ENVIRONMENTAL POLLUTANTS AND BIOAVAILABILITY
2025, VOL. 37, NO. 1, 2474007
https://doi.org/10.1080/26395940.2025.2474007

Environmental factors influencing bioaccumulation of xenobiotic metals in freshwater turtles

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ABSTRACT

Human-induced land use practices can result in the bioaccumulation of metals in turtles. We assessed relationships between land use and pollutant concentrations in painted turtles (*Chrysemys picta*) and snapping turtles (*Chelydra serpentina*) through non-destructive tissue sampling across 22 sites within a mixed-use watershed in north-central West Virginia, U.S.A.. Metal concentrations were consistently higher in nails than in blood. Selenium levels in soil and turtle nails were positively correlated with proximity to mine land. Lead levels in turtle nails were positively correlated with Pb concentrations in soil. All samples had Hg levels that exceeded consumption limits set by the United States Environmental Protection Agency.

ARTICLE HISTORY
Received 23 November 2024
Accepted 25 February 2025

KEYWORDS
Acid mine drainage; snapping turtle (*Chelydra serpentina*); painted turtle (*Chrysemys picta*); methylmercury; Wetland

Introduction

Anthropogenic land use changes such as surface coal mining and increased vehicular traffic density can introduce chemical elements that may not naturally occur in nearby wetland systems, such as selenium (Se), cadmium (Cd), lead (Pb), and zinc (Zn [1–3]. Other trace elements such as chromium (Cr), copper (Cu), and titanium have been identified in higher concentrations in groundwater surrounded by agricultural fields compared to sites tested in bottomland hardwood forests [4]. Mercury (Hg) deposition and runoff into wetlands increases the rate of methylation, which increases the bioavailability of Hg and associated health risks to plants and animals in a system [5]. Land use practices also alter metal concentrations found in soil [6], which can indirectly impact organisms such as benthic macroinvertebrates [7], fishes [8], and turtles [9].

Xenobiotic concentrations in fish species that are commonly harvested for consumption have significant implications for human health, which leads to the heavy monitoring and regulation of fish that is found in ecotoxicology literature. Consumable species of turtles like common snapping turtles (*Chelydra serpentina*), however, do not have strict harvest or consumption guidelines in states like West Virginia, where there are only daily creel limit and possession limit restrictions [10]. Due to their long lifespan, high trophic position, and diet preferences, freshwater turtles have an increased likelihood of accumulating metals over time Grillitsch and Schiesari 2010 [11,12]. This has significant implications for human health, especially with concentrations of metals like Hg found in snapping turtles [13–15]. For example, monitored total Hg levels accumulated in a population of snapping turtles in New York showed that 61% of muscle samples had Hg levels higher than the U.S. Environmental Protection Agency (USEPA) limits [12].

Blood and nail Hg and Pb concentrations are positively correlated with concentrations in consumable parts of turtles (e.g. muscle tissue [11,16]. Omnivorous turtle species, such as snapping turtles, had higher concentrations of metals than more herbivorous species (e.g. painted turtles [*Chrysemys picta*] and sliders [*Trachemys* spp.]) within the same wetland [16]. Species of other taxa have exhibited this relationship as well. For example, small mammals found in grasslands had different concentrations of Cd and Cu in their tissues based on diet variation (insectivorous > herbivorous > frugivorous [17].

Biological characteristics such as size [18,19] and sex [20] affect metal accumulation in turtles. However, the literature is inconsistent with respect to accumulation patterns between sexes. Some studies have concluded that because females transfer contaminants to their offspring, adult male turtles have higher concentrations than females of the same species [16]. Other studies showed that metal concentrations vary between sexes due to differences in food consumed [11]. Alternatively, some studies concluded no correlation between sex and metal bioaccumulation [12,18,19,21,22]. This pattern of variation may be due

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to differences in studies such as target species, wetland condition, and site-specific wetland factors such as hydroperiod and food web complexity [23].

Bioaccumulation of metals is not without consequences for turtles. Non-essential metals such as Hg reduce fertility and increase embryonic mortality rates [24]. Lead contamination negatively influences the survival of young turtles and affects their righting ability [25]. Studies indicate that snapping turtle hatchling survival is affected by nest substrate Hg contamination in addition to maternal transfer [20,26].

The purpose of this study was to assess relationships between surrounding land use practices and concentrations of xenobiotic metals (i.e. Cd, Cr, Hg, Pb, Se, and Zn) accumulated in freshwater turtles in wetlands and streams within a mixed-use (hardwood forest and agriculture) watershed impacted by coal mining in north-central West Virginia, U.S.A.. The concentration of metals was quantified among different size classes of snapping turtles and painted turtles, two of the most common freshwater turtle species in the eastern United States [14]. We hypothesized that wetland distance to anthropogenic land use types (i.e. agriculture, roads, mine land) would be negatively correlated with metal concentrations in turtle tissues and metal concentrations in wetland soil. We also hypothesized that metal concentrations would be higher in nail tissue samples compared to blood samples for both species, snapping turtles would have greater metal concentrations than painted turtles due to their larger size, and turtle body condition would be negatively associated with metal concentrations found in turtle tissue.

Methods

Study sites

Twenty-nine wetlands were surveyed in the Upper Deckers Creek watershed, a hydrologic unit code 12 watershed that covers 7,778 ha in Preston County, West Virginia, U.S.A., and encompasses 89.9 km of the stream (Deckers Creek headwaters and its tributaries; Figure 1). Deciduous forests and wetlands (freshwater emergent wetlands [18%], freshwater ponds [42%], freshwater forested/shrub wetlands [25%], and riverine systems [15%]) have been highly fragmented from agriculture, coal mining, industry, and residential development [27].

The primary causes of water pollution in the Deckers Creek watershed include acid mine drainage (AMD), sediment, and lead (Denicola et al. 2014). Since

