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Using portable x-ray fluorescence with biological collections to broaden ecotoxicology research

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Abstract

In ecotoxicology, many studies have identified the effects of specific toxicants on cells, tissues, and individual organisms. However, large-scale and/or long-term studies that focus on wild populations, diverse taxonomic communities, and complex ecosystems are rare because of sampling and analytical constraints. Ecotoxicology stands to benefit from approaches that expand, quicken, economize, and simplify sample procurement and chemical analysis to address broader questions at the scale of populations, communities, and ecosystems. Here, we outline a strategy for achieving larger sample sizes and increasing the spatial, temporal, and taxonomic breadth of studies; a strategy that pairs biological collections with advanced chemical analyzers. Biological collections contain a wide range of preserved specimens that reflect the toxicological conditions of the times and places from which each specimen was collected, while the latest chemical analyzers provide an efficient means to analyze specimens. We demonstrate this capacity by developing a method to measure metal(loid) contamination in bird specimens using a portable x-ray fluorescence (pXRF) analyzer. We experimentally and qualitatively optimized a method for rapid, consistent, and non-destructive measurements of a focal contaminant of environmental health interest, lead, quantifying its bioaccumulation in the bones of bird specimens. We developed an analytical setup and procedure to tractably sample thousands of bird specimens through geographic space and time, and across the taxonomic breadth of birds. This method highlights how methods that pair collections with advanced analyzers can answer questions about pollution and contamination on ecologically relevant scales. This method can be readily modified to investigate other taxa and contaminants.

1. INTRODUCTION

A central goal of ecotoxicology is to address toxicological questions about large-scale, complex processes at higher levels of biological organization (Newman & Unger 2003, Beldon 2020). To tackle “big picture” questions, ecotoxicologists rely on bottom-up, reductionist approaches (which focus on molecules, cells, tissues, and whole organisms, usually in controlled lab settings) as well as top-down, holistic approaches (which focus on populations, communities, and ecosystems in real-world settings). While both approaches are necessary to answer many ecotoxicology questions, top-down, holistic approaches are scarcer because of the challenges associated with obtaining appropriate data at the necessary scale and scope of ecosystem-level questions (Taub 1989, Chapman 2002, Beketov & Liess 2012). As a result, we have partial answers to important ecosystem-level questions about the role of toxicants in environmental and macroecological processes, which include questions like how pollution affects biodiversity, how toxicants impact ecosystem functions, how contamination varies over large spatial and temporal scales, and how to promote the health of populations in the presence of multiple contaminants and stressors (Eggen et al. 2004, Beketov & Liess 2012, Vighi & Villa 2013, Köhler & Triebskorn 2013, Pesce et al. 2025).

There are several reasons why studies that tackle these questions are scarce in ecotoxicology and related fields (*see* Kareiva et al. 1996, Beketov & Liess 2012). Of the various factors that limit the scale

and scope of ecotoxicology studies, many are practical concerns; it is often too difficult, too costly, and/or unethical to obtain and analyze the necessary volumes and/or types of samples. Ecotoxicology studies that involve wild populations, diverse biotic communities, and/or complex ecosystems often require large sample sizes, samples of various types and species, geographically widespread sampling, or long-term monitoring (Rosner et al. 2024). Additionally, sample analyses (i.e., detecting and quantifying toxicants and other substances within biological or environmental samples) are often expensive, complicated, destructive (to the sample), slow (low-throughput), or unsustainable (in terms of waste generation; Hussain et al. 2023). Ecotoxicology and related fields are thus continually in need of approaches that can expand, quicken, economize, or simplify sample procurement and analysis. Here, we highlight a strategy that allows ecotoxicology researchers to increase sample sizes, assemble diverse sample sets across space and time, and increase the rate of data collection, allowing for larger and broader datasets to be generated. This strategy involves pairing two distinct resources: biological collections and the latest iterations of chemical compositional analyzers.

Biological collections (which include natural history collections, herbaria, culture collections, biorepositories, etc.) represent a vast scientific resource that serves several functions (NASEM 2020). Globally, hundreds of collections contain hundreds of millions of plant, animal, and microbial specimens, most of which were previously collected for systematics or biodiversity research. These collections contain specimens that reflect the environmental conditions of the times and places in which the organisms lived, and these specimens can now help answer new questions and address contemporary problems (Suarez & Tsutsui 2004, Pyke & Ehrlich 2010, Schmitt et al. 2018, DuBay et al. 2025). Specimens that are time-stamped and georeferenced are useful for ecotoxicology and pollution studies that seek to construct long-term timelines of environmental contamination at local, regional, and continental scales in the absence of historical pollution monitoring data (e.g., Snell-Rood et al. 2024, DuBay et al. 2025). For these studies, biological collections provide a level of spatial, temporal, and taxonomic breadth and specificity that would be difficult or impossible to attain via contemporary field- and lab-based methods. For ecotoxicology and pollution research in particular, collections are rich environmental archives that can now be utilized more thoroughly given the recent advances in chemical compositional analyzers.

Chemical compositional analyzers have advanced significantly in recent decades and have been trending towards more portable designs, shorter analysis times, and fewer sample preparation requirements (Leary et al. 2016, Asif et al. 2020, Filipoiu et al. 2022). Toxicology-related fields have much to gain from these advancements. For example, portable analyzers provide options for *in situ* field and on-site measurements of toxic elements and compounds, which reduce the need for sample collection and lab work (Senesi et al. 2021, Frydrych and Jurowski 2025), as well as options for quick, high-throughput sampling and analyses (Massos & Turner 2017, Brandis et al. 2024). Spectrometric analyzers, which include infrared-, ultraviolet-, and x-ray-based spectrometers, are especially useful because many are designed to work non-destructively, without altering or damaging the samples of interest (Otsuki et al. 2023). Portable, rapid, and non-destructive analyzers, when paired with biological collections, enable widespread toxicological sampling because such analyzers can (1) be taken to

multiple collections or specimen storage locations, (2) feasibly analyze toxicants within the numerous potential samples contained within large collections, and (3) help preserve the integrity of museum specimens compared to analyzers that require sample alterations (such as tissue removals or ablations). A certain class of spectrometers known as portable x-ray fluorescence (pXRF) analyzers have all three of these features and can detect and quantify most elements within a variety of sample types (Frydrych and Jurowski 2023). For these reasons, pXRF analyzers have garnered much attention as chemical analysis tools in museum settings (Dussubieux et al. 2005, Liritzis et al. 2018), but their potential to connect toxicological research and biological collections has yet to be fully realized.

In this study, we develop a pXRF-based method for analyzing toxic metal(loid) burdens in wild birds that have been preserved as natural history specimens. This method demonstrates how elemental toxicants can be quantified in organisms over large spatial and temporal scales to address toxicological questions at ecosystem and macroecological levels. pXRF analyzers are handheld devices that readily detect toxic metal(loid)s like lead (Pb), mercury (Hg), arsenic (As), and cadmium (Cd). Here, we focus on developing methods to quantify Pb concentrations in birds, but these methods can be used to investigate other contaminants in other taxa. We focus on Pb because of its historical and global significance as a persistent, bioaccumulative, and toxic (PBT) chemical that is biologically non-essential and harmful to most exposed organisms, even at low doses (Check and Marteel-Parrish 2013, Assi et al. 2016, Raj and Das 2023). In birds and other vertebrates, Pb can bioaccumulate in many organs and tissue types (e.g., De Francisco et al. 2003, Markowski et al. 2013, Khwankitrittikul et al. 2024), but Pb is largely stored in bones, so bone Pb levels reflect chronic or lifetime Pb exposure (García-Fernández et al. 1997, Ethier et al. 2007). We focus on birds for two reasons. First, there are millions of vouchered bird specimens in natural history collections worldwide (Marini et al. 2020, Norris et al. 2024, DuBay et al. 2025), including skeletal specimens, soft tissues, eggs, and “study skins,” which are whole birds preserved by removing the axial skeleton and internal organs, and stuffing the major cavities, leaving many of the feathers, claws, and appendicular skeleton in place. Second, birds exhibit a wide range of ecologies and life-history strategies; they occur in different habitats, feed across different trophic levels, and some live in close proximity to humans, making these specimens useful for questions regarding ecosystem functionality and health in the presence of toxicants (Egwumah et al. 2017, Schmitt et al. 2018).

A key challenge in studies using pXRF technology has been repurposing the analyzers for non-standard sample types. Commercial pXRF analyzers are largely designed to analyze manufactured metals, minerals, soils, and certain consumer goods (Piorek 1997, McComb et al. 2014), whose physical properties can be accounted for during the onboard analysis of x-ray signals and the final calculations of metal(loid) concentrations However, these analyzers have been successfully repurposed to analyze many non-standard samples (e.g., food, animal tissues; McComb et al. 2014, Frydrych and Jurowski 2023) because initial pXRF readings can be corrected and refined based on the element(s) of interest and the physical properties of the sample/matrix (Rouillon and Taylor 2016, Brandis et al. 2024). In previous work, we developed methods to calibrate pXRF analyzers and correct pXRF data to quantify Pb in bone tissue, validating these methods against more established analytical techniques including K-shell x-ray fluorescence (KXRF) and inductively coupled plasma mass spectrometry (ICP-MS; Specht et

al. 2014, 2018, 2019). Yet, while the spectrometric performance of pXRF analyzers can be optimized for biological tissues and structures, standardizable protocols are still needed to make full use of natural history specimens. Here, we develop a protocol that accommodates morphological variations (bird skins vary widely in terms of size, shape, and preparation style), sample complexity (study skins are whole body preparations that include various appendages and many intricate structures), and fragility (skins can easily be preserved for over a century but are still susceptible to mechanical damage during handling). Innovative uses of pXRF and the research potential of bird specimens underscore the need for a robust, repeatable, and specimen-safe analytical protocol.

By pairing pXRF analyzers with bird collections, we develop and evaluate a protocol and analytical pipeline that is portable, cost-effective, high-throughput, non-destructive, and useful for building spatially and temporally robust datasets of environmental contamination. This method (and the general strategy that it represents) has implications for expanding the capacity of ecotoxicology research to answer pressing, large-scale, ecological and environmental questions that have previously been challenging to address.

2. METHODS

We first identified specific features of a protocol that would facilitate generating more extensive ecotoxicology datasets. This protocol has two major components: (1) the hardware and apparatuses needed to carry out the measurements, which we call the analytical setup, and (2) the instructions for the x-ray scanning process, which we call the analysis procedure. We performed a series of assessments and experiments to optimize the analytical setup (in terms of *portability*, *versatility*, *adjustability*, and *reproducibility*) and the analysis procedure (in terms of *rapidity* and *consistency*). Below, we present details of the assessments and experiments conducted using a pXRF analyzer and the bird specimen collection at the University of Texas, Arlington.

2.1. Optimizing the analytical setup

To be useful for studies involving travel to and from various collections, the entire analytical setup must be *portable*. The analytical setup includes the pXRF analyzer as well as auxiliary devices to hold the analyzer, support the bird specimens, and facilitate data collection. The analyzer we used (a Niton XL3t GOLDD+, an energy dispersive analyzer from Thermo Fischer Scientific Inc.) is portable and field-rugged by design, so we focused on assessing hardware and devices that could perform the necessary auxiliary functions but that were also lightweight, easy to assemble and disassemble, and able to be consolidated into carriable containers.

