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RESEARCH ARTICLE

Trace elements and heavy metals in black vultures (*Coragyps atratus*) and turkey vultures (*Cathartes aura*) in the southeastern United States

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Abstract

Many vulture species worldwide are declining at alarming rates due to a variety of anthropogenic causes, including exposure to pollutants and pharmaceuticals through consumption of contaminated carrion. However, little is known about the extent to which vultures are exposed to various contaminants as well as toxicity thresholds for trace elements and heavy metals. Our objective was to quantify levels of trace elements and heavy metals within black vulture (*Coragyps atratus*) and turkey vulture (*Cathartes aura*) tissues to determine the extent to which populations in the Southeastern United States are exposed to carrion that contains high levels of contaminants. We collected 34 black vulture liver samples and examined differences in trace element and heavy metal concentrations between sexes and age classes (adult and juvenile). Further, we collected 81 blood and 42 feather samples from additional black and turkey vultures and compared differences between species and age classes. We found similar element concentrations between juvenile and adult black vultures with the exception of Cu, where levels were higher in juveniles compared to adults. However, we did observe substantial differences in element concentrations between species for both blood and feather samples, with black vultures generally having higher concentrations of most elements. Our data revealed higher element levels in both species compared to toxicity thresholds found in other bird of prey species, such as blood and liver toxicity threshold suggestions for Pb poisoning in Falconiformes. Further, while average contaminant levels were generally low, extreme outliers were observed for some elements, including Pb, suggesting some individuals were exposed to high levels of potentially toxic elements. More research is needed to better understand contaminant exposure in black and turkey vultures across a broader geographic region, as well as elucidate toxicity thresholds and non-lethal impacts of contaminant exposure in these species.

Keywords Avian health · Contaminants · Environmental toxicants · Exposure · Scavengers · Wildlife health

Introduction

Vultures are a globally distributed taxa consisting of long-lived obligate carrion feeders that occupy high trophic positions within terrestrial food webs (Buckley et al. 2022). As obligate scavengers, vultures rely almost entirely on carrion as a food source. As a result, vultures and other scavengers fill necessary ecological roles within the environment and facilitate nutrient distribution, altered disease dynamics, linkage of food webs, and removal of carrion from the environment (Beasley et al. 2015; DeVault et al. 2016; Kirk and Mossman 2020; Buckley et al. 2022). For example, in the absence of vultures, carrion resides in the landscape longer, allowing time for more mammal scavengers to visit and consume carrion which could increase the transfer of disease between mammals and, subsequently, humans (Ogada et al. 2012b). However, as a group, vultures are currently facing

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numerous extinction threats, with 72% of vulture species globally experiencing population declines and over half considered endangered or critically endangered (Ogada et al. 2012a; IUCN 2022). The decline in vulture populations is largely due to human activities and the bioaccumulation of anthropogenic contaminants introduced into the environment (Ogada et al. 2012a; DeVault et al. 2016; Plaza and Lambertucci 2019; Ives et al. 2022). Indeed, 95% of reported deaths in threatened or near-threatened vulture species have been linked to dietary toxins (Buechley and Sekercioglu 2016).

Since vultures occupy a high trophic level and feed on carrion, they are especially vulnerable to exposure to heavy metals, trace elements, and other environmental contaminants that can bioaccumulate in their tissues and create health problems such as lead toxicosis and even mortality (Ogada et al. 2012b; Yordy et al. 2013). Further, many vulture species are communal feeders, which can result in multiple individuals being exposed to contaminants from a single contaminated carcass (Buckley 1996; Plaza and Lambertucci 2019). Vultures also have relatively long lifespans, allowing time for toxins to build up to greater levels compared to shorter-lived scavengers (Gangoso et al. 2009). Declines in vulture populations due to bioaccumulation of dietary toxins introduced via carrion can be found worldwide (Ogada et al. 2012b; Ogada et al. 2016). This bioaccumulation can limit individual reproductive success, immune responses, and life span. For example, the drastic decrease in vulture populations in India has been attributed to consumption of livestock treated with veterinary drug diclofenac (Ogada et al. 2012b). Similarly, vulture populations in Africa have declined due to both intentional killings (poachers poisoning leftover kills to avoid the attention generated by circling vultures), unintentional killings (feeding on carcasses poisoned by farmers to kill livestock predators), traditional medicine, and other anthropogenic causes (Ogada et al. 2016).

