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FACTORS AFFECTING THE ACCUMULATION OF HEAVY METALS IN PASSERINE BIRDS AT THE TAR CREEK SUPERFUND SITE

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FACTORS AFFECTING THE ACCUMULATION OF HEAVY METALS IN PASSERINE BIRDS AT THE TAR CREEK SUPERFUND SITE

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BY THE COMMITTEE CONSISTING OF

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Table of Contents

ABSTRACT:

Exposure to lead (Pb), cadmium (Cd), and zinc (Zn) at the Tri-State Mining District and more specifically within the Tar Creek Superfund Site (TCSFS) is an ongoing issue for both humans and wildlife in the area. This study aims to find the concentrations of these metals in passerine birds living in and around this area, as well as to elucidate the effects of factors such as capture location, species, and migratory strategy on the tissue loads of these contaminants. To this end, Carolina Chickadees (*Poecille carolinensis*), Northern Cardinals (*Cardinalis cardinalis*), Tufted Titmice (*Baeopholus bicolor*), White-Throated Sparrows (*Zonotrichia albicolis*), Indigo Buntings (*Passerina cyanea*), and Painted Buntings (*Passerina cirris*) were captured over a period of several months and feather and whole blood samples were obtained and tested for Pb, Cd, and Zn. The results of this study showed that the capture site had the strongest influence over the feather concentrations of all three metals and the blood concentrations of Pb. In all these, concentrations at the contaminated sites were significantly higher than reference values. Zn in blood showed very different trends, with migratory strategy explaining much more of the variation in the data for blood Zn concentrations, showing that the summer migrants had lower Zn loads. The blood Zn results also showed the Reference site birds having the highest Zn concentrations. Overall, these results suggest that passerines at the TCSFS continue to suffer from heightened levels of heavy metals, especially at locations with closer proximity to chat piles and roads.

INTRODUCTION:

Anthropogenic pollution is one of the main drivers of worldwide biodiversity loss (Groh et al., 2022; Jaureguiberry et al., 2022; Sigmund et al., 2023) and is a major stressor that has contributed to the nearly 30% loss of species since the 1500s (Isbell et al., 2023). These pollutants can come from a variety of sources, such as agriculture, manufacturing, construction, and mining (Rattner, 2009) and can have various chronic and acute effects on the human and non-human organisms living in the areas around the pollution. The ongoing threats of pollution throughout the world and the threat it poses to ecosystems makes it imperative to monitor how wildlife are being affected by pollutants in the environment.

Birds were among the first species to be used in the field of ecotoxicology, with studies dating back to the late nineteenth century (Rattner, 2009). Like all wildlife, birds all over the world are threatened by pollution that results from anthropogenic activities and understanding how exactly pollutants are affecting them is a top priority in avian conservation (Richard et al., 2021). Besides simply conserving bird species themselves, many birds are considered sentinel species, meaning that by studying avian exposure to pollution we can monitor entire ecosystems (Smits and Fernie, 2013). Moreover, the ecological ubiquity of birds allows them to serve as sentinels across ecosystem types, including terrestrial, aquatic, and aerial habitats (Barton et al., 2023; Scheuhammer, 1987). Finally, since birds are highly visible to both laypeople and researchers, they are uniquely suitable to be used as both sentinels of pollution and as an ambassador species that shed light on the dangers of environmental pollution (Quinn et al., 2011).

Heavy metals, such as lead (Pb), cadmium (Cd), zinc (Zn), mercury (Hg), and arsenic (As), are an important class of environmental pollutants that can negatively affect birds. These metals occur naturally and are naturally prevalent in some ecosystems, such as Hg in the Amazon River Basin (Silva-Forsberg et al., 1999). However, most exposure to high concentrations of these metals, both in humans and wildlife, is due to human activities, such as manufacturing and mining (Edelstein and Ben-Hur, 2018). The Tri-State Mining Area, located at the convergence of the Kansas, Arkansas, and Oklahoma state lines, is one of the most heavily contaminated locations in the continental United States. This area includes the Tar Creek Superfund Site (TCSFS), which is primarily contaminated with Pb, Cd, and Zn due to mining activities that took place there from the late nineteenth century to the mid-twentieth century.

Mining at the TCSFS has ceased, but the sediments in the area are still heavily contaminated with Pb and other heavy metals, with large piles of mine tailings, called chat, dotting the landscape. Tar Creek was added to the National Priorities List and became a superfund site in 1983 (US EPA, 2025). While some cleanup has taken place, heavy metals are still prevalent in the area. Soils in Pitcher, a town within the TCSFS, have elevated levels of heavy metals compared to soils in surrounding areas (Beattie et al. 2017). Besides the contaminated sediment covering the land, the water in and around the TCSFS is also contaminated with heavy metals. After the mines were abandoned and the mining companies stopped pumping the water out of them, they filled with water and began draining into Tar Creek, the waterway that gives the TCSFS its name. In Pitcher, the Pb concentrations in stream sediments are about 14 times the natural background level in surrounding areas and Zn concentrations are about 24 times higher than background levels (Potra et al. 2017).

Though some cleanup has taken place due to the designation of the area as a Superfund Site, wildlife and humans in the area continue to cope with elevated heavy metal concentrations. About a decade ago, the town of Pitcher, which was located within the TCSFS was evacuated following reports of high Pb levels and elevated frequencies of learning disabilities in children living in the area (Hu et al., 2007), though the ongoing danger of sinkholes was also a critical part of this decision. Tissue sampling of birds within the TCSFS and elsewhere within the Tri-State Mining District indicates that they continue to cope with elevated levels of heavy metals (Beyer et al., 2004, Beyer et al., 2013; LePage 2020). There has also been some research on non-avian species in the area that also show elevated heavy metals in benthic invertebrates (Miess et al., 2025) and mammals (Phelps and McBee, 2009), though studies in red-eared sliders (*Trachemys scripta*) indicate no difference in tissue Pb concentrations between individuals from the TCSFS and individuals from a reference location (Hays and McBee, 2007).

Acute exposure to heavy metals has clear detrimental effects on avian fauna. For example, across North America, Pb poisoning accounts for 11–49% of adult Common Loon (*Gavia immer*) mortality (Grade et al., 2019) and around 20% of California Condors (*Gymnogyps californianis*) have blood Pb concentrations that warrant clinical intervention (Finkelstein et al., 2012). Additionally, the TCSFS is the only place in which acute Zn poisoning has been seen in free-living birds, leading to pancreatitis in waterfowl (Beyer et al., 2004; Sileo et al., 2003). Besides these more dramatic effects, chronic exposure to heavy metals, such as the Pb, Zn, and Cd prevalent at this site can have a host of more subtle adverse effects, including effects on immune function, growth, reproduction, and a host of other health biomarkers (Burger, 2008; Vallverdú-Coll et al., 2019; Williams et al., 2018).

The TCSFS serves as habitat to a variety of bird species including both residents and migrants. Whereas residents are exposed to contaminants in the TCSFS year round, exposure of migratory species would likely be much reduced, as they migrate to wintering or breeding grounds that may be uncontaminated. The migratory escape hypothesis posits that migration serves as a mechanism that can mitigate the effects of disease and parasites as migrants move quickly among different habitats during different life stages (Altizer et al., 2011; Bush and Clayton, 2018). There has been some discussion and findings to support the idea that migratory escape may also pertain to environmental pollutants (Klaassen et al., 2012; Cooper et al., 2017; Durkalec et al., 2022). However, the results of these studies are inconsistent with each other and show differences between metals in terms of how migration affects individuals' uptake. Durkalec found that Cd was actually higher in migratory birds and that Pb was not different between migratory and nonmigratory birds, and another study found that Cd, Zn, and Pb concentrations in feathers and blood were not different between a migratory and nonmigratory species (Cooper et al., 2017). There has been some work done at the TCSFS indicating that migratory birds may have lower metal loads than nonmigratory birds (LePage, 2020), but these conclusions were drawn using a limited number of birds and only summer migrants.

The present study seeks to provide current data on heavy metal exposure of birds in and around the TCSFS. More specifically, we sought to determine how different life histories (i.e., migratory vs. sedentary) and capture location interact to influence the accumulation of Pb, Zn, and Cd in blood and feathers of passerine birds, and to assess whether these metals are being accumulated in potentially harmful concentrations. Our use of both blood and feathers enables us to differentiate between the effects of short-term and long-term exposure to these heavy metals with regards to the factors listed above. We also captured birds both within and nearby the contaminated area to determine the extent to which heavy metal contamination at the TCSFS can spread from its source. Finally, we captured a subset of migratory birds at different time points during the breeding season to test for heavy metal accumulation over time. We predict that there will be higher metal concentrations in the tissues of birds living in closer proximity to the more contaminated former mining area and that metal loads will increase over the course of the birds' exposure period. We also predict that there will be lower metal loads in migrants and that species with the same migratory strategy (i.e., resident, summer migrant, winter migrant) will not differ from each other in terms of metal exposure.

METHODS:

Study Sites:

We captured and sampled birds in five different location groupings. Within the presumably more contaminated area (TCSFS) we captured birds within the Mineral Heights neighborhood (an abandoned residential area with remnant house foundations and paved roads), and within a network of gravel roads (henceforth referred to as the Roadside site) that pass among the chat piles in the area (Figure 1). These sites differ in that Mineral Heights sees very little vehicle traffic and the roads are paved, whereas the gravel roads in the Roadside site are used daily by a fleet of trucks hauling chat within and away from the TCSFS. We also sampled at two sites downstream along Tar Creek (Figure 1) at a remediation site in Commerce, OK and a private property in Miami Oklahoma downstream of both the TCSFS and the remediation site, hereafter they are referred to as Remediated and Downstream, respectively (Figure 1). The Remediated site is managed by the University of Oklahoma Center for Restoration of Ecosystems and Watersheds and consists of a series of ponds that funnel water out of a former mining tunnel and passively treat the water as it flows through the pond system; thus the water near the entrance ponds is more polluted than the water exiting the system and flowing into Tar Creek (Nairn et al. 2011). Finally, we captured birds at uncontaminated reference sites on private properties and along roadsides in an area approximately 40 km Southeast of the TCSFS. This rural area is completely outside of the Tar Creek watershed and parts of it served as a reference site for a previous study of birds in the TCSFS (LePage, 2020).

Field Methods:

We targeted seven common species for heavy metal sampling. We selected three species that were year-round residents: Carolina Chickadees (*Poecile carolinensis*, abbreviated CACH), Tufted Titmouse (*Baeolophus bicolor*, TUTI), and Northern Cardinals (*Cardinalis cardinalis*, NOCA). We selected two species that were winter residents: White-Throated Sparrow (*Zonotrichia albicollis*, WTSP) and Dark-Eyed Junco (*Junco hyemalis*, DEJU); and two species that were summer residents: Indigo Bunting (*Passerina cyanea*, INBU) and Painted Bunting (*Passerina cirris*, PABU). For year-round and winter residents, birds were baited to capture sites where we used mist nets to capture them. For summer residents we used a combination of pre-recorded song and visual decoys to lure birds into a mist net or spring-loaded flip trap.