Given that the analytical setup is meant to be transported frequently and used in a wide variety of workspaces, its design must enable reliable data collection regardless of local conditions. We were most concerned about “substrate effects” which refer to how elemental readings of a target material can be skewed significantly and unintentionally by other, non-target materials underneath or in the immediate

vicinity of the analyzer's x-ray beam (Kalnicky & Singhvi 2001). We expected that bird specimens would have to be laid on flat surfaces during pXRF analysis (to avoid damage to the specimen), and we suspected that the underlying surfaces (i.e., substrates) could skew the final elemental readings of specimens, especially those of small bird specimens with thin body parts. To determine whether pXRF measurements of small bird specimens could be affected by substrate effects, we repeatedly measured bone Pb (μg Pb g^{-1} bone tissue) in a small bird skin specimen (a house sparrow; *Passer domesticus* L.) while it was directly on top of various substrates (a piece of metal shelving, wood from a countertop, and plastic-polymer from a table). Without moving the specimen or the analyzer, we repeatedly switched the type of material that was underneath the specimen until we obtained three bone Pb readings to associate with each substrate material. We also took readings while nothing underlaid the specimen (i.e., when there was about 1 m of empty space directly between the specimen and the work surface). We obtained all readings using 3-minute x-ray scans in the analyzer's "soils" analysis mode, and we corrected the Pb data to more accurately indicate bone tissue readings using the method developed by Specht et al. 2014 and Specht et al. 2018 (described in more detail below). Finally, we determined if and how the corrected bone Pb readings over empty space differed from readings taken over metal, wood, and plastic surfaces using a Kruskal-Wallis test ($\alpha = 0.05$).

Discovering substrate effects during the analysis of bird specimens would have direct implications for the final design of our analytical setup. More specifically, the analytical setup would have to ensure that no substrates or other non-target materials are close enough to the specimens to critically skew the elemental readings. In practice, other materials will always be in the general vicinity of the specimen being analyzed, so we prepared a follow-up experiment that determined how far typical substate materials must be from the analyzer's x-ray detector to dependably preclude their detection. We attempted several measurements while the analyzer was fixed at 0, 1, 5, and 10 cm above either metal, wood, plastic, or concrete surfaces (which represented surface material types that are commonly found in areas used for data collection). We attempted three measurements of each of the four material types at each of the four test heights (48 measurement attempts total), and we utilized a built-in quality control/safety function to determine if the analyzer was detecting the materials: when the material in the beam path is too far away to be reliably analyzed, the analyzer automatically aborts the analysis within a few seconds (due to an insufficient x-ray count reaching the detector). We recorded the minimum distance at which the analyzer failed to register the test materials 100% of the time, regardless of material type. We later used this information to design *versatile* analytical setups that could prevent critical substrate effects and enable users to implement the same protocol in different locations and workspaces.

For the analytical setup to accommodate a wide variety of bird specimens, it must be highly *adjustable*. Bird skin specimens vary widely in terms of shape, size, preparation style, and overall fragility. We assessed several apparatuses that could safely support various skin specimens while simultaneously allowing the user to easily reposition the analyzer to be in stable, direct or near direct contact with the sample (reliable pXRF measurements require the analyzer to be in direct or near direct contact with the sample and remain completely still during x-ray scanning; Ravansari et al. 2020). We trialed holding the

analyzer manually by its handgrip, inserting the analyzer into a brand-specific mobile test stand (Thermo Scientific Inc., part number 430 – 032), and fixing the analyzer in place with a laboratory support stand and clamp system.

After considering setup designs that could achieve *portability*, *versatility*, and *adjustability*, we lastly considered *reproducibility* or how easily the setups could be recreated by other researchers. We specifically assessed the cost, availability, simplicity, modularity, and modifiability of the analytical setup. While testing various setup designs and apparatuses, we also investigated ways to optimize the pXRF scanning procedure so that it is conducive to large-scale, collections-based ecotoxicology studies.

2.2. Optimizing the analysis procedure

Along with a suitable analytical setup, any large-scale, collections-based ecotoxicology study would also need a *rapid* data collection procedure to take full advantage of the numerous specimens found in biological collections. We determined the minimum scanning time necessary to obtain relatively precise elemental readings from complex biological samples such as bird skin specimens. Using a prototype analytical setup that properly positioned and held both the pXRF analyzer and the skin specimens, we performed replicate bone Pb measurements on ten bird specimens of various species and sizes, with total body lengths ranging from 5 to 95 cm from tip of the bill to the tip of the tail feathers. On each specimen, we targeted the midpoint of the left or right tarsometatarsus (the long leg bone directly superior to the toes). Then, without moving the specimen or the analyzer, we performed pXRF scans lasting 1, 2, 3, 4, and 5 minutes in random orders. As done earlier, we corrected the raw Pb readings to bone Pb readings (described in more detail below). Each corrected reading included a Pb concentration value (μg Pb g^{-1} bone tissue) and an uncertainty value. The uncertainty values are equivalent to two standard deviations (2σ) and reflect the precision of each reading. To determine if scanning time has a significant effect on the precision of bone Pb readings, we compared the uncertainty values from the five scanning times for each specimen using a Friedman test ($\alpha = 0.05$). We used this repeated-measures test because each bird specimen was measured five times. Then, to assess how *rapidly* pXRF measurements can be performed without sacrificing precision, we noted the scanning time after which uncertainty values begin to level off (i.e., the scanning time that balances analytical precision and efficient data collection).

We also attempted to optimize *consistency* with regards to the readings obtained from any given body part on a given specimen. We suspected that, even if an individual reading had relatively high precision, we might obtain different data when trying to replicate that measurement later. More specifically, we suspected that inconsistencies in readings could be introduced from three sources: (1) the pXRF analyzer itself, (2) bodily variations within the specimens, and (3) the human user. In the interest of developing a repeatable and standardizable method, we thus conducted tests to quantify how much measurement variation is typically produced by each of these factors.

To assess the consistency of the pXRF analyzer itself, we performed three consecutive scans on the midpoint of a tarsometatarsi on ten bird specimens. We kept the scanning time constant at 3 minutes,

and we left the analyzer and specimen completely still until all replicate measurements were complete, thereby eliminating the possibility that the consistency of the measurements was influenced by bodily variations in the specimens or by the position of the analyzer. After correcting the data for bone Pb measurements, we determined if each corrected concentration reading fell outside the uncertainty range of its other two replicate readings. A concentration reading falling within this uncertainty range suggested that the reading was indeed predictable by the replicate readings, which suggested that the reading was consistent with the others. To quantify the consistency of the pXRF analyzer, we calculated how frequently all readings were consistent with their replicate readings (as a percentage).

We also evaluated how variations within and among the specimens themselves could affect the consistency of pXRF analyses. Our standard protocol for assessing metal bioburdens in bird specimens must somehow account for the possibility that toxic metals are unevenly distributed throughout the skeleton. Our protocol must also account for the fact that specimens vary considerably in the number and types of possible scanning locations (due to bird morphology and specimen preparation style). We examined 19 bird skin specimens of various species and body sizes (**Supplementary Table 1**), and we identified 60 body locations that could be reliably scanned on at least some of the specimens. The possible targets included feathers, claws, and body sections inclusive of bone. Depending on bird morphology and preparation style, scan locations may include distinct sections of larger structures (e.g., the distal end of the tibiotarsus leg bone, or the midpoint of a feather shaft), whole structures or appendages (e.g., the entire length of the tibiotarsus or feather), or groupings of structures and appendages that were too small and close to each other to be analyzed separately (e.g., the intertwined toes of some small bird specimens). After scanning all accessible body locations on all specimens, we then determined which locations were most frequently accessible on our specimens. We also ranked Pb readings for each specimen individually and determined which locations, across all specimens, most often ranked highly in terms of Pb content. We used this information to identify the overall best scanning locations on bird specimens, i.e., the body locations that (1) were accessible on most specimens, so that exact scanning location could be standardized within or across studies, and (2) usually have a high Pb content compared to other body locations on a given specimen, so to improve our chances of detecting Pb in animals with low overall bioburdens (by avoiding locations that are more likely to have non-detectable amounts of Pb).

After determining which body locations were the most accessible for pXRF scanning and that which had among the highest Pb concentrations within specimens, we tested how consistently a human operator could target these locations (i.e., how consistently someone could replicate a measurement and obtain a reading similar to the previous readings). While operator inconsistencies may be negligible when performing straightforward pXRF analyses of simple, standard samples, it is possible that operator inconsistencies could significantly affect analyses of samples that are complex, intricate, or otherwise non-standard for pXRF work (Dussubieux et al. 2005), as would be the case for many biological specimens. To assess human operator effects, we performed additional scans on the three body locations that were consistently accessible and had relatively high Pb concentrations and thus were promising candidates as standard scanning locations: the frontal skull, the joint directly anterior to the

toes, and the joint between the tibiotarsus and tarsometatarsus (the “intertarsal joint”). We scanned these locations three times each on a subset of 10 specimens, being sure to move and reposition the scanner and specimen between each replicate measurement. We then calculated the frequency at which the corrected concentration readings fell inside the calculated uncertainty ranges of its other two replicate readings. We presumed that body locations whose pXRF readings more frequently fell inside the ranges predicted by their replicate readings were inherently more suitable for producing consistent results.

2.3. Statistical analyses

We performed all statistical tests, including the Kruskal-Wallis test to detect substrate effects and the Friedman test to judge scanning time effects, using R version 4.5.1 (R Project for Statistical Computing).

3. RESULTS & DISCUSSION

Our main objective for this study was to develop an optimized method for setting up and executing pXRF analyses on bird skin specimens. In the end, we developed a method that incorporated all the major features that can make a protocol more useful for large-scale, collections-based, ecotoxicology studies (i.e., *portability*, *versatility*, *adjustability*, *reproducibility*, *rapidity*, and *consistency*). Here, instead of describing the results of each test sequentially, we describe a finalized, stepwise process for completing pXRF measurements of bone metal(loid) concentrations in bird skin specimens. As we describe the steps in the order in which they would generally be completed, we discuss the findings of specific experiments and assessments that led us to adopt certain materials, devices, and procedures. We also discuss potential modifications to the analytical setup and procedure that might better serve different research goals.

3.1. Selecting the workstation:

We found that a complete workstation (Fig. 1a) can be set up comfortably on a variety of flat, stable surfaces within an area of about 1 m^2 (e.g., lab benches, countertops, sturdy folding tables) and in any area where specimens can be safely examined (e.g., collections areas, research labs, indoor classrooms). Proximity to electrical outlets was helpful for data collection sessions lasting more than a few hours, but this was not always necessary because pXRF analyzers and laptops can be battery-powered. For work in collections, we always used batteries and external power simultaneously to ensure that the pXRF analyzers and computers did not unexpectedly lose power during extended data collection sessions. However, the battery power options (combined with the field-rugged designs of many pXRF analyzers) make it possible to set up a complete workstation outdoors. In any case, we recommended recording the power source for each data collection session in case this affects instrument performance (Goodale et al. 2012, Ravansari et al. 2020, Johnson et al. 2024).

3.2. Setting up the analysis platform:

Supporting bird specimens on a simple, elevated platform (Fig. 1b) works well as a standard practice. The platform design we report here allows for long-distance transport, analysis of both large and small specimens, and x-ray readings that are not extraneously impacted by other nearby materials. We placed specimens on a 60 × 30 × 0.3-cm sheet of clear polycarbonate. Polycarbonate sheets are easy to clean/decontaminate, relatively easy to cut to shape, durable and strong enough to hold many types of samples and specimens, and lightweight (which enhances *portability*). However, suitable platforms could be made from other materials with different properties, especially if portability is not a concern.

Regarding size, one 60 × 30-cm sheet was often large enough to hold specimens with total body lengths less than 50 cm (hawk-size or smaller), but when analyzing larger specimens, we set up a second platform on one side of the first platform to double the total platform area. Using two medium-sized platforms instead of one large platform also enhances modularity and thus *portability* because two medium-sized platforms are easier to pack than a single large platform. However, we note that the size and number of platforms can be easily changed to fit the needs of different projects. For example, building only one small platform would be adequate for a project working solely with small bird specimens or other small samples.