Within North America, three species of vultures occur (black vulture (*Coragyps atratus*), turkey vulture (*Cathartes aura*), and the California condor (*Gymnogyps californianus*)), all of which experience threats to their populations, such as habitat loss, microtrash consumption, trapping and shooting, aircraft collisions, utility line collisions, wildfire, and exposure to contaminants (such as lead, mercury, and DDT) (Parmalee 1954; Wilbur 1978; Kiff 2000; Blackwell and Wright 2006; Walters et al. 2010; Ogada et al. 2012b; Kelly et al. 2015). In particular, California condors experienced such severe threats and declining populations throughout the nineteenth and twentieth centuries that by 1987 they were considered extinct in the wild and required human intervention through intensive captive breeding programs in efforts to increase populations (Walters et al. 2010). While all of the aforementioned threats contributed to this occurring, the impact of the introduction of DTT into the landscape and lead poisoning through ingestion of lead fragments was a leading cause in declining populations (Finkelstein et al. 2012; Tubbs 2016). Condor populations have since increased due to intensive recovery efforts and consistent intervention to mitigate lead toxicosis, although exposure to lead and other contaminants remain the primary threat to the recovery of this species (Johnson et al. 2014; Finkelstein et al. 2020). Similarly, although black vultures and turkey vultures are listed as species of least concern on the IUCN Red List and have seen population increases since the late 1900s, contaminant exposure remains a threat and there is little research regarding the current impact of contaminants on either species (IUCN 2022).

Black vultures range throughout the Southeastern United States but recently have expanded their range into the Northeastern and Midwestern United States (Kluever et al. 2020). Turkey vultures share a similar distribution but extend as far west as California and as far north as southern Canada (Kiff 2000). This increase in range coupled with the increasing human population means there is a greater chance for contaminant exposure from anthropogenic sources such as landfills (Novaes and Cintra 2015; Borges-Ramírez et al. 2021). Both vulture species have been able to adapt to the growing human population in the USA and to anthropogenic changes in their environment, roosting closer to roads to forage on roadkill and foraging at landfills (Prior 1990; Holland et al. 2019; Hill et al. 2021). Thus, there is a critical need to quantify vulture exposure to myriad contaminants to better elucidate and mitigate risks to expanding populations.

Although there have been studies exploring the effects of long-term exposure to certain heavy metals such as lead in avian predators (Behmke et al. 2015), there is little information on toxic thresholds for many elements and contaminants of concern for vultures. Further, while exposure to heavy metals and trace elements can ultimately lead to death, it can also lead to less apparent health problems such as bone mineralization, reduced muscle and fat concentrations, organ damage, and internal lesions (Beasley et al. 2015). Heavy metals and trace elements can accumulate in various tissues of birds, and certain elements tend to bioaccumulate within specific organs of the body. For example, elements such as cadmium accumulate mostly in the liver and kidneys, while others, such as lead, mainly accumulate in bones (Scheuhammer 1987). The liver usually contains higher heavy metal concentrations because metals are transported to the liver before other soft tissue such as the kidneys (Ek et al. 2004). However, for species that are protected or not routinely harvested, like vultures, assessments of organ contaminant concentrations may not be feasible, and thus, there is a critical need to quantify relationships between lethally collected samples (e.g., liver, kidney, bones) and non-lethal samples (e.g., blood, feathers) to evaluate the efficacy of non-lethal samples in quantifying contaminant levels.

The objective of this study, therefore, was to quantify levels of a broad suite of trace elements and heavy metals within various tissues of black and turkey vultures to determine the extent to which populations in the Southeastern United States are exposed to contaminants. We compared levels of trace elements and heavy metals between age classes and species for both black and turkey vulture blood and feather samples and age class and sex for black vulture liver tissue samples. We predicted adult black vulture liver samples would have higher levels of trace elements and heavy metals, especially elements that bioaccumulate in the liver such as Cd, Pb, Cr, and Hg, compared to juveniles due to the long-term bioaccumulation within an individual's soft tissue over their life span (Yordy et al. 2013; Ozaki et al. 2023). We also predicted female black vultures would have higher levels of trace elements and heavy metals compared to males due to their more varied foraging habits throughout their reproductive cycle (Negro et al. 2002; Holland et al. 2017). As black vultures have foraging habits that have the potential to expose multiple individuals to contaminants (such as being communal feeders and foraging at landfills) and often feed on larger carrion (Byrne et al. 2019), we predicted black vultures would have higher levels of trace elements and heavy metals compared to turkey vultures (Coleman and Fraser 1987; Buckley et al. 2022).