All target species had blood samples taken from the brachial vein (~50 to ~200 microliters) using a needle prick and a non-heparinized capillary tube. The blood was then immediately transferred to a 1.5 mL Betkon-Dickinson K2 EDTA Microtainer. For each bird captured we also performed a series of morphometric measurements and collected the right or left innermost primary to use for metal analysis. We used innermost primaries because these feathers have the least inter-feather and intra-individual variation in terms of metal accumulation and often show the highest metal concentrations (Dauwe et al., 2003; Roque et al., 2016). The birds used in this study were all passerines occupying similar niches, and as such should have similar metal exposure and accumulation (Abbasi et al., 2015). Sampling was conducted under banding permit 23215 and an Oklahoma State Scientific Collecting Permit. Sampling protocols were reviewed and approved by the University of Oklahoma Institutional Animal Care and Use Committee.

Although we sought to capture representatives of all species at all study locations, we failed to do so in some cases, either because the species was elusive or not detected. We only captured DEJU at the Reference, Downstream, and Remediated locations. Hence, we excluded this species from all formal analyses as there were no samples from contaminated areas. We also failed to capture any PABU at the Remediated and Downstream locations, and there were no CACH from the Mineral Heights location or WTSP from the Remediated location. We still included these species in our analyses as there were still samples from both the Reference sites and contaminated areas.

Metal Analysis:

Blood and feather samples were analyzed at the Michigan State University Veterinary Diagnostic Laboratory (MSU VDL). Elemental analysis for blood was conducted following the methods of Wahlen et al., 2005 using an Aligent 7900 Inductively-Coupled Plasma Mass Spectrometer with standards sourced from Inorganic Ventures (Christiansburg, Virginia). Elemental concentrations were calibrated using a 6-point linear curve of the analyte-internal standard response ratio. Blood samples that were not sufficiently large were excluded from this analysis and the method limits of detection (MLOD) were determined by the volume of blood that could be analyzed.

Because of some sampling issues, blood samples from INBU and PABU taken in the summer had to be analyzed as dried samples and were therefore reported using dry weight. All values were converted to wet weight using the conversion factor of multiplying the dry weight concentrations by 0.2 after ensuring units were the same as the wet weight values (Scanlon, 1982), which has been broadly utilized in other studies (Franson and Pain, 2011; Hoffman et al., 2006; Schulwitz et al., 2015; Wayland et al., 1999; Wayland and Scheuhammer, 2011)

The necessity for small blood samples resulted in higher MLODs for some samples, meaning that some metals had high proportions of non-detects in the data. In Pb, 25.77% of samples were below the MLOD, in Zn, only 3.24% of samples were below the MLOD, but in Cd, 80.61% of samples were below the MLOD. For Pb, 52% of these non-detects were at the reference site, 20% were at the remediation site, 14% were in Mineral Heights, 10% were at the downstream site, and 4% were from roadside sampling locations. For Zn, there were no non-detects at the reference site, the Mineral Heights and Roadside sites both made up 33.33% of the non-detects, respectively, and the Downstream and Remediated locations both made up 16.67% of the non-detects, respectively. In Cd, the Reference site accounted for 30.38% of the non-detects, Mineral Heights accounted for 22.78%, Roadside sites accounted for 19.62%, Downstream accounted for 18.99%, and the Remediated site accounted for 8.23% of non-detects. However, because over 80% of Cd results were below the MLOD, blood Cd concentrations were not used in statistical analysis (Hwang et al., 2023). MLOD values were replaced by half the MLOD for data analysis, which is a broadly-utilized method in toxicological studies (Shoari and Dubé, 2018).

Feather analysis was conducted using standard methods at the MSU VDL. Feathers were first washed using a series of acetone and water rinses, wherein they were agitated in clean reagent grade acetone for two minutes twice, the process was then repeated twice with ultra-pure water, and then again once with reagent grade acetone. After this, the feathers were dried overnight and then saturated with nitric acid and digested in a 95°C oven. The digested samples were diluted with water to 100 times the dried feather mass and analyzed using an Aligent 7900 Inductively Coupled Plasma Mass Spectrometer, also according to the methods of Whalen et al., 2005. Elemental concentrations were calibrated using a 6-point linear curve of the analyte-internal standard response ratio, also using standards from Inorganic Ventures (Christiansburg, Virginia). Results were reported as μg/g. For feathers, none of the concentrations fell below the MLOD

Data Analysis:

Data were analyzed using a combination of conditional inference trees and ANOVAs. Conditional inference trees were used to determine the hierarchical predictor structure of the following variables: site (Mineral Heights, Roadside, Remediation, Downstream, and Reference), species (CACH, INBU, NOCA, PABU, TUTI, WTSP), and life history strategy (summer migrant, winter migrant, or resident), with respect to their effect on tissue metal concentrations. Separate analyses were done for Pb, Cd, and Zn in feathers. We also conducted separate analyses for Pb and Zn in blood, but not Cd as we lacked sufficient lab results for any meaningful analyses.

Data were first log-transformed to decrease variation and increase normality for the analysis. Conditional inference trees were constructed using the ctree function in the partykit library in R, which was chosen due to the robustness of the binary recursive partitioning and the ability to model nonlinear data containing variables that are suspected of interacting in a hierarchical fashion (De'ath and Fabricus, 2000; Olden et al. 2008). The ctree methodology also differs from other conditional inference tree construction methods in R due to its use of multiple test procedures to approve or dismiss splits and minimize overfitting (Hothorn et al., 2006). The significance threshold used for the trees was $\alpha=0.05$ and the size of terminal nodes were constrained to reduce instability in small partitions.

Variables that were found to have significant effect on metal concentrations using the conditional inference trees were analyzed using complementary ANOVAs to determine effect sizes and further investigate the differences between groups. For ANOVAs that revealed a significant difference between factors, a post hoc Tukey's HSD test was utilized to determine differences between individual pairings of factors. Correlations between the metals were determined using a spearman's rank correlation and visualized using scatter plot matrices for each tissue type.

As a separate analysis we compared blood samples of the two summer migrant species (PABU and INBU) that were collected early and late in the breeding season to test for accumulation of heavy metals during the time spent in the contaminated area. For this test we used simple ANOVAs–one each for Pb and Zn, with site, species, and sample time (encoded as a binary: early or late) as dependent variables. This comparison was limited to summer migrants as our sampling of winter migrants occurred when they had already been in the area for several months (i.e., we did not have early and late samples), and we did not use feathers for this analysis because Pb and Zn are only integrated into feathers during molt.

RESULTS:

Metal concentrations in both tissues varied widely. In feathers, Pb ranged from 0.0543µg/g to 63.603 µg/g with a mean of 2.775 µg/g, Zn ranged from 100.3 µg/g to 598.1 µg/g with a mean of 182.0 µg/g, and Cd ranged from 0.025 µg/g to 17.885µg/g with a mean of 0.596 µg/g. For feathers, 18% of samples fell above the accepted toxicity threshold level for Pb of 4 µg/g (Burger and Gochfield, 2000; Espejo et al., 2018) (Table 1) and 4% fell above the accepted toxicity threshold level for Cd of 2 µg/g (Burger and Gochfield, 2004; Carvalho et al., 2013) (Table 2). Interpreting toxicity thresholds in feather Zn concentration is complicated, but the values reported here are very similar to other studies of passerines that did not report toxic effects (Chatelain et al., 2021; Cooper et al., 2017) (Table 3).

In blood, Pb ranged from 9.613 ppb to 715 ppb with a mean of 91.992 ppb, with 4% of samples falling above 200 ppb threshold for subclinical poisoning in Anseriformes, Falconiformes, and Columbiformes (Franson and Pain, 2011) (Table 4). Zn ranged from 0.5 to 24.383 ppm with a mean of 5.643, and 9% of samples fall above concentrations that have caused Zn-related pancreatitis at the TCSFS in the past (Sileo et al., 2003) (Table 5), though these effects can be very species-dependent. Cd ranged from 0.1 to 100 ppb with a mean of 13.799.

Effects of Factors on Metal Concentrations in Feathers:

Lead in Feathers:

The conditional inference tree for Pb in feather samples (Figure 2) indicated an initial split between the contaminated sites (Mineral Heights and the nearby Roadsides) and the other sampling areas. Within the contaminated sample the tree specified a second split between

Mineral Heights and Roadside, with subsequent groupings according to species in Mineral Heights and life history strategy in the Roadside samples. Among the samples from the non-contaminated sites, the tree indicated a split between the Reference site and the other two locations (Remediated and Downstream), which likely indicates some degree of Pb exposure downstream from the contaminated area. There were subsequent splits in each group according to species. An ANOVA that compared sampling sites with data from all species included confirmed that there were significant differences between sites ($p < 0.001$), with elevated levels associated with Roadside and Mineral Heights (Figure 3). Pairwise comparisons between the sites revealed significant differences between all the sites besides Downstream and Remediated. The ANOVAs showed no significant differences between species or migratory strategies.

Zinc in Feathers:

The conditional inference trees for Zn (Figure 4) also indicated an initial split between the contaminated sites (Mineral Heights and the nearby Roadsides) and the other sampling areas. The Mineral Heights and Roadside grouping was then split along species lines, with CACH and TUTI having higher Zn concentrations than the other species. The Control, Downstream, and Remediated grouping was then split again by region, with the Downstream region splitting from the other two. Both groupings were then split along species lines, with the Downstream region showing higher concentrations in CACH, INBU, and WTSP than in NOCA and TUTI. The NOCA at the Control and Remediated regions had lower Zn concentrations than the other species. An ANOVA comparing Zn concentrations between regions with the species combined showed that there were significant differences between sites ($p < 0.001$). Pairwise comparisons made using a Tukey's HSD test revealed differences between every site besides the remediated and control sites and the remediated and downstream sites (Figure 3). Similar to the Pb results, there were no significant differences between species or migratory strategies revealed by the ANOVAs.

Cadmium in Feathers:

The conditional inference tree for Cd in the feathers differs slightly from the Zn and Pb trees. Here, the primary split was between the Reference site and every other site, with the Reference having lower Cd values than the other sites (Figure 5). The other sites then split between Roadside and all the other regions, with Roadside generally having higher Cd values. Roadside sites were then split by migratory strategy, with Resident birds having higher Cd concentrations than both summer and winter migrants, which were split by species with PABU and WTSP having higher Cd concentrations than INBU. The Downstream, Mineral Heights, and Remediated grouping were then split again by region, with Downstream having lower Cd concentrations than Mineral Heights and Remediated, which were split by species, with INBU, PABU, and WTSP having lower Cd than CACH, TUTI, and NOCA, which were again split with NOCA having lower Cd concentrations than CACH and TUTI. An ANOVA comparing different sites across all species revealed significant differences ($p < 0.001$) (Figure 3), and the pairwise comparison test showed that each site was significantly different from all others, besides Mineral Heights and Remediated. Additionally, an ANOVA comparing migratory strategies revealed significant differences ($p = 0.019$). However, a Tukey's HSD test revealed that only the resident and summer migrant birds were significantly different from each other ($p = 0.014$). The ANOVA did not show a significant difference among species.