We found that elevating the platform (Fig. 1b) and positioning the specimens over a small hole in the platform (Fig. 1c) was useful for avoiding the confounding effects of non-target materials near the specimen. Our tests revealed that pXRF readings of small bird specimens can indeed be skewed by materials directly underneath the specimen (i.e., by substrate effects). Bone Pb concentration readings were significantly different when different substrates (common worksurface materials) underlaid the specimen ($p < 0.001$, $H = 10.421$, $df = 3$; Fig. 2). This confirms that many of the possible scan targets on a bird specimen do not meet the assumption of “infinite thickness” or “infinite depth.” In other words, the target tissue (in this case, a leg bone) allowed some of the x-rays from the analyzer to pass through and interact with the materials beyond (in this case, common tabletop substrates). While it is possible and often feasible to correct readings from samples that are not infinitely thick and that are affected by certain substrates, it is less feasible to correct readings when the substrates are constantly changing or when the x-rays signals from the substrates overshadow the signals from the sample of interest (as would be the case when the substrates are thick, high-density objects like tabletops). Thus, for the *versatility* to work in different spaces, we opt for an elevated platform that eliminates these confoundments by positioning the specimens over adequate empty space.

To minimize the size of the platform and thus further enhance its *portability*, we determined the minimum height the platform must be to reliably preclude substrate effects. We found that our pXRF analyzer was always able to detect common substrate materials that were 1 cm away from the beam source, sometimes able to detect substrates that were 5 cm away, and never able to detect substrates that were 10 cm (Table 1). From this, we inferred that about 10 cm of empty space is needed between the bottom of specimen and the underlying worksurface to eliminate the possibility of substrate effects. We then attached six 10-cm legs to the undersides of the polycarbonate sheets (Fig. 1b). We made the legs from 28-mm diameter wooden dowels, which were lightweight and easy to cut to length. We

inserted the top of each leg into a 100-mL cylinder bumper which was taped to the underside of the polycarbonate sheet (Fig. 1b). The bumpers provide fixed insertion points that hold the tops of the legs in place. We inserted the bottom of each leg into a plastic graduated cylinder base made for 100-mL graduated cylinders to form a stable footing for the leg (Fig. 1b). Temporarily taping the cylinder bases to the worksurface substantially increased the stability of platform.

Table 1
Detection frequencies of different substrate materials by the pXRF analyzer at different distances from the analyzer's x-ray beam source and x-ray detector. While the analyzer was fixed at specified heights above the materials, we attempted 3-minute pre-programmed x-ray scans of the materials. When the fluorescent x-ray signals from the material are too weak for analysis, the analyzer automatically aborts the analysis after a few seconds of scanning. Shown are the instances in which the material was successfully detected (analysis completed) or not detected (analysis aborted)

Substrate Material Type	**Replicate Number**	**Distance between analyzer and substrate material**			
		0 cm	**1 cm**	**5 cm**	**10 cm**
Wood	1	Detected	Detected	Detected	Not detected
Wood	2	Detected	Detected	Detected	Not detected
Wood	3	Detected	Detected	Detected	Not detected
Concrete	1	Detected	Detected	Detected	Not detected
Concrete	2	Detected	Detected	Not detected	Not detected
Concrete	3	Detected	Detected	Not detected	Not detected
Benchtop	1	Detected	Detected	Not detected	Not detected
Benchtop	2	Detected	Detected	Not detected	Not detected
Benchtop	3	Detected	Detected	Not detected	Not detected
Metal	1	Detected	Detected	Not detected	Not detected
Metal	2	Detected	Detected	Not detected	Not detected
Metal	3	Detected	Detected	Not detected	Not detected
Detection Frequency		**100%**	**100%**	**33.3%**	**0%**

To eliminate possible substrate effects from the polycarbonate platform itself, we cut holes in the platform over which to center the target area of the specimen (Fig. 1c). We usually positioned bird skin specimens over a 57-mm diameter hole that we cut out of the polycarbonate using a drill with a hole saw bit (Fig. 1c). Just as the height of the platform created sufficient empty space between the analyzer and the worksurface, this hole provided sufficient empty space around the target area of the specimen while keeping the rest of the specimen fully supported from beneath. However, we often positioned specimens that were sparrow-sized or smaller over a 38-mm diameter hole (cut out of the same sheet and several centimeters away from the first hole) so that the specimen would not fall through the hole or suffer undue physical strain when we placed the pXRF unit on top of the specimen (Fig. 1c). In either case, this setup allowed consistent measurements in a variety of workstations and atop a variety of worksurfaces, thereby achieving *versatility*.

It is important to note that a holed, elevated platform may not be necessary when scanning some specimens. Some target areas on some bird specimens (e.g., large specimens of cranes, pelicans, and vultures) may already meet the assumption of infinite thickness and/or may sit several centimeters above the worksurface when laid down. Yet, in cases like these, it is important to consider the specific sample type (surface area, thickness, and density), platform type (material composition, thickness, proximity to sample), and data correction methods. If researchers want to analyze specimens that are directly on top of or contained by other materials, it is advisable to use materials that are not readily detected by pXRF analyzers, such as low-density pieces of polystyrene (*e.g.*, Styrofoam) and thin pieces polyethylene terephthalate (e.g., Mylar film; Zambito et al. 2016). Otherwise, in the interest of standardization, we recommend analyzing samples in a way the eliminates or nearly eliminates the possibility of signal interference from substrates or other non-target materials, as is the case with our holed, elevated platform.

3.3. Calibrating, positioning, and stabilizing the pXRF analyzer:

We used a pXRF model that has been previously shown to be suitable for the measurement of metal burdens in biological samples, including avian bone samples – a Niton XL3t GOLDD+ analyzer (Specht et al. 2018, Fig. 1a). Prior to bird specimen analyses, we set the analyzer to the “soils” analysis mode, which uses a tube voltage of 50 kV, current of 40 μA, and a silver-iron filter combination. Given our specific interest in Pb, we set the device to only measure a small set of elements including Pb. That is, we only selected “Main Range” elements for analysis, using the “Elemental Range” options in the “Advanced” settings (other sets of elements can be analyzed in addition to or instead of the “Main Range” set, but this was unnecessary for this study and would often require additional time for scanning).

Then, we calibrated our pXRF analyzer to account for how our unique instrument detects our pollutant of interest (Pb) in solid, bone-like samples, following Specht et al. (2014). In brief, we recorded readings and spectral data from our instrument during calibration scans of Pb-doped bone “phantoms,” which were composed of Plaster of Paris and ranged between 0 and 100 ppm Pb. We also ran calibrations to

account for the effect of skin by measuring the bone phantoms while they were covered with different thicknesses of lucite. Plaster of Paris and lucite effectively mimic the XRF spectral signal and x-ray attenuation effects of bone and skin, respectively (Specht et al. 2014). We performed these calibrations before analyzing bird specimens, but it is useful to note that these calibrations can be done post hoc as long as the original readings do not contain critical amounts of erroneous signal interference.

While pXRF devices are mostly designed for handheld operation and can take measurements in a wide variety of orientations and configurations, we found it most useful to position our pXRF analyzer above the specimen (with the scanning apparatus and x-ray beam pointing down; Fig. 1a). This way, we could gently pin the study specimen between the platform and analyzer, thus ensuring that x-ray beam source is positioned within a few millimeters of the targeted body part (which is critical for reliable pXRF measurements). We found that simply laying a skin specimen on top of the analyzer's x-ray source made it difficult to see the legs and position them close enough to the x-ray beam source. Furthermore, pointing the open x-ray beam down toward the tabletop and centering the analyzer over a wide, solid platform helps mitigate risks associated with using x-ray producing devices because the beam is never directed at the operator nor is it likely that the operator will ever come between the x-ray beam source and the tabletop or be within range of diffracted beams or scatter/leaked radiation (as long as the operator's legs are not kept under the table or worksurface). However, it should be noted that pXRF dosimetry studies show that the radiation exposure risk are low and well within safe limits of exposure (Rouillon et al. 2015), so the risks associated with indirect irradiation are inherently low. If radiation shielding is desired, panels can be added under and to the sides of the analysis platform.

Keeping the analyzer and the specimen still during measurements was also critical, so we fixed the analyzer above the specimen using a laboratory support stand system (Fig. 1a). We simply duct taped exercise weight plates to the base of the support stand so that the total weight was increased to about 5 kg. We fastened a heavy-duty 3-pronged clamp to the support rod using swivel-type clamp holder, and then we closed the clamp prongs around the handgrip of the analyzer. In this configuration, the weight of the analyzer and its battery was counterbalanced by the support base (so that it does not tip over), and we could freely raise, lower, rotate, and tilt the analyzer, as well as move the entire stand (thus achieving *adjustability*). Simply re-tightening the clamps after each adjustment and before the scanning process ensured that the analyzer did not move or rest its weight on the specimen during the analysis.

On the Niton XL3t GOLDD+ pXRF analyzer model, the part that contacts the sample (where the x-ray beam source and detector are housed, sometimes the called the "nose") is broad and flat because it was largely designed for analyzing bulk materials with simple geometries and large and/or flat surfaces. This design is admittedly not ideal for measuring bird skins, which can be geometrically complex and can contain many small, intricate structures. However, because the support stand system was highly *adjustable*, we could normally position the scanning apparatus well within 5 mm of the target tissues (e.g., of the legs, feet, head, bill, or tail feathers), which along with stillness, is critical for reliable pXRF measurements. We acknowledge that pXRF units could be held more efficiently by more sophisticated or

custom-made equipment, but standard laboratory support stand systems are cheap, readily available, and familiar to many researchers, thereby enhancing *reproducibility*.

We also confirmed that, using the laboratory support stand and clamp system, the analyzer could easily be repositioned to sit under the platform with the x-ray beam pointing up. This configuration requires the platform to be raised from 10 cm to about 30 cm above the worksurface (either by stacking objects under the 10-cm platform legs or using longer platform legs), but again, we do not recommend this configuration when analyzing whole study skins. We only recommend using this setup when analyzing samples that can be laid flush on top of the scanning apparatus (e.g., disarticulated bones, bone fragments, whole eggshells, eggshell fragments, or other samples that can be manipulated into multiple positions). Overall, the laboratory support stand system was highly *adjustable*, and helped us comfortably analyze intricate structures on a wide variety of bird specimens while also achieving stability and avoiding damage to all specimens. Holding the pXRF unit by hand is another option, but a mechanical holder of some type will usually be necessary for projects that require precise targeting (of small anatomical structures), relatively long scan times, and/or numerous measurements (because holding the analyzer manually still for extended periods of time is not usually feasible). Also, we do not recommend using the mobile test stand (Thermo Scientific Inc., part number 430 – 032) for bird specimens because most specimens will not fit in the sample chamber, and the chamber itself may introduce substrate effects if the bird specimens do not meet assumptions of infinite thickness.

3.4. Positioning the bird specimen and targeting specific body locations:

The last major consideration before executing the x-ray scan is which body part(s) to target. During normal operation, the Niton XL3t GOLDD+ analyzer can scan an area as small as 3 mm^2, so theoretically, many discrete parts of a bird specimen can be scanned separately. Although, in practice, the scanning process is impacted by several factors, including how the analyzer is put into contact with the specimen and where the target tissues are located on the specimen. By scanning all available body locations on a wide variety of bird skin specimens using the methods described above, we learned which body locations and tissue types are the most amendable to pXRF analysis. Soft tissues and body fluids are generally absent from study skin specimens, but we found that, on many specimens, at least some of the claws, feathers, and bones can be scanned successfully. For future studies, the type of tissue(s) and the exact body part(s) being scanned should reflect the project goals. Claws can be scanned somewhat easily on large bird specimens (hawk-sized or larger) or using disarticulated remains. Yet, when using a portable analyzer, it is usually difficult to precisely scan claws on smaller bird specimens and on specimens whose toes are curled or bunched together. Also, while feathers cover most of the body on bird specimens, relatively few feathers can be analyzed discretely. Body and wing feathers are not usually amenable to scanning because they are usually overlaying other materials. Feathers are often thin, low-density, and protein-based, so their already weak x-ray signatures will often be critically overshadowed by underlying materials (i.e., skin, bones, materials used to stuff the body cavities during specimen preparation). The exceptions are the distal portions of the tail feathers, which are often easy to

scan when they extend beyond the feet and can be scanned separately from all other tissues. The same is true for tail and wing feathers that have been spread out or separated from the body, but this is not the case for most skin specimens. For the current study, we decided that targeting bone tissue is best because our test analyte, Pb, is largely stored in the bones of birds (Garcia-Fernandez et al. 1997, Ethier et al. 2007), and scanning bones on skin specimens presents many practical challenges, thus forcing us to develop a more robust methodological starting point that can be scaled back for future studies. Several remnant bones are present in typical skin specimens, so we systematically determined which body locations should be prioritized for bone Pb analysis and which locations should be avoided.