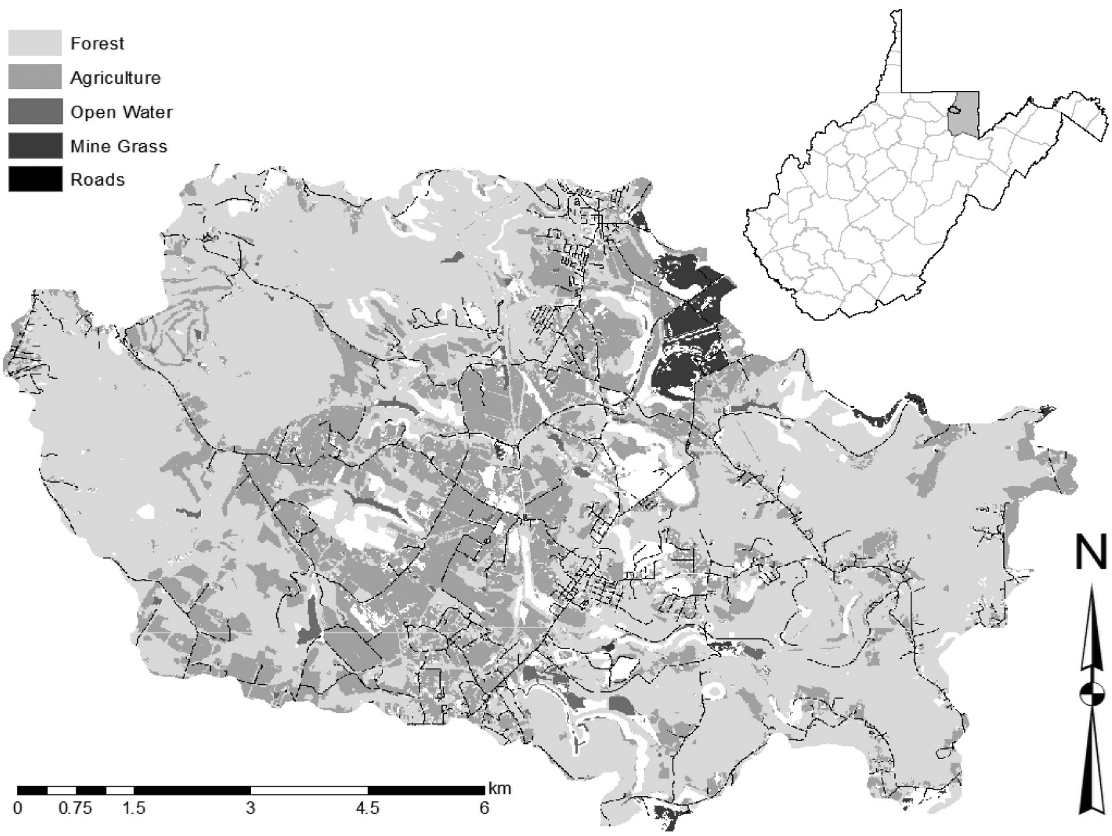

Figure 1. Mosaic of land use within the Upper Deckers Creek watershed in Preston County, West Virginia, U.S.A. (inset). Twenty-six wetlands and three stream locations were surveyed during 2019 for freshwater turtles from 18 April to 19 May (spring), 1 July to 31 July (summer), and 5 October to 1 November (fall). Wetlands were predominantly surrounded by forested ($n = 13$) or agricultural ($n = 13$) land within 1 km, while three sites had an even combination of both. Eight sites were associated with West Virginia University, 11 were privately owned, and 10 wetlands were in wildlife management areas.

the turn of the century, the earliest recording of mine activity in the watershed was the Richard Mine in 1903 [28]. Deckers Creek watershed had many active underground and surface mines that soon led to AMD. Untreated water flowed into the creek before the Clean Water Act and Surface Mining Control and Reclamation Act (SMCRA) in 1977, reducing pH [29]. Stream flow is highest in February and March and lowest in August and September [30]. Conversion of the landscape from hardwood forest and wetlands to agriculture eliminated riparian vegetation, which increased runoff and sediment accumulation (Denicola et al. 2014). However, wetland, riparian, and stream restoration designed to reduce sedimentation and livestock impacts are ongoing [27,31]. Lead impacts in Deckers Creek are not as widespread as other metals; however, a plumbing manufacturing plant operating in the 1960s and 1970s near the Fairfax Pond-Rehe Wildlife Management Area caused Pb to be exposed to Deckers Creek [32].

Eleven sites were privately owned, eight sites were associated with the West Virginia University JW Ruby Research, Education, and Outreach Center (REOC), and ten sites were part of the West Virginia Division of Natural Resources wildlife management areas. Surveyed sites varied in size from 0.04 to 7.45 ha. Sites were surrounded within 1 km by predominantly forests ($n = 13$), agriculture ($n = 13$), or an approximately even amount of forest and agricultural land ($n = 3$). Management varied among the 29 survey sites (e.g. mowing edges, direct cattle access, chemical application, wetland plant removal).

Turtle capture and processing

At each site, 10 aquatic hoop net traps of alternating sizes (0.91 m diameter and 0.76 m diameter [33,34]; were set at least 10 m apart for three consecutive days during three trapping sessions in 2019, including spring (18 April to 19 May), summer (1 July to 31 July), and fall (5 October to 1 November). Traps were either set in a straight line along one side of the wetland (for larger wetland sites) or across the entire wetland perimeter when accessible (for smaller wetland sites).

Traps were set in streams at least 25 m apart. In locations where the channel was too narrow or the water level was too shallow, the distance between traps varied as necessary to ensure each trap was deployed. Each trap was baited with a half-can of sardines in a container so the scent could escape and the sardines would not be consumed, and the bait was replaced every 24 hours [35]. Each trap had a flotation device to allow captured individuals constant access to air.

Captured turtles were weighed with hanging scales to the nearest 5 g for individuals <600 g, 100 g for individuals ≤10 kg, and 500 g for individuals >10 kg. Morphometric data, including midline carapace length (MCL) and width, plastron length and width, and body depth, were measured with calipers to the nearest 1 mm [36]. Pre-cloacal tail and posterior plastral scute lengths were measured on snapping turtles to determine sex [14,37]. Unique shell markings in the marginal scutes with a hand file or rotary tool provided distinct identification [38]. After processing, each turtle was photographed and released near their location of capture.

Tissue and soil sample collection

Turtle tissue samples were analyzed from 22 of the 29 sites surveyed for xenobiotic metals in the environment (i.e. soil), chosen based on surrounding wetland landscape type (i.e. agriculture, forest, or near equal) and tissue sample quantity and quality (i.e. absence of lymph in blood). One milliliter of blood from the dorsal or ventral caudal vein [39] and 1–2 mm trimmings from 3–4 hind toenails were collected onsite from each captured individual for analysis [11,40]. Blood samples were collected using 3 mL non-heparinized syringes, and nail samples were collected using human or dog nail clippers, depending on the size of the turtle. Samples were placed in 1.5 mL Eppendorf tubes, stored in a cooler, and transferred to a freezer at −20°C immediately upon return from the field until analysis.

Given that water metal concentrations such as Cd and Cr can seasonally fluctuate [41] compared to the slow transformation to stable forms of metals in soil [42], two surface soil samples (0–10 cm depth) were collected bordering the water line of each wetland using a 5-cm diameter handheld PVC core sampler to quantify a baseline of stable, immobile environmental concentrations of metals [16,43–45]. The PVC corer reduced potential metal soil sample contamination risk compared to a conventional steel soil sampler [44].