Effects of Factors on Metal Concentrations in Whole Blood:

Lead in Blood:

The conditional inference tree for Pb in blood samples indicated an initial split between the more predictably contaminated sites (Downstream, Mineral Heights, and Roadside) and the other sites (Remediated, Reference) (Figure 6), which is similar to the pattern seen in feather Pb concentrations. Within the less contaminated sites, the samples were then split by migratory strategy, with summer migrants having generally lower Pb concentrations, but more variation, and the residents and winter migrants having generally higher Pb concentrations. The resident and winter migrant grouping was then again split by site, with Reference birds having higher Pb concentrations than birds from the Remediated area. In the more contaminated sites, the samples were again split by site, with Roadside birds having higher concentrations than those at the Downstream and Mineral Heights sites. The Downstream/Mineral Heights grouping was then split by migratory strategy, with winter migrants having higher Pb concentrations than residents or summer migrants. An ANOVA revealed a significant difference between blood Pb concentrations for different species ($p < 0.001$) (Figure 7), sites ($p < 0.001$) (Figure 8), and migratory strategies ($p < 0.001$). Within the species, a Tukey's HSD test revealed significant differences between PABU and NOCA, TUTI, and WTSP, as well as differences between WTSP and INBU. For the sites, all pairings in the Tukey's HSD test revealed significant differences except for those between Control and Remediated, Control and Downstream, and Downstream and Mineral Heights. There was also a significant difference between each pairing of migratory strategies.

Zinc in Blood:

The conditional inference tree for Zn in blood revealed an initial split between migratory strategies, with residents and winter migrants generally having higher Zn concentrations than the summer migrants (Figure 9). The residents and winter migrants were then split along sites, with

Mineral Heights, Remediated, and Roadside locations having lower Zn concentrations than the Reference and Downstream sampling locations. The Reference and Downstream grouping was then split again by site, with the Reference birds having higher Zn concentrations than the Downstream ones. An ANOVA comparing the species without differentiating by site revealed significant differences ($p < 0.001$) (Figure 7), as well as an ANOVA comparing sites ($p < 0.001$) (Figure 8), and an ANOVA comparing migratory strategies ($p < 0.001$). For species, Tukey's HSD test revealed significant differences between INBU and NOCA; INBU and TUTI, PABU and NOCA; and PABU and TUTI. Pairwise comparisons of sites revealed significant differences between Control and Mineral Heights, Remediated, and Roadside, respectively, and Downstream and Roadside and Remediated, respectively. The Tukey's HSD test for migratory strategy showed significant differences between summer migrants and both resident birds and winter migrants.

Correlations Between Metals:

In feathers, the log-transformed concentrations of the three metals we evaluated (Pb, Cd, and Zn) correlated very strongly together within individuals, with a spearman's rank correlation revealing all pairings to be significantly correlated with each other (Zn/Pb $\rho = 0.7337$, $p < 0.001$; Zn/Cd $\rho = 0.7042$, $p < 0.001$; Pb/Cd $\rho = 0.8218$, $p < 0.001$) (Figure 10). This indicates that these metals were likely accumulated through similar sources and mechanisms in each individual. The correlation between Zn and Pb in blood is much weaker, though still significant ($\rho = 0.3246$, $p < 0.001$) (Figure 11). We did not test for correlations with Cd in blood samples as we lacked sufficient measurements.

Metal Accumulation Over Time:

We used a restricted data set to address whether heavy metals accumulate in the tissues of birds in the TCSFS. First, we only considered migratory species, as they presumably arrived in the TCSFS with low metal loads. Second, we only had early and late season samples for the two summer migrant species (PABU and INBU). Third, we only considered samples from the contaminated areas (Road and Mineral Heights). Fourth, we only used blood-based measures, as feather values would be static throughout the season. And finally, we excluded Cd from the analysis because all of the early season samples were below detectability. For Pb, there was a similar problem with a high proportion of values below detection levels in the early season sample, but the detection thresholds were relatively low, such that using half-detection-level values was more meaningful.

There was no significant difference between early and late samples with respect to Pb ($F_{1,39} = 2.379$, $p = 0.131$), although the late season mean (116.0 ± SE) was higher than the early season mean (85.4 ± SE). With regard to Zn, an initial analysis also showed no significant effect ($F_{1,39} = 1.70$, $p = 0.2$) (Figure 12). However, there was a low outlier among the INBUs captured in late summer. This sample was below the Zn detection limit and yielded a half DT value of 0.5 µg/g. With this apparent outlier removed, the difference between early and late samples was significant ($F_{1,38} = 12.32$, $p = 0.001$). This low outlier was the only sample below the detection threshold in the data subset used for early and late detection. We suspect that this individual may not have spent the entire summer in the TCSFS, and did not have sufficient time to accumulate heavy metals in the blood. The Pb measurement for this individual was not below the detection threshold, but at 32 µg/g, it was the second lowest Pb sample from late summer and was comparable or less than samples from uncontaminated areas.

DISCUSSION:

For the feather metal concentrations, the hierarchical structure produced by conditional inference trees all followed the general pattern of site differences being responsible for early splits and species differences and migratory strategies being responsible for splits nearer to the terminal nodes. This indicates that the site explained the largest share of variation in metal exposure and that taxonomic exposure is dependent on the spatial context. Besides this overarching hierarchical pattern, it is also notable that the primary split in both Pb and Zn resulted in the partitioning of Mineral Heights and Roadside, the sites in the more contaminated regions of the TCSFS, from the other sites, and that in Cd, the primary split was between the Reference sites and the other sampling locations. The simpler comparisons between these variables using ANOVAs revealed similar patterns in differences between sites, species, and migratory strategies, though it lacked the more hierarchical large-scale patterns that showed the relationships between these variables. These patterns align with our general predictions about heavy metal exposure across study sites.

The analyses for Pb concentrations in blood showed similar patterns to those for feathers. The blood Pb concentrations followed a similar hierarchical pattern in the conditional inference trees, with the site being responsible for the partitioning at the primary node, though in the blood Pb results the Downstream site was grouped with Mineral Heights and Roadside at the first node split, unlike the feather Pb concentrations, which grouped Downstream with Control and Remediated. However, node 7 of the blood Pb conditional inference tree resulted in splits between the combined results of Downstream and Mineral Heights and Roadside's individual results. The ANOVAs for blood Pb also supported this, with no significant differences between

Mineral Heights and Downstream. However, this analysis also showed no significant differences between blood Pb concentrations at Reference and Downstream even though the primary split of the conditional inference tree separates the sites. The blood Zn concentrations were generally higher at the Reference site and did not follow the same general hierarchical pattern as the other metals, with the primary split separating Winter Migrants and Residents from Summer Migrants.

The evaluation of how time of year affects blood heavy metal concentrations in migrants showed similar trends between Zn and Pb, with both being slightly higher in birds captured in late summer, when they had been around the TCSFS longer, than early summer, when they had recently arrived at the TCSFS. However, this difference was only significant for Zn and not for Pb.

Heavy metal concentrations in feathers indicated toxic levels of exposure across the species sampled, especially at the more contaminated sites. 18% of the samples we obtained exceeded the toxicity threshold of Pb in feathers, which is 4 µg/g (Burger and Gochfield, 2000; Espejo et al., 2018), and all of the samples that exceeded this threshold were obtained at either Mineral Heights or Roadside sampling locations, with 57% of Roadside birds exceeding this concentration (Table 1). Burger and Gochfield (2004) put the threshold for Cd toxicity at 2 µg/g, which is also used as a toxicity threshold in other studies (Carvalho et al., 2013). Only 4% of our feather samples exceed this metric, though all samples that exceeded this threshold were from the Roadside site. However, adverse effects, such as reduced growth rates, have been noticed at concentrations ranging from 0.1 to 2 µg/g (Gruz et al., 2019; Spahn and Sherry, 1999). Overall, sensitivity to Cd is highly species-dependent (Burger, 2008). There may be different toxic thresholds for passerines, and we use the threshold of 2 µg/g here only to aid interpretability (Table 2). Still, the feather Cd concentration values reported here are substantially higher than those reported in a study where the diets of European Starlings (*Sturnus vulgaris*) were supplemented with doses of Cd above the threshold known to cause damage to both renal and reproductive endpoints (Pilastro et al., 1993; Stanton et al., 2010). There is limited reporting on toxicity thresholds for Zn in feathers, but the concentrations we found are similar to those found in other studies with passerine birds (Chatelain et al., 2021; Cooper et al., 2017). Our values are most comparable to those that Cooper et al. (2017) found at the U.S. Department of Energy's Savannah River Site (SRS) south of Aiken, South Carolina, which was exploited for production of materials used for nuclear weapons in the 1950s. In addition, all but 3% of Zn concentrations in feathers were lower than those that Chatelain et al. (2021) reported at their non-urbanized, baseline Zn concentration sites (approximately 350 μg/g) (Table 3). All the samples that exceeded this threshold were from the Roadside site. However, our Zn concentrations were also generally higher than concentrations reported in other species from uncontaminated areas (Anbazhagan et al., 2021), though none of the species from that study were Passerines. Overall, there is limited evidence to say that Zn concentrations in feathers from the birds in this study are above or below thresholds of toxicity because Zn results from other studies are so variable.

Evidence of heavy metal contamination in blood samples was reduced, although there were still levels that exceeded accepted toxicity thresholds. In birds, broad hematological effects are noticed at blood Pb concentrations over 710 ppb (Buekers et al., 2009), which was only exceeded by one sample from Mineral Heights. However, minimum concentrations in blood with negative effects on delta-aminolevulinate dehydratase, an enzyme involved in heme production, range from 20 ppb to 210 ppb (Buekers et al., 2009; Beyer et all., 2000; Henny et al., 1991), though this is species- and endpoint-dependent as some conservationists place 100 ppb as the maximum safe concentration and 450 ppb as the concentration requiring clinical intervention

(Finkelstein et al., 2012; Williams et al., 2018). Though thresholds for Passerines may be disputed, Franson and Pain (2011), developed a framework for interpreting toxicity thresholds for Pb in Anseriformes, Falconiformes, and Columbiformes, which range from 200 ppb to 2000 ppb for subclinical effects. The lower value for the range in which subclinical effects can be seen for all three orders was 200 ppb, which was the threshold used in the present study to determine that 4% of blood samples were above toxic levels and that 34% of birds from the Roadside and 10% of birds from Mineral Heights had toxic exposure (Table 4). There is limited information regarding Cd toxicity thresholds in blood, but a study in mallards found that adverse effects were not noticed at concentrations below 260 ppb (White and Finley, 1978) and a study in pheasants showed that negative effects occurred at concentrations as low as 170 ppb (Świergosz and Kowalska, 2000; Wayland and Scheuhammer, 2011). Thresholds for Zn toxicity in birds are poorly defined, but the mean concentration reported in a study in which birds that died from pancreatitis linked to Zn toxicity at the TCSFS was the equivalent of 14.2 ppm wet weight. (Sileo et al., 2003), which 9% of the birds from this study exceeded and 18% of the Reference birds exceeded (Table 5). However, Zn toxicity thresholds may vary among species, so the concentrations reported here may not be indicative of a biologically significant heightening of Zn concentrations.