We found that remnant bones in the wings, neck, and torso are usually not scannable because they are difficult to visually pinpoint and/or difficult to scan separately other body parts. In other body regions including the head, bill, legs, and feet, we identified 56 body locations inclusive of bone that could be scanned on at least some of our bird specimens. By scanning all available locations on each specimen, we first found that Pb concentrations vary throughout individual skeletons and within individual bones (Fig. 3, **Supplementary Table 2**), which is consistent with previous bone Pb studies (e.g., Cretacci and Parson 2010, Pemmer et al. 2013, Ishii et al. 2018). This uneven distribution of Pb between and within bones reflects the complex ways in which vertebrates store Pb throughout their bodies, and it suggests that there is no simple way of characterizing Pb body burdens, regardless of the analytical methods being used (Barbosa et al. 2005). Therefore, we recommend different sampling strategies depending on the project goals and designs. When conducting pXRF studies that are more limited in scale and scope, we recommend completing multiple scans on each specimen to characterize their bioburdens more completely. Of course, multiple measurements on irreplaceable specimens are only advisable with non-destructive techniques like pXRF—destructive measurements should be limited on specimens. For larger-scale and broader-scope studies in which it would be difficult to obtain multiple measurements from every specimen, we recommend targeting one body location that is most likely to yield reliable and pertinent information about bioburdens (*sensu* Hammond et al. 1992). To this end, we assessed all 56 body locations inclusive of bone in terms of (1) how *consistently* each location can be scanned with a pXRF device, and (2) how each location ranked in terms of Pb readings.

We used multiple tests to determine which body sections inclusive of bone could be scanned most *consistently*. We first confirmed that the analyzer itself was consistent. When we kept the analyzer and specimen completely still and performed three consecutive measurements of bone Pb in the tarsometatarsi in a diverse, representative subset of bird specimens (n = 10), the Pb readings were consistent 98.8% of the time. This indicates that, even when scanning non-standard samples, the analyzer is highly consistent if basic scanning requirements are met and if scan parameters are kept constant. Next, we determined which body parts were most consistently accessible on typical bird specimens. We found that relatively few body locations could be consistently accessed (i.e., put in proper contact with the analyzer's scanning apparatus on at least 90% of specimens in our representative subset of 20 specimens; Table 2). We noticed that the accessibility of certain scanning locations was greatly influenced by preparation style and morphology (body size). Specimens that were prepared so that the legs are mostly laid flat and are not tightly bunched together have more scannable

body sections than specimens that were prepared more erroneously. Furthermore, when scanning locations on the legs, scanning was generally easier on whichever leg was crossed and fixed on top during specimen preparation (the tarsometatarsi are generally crossed one over the other and tied together with string for added stability). Our bird specimens usually had their left legs crossed on top, which explains why locations on the left legs were more likely to be accessible for scanning (Table 2). Regarding morphology, it is generally easier to target many specific sections or structures on larger birds, simply because these parts are larger and there is more space between them. For example, on large specimens, it is possible to separately scan the distal, proximal, and middle *sections* of long bones or to scan a *single* toe. Yet, with smaller birds, it may only be feasible to have more generalized scan targets (e.g., the left tarsometatarsus *as a whole* or a *whole* foot). Due to these patterns and constraints, we calculated the most consistently scannable body locations to be the frontal skull, the joint directly anterior to the toes, the point at which the tarsometatarsi are crossed, and the intertarsal joint. We then determined which body part among these four parts can be most consistently scanned by a human operator.

Table 2

Assessment of possible pXRF scan targets on bird skin specimens. Possible scan targets were body sections that were inclusive of bone (to enable bone Pb measurements). Using a representative subset of 19 bird specimens of various species and morphologies, we calculated the overall likelihood that a body section will be available for proper scanning (i.e., the percentage of specimens on which that body section, as described, could successfully be put in precise, stable, and direct contact with the analyzer's scanning apparatus). We also calculated the overall likelihood that a body section will yield a relatively high Pb reading compared to other sections on a given specimen (i.e., the percentage of times the body section's Pb concentration ranked in the top 25% of all successful Pb readings for a given specimen). Shown are the 25 body locations (out of 56 total locations) that ranked most highly in accessibility and relative Pb concentrations. Percentages are rounded to the nearest whole number.

Body location	**Likelihood of being accessible for proper scanning (%)**	**Likelihood of getting a relatively high Pb concentration reading (%)**
left intertarsal joint	95	68
Joint connecting left phalanges	89	42
left stacked phalanges	89	42
Forehead	100	42
Joint connecting right phalanges	89	37
distal left tibiotarsus	63	26
right intertarsal joint	95	26
right stacked phalanges	89	26
tip of upper and lower bill	47	21
base of upper and lower bill	37	16
proximal left tarsometatarsus	42	11
proximal half of left tarsometatarsus	47	11
proximal right tibiotarsus	21	11
upper and lower bill	42	11
distal-mid left tibiotarsus	11	5
proximal-mid left tarsometatarsus	11	5
Crossing of tarsometatarsi	100	5

Body location	Likelihood of being accessible for proper scanning (%)	Likelihood of getting a relatively high Pb concentration reading (%)
midpoint left tarsometatarsus	26	5
distal half of left tarsometatarsus	32	5
left single phalange	16	5
feet together	11	5
proximal-mid right tibiotarsus	11	5
distal right tibiotarsus	58	5
proximal half of right tarsometatarsus	53	5
midpoint right tarsometatarsus	16	5

We initially suspected that the operator can heavily influence pXRF results (Dussubieux et al. 2005) and that some body parts would be easier to scan in a consistent way than other body parts. We found it easiest to replicate Pb readings of the intertarsal joints. When we scanned the intertarsal joints, bone Pb concentrations measured independently on three separate occasions were most likely to be consistent (i.e., 70% of the time, the bone Pb measurement fell within the uncertainty ranges of the replicate measurements; Table 2). Replicate measurements were much less consistent for measurements of the frontal skull (30% consistent), the joint connecting the toes (20%), and the crosspoint of the tarsometatarsi (5%). We attribute the greater consistency in the intertarsal joint measurements to how easily this structure can be visually pinpointed. Using the analyzer's on-board video camera and anatomical landmarks on the joint, an operator can more precisely position the center of the intertarsal joint in the path of the x-ray beam, which we determined for our instrument to always fall in the upper-right quadrant of the camera's field of view (Fig. 4). We recommend that x-ray beam path of each pXRF device be mapped before commencing a new study (perhaps using x-ray paper or systematically testing how the instrument detects smalls objects in various locations within the normal field of view). X-ray beam mapping is likely unnecessary for large, homogenous samples with broad, flat surfaces, but it is important when the study involves challenging, non-standard samples (such as many biological specimens). Yet, while intertarsal joints are inherently easier to analyze in a consistent way, perfectly replicating an x-ray scan, in terms of the angles and distances between the analyzer and specimen, is not possible to do manually. This source of user error, combined with the minor but unavoidable uncertainty associated with probabilistic XRF readings and the fine-scale variations in Pb content that exist within bones (Cretacci and Parson 2010, Ishii et al. 2018), likely explain why our measurement consistency did

not exceed 70%. Still, our results show that measurement consistency and reproducibility can be greatly improved by developing precise, standardized protocols around the inherent features of the specimens.

In addition to assessing how consistently that a body location can be scanned with a pXRF device, we also assessed how each location ranked in terms of Pb readings within an individual specimen. We found that intertarsal joints often had high Pb concentrations compared to other skeletal locations on a given specimen (Table 2), and this may further justify targeting this body location over other locations. High concentrations of Pb in this location generally aligns with previous studies of Pb bioaccumulation which found that Pb tends to accumulate more near the ends of long bones (i.e., the epiphyses; Cretacci & Parsons 2010, Ishii et al. 2018), and the intertarsal joint we targeted includes in the distal epiphysis of the tibiotarsus and the proximal epiphysis of the tarsometatarsus. Relatively high Pb deposition in these joints may also be explained by higher proportions of trabecular bone and/or residual bone marrow, which, during life, may receive more Pb from circulating blood compared to more slow-growing cortical bone (Cretacci & Parsons 2010, Ishii et al. 2018). However, we did not measure the different types of bone tissue in the intertarsal joints in our specimens. Regardless of the reasons that intertarsal joints rank highly in terms of Pb content, the relatively high concentrations of Pb in these joints suggest that specifically targeting these joints can increase the likelihood of successfully detecting Pb in specimens with otherwise low bioburdens.

However, we noticed that no body part was perfect in terms of their availability on specimens, relative Pb content, or the ease at which consistent analyses can be performed. In this study, we encountered specimens that had many feathers directly on the intertarsal joint or had flank feathers covering the joints. This adds some difficulty to the process, but in most cases, we could simply move feathers out of the way of video camera or position the analyzer on the joint by feel. In either case, feathers and skin overlaying or underlaying the joint generally do not critically disrupt underlying bone measurements (Specht et al. 2019; *see also* NIST*a and* NIST*b for attenuation coefficients for bone and soft tissue).* In the rare cases in which we were unable to target an intertarsal joint, we alternatively targeted the other intertarsal joint, the point where the tarsometatarsi were crossed, or the joint where the toes meet (at the distal end of the tarsometatarsus). In future projects, decisions about scanning locations may be influenced by concerns that pXRF irradiations may interfere with other uses of the specimens, such as providing tissues for DNA analysis. While it should be noted that these most XRF analyses do not impart enough radiation to affect the recovery of even degraded ancient DNA (Fehren-Schmitz et al. 2016, Immel et al. 2016), we still recommend that all future projects carefully consider the model-specific features of their analyzer, the morphology of their specimens, and all potentially competing research goals.

3.5. Executing the pXRF analyses:

While the pXRF analyzer can be operated manually with the pull trigger on the handgrip, we remotely controlled the analyzer from a laptop to keep the analyzer still and make use of a function that allowed us to pre-program the exact duration of the analysis. We connected the analyzer to the PC laptop via a USB-mini data cable and controlled the analyzer with the free proprietary Niton software (Niton XL2/XL2

Plus/XL3/DXL- NDT/NDTr PC Companion Software Version 8.5.2.22). We conducted all bone scans in the analyzer's "soils" mode targeting "main range" elements, which programs the instrument to quantify 18 elements, including Pb, and is the same mode in which we calibrated the instrument for bone analysis (*see above*).

To assess metal burdens in avian bones, we perform x-ray scans lasting three minutes (180 seconds). While longer scan times may produce more precise pXRF data for some sample types (Zhang et al. 2021), our tests showed that, for bird specimens, 3-minute scans allowed the analyzer to achieve adequate measurement precision (as measured by relatively small uncertainty values; Fig. 5). The leveling off in measurement quality after 3 minutes aligns with previous pXRF research on other sample types (Newlander et al. 2015). Of equal importance, 3-minute scans allow numerous samples to be analyzed over the course of a day, which is critical for projects attempting to make full use of large biological collections. Members of our research team, working alone and with one analyzer, can typically analyze between 25–50 skins in a normal workday. Our sampling rates were increased when another team member and/or analyzer was added to the workflow, when working with specimens that are inherently easier to scan (*see* Section 3.4), and when working with a series of similar specimens (which means fewer adjustments are needed between specimens). Sampling rates were slowed when we constantly needed to make large adjustments between specimens, when we needed to collect additional data about each specimen (e.g., morphological or life stage information), when collections were less organized (physically or digitally), and when specimens were less accessible or conveniently stored. Overall, considering most of the factors that affect data collection, 3-minute scans were an acceptable compromise between analytical precision and faster sampling rates (*rapidity*).