Materials and methods

Study area

Blood and feather samples were collected from black and turkey vultures live-captured at the Savannah River Site (SRS), located near Aiken, South Carolina, along the eastern border of Georgia. The SRS is a limited-access nuclear research facility that is owned and maintained by the United States Department of Energy. The SRS is primarily forested but has multiple industrial facilities on the property, such as landfills and decommissioned nuclear reactors. Forested areas within SRS included planted pine forests, upland and bottomland hardwood forests, and wetlands, with abundant populations of numerous mid- to large-sized mammals such as white-tailed deer (*Odocoileus virginianus*), wild pigs (*Sus scrofa*), raccoons (*Procyon lotor*), and opossums (*Didelphis virginiana*) (White and Gaines 2000). Despite having these facilities on site, the majority of the SRS is undisturbed. In addition, liver samples were collected opportunistically from black vultures captured throughout South Carolina and Florida by nuisance control operators.

Sample collection

Blood and feather samples were collected in the summer of 2013 and the spring of 2014 at the Savannah River Site (SRS) following the methods outlined in Holland et al. (2017). Briefly, black and turkey vultures were trapped using a compressed-air-powered cannon net over a carrion bait site. We used wild pig carrion for this study, as wild pigs are removed year-round on the SRS (VerCauteren et al. 2020) and commonly consumed by both black and turkey vultures (Turner et al. 2017). Samples were taken from each bird, and age class (determined by coloration and wrinkling of the head), body condition, location, collection date, and handling time were recorded (Kirk and Mossman 2020; Buckley et al. 2022). We were unable to distinguish between males and females for live-trapped individuals and thus sex was not included in any analyses of blood and feather samples. One to three feathers were taken directly from the breast area and stored in Whirl-Pak® bags (Whirl-Pak®, Madison, WI, USA). Approximately 20 mL of blood was taken in trace-metal-grade heparin tubes to avoid clotting and frozen upon return to the lab. All vulture capture and handling was conducted in accordance with the University of Georgia Animal Care and Use Committee under protocol no. A2013 02-004-Y2-A2, and samples were collected with approval of appropriate state and federal permits. Liver samples were collected in 2020 during necropsies of black vultures euthanized as a part of a nuisance control program in South Carolina and Florida conducted by the United States Department of Agriculture, Animal Plant Health Inspection Service, Wildlife Services. No vultures were euthanized specifically for the purposes of this research. We determined the sex of all necropsied vultures.

Sample preparation

Blood samples were weighed and transferred into a metal-grade centrifuge tube and spun down for 5 s. The samples were then placed into a −80 °C freezer until solidified and then freeze-dried. Feather samples were weighed and cleaned with a 5% Citranox dilution and rinsed with Milli-Q® water to clean residue and debris from the feather that would potentially affect contaminant levels (Sigma-Aldrich, St. Louis, MO, USA). Feather samples were then freeze-dried, cut into smaller pieces to homogenize the sample, and then weighed again. All the original feather sample was used for analysis. Liver samples were weighed, freeze-dried, ground to homogenize, and then weighed again.

Trace element analysis—blood and feather

Blood and feather samples were analyzed for the following trace elements: vanadium (V), chromium (Cr), manganese

(Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), strontium (Sr), lead (Pb), mercury (Hg), cadmium (Cd), thorium (Th), and uranium (U). To analyze for all elements except Hg, feather and blood samples were placed into a 75–85 °C sand bath for 5 min. Each sample then received 1 mL of HNO_3 and 1 mL of 30% hydrogen peroxide while remaining in the sand bath. After 1 h, another 1 mL of HNO3 was added, and the samples were digested for an additional 30 min, or until fully digested (fully liquidized). Once the samples were digested, 3 mL of Milli-Q was added and diluted 1:10 in new tubes; this made the final sample acid concentration 5%. The samples were then analyzed using inductively coupled plasma mass spectrometry (ICP-MS) (Nexlon 300x ICP-MS; Perkins Elmer, Norwalk, CT, USA). Two blank samples and two certified reference material samples (CRM; TORT-3, National Research Council of Canada, Ottawa, ON) were included with each digestion for quality control to calibrate the machine and eliminate any possibility of non-conforming results. Method detection limits (MDLs) for each element were calculated using dry mass weights and are as follows: V = 0.0714 mg/kg, Cr = 0.0755 mg/kg, Mn = 0.1087 mg/kg, Fe = 7.7226 mg/kg, Co = 0.0738 mg/kg, Ni = 0.2207 mg/kg, Cu = 0.2967 mg/kg, Zn = 0.2402 mg/kg, As = 0.0915 mg/kg, Se = 0.7553 mg/kg, Sr = 0.0934 mg/kg, Cd = 0.0725 mg/kg, Th = 2.2146 mg/kg, Pb = 0.0682 mg/kg, and U = 0.0847 mg/kg.