The differences between heavy metal dynamics in feathers and blood plays an important role in interpreting the patterns exhibited in these results. In general, blood represents current exposure to heavy metals and feathers represent longer term-metal exposure (Golden et al. 2003). However, there are key differences between these metals in terms of what blood components they are bound to and how they circulate throughout the body. Once Pb enters circulation through either inhalation or dietary exposure, the majority is sequestered into bone and other tissues and a small amount remains in circulation, primarily bound to erythrocytes, which shortens their life cycles (Flora et al., 2012; Williams et al., 2018). In one bird species, California Condors (*Gymnogyps californianus*), Pb has a half life in blood of 13 days (Williams et al., 2018). After sequestration in bone and tissues, Pb can be mobilized by stress or calcium mobilization, as happens during egg laying (Burger, 1994). Conversely, Zn is an essential element for many cellular processes, so there is rapid exchange between plasma and tissues that is tightly homeostatically controlled and regulated by metallothioneins and other enzymes, such as ZIP and ZnT transporters (Hambidge et al., 2010). This means that blood Zn is less sensitive to moderate environmental variation. Cd circulates in the blood primarily bound to metallothioneins and other plasma proteins, reflecting primarily recent exposure but also longer-term tissue stores (Satarug et al., 2009). However, increased Cd exposure can both upregulate and displace Zn from metallothionein, thereby altering Zn metabolism and distribution of Zn between blood and other tissues (Berglund et al., 2011; Chmielnicka and Sowa, 1996; Honda et al., 1990).

Though these metals can be excreted through a variety of methods (e.g. feces and eggs), feathers have long been studied as another excretory route for metals and an important biomarker of metal concentrations circulating in the body during feather growth (Furness et al., 1986; Goede and DeBruin, 1986; Monteiro and Furness, 1995). During feather growth, metals circulating in the blood, due to diet or tissue store mobilization, are incorporated into the keratin structures of feathers (Markowski et al., 2014), meaning that feather concentrations of heavy metals reflect blood concentrations at the time of their growth and remain stable after (Jaspers et al., 2004). Though some studies indicate that Pb and Cd can continue to increase after feathers stop growing, this is due to exogenous contamination (Bianchi et al., 2008), which is minimized in the present study through acetone and water rinses that preceded chemical analysis. However, since molt takes place over a period of several weeks, a significant amount of metal can be deposited in feathers through reuptake into blood from tissues. In fact, feathers can act as a metal sink, with higher concentrations of metals found in growing feathers than blood at any particular time (Burger and Gochfield, 1992) and internal tissue concentrations of metals can drop as metals are sequestered into feathers (Dauwe et al., 2003).

The analyses for Zn concentrations in blood revealed patterns that were notably different from those for Zn in feathers and Pb in blood. Unlike all the other conditional inference trees, the primary split occurred between the different migratory strategies, with Residents and Winter Migrants being grouped separately from Summer Migrants. While the secondary partition in the tree occurred along site lines, it showed that the Reference and Downstream sites had higher Zn concentrations in general than the other sites, which is very different from the feather results, which showed higher concentrations at the Roadside and Mineral Heights locations. The contrast between the results from the feathers and blood is likely due to feathers being indicative of longer-term exposure and blood being more indicative of more recent exposure (Golden et al., 2003).

The homeostatic control of Zn discussed above, as well as the effects of Cd on Zn transport and uptake in tissues could be a potential explanation for the unexpected patterns noticed in the blood Zn concentrations. Though the blood Cd concentrations primarily fell outside of the MLOD, the feather concentrations indicate that there was increased Cd exposure at all of the non-Reference sites and primarily at the Roadside sampling locations. This could have affected the blood concentrations of Zn through altering its transport with metallothionein. Additionally, Markowski et al., 2014 posits that higher Cd could affect Zn sequestration in feathers, which could offer additional explanation for the notable differences between the patterns of Zn in blood and Zn in feathers. However, a recent study in white-footed mice (*Peromyscus leucopus*) at the TCSFS showed similar results of Zn not being higher within the TCSFS than at a reference site but also determined that there was not upregulation of Metallothionein-1 within the TCSFS (Elturki, 2025), though this may not be directly comparable since these results were from liver tissues of a mammal.

Zn concentrations in wildlife can also be affected by agricultural practices, which may be the case here since the Reference locations were in very rural, predominantly agricultural areas. Zn is often added to fertilizers to correct soil deficiencies (Nielsen, 2012) and also added to livestock feed to correct dietary deficiencies (Cai et al., 2025), which results in increased concentrations in manure that is added to agricultural fields (Foust et al., 2018). Pesticides such as Zn phosphide can also increase avian exposure to Zn (Jacob and Leukers, 2008). A recent paper investigating Zn concentrations in house sparrows also had results similar to the ones seen here, with increased Zn concentrations in tissues in rural areas (Haddadi et al., 2025).

These and other results show limited support for the migratory escape hypothesis. If the hypothesis were supported and migratory strategy played a large role in mitigating exposure to environmental pollutants, we would expect to see Migratory Strategy playing a stronger role in the structure of the conditional inference trees. The only contaminant and tissue that shows support for this hypothesis is Cd concentrations in feathers. In blood Zn, winter migrants and residents had higher concentrations than summer migrants, which does not directly support the migratory escape hypothesis but offers additional evidence that metal concentrations in blood increases with the amount of time spent at the TCSFS since the winter migrants were captured at a time in their annual cycle when they would have been at the TCSFS for longer than even the later group of summer residents (Figure 13). However, nonmigratory species were generally more likely to exceed toxicity thresholds in feathers (Tables 1-3), which shows some support for the migratory escape hypothesis. The unclear effect of migratory strategy is consistent with other studies that have investigated migratory escape as it relates to pollution (Cooper et al., 2017; Durkalec et al., 2022)

It is important to interpret our use of the innermost primary feather to evaluate heavy metal exposure at the TCSFS in light of the molt schedules of the birds we sampled. Feathers are inert tissue during most of the annual cycle, and heavy metals are integrated into feather tissue only when feathers are being grown, which happens in discrete periods during the annual cycle. Indigo Buntings replace primaries as part of the definitive basic molt or, in first year birds, the definitive pre-basic molt (Payne, 2020). This molt is initiated during or just after pre-breeding (fall) migration, with the first primary being among the first feathers replaced. Painted Buntings also replace primaries as part of the definitive basic molt (or definitive pre-basic molt in first year birds; Lowther et al., 2020). However, molt generally overlaps with migration or occurs as part of a molt-migration strategy (Lowther et al., 2020; Rohwer, 2013). Stable isotope analyses indicate that there is substantial carryover of the chemical signal from the breeding ground among the innermost primaries, even if they were molted elsewhere (Bridge et al., 2011)

For White-Throated Sparrows, there is a preformative molt that occurs on the wintering grounds in March, wherein the birds replace mostly head feathers as well as some tertials and remiges. However primary molt occurs on the breeding grounds, generally after breeding occurs. Therefore, it is interesting that we see elevated Pb levels in WTSP that were sampled in contaminated areas. We reason that heavy metals accumulated during the winter at TCSFS were sequestered within the bones, liver, and perhaps other living tissues until they could be effectively excreted into growing feathers. This finding agrees with other research that suggests birds use feather molt as one of the primary mechanisms for excreting toxins, and that toxins may accumulate and remain in living tissues for long periods in anticipation of the next opportunity to remove them through feather molt (Burger and Gochfield, 1992). However, this differs from Burger's other 1992 article that showed that feathers grown in a less contaminated area had lower metal concentrations, though these findings were for mercury in contour feathers. The same publication (Burger 1992) found no difference between Pb in those same birds, but neither set of feathers were grown in a known Pb-contaminated area.

While the species used in this study generally have similar niches and are all small passerines, slight differences between them may affect their uptake of heavy metals. In other studies, there have been links between a granivorous or omnivorous diet and higher feather Pb concentrations (Durkalec et al. 2022), which may account for some of the differences between species seen here. However, these birds are all primarily omnivorous and no stable isotope analysis was conducted to determine trophic levels. Notably, metal concentrations in CACH feathers at the Roadside sites exceeded these thresholds of toxicity at nearly double the rates that it was exceeded in other species (Tables 1-3), This may be due to the smaller size and higher relative metabolic rates of CACH, which could increase rates of metal uptake into tissues through their increased food intake relative to their size (Glazier, 2010). The same pattern was not exhibited in blood Pb and Zn concentrations, and in fact CACH had slightly lower concentrations of these metals in their blood than NOCA and TUTI. This could indicate some difference in seasonal metal uptake between these species, but could also be due to the small size of CACH (~10g for CACH vs ~45g for NOCA and ~22g for TUTI (Halkin et al., 2021; Mostrom et al., 2020; Ritchison et al., 2020)) necessitating that smaller blood samples be taken, which would lead to higher MLODs for that species.

Though the results discussed here show some substantial differences between study sites and species, there are some important limitations to the sampling procedures we used. One is that some species were either undetected or not caught at certain locations (CACH at Mineral Heights, PABU at Remediated and Downstream, WTSP at Remediated). These species were still included in analyses. However, the absence of two of the migratory species from the Remediated site could have an effect on the results of the conditional inference trees and the ANOVAs comparing each site. The absence of CACH at Mineral Heights could also mean that we're missing some important information about the differences between the two contaminated sites since at the Roadside site CACH were more likely to exceed thresholds of toxicity than other species.

It was also notable that PABU were completely undetected at two of our sampling locations and that their absence at the original reference site used in a previous study (LePage, 2020) necessitated sampling at additional reference locations. This is odd because these locations exhibit some of the key characteristics of PABU breeding habitat, such as having strips of woodland and shrubland interspersed with open fields (Lowther et al., 2020; Plappert et al., 2024). Their absence could be due to the entire sampling region being at the edge of the PABU breeding range (Lowther et al., 2020), but the reason for the selection of the highly-contaminated TCSFS by this species over nearby suitable habitat remains a mystery.

Another limitation of our sampling procedure is that the WTSP grouping and the winter migrant grouping contain the exact same individuals. This could have worsened nesting effects on the conditional inference trees and interfered with our interpretation of the ANOVAs since two of our methods of grouping individual birds had groups that were completely the same. Though DEJU, another winter migrant, were caught at Reference, Remediated, and Downstream, we were unable to capture any at the more contaminated sites despite seeing flocks in the areas in which we set up mist nets. Thus, we excluded them from analysis. For results of this study pertaining to winter migrants, it is important to interpret them with the caveat that they may be WTSP-specific and not applicable to all winter migrants.

The prevalence of blood samples that fell below the MLOD could also have an effect on the results of this study, as well as the interpretation of those results. For example, since the majority of the cadmium analyses in blood resulted in sub-MLOD values, we were unable to more concretely explore the connections between Cd and Zn circulation in these birds. Additionally, since the MLOD is dependent on the size of the blood sample, with a higher MLOD for smaller blood samples, there were more samples that fell below the MLOD for the smaller species used in this study.

Finally, the samples from the summer had to be analyzed in conjunction with an acid digestion process because they had become clotted before reaching the MSU VDL. Though we attempted to achieve uniformity in sampling protocol, this could have occurred due to the warm temperatures that these samples were taken under causing more rapid clotting of samples via both increased enzyme and platelet activity and more rapid evaporation (Valeri et al., 1995; Wolberg et al., 2004). Because of the difference in analytical methods, blood samples from the summer were reported as dry weight and converted to wet weight via Scanlon's (1982) conversion factor. This conversion factor, while broadly used across bird species (Franson and Pain, 2011; Hoffman et al., 2006; Schulwitz et al., 2015; Wayland et al., 1999; Wayland and

Scheuhammer, 2011), was derived from Mallards (*Anas platyrhynchos*), which may have slightly different ratios between wet and dry weight of tissues than the passerines used in this study.

