Environ Monit Assess (2021) 193:35
https://doi.org/10.1007/s10661-020-08804-2

Bird feathers are potential biomonitors for airborne elemental carbon

Claire Pitre · Alexandra G. Ponette-González · Jenna E. Rindy · Anna Lee · Dornith Doherty · Matthew Fry · Jeff A. Johnson

Received: 5 July 2020 / Accepted: 10 December 2020

Abstract Birds can serve as effective biomonitors of air pollution, yet few studies have quantified external particulate matter accumulation on bird feathers. Biomonitoring of airborne elemental carbon (EC) is of critical significance because EC is a component of particulate matter with adverse effects on air quality and human health. To assess their effectiveness for use in EC monitoring, we compared EC accumulation on bird feathers at two sites that differed in vehicular traffic volume in an urban environment within the Dallas-Fort Worth Metropolitan Area, USA. Moulted flight feathers from domestic chickens were experimentally exposed to ambient EC pollution for 5 days in two urban microenvironments 1.5 km distant from each other that differed in traffic volume—adjacent to an interstate highway and a university campus bus stop. Feathers near the highway accumulated approximately eight times more EC (307 ± 34 μg m^{-2} day^{-1}), on average, than feathers near the bus stop (40 ± 9 μg m^{-2} day^{-1}). These findings indicate that EC accumulation on feathers varies over short distances within urban areas and that bird feathers potentially can be used for biomonitoring airborne EC.

C. Pitre · A. G. Ponette-González (✉) · J. E. Rindy · M. Fry
Department of Geography and the Environment, University of North Texas, 1155 Union Circle #305279, Denton, TX, USA
e-mail: alexandra.ponette@unt.edu

C. Pitre
e-mail: claire.pitre@student.hu-berlin.de

J. E. Rindy
e-mail: jer0275@bu.edu

M. Fry
e-mail: matthew.fry@unt.edu

A. Lee · D. Doherty
Department of Studio Art, University of North Texas, Denton, TX
1155 Union Circle #305100, USA

D. Doherty
e-mail: dornith.doherty@unt.edu

J. A. Johnson
Department of Biological Sciences, University of North Texas, 1155 Union Circle #310559, Denton, TX, USA
e-mail: jeff.johnson@wcof-wy.org

Present Address:
C. Pitre
Albrecht Daniel Thaer - Institute for Agricultural and Horticultural Sciences, Humboldt University of Berlin, Invalidenstraße 42, 10115 Berlin, Germany

Present Address:
J. E. Rindy
Department of Biology, Boston University, 5 Cummington Mall, Boston, MA, USA

Present Address:
J. A. Johnson
Wolf Creek Operating Foundation, 1026 Soldier Creek Road, Wolf, WY 82844, USA

Published online: 06 January 2021

Keywords Black carbon · Cities · External contamination · Intra-urban variability · Particulate matter · Soot

Introduction

Birds are ubiquitous and, in some cases, valuable biomonitors of environmental pollution (Jaspers et al. 2019; Pollack et al. 2017; Wolterbeek 2002). Bird feathers, specifically, have been widely used to track bioaccumulation of pollutants in rural, urban, agricultural, and industrial environments (Cai and Calisi 2016; Carneiro et al. 2016; Jaspers et al. 2009; Schulwitz et al. 2015), in part because feathers can often be sampled non-destructively (Jaspers et al. 2007; Movalli et al. 2017). Many of these studies focus on the bioaccumulation of heavy metals (e.g., mercury, lead) or organic pollutants due to their toxicity to humans and birds (e.g., Cai and Calisi 2016; Eulaers et al. 2011; Gasparini et al. 2014; Sun et al. 2019).

Comparatively few studies investigate the external accumulation of airborne particulate matter on bird feathers, with most, if not all, focused on removing external contaminants to improve the accuracy of internal pollutant concentration estimates (e.g., Borghesi et al. 2016; Cardiel et al. 2011). Nevertheless, feather washing experiments and imaging show that non-trivial amounts of particulates can accumulate on feather vanes and, to a lesser extent, on the shafts (Dauwe et al. 2003; DuBay and Fuldner 2017). Furthermore, some airborne particulate elements, such as iron and aluminum, are preferentially retained on feather surfaces (Borghesi et al. 2016; Howell et al. 2017). These findings suggest that bird feathers could serve as an effective tool for biomonitoring particulate matter air pollution.