Trace element analysis—liver

We analyzed liver samples for the following trace elements: Hg, Ni, Cu, Zn, As, Se, Cd, Pb, and U. To analyze for all elements except Hg, liver samples were microwave digested (MARSX Xpress, CEM, Corporation, Matthews, NC, USA) using 250 mg of the liver sample and 10.0 ml of 70% nitric acid following EPA method 3052 (USEPA 1996). Once the liver sample was digested, it was transferred to a 15-mL tube and 5 mL of Milli-Q water was then added, bringing the volume to 15 mL. The samples were then analyzed at the Savannah River Ecology Laboratory using ICP-MS (Nexlon 300x ICP-MS; Perkins Elmer, Norwalk, CT, USA) with a 1:10 dilution factor (1.0 mL sample to 9.0 mL Milli-Q water). MDLs for each element were calculated on a dry mass basis and are as follows: Ni (0.0546 mg/kg), Cu (0.0893 mg/kg), Zn (0.127 mg/kg), Cd (0.0109 mg/kg), Pb (0.0114 mg/kg), U (0.0117 mg/kg), As (0.0489 mg/kg), and Se (1.68 mg/kg). CRM, acid blanks, and sample duplicates were included with each digestion for quality control to eliminate any possibility of non-conforming results (TORT-3, National Research Council of Canada, Ottawa, ON). Percent recovery of CRM between 80 and 120% are considered quality and are included in the data set. If samples were not within that range, they were reanalyzed to ensure correct recovery measurements and taken out of the data set if the recovery came back out of range again.

Mercury analysis

Mercury (Hg) was analyzed following the same protocol for all three sample types. We weighed 10–15 mg of each of the freeze-dried sample for direct Hg analysis (DMA-80; Milestone, Shelton, CT, USA) following EPA method 7473 (USEPA 1998). Each sample was subjected to thermal decomposition, catalytic conversion, amalgamation, and atomic absorption spectrophotometry using the direct mercury analyzer. For quality assurance, a duplicate, blank, and two standard reference materials were used within each set of 10 samples (TORT-3 and PACS-2, National Research Council of Canada, Ottawa, ON). Duplicates with <20% relative percent difference from each other were considered acceptable. The MDL for Hg for liver samples was 0.155 mg/kg and was calculated as threefold the standard deviation of the procedural blanks. The MDL for blood and feather samples was Hg = 0.6060 mg/kg and was calculated using dry mass weights. Percent recovery of CRM between 80 and 120% is considered quality and samples within this range were included in the analysis. If samples were not within that range, they were reanalyzed to ensure correct recovery measurements and taken out of the dataset if the recovery came back out of range again. Blanks were included to test for contamination in sample processing.

Statistical analysis

The three main datasets (black vulture liver, black and turkey vulture blood, and black and turkey vulture feather) were delineated into six subcategories for comparison: (1) females vs. males (black vulture liver), (2) juveniles vs. adults (black vulture liver), (3) black vultures vs. turkey vultures (blood), (4) juveniles vs. adults (blood), (5) black vultures vs. turkey vultures (feather), and (6) juveniles vs. adults (feather) to determine the average, standard error, minimum, and maximum values for each element. For the black vulture liver data, we evaluated differences in trace element levels between age classes and sexes. No turkey vulture liver samples were available to compare element levels between species. For the blood and feather data, we tested for differences between species and age classes but did not have sufficient data to make comparisons between males and females. Each element was tested for normality using a Shapiro-Wilks test and for homogeneity of variance using a Levene test. Elements that had a non-normal distribution were log-transformed.

Elements in which >50% of the samples were below the MDL were excluded from analysis. If an element had <50% of the samples below the MDL, samples below the MDL were replaced with half of the MDL value for the specific element (Fletcher et al. 2014). We tested for differences in element concentrations between age classes (blood, feather, and liver), sex (liver), and species (blood and feather) using separate multivariate analysis of variance (MANOVA) models. If MANOVA tests revealed significant differences among the elements ($p \leq 0.05$), we then ran pair-wise analysis of variance (ANOVA) models to determine which specific elements were statistically different from each other within each comparison subcategory. Program R was used for all statistical tests using R packages "ggplot2" and "car" (version 4.1.1., R Core Team 2021; Wickham 2016, Fox and Weisberg 2019).

Results

We analyzed 34 black vulture liver samples, 80 blood samples (39 black vultures and 41 turkey vultures), and 42 feather samples (20 black vultures and 22 turkey vultures). For the black vulture liver samples, Ni, U, and As were excluded from statistical analysis as >50% of samples contained levels that fell below the MDL. Similarly, elements Co, Cd, Th, and U were excluded from analyses of blood samples, and Cd, Th, and U were excluded from analyses of feather samples. All reported sample trace element and heavy metal levels are dry weight (dw).