Since many of the highest concentrations of heavy metals were observed at the Roadside sampling locations, it may indicate that the heavier vehicle traffic on the gravel roads and the mobilization of dust may be resulting in higher exposure to heavy metals by wildlife in the TCSFS. Much of this traffic is due to ongoing remediation efforts and the work of the Quapaw tribe to reduce the volume of exposed chat within the TCSFS. Every year, thousands of tons of chat are removed from chat piles and bases and sorted as either salable or non-salable material. Salable materials are removed from the TCSFS and further processed into materials, such as asphalt, and non-salable materials are brought to the Central Mill repository, which will be capped once cleanup efforts are completed. As of 2024, 9.6 million tons of mine waste and contaminated soil have been deposited at the Central Mill and over 2 million tons of chat have been sold to chat processors (US EPA, 2025). Many of the roads running through the TCSFS are unpaved, which contributes to the dust problem. Furthermore, studies have shown that the most toxic portions of the chat are the fine particles (Schaider et al., 2007), which are the most prone to becoming airborne (Zota et al., 2012). This finer particulate chat is usually sequestered in the middle of the chat piles, but it can be exposed by excavation, which happens during the chat removal stage of remediation.

Many measures are currently taken to reduce the amount of dust mobilized by the remediation, such as spraying the roads with water, particularly on dry days (US EPA, 2025). Dust reduction activities are also undertaken at chat excavation sites. Dust could further be reduced by paving unpaved roads and utilizing cover crops over chat piles and along roadsides, though these solutions are possibly either cost-prohibitive or otherwise impractical. It would be very difficult to find a cover crop that could thrive on the chat piles without extensive amounts of human intervention and it could also increase the difficulty in excavating the chat piles. Since the towns that were within the TCSFS no longer exist and most of the traffic is isolated to remediation efforts, justification for paving many of the roads is limited.

The unintended consequences of conservation work has been broadly discussed in terms of species introduction or reintroduction (Pearson et al., 2022) and in the context of dam removal (Bellmore et al., 2019). However, the unintended results of contaminant removal have been minimally studied and are likely highly context-dependent. Though the increased exposure to wildlife along the roads may be a negative by-product of that remediation work, it may be a necessary part of the long-term cleanup of the area.

Furthermore, the concentrations of metals in the tissues of birds along Tar Creek but outside of the core contaminated area indicate that some remediation efforts have been successful in reducing the exposure to hazardous metals downstream of the contaminated site. Though the metal concentrations in Tar Creek can change based on outflow rates from underground mines and recent precipitation events, the fact that feather concentrations of Pb and Zn were lower at the Remediated and Downstream site and even comparable to Reference values in terms of Zn at the Downstream site indicates that the cleanup efforts by both the Quapaw and the University of Oklahoma Center for Restoration of Ecosystems and Watersheds are having broadly positive effects wildlife exposure in the region.

While it may seem that birds have little to do with environmental justice, just monitoring exposure to environmental pollutants can help to mobilize action and legislation that can lead to the cleanup of polluted areas (Racz and Rish, 2021). For both humans and wildlife, there is no safe level of Pb exposure (Grandjean, 2010), meaning that any exposure to Pb could be hazardous to organisms' health. In fact, historical Pb pollution can lead to more than individual health effects, it can contribute to region-wide aggression and crime rates (Mielke and Zahran, 2012; Taylor et al. 2016).

Indigenous people are also often more affected by environmental pollution (Fernández-Llamazares et al. 2020), which holds true at this particular location. Ottawa county, Oklahoma, where the TCSFS is located, is home to nine Native American Tribes. Most of the mining occurred on land leased from the Quapaw tribe, with only one-sixth of the landowners in the tribe ever receiving lease payments or mining royalties despite most of the tribal members needing to contend with the environmental contamination caused by the mining (Everett, 2009). Additionally, children living in and near the TCSFS have higher Pb exposures than other children in the state, which could make them prone to learning disabilities and other negative health effects (Hu et al. 2007).

Over one hundred-thirty years of mining in Northeast Oklahoma has degraded the environment (Evertett, 2009), and the repercussions of this damage will continue for the coming decades. Though Pb is now widely acknowledged as an environmental hazard, It is imperative that Pb and other hazardous materials remain tightly regulated to minimize exposure to humans, wildlife, and ecosystems.

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FIGURES:

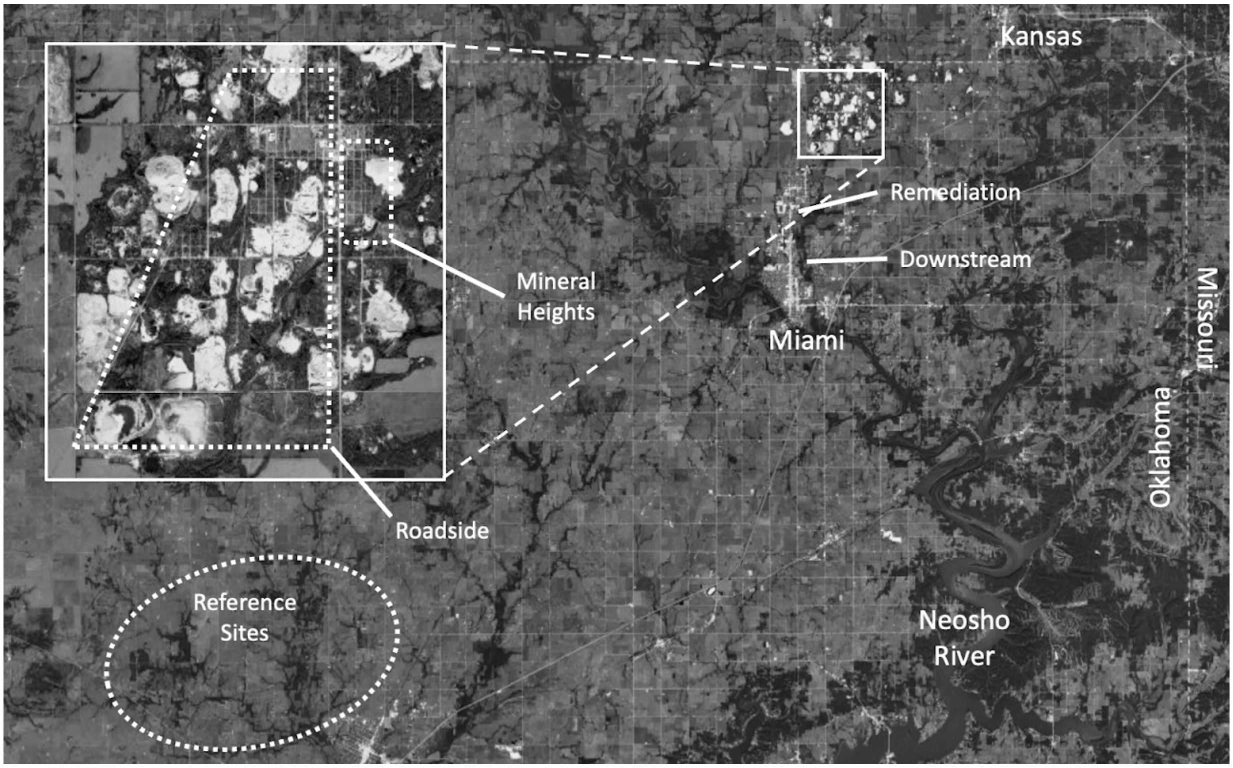

Figure 1:

Map showing the sampling locations used in this study. The Contaminated Sites encompass the former towns of Picher, Cardin, and Douthat, with Mineral Heights being a neighborhood of Pitcher. Tar Creek flows through the Contaminated Sites past the Remediation and Downstream sites through Miami into the Neosho River. Note that the light spots within the inlay are chat piles.

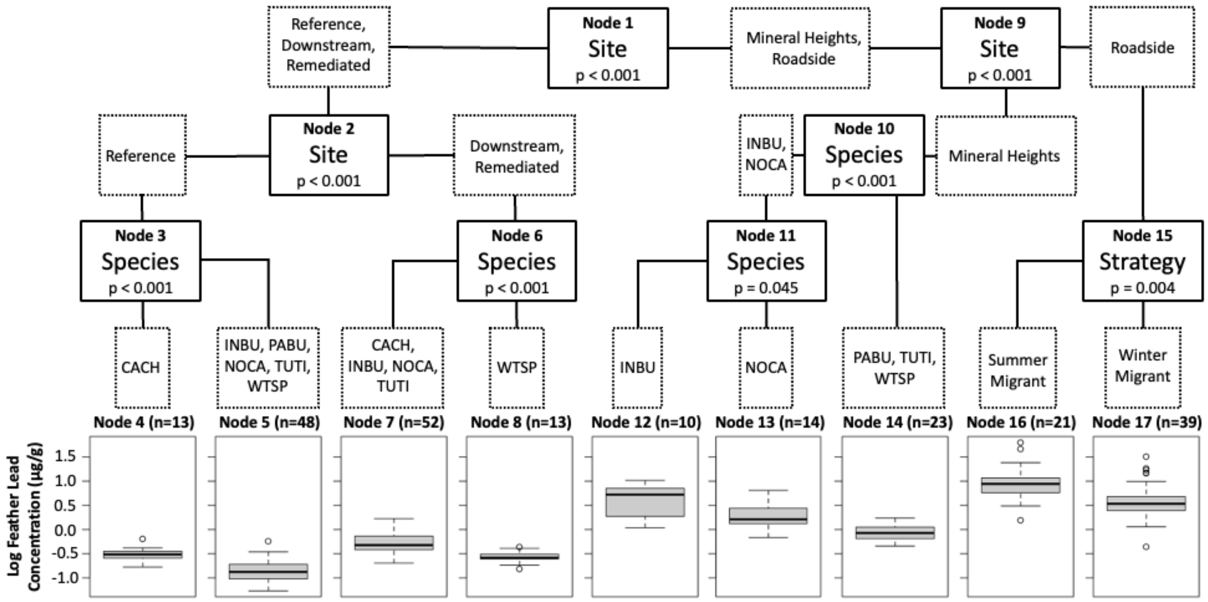

Figure 2:

The conditional inference tree structure of log-transformed feather Pb concentrations (µg/g). Factors being split are listed with each node, as well as the p-value for the comparison between the groups being split. Each grouping then undergoes additional splits before reaching terminal nodes. Earlier splits denote groups that explain more of the data structure than later splits. Terminal nodes include boxplots of the values included in the node, as well as the number of observations included in that node.

