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Impervious Surface Is Not a Strong Predictor of Contaminant Accumulation in Freshwater Turtles in a Rapidly Urbanizing Region

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Abstract

Due to the relatively long lifespan and resilience of adults to environmental stressors, freshwater turtles are characterized as bioaccumulators of chronic contaminant exposure in urban ecosystems. Urbanization increases pollutants, resulting in subsequent runoff into streams. We evaluated the relationship between percent impervious surface and contaminant concentrations in turtles from 20 wetlands in Wake County, North Carolina, USA, one of the fastest-growing counties in the United States. We evaluated the concentrations of eight environmental contaminants known to cause human and environmental health issues listed under the U.S. Environmental Protection Agency's Resource Conservation and Recovery Act: arsenic (As), barium (Ba), cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg), selenium (Se), and silver (Ag), as well as vanadium (V) and copper (Cu) due to their presence in urban environments and bioaccumulation, in the blood and claws from *Chelydra serpentina* and *Trachemys scripta*. All contaminants, except for Cd and Ag, were detected in both species and both tissue types. Carnivorous *Chelydra serpentina* exhibited higher concentrations of Se and Hg than omnivorous *Trachemys scripta*. Partial redundancy analysis indicated that species accounted for more variance in the data than % impervious surface at a 2200-m scale. Robust mixed-effects models showed that % impervious surface was not correlated with contaminant concentrations in either species. Although we documented no relationship between urbanization and contaminant concentrations, we recommend additional research to investigate the effects of urbanization over time in this rapidly developing region.

Keywords: environmental contaminants; urbanization; Testudines; freshwater turtles

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1. Introduction

Globally, more than half of the world's human population resides in urban areas, driving landscape transformations [1]. Urban development is essential for addressing the challenges of human population growth, but it is equally important to consider how these landscape changes impact wildlife and overall ecosystem health. One of the ecosystem health concerns are emissions of pollutants. Sources of pollutants in urban areas include industrial activity, transportation, and domestic waste that can release contaminants into

soil and aquatic ecosystems [2]. In rapidly urbanizing areas, the expansion of impervious surface area contributes to increased runoff into bodies of water, leading to reduced water quality and heightened levels of pollutants [3–5]. Impervious surface runoff commonly carries pollutants, including metals like zinc, cadmium, chromium, copper, and lead, as well as polycyclic aromatic hydrocarbons (PAHs) such as phenanthrene, pyrene, and fluoranthene [6,7].

Urban wildlife, including freshwater turtles, can be adversely affected through the consumption of contaminated food and the bioaccumulation of contaminants in eggs laid in contaminated soil [2]. Turtles are widely regarded as effective environmental indicators and have been shown to reflect environmental contamination [8]. Because of their longevity and relatively high tolerance to starvation, adult freshwater turtles can appear resilient by surviving in polluted waters, accumulating contaminants over extended periods [9–11]. Turtles have shown the ability to persist in degraded environments, where permanent water and food resources are provided by anthropogenic waste [12]. They can bioaccumulate contaminants in various tissues over time [9,13,14]. Additionally, high biomass, wide distribution, and representation across various trophic levels (i.e., primary, secondary and tertiary consumers) further enhance the turtle's role in evaluating environmental health [14,15]. Research on the accumulation of contaminants, including metals and metalloids, in freshwater turtle tissues can indicate elevated levels of environmental contamination from anthropogenic sources [16–20]. Research has shown that contaminant accumulation can lead to notable sublethal effects in turtles and that adverse effects are often more severe in juvenile turtles [21–23]. Overall, pollutant accumulation in turtles has caused kidney damage, oxidative stress, genotoxicity and a reduction in reproductive success, immunosuppression, and deformities [24–28].

Assessing environmental contaminants in urban regions through freshwater turtles represents a growing area of research. We evaluated the accumulation of environmental contaminants in two freshwater turtles, common snapping turtle (*Chelydra serpentina*) and slider turtle (*Trachemys scripta*), inhabiting Wake County, North Carolina, one of the fastest growing counties in the United States of America [29]. The assessment of contaminants in turtles has been conducted using various methods, including the analysis of blood, claws, muscle, liver, and eggs [30–32]. Here, we focused on two tissue types, blood and claw. Collecting blood and claws is a non-lethal sampling approach to monitoring contaminants in comparison to other tissue types such as liver or muscle [14,33].

We assessed the concentrations of ten contaminants, eight listed under the U.S. Environmental Protection Agency (US EPA) Resource Conservation and Recovery Act (RCRA), which included arsenic (As), barium (Ba), cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg), selenium (Se), and silver (Ag). The RCRA gives federal and state entities the authority to monitor and manage hazardous waste and provides regulatory limits to these eight contaminants in wastewater and leachates because of their potential risks to human health and the environment. Because pollution in urban environments spans beyond the RCRA 8 list of elements, we included two additional pollutants in our analysis. Vanadium was included due to its presence in urban air [34], urban soils [35], and diesel vehicle emissions [36]. Copper was included due to its presence in urban soil near areas with heavy traffic [37], urban agricultural practices, solid waste [38], and deposition from vehicle brake pad wear in urban stormwater [39]. We aimed to investigate whether variables associated with urbanization were potentially related to environmental contaminants in freshwater turtles. We primarily focused on the effects of imperviousness as a coarse proxy for urbanization due to its known contribution to increased stormwater runoff into bodies of water in urban environments. We hypothesized that the contaminant concentration in turtle

tissues will be positively correlated to the percent impervious surface area surrounding the water bodies.

