Assessment of Heavy Metal and Metalloid Concentrations at Horicon National Wildlife Refuge

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Abstract

Anthropogenic inputs of heavy metals and metalloids pose a risk to wetland ecosystems due to their long retention time in sediment, high toxicity at low concentrations, and ability to biomagnify in the food chain. Our study involved an extensive monitoring effort for seven heavy metals (cadmium:Cd, chromium:Cr, copper:Cu, mercury:Hg, nickel:Ni, lead:Pb, zinc:Zn) and one metalloid (arsenic:As) in sediment, roots of the invasive hybrid cattail (*Typha x glauca*), and livers from muskrats (*Ondatra zibethicus*) at Horicon National Wildlife Refuge, a wetland of international importance in southeastern Wisconsin, United States. Overall, our comparison to literature values and thresholds led us to conclude that heavy metals and metalloids pose a low risk to the refuge. The highest concentrations were found in the sediment, followed by *T. x glauca* roots, and with negligible concentrations in muskrat liver tissue for all but the essential metals Cu, Ni and Zn, indicating low biomagnification in this food chain. A spatial analysis using GIS revealed hotspots for Cd, Cr, Cu, Ni, and Zn in sediment in one particular subplot, which we hypothesize may be from runoff of agricultural amendments. However, since concentrations in sediment were similar to or lower than concentrations found in a prior survey from 1990, there may have been improvement over the last three decades. Overall, while anthropogenic influences are present, we recommend that our relatively low concentrations be used as healthy points of comparison regarding risk to plants and mammals for others conducting metal and metalloid surveys on wetlands.

Introduction

Heavy metals and metalloids are an important class of environmental pollutants. While in small quantities some are essential for biological functioning in plants and animals (i.e., copper, nickel, and zinc), they are known for their high toxicity (Alloway, 2013). In addition, they cannot be degraded by biological processes, which can lead to accumulation in the environment (Sidhu, 2016). While traditionally ill-defined, one standardized definition of heavy metals posits that they must have atomic numbers greater than 20, densities greater than 5 g cm^{-3}, in addition to being naturally occurring metals (Ali & Khan, 2019). Metalloids are less well-defined but tend to have the physical appearance and properties of metals while behaving chemically like non-metals (Atkins & Jones, 1997). Of the 51 heavy metals and six metalloids that fit these criteria, the most common soil contaminants on a global scale are the metalloid arsenic (As) and the heavy metals cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb) and zinc (Zn) (He et al., 2015; Wai et al., 2016). While these elements occur in the Earth's crust and are released naturally through weathering (Tchounwou et al., 2012), unprecedented anthropogenic inputs primarily through fossil fuel combustion, industrial emissions, pesticide and fertilizer application, and mining have led to consequences worldwide (Han et al., 2002). A metal's species is the most important factor influencing its mobility and bioavailability and thus toxicity to organisms which, in turn, is influenced by factors such as soil composition, dissolved oxygen, and pH (Beyer & Meador, 2011).

Wetlands can aid with metal and metalloid pollution with mechanisms including sequestration in sediment and plants, or transformation into more innocuous species through microbial activity and/or plant mechanisms (Rebello et al., 2021). However, wetlands also support a large number of trophic levels, which makes higher organisms susceptible to toxicological effects resulting from biomagnification (Ali & Khan, 2019). One such species that may be at risk is the muskrat (*Ondatra zibethicus*), a common, primarily herbivorous, semi-aquatic mammal that is declining across North America for undetermined reasons (Sadowski & Bowman, 2021). One potential contributing factor is contaminants including heavy metals and metalloids, which may be introduced to their diet through the ingestion of cattail roots (Erickson & Lindzey, 1983). Two common cattail species found in North American wetlands, *Typha angustifolia* and *Typha latifolia*, have been shown to accumulate Cd, Cr, Cu, Hg, Ni, and Zn at concentrations higher than in the surrounding sediment, with the addition of Pb in *T. latifolia* (Bonanno & Cirelli, 2017; Chandra & Yadav, 2011; Klink et al., 2013). The cross between these two species results in the hybrid cattail, *Typha x glauca*, which forms dense monotypic stands that produce copious amounts of litter (Larkin et al., 2012). As a result, it tends to outcompete both of its parent species (Larkin et al., 2012). If it differs from its parent species in its metal uptake capacity, it could alter metal and metalloid cycling in wetlands. Some lines of evidence suggest that muskrats may preferentially feed on hybrid cattail over its parent species. Larreur et al., (2020) showed higher muskrat occupancy with greater *T. x glauca* coverage, while Droste (2015) demonstrated that hybrid cattail is easier for muskrats to metabolize and is more nutritious (Droste, 2015; Larreur et al., 2020).

In the present study, we took a closer look at the potential trophic transfer pathway from sediment to the roots of hybrid cattail to the liver tissues of muskrats at Horicon National Wildlife Refuge (HNWR) in southeastern Wisconsin. The refuge has been designated as a wetland of international importance by the Ramsar Convention of the United Nations due to its importance for maintaining biological diversity in the region. *T. x glauca* is the dominant plant species on the refuge, growing primarily on poorly drained organic-rich, muddy sediment (Staffen, 2012). Muskrats are abundant and a select number of certified trappers can harvest them under refuge regulations. In 1990, two to three sediment samples were taken at two sites from 0 to 4 inches and analyzed for concentrations of seven heavy metals (Cd, Cr, Cu, Hg, Pb, Ni, Zn) and one metalloid (As) via inductively coupled plasma optical emission spectrometry (ICP-OES) (Warner, 2012; J. Killian, personal communications, May 26, 2021). Concentrations tended to be higher at the northern site ($n = 3$), ranging on average from 0.12 mg/kg for Hg to 95 mg/kg for Zn compared to 0.04 mg/kg for Hg and 23 mg/kg for Zn at the southern site ($n = 2$) (Warner, 2012). We expanded the 1990 sampling effort to cover a greater area of the refuge and include an analysis of *T. x glauca* roots and muskrat livers in addition to sediment. Our main goals were to: 1) characterize the element concentrations in all three sample types and compare them to literature values and thresholds to assess risk, 2) investigate uptake by *T. x glauca* to provide evidence for or against a proposed trophic transfer pathway for metal and metalloids in muskrats, 3) consider spatial variation to gain clues to potential sources of any elevated concentrations, and 4) compare current sediment concentrations to concentrations measured in 1990.

Materials And Methods

STUDY AREA

Sampling took place at Horicon National Wildlife Refuge which is situated on the west branch of the Rock River in southeastern Wisconsin (43° 34' N, -88° 37' W). The refuge is divided into 18 impoundments whose water levels can be manipulated separately. We collected samples from four plots across three impoundments that ranged from the North to the South end of the refuge: Radke, Teal, Main Pool North (MPN) and Main Pool South (MPS), each of which were assigned three 65-acre subplots (Figure 1). Radke, at 735 acres, is the northmost impoundment and has major inflows from the city of Waupun to the north, which may contribute metal and metalloid pollutants through urban runoff, sewage, and several large industrial sources. Teal, at 958 acres, borders Radke to the south and has high agricultural input from the east. Both Radke and Teal, along with all other unsurveyed impoundments drain into Main Pool, the largest water body at 12,193 acres. Therefore, Main Pool represents an amalgamation of various metal/metalloid sources. The refuge's bedrock primarily consists of dolostone, with a small portion in the southeast corner also containing shale (Figure 1) (United States Geological Survey, 2012).

SAMPLE COLLECTION

Collection of sediment, *Typha x glauca* roots, and muskrat livers took place over five days between March 2 and March 14, 2021. Local trappers set steel body grip traps following the Wisconsin Department of Natural Resources Wisconsin trapping regulations in each of the nine subplots (Wisconsin Department of Natural Resources, 2020). From each subplot, they donated three adult muskrats (> 1 year old as determined by size) to the project. Animals were skinned in the field and the whole remaining carcasses were bagged in individual Ziploc® bags. At each location where a muskrat was taken (N = 36), S. Woody collected and bagged sediment (N = 36) and *T. x glauca* roots (N = 35) in plastic ziptop bags from Uline. Sediment was obtained from between 10–20 centimeters below the top sediment layer using a 2-inch diameter plastic homemade corer. Between samples, the corer was rinsed with Liquinox® followed by deionized water. *Typha x glauca* was uprooted with the aid of a chisel, and a 6-inch section of rhizome with attached root hairs was cut from the bottom of the plant using a steel hatchet (manufacturer Otakumod). All samples were transferred to the University of Wisconsin Oshkosh in coolers and were stored in a freezer within eight hours of collection until further processing.

LAB ANALYSIS

Lab work was conducted at the Wisconsin State Laboratory of Hygiene Trace Elements Clean Lab in Madison, Wisconsin. Sediment was thawed in a fridge for three days prior to processing. The entire contents of each bag were poured into a 250-micron nylon sieve (manufacturer Wildco) and the fine contents were collected in a polypropylene tube from Falcon® and re-frozen. The tubes were then placed in a freeze drier for one week. The dried sediment was ground into a fine dust using an agate mortar and pestle. Cattail rhizomes with attached roots were hand-washed with Type II water to remove visible dirt particles and then subsequently freeze dried for three days or until dry. The dried cattail roots were separated from the rhizome, cut into fine pieces with ceramic scissors, and placed into polypropylene tubes. Muskrat carcasses were thawed in a refrigerator for three days prior to dissection. Livers were removed from each animal using ceramic scissors and scalpels (manufactured by Slice Industrial), homogenized by chopping into fine pieces with a ceramic knife (manufactured by Vicera), and refrozen until subsequent analysis. In all preparation procedures, any reusable tools were cleaned using dilute trace metal grade nitric acid and Type I reagent water.

All samples underwent a similar digestion process based on methods by Giesy & Wiener (1977), with some adjustments made depending on sample type. Sub-samples were weighed on a Mettler XPE205 analytical balance (0.95 – 0.105 g. dried sediment, 0.1–0.2 g. dried cattail root, 0.045–0.055 g. wet, thawed liver) and 1.0–2.0 mL concentrated Optima grade nitric acid (HNO_3) was added to all tubes and allowed to pre-digest overnight. Samples were placed in a digestion block at 95°C for 1 hour to dissolve the material. After cooling, 0.5 mL of 30% Optima grade hydrogen peroxide (H_2O_2) was added to each tube and returned to the hot block for half an hour. This step was repeated with half of the cattail samples and all sediment samples due to the persistence of fine solids. Half of the cattail samples received an additional 0.5 mL of hydrochloric acid (HCl) and 45 minutes of hot block heating. Sediment samples received an additional 2 mL of Optima HNO_3 and were returned to the hot block for 45 minutes followed by one final addition of 1.5 mL Optima HCl and one more hour of hot block heating. After cooling, samples were brought up to a final volume of 50 mL with Type I reagent water for analysis by a high resolution inductively coupled plasma mass spectrophotometer (HR-ICP-MS) using a Thermo Scientific Element 2.

The digests of each sample type were split into two batches. Each batch was processed along with two standard reference materials (SRMs) certified by the National Institute of Standards and Technology or National Research Council of Canada: sediment (Buffalo River Sediment, Ref. No. 2704 and Marine Sediment, Ref. No. 2702), cattail (Apple Leaves, Ref. No. 1515 and Tomato Leaves, Ref. No. 1573), and muskrat liver (Bovine Liver, Ref. No. 1577b, and Dogfish Liver, Ref. No. DOLT-3) (National Institute of Standards and Technology, 2022; National Resource Council Canada, 2022). Also included with each batch were three reagent blanks (Optima grade HNO_3 only) and one fortified blank (Optima grade HNO3 with added spike solution containing elements of interest). Precision and accuracy of the preparation and analysis were assessed by preparing duplicate and matrix spike controls at a frequency of 10%, selected from samples of sufficient mass. Most average recoveries for the SRMs, average percent differences between digestion and analytical duplicates, and matrix spike recoveries fell in the acceptable ranges from 80–120%, 0–25%, and 75–125% respectively with some exceptions (Tables S1 – S3). Concentrations measured from the diluted digests were blank corrected relative to the average concentrations of digested reagent blanks.

STATISTICAL ANALYSES

Limits of detection (LOD) for the specific instrument used are listed in Table 1. The dataset was adjusted so that any values under the LOD were set to the LOD. Of the 36 liver samples, 22 fell under the LOD for

As, 36 for Cr, 25 for Hg, and 35 for Ni. Of the 35 cattail samples, 2 fell under the LOD for Ni. Of the 36 sediment samples, 23 fell under the LOD for Hg. We conducted all statistical tests in R (RStudio Team, 2021). For all analyses involving liver, only Cu and Zn were studied as they were the only elements that accumulated in muskrat liver tissues at concentrations great enough to analyze. T tests were run prior to these analyses to determine if there were sex differences in element concentrations in muskrat livers. Tests relying on probability distributions to determine significance were two-tailed and $\alpha = 0.05$, except for the bonferroni adjusted *t*-test plot and subplot comparisons where $\alpha = 0.01$ and $\alpha = 0.017$, respectively.

Table 1
Limits of Detection for the Specific Instrument Used for HR-ICP-MS

	As	**Cd**	**Cr**	**Cu**	**Hg**	**Ni**	**Pb**	**Zn**
Sediment	0.14	0.05	0.29	0.15	0.06	2.37	0.02	0.64
Cattail	0.01	0.01	0.03	0.01	0.01	0.24	0.002	0.06
Liver	0.005	0.002	0.01	0.01	0.002	0.08	0.001	0.02

Instrument detection limits with units in mg/kg on a dry weight basis for sediment and roots of hybrid cattail and on a wet weight basis for muskrat livers.

Correlations between concentrations in different sample types were performed for variables that exceeded safe thresholds for plants and/or mammals (U.S. Environmental Protection Agency, 2005). Assumptions of linearity and absence of outliers were assessed visually with scatterplots and boxplots respectively, while assumptions of normality were checked with shapiro-wilk tests and homogeneity of variance with bartlett tests. Pearson’s correlations were used for elements where all assumptions were met with or without log transformation, while spearman’s correlations were used for those where they were not. To better understand potential trophic transfer, we calculated three types of bioconcentration factors (BCFs) as ratios between the sample types: 1)

$\text{Concentration}_{\text{root}}$:$\text{Concentration}_{\text{sediment}}$, 2) C_{liver}:C_{root}, and 3) C_{liver}:C_{sediment}. So that all sample types were being compared on a dry weight basis, we used the following formula to convert the muskrat liver concentrations from wet to dry weight prior to BCF concentrations based on a literature value of liver moisture content in rats of 72.2% (Cieslar et al., 1998):
Wet weight = Dry weight * (1/0.278)

To assess spatial variation of element concentrations in each sample type, we used nested ANOVAs with concentration of the element of interest in sediment, cattail, or muskrat livers (mg/kg, as an integer) as the dependent variable and subplot (factor with nine levels) nested within plot (factor with four levels: MPN, MPS, Radke, Teal) as the independent variable. We ran the test for all elements, even where normality and/or equal variance were not met after transformation (Hg in sediment and cattail were not normal, Cd and Cr in sediment and As and Zn in cattail did not meet equal variance assumption). For elements where significance was shown between plots in the ANOVA, we followed up with bonferroni corrected t-tests between each possible combination of plots to see between which plots the differences lie. Hotspot analyses in ArcGIS Online were used in conjunction with the ANOVAs to visualize locations where concentrations might be higher than other sampling locations (ESRI, 2021). Finally, *t* tests, or wilcox tests where normality and/or equal variance assumptions were not met as assessed by shapiro-wilk and bartlett tests, were used to assess differences in element concentrations in sediment between years (factor with 2 levels: 1990 and 2021). The two sites where samples were collected during both years, Main Pool North and Main Pool South, were analyzed separately.

Results And Discussion

CHARACTERIZATION OF ELEMENT CONCENTRATIONS AND COMPARISON TO LITERATURE

The sex ratio of collected muskrats was 13M:23F and concentrations did not differ based on sex for Cu ($t = -0.01$, $p = 0.99$), nor for Zn ($t = 0.784$, $p = 0.44$). The concentrations (based on wet weight in mg/kg) for each element in muskrat livers are as follows: arsenic ranged from 0.005–0.02, cadmium 0.002–0.02, chromium 0.01–0.5, copper 1.34–3.65, mercury 0.002–0.02, nickel 0.08–0.19, lead 0.0007–0.02, and zinc 19.37–30.98 (Fig. 2). Copper and zinc, both essential metals, were the only elements accumulating in muskrat livers at levels that were noticeably higher than the instrument detection limits. While no experimental heavy metal/metalloid toxicology studies have been conducted on muskrats, such studies have been conducted with similar species. Mink with Zn liver levels up to 212 mg/kg wet weight experienced no adverse effects (Aulerich et al., 1991), which is far above the 30.98 mg/kg observed in muskrats on the refuge. For Cu, excretory capacity in rats was not overwhelmed until liver concentrations exceeded 20 mg/kg wet weight (Milne & Weswig, 1968), far exceeding the maximum concentration found in the present study of 3.65 mg/kg (Fig. 2). In an observational study, concentrations of essential metals Cu and Zn in livers of muskrats collected near a smelter were comparable to the present study, ranging from 1–2.6 and 18.4–27.4 mg/kg wet weight respectively, while concentrations of non-essential metals Hg (non-detectable – 0.22) and Pb (0.27–0.96 mg/kg wet weight) were higher than in the present study (Blus et al., 1987). Based on these comparisons, neither Zn, Cu, nor any of the other elements studied are presumed to be at levels harmful for muskrats on the refuge.

The concentrations (based on dry weight in mg/kg) for each element in hybrid cattail are as follows: arsenic ranged from 0.19–11.5, cadmium 0.03–1.06, chromium 0.05–2.64, copper 1.82–59.25, mercury 0.006–0.02, nickel 0.2–4.67, lead 0.83–42.9, and zinc 2.01–53.66 (Fig. 3). Levels found in *T. x glauca* roots tended to be lower than in other *Typha* species (*T. latifolia, T. angustifolia, T. domingensis*) growing in contaminated wetlands (Bonanno & Cirelli, 2017). For instance, during their first collection period, average concentrations in roots of *T. latifolia* growing at the inflow point of a constructed wetland near a landfill had higher mean root concentrations than in the present study for the following elements in mg/kg dry weight: As (39.22 ± 2.68), Cr (9.00 ± 1.02), Cu (38.3 ± 4.66), Ni (12.6 ± 1.99) and Zn (126 ± 10.68) (Salem et al., 2014). As *Typha* species tend to have generally high resilience to heavy metals and metalloids and since concentrations in our hybrid cattail roots tended to be lower than its parent species in contaminated wetlands, there is no presumed harm to this species on the refuge (Bonanno & Cirelli, 2017).

The concentrations (based on dry weight in mg/kg) for each element in sediment were as follows: arsenic ranged from 1.27–11.5, cadmium 0.16–2.83, chromium 2.58–19.68, copper 5.51–25.83, nickel 4.54–22.31, lead 3.28–42.42, and zinc 6.8–82.17 (Fig. 4). We compared the sediment concentrations to two different EPA thresholds, the protective soil screening levels for plants, and mammals, which were given on a dry weight basis in mg/kg (U.S. Environmental Protection Agency, 2005). Where data did not exist from the EPA, we used the Texas Commission on Environmental Quality's soil screening benchmarks for the protection of soil invertebrates or plants given in the same units (Texas Commission on Environmental Quality, 2021). Concentrations exceeded the soil screening level for plants for Cr only, of which all 36 sediment samples exceeded the threshold of 1 mg/kg. Concentrations in some sediment samples exceeded the soil screening levels for mammals for Cd and Zn. For Cd, 30 of 36 sediment samples exceeded the threshold of 0.36 mg/kg, while for Zn just one sediment sample exceeded the threshold of 79 mg/kg in MPN at 82.17 mg/kg in MPN. Overall, based on these thresholds, Zn is of low concern as only one sample exceeded the mammal threshold and only by a small margin. However, many samples exceeded the safe threshold for Cr in plants and for Cd in mammals.

Chromium does not have any known biological role in plant physiology and can impede growth and metabolic processes (Sharma et al., 2020). However, wetland plants have been shown to have high tolerance to metal contamination by sequestering them in the vacuoles of root cells thus preventing translocation to the aerial parts of the plant (Sharma et al., 2020). In the present study, a significant, but moderate positive correlation between Cr in the sediment and Cr in hybrid cattail roots ($r = 0.56$, $p < 0.001$) provides evidence that some but not all Cr in the sediment is bioavailable for uptake by *T. x glauca*. However, the Cr concentrations observed in hybrid cattail roots are not at levels presumed to cause harm. One study found no evidence of adverse effects in *T. latifolia* with maximum concentrations of 6.75 ± 1.20 mg/kg Cr in its roots, which is about three times higher than the maximum concentration we observed in *T. x glauca* roots at 2.64 mg/kg (Bonanno & Cirelli, 2017). Therefore, although the EPA suggests negative effects to plants at the current Cr levels in sediment, we do not suspect harm to hybrid cattail, which may extend to other resilient wetland plant species.

Cadmium has no known biological function and effects of Cd toxicity in mammals include reduction of food and water intake, growth depression, renal dysfunction, osteoporosis, hypertension, anemia, bleaching of incisors, and cancers (Cooke, 2011). While most of the Cd concentrations seen in soil samples in the current study surpassed the EPA's safe level for mammals of 0.36 mg/kg, levels in muskrat livers were negligible, ranging from just 0.002–0.02 mg/kg. While the liver is a main organ of accumulation for many metals, Cd has been shown to be 2–8 times higher in kidneys than in livers of small mammals (Cooke, 2011). Nevertheless, even at levels eight times higher than observed, liver concentrations from muskrats in the current study are still far below what is considered a sub-lethal effect level of 10 mg/kg (wet weight) in livers of vertebrates (Peakall & Burger, 2003). While other mammals could possibly be experiencing detrimental effects of Cd, since muskrats are generalist feeders, we would expect them to be fairly representative at least of other non-carnivorous wetland mammalian species.

TROPHIC TRANSFER

Bioconcentration factors (BFs) between sample types were calculated to provide insight into the potential trophic transfer pathway from sediment to hybrid cattail roots to muskrat livers. Typically, higher BF values imply a greater capacity for bioaccumulation, with BF values exceeding 1 indicating that a species could act as a hyperaccumulator of trace elements (Zhang et al., 2002). The elements showed the following decreasing trends in BFs (mean values), with asterisks representing those with BFs greater than 1:

- BF (root:sediment): Cu* > Pb > Zn > As > Cd > Hg > Ni > Cr
- BF (liver:root): Zn* > Cu*
- BF (liver:sediment): Zn* > Cu

Between root and sediment, the only element with an average BF exceeding one was Cu (1.01), indicating that *T. x glauca* was taking up at least as much Cu as was present in the surrounding sediment. Other notably high BFs over 0.5 included Pb (0.86) and Zn (0.81). Bioconcentration factors for the other elements decreased in the following order: As 0.49, Cd 0.41, Hg 0.17, Ni 0.12, and Cr 0.08. The results of BFs between root and sediment contrast with a previous study, which showed BFs in *T. domingensis*, *T. latifolia*, and *T. angustifolia* in a different order as follows: Hg > Ni > Cd > Zn > As > Cr > Pb > Cu, with Hg and Ni showing BFs greater than one (Bonanno & Cirelli, 2017). However, it is noteworthy that the ranges found in our sediment samples were fairly small, especially for As (1.27 to 11.5), Cd (0.03–1.06 mg/kg), and Hg (0.06 to 0.11), which may not have been sufficient to cause detectable differences in hybrid cattail roots (Fig. 4). The difference in BFs between the studies may also reflect differences in environmental conditions that would impact bioavailability of the studied elements for uptake by hybrid cattail. Alternatively, our results may provide evidence that *T. x glauca* differs in its potential to take up heavy metals and metalloids compared to other species in its genus. These results should encourage further lab and field research, as a true difference in metal uptake capacity between the hybrid cattail and