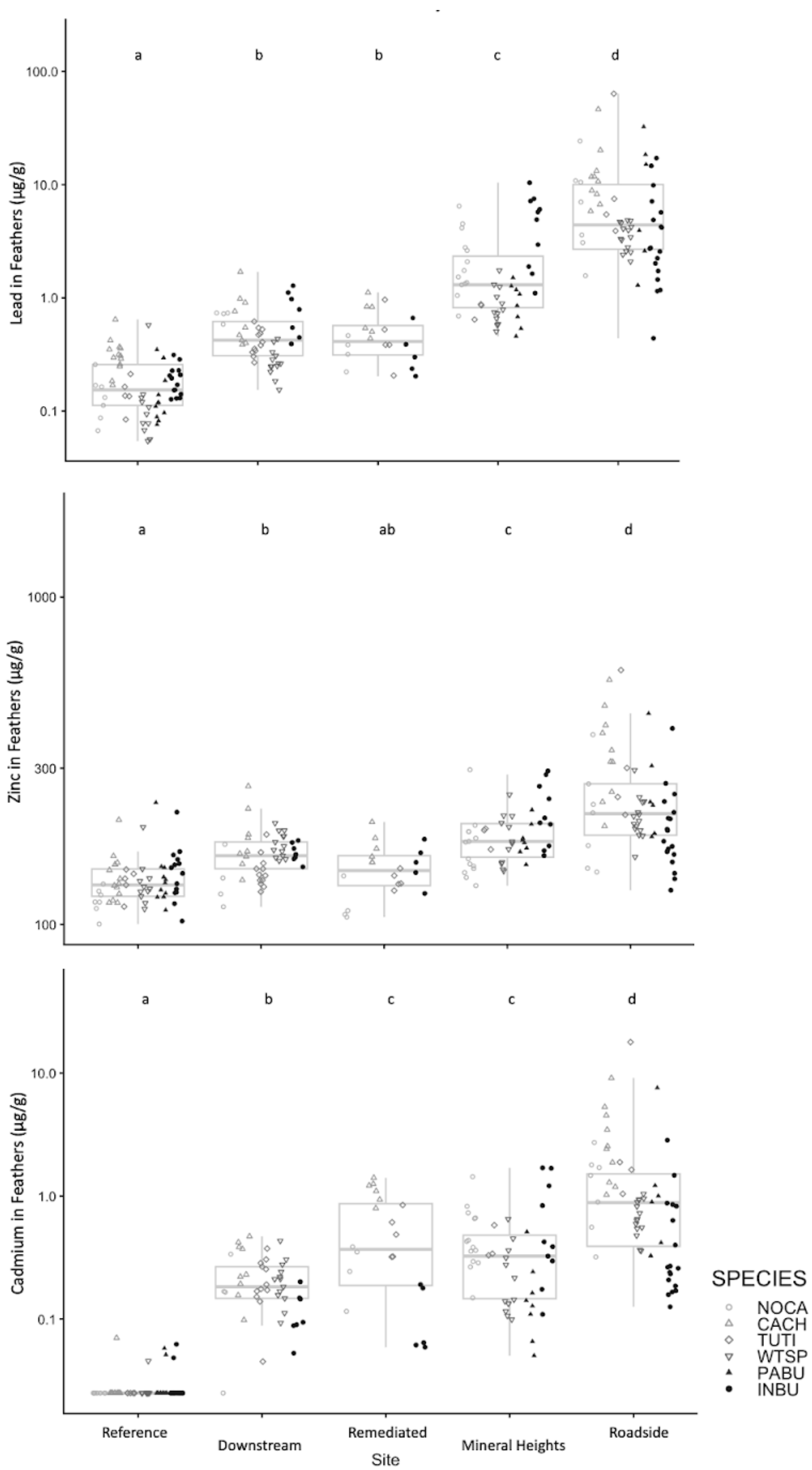

Figure 3:

Boxplots showing the concentration of Pb, Zn, and Cd in feathers (µg/g) by study site. All datapoints are shown with shape denoting the species. Connecting letters as a result of the

Tukey's HSD test are included at the top of each graph, sites that are significantly different from each other are labeled with separate letters. Letters are assigned in alphabetical order starting with the group with the lowest mean.

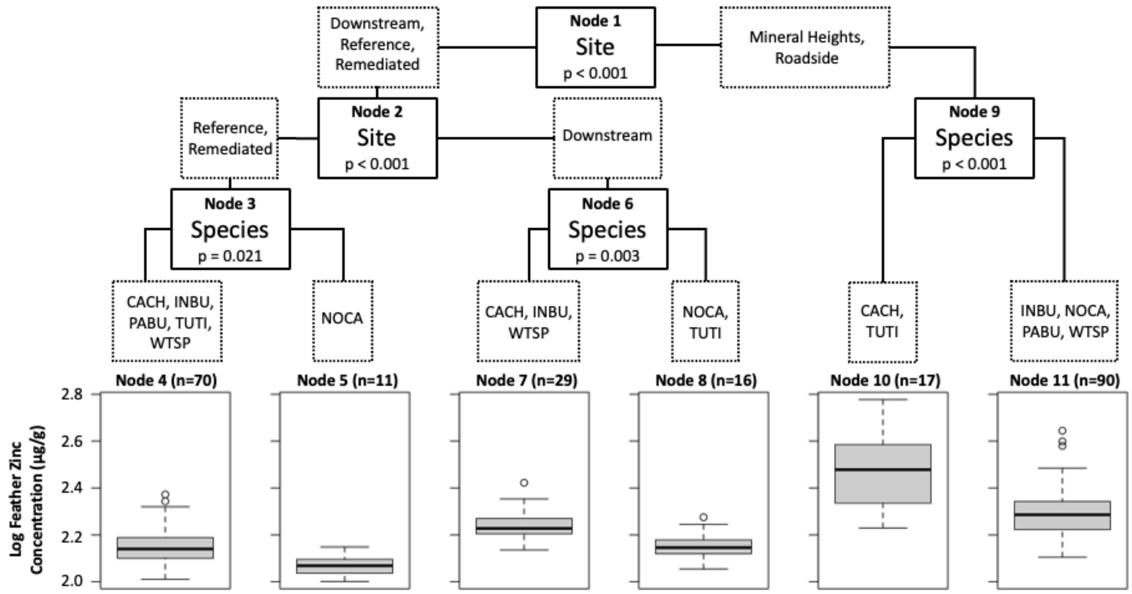

Figure 4:

The conditional inference tree structure of log-transformed feather Zn concentrations (µg/g). Factors being split are listed with each node, as well as the p-value for the comparison between the groups being split. Each grouping then undergoes additional splits before reaching terminal nodes. Earlier splits denote groups that explain more of the data structure than later splits. Terminal nodes include boxplots of the values included in the node, as well as the number of observations included in that node.

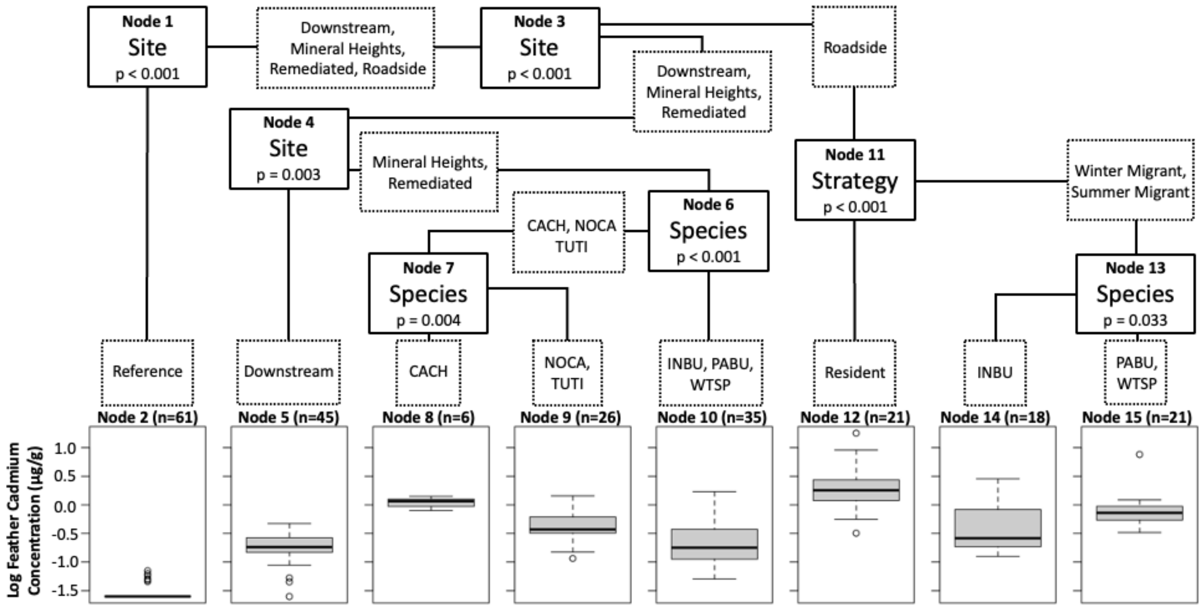

Figure 5:

The conditional inference tree structure of log-transformed feather Cd concentrations (µg/g). Factors being split are listed with each node, as well as the p-value for the comparison between the groups being split. Each grouping then undergoes additional splits before reaching terminal nodes. Earlier splits denote groups that explain more of the data structure than later splits. Terminal nodes include boxplots of the values included in the node, as well as the number of observations included in that node.

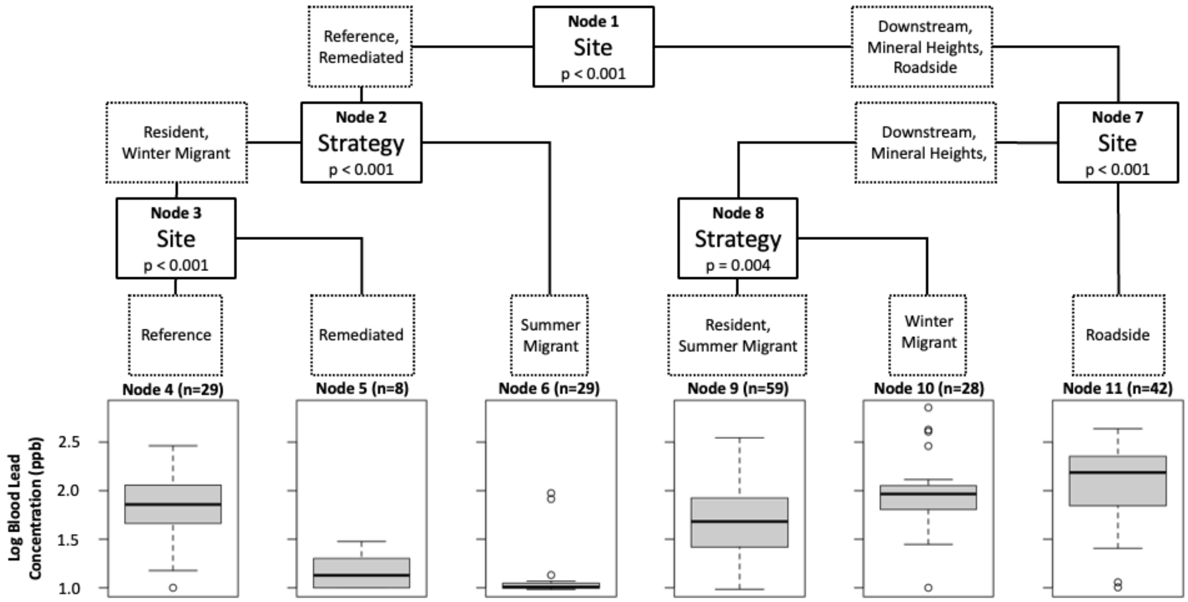

Figure 6:

The conditional inference tree structure of log-transformed blood Pb concentrations (ppb). Factors being split are listed with each node, as well as the p-value for the comparison between the groups being split. Each grouping then undergoes additional splits before reaching terminal nodes. Earlier splits denote groups that explain more of the data structure than later splits. Terminal nodes include boxplots of the values included in the node, as well as the number of observations included in that node.