2. Materials and Methods

2.1. Study Sites

Wake County, North Carolina, supports a diverse assemblage of native freshwater turtle species, including the painted turtle (*Chrysemys picta*), pond slider (*Trachemys scripta*), river cooter (*Pseudemys concinna*), eastern mud turtle (*Kinosternon subrubrum*), common musk turtle (*Sternotherus odoratus*), common snapping turtle (*Chelydra serpentina*), and the spotted turtle (*Clemmys guttata*), which is a Species of Greatest Conservation Need [40]. For this study, we collected tissue samples from the two most frequently captured large-sized turtles, *Trachemys scripta* and *Chelydra serpentina*. We sampled 20 wetlands throughout Wake County, consisting of varying levels of urbanization (Figure 1). The 20 sites included one managed by Knightdale High School, two by North Carolina State University, two by the Town of Garner, one by Triangle Land Conservancy, six by the City of Raleigh, and eight by the Wake County Parks, Recreation, and Open Space Program. All sites were open to the public.

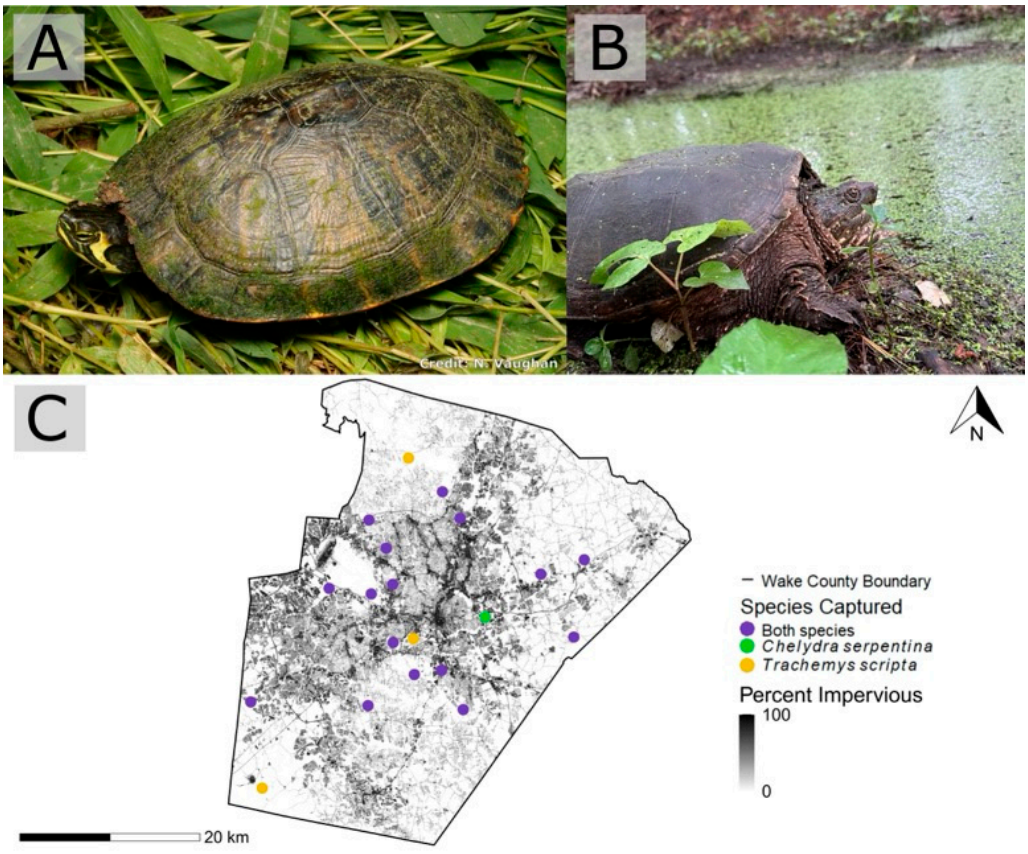

Figure 1. Photographs of *Trachemys scripta* (**A**) and *Chelydra serpentina* (**B**) surveyed across 20 sites in Wake County, North Carolina, USA, during 2023–2024 (**C**). Survey locations are overlaid on the 2023 percent impervious surface layer from the National Land Cover Database (NLCD).

2.2. Turtle Surveys

We sampled freshwater turtles during the following time periods: March through May (3 sites), July through September (6 sites), and November (1 site) of 2023, as well as May and June of 2024 (10 sites). In the Piedmont ecoregion of North Carolina, freshwater turtles are known to begin their activity in March, with a peak from May to July, and extending into October. We could not access one site until November because the area was closed due

to construction. Logistical constraints prevented us from surveying all sites within a shorter time span, but given that turtles were active during all survey months and presumably feeding, we assumed that seasonal heterogeneity did not have a significant effect on our analyses. Ten large hoop net traps [41] and five smaller hoop net traps [42] were used for all sites except one (see details below). The large hoop net traps were single opening, collapsible, wide-mouthed traps with four interconnected hoops (50.8 cm diameter, 2.5-cm mesh size), held open by handmade wooden poles placed between the first and last hoop on each side of the mouth. The small traps were also collapsible and had a wire frame, double openings, a 30-cm diameter, and a 0.39-cm mesh size.