Liver samples

Two black vultures had elevated Pb liver levels that were considered outliers with levels of 2628.10 mg/kg dw and 30.35 mg/kg dw. The average Pb level of the liver samples excluding the two outliers was 1.48 mg/kg dw (±0.32 SE). One individual had considerably higher Hg levels (6.01 mg/kg dw) than other individuals tested. The average Hg level of the liver samples excluding that outlier was 0.59 mg/kg dw (±0.18 SE). All outlier samples were reanalyzed in the lab with a new dilution, which confirmed the high concentrations of those elements. These outliers were subsequently excluded from the MANOVA analyses.

MANOVA tests for black vulture liver samples revealed a significant difference in trace element concentrations between age classes ($p = 0.015$), with juveniles ($n = 5$) having higher element levels compared to adults ($n = 25$). The subsequent pairwise ANOVA for age class revealed only Cu ($p = 0.003$) differed significantly between adults and juveniles. Juveniles had an average Cu level (mean = 118.95 mg/kg dw ± 52.30 se) that was 311% higher than that of adults (mean = 38.20 mg/kg dw ± 2.54 se) (Table 1). Average Pb was generally higher in adults than juveniles (adult mean = 1.72 mg/kg dw ± 0.39 se, juvenile mean = 0.75 mg/kg dw ± 0.11 se), although this difference was not significant ($p = 0.344$) (Table 1). Between sexes, there was not a statistical difference in any trace element or heavy metal concentration (MANOVA $p = 0.144$).

Blood samples

Our MANOVA test for blood samples indicated element levels between black vultures and turkey vultures were significantly different ($p < 0.001$), with black vultures having higher trace element and heavy metal concentrations on average compared to turkey vultures. The ANOVA for species revealed that Cu, Zn, Pb, Hg, Cr, Mn, Fe, Co, As, Ni, and V differed significantly between the two species (Cu $p = 0.004$, Zn $p < 0.001$, Pb $p = 0.028$, Hg: $p < 0.001$, Cr $p = 0.004$, Mn $p = 0.011$, Fe $p < 0.001$, Co $p = 0.020$, As $p < 0.001$, Ni $p = 0.004$, V $p = 0.014$). Black vulture blood samples had higher Cu, Pb, Cr, Fe, As, Ni, and V than turkey vulture blood samples (Table 2). Turkey vulture blood samples had higher averages of Zn, Hg, Se, and Mn than black vulture blood samples (Table 2). However, there was no difference in element levels between age classes for either species (black vulture $p = 0.126$, turkey vulture $p = 0.682$).

Feather samples

Similar to blood samples, our MANOVA test for feather samples revealed element levels differed between black vultures and turkey vultures ($p = 0.004$), with black vultures having higher trace element and heavy metal concentrations on average compared to turkey vultures. The ANOVA for

Table 1 Mean, standard error, minimum, and maximum trace element and heavy metal concentrations (mg/kg dw) in adult ($n = 25$) and juvenile ($n = 5$) black vulture (*Coragyps atratus*) liver samples. Samples were collected in 2020 from South Carolina and Florida, USA

Adult					Juvenile			
Element	Mean	SE	Min	Max	Mean	SE	Min	Max
Cu	38.20	2.54	19.94	66.98	118.95	52.30	24.40	302.43
Zn	113.03	2.58	85.52	137.83	110.25	4.71	96.00	124.65
Cd	0.14	0.04	0.04	0.90	0.18	0.08	0.03	0.50
Pb	1.72	0.39	0.20	7.45	0.75	0.11	0.47	1.13
Se	3.21	0.14	1.90	4.63	3.62	0.30	2.89	4.45
Hg	0.40	0.05	0.05	1.27	0.50	0.14	0.19	0.93

Table 2 Mean, standard error, minimum, and maximum trace element and heavy metal concentrations (mg/kg dw) in blood samples from black vultures (*Coragyps atratus*; $n = 39$) and turkey vultures (*Cathartes aura*; $n = 41$). Samples were collected in summer of 2013 and spring of 2014 at the Savannah River Site in South Carolina, USA