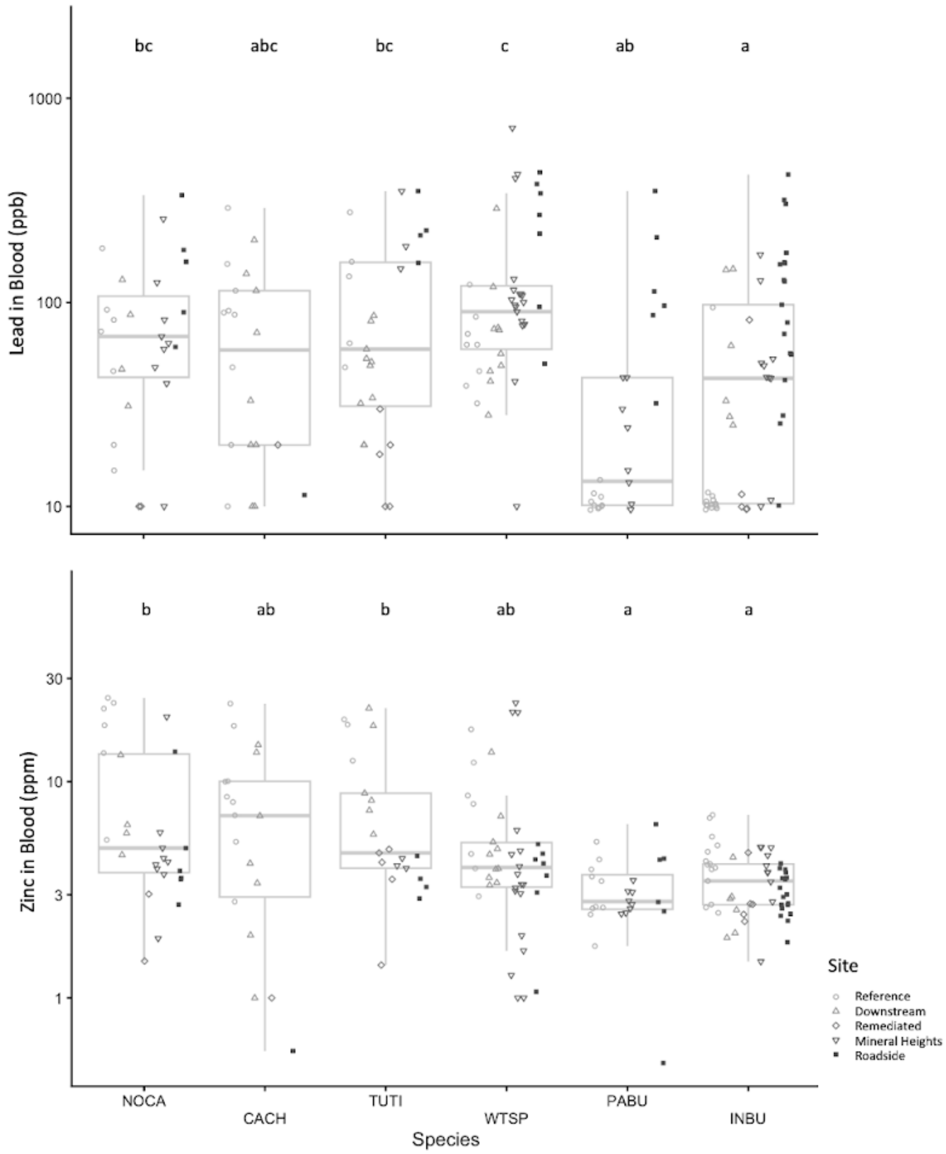

Figure 7:

Boxplot showing the concentration of Pb (ppb) and Zn (ppm) in blood by species. All datapoints are shown with shape denoting the study site. Connecting letters as a result of the Tukey's HSD test are included at the top of the graph, species that are significantly different from each other are labeled with separate letters. Letters are assigned in alphabetical order starting with the group with the lowest mean.

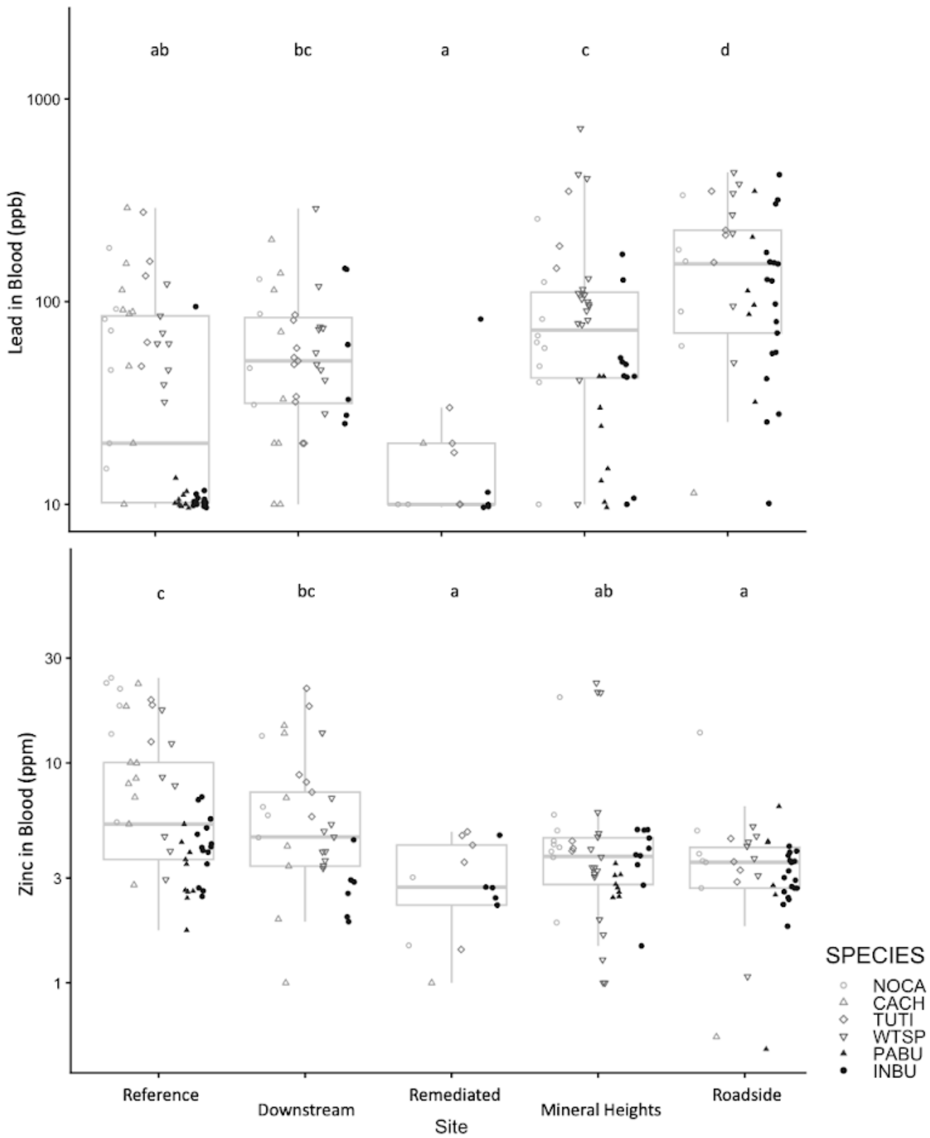

Figure 8:

Boxplot showing the concentration of Pb (ppb) and Zn (ppm) in blood by study site. All datapoints are shown with shape denoting the species. Connecting letters as a result of the Tukey's HSD test are included at the top of the graphs, sites that are significantly different from each other are labeled with separate letters. Letters are assigned in alphabetical order starting with the group with the lowest mean.

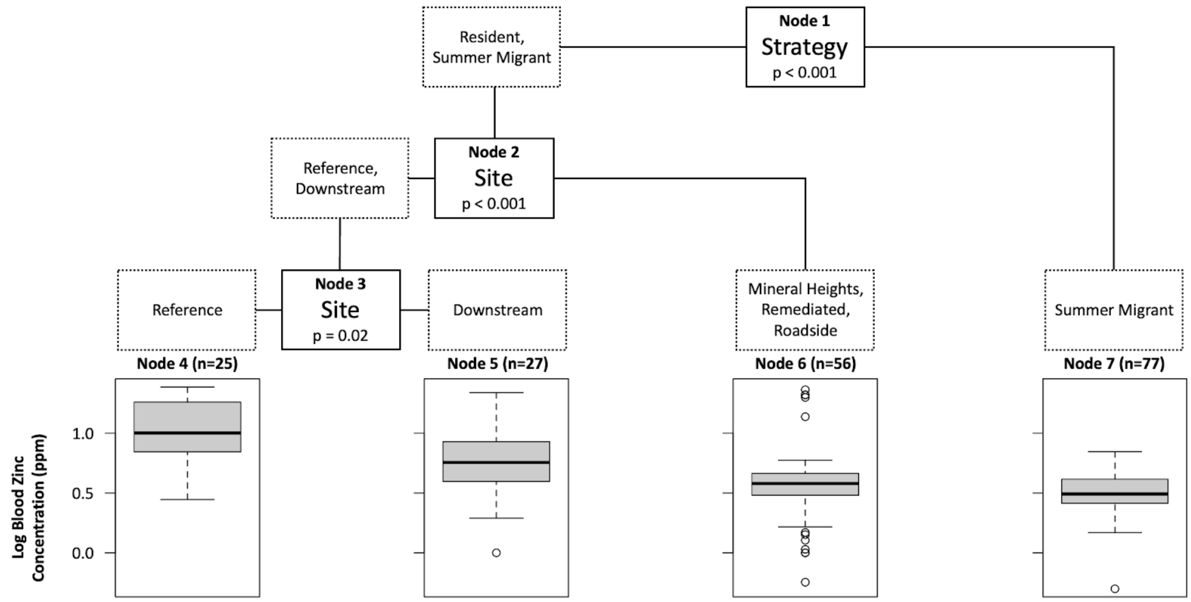

Figure 9:

The conditional inference tree structure of log-transformed blood Zn concentrations (ppm). Factors being split are listed with each node, as well as the p-value for the comparison between the groups being split. Each grouping then undergoes additional splits before reaching terminal nodes. Earlier splits denote groups that explain more of the data structure than later splits. Terminal nodes include boxplots of the values included in the node, as well as the number of observations included in that node.

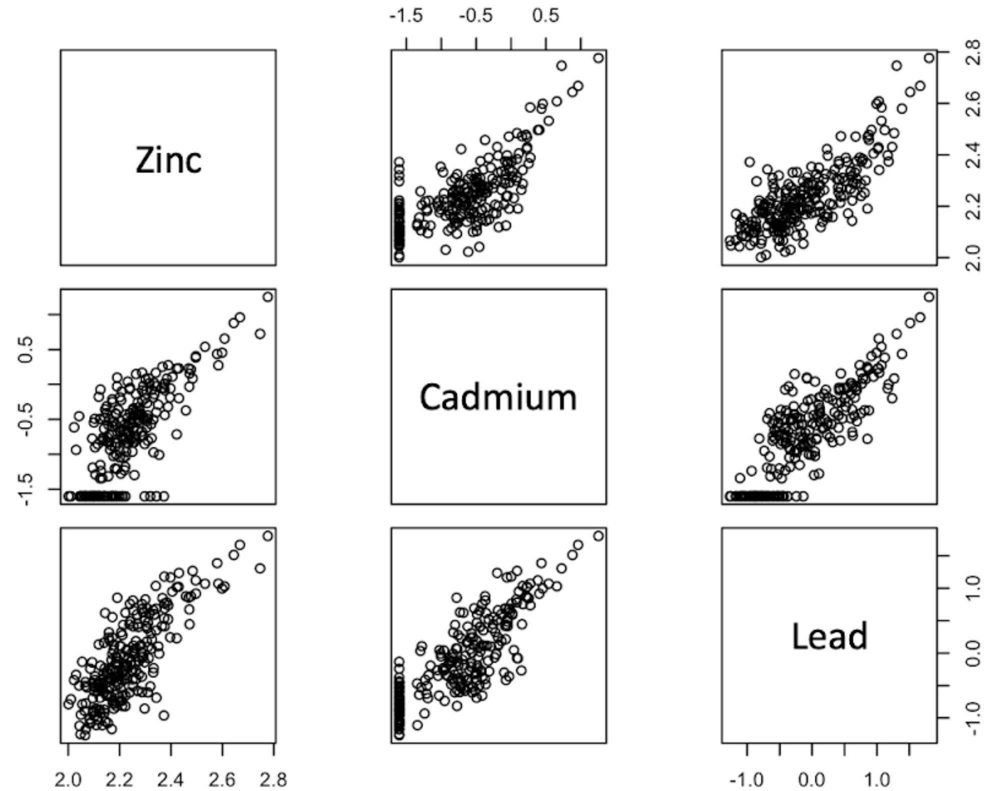

Figure 10:

Correlations between log-transformed Zn, Cd, and Pb concentrations (µg/g) in feathers.