We baited all traps with ½ can of sardines in oil placed in perforated bottles to allow for scent dispersal but not consumption, and re-baited every other day. A flotation device (an empty 2-L bottle or a pool noodle) was placed inside of each trap to prevent turtle drowning. We tied each trap to live, sturdy vegetation for security, with GPS location recorded. We positioned traps away from frequently accessed public areas and concealed, if possible. We checked traps 1–2 times per day. All sites, except one, were trapped consistently over four consecutive days, resulting in 60 trap-days per site. The only site trapped in November was sampled for a single day using 12 large traps.

We measured the straight-line carapace length to the nearest millimeter (CL) of each turtle and marked all turtles by notching marginal scutes [43] or inserting a PIT tag [44]. Approximately 0.2–0.4 mL of blood was drawn from the femoral vein using a sterile non-heparinized syringe, never exceeding 10% of the turtle's blood volume [45]. We collected claw samples using a dog nail trimmer to acquire 3–4 claws from the hind legs. All samples were collected in empty sterile 1.8-mL Nunc and Eppendorf tubes placed in a cooler with ice in the field, then transported to the laboratory and stored at approximately 2 °C in a refrigerator. Because we did not use anticoagulants, blood was allowed to coagulate and dry; therefore, dry weight was used to measure the final mass of sample collected. Laboratory analyses occurred ~4–6 months after collection. We released all turtles captured after processing at their capture location. Because all turtles were marked, we never sampled the same turtle more than once even if recaptured. The study was conducted under the North Carolina Wildlife Resources Commission Scientific Research Permit No. 22-SC01527 and SWC24000030, as well as North Carolina State University Institutional Animal Care and Use Committee Protocol No. 22-403-W.

2.3. Assessment of Urbanization

We obtained impervious surface data with (30-m × 30-m resolution) from the 2023 National Land Cover Database (NLCD) [46] and used 2200-m buffers around each study site (i.e., mid-point of the trap line) to calculate the mean percent imperviousness. For each buffer, the raster values inside the buffer were extracted, and the frequency of each unique pixel value was counted. These unique values were then multiplied by their respective pixel counts and summed, and the result was divided by the total number of pixels within the buffer. The 2200-m buffer size encapsulated the distances that the two focal species of turtles can travel. For example, *C. serpentina* has been observed traveling up to 2200 m overland [47], whereas individuals in the genus *Trachemys* can travel as far as 1400 m to reach nesting sites [48].

In addition to imperviousness, we examined additional metrics of urbanization. We calculated the percentage of urban cover by reclassifying the 2023 NLCD land cover classes of low-, medium-, and high-intensity development, as well as developed open space, as "urban". Percentages were calculated as the number of urban pixels divided by the total number of pixels across all land cover classes within the 2200-m spatial scale around a site multiplied by 100. Road density was calculated by converting the vector road data into a

raster layer (with 10-m × 10-m resolution), where the value of the raster represented the length of the road passing through it. The pixels occurring within each buffer around a site were extracted, and the road length (pixel values) within the 2200-m buffer was summed. These calculations were completed using the Raster [49], sf [50,51], and spatstat.geom [52] packages in R version 4.5.0 [53]. We documented that all three metrics (imperviousness, developed land, and road density) were highly correlated ($|r| > 0.7$).

2.4. Laboratory Analyses

All blood and claw samples were analyzed by the Research Triangle Institute (RTI International in Durham, NC). The samples were analyzed for the RCRA 8 elements, plus V and Cu. Before sample digestion, the claws were cleaned to remove any sediment or other attached debris using a procedure derived from the International Atomic Energy Agency (IAEA, Vienna, Austria) [54]. Samples were placed into a glass tube labeled with the appropriate sample identification. Approximately 2 mL of acetone was added to each sample tube and vortexed for five seconds with a homogenizer (Daigger Vortex Genie 2, Scientific Industries Inc., New York, NY, USA) set to power level 3.5. Samples were soaked for five minutes, and then acetone was decanted. Next, 2 mL of deionized water was added to each sample tube and vortexed for five seconds on the same power level. Afterward, samples were soaked for 20 min. Once all samples were soaked, water was decanted from the tubes, carefully ensuring that the claws did not exit the tube. Each sample was rinsed and vortexed five times to ensure all sediment or other debris had been removed from the claws. Then, samples were air-dried for one hour. Once samples were dry, each sample was weighed (Mettler Toledo AB104 Analytical Balance; Mettler-Toledo LLC, Columbus, OH, USA) in a separate weigh boat, adding one claw until the total sample weight had been recorded. To ensure sufficient sample mass for analysis, claw samples of the same species, sex, and site were combined if the weight of each individual sample was less than 0.03 g. This approach ensured that the detection limit (DL), 4 ng/g, would be exceeded. Each sample was placed into a closed MoldPro MP-123PN, 15-mL digestion tube (MoldPro Inc., Swanzey, NH, USA) and transferred to RTI International for contaminant analysis. All dry coagulated blood samples were weighed and transferred to the same type of tube as those used for the claw samples. The procedure involved weighing the empty tube, adding the blood, and then subtracting the initial weight to obtain the weight of the blood alone. Both blood and claw samples were subsequently stored in a refrigerator at 2.0 °C until analysis.