Elemental carbon (EC) is a component of particulate matter that is emitted directly into the atmosphere from fossil fuel combustion and biomass burning. Elemental carbon exhibits spatially heterogeneous concentrations in (Apte et al. 2017; Caubel et al. 2019) and among urban atmospheres (Barrett et al. 2019), where it degrades air quality and is harmful to human health (Janssen et al. 2012; Jia et al. 2020). Utilizing bird feathers as biomonitors of atmospheric EC could improve current understanding of spatial variability in urban particulate matter air pollution and its implications for environmental and human health.

We conducted a pilot study to quantify differences in EC accumulated on feather surfaces in two microenvironments within the City of Denton, Texas, located in the Dallas-Fort Worth Metropolitan Area, USA. Monitoring conducted by the Chemical Speciation Network (CSN 2020) indicates that average annual atmospheric EC concentrations in the City of Dallas (65 km southeast of Denton) are well above background levels (2018 mean EC = 0.52 μg cm^{-3}; max EC = 1.84 μg cm^{-3}). We evaluated if feathers could capture differences in EC pollution at two urban sites separated by just 1.5 km and with different traffic volumes and therefore expected potential exposure to particulate matter and EC levels in their immediate locations (e.g., Luce et al. 2020). We expected to find higher EC accumulation on feathers at the site with higher traffic volume.

Materials and methods

Sample collection and preparation

A total of 59 naturally moulted (i.e., not plucked) chicken (*Gallus gallus domesticus*) feathers were obtained from two commercial farms (n = 29 and n = 30) in the USA to obtain adequate sample sizes. We selected primary feathers (i.e., flight feathers) to limit potential variation in EC accumulation resulting from differences in feather type (e.g., Peterson et al. 2019) and to enable comparison among individuals (Martínez et al. 2012). Upon receipt, feathers were stored at room temperature in a dust-free glovebox to prevent any further contamination until sample preparation.

All glassware was soaked for 24 h in 10% nitric acid and baked in a muffle furnace at 450 °C for 5 h prior to feather washing. Feathers were thoroughly washed prior to experimental exposure to remove any adhered particles on their surface using a two-step process similar to that of Dauwe et al. (2003). The first step of the washing process consisted of removing externally deposited surface particles by sonicating each feather individually in 300 ml of double-deionized (DDI) water for 5 min, and then rotating the feather vertically and sonicating for an additional 5 min. Subsequently, each feather was rinsed three times on each side with acetone to remove particles remaining after sonication. The acetone rinsate was added to the DDI rinsate for a total sample of ~ 330 ml. We added 4.5 g of salt (1.5 g $(NH_4)H_2PO_4$ per 100 ml DDI) to each sample and then sonicated the sample for

10 min to increase EC recovery (Torres et al. 2014). Samples were transferred to glass bottles and refrigerated overnight.

After each individual feather was washed, glassware was thoroughly rinsed with DDI, and metal forceps and spatulas were cleaned with acetone. Feathers were placed in the glovebox to dry at room temperature. We then measured the length and width of each feather before photographing. The surface area of both sides of each feather was calculated using the open-source software ImageJ (Rasband and Ferreira 2011), with feather surface area equal to the average of both measurements.

Experimental exposure to ambient atmospheric EC

A total of 29 or 30 feathers were attached to plastic mesh screens mounted on two identical ~ 90 × 90 cm frames, with each feather spaced 15 cm apart (Fig. 1). The screens were washed with deionized water prior to attaching the feathers. Each feather was positioned vertically on the screens and tied to the mesh at two points along the feather shaft using cotton sewing thread. Feathers were secured to the mesh screen in rows of six feathers each, with 15 cm between each row. Given that we obtained feathers from two different sources, we attached the feathers to each screen by alternating the sequence of feathers by their source. Screens were then wrapped and secured in new plastic immediately after feathers were attached to prevent contamination before deployment.

The frames were installed 60 cm above the ground on 13 February 2019 at two sites in the City of Denton, Texas, differing in average annual daily traffic (AADT; NCTCOG 2020). The frame with 29 feathers was ~ 30 m from Interstate I-35E (2018 AADT = 82,287, speed limit = 105 km h^{-1}), and the frame with 30 feathers was located ~ 10 m from a bus stop on the University of North Texas main campus (2014 AADT = 2,584, speed limit = 30 km h^{-1}). The frames were installed with the feathers facing oncoming traffic. Feathers were exposed to ambient conditions for five consecutive dry days. After the fifth day of exposure, screens were removed and covered in plastic for transport to the lab for feather EC extraction and analysis.