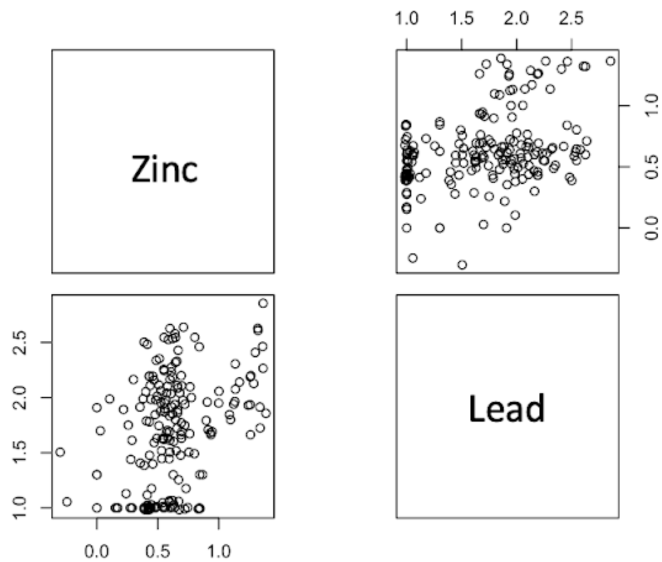

Figure 11:

The correlations between log-transformed Pb (ppb) and Zn (ppm) measured in blood

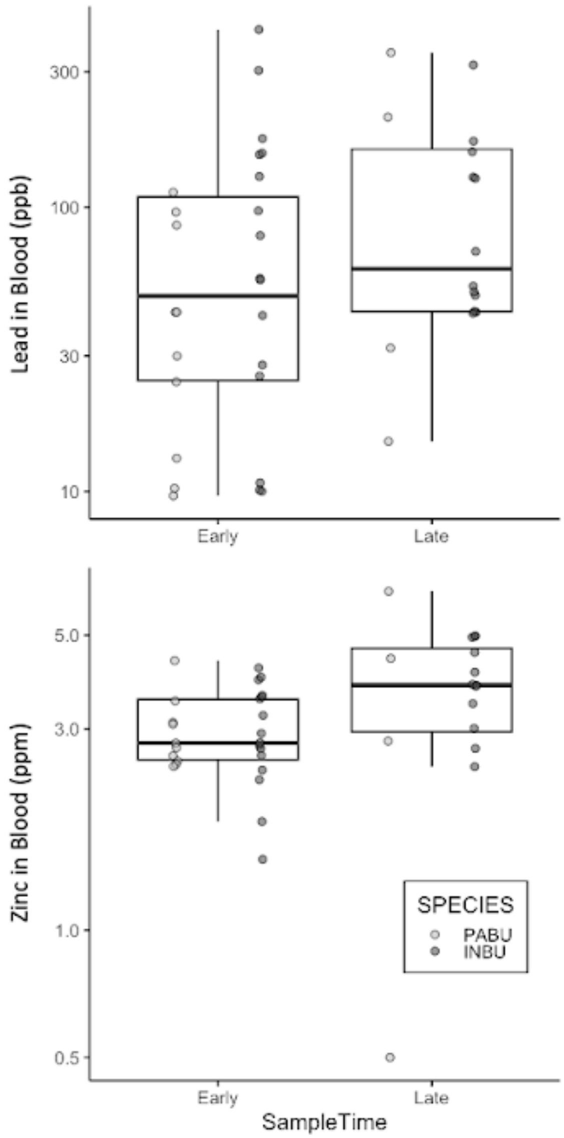

Figure 12:

Boxplot showing the comparison of Pb (ppb) and Zn (ppm) in blood of PABU and INBU from an early (May-Early June) and late (mid-July) timepoint in the summer. Note the low outlier for the late summer sample.

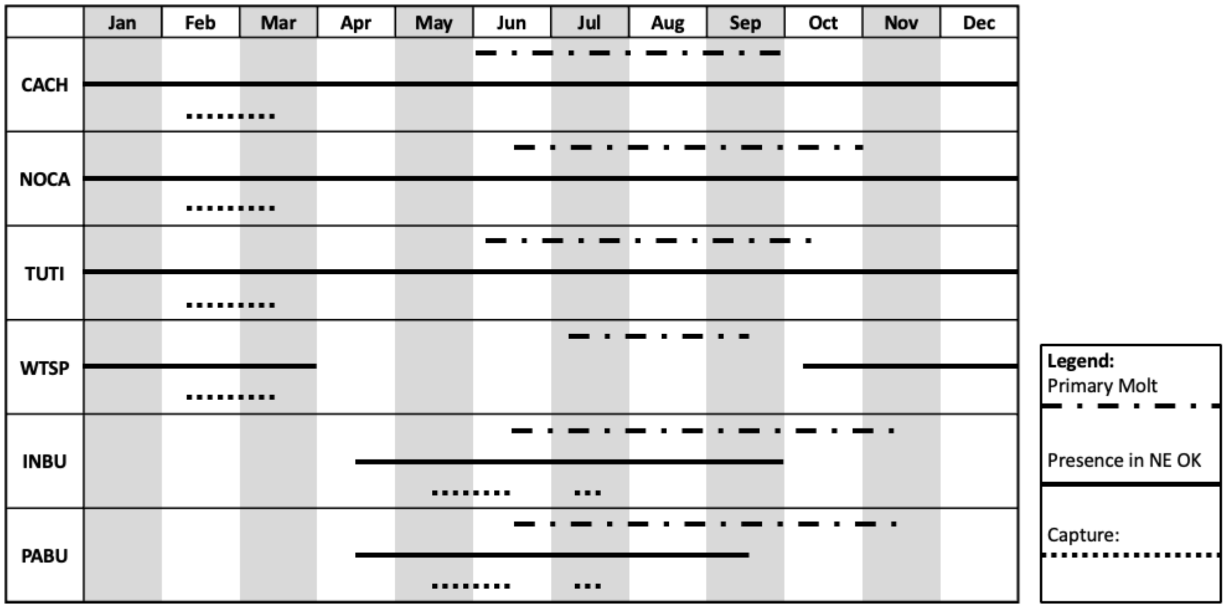

Figure 13:

Graphic showing the general time of year that each species in this study undergoes its primary molt, when it is present in the TCSFS, and when we sampled individuals of it as part of this study. Molt and migratory information obtained from the Cornell Lab of Ornithology's Birds of the World account for each species: CACH (Mostrom et al., 2020), NOCA (Halkin et al., 2021), TUTI (Ritchison et al., 2020), WTSP (Falls and Kopachena, 2020), INBU (Payne, 2020), PABU (Lowther et al., 2020).

TABLES:

Proportion of Feather Samples Over 4µg/g Pb							
	CACH	NOCA	TUTI	WTSP	INBU	PABU	**All Species**
Roadside	1.00	0.57	0.75	0.40	0.44	0.57	**0.57**
Mineral Heights	N/A	0.21	0	0	0.60	0	**0.19**
Downstream	0	0	0	0	0	N/A	**0**
Remediated	0	0	0	N/A	0	N/A	**0**
Reference	0	0	0	0	0	0	**0**
All Sites	**0.26**	**0.19**	**0.10**	**0.12**	**0.26**	**0.12**	**0.18**

Table 1:

Proportion of feather Pb concentrations from each species and site that exceed the threshold for toxicity.

Proportion of Feather Samples Over 2µg/g Cd							
	CACH	NOCA	TUTI	WTSP	INBU	PABU	**All Species**
Roadside	0.60	0.14	0.25	0	0.06	0.17	**0.17**
Mineral Heights	N/A	0	0	0	0	0	**0**
Downstream	0	0	0	0	0	N/A	**0**
Remediated	0	0	0	N/A	0	N/A	**0**
Reference	0	0	0	0	0	0	**0**
All Sites	**0.16**	**0.03**	**0.03**	**0**	**0.02**	**0.04**	**0.04**

Table 2:

Proportion of feather Cd concentrations from each species and site that exceed the threshold for toxicity.

Proportion of Feather Samples Over 350μg/g Zn							
	CACH	NOCA	TUTI	WTSP	INBU	PABU	**All Species**
Roadside	0.40	0.14	0.25	0	0.05	0.17	**0.13**
Mineral Heights	N/A	0	0	0	0	0	**0**
Downstream	0	0	0	0	0	N/A	**0**
Remediated	0	0	0	N/A	0	N/A	**0**
Reference	0	0	0	0	0	0	**0**
All Sites	**0.10**	**0.03**	**0.03**	**0**	**0.04**	**0.02**	**0.03**

Table 3:

Proportion of feather Zn concentrations that exceed concentrations found at non-industrialized areas.

Proportion of Blood Samples Over 200ppb Pb							
	CACH	NOCA	TUTI	WTSP	INBU	PABU	**All Species**
Roadside	0	0.20	0.75	0.71	0.17	0.33	**0.34**
Mineral Heights	N/A	0.11	0.33	0.17	0	0	**0.10**
Downstream	0.11	0	0	0.10	0	N/A	**0.05**
Remediated	0	0	0	N/A	0	N/A	**0**
Reference	0.11	0	0.2	0	0	0	**0.04**
All Sites	**0.10**	**0.07**	**0.19**	**0.21**	**0.06**	**0.08**	**0.04**

Table 4:

Proportion of blood Pb concentrations from each site and species that falls above a possible threshold for toxicity.

Proportion of Blood Samples Over 14.2ppm Zn							
	CACH	NOCA	TUTI	WTSP	INBU	PABU	**All Species**
Roadside	0	0	0	0	0	0	**0**
Mineral Heights	N/A	0.11	0	0.17	0	0	**0.08**
Downstream	0.14	0	0.33	0	0	N/A	**0.09**
Remediated	0	0	0	N/A	0	N/A	**0**
Reference	0.22	0.67	0.67	0.14	0	0	**0.18**
All Sites	**0.17**	**0.19**	**0.19**	**0.10**	**0**	**0**	**0.09**

Table 5:

Proportion of blood Zn concentrations for each site and species that falls above a possible threshold of toxicity.