While scans lasting three minutes are common in pXRF studies to date, it should be noted that, even for ecotoxicology studies, shorter scans may be sufficient in some cases while longer scan times may be necessary in other cases, depending on the sample type, analyzer capabilities, and project goals (Brandis et al. 2024). Yet, our general advice for large-scale or novel ecotoxicology studies is to be cautious of scan times much shorter than three minutes (e.g., 60 seconds) because these may be insufficient for highly variable, non-standard samples. We also advise that longer scan times should not be immediately disregarded because even scans lasting several minutes will still be substantially quicker than other element analysis methods such as ICP-MS and atomic absorption spectroscopy (AAS; Frydrych and Jurowski 2023).

During or after a scan was complete, we recorded the analysis number (automatically displayed by the analyzer), identifying information for the specimen (e.g., collection catalog number), and the exact location of the scan (e.g., left intertarsal joint, right intertarsal joint). Also, while not always necessary, we recorded the preliminary ("raw") measurement for at least one of the metals of interest (in ppm or as "below limit of detection"). These measurements are displayed automatically on the analyzer when the analysis is finished, and it provides an additional data point to later connect the results to a particular specimen. Keeping track of some raw data also helps operators notice trends among multiple samples and notice erroneous or suspicious readings (which likely need to be repeated). Lastly, we recorded

additional information whenever we had to deviate from the standard protocol or when we encountered difficulties or abnormalities associated with the specimen.

3.6. Correcting the raw data to accurately reflect metal concentrations in bones:

After completing the x-ray scanning, we downloaded the data from the pXRF and corrected each Pb measurement to more accurately estimate bone Pb concentrations. We used a previously validated data correction method that involves analyzing the full spectral data of each measurement, and fitting the spectral peaks associated with Pb with a Gaussian peak and an exponential background (Specht et al. 2014). The final corrected data were bone Pb concentrations for each bird specimen in ppm along with uncertainty values (95% confidence intervals). We performed all data corrections and peak fitting using MatLab. This same type of correction can be applied to other metals after the appropriate calibrations are performed, so this method is not restricted to Pb.

4. CONCLUSIONS AND FUTURE DIRECTIONS

We describe here how pairing advanced chemical analyzers and biological collections is a strategy that can expand the scale, scope, and ecological relevance of ecotoxicology studies, which is urgently needed if the field is to keep pace with the mounting environmental and health issues associated with chemical toxicants (Artigas et al. 2012, Franco 2025). We present a pXRF-based method to measure metal(loid) bioburdens in a common type of museum specimen, bird skins. This unlocks the enormous potential of natural history collections worldwide to help address a variety of pressing ecotoxicology questions. Our method builds on previous studies that have validated the use of pXRF to quantify metal bioaccumulation in vertebrate skeletons (e.g., Specht et al. 2018). Like these studies, our method leverages the important features of pXRF elemental analyzers, namely their rugged and portable design, safe and cost-effective operation, and rapid and non-destructive sample analysis. Using our suggested hardware and procedural recommendations, researchers can: (1) easily recreate and transport the analytical setup, (2) avoid confounding factors that might otherwise affect sensitive elemental analyses, (3) accommodate diverse samples housed in large biological collections, and (4) generate extensive and reliable toxicological datasets that have been previously costly and time consuming to generate. Also, while we focused on developing a protocol for measuring bone Pb levels using a single location on the legs of bird specimens, we show that this protocol can be easily modified to meet other research goals.

Our analytical setup and analysis procedure can be modified to investigate different metals, tissue types, and taxa, and can be used in various settings. In our study, we set our analyzer to measure concentrations of 18 metals and metalloids, including other potentially toxic elements (e.g., tungsten, uranium) as well as biologically essential elements (e.g., iron, zinc), which highlights how additional elements besides Pb could be studied along with multi-metal profiles, elemental fingerprints, and “metallomics” (Purwadi et al. 2022, Frydrych and Jurowski 2025). However, under different settings and with varying levels of sensitivity, our analyzer model can measure up to 33 elements, including other

“heavy metals” like cadmium (Cd) as well as relatively light elements like sulfur (S), potassium (K), and calcium (Ca). In any case, obtaining reliable absolute values of elemental concentrations is contingent on proper instrument calibration, optimizing the analytical settings, and effective data correction procedures and pipelines, which must be considered for each unique analyzer and sample set. Furthermore, irremediable contamination, such as arsenic or mercury preservatives (which were historically applied to some biological specimens) may preclude or complicate measuring the bioaccumulation of those elements (but measuring those contaminants via pXRF can be useful for other purposes, like safety and provenance testing; Block et al. 2007, Cramer et al. 2023). Study skins, like the bird specimens that we analyzed, present other tissue types for testing as well, including feathers and claws, some of which can be isolated for pXRF scanning, depending on their size, morphology, and preparation. In addition, zoological specimens other than birds and botanical specimens may be amenable to pXRF analysis (Purwadi et al. 2022, Brandis et al. 2024). The general methodology that we propose here could also be applied to collections other than formal natural history collections, like informal, incidental, crowdsourced, project-specific, and personally-maintained archives as well as specimens kept for non-research purposes, as done by veterinary hospitals, rehabilitation centers, governmental agencies, and regulatory bodies (e.g., Mukhtar et al. 2020, Pay et al. 2021, Mastick et al. 2024). Lastly, thinking beyond collections and preserved specimens, previous studies have demonstrated the feasibility of *in vivo* pXRF analysis of live animals (Grier et al. 2025) and plants (Montanha et al. 2020), and it is possible to incorporate pXRF tissue analysis into field studies and live wildlife studies.

Strategically pairing biological collections and advanced chemical analyzers can bolster a wide variety of ecotoxicology studies. For example, organismal studies looking at metal exposures, bioburdens, and bioaccumulation can be investigated directly when our method is applied to bird skins or other x-ray scannable specimen types, but investigating macro-scale processes and higher levels of biological organization will also be possible. Population-level studies regarding metal pollution and avian reproductive success, demographic trends, and/or migration (e.g., Hanley et al. 2022, Slabe et al. 2022, Hitt et al. 2023) would benefit from large, species-specific “series” housed in collections that, in many cases, reflect certain populations over several generations. Analyzing metal bioburdens in specimens of several species from within a defined area could help reveal community-level processes, such as biotransferance, biomagnification, biodilution, or food web alterations as well as allow for inter-species and inter-guild comparisons of pollution exposure and responses (based on ecological traits and life-history strategies). This would be especially useful for Pb research as relatively little is known about how Pb affects higher-level ecological processes, such as population declines and trophic transfers (Pain et al. 2019, Saidon et al. 2024). Ecosystem-level processes such as bioconcentration of contaminants by birds from water, soil, etc. would be feasible with additional analyses of environmental samples or with preexisting data. Linking metal bioburdens in animals to certain ecosystem functions and services like seed dispersal, pest control, and nutrient cycling would also be possible with additional data. With pXRF analyzers, toxicological data could be easily attached to a large proportion of the world’s bird specimens,

thereby enabling studies involving historical metal pollution timelines, pollution mapping, and environmental and public health evaluations.

DECLARATIONS

Ethics Approval:

No ethical approval was required; no live animals were used during this study or euthanized for this study.

Consent to Participate:

Not applicable; no human subjects were involved in this study.

Consent to Publish:

Not applicable; no human subjects were involved in this study.

Competing Interests:

Dr. Aaron Specht is an Editor of ESPR but did not have an active role in the submission. The authors declare no other conflicts of interest.

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Author Contributions:

Conceptualization: Jordan Brown, Shane DuBay, Aaron Specht; Methodology: Jordan Brown, Melissa Salazar, Shane DuBay, Aaron Specht; Formal analysis and investigation: Jordan Brown, Melissa Salazar, Baiyinah Abdullah, Shane DuBay; Writing - original draft preparation: Jordan Brown; Writing - review and editing: Jordan Brown, Shane DuBay, Aaron Specht, Melissa Salazar, Baiyinah Abdullah; Funding acquisition: Jordan Brown, Shane DuBay; Resources: Shane DuBay; Supervision: Jordan Brown, Shane DuBay

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Data availability Statement:

The manuscript has associated data included in the body of the article and supplementary tables.

REFERENCES

1. Assi MA, Hezmee MNM, Haron AW, Sabri MYM, Rajion MA (2016) The detrimental effects of lead on human and animal health. Vet World 9(6):660–671. https://doi.org/10.14202/vetworld.2016.660-671
2. Asif AKM, Abdur Razzaque A, Zayedul Hasan M (2020) A brief overview of different analytical techniques for material and chemical analysis. Int J Instrum Technol 7(1):1–12. https://doi.org/10.5923/j.instrument.20200701.01
3. Beketov MA, Liess M (2012) Ecotoxicology and macroecology – time for integration. Environ Pollut 162:247–254. https://doi.org/10.1016/j.envpol.2011.11.011
4. Brandis KJ, Francis RJ, Zawada KJA, Hasselerharm CD, Ramp D (2024) Advancing the application of pXRF for animal samples. PLoS ONE. https://doi.org/10.1371/journal.pone.0297830
5. Chapman PM (2002) Integrating toxicology and ecology: putting the “eco” into ecotoxicology. Mar Pollut Bull 44:7–15. https://doi.org/10.1016/S0025-326X(01)00253-3
6. Check L, Marteel-Parrish A (2013) The fate and behavior of persistent, bioaccumulative, and toxic (PBT) chemicals: examining lead (Pb) as a PBT metal. Rev Environ Health 28(2–3):85–96. https://doi.org/10.1515/reveh-2013-0005
7. Cretacci Y, Parsons PJ (2010) Localized accumulation of lead within and among bones from lead-dosed goats. Environ Res 110(1):26–32. https://doi.org/10.1016/j.envres.2009.09.005
8. De Francisco N, Ruiz Troya JD, Agüera EI (2003) Lead and lead toxicity in domestic and free living birds. Avian Pathol 32:3–13. https://doi.org/10.1080/0307945021000070660
9. DuBay SG, Weeks BC, Davis-Kean PE, Fuldner C, Harris NC, Hughes S, O’Brien B, Perkins M, Weyant C (2025) Measuring historical pollution: Natural history collections as tools for public health and environmental justice research. *Proc. Natl. Acad. Sci. USA* 122(24):e2403781122. https://doi.org/10.1073/pnas.2403781122
10. Dussubieux L, Pinchin SE, Tsang JS, Tumosa CS (2005) Non-destructive elemental analysis: reliability of a portable x-ray fluorescence spectrometer for museum applications. 14th Triennial Meeting, ICOM, The Hague, 2005, pp. 766–773. Preprints Volume II
11. Eggen RIL, Behra R, Burkhardt-Holm P, Escher BI, Schweigert N (2004) Challenges in ecotoxicology. Environ Sci Technol 38(3):58A–64. https://doi.org/10.1021/es040349c. A
12. Egwumah AF, Egwumah PO, Edet D (2017) Paramount roles of wild birds as bioindicators of contamination. Int J Avian Wildl Biol 2(1):194–200. https://doi.org/10.15406/ijawb.2017.02.00041