Environmental data

Wetland boundaries were digitized using ESRI World Imagery in ArcGIS (version 10.7, ESRI, Redlands, California), and adjacent land use data were retrieved from the 2011 National Land Cover Data Set (NLCD [46]. Hay, pasture, and cultivated cropland use types were combined and defined as agriculture. Distance to nearby mine land and roads (in meters) was calculated using the Near function in ArcMap. To identify mine land locations, current mining activity as of 2011 (NLCD 'mine grass' and 'mine barren') was combined with land that was mined before the SMCRA (NLCD 'Pre-SMCRA grass' and 'Pre-SMCRA barren'). Road locations were derived from the U.S. Census Bureau 2011 roads database (https://www.census.gov/geographies/mapping-files/time-series/geo/tiger-line-file.html).

Table 1. Number of tissue samples analyzed from snapping turtles (*Chelydra* serpentina) and painted turtles (Chrysemys picta) captured during 2019 within Upper Deckers Creek watershed in Preston County, West Virginia, U.S.A.. The number of turtles varied by how many individuals were captured and the quality of the tissue sample (i.e. large enough blood samples without lymph). Juvenile individuals were not sampled as commonly due to hoop-net trap size bias [34].

	Nail samples			Blood samples			
	J	M	F	J	M	F	Total
Snapping turtle	3	13	14	0	11	5	59
Painted turtle	7	15	9	5	34	12	69
Total	10	28	23	5	45	17	128

Sample analyses

Tissues from 61 painted turtles (range MCL = 51–180 mm, median MCL = 139 mm) and 33 snapping turtles (range MCL = 113–386 mm, median MCL = 257 mm) across 22 sites were analyzed (Table 1) and categorized based on dominant land use surrounding the wetland in which the turtles were captured (Table 2). Some turtles had both tissue types analyzed, while others had either blood or nail samples. Samples were analyzed for concentrations of Cd, Cr, Hg, Pb, Se, and Zn. This suite of metals was chosen due to their status as metals and metalloids of primary interest, according to USEPA [47]. The history of coal mining, agriculture, and development in the Upper Deckers Creek watershed was also informative in identifying metals for analysis. Total Hg was measured as a proxy for methylmercury (MeHg) because 70–100% of Hg in turtle blood is methylated [18,23,48].

The West Virginia University National Research Center for Coal and Energy Analytical Lab analyzed all tissue and soil samples for each site. Acid digestion using nitric acid and dilution to 100 mL was applied to blood samples before analysis, and all samples were analyzed according to the USEPA method 3050B with a Perkin Elmer Nexlon 2000 inductively coupled plasma mass spectrometer (Waltham, Massachusetts [49]. A linear calibration was completed using a blank (1% HNO_3) for quality assurance. A check-verification standard and a blank were run after the calibration (±10% recovery). Additional blanks were run after every 20 injections and at the end of each run with all blanks resulting in concentrations below the aqueous level of detection for each metal analyzed: 0.000025 mg/kg for Cd, 0.000018 mg/kg for Cr, 0.000003 mg/kg for Hg and Pb, 0.000185 mg/kg for Se, and 0.000031 mg/kg for Zn. After soil metal analysis, one site was considered an outlier due to the amount of Pb and Zn (457.26 and 904.98 mg/kg, respectively), possibly due to measurement error or sample contamination, and was removed from further analysis to avoid skewing due to the regression-based nature of the redundancy analyses [50].

Statistical analyses

Statistical analyses were conducted using R statistical software [51]. To investigate relationships between land use practices, wetland soil metal concentrations, and turtle metal concentrations, partial redundancy analyses (pRDA) or redundancy analyses (RDA) were performed using Cd, Cr, Hg, Pb, Se, and Zn concentrations for each tissue or soil sample as response variables [52,53]. Redundancy analysis is an extension of principal component analysis to include predictor variables, thus allowing for the estimation of the influence of environmental predictors on a suite of response variables in a single analysis [52]. Surrounding landscape (percent agriculture and distance to nearest mines and roads) and soil metal concentration variables were used as predictors in the analyses. During preliminary analyses, turtle size and sex classes were found to be correlated with heavy metal concentrations. To ensure that size-related variance was not attributed to the environmental predictors, the influence of MCL and sex class was partialled out in the turtle response models.

For each analysis, response variables were standardized to give each metal equal weight. Separate pRDA analyses were performed for each turtle species and tissue type to maximize the interpretability of

Table 2. Number of tissue samples analyzed from snapping turtles (*Chelydra serpentina*) and painted turtles (*Chrysemys picta*) captured during 2019 within the Upper Deckers Creek watershed in Preston County, West Virginia, U.S.A.. Of the 128 tissue samples collected, 22 wetlands were represented: 8 wetlands had forest, 11 wetlands had agriculture (Ag), and three wetlands had both forest and agriculture as the dominant land use types within 1 km of the wetland.

	Nail samples			Blood samples			
	Ag	Forest	Both	Ag	Forest	Both	Total
Snapping turtle	13	10	7	9	6	1	46
Painted turtle	12	9	8	27	19	7	82
Total	25	19	15	36	25	8	128

relationships among turtle metal concentrations, environmental variables, and soil metal concentrations. An additional RDA was run to assess relationships between soil metal concentrations and surrounding land use practices. Prior to RDA analyses, detrended correspondence analyses were performed to ensure gradient lengths were short (≤4), and thus, a linear rather than unimodal model was appropriate for the data [52]. We report variance explained using the adjusted R^2, which corrects for variance explained by random chance. When the explained variance is low, adjusted R^2 values can be negative.

Relationships between turtle metal concentrations and turtle health were investigated using body condition index (BCI) scores as the response variable. BCI scores represented residual values from a linear regression using log-transformed MCL and weight [54]. Individuals with a positive BCI score have greater than expected weight for the length of the individual, and vice versa [54]. Candidate models were tested separately based on tissue type, and predictors included species and concentrations of each heavy metal. Metal concentrations were standardized to ensure equal weight across metals and turtle species. Additive models were considered if the individual predictor models had greater support than the null model. Candidate models were ranked using the Akaike Information Criterion corrected for small sample size (AICc). Models with ΔAICc <2 were considered to have strong support [55,56]. For supported models, we considered individual predictors to have strong support when their 85% confidence intervals did not overlap 0 [57].

Results

Land use composition across sites

The mean percent of agriculture within 30 m of each of the 29 sites surveyed in 2019 varied between 0 and 79% (Table 3). Sites with forest as the predominant land use were primarily associated with wildlife management areas. Sites with agriculture as the dominant land use were associated mainly with the REOC or private farms where the land was used for hay or pasture. Distance from roads (0—419 m) and mine (0—935 m) land varied greatly between sites (Table 3).