At RTI International, a 0.5-g aliquot was taken from each of the blood samples and claw weights were recorded. Samples were processed using a modified version of the U.S. EPA Method 3050B to account for the sample size and 5 mL final volume needed to achieve the best possible analytical detection. Then, 0.5 mL of HNO_3 was added to the extraction tubes. The samples were left loosely capped in a fume hood for 30 min to allow the nitric acid to react with the samples. The samples were then heated at 95 °C in an SCP Science DigiPrep block digestion unit (Montreal, QC, Canada) and allowed to reflux for one hour. The samples were cooled, and 1.0 mL of 30% of H_2O_2 was added. The samples were heated at 95 °C loosely capped to allow reflux for thirty minutes. The samples were cooled and brought to a final volume of 5 mL using deionized water and vortex mixed. Analysis was performed on a Thermo RQ Inductively Coupled Plasma Mass Spectrometer (Thermo Fisher Scientific, Waltham, MS, USA) equipped with an Elemental Scientific Incorporated (ESI) FAST autosampler system (Omaha, NE, USA). Au was employed as a chelating agent to improve Hg recovery, and the limited sample volume allowed for the application of only a single digestion method. As part of the quality assurance/quality control protocol, analytical blanks were analyzed with each batch of samples and an internal standard was added on-line using the FAST system. Contaminant concentrations in blanks were not

detected, and recoveries of the internal standard ranged from 80 to 120%. Analysis followed U.S. EPA Method 200.8. No duplicate samples or spiked samples were analyzed due to the limited sample masses. To ensure analytical rigor despite these constraints, we used NIST-traceable reagent blank spikes to assess method performance, performed continuing calibration designations (continuing calibration verification [CCV], continuing calibration blank [CCB]), and assessed the calibration curve every ten samples using standards and blanks. A standard reference material was run to ensure accuracy (precision plus bias). We assessed recoveries with reagent blank spikes using NIST-traceable standards (Inorganic Ventures, Christiansburg, VA, USA). The detection limit was calculated using the average sample mass, and we reported the mean value (4 ng/g) for ease of comprehension and to provide a standardized value. Across the dataset, detection limits ranged from 2 to 8 ng/g, with 4 ng/g serving as the representative average.

2.5. Statistical Analyses

We performed all statistical analyses in R version 4.5.0 [53]. For contaminants for which fewer than 50% of samples were below the detection limit (DL), values below the DL were assigned a constant value equal to one-half of the DL (2 ng/g) for statistical analyses [55]. Prior to any analyses, mean % imperviousness values were scaled to have a mean of 0 and a standard deviation of 1 before analyses. In addition, the response variables were log-transformed to improve normality and stabilize variance. We used partial redundancy analysis (pRDA) in package vegan [56] to examine the effects of mean % imperviousness on contaminant concentrations while using species as a conditioning factor (i.e., a grouping variable) to account for inherent dietary differences among these groups. pRDA is a multivariate statistical approach that allows for the simultaneous analysis of all contaminants in relation to the predictor variables. Two RDA analyses were performed, one for blood and one for claw samples.

We used robust linear mixed-effects models to evaluate the relationship between % imperviousness and concentration of each contaminant individually. We fit the models using the rlmer function from the robustlmm package in R [57]. We used scaled mean % imperviousness as a fixed effect representing the level of urbanization and site as a random effect to account for site-level variability. The rlmer function reduces the impact of outliers and extreme values, ensuring more reliable parameter estimates when data may not meet traditional linear model assumptions, which was the case with our data. We constructed separate models for each contaminant, species, and tissue type. Because robust linear mixed-effects models do not produce *p*-values, we assessed the significance of impervious surface cover by examining the back-transformed 95% confidence intervals (CIs) for the models' coefficients (i.e., exp beta), with the effect considered significant if the CIs did not overlap 1.

We used a Gamma generalized linear model with a log link to evaluate the correlation between turtle size (CL) and contaminant concentrations in blood for each species. We used the same models to test the difference in contaminant concentrations in blood and claw between the two species. Only samples with concentrations above DL were included in all analyses. We used Bonferroni corrected *p*-values to interpret the results, inferring statistical significance when $\alpha > 0.05$.