	Black Vulture				Turkey Vulture			
Element	Mean (mg/kg)	SE	Min	Max	Mean (mg/kg)	SE	Min	Max
V	0.14	0.01	0.07	0.24	0.11	0.01	0.06	0.27
Cr	1.54	0.06	0.64	2.51	1.30	0.08	0.70	3.40
Mn	0.14	0.02	0.05	0.85	0.19	0.02	0.07	0.66
Fe	2376.36	69.16	1155.85	3437.56	2041.88	57.92	1285.67	2576.11
Ni	0.31	0.02	0.09	0.66	0.26	0.05	0.09	2.25
Cu	2.25	0.14	0.65	5.29	1.72	0.13	0.66	3.49
Zn	19.66	0.87	8.50	36.75	25.16	1.00	15.37	39.91
As	0.16	0.01	0.06	0.50	0.12	0.01	0.06	0.21
Se	2.91	0.12	1.05	4.70	3.18	0.16	1.84	5.96
Sr	0.14	0.04	0.03	1.46	0.10	0.01	0.04	0.28
Pb	1.12	0.33	0.18	13.52	0.66	0.10	0.10	3.75
Hg	0.56	0.06	0.06	1.86	1.12	0.13	0.19	4.93

species revealed that Cu, Se, Hg, Mn, Fe, Co, As, Sr, and V all were significantly different between the two species (Cu $p = 0.011$, Se $p = <0.001$, Hg $p = 0.001$, Mn $p = < 0.001$, Fe $p = <0.001$, Co $p = 0.011$, As $p = 0.020$, Sr $p = 0.006$, V $p = <0.001$). Black vulture feather samples had higher averages for Cu, Mn, Fe, Co, As, Sr, and V than turkey vulture feather samples (Table 3). Turkey vulture feather samples had higher averages of Se and Hg than black vulture feather samples (Table 3). For both black and turkey vultures, there was no difference in element levels between age classes (black vulture $p = 0.459$, turkey vulture $p = 0.512$).

Discussion

Our results revealed black and turkey vultures exhibited differential contaminant burdens in feather and blood samples for several trace elements, with black vultures generally having higher trace element and heavy metal concentrations than turkey vultures. Despite the propensity of some contaminants to bioaccumulate with age, only liver concentrations of Cu differed between juveniles and adults in our study. Although average contaminant levels were generally low for most elements, extreme outliers were observed for some elements, including Pb, suggesting some individuals were exposed to high levels of potentially toxic elements. Collectively, these results suggest black vultures may be more likely to consume food sources that are higher in trace element and heavy metal levels than turkey vultures within some ecosystems where the species co-occur Tables 4, 5, 6.

Despite both species being obligate scavengers, our results demonstrated black vultures and turkey vultures sampled from the same study region differed in element levels in both feather and blood samples, with black vultures on average having higher element levels than turkey vultures, supporting our prediction. Differences in feeding

Table 3 Mean, standard error, minimum, and maximum trace element and heavy metal concentrations (mg/kg dw) in feather samples from black vultures (*Coragyps atratus*; $n = 20$) and turkey vultures (*Cathartes aura*; $n = 22$). Samples were collected in summer 2013 and spring of 2014 at the Savannah River Site in South Carolina, USA

	Black Vulture				Turkey Vulture			
Element	Mean (mg/kg)	SE	Min	Max	Mean (mg/kg)	SE	Min	Max
V	0.95	0.09	0.38	1.94	0.44	0.05	0.11	0.934
Cr	3.46	0.40	1.67	8.76	3.41	0.33	1.27	6.968
Mn	75.04	10.19	15.67	168.95	28.55	4.37	3.68	75.34
Fe	375.37	39.92	109.48	655.25	129.45	14.67	37.54	292.96
Co	1.11	0.60	0.08	12.54	0.37	0.11	0.04	2.56
Ni	1.34	0.23	0.52	4.43	6.77	5.72	0.31	126.80
Cu	4.81	0.24	3.71	7.89	4.03	0.21	2.49	6.36
Zn	162.33	7.58	119.63	231.91	155.96	15.5	70.17	448.79
As	0.21	0.02	0.05	0.43	0.14	0.03	0.05	0.56
Se	1.02	0.22	0.38	4.91	1.50	0.12	0.38	2.58
Sr	4.30	0.48	1.39	9.50	2.60	0.27	0.91	5.56
Pb	3.45	0.63	0.73	14.54	2.69	0.51	0.90	9.42
Hg	0.859	0.13	0.07	2.21	1.81	0.24	0.45	4.11

Table 4 Summary of the *p* values for black vulture liver MANOVA analysis for each covariate (bolded) and ANOVA analysis of significant elements for the respective covariate (p <0.05). Samples were collected necropsied October 2020 and were collected from South Carolina and Florida

Covariate	Element	*p* value
Sex		0.144
Age class		**0.015**
	Cu	0.003

Table 5 Summary of the *p* values for blood MANOVA analysis for each covariate (bolded) and ANOVA analysis of significant elements for the respective covariate (p <0.05). Samples were collected summer of 2013 and spring of 2014 at the Savannah River Site in South Carolina

