sigma-Aldrich) to remove any gross surface contamination, which might have resulted from previous sample handling. X-ray spectra and X-ray fluorescence images were obtained on beamline X27-A at the National Synchrotron Light Source, Brookhaven National Laboratories, Upton, NY, using a 13-element detector and an exciting beam energy of 16.2 keV. This energy was sufficient to excite X-ray fluorescence from the elements of interest in this analysis. Data were collected from a spot 6 microns in the vertical direction and 17 microns in the horizontal direction using a collection time of 0.3 s. During imaging, the sample stage was moved 6 microns in the vertical direction and 17 microns in the horizontal for each step, ensuring a continuous image the count time allowed collection elemental images in reasonable time.

No attempt was made to obtain absolute element concentrations using the SXRF data because the fluorescence yield will depend on the density of the medium and its thickness, and both vary significantly across the regions of the feather from which fluorescence data was obtained.

Environmental scanning electron microscopy

The male ring-billed gull primary feather was examined using a FEI Company Quanta 200F field emission environmental scanning electron microscope (FE-ESEM) under low vacuum (75 Pa) at 12.5 kV. The FE-SEM is equipped with a Schottky field emission gun providing optimal spatial resolution. Samples were prepared in a clean room by sectioning a 4-mm piece of the feather adjacent to the rachis (central spine). The section morphology includes filamentous structures termed barbs and barbules. The feather section was examined using backscattered electron and energy

dispersive spectroscopy (EDS) analysis to determine both spatial surface morphology and elemental composition; quantitative element composition for selected metal-rich particles adhering to the feather surface was estimated using the software supplied with the ESEM instrument.

Results and discussion

SEM-EDS analysis of feather barbules

The male ring-billed gull feather was examined using an environmental SEM to document its surface morphology and chemistry.^[11] Under low magnification, the feather displayed significant surface area comprised of a network of radiating barbules (angled at 45°) arising from the barbs (Fig. 1). Some barbules terminate with hooklet structures, which tend to trap dander and dust debris. The entire barbule network comprises a series of troughs and trenches (Fig. 1). Close examination revealed significant entrainment of dust particulates confined to the troughs located between the individual barbules. Analysis of these dust particulates suggests two types of morphologies with different chemical compositions. The two predominant forms consist of distinct spherical and irregular forms (Figs 2 and 3). The spherical particles (Fig. 2) contain two distinct iron oxide species indicative of anthropogenic smelting products.^[12] These iron oxides consist of iron-rich particles Fe (83.4 wt%), O (8.30 wt%), N (4.46 wt%) and S (3.81 wt%), and iron-poor particles Fe (59.8 wt%), O (38.1 wt%) and S (2.08 wt%). The irregular dust particles (Fig. 3) have varied chemical compositions consisting of zinc and lead oxides.

Importantly, the SEM-EDS analysis revealed that the dust particles are readily distinguished so that elements present in them may be differentiated from those present in the structure of the feather itself. While there appears to be material of detrital origin in the feathers, the spherical iron-rich particles likely originate from nearby smelting operations.^[12]

SRA analysis of feathers

We obtained X-ray fluorescence spectra from random sites on the male feather (Fig. 4). With these results, X-ray fluorescence images were obtained for the Ca $K\alpha$, Zn $K\alpha$, Pb $L\alpha$ /As $K\alpha$, Pb $L\beta$,

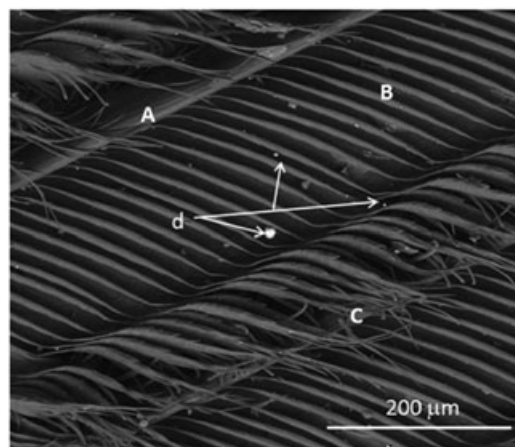


Figure 1. Electron micrograph of a ring-billed gull feather showing the (A) barb, (B) barbules and (C) associated hooklets (proximal filaments). Particles (d) were identified adjacent to the barbules structures.