3. Results

Percent imperviousness per site ranged from 3% to 36% (mean = 18%; median = 16%; Figure 1). We captured *Trachemys scripta* at 19 of the 20 sampled sites, whereas we captured *Chelydra serpentina* at 17 sites. The relative abundance of *Trachemys scripta* ranged from 0 to 31 per site, and *Chelydra serpentina* ranged from 0 to 10 per site. We collected claw samples

from 57 *Chelydra serpentina* and 151 *Trachemys scripta*. After combining claw samples to meet the minimum sample weight requirement, the total number of samples was 155, 55 *Chelydra serpentina* and 100 *Trachemys scripta* (Supplemental Table S1). Blood samples did not require combining and totaled 197 (47 *Chelydra serpentina* and 150 *Trachemys scripta*). For claw samples, both species had no analytical result less than the DL for any contaminant measured other than Ag and Cd. Only five claw samples from *Chelydra serpentina* and seven from *Trachemys scripta* were measured above the DL for Ag. Seven claw samples from *Chelydra serpentina* and five claw samples from *Trachemys scripta* were above the DL for Cd. Therefore, Ag and Cd were not included in the statistical analysis.

For *Chelydra serpentina*, no blood samples were measured above the DL for Ag and Cd, whereas for *Trachemys scripta*, only one blood sample measured above the DL for both Ag and Cd (Table 1). Both species had most samples above the DL for the remaining eight contaminants except for V (Table 1). *Chelydra serpentina* had 35 out of 47 samples below the DL for V, and *Trachemys scripta* had 78 out of 150 samples below the DL. For both species, Ba, Pb, Se, and Cu had all, or nearly all, samples above DL (Table S1). Both species had approximately 30–40% of samples below the DL for Cr and As. The greatest difference between the species was Hg concentration, where *Chelydra serpentina* had only 5% of samples below DL and *Trachemys scripta* had 30% of samples below DL (Table 1). For *Chelydra serpentina*, the median blood concentrations of contaminants ranked from highest to lowest was as follows: Cu, Se, Ba, Hg, Pb, Cr, As, and V (Table 1). In contrast, for *Trachemys scripta*, the mean blood concentrations were ranked as: Cu, Ba, Se, Pb, Hg, As, Cr, V, and Cd (Table 1). Regarding claw concentrations, *Chelydra serpentina* exhibited median contaminant values ranked from highest to lowest as follows: Ba, Hg, Se, Cu As, Cr, V, Pb, Ag, and Cd (Table 2). For *Trachemys scripta*, median claw contaminant concentrations were ranked as: Ba, Cu, As, Hg Se, V, Cr, Pb, Ag, and Cd (Table 2).

Table 1. Median (range) contaminant concentrations (ng/g) in blood samples from two freshwater turtle species in Wake County, North Carolina, USA, collected in 2023 and 2024. Sample size (*n*) indicates the number of samples with concentrations above the detection limits; samples below detection limits were excluded from descriptive statistics. A total of 47 *Chelydra serpentina* and 150 *Trachemys scripta* samples were analyzed.

	Chelydra serpentina	***n***	***Trachemys scripta***	***n***
Ag	Na	0	14.42	1
As	7.12 (4.17–49.35)	30	9.14 (4.15–27.26)	93
Ba	84.43 (18.87–675.03)	47	98.93 (16.61–1386.2)	150
Cd	Na	0	4.05	1
Cr	7.68 (4.04–46.31)	31	7.02 (4.04–31.38)	109
Cu	372.38 (163.11–521.03)	47	426.09 (210.66–1161.1)	150
Hg	32.85 (4.96–395.93)	45	12.09 (4.11–122.66)	105
Pb	26.32 (4.44–143.98)	46	14.46 (4.24–72.67)	149
Se	145.84 (27.05–312.9)	47	60.56 (18.55–197.23)	150
V	5.72 (4.23–10.67)	12	5.49 (4.01–20.57)	72

Species explained 9.6% of the total variance in blood contaminant concentrations according to the partial RDA. After accounting for species, impervious surface explained an additional 1.6% of the variance, leaving 88.8% unexplained. For claw analysis, species accounted for 10.3% of the total variance. Impervious surface explained only 0.9% of the total variance, with 88.8% remaining unexplained. Robust mixed-effects models consistently indicated no effect of % imperviousness on contaminant concentrations in both species and tissue types (Figure 2).

Table 2. Median (range) contaminant concentrations (ng/g) in claw samples from two freshwater turtle species in Wake County, North Carolina, USA, collected in 2023 and 2024. Sample size (*n*) indicates the number of samples with concentrations above the detection limits; samples below detection limits were excluded from descriptive statistics. A total of 55 *Chelydra serpentina* and 100 *Trachemys scripta* samples were analyzed.

	Chelydra serpentina	***n***	***Trachemys scripta***	***n***
Ag	13.93 (5.96–588.26)	5	17.28 (4.67–64.59)	7
As	178.53 (49.9–1323.1)	55	206.02 (15.2–2922.8)	100
Ba	2758.05 (249.2–137,352.8)	55	19,571.5 (651–232,487.1)	100
Cd	4.55 (4.11–7.74)	7	6.45 (4.19–12.56)	5
Cr	169.53 (49.8–745.3)	55	150.49 (50.7–662.7)	100
Cu	306.53 (106–2469.9)	55	439.03 (201.2–1498.6)	100
Hg	748.54 (138.6–7326.7)	55	188.04 (19.6–4383)	100
Pb	101.36 (15.63–688.65)	55	125.33 (23.4–729.7)	100
Se	331.5 (161.9–609)	55	184.14 (81.3–410.8)	100
V	134.15 (33.2–1263.1)	55	155.31 (22.1–756.4)	100