13. Ethier ALM, Braune BM, Scheuhammer AM, Bond DE (2007) Comparison of lead residues among avian bones. Environ Pollut 145(3):915–919. https://doi.org/10.1016/j.envpol.2006.05.001
14. Fehren-Schmitz L, Kapp J, Ziegler KL, Harkins KM, Aronsen GP, Conlogue G (2016) An investigation into the effects of x-ray on the recovery of ancient DNA from skeletal remains. J Archaeol Sci 76:1–8. http://dx.doi.org/10.1016/j.jas.2016.10.005
15. Filipoiu DC, Bungau SG, Endres L, Negru PA, Bungua AF, Pasca B, Radu AF, Tarce AG, Bogdan MA, Behl T, Nechifor AC, ul Hassan SS, Tit DM (2022) Characterization of the toxicological impact of heavy metals on human health in conjunction with modern analytical methods. Toxics 10:716. https://doi.org/10.3390/toxics10120716
16. Frydrych A, Jurowski K (2023) Portable X-ray fluorescence (pXRF) as a powerful and trending analytical tool for in situ food samples analysis: A comprehensive review of application - State of the art. Trends Anal Chem 166:117165. https://doi.org/10.1016/j.trac.2023.117165
17. Frydrych A, Jurowski K (2025) Direct, rapid, non-destructive and 'white' multielemental toxicological analysis of hazardous elements with health risk assessment in candies and wrappers from Polish flea markets using portable XRF spectroscopy. Food Chem Toxicol 196:115208. https://doi.org/10.1016/j.fct.2024.115208
18. García-Fernández AJ, Motas-Guzmán M, Navas I, María-Mojica P, Luna A, Sánchez-García JA (1997) Environmental exposure and distribution of lead in four species of raptors in Southeastern Spain. Arch Environ Contam Toxicol 33(1):76–82. https://doi.org/10.1007/s002449900226
19. Goodale N, Bailey DG, Jones GT, Prescott C, Scholz E, Stagliano N, Lewis C (2012) pXRF: a study of inter-instrument performance. J Archaeol Sci 39(4):875–883. https://doi.org/10.1016/j.jas.2011.10.014
20. Grier T, Costa SS, Faber B, Cooper S, Dean K, Martin M, Jackson BP, Katzner TE, Specht AJ (2025) Feasibility and accuracy of In Vivo XRF bone lead assessment wild birds: An example with black vultures, Coragyps atratus. J Trace Elem Med Biol 91:127730. https://doi.org/10.1016/j.jtemb.2025.127730
21. Hammond PB, Jones MM, Succop PA, Singh PK, Basinger MA (1992) Validity of femur and tibia as surrogates for total skeletal burden of lead. Toxicol Methods 2(3):169–176. https://doi.org/10.3109/15376519209066103
22. Hanley BJ, Dhondt AA, Forzán MJ, Bunting EM, Pokras MA, Hynes KP, Dominguez-Villegas E, Schuler KL (2020) Environmental lead reduces the resilience of bald eagle populations. J Wildl Manage 86:e22177. https://doi.org/10.1002/jwmg.22177
23. Hitt LG, Khalil S, Blanchette A, Finkelstein ME, Iverson ENK, McClelland SC, Ribeiro RD, Karubian J (2023) Lead exposure is correlated with reduced nesting success of an urban songbird. Environ Res 227:115711. https://doi.org/10.1016/j.envres.2023.115711
24. Hussain CM, Hussain CG, Keçili R (2023) White analytical chemistry approaches for analytical and bioanalytical techniques: applications and challenges. Trends Anal Chem 159:116905. https://doi.org/10.1016/j.trac.2022.116905

25. Immel A, Le Cabec A, Bonazzi M, Herbig A, Temming H, Schuenemann VJ, Bos KI, Langbein F, Harvati K, Bridault A, Pion G, Julien M, Krotova O, Conard NJ, Münzel SC, Drucker DG, Viola B, Hublin J, Tafforeau P, Krause J (2016) Effect of x-ray irradiation on ancient DNA in sub-fossil bones – guidelines for safe x-ray imaging. Sci Rep 6:32969. https://doi.org/10.1038/srep32969
26. Ishii C, Nakayama SMM, Kataba A, Ikenaka Y, Saito K, Watanabe Y, Makino Y, Matsukawa T, Kubota A, Yokoyama K, Mizukawa H, Hirata T, Ishizuka M (2018) Characterization and imaging of lead distribution in bones of lead-exposed birds by ICP-MS and LA-ICP-MS. Chemosphere 212:994–1001. https://doi.org/10.1016/j.chemosphere.2018.08.149
27. Johnson K, Quinn CP, Goodale N, Conrey R (2024) Best practices for publishing pXRF analyses. Adv Archaeol Pract 12(2):156–162. https://doi.org/10.1017/aap.2024.6
28. Kalnicky DJ, Singhvi R (2001) Field portable XRF analysis of environmental samples. J Hazard Mater 83:93–122. https://doi.org/10.1016/S0304-3894(00)00330-7
29. Kareiva P, Stark J, Wennergreen U (1996) Using demographic theory, community ecology and spatial models to illuminate ecotoxicology. In: Baird DJ, Maltby L, Greig-Smith PW, Douben PET (eds) ECOtoxicology: Ecological Dimensions. Chapman and Hall, London, pp 13–23
30. Khwankitrittikul P, Poapolathep A, Poapolathep S, Prasanwong C, Kulprasertsri S, Khidkhan K (2024) Species differences and tissue distribution of heavy metal residues in wild birds. Animals 14:308. https://doi.org/10.3390/ani14020308
31. Köhler HR, Triebskorn R (2013) Wildlife ecotoxicology of pesticides: can we track effects to the population level and beyond? Science 341:759–765. https://doi.org/10.1126/science.1237591
32. Leary PE, Dobson GS, Reffner JA (2016) Development and applications of portable gas chromatography-mass spectrometry for emergency responders, the military, and law-enforcement organizations. *Appl. Spectrosc.* 70(5):888–896. https://doi.org/0.1177/0003702816638294
33. Liritzis I, Zacharias N, Papageorgiou I, Tsaroucha A, Palamara E (2018) Characterisation and analyses of museum objects using pXRF: An application from the Delphi Museum, Greece. Studia Antiqua et Archaeologica 24:31–50. https://doi.org/10.47743/saa-2018-24-1-2
34. Markowski M, Kaliński A, Skwarska J, Wawrzyniak J, Bańbura M, Markowski J, Zieliński P, Bańbura J (2013) Avian feathers as bioindicators of the exposure to heavy metal contamination of food. Bull Environ Contam Toxicol 91:302–305. https://doi.org/10.1007/s00128-013-1065-9
35. Massos A, Turner A (2017) Cadmium, lead and bromine in beached microplastics. Environ Pollut 227:139–145. http://dx.doi.org/10.1016/j.envpol.2017.04.034
36. McComb JQ, Rogers C, Han FX, Tchounwou PB (2014) Rapid screening of heavy metals and trace elements in environmental samples using portable X-ray fluorescence spectrometer, a comparative study. Water Air Soil Pollut 225(12). https://doi.org/10.1007/s11270-014-2169-5
37. Montanha GS, Rodrigues ES, Marques JPR, de Almeida E, Dos Reis AR, Pereira de Carvalho HW (2020) X-ray fluorescence spectroscopy (XRF) applied to plant science: challenges towards *in vivo* analysis of plants. Metallomics 12(2):183–192. https://doi.org/10.1039/c9mt00237e

38. Mukhtar H, Chan CY, Lin YP, Lin CM (2020) Assessing the association and predictability of heavy metals in avian organs, feathers, and bones using crowdsourced samples. Chemosphere 252:126583. https://doi.org/10.1016/j.chemosphere.2020.126583
39. [NASEM] National Academies of Sciences, Engineering, and Medicine (2020) Biological Collections: Ensuring Critical Research and Education for the 21st Century. National Academies
40. Newlander K, Goodale N, Jones GT, Bailey DG (2015) Empirical study of the effect of count time on the precision and accuracy of pXRF data. J Archaeol Sci Rep 3:534–548. http://dx.doi.org/10.1016/j.jasrep.2015.07.007
41. Newman MC, Unger MA (2003) Fundamentals of Ecotoxicology. Lewis, Boca Raton, FL
42. NISTa X-ray Mass Attenuation Coefficients—Bone, Cortical. Available online: https://physics.nist.gov/PhysRefData/XrayMassCoef/ComTab/bone.html. Accessed on 10 November 2025
43. NISTb X-ray Mass Attenuation Coefficients—Tissue, Soft. Available online: https://physics.nist.gov/PhysRefData/XrayMassCoef/ComTab/tissue.html. Accessed on 10 November 2025
44. Norris K, Bond AL, Coooper JH, Adams MP, Van Grouw H, White J, Stervander M, Russell DGD, Loader SP (2024) Unlocking avian museum collections to enable and advance environmental change research. Ibis 166:315–322. https://doi.org/10.1111/ibi.13271
45. Otsuki A, Jose S, Mohan M, Thomas S, Editors (2023) Non-Destructive Material Characterization Methods. Elsevier, Amsterdam, The Netherlands
46. Pain DJ, Mateo R, Green RE (2019) Effects of lead from ammunition on birds and other wildlife: A review and update. Ambio 48:935–953. https://doi.org/10.1007/s13280-019-01159-0
47. Pemmer B, Roschger A, Wastl A, Hofstaetter JG, Wobrauschek P, Simon R, Thaler HW, Roschger P, Klaushofer K, Streli C (2013) Spatial distribution of the trace elements zinc, strontium and lead in human bone tissue. Bone 57:184–193. http://dx.doi.org/10.1016/j.bone.2013.07.038
48. Pesce S, Bérard A, Coutellec MA, Hedde M, Langlais–Hesse A, Larras F, Leenhardt S, Mongruel R, Munaron D, Sabater S, Gallai N (2025) Linking ecotoxicological effects on biodiversity and ecosystem functions to impairment of ecosystem services is a challenge: an illustration with the case of plant protection products. Environ Sci Pollut Res Int 32:2773–2785. https://doi.org/10.1007/s11356-023-29128-x
49. Piorek S (1997) Field portable x-ray fluorescence spectrometry: past, present, and future. Field Anal Chem Technol 1(6):317–329. https://doi.org/10.1002/(SICI)1520-6521:6%3C317::AID-FACT2%3E3.0.CO;2-N
50. Pyke GH, Ehrlich PR (2010) Biological collections and ecological/environmental research: a review, some observations and a look to the future. Biol Rev 85(2):247–266. https://doi.org/10.1111/j.1469-185X.2009.00098.x
51. Raj K, Das AP (2023) Lead pollution: impact on environment and human health and approach for a sustainable solution. Environ Chem Ecotoxicol 5:79–85.

https://doi.org/10.1016/j.enceco.2023.02.001

52. Ravansari R, Wilson SC, Tighe M (2020) Portable x-ray fluorescence for environmental assessment of soils: not just a point and shoot method. Environ Int 134:105250. https://doi.org/10.1016/j.envint.2019.105250
53. Rosner A, Ballarin L, Barnay-Verdier S, Borisenko I, Drago L, Drobne D, Eliso MC, Harbuzov Z, Grimaldi A, Guy-Haim T, Karahan A, Lynch I, Lionetto MG, Martinez P, Mehennaoui K, Ozcan EO, Pinsino A, Paz G, Rinkevich B, Spagnuolo A, Sugni M, Cambier S (2024) A broad-taxa approach as an important concept in ecotoxicological studies and pollution monitoring. Biol Rev 99:131–176. https://doi.org/10.1111/brv.13015
54. Rouillon M, Kristensen LJ, Gore DB (2015) Handheld x-ray fluorescence spectrometers: Radiation exposure risks of matrix-specific measurement scenarios. Appl Spectrosc 69(7):815–822. https://doi.org/10.1366/14-07809
55. Rouillon M, Taylor MP (2016) Can field portable x-ray fluorescence (pXRF) produce high quality data for application in environmental contamination research? Environ Pollut 214:255–264. https://doi.org/10.1016/j.envpol.2016.03.055
56. Saidon NB, Szabó R, Budai P, Lehel J (2024) Trophic transfer and biomagnification potential of environmental contaminants (heavy metals) in aquatic ecosystems. Environ Pollut 340:122815. https://doi.org/10.1016/j.envpol.2023.122815
57. Schmitt CJ, Cook JA, Zamudio KR, Edwards SV (2018) Museum specimens of terrestrial vertebrates are sensitive indicators of environmental change in the Anthropocene. Philos Trans R Soc B 374:20170387. https://doi.org/10.1098/rstb.2017.0387
58. Senesi GS, Harmon RS, Hark RR (2021) Field-portable and handheld laser-induced breakdown spectroscopy: Historical review, current status and future prospects. Spectrochim Acta B Spectrosc 175:106013. https://doi.org/10.1016/j.sab.2020.106013
59. Slabe VA, Anderson JT, Millsap BA, Cooper JL, Harmata AR, Restani M, Crandall RH, Bodenstein B, Bloom PH, Booms T, Buchweitz J, Culver R, Dickerson K, Domenech R, Dominguez-Villegas E, Driscoll D 1, Smith BW, Lockhart MJ, McRuer D, Miller TA, Ortiz PA, Rogers K, Schwarz M, Turley N, Woodbridge B, Finkelstein ME, Triana CA, DeSorbo CR, Katzner TE (2022) Demographic implications of lead poisoning for eagles across North America. Science 375:779–782. https://doi.org/10.1126/science.abj3068
60. Snell-Rood EC, Kjaer SJ, Marek-Spartz M, Devitz AC, Jansa SA (2024) Pronounced declines in heavy metal burdens of Minnesotan mammals over the last century. Environ Sci Pollut Res 31:52473–52484. https://doi.org/10.1007/s11356-024-34667-y
61. Specht AJ, Weisskopf M, Nie LH (2014) Portable XRF technology to quantify Pb in bone in vivo. J Biomark 2014:398032. https://doi.org/10.1155/2014/398032
62. Specht AJ, Parish CN, Wallens EK, Watson RT, Nie LH, Weisskopf MG (2018) Feasibility of a portable x-ray fluorescence device for bone lead measurements of condor bones. Sci Total Environ 615:398–403. https://doi.org/10.1016/j.scitotenv.2017.09.123