Comparing turtle species

All metals were detected in toenail tissue samples. Cadmium was not detected in 29 painted turtle blood samples (54.7%), and Cr was not detected in 39 painted turtle blood samples (73.5%). Six snapping turtle blood samples (37.5%) also had no detectable Cd or Cr. Painted turtle nail concentrations were 0.88 to 1.85 times higher than snapping turtles. Snapping turtle blood concentrations of xenobiotic metals were as much as four times greater than painted turtle blood concentrations (Table 4). The order of mean concentrations for painted turtle blood samples was Zn > Se > Hg ≥ Pb > Cr ≥ Cd, and the mean nail concentration order was Zn > Se > Cr > Pb > Hg > Cd. The order of mean concentrations for snapping turtle blood samples was Zn > Se > Pb > Hg > Cr > Cd, and the mean nail concentration order was Zn > Se > Cr > Pb ≥ Hg > Cd.

Table 3. Descriptive statistics for percent agriculture within 30 m of each wetland and distance to nearest mine land and roads for 29 wetlands in the Upper Deckers Creek watershed in Preston County, West Virginia, U.S.A., sampled for soil analysis in 2019. Tissues from turtles surveyed in 22 sites were used for turtle tissue analysis. Values reported are distances in meters for all parameters but percent agriculture. Agriculture values were calculated using the 2011 National land use/Land cover dataset provided by the West Virginia GIS technical center [46].

Type	Min	Max	Mean	SE
Soil analysis				
Ag within 30 m	0.00	79.95	23.41	4.93
Mine land	0.00	934.63	331.74	60.89
Roads	0.00	418.86	78.53	17.38
Turtle analysis				
Ag within 30 m	0.00	79.10	26.54	5.72
Mine land	0.00	934.63	363.09	72.77
Roads	0.00	252.41	77.17	15.47

Table 4. Mean (±SE) concentrations (mg/kg) for each metal tested in nail and blood samples from snapping turtles (*Chelydra serpentina*) and painted turtles (*Chrysemys picta*) across three trapping sessions during 2019 in the Upper Deckers Creek watershed in Preston County, West Virginia, U.S.A.. Twenty-two wetland locations were surveyed from 18 April to 19 May (spring), 1 July to 31 July (summer), and 5 October to 1 November (fall).

		Cd	Cr	Hg	Pb	Se	Zn
Snapping turtle	Nail	0.02 (0.004)	0.99 (0.14)	0.42 (0.06)	0.44 (0.08)	1.04 (0.19)	203.72 (9.30)
	Blood	0.0001 (0.00006)	0.0003 (0.0002)	0.005 (0.001)	0.02 (0.01)	0.42 (0.10)	3.94 (0.75)
Painted turtle	Nail	0.07 (0.19)	1.63 (0.35)	0.39 (0.075)	0.41 (0.08)	1.77 (0.31)	254.79 (17.08)
	Blood	0.0001 (0.00003)	0.0002 (0.00005)	0.007 (0.0001)	0.005 (0.001)	0.37 (0.04)	1.47 (0.15)

Of the 128 samples that were analyzed for Hg, 34 nail samples (16 painted turtles and 18 snapping turtles) exceeded both the 0.0001 mg/kg human consumption limit set by the EPA [58], as well as the 0.3 mg/kg fish tissue criterion that was established by the EPA in the Clean Water Act (Section 304[a] [5,59]. Lower threshold concentrations for MeHg were estimated to be at least 70% of Hg detected [18], and 19 of the 34 nail samples (10 painted turtles and nine snapping turtles) had MeHg levels exceeding both EPA limits (Figure 2).

Painted turtle redundancy analyses

Landscape predictors explained 3.9% of the variance in painted turtle blood concentrations after partialling out the effect of MCL and sex class (1.1%; Figure 3a). Selenium and Cr were negatively correlated with percent agriculture and distance from mine land. Turtles sampled from wetlands farther from roads had higher Cd, Pb, and Zn concentrations, while increased distance from roads resulted in lower Cr and Se concentrations in turtle blood. Wetland soil concentrations explained 3.4% of the variance in painted turtle blood concentrations after partialling out MCL and sex class (−1.2%; Figure 4a). Concentrations of Hg and Pb in turtles were negatively related to Hg and Pb concentrations in wetland soil, respectively, while Cd and Cr exhibited strong positive relationships with soil Cd and Cr concentrations, respectively. Interestingly, soil Cr had a strong negative relationship with turtle Cd. Selenium and Zn also exhibited positive relationships between soil and turtle blood concentrations.

Landscape predictors explained 6.6% of the variance in painted turtle nail concentrations after partialling out the effect of MCL and sex class (20.1%; Figure 3b). Concentrations of Se and Zn in painted turtle nail samples were negatively related to percent agriculture within 30 m, while Cd, Cr, and Pb exhibited a strong positive relationship. Mercury concentrations were positively related to distance from mine land and roads. Turtle nail Zn concentrations had a strong positive relationship with distance from roads. Wetland soil concentrations explained 7.5% of the variance in painted turtle nail concentrations after partialling out the effect of MCL and sex class (18.3%; Figure 4b). Turtle Se and Zn were positively related to soil Pb, while Cd, Cr, and Hg showed a strong negative relationship between turtle and respective soil concentrations.