Covariate	Element	*p* value
Species		**<0.001**
	Cu	0.004
	Zn	<0.001
	Pb	0.028
	Hg	<0.001
	Cr	0.004
	Mn	0.011
	Fe	<0.001
	Co	0.020
	As	<0.001
	Ni	0.004
	V	0.014
Age class (black vulture)		0.459
Age class (turkey vulture)		0.512

Table 6 Summary of the *p* values for feather MANOVA analysis for each covariate (bolded) and ANOVA analysis of significant elements for the respective covariate (p <0.05). Samples were collected summer of 2013 and spring of 2014 at the Savannah River Site in South Carolina

Covariate	Element	*p* value
Species		**0.004**
	Cu	0.011
	Se	<0.001
	Hg	0.001
	Mn	<0.001
	Fe	<0.001
	Co	0.011
	As	0.02
	Sr	0.006
	V	<0.001
Age class (black vulture)		**0.459**
	V	0.004
	As	0.002
	Co	0.03
	Fe	0.018
Age class (turkey vulture)		0.512

and foraging habits between the two species could account for disparities in feather and blood element levels. Black vultures are communal feeders, have a longer foraging time compared to turkey vultures, and often forage at landfills (Coleman and Fraser 1987; Buckley et al. 2022). Further, black vultures in urban areas often feed at garbage dumps and on livestock whereas turkey vultures are more likely to forage on smaller, wild animals in areas less populated by humans (Prior 1990; Avery and Cummings 2004) and are better able to exploit wild carrion within forested areas (Byrne et al. 2019). Feeding in garbage dumps and other anthropogenic sources could increase exposure to certain elements at higher levels than would normally be introduced in a black vulture's natural diet. Birds that nest and forage near landfills have elevated levels of metals such as Pb, Hg, Fe, and As, elements that can cause health issues in large quantities (de la Casa-Resino et al. 2014). Feeding on livestock that has been treated with medicine or dietary supplements could also potentially expose black vultures to pharmaceuticals, as was seen with vultures in India feeding on deceased cattle treated with the veterinary medicine dioflenac (Ogada et al. 2012b). Furthermore, black vultures are more social than turkey vultures, and a vulture from a communal roost will often return with other roost members to feed on the same large carcass for multiple days (Rabenold 1987). If the carcass has elevated levels of trace elements and heavy metals, this communal foraging behavior has the potential to impact numerous individuals within a population. A longer foraging time at a single carcass would also increase the consumption of the contaminated carcass, subsequently increasing the accumulation of contaminants by black vultures. Since turkey vultures usually eat smaller, wild carrion and have a shorter length of stay at a carcass than black vultures, turkey vultures may be at less risk of exposure to contaminated carrion (Prior 1990, Avery and Cummings 2004).

Our results demonstrated a difference in trace element concentrations between age classes in black vulture liver samples. However, the only element that was significantly different between adults and juveniles was Cu. This difference was due to high copper levels in two juveniles, both of which were collected from South Carolina. This differed from our expectations, as we predicted adults would have higher element levels as a result of bioaccumulation as the birds aged (Yordy et al. 2013). Due to the large home ranges of vultures (Holland et al. 2017) and the inability to identify specific foraging areas where the individuals might have been exposed to high copper, we are unable to speculate why these particular birds had higher copper levels, although this may be due in part to the small sample size of juveniles ($n = 5$) compared to adults ($n = 25$).

Exposure to lead is a concern for many scavenging bird species, such as vultures, condors, and eagles, as these species are at risk of ingesting lead fragments through discarded offal and other un-obtained carcass remains (Stroud and Hunt 2009). Lead poisoning was a main component in the decline of California condors and remains a primary threat to the recovery of this species (Plaza and Lambertucci 2019; Finkelstein et al. 2020). Exposure to lead can cause multiple health problems and ultimately result in mortality if there is no intervention (Krone 2018). Studies suggest that Pb liver levels >6 mg/kg dw indicate abnormally high exposure to Pb (<6 mg/kg dw indicate normal background levels) and Pb liver levels >20 mg/kg dw indicates acute exposure and absorption of lead (Pain et al. 1995). Our results revealed several black vultures with liver lead levels either close to, or higher than, 6 mg/kg dw. However, there are no known lead toxicity thresholds specifically for vulture species and multiple experiments on vultures have revealed lead concentrations at higher blood and liver levels than other birds of prey (Pattee et al. 2006). One study that experimentally dosed turkey vultures with lead found that liver levels of the vultures that died or were euthanized due to lead toxicosis symptoms were higher than that of other Falconiformes (Carpenter et al. 2003). Our blood and feather samples were taken from living vultures which could illustrate black and turkey vulture's tolerance for elevated lead levels, but health metrics were not available to confirm their overall health and long-term survival. Two black vultures in our study were found to have highly elevated liver lead levels, with concentrations in one individual exceeding 2600 mg/kg dw. This extreme outlier was collected from a Floridian black vulture liver sample of unknown sex. As this vulture was collected from Florida as a part of a nuisance control program, the health of this bird was unknown. Euthanasia methods in Florida included firearms, and thus, this high lead level could be due to a lead fragment from ammunition; the copper level for this individual was also elevated (360.67 mg/kg dw) which could possibly be caused by ammunition. However, the liver itself did not appear to be shot or impacted from a bullet fragment when performing the necropsy and the sample was analyzed twice to confirm element concentrations.