63. Specht AJ, Kirchner KE, Weisskopt MG, Pokras MA (2019) Lead exposure biomarkers in the Common Loon. Sci Total Environ 647:639–644. https://doi.org/10.1016/j.scitotenv.2018.08.043
64. Suarez AV, Tsutsui ND (2004) The value of museum collections for research and society. Bioscience 54(1):66–74. https://doi.org/10.1641/0006-3568(2004)054[0066:TVOMCF]2.0.CO;2
65. Vighi M, Villa S (2013) Ecotoxicology: The challenges for the 21st century. Toxics 1:18–35. https://doi.org/10.3390/toxics1010018

Figures

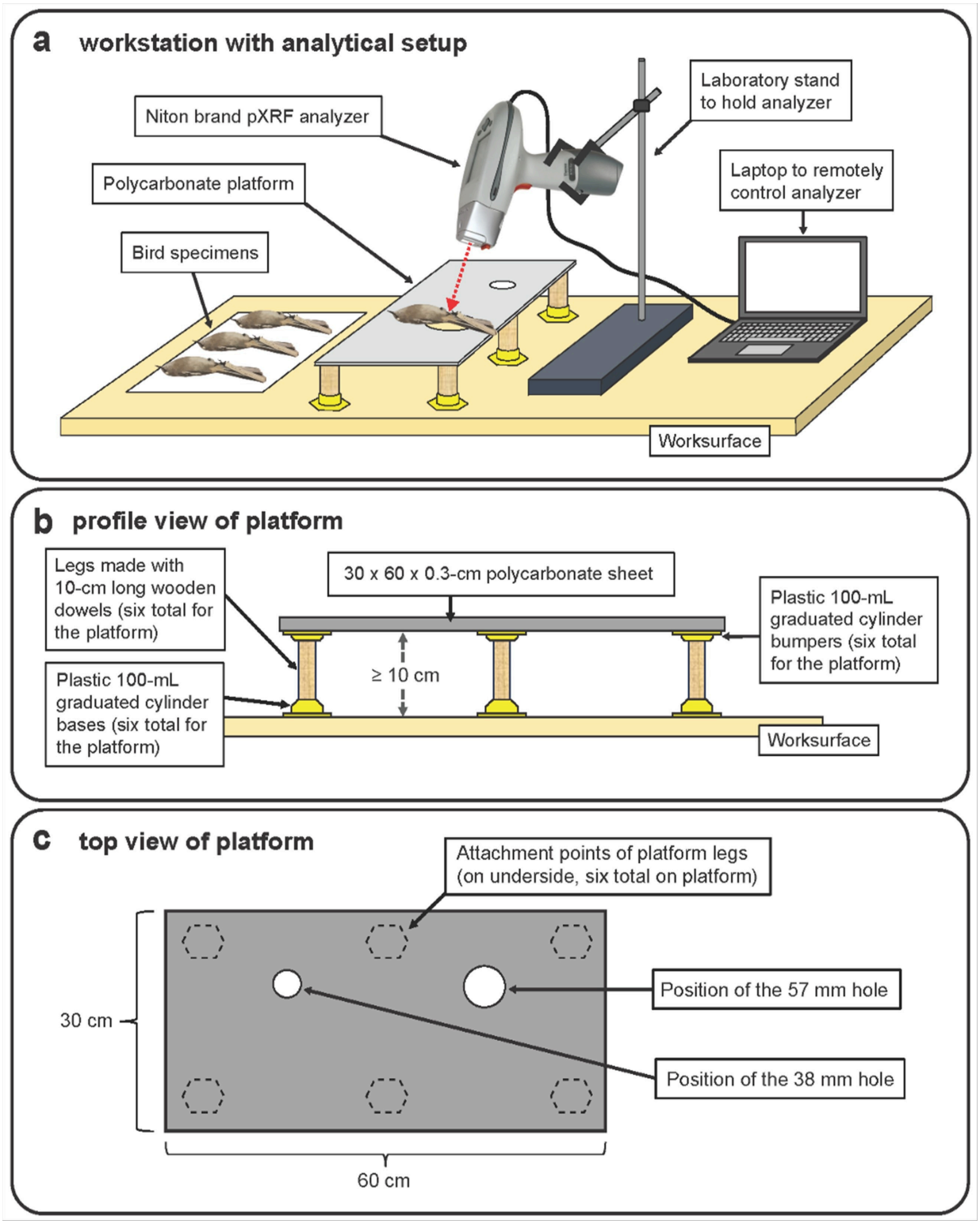


Figure 1

Diagrams of the analytical setup used to chemically analyze bird specimens with a portable x-ray fluorescence (pXRF) analyzer. Shown is a complete workstation with bird specimens and the analytical setup arranged on a typical worksurface (**a**). Here, the x-ray beam that emanates from the analyzer is stylized as a red arrow. In practice, the beam is invisible, and analyzer is held in direct contact with the body part being analyzed. Also shown is a profile view of the custom-made platform which holds the bird

specimens during pXRF scanning (**b**) and the top view of the platform, showing the position of the holes that facilitate x-ray readings of specimens (**c**), along with the pertinent dimensions of each component

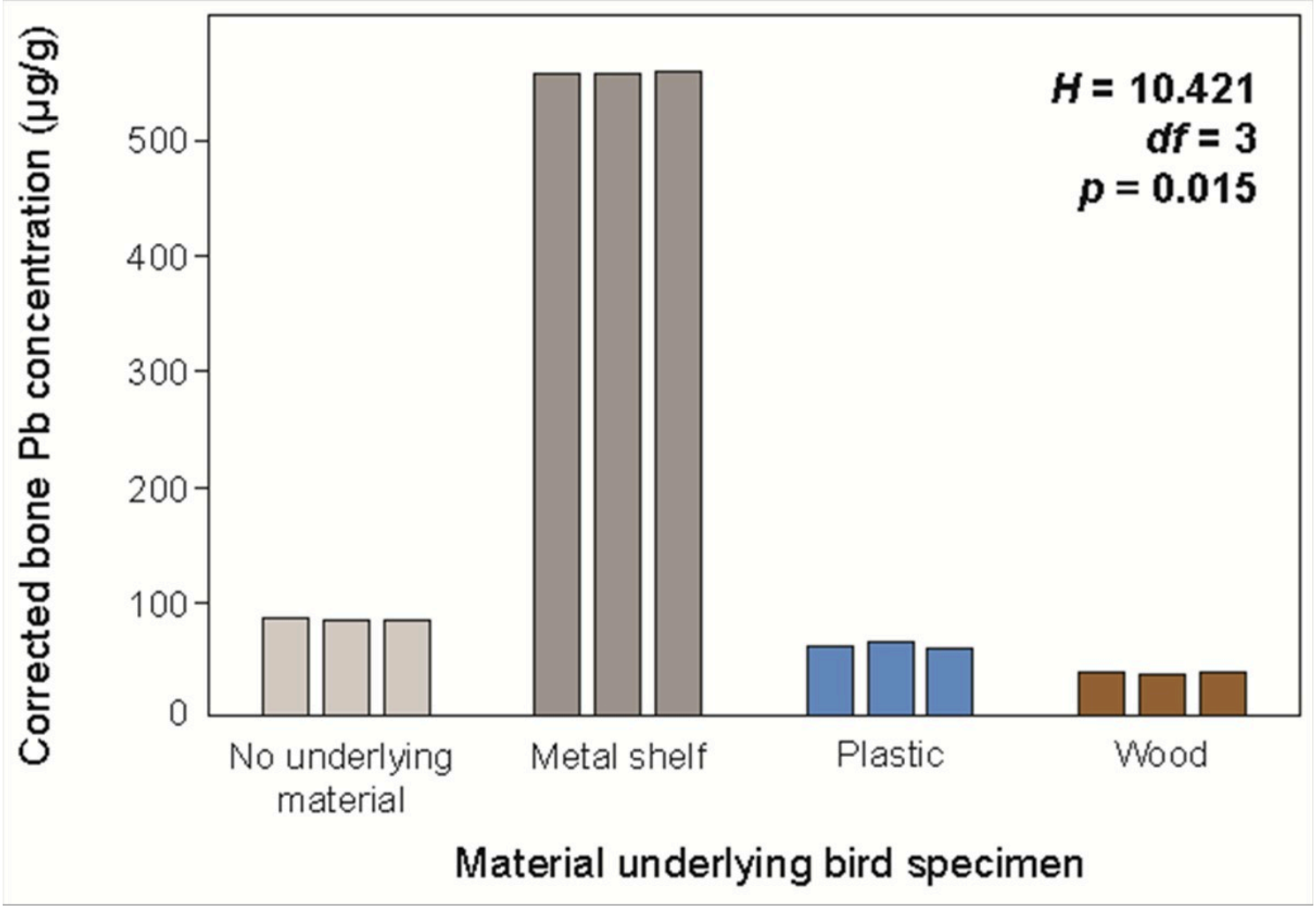


Figure 2

Clustered bar chart of corrected bone Pb concentrations as measured at the same point on the leg bone of small bird specimen with a pXRF analyzer. We measured bone Pb concentrations three times while the specimen was directly on top three different materials as well as with no material directly underneath. We determined if pXRF readings were significantly skewed by the underlying material (i.e., were impacted by “substrate effects”) with a Kruskal-Wallis test (α = 0.05)