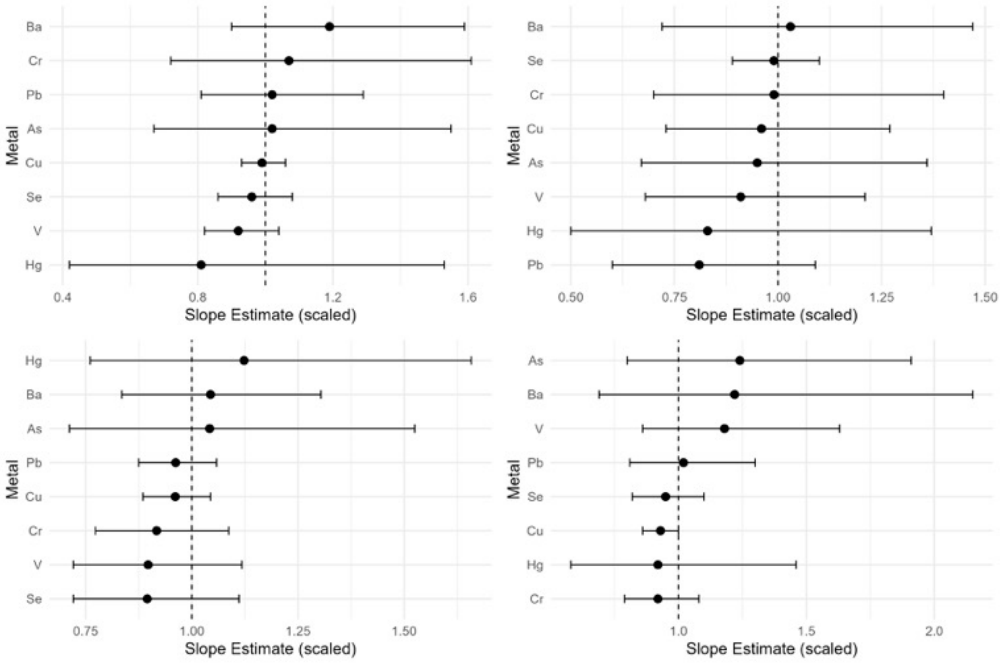

Figure 2. Results of robust mixed effect models showing slope estimates (black dots) and 95% confidence intervals (CIs) for the effect of percent of impervious surface on contaminant concentrations in blood (top left) and claw (top right) of *Chelydra serpentina*, and blood (bottom left) and claw (bottom right) of *Trachemys scripta*. The explanatory variable (percent impervious surface) was scaled, and the response variable was log-transformed prior to analysis. Slope estimated were back-transformed; effects were considered significant when 95% CIs did not overlap 1.

A significant negative correlation between turtle size and As concentrations was observed in *Trachemys scripta* ($p < 0.05$) while there was a significant positive correlation between turtle size and Hg concentrations ($p < 0.05$; Table 3) The rest of the analyses showed no significant relationship to turtle size. *Trachemys scripta* showed significantly lower concentrations of Hg, Pb, and Se in blood in comparison to *Chelydra serpentina* ($p < 0.01$), while the Cu concentrations were significantly higher ($p < 0.01$; Table 4). We saw the same significant trend for Hg and Se in claws ($p < 0.01$), while Ba was significantly higher in *Trachemys scripta* claws ($p < 0.01$; Table 4).

Table 3. Summary of generalized linear models examining the relationship between turtle size (carapace length) and contaminant concentrations in blood of *Chelydra serpentina* and *Trachemys scripta* collected in Wake County, North Carolina, USA, in 2023 and 2024. Asterisks (*) denote statistical significance.

Contaminant	Species	Estimate	SE	t	Bonf. *p*
As	*C. serpentina*	0.003	0.002	1.181	1
Ba	*C. serpentina*	−0.0003	0.002	−0.13	1
Cr	*C. serpentina*	−0.004	0.002	−1.461	1
Cu	*C. serpentina*	0.0007	0.0005	1.299	1
Hg	*C. serpentina*	0.002	0.003	0.551	1
Pb	*C. serpentina*	0.0001	0.002	0.073	1
Se	*C. serpentina*	0.0009	0.0008	1.104	1
V	*C. serpentina*	−0.003	0.001	−3.097	0.09
As	*T. scripta*	−0.006	0.002	−3.598	0.004 *
Ba	*T. scripta*	0.007	0.004	1.91	0.464
Cr	*T. scripta*	−0.004	0.002	−1.917	0.463
Cu	*T. scripta*	−0.002	0.0008	−2.15	0.266
Hg	*T. scripta*	0.011	0.004	3.037	0.024 *
Pb	*T. scripta*	0.001	0.002	0.488	1
Se	*T. scripta*	0.001	0.001	0.874	1
V	*T. scripta*	0.002	0.002	0.845	1

Table 4. Summary of generalized linear models examining differences in contaminant concentrations in blood and claw between *Chelydra serpentina* and *Trachemys scripta* sampled in Wake County, North Carolina, USA, in 2023 and 2024. *Chelydra serpentina* was used as the reference category. Asterisks (*) denote statistical significance.