Although we collected blood and feather samples from the same individuals, we limited our analyses to within-sample type comparisons due to differences in contaminant circulation and accumulation between these tissue types. Specifically, feather samples represent contamination levels present while the feather is actively growing and receiving blood and nutrients from the dermal pulp. Once the feather is done growing, the feather will become isolated from the dermal pulp and will no longer receive additional nutrients or blood and thus may not be reflective of blood contaminant concentrations if sampled outside of the brief period of growth (Prum and Williamson 2001). While feathers may not be accurate surrogate samples for predicting blood contaminant levels when not collected during the feather's growth period, studies have found evidence that feathers are a useful indicator for other contaminants like blood persistent organic pollutants (POP) (Pacyna-Kuchta 2023). Further, utilizing both blood and feather samples in conjunction gives a more complete contaminant exposure history of the individual rather than analyzing either sample type individually. For example, Finkelstein et al. (2010) was able to use estimated feather growth of primary and rectrices feathers to estimate lead exposure events that were not reflected by blood monitoring efforts of California condors. Interestingly, many of the elements evaluated in our study had higher average concentrations in feathers than in blood for both species. This difference could be a reflection of contaminant exposure events prior to the collections of both sample types and could indicate greater exposure than shown by blood samples alone.

There is little to no information on contaminant poisoning in either turkey vultures or black vultures. The few existing contaminant studies focused on turkey vultures and limited elements of interest such as Pb, Cd, and Hg (Carpenter et al. 2003; Di Marzio et al. 2018). These studies have shown that turkey vultures have a much higher threshold for trace element exposure than other vulture and raptor species (Carpenter et al. 2003; Franson and Pain 2011). Cadmium is a heavy metal that can pose health risks at high levels in birds (Scheuhammer 1987). Durkalec et al. (2023) evaluated heavy metals and trace elements in livers from 36 white-tailed eagles (*Haliaeetus albicilla*) found in Poland and reported mean liver Cd levels of 0.171 mg/kg dw and a range of 0.039–0.470 mg/kg dw. Our black vulture liver samples had a mean of 0.14 kg/mg dw and ranged from 0.04–0.90 mg/kg dw, which is considerably higher than Durkalec et al.'s findings. However, Ozaki et al. (2023) collected liver samples from common buzzards (*Buteo buteo*) in the UK and reported a mean Cd level of 1.60 mg/kg dw and a range of 0.02–19.46 mg/kg dw. Another study collected turkey vulture liver samples from a landfill in Chile and reported mean Cd levels of 5.24 µg/g dw (SD 8.00, range 0.49 to 19.70 ug/g dw) (Valladares et al. 2012). Although this is a valuable information, it is difficult to find consistent toxicity thresholds in vulture species for most elements, and there are other elements that pose health risks to vultures at high levels, many of which have not been researched in either species (Scheuhammer 1987). Both black and turkey vultures are slowly expanding their range and moving closer to urban areas (Avery 2004) where they are adapting to feed in landfills, potentially increasing their exposure to pollutants within the Southeastern United States (Holland et al.

2019). This will expose vultures to higher levels of anthropogenic contaminants and could lead to potential health effects in vultures. However, element and metal toxicity thresholds in both species are poorly understood (Plaza et al. 2020), and more research must be done to elucidate the element tolerance for a broad suite of anthropogenic contaminants.

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Data Availability Data is available upon reasonable request to the authors.

Declarations

All vulture capture and handling was conducted in accordance with the University of Georgia Animal Care and Use Committee under protocol no. A2013 02-004-Y2-A2 and samples were collected with approval of appropriate state and federal permits.

Consent to participate This research did not involve human participants.

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