Sample extraction and analysis

The same two-step washing method used prior to exposure was used to extract EC particles from feathers after exposure. However, instead of processing each feather individually, two adjacent feathers (i.e., from different

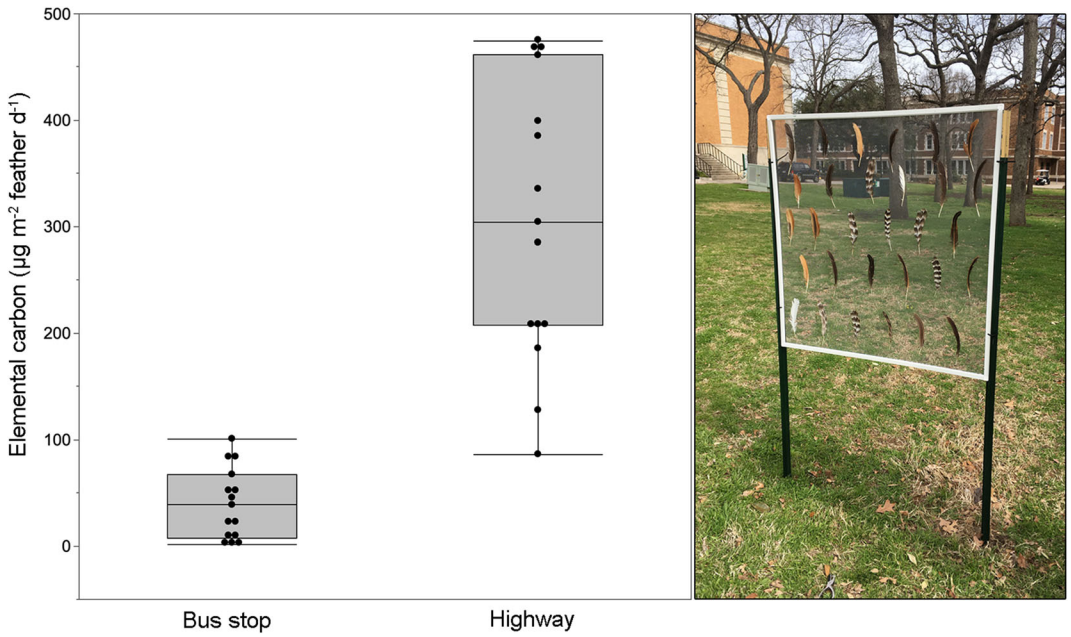

Fig. 1 Left: Elemental carbon accumulation (µg m^{-2} feather day^{-1}) on chicken feathers at a university bus stop and highway site. Each point represents a pooled sample of two feathers. Right: Frame with chicken feathers mounted at a university bus stop in Denton, TX, USA

sources) were pooled into one sample, for a total of 15 samples from each frame. This approach was used to (1) eliminate potential bias due to feathers originating from two sources, and (2) ensure that EC levels would be above instrument detection limit (0.2 μg per cm^2 of filter). Following extraction, samples were filtered through an Advantec 13-mm filter funnel into a Buchner flask over a 1.3 cm^2 punch of Pall quartz-fiber filter (Pall Corp., Washington, NY) and subsequently left to dry in a desiccator for 24 h. Filters were then analyzed on a Sunset OC/EC Aerosol Analyzer to determine the EC mass on each filter. For quality assurance and quality control, one oven blank was used to ensure a clean instrument at the beginning of each sample analysis day. Additionally, two sucrose spikes (35.16 μg carbon) were analyzed after every 10 feather samples.

Statistical analysis

Because external EC accumulation is a time-dependent process and varies with feather size, EC mass was divided by the surface area (cm^2) of each feather and by the number of days exposed ($n = 5$) to calculate the quantity of EC accumulated on feathers per unit surface area per day for each location (Rindy et al. 2019). The data were tested for normality using a Shapiro-Wilk test. The data were normally distributed, and therefore, we used a two-sample *t* test to test for mean differences in EC accumulation between the two sites using JMP. v14 software (SAS Institute Inc.). Significance was set at $p < 0.05$. Data are reported in μg m^{-2} day^{-1} and values reported in the Results section are means ± SE.