Contaminant	Species	Tissue	Estimate	SE	t	Bonf. *p*
As	*T. scripta*	Blood	0.05	0.125	0.401	1
Ba	*T. scripta*	Blood	−0.0002	0.189	−0.001	1
Cr	*T. scripta*	Blood	−0.195	0.136	−1.435	1
Cu	*T. scripta*	Blood	0.208	0.047	4.404	<0.01 *
Hg	*T. scripta*	Blood	−1.056	0.209	−5.056	<0.01 *
Pb	*T. scripta*	Blood	−0.556	0.122	−4.547	<0.01 *
Se	*T. scripta*	Blood	−0.754	0.080	−9.392	<0.01 *
V	*T. scripta*	Blood	0.068	0.144	0.472	1
As	*T. scripta*	Claw	0.099	0.185	0.535	1
Ba	*T. scripta*	Claw	1.078	0.260	4.145	<0.01 *
Cr	*T. scripta*	Claw	−0.178	0.109	−1.642	0.82
Cu	*T. scripta*	Claw	0.149	0.109	1.365	1
Hg	*T. scripta*	Claw	−1.187	0.231	−5.133	<0.01 *
Pb	*T. scripta*	Claw	−0.067	0.136	−0.49	1
Se	*T. scripta*	Claw	−0.576	0.051	−11.26	<0.01 *
V	*T. scripta*	Claw	−0.295	−2.126	0.035	0.28

4. Discussion

This study investigated the concentrations of environmental contaminants in the blood and claws of freshwater turtles across Wake County, North Carolina, a region characterized by rapid and ongoing urbanization. We used a broad landscape level urbanization metric, impervious surface, under the premise that increased impervious cover is positively correlated with runoff and greater pollutant influx into environments occupied by turtles. In our study, this urbanization metric was a poor predictor of contaminant accumulation in turtles. Overall, multiple factors and their interactions likely contributed to the large proportion of unexplained variability, reflecting the complexity of contaminant sources and

pathways. One possibility is that the urbanization metric used here did not have sufficient resolution to capture the complex dynamics of contaminant release into the environment. For example, a study in the Raleigh-Durham metropolitan area, which included our general study area, compared seven streams with similar amounts of pavement and reported a wide range of subsurface and surface hydrologic connectivity between the pavements and their receiving streams [58]. Second, contaminants can originate from various point and non-point sources. Non-point pollution is particularly difficult to identify, including car emissions and nutrient, household, and commercial waste. The gradual phase-out of leaded gasoline, which began in the 1970s and was fully banned in 1996, could contribute to the decline of some contaminants, especially Pb, on roadways [59]. Finally, freshwater turtles are relatively long-lived (approximately 20 to 70 years) and exhibit high mobility [60], complicating the establishment of direct links between contaminant accumulation and local environmental conditions. The mobility of turtles indicate that contaminants detected in their tissues, especially in their claws, may stem from various locations and sources within the surrounding environment. This mobility may, in turn, reduce the suitability of turtles as a model taxon for detecting the effects of urbanization at this spatial scale.

Concentrations of some contaminants differed between the two species, likely due to inherent differences in diet. Concentrations of Se and Hg in blood and claw were higher in *Chelydra serpentina* than in *Trachemys scripta*, likely attributable to the primarily carnivorous diet of *Chelydra serpentina*, mostly consisting of fish [59]. Elevated levels of Hg and Se have been linked to fish consumption [61–64]. In contrast, while *Trachemys scripta* are generally considered omnivorous, they exhibit an ontogenetic diet shift, with older individuals being more herbivorous [65]. Because As and Ba have been detected in plant tissues, which accumulate through soil uptake [66–68], herbivorous turtles may accumulate these contaminants through the consumption of plants. However, only Ba in the claw samples of *Trachemys scripta* was significantly higher than in *Chelydra serpentina*.

Our results indicate that factors beyond turtle size, such as blood turnover rate and the distribution of contaminants among different tissues, may play roles in determining contaminant concentrations. Because claw samples had to be pooled to meet the minimum weight requirement for analysis, individual-level variability was lost. As a result, we were unable to evaluate relationships between contaminant concentrations in claws and turtle size; however, these relationships were assessed using blood samples. Generally, there was little evidence that turtle size correlated with the contaminant concentrations. Two exceptions were a significant negative relationship for As and significant positive relationship for Hg in *Trachemys scripta*. This finding was contrary to our expectations given the ontogenetic diet shift in *Trachemys scripta*. However, all turtles in our study were adults, and turtle size does not always correlate with age [69]. In addition, larger turtles may distribute the same amount of contaminant more widely throughout their body compared to smaller individuals. As a result, larger turtles may have lower contaminant concentrations in the same tissue type than smaller turtles [70]. Further research is needed to examine the ecological and physiological factors that influence the bioaccumulation of contaminants in these species.