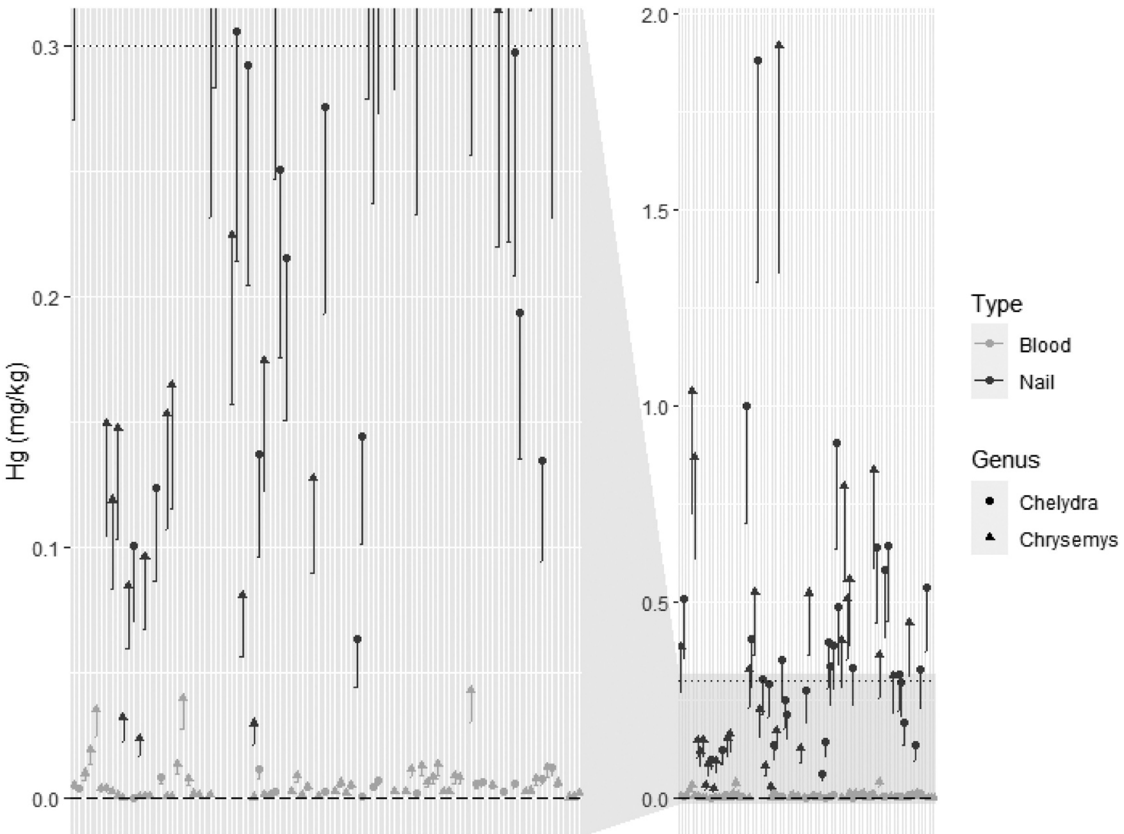

Figure 2. Total mercury (Hg) concentrations were found in nail and blood tissue samples from snapping turtles (*Chelydra serpentina*) and painted turtles (*Chrysemys picta*) in the Upper Deckers Creek watershed in Preston County, West Virginia, U.S.A., 2019. Lower bars for each point indicate the lower threshold for the estimated MeHg concentration defined by Bergeron et al. [18] as 70–100% of Hg being methylated. The zoomed subsection emphasizes samples between the 0.0001 mg/kg recommended daily consumption level (dashed line [58,59]); and the 0.3 mg/kg fish tissue criterion (dotted line [59]).

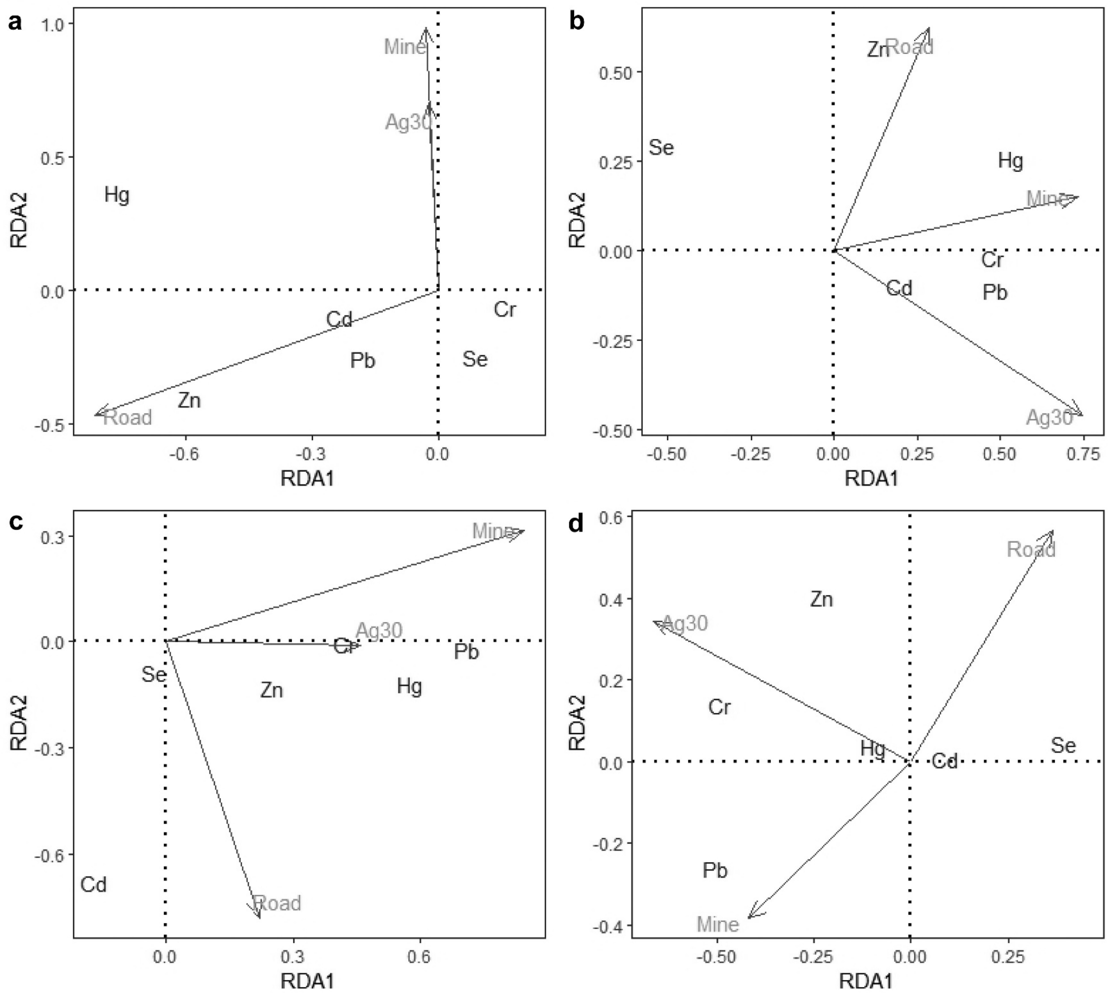

Figure 3. Redundancy analysis (RDA) biplots relating land use to painted turtle (*Chrysemys picta*; A) blood and (B) nails, and snapping turtle (*Chelydra serpentina*; C) blood and (D) nail Cd, Cr, Pb, Hg, Pb, Se, and Zn concentrations from individuals captured in the Upper Deckers Creek watershed in Preston County, West Virginia, U.S.A., during 2019. Response variables for each RDA are the six metals analyzed in turtles. The predictors included are straight-line carapace length (MCL), percent agriculture within 30 m, and distance from the nearest mine land and roads. Vectors describe the direction (positive or negative) and magnitude (strong or weak) of the relationships between predictors and response variables.

Snapping turtle redundancy analyses

Landscape predictors had no explanatory power (adjusted $R^2 = -0.033$) for snapping turtle blood concentrations after partialling out the effect of MCL and sex class (5.6%; Figure 3c). Blood Cd and Se concentrations had a strong negative relationship with percent agriculture and distance from mine land, while Cr, Hg, Pb, and Zn levels had a positive relationship. Despite the small sample size of snapping turtle blood, wetland soil concentrations improved the explained variance for snapping turtle blood concentrations (11.6%) after partialling out the effect of MCL and sex class (−1.3%; Figure 4c). Lead, Hg, Se, Pb, and Zn concentrations in turtles were negatively related to their respective soil concentrations. Blood Cr also exhibited a weak negative relationship with soil Cr.

Landscape predictors had no explanatory power (adjusted $R^2 = -0.027$) for snapping turtle nail concentrations after partialling out the effects of MCL and sex class (−4.8%; Figure 3d), whereas wetland soil concentrations explained 9.9% after partialling out demographic effects (0.3%; Figure 4d). Distance from mine land was negatively related to turtle Cd, Hg, and Se, whereas distance from roads was negatively associated with Hg and Pb. Chromium and Zn had a strong positive relationship with percent agriculture. Regarding soil concentrations, there was a negative relationship between Cr and Pb in turtle nails and Cr and Pb concentrations in wetland soil, respectively. Like painted turtles, there was a strong positive relationship between soil Se and Se levels in turtle nails.

Soil redundancy analyses

All metals were detected in soil samples of the 29 sites surveyed. The RDA that described the relationship between landscape parameters and wetland soil concentrations explained 12.0% of the total variance (Figure 5). Distance from roads had a strong positive correlation with Zn and Se in soil. There was a strong

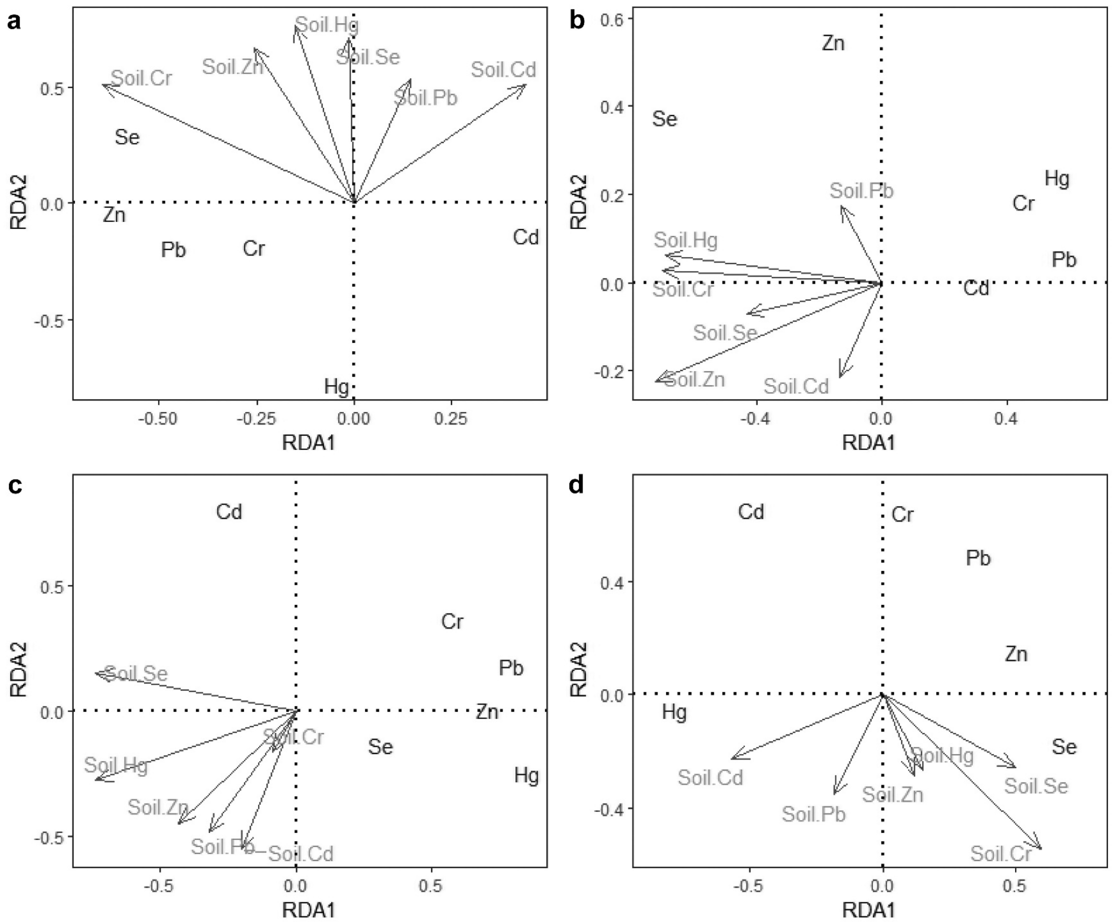

Figure 4. Redundancy analysis (RDA) biplots relating soil metal concentrations to painted turtle (*Chrysemys picta*; A) blood and (B) nail and snapping turtle (*Chelydra serpentina*; C) blood and (D) nail Cd, Cr, Pb, Hg, Pb, Se, and Zn concentrations from individuals captured in the Upper Deckers Creek watershed in Preston County, West Virginia, U.S.A., during 2019. Response variables for each RDA are the six metals analyzed in turtles, and predictors included are straight-line carapace length (MCL) and the six metals analyzed in soil. Vectors describe the direction (positive or negative) and magnitude (strong or weak) of the relationships between predictors and response variables.

positive relationship between distance from mine land and Cd, Pb, and Hg in soil, while wetland soil Cr concentrations were lower in wetlands farther from mine land. Most metals found in soil were negatively related to percent agriculture within 30 m.

Body condition index

Residual weight values (difference between log-transformed actual weight and expected weight) varied based on tissue type and turtle species. Concentrations in painted turtle blood led to residuals of −3.36 to 3.06 and painted turtle nail concentrations led to residuals of −3.16 to 2.26. Concentrations in snapping turtle blood led to residuals of −0.88 to 2.74, while snapping turtle nail concentrations led to residuals of −1.5 to 4.05. For both the blood and nail analyses, the model selections did not support the species-specific effects of the xenobiotic metals on BCI (Table 5). The most supported candidate model for the blood analyses was Cd ($w_i = 0.37$), followed by the null model ($w_i = 0.19$). The most supported candidate model for nail metal concentrations was the null model ($w_i = 0.23$), although Zn, Hg, Se, Cd, and Cr all had strong support (ΔAICc <2). Cadmium concentrations in turtle blood had a negative relationship with BCI (coef. = −0.197; CI: −0.35 to −0.04).

Discussion

Difference between turtle species and tissue types

Results showed that all metals were higher in nails than blood, as expected, but concentration differences between species varied across metals, which could potentially be attributed to dietary differences [60–62] and the temporal inference of each tissue type – nails reflecting long-term accumulation [11,63] and blood reflecting recent accumulation [64,65]. Although both painted turtles and snapping turtles are omnivorous generalists [14], snapping turtles can bioaccumulate higher short-term levels of metals due to their larger prey items, including fish, other turtles, and small waterfowl [14]. High blood Hg

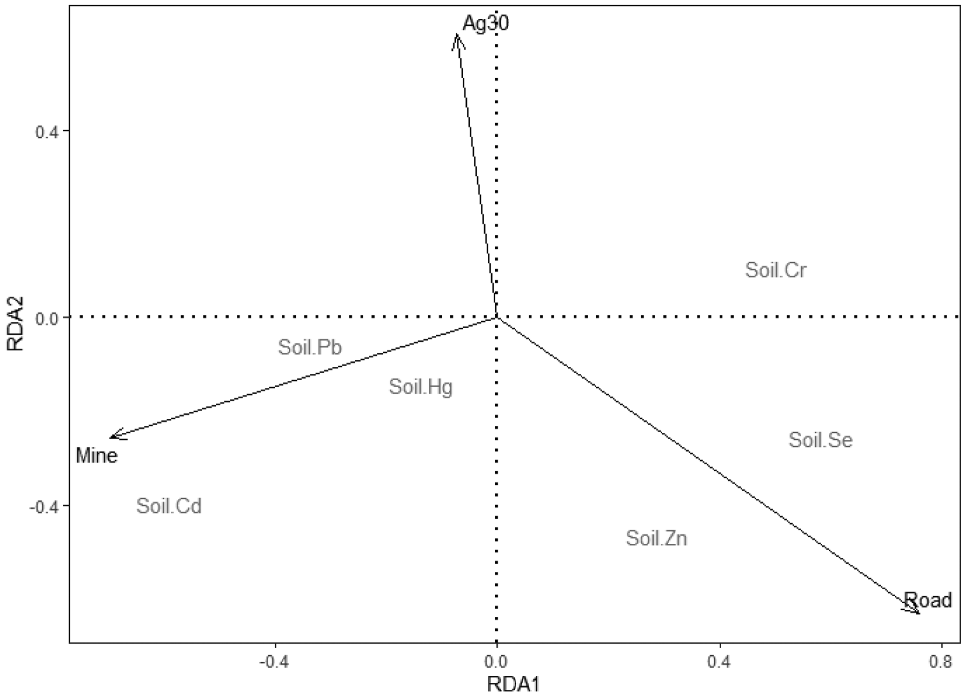

Figure 5. Soil redundancy analysis (RDA) biplot to relate soil Cd, Cr, Pb, Hg, Pb, Se, and Zn concentrations with surrounding land use practices. Soil samples were analyzed from 28 wetland sites (one outlier site omitted) in the Upper Deckers Creek watershed in Preston County, West Virginia, U.S.A., 2019. The multivariate response variable was the six metals analyzed in wetland soil. Land use variables were predictors, including distance to nearby roads and mine land. Vectors describe the direction (positive or negative) and magnitude (strong or weak) of the relationships between predictors and response variables.

Table 5. Candidate linear models for body condition indices of snapping turtles (*Chelydra serpentina*) and painted turtles (*Chrysemys picta*) from the Upper Deckers Creek watershed in Preston County, West Virginia, U.S.A., based on xenobiotic metal bioaccumulation in blood and nail tissues the Akaike information criterion corrected for small sample size (AICc), change in AICc from the top model (ΔAICc), and model weight (w_i). Additive models were tested for individual variables that had greater support than the null model (.).

Model	AICc	ΔAICc	w_i
Blood			
Cd	**214.24**	**0.00**	**0.37**
(.)	**215.52**	**1.28**	**0.19**
Pb	217.36	3.12	0.08
Hg	217.52	3.27	0.07
Cr	217.61	3.37	0.07
Zn	217.62	3.38	0.07
Se	217.68	3.44	0.07
Cd * Species	218.56	4.31	0.04
Pb * Species	220.75	6.51	0.01
Hg * Species	221.36	7.11	0.01
Zn * Species	221.58	7.34	0.01
Se * Species	222.01	7.77	0.01
Cr * Species	222.08	7.83	0.01
Cd + Cr + Hg + Se + Pb + Zn	224.77	10.53	0.00
Cd * Species + Cr * Species + Hg * Species + Se * Species + Pb * Species + Zn * Species	242.22	27.98	0.00
Nail			
(.)	187.13	0.00	0.23
Zn	187.79	0.66	0.17
Hg	188.51	1.38	0.12
Se	188.58	1.45	0.11
Cd	188.67	1.54	0.11
Cr	188.70	1.58	0.11
Pb	189.28	2.15	0.08
Zn * Species	192.43	5.30	0.02
Hg * Species	192.76	5.64	0.01
Se * Species	193.19	6.07	0.01
Cr * Species	193.30	6.18	0.01
Cd * Species	193.33	6.20	0.01
Pb * Species	193.55	6.42	0.01
Cd + Cr + Hg + Se + Pb + Zn	194.65	7.52	0.01
Cd * Species + Cr * Species + Hg * Species + Se * Species + Pb * Species + Zn * Species	211.56	24.44	0.00

concentrations can be attributed to dietary Hg uptake [11] or additional factors such as body mass [20,66]; however, nail Hg can be significantly higher than blood concentrations due to the high affinity for Hg that is found in keratinized tissue [11].

Selenium concentrations were higher in painted turtle nails relative to snapping turtle nails, unlike other studies that compared Se concentrations in species across trophic levels. In Tennessee, there were no significant differences in Se bioaccumulation found between snapping turtles, map turtles (*Graptemys* spp.), musk turtles (*Sternotherus odoratus*), and red-eared sliders (*Trachemys scripta elegans* [67]. A similar study in Virginia found that feeding ecology was related to MeHg and Se bioaccumulation (snapping turtles ≥ musk turtles > painted turtles > northern red-bellied cooter [*Pseudemys rubriventris*]), where more carnivorous omnivores like snapping turtles accumulated higher concentrations than other omnivores within the same system [18]. The partial RDA model for snapping turtle nails indicated, however, that more variance was explained using demographic predictors.

Zinc concentrations in the present study were as high as 400-fold greater in nails than blood and 40-fold greater than any other metal tested, similar to what was found in a population of Mediterranean pond turtles (*Mauremys leprosa*) in a contaminated mining district in Spain (Zn > Cu > Pb > Hg > Cd [68]. High concentrations of Zn across species may be attributable to the essential role of Zn in turtle development, and it is found in all tissue types due to affinity toward blood plasma [69]. Despite the potentially toxic effects that could occur in organisms with high levels of Zn [69], it has been reported to not be of consequence to turtles due to the metabolic equilibrium of Zn uptake and excretion [70]. Homeostatic regulation of Zn has been observed in Hawksbill sea turtles (*Eretmochelys imbricata* [69], green turtles (*Chelonia mydas* [71], and Caspian pond turtles (*Mauremys caspica* [72]; to prevent toxic effects. Other metals such as Cu, Cd, and Hg undergo similar regulation to reach an equilibrium [73] or detoxification processes involving metallothionein [74]. These equilibria being reached, along with other adaptations like avoidance behavior [75], could explain why there was no significant relationship between body condition and accumulation of all metals but Cd in this study. The effects of chronic Cd accumulation in green turtles were similar to those of this study in that contaminated turtles exhibited immunosuppression, which lowered their body condition [76]. Conversely, Yu et al. [77] found no differences in body condition when comparing populations of snapping turtles and red-eared sliders above (no exposure) and below (exposure to Cu, Cd, Cr, Pb, and Hg) a gaseous diffusion plant.

Land use effects on turtle heavy metal concentrations

Cadmium, Se, and Zn were negatively related to distance from mine land across species and tissue types, which was expected due to coal runoff and other contaminants associated with mining activity [2,78]. Similar Se patterns were found in mud turtle (*Kinosternon subrubrum*) blood at the Savannah River Site in Georgia, where heavy impacts from coal combustion residue were present [79]. Interestingly, Se was the only metal in the current study that showed a linear relationship where the distance from mining was positively related to Se levels in soil, and soil Se concentrations showed a positive relationship with concentrations found in turtle nails. Natural processes such as dietary uptake [80] or maternal transfer [20] likely contribute to this relationship. Like Se, Zn is an essential metal for turtle egg development Storelli and Marcotrigiano [81] and overall health [82]. Huang, Chen, and Huang [83] found the optimum level of Zn in Chinese softshell turtles (*Pelodiscus sinensis*) is 35–46 mg/kg, so possible variation in diet items from wetlands surrounded by different land use types may be considered in future studies as the same study found concentrations of metals like iron and Se were negatively affected by Zn concentrations in carapace tissue [83].

Study results showed that turtles had higher concentrations of Pb in wetlands further from roads, which is opposite from the relationship between Pb and roads that has been reported in diamondback terrapins (*Malaclemys terrapin*) and plants [84] Burger [85] [1]. The lower concentrations of Pb in turtles from wetlands adjacent to roads compared to wetlands farther from roads may be due to the low vehicular traffic observed in the area during the study, as most contaminants affiliated with roads are from vehicle wear [1,86,87]. This unexpected relationship between Pb and proximity to roads could imply a possible need for further analyses, including land use interactions (e.g. wetlands closer to mine land are naturally farther from roads). Residual effects in wetland systems from mine lands could be a factor as well, regardless of how long post-reclamation [88]. This current study observed a weak negative correlation between wetland proximity from roads and mine land (Pearson correlation coefficient = –0.25). Higher Pb concentrations than expected in turtles from wetlands farther from roads could be due to road factors that were not measured in this study, such as road age, vehicle traffic density, and vehicular speed [89]. Other metals associated with roads, such as Cd and Cr in turtles, also showed positive or weak negative relationships with proximity to roads, which could be because trace amounts were detected in all sample types across species. However, most turtles use multiple wetlands during a year, so the capture location may not be indicative of where they spend all of their time [35]. Because of their long-lived nature, metal concentrations of turtles could reflect historical land use, but the duration of Pb body burdens on individual turtles would need to be investigated further.

Some historic problem mining areas within the watershed have since been reclaimed (34.8 ha). Still, about 75% of mines that were permitted around the 1990s (278.7 ha) have been completely released [90], meaning the area is not actively mined and is defined as 'released acres' [91]. Whether or not this released land has been reclaimed is uncertain. Depending on the number of years since reclamation, turtles could be reflecting past contamination due to their long lifespan.

Land use effects on soil concentrations

The negative relationship between agriculture within 30 m of wetlands and soil metals (i.e. Hg, Pb, and Zn) was unexpected based on previous studies (e.g [92,93]. Opposite trends were found in New York and Connecticut, where pastures had higher concentrations of Hg in the A soil horizon [94]. The negative pattern this current study observed may result from metal uptake by plants and subsequent removal through agricultural practices (i.e. phytoremediation [95; 96]. Separating hay and pasture from cultivated crops rather than analyzing all as 'agriculture' could be further investigated to determine differences in metal contamination between land use practices (i.e. livestock introduction compared to seasonal removal of crops).

Conclusions

Results from this investigation indicate that Cd, Cr, Pb, Hg, Pb, Se, and Zn are present in wetland soils within the Upper Deckers Creek watershed. Landowners could consider management practices that limit the harmful long-term effects of heavy metal bioaccumulation that could affect freshwater turtles. Coal mining activity, whether current or historic, has impacted the environment for decades. Point source pollutants have the potential to manifest in organisms such as freshwater turtles and are an important consideration when implementing and managing wetlands and riparian buffer zones. Finally, turtle harvesting limits may need further investigation to ensure the safety of residents who consume turtles within the study watershed and similarly managed watersheds globally. Whether or not snapping turtles are being captured for consumption in this region, the presence of metals is an indication of the overall ecosystem status, which could reflect the status of taxa that are regularly captured for consumption like fish.

Acknowledgments

We thank the National Research Center for Coal and Energy for analyzing tissue samples and the West Virginia University Institute of Water Security and Science for providing logistic support. We thank J. Mota, J. Gordon, M. Tenney, and A. Gulette for field assistance, and we thank the various landowners and the WV Division of Natural Resources for permission to research their properties.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the National Science Foundation under Grant [OIA-1458952]; USDA National Institute of Food and Agriculture McIntire Stennis under Grant [WVA00812]; Society of Wetland Scientists Student Research Grant; West Virginia University David and Catherine Samuel Wildlife Graduate Fund; the West Virginia Agricultural and Forestry Experiment Station, and the West Virginia University Natural History Museum. The USDA National Institute of Food and Agriculture, McIntire Stennis project [WVA00820], the West Virginia Agricultural and Forestry Experiment Station, and the USDA Forest Service Northern Research Station supported Donald Brown during the data collection phase of the study. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government. The findings and conclusions in this publication are those of the authors and should not be construed to represent any official USDA or U.S. Government determination or policy.

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