Results

In total, 27 of the 30 samples (90%) accumulated EC at levels above instrument detection limit. The three samples measuring below detection limit were all from the bus stop site. Mean EC accumulation on feathers at the highway site (307 ± 34 μg m^{-2} day^{-1}) was nearly eight times higher than at the bus stop site (40 ± 9 μg m^{-2} d^{-1}; $p < 0.0001$; Fig. 1). Feathers at the bus stop site exhibited EC accumulations ranging from ~ 1 to 100 μg m^{-2} day^{-1} whereas feathers at the highway site showed a much broader range of values (86–475 μg m^{-2} day^{-1}) with the majority (80%) of highway accumulation values greater than 200 μg m^{-2} day^{-1}.

Discussion

Our findings show that EC accumulation on bird feathers can vary dramatically by location in urban environments. It is well established that atmospheric EC concentrations decrease exponentially with distance from major highways (Apte et al. 2017), with EC concentrations generally highest within the first 50 m (Zhu et al. 2002). Thus, we were not surprised to find that feathers exposed ~ 30 m from the highway accumulated significantly more EC compared to feathers exposed near the bus stop. Moreover, the two sites differed ~ 30-fold in vehicular traffic volume. We know of only one other study that has quantified EC on bird feathers. Using photometric reflectance data from > 1,300 bird specimens, Dubay and Fuldner (2017) showed that EC on the plumage of museum specimens was positively correlated with black carbon emissions in the USA during the first half of the twentieth century. Although the photometric reflectance data are not directly comparable to this study's EC measurements, the correlation supports our hypothesis that feather surface EC accumulation can reflect differences in potential local exposure concentrations. Taken together, these studies suggest that atmospheric EC concentrations were important in driving the difference in feather EC accumulation between the two urban study sites.

Recent research investigating how vegetation accumulates EC on leaf surfaces in the same study area indicates that bird feathers accumulate considerably less EC than live oak (*Quercus virginiana*) leaves (Rindy et al. 2019). While live oak leaves were not sampled at the highway site, feathers accumulated 12-fold less EC compared to leaves sampled at the same bus stop site and during the same month. Despite the caveat of limited sampling, this difference in substrate type raises an interesting question: why might leaves accumulate more EC than feathers? Although addressing this question was outside the scope of this pilot research, it is worth noting that EC was extracted from leaf and feather surfaces using different washing protocols. To remove surface-deposited ("on-surface") EC, the leaves were rinsed with 250 ml of DDI water and then sonicated for 15 min whereas the feathers were sonicated for 10 min and subsequently rinsed with acetone. Unlike the protocol for leaves, standardized procedures for removing particulate matter and many other pollutants from feathers are not well established (Jaspers et al. 2019). Indeed, washing protocols have been found to

vary in their effectiveness in removing externally deposited particles from bird feathers (Borghesi et al. 2016). Additional development and assessment of methods for removing externally accumulated EC particles from feather surfaces are needed. In sum, this research highlights the potential for considerable site and organismal-level differences in EC accumulation, warranting further research to identify the important factors that may influence those differences.

Future research

According to Wolterbeek (2002), biomonitors of trace elements in atmospheric particles and deposition should concentrate the element of interest and reflect ambient conditions. Thus, based on our findings, we contend that bird feathers can represent a potential tool for biomonitoring airborne EC particles. After only 5 days, EC on 90% of feather samples each consisting of two flight feathers was above minimum detection limit, and average accumulation rates reflected major differences in vehicular traffic volume between the two microenvironments. While our results show an 8-fold difference between sites, more accurate estimates of EC accumulation on bird feathers using a similar pre-post experimental design will require quantification of background EC on a subset of feathers after the initial washing procedure. Similar to biomonitoring of internally accumulated contaminants (Jaspers et al. 2019), several additional factors also merit further investigation to better understand the potential variability in external accumulation of particles and other contaminants that may exist among bird feathers.