Although published studies on the exact turtle species used in our research are limited, existing research on similar species provides a useful basis for comparison. It is important to note that studies of the same or similar species may vary in their sample collection techniques, preparation and cleaning methods, and laboratory procedures. One study measured Pb and Hg levels in the blood of captive-raised 3- to 4-year-old alligator snapping turtles (*Macrochelys temminckii*) in Louisiana [71]. The average Hg concentration was 20 ng/g, while the Pb levels were all $\leq$20 ng/g, preventing mean calculations due to Pb being below the detection limit according to their laboratory procedures [71]. In our

study, average Hg concentration for the most comparable species, *Chelydra serpentina*, was substantially higher, averaging 395.93 ng/g. In Brazil, Geoffroy's side-necked turtles (*Phrynops geoffroanus*) exhibited serum levels of Cu (2194 ng/g) and Pb (1150 ng/g), the highest ever recorded for any reptile [20]. The values from our study were much lower, with the highest Cu concentration detected in *Trachemys scripta* at 1161 ng/g, and the highest Pb concentration detected in *Chelydra serpentina* at 144.0 ng/g.

Studies on metal and metalloid concentrations in turtle claws have also shown varying levels across species. Painted turtles (*Chrysemys picta*) from Lake Michigan had average Cr levels below 10,000 ng/g, Pb under 5000 ng/g, and Cd at levels that were undetectable [70]. The average Cr concentrations that we detected were lower, ranging from 180.3 ng/g in *Trachemys scripta* to 215.5 ng/g in *Chelydra serpentina.* The Pb concentrations we detected in claws were also lower, ranging from 163.7 ng/g in *Trachemys scripta* to 175 ng/g in *Chelydra serpentina*, while average Cd ranged from 2.25 ng/g in *Trachemys scripta* to 2.39 ng/g in *Chelydra serpentina*. In another study, the claws of European pond turtles (*Emys orbicularis*) from Brenne Natural Park, France, were evaluated for 14 metals, revealing only Hg above the detection limit, due to the small mass of turtle claws, with a mean concentration of 1346 ng/g [72]. The Hg concentrations in our study were lower, ranging from 378.1 ng/g in *Trachemys scripta* to 1239.6 ng/g in *Chelydra serpentina*.

Using the same field and laboratory techniques, turtles from our study exhibited lower levels of most contaminants than turtles in the Permian Basin of southeast New Mexico [73]. The most direct comparison is between *Trachemys scripta* from New Mexico and *Trachemys scripta* from North Carolina. In claws, the mean As concentration was more than two times higher in the Delaware River (707 ng/g) of New Mexico than in Wake County (292 ng/g), North Carolina. The Delaware River also had higher Cr, Hg, and Se levels relative to this study, with Cr and Hg approximately twice as high in New Mexico samples [73]. Claw Se from the Delaware River (792 ng/g) was approximately four times higher than Se in this study (191.1 ng/g). Blood samples from the Delaware River had notably higher levels of As (76 ng/g) and Se (700 ng/g) than this study (As = 10.66 ng/g and Se = 68.4 ng/g). The only instance where the Wake County turtles had higher levels of both blood and claw contaminant concentrations was for Ba; the New Mexico claw Ba ranged from 1215 ng/g to 3464 ng/g across all rivers, whereas the Wake County mean was much higher at 28,479 ng/g. Similarly, blood Ba concentrations in New Mexico turtles ranged from 27.6 ng/g to 66 ng/g, and the Wake County mean was 162.5 ng/g. Although Ba is a naturally occurring element found in mineral deposits, erosion can increase its release into the surrounding soil and water. We suggest that the higher Ba concentrations in Wake County are linked to the more abundant aquatic vegetation at study sites than in New Mexico, where aquatic plants were rarely observed, despite being a primary food source for *Trachemys scripta*. It is important to note that the geology, plant composition, and water type (i.e., lotic in NM and lentic in NC) differed significantly between the two studies. Additionally, New Mexico rivers face challenges related to the oil and gas industry, including oil spills, fracking, and salinization [74,75]. Overall, the relatively lower contaminant concentrations in Wake County may indicate that parks and other greenspace conservation efforts in the region may buffer some aquatic systems from the effects of urbanization.

5. Conclusions and Future Directions

Assessing the condition of water resources is important for understanding the factors influencing the health of turtles, humans, and the broader ecosystem. Urban growth may lead to the increased use of public outdoor spaces, highlighting the importance of monitoring the pollutants affecting these areas. Given the limited data on contaminant

bioaccumulation in the region [57], this study provides an important baseline for future research. Our study indicates that impervious surface alone is not an adequate proxy for contaminant exposure in freshwater turtles. Future research should directly quantify contaminants in water and soil across our study sites to provide a more comprehensive assessment of exposure pathways. Because the shift to unleaded gasoline relied on the addition of catalytic converters made from platinum metals (PGM, e.g., platinum, palladium), PGMs are now being deposited along roadways instead of lead. Therefore, future studies should expand the breadth of pollutants evaluated. Future studies should also explore how surface and subsurface hydrologic connectivity may affect the spatial distribution of pollutants in the environment and their bioaccumulation in wildlife, as well as any additional factors not considered here to explain observed variability in contaminant accumulation in turtle tissues.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d18030131/s1; Table S1. Raw data for concentrations of each contaminant per sample, including site codes, urbanization metrics, and turtle size.

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