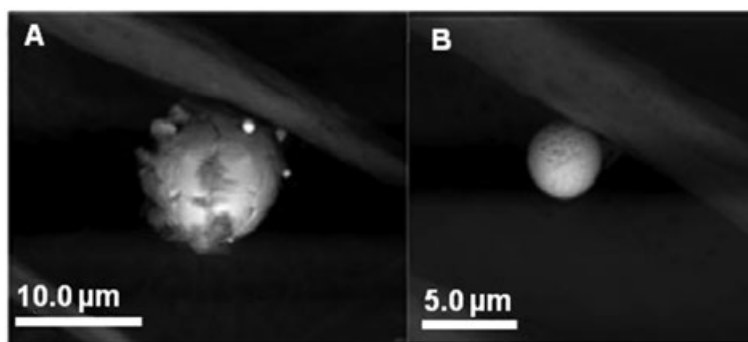


Figure 2. Secondary electron images showing smelted derived dust particles attached to feather barbules. The spherical particles show two distinct chemistries: (A) Fe-poor, Fe (59.8% by wt), O (31.8% by wt) and S (2.1% by wt); (B) Fe-rich, Fe (82.9% by wt), O (8.8% by wt), N (4.5% by wt) and S (3.8% by wt).

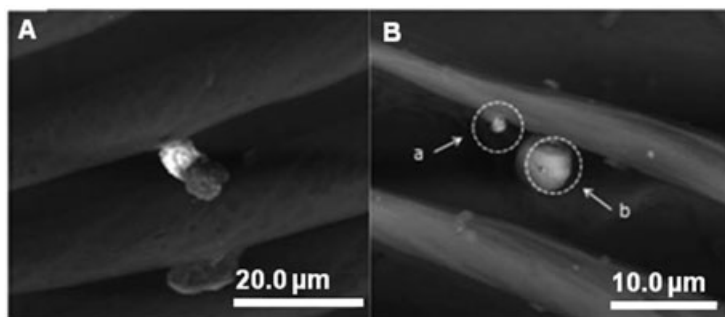


Figure 3. ESEM images showing other smelting morphologies associated with feather barbules. The angular sub-rounded particles are less abundant, appearing singly or as fused aggregates. (A) One of these is silica while the high-contrast image is lead oxide. (B) shows two adjacent particles (A) presents a Zn–Fe–S–O matrix, Zn (73.0% by wt), Fe (6.1% by wt), S (7.0% by wt) and O (13.9% by wt). (B) is Fe rich, Fe (85.5% by wt), O (10.4 by wt) and S (4.0% by wt).

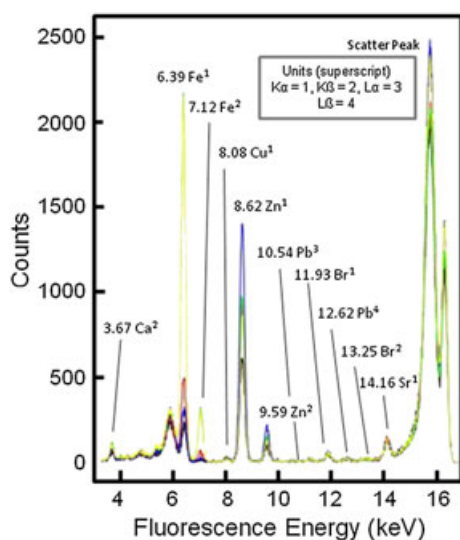


Figure 4. Repeated X-ray Fluorescence spectra collected from random sites on one male feather.