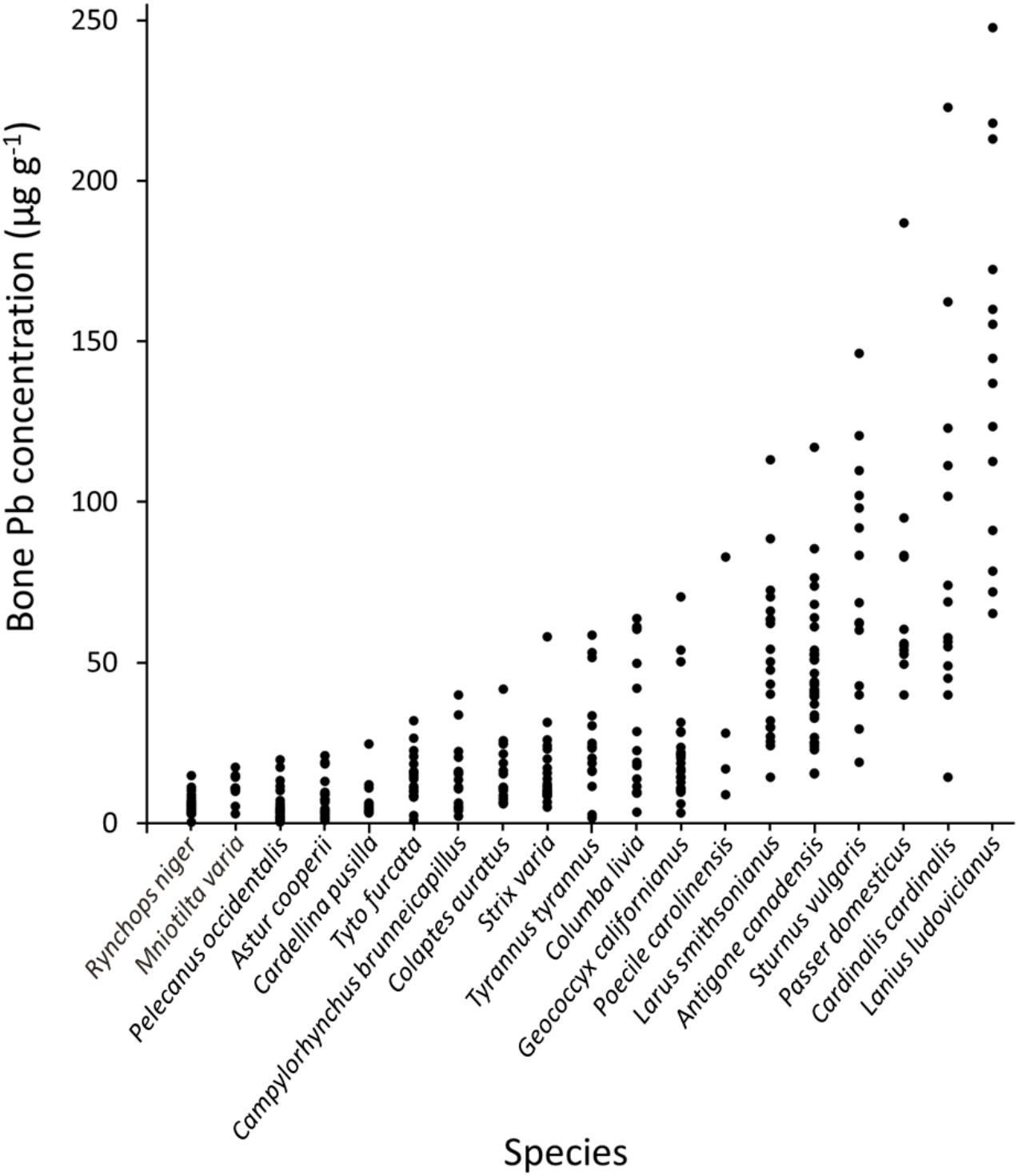


Figure 3

Bone Pb concentrations measured by portable x-ray fluorescence (pXRF) at various locations throughout skeletons of preserved bird “study skin” specimens. Specimens (*n* = 19) represent distinct species and various morphologies, ecological traits, and Pb exposure histories. Specimens were collected between 1963-1980 from various localities within the US and Mexico, including from urbanized areas before milestone pollution regulations. The original Pb readings were obtained via 3-minute pXRF scans but

were further corrected to account for the unique properties of the analyzer and the biological tissues being measured.

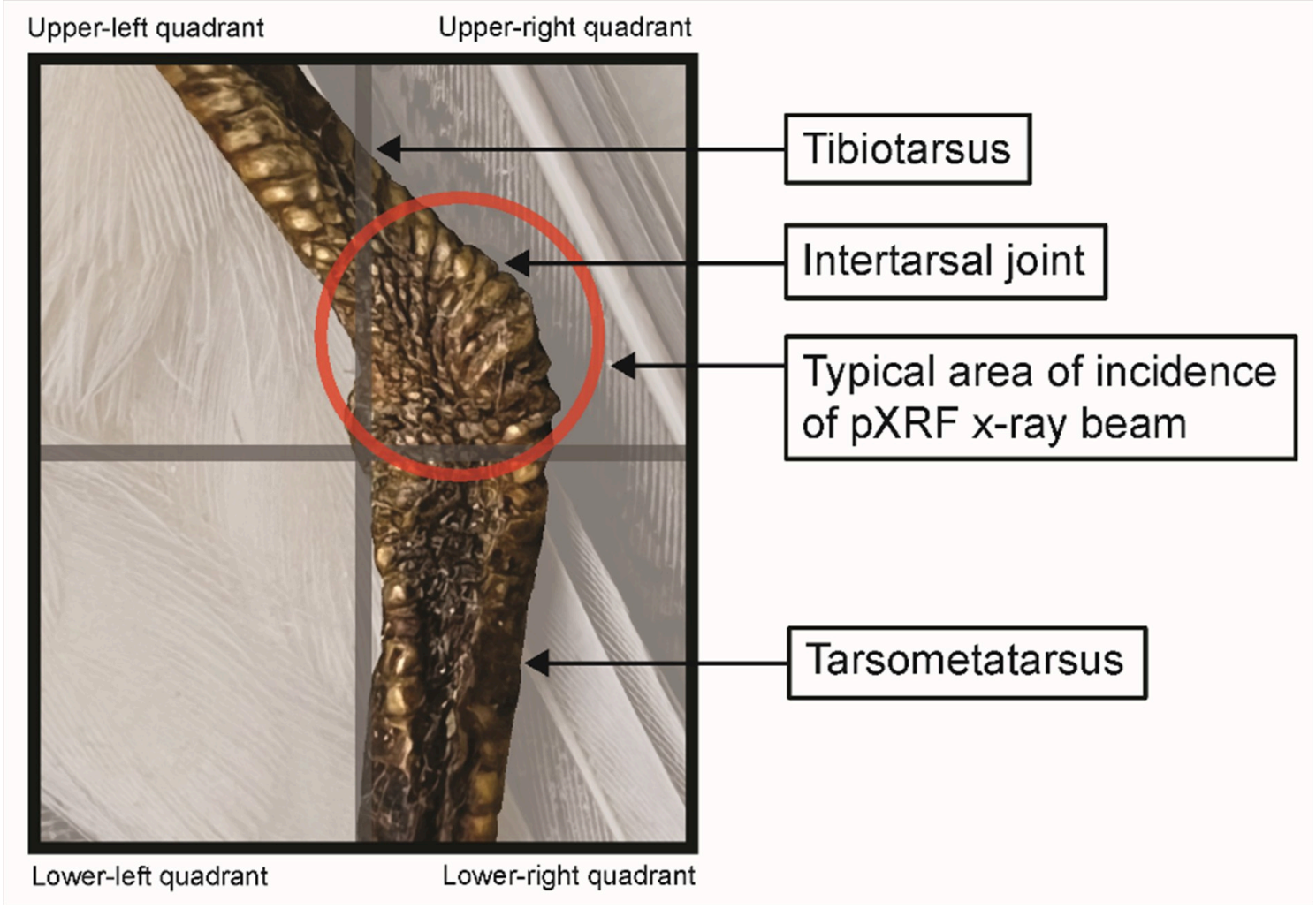


Figure 4

Generalized example of targeting the intertarsal joint for pXRF analysis. The black outlined rectangle shows the field of view through the pXRF analyzer's on-board video camera. For our method, the intertarsal joint is mostly centered in the upper-right quadrant of the field of view so that the approximate center of the joint is irradiated by the analyzer's x-ray beam (general area of incidence outlined as a red circle)

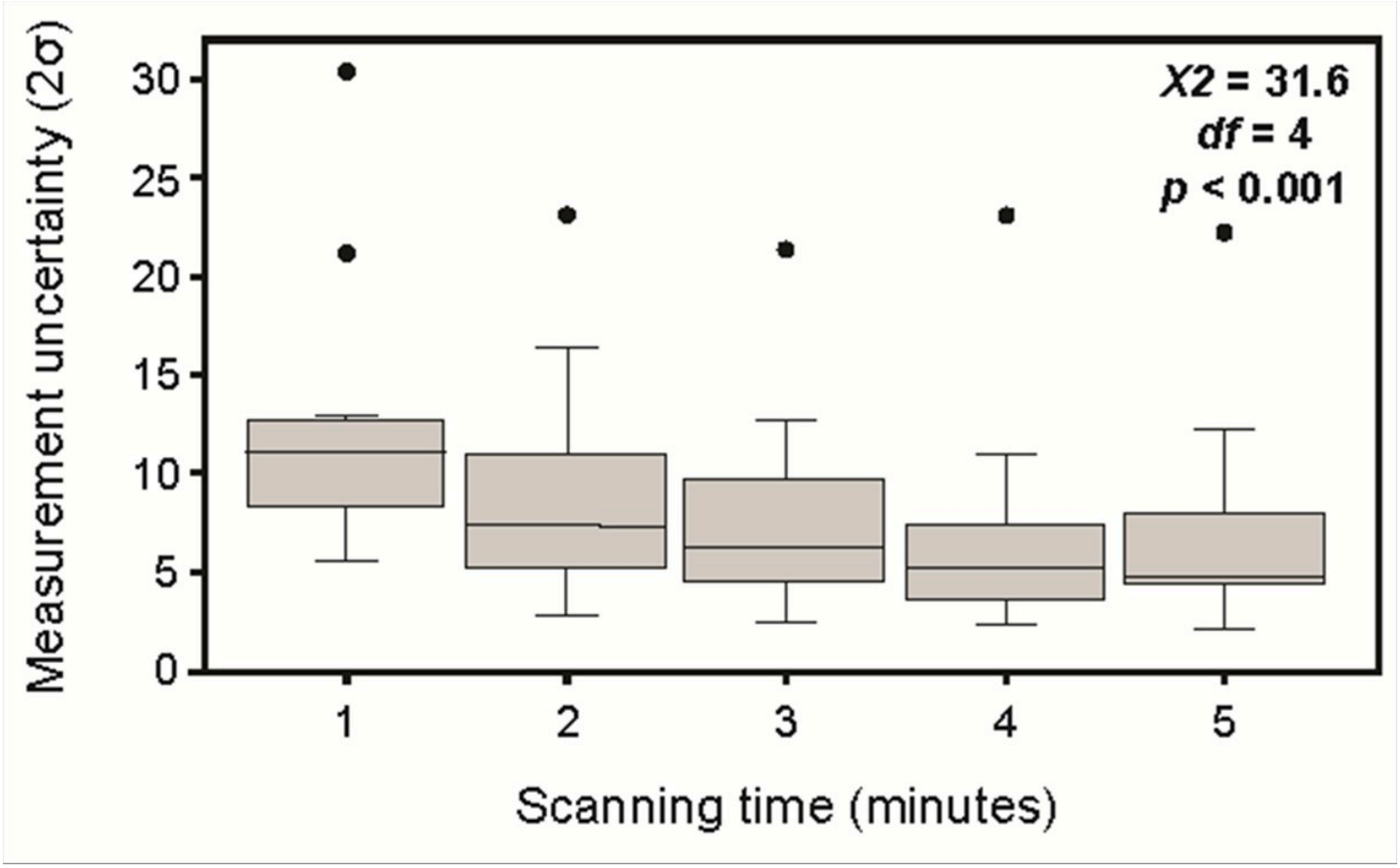


Figure 5

The effect of scanning time on the uncertainty (precision) of pXRF measurements performed on the bones of bird specimens. Each pXRF reading of metal concentrations is accompanied by an uncertainty value (equivalent to two standard deviations around the mean), so shown here are the medians, quartiles, maximum, and minimums of the uncertainty values that accompanied each concentration reading. We determined if uncertainty of pXRF readings were significantly skewed affected by scanning time with a Friedman test (α = 0.05)

Supplementary Files

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