First, feather type (e.g., flight, contour, or down) has been shown to affect bioaccumulation levels of mercury and other pollutants (e.g., Bortolotti 2010; Jaspers et al. 2009; Peterson et al. 2019) and presumably affects external particulate matter accumulation as well. Similar to tree leaves (e.g., Muhammad et al. 2019), feather types exhibit differences in structural properties (e.g., size, shape, roughness) that likely influence accumulation of externally deposited particles. In primary feathers, for example, the presence and quantity of barbules and hooklets along feather barbs could potentially increase external EC accumulation compared to other types of feathers due to increased surface area and adherence properties. Feather position on the bird's body can also affect exposure to airborne particles. Greater exposure generally leads to higher rates of atmospheric dry particle deposition in plant canopies (Griffith et al. 2015), a pattern we might also expect in feathers with respect to their position and location on the bird's body. Previous studies have also indicated that preening and the presence of preen oil specifically may increase external particle contamination (Jaspers et al. 2011). In this study, it is possible that the acetone rinse used to wash feathers prior to exposure may have removed preen oil from feathers (see Jaspers et al. 2011), resulting in decreased EC particle accumulation upon exposure to ambient conditions. Future research should consider the role of preening and preen oil on external EC accumulation. Improved understanding of variation in EC accumulation among feather types and their location on the body is therefore important for guiding feather sampling and processing, and interpretation of results (Peterson et al. 2019).

Second, determination of feather age is critical for accurate estimates of external particulate matter accumulation on bird feathers. In the case of pollutants that are transferred to feathers via the bloodstream during feather growth, pollutant deposition ceases when the feather stops growing. In contrast, feathers may behave like leaf surfaces with respect to external accumulation of particles (Cai et al. 2017). In this case, feathers would externally accumulate particles with increasing exposure to the atmosphere even after feather growth has ceased, at least up to a point where feathers reach a dynamic equilibrium, and no additional particles are deposited. For many wild bird species, determination of exact feather age remains a challenge (Bortolotti 2010), although plumage characteristics may be used to distinguish feather age class in reference to well-characterized molt cycle patterns (e.g., Flinks and Salewski 2012). In the latter case, particulate matter accumulation could be quantified in relative terms by examining the ratio of accumulation in old to young feathers.

Finally, future research should investigate the extent to which EC accumulation on feathers reflects environmental variations in atmospheric pollutant emissions or concentrations due to site-specific factors such as topography and meteorology (e.g., humidity, rainfall). Co-located sampling of atmospheric concentrations and accumulation (e.g., see Gillooly et al. 2019) could be used to this end. It also will be important to collect data on movement patterns of sampled birds using technology such as archival GPS tags (Maynard and Ronconi 2018; Rose et al. 2006) to investigate how changes in

location and hence exposure to particulate matter may vary among individuals and bird species depending on their foraging activities.

We conclude from this pilot research that bird feathers can represent a potentially promising tool for biomonitoring airborne EC. Additional research is needed to assess how EC accumulation varies by feather type, feather age, and with atmospheric concentrations, among other factors (e.g., bird movement or species). Investigation of these factors is necessary to determine the suitability of feathers as biomonitors, especially in urban environments where airborne EC and other particulate matter pollution is a growing problem. Exploration and development of this technique could potentially lead to better understanding of spatial variability in airborne EC across urban environments.

Acknowledgments We thank the Pitre family for collecting moulted feathers used in this study. We are also grateful to David Hoeinghaus for generously providing laboratory resources needed to conduct this research, and to Brian Wheeler and the grounds staff at the University of North Texas for campus support. We are indebted to three anonymous reviewers for providing excellent feedback on our manuscript.

Authors' contributions Pitre: Conceptualization, methodology, formal analysis, investigation, writing (original draft). Ponette-González: Conceptualization, methodology, resources, writing (original draft), visualization, supervision, project administration, funding acquisition. Doherty: Writing (review and editing), visualization, supervision, funding acquisition. Lee: Methodology, writing (review and editing), visualization. Rindy: Methodology, formal analysis, writing (review and editing). Fry: Methodology, writing (review and editing), supervision, funding acquisition. Johnson: Conceptualization, methodology, writing (review and editing), supervision.

Funding This work was supported by the National Science Foundation (CAREER #1552410 to AGPG), the University of North Texas Office of the Provost, Honors College, College of Visual Arts and Design, College of Liberal Arts and Social Sciences, the Department of Geography and the Environment, and the Department of Studio Art. Sponsors played no role in the collection, analysis and interpretation of data, in the writing of the report, or in the decision to submit the article for publication.

Data availability The dataset related to this article will be hosted on the Knowledge Network for Biocomplexity.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Code availability Not applicable.

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