Sr $K\alpha$ emission lines in all subsequent image patterns. Given the complexity of the matrix with its varying chemical composition, thickness and density within the areas from which SXRF was obtained, no effort has been made to obtain quantitative data. In addition, no elements with atomic number less than Ca could be detected under the conditions used. We estimate detection limits to be in the high ppb to low ppm range^[13]

Localized high concentrations of metals such as Ca and Fe were not associated with any recognizable feather structures and most likely represent residual detrital particles such as those observed in the FE-SEM study. The washing technique used most likely did not remove all the detritus. Interestingly, Ca, Fe, Zn and Cu assist in the production of pigments, with a general effect on murine and avian melanocytes.^[5,6,14–16] Moreover, there is evidence to support a close relationship between metals and melanin-based feather colour, suggesting that feathers may be useful for monitoring metal pollution.^[4,17,18] Our data suggest that SRA conducted on feathers may be an effective means of monitoring metal pollution.

The element distribution maps for Ca, Fe, Zn and Sr (Fig. 5) illustrate the utility of this method for detecting a variety of metals across the feather; however, a more rigorous examination would be required to account for the variability in feather

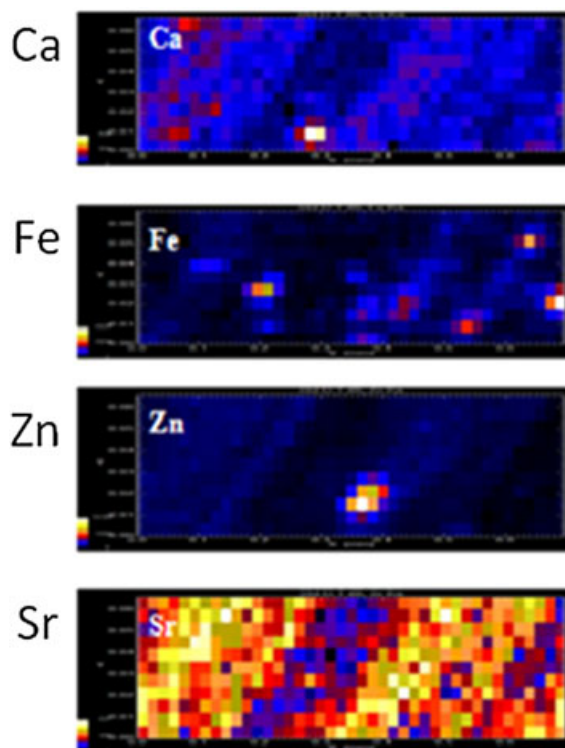


Figure 5. Typical X-ray Fluorescence maps for Ca, Zn, Fe and Sr from gull feathers. Isolated intense spots for Ca, Zn and Fe are probably detrital.

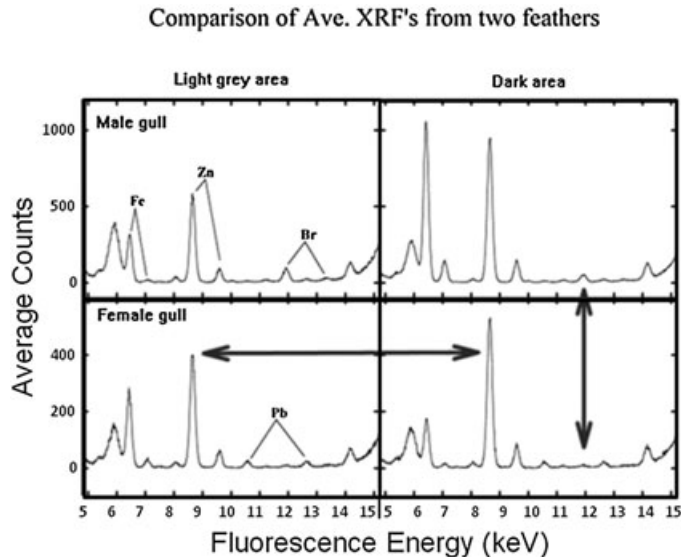


Figure 6. X-ray fluorescence spectra from light and dark areas (left and right panels respectively) of male and female gull feathers (upper and lower panels respectively). Note that Zn and Fe fluorescence was highest in the dark feather regions, traces of Br were found in both light and dark areas of both feathers, while Pb was highest in the female feather.

thickness and density. Our results show that Ca and Sr track each other exactly, except in areas associated with surface particles as evidenced by the ESEM pictures, as expected from their similar chemistry. Zn and Fe also show small regions of more intense X-ray fluorescence, once again possibly surface particles; there is also a lower level fluoresce that appears to be associated with

the feather structure. The larger areas within the image that show a uniform distribution of Ca, Sr, Zn and potentially Fe suggest that these elements are integral parts of the feather structure. This distribution is especially striking for Ca and Sr. The average XRF (Fig. 6) suggests that the dark portion of the feathers is enriched with Zn, an observation consistent with other

observations,^[17] as well as Fe, which has not been reported previously. Interestingly, the female feather appears to have a higher Pb concentration, possibly indicative of local pollution.^[19] This finding is consistent with the observation that female birds tend to accumulate Pb at a higher rate than males, particularly during egg laying.^[20]

Melanin deposition may enable birds to expel metals from their bodies by sequestering them into growing feathers,^[5] which is particularly important for potentially harmful metals. Melanin is a powerful ligand^[21] that easily binds to metals and serves as protection against parasitic organisms.^[22–24] Because feathers are metabolically inert and melanin pigmentation is nearly ubiquitous among avian families,^[25] the hypothesis that melanin serves to sequester potentially harmful agents away from the body^[5,21] is particularly compelling. We found Pb in both the melanin and non-melanin areas; however, we cannot speculate about the concentrations between these regions.

Similarly, bromine appears to be present in all portions of both feathers. Little is known of the physiological role of bromine in humans, though it is an essential trace element.^[26] Concerns have been raised about the safety of the brominated persistent organic compounds, which are now in widespread use, and bird feathers might be an effective tool to track the environmental distribution of these pollutants.^[27,28] We suggest that bromine was found ubiquitously across the feather because it was concentrated in the preen oil, and then distributed across the feather as the birds preened. Our approach may be especially efficient for these oil-soluble pollutants that are present in preen-gland oil and therefore distributed across the entire feather, from which they may be collected with minimal disturbance.^[29] We suggest that future research use quantitative approach to examine the concentrations of contaminants within the feather, and determine the types of elements that melanin efficiently sequesters from the body. Repeated washing of the feathers was not undertaken because bromine in the preen oil has been suggested elsewhere^[29] and penetration of oil into the feather may be too deep to remove easily. Future studies examining a larger number of feathers may benefit from these extra steps.

Our findings suggest that E-SEM and SRA are valuable complementary techniques for identifying the presence and origin of metals in bird feathers. In particular, E-SEM allowed for the characterization of iron-rich smelting byproducts on the surface of the feather, whereas SRA identified Ca, Sr, Zn and potentially Fe as integral parts of the feather structure. We show more Zn and Fe in the darker, rather than lighter, portions of the feathers, and this is consistent with metal involvement in melanin colouration. Our results support the hypothesis that melanin sequesters metals out of the body and into growing feathers, but suggest that certain metals have a higher affinity for melanin binding. We suggest that this would be a valuable avenue for future research and that the technique we outline provides an ideal method. Our study shows how feathers may provide a powerful tool for monitoring pollution by persistent organobromine compounds. Future research should refine this technique to provide more detailed elemental maps within individual feathers and use SRA to rapidly screen and identify sources of environmental bromine in bird feathers. Overall, these findings show that SRA and perhaps E-SEM have important roles to play in the use of feathers as bio-indicators of environmental stress.

Conclusions

The results show that SRA is an effective technique for studying metals in bird feathers, especially when combined with SEM. Using this technique, we detected local lead pollution in the female feathers, which possibly resulted from nesting location. Moreover, bromine, now a universal pollutant, was detected in both melanized and non-melanized portions of male and female feathers. We suggest that the bromine deposited on the feather originated within the oil from the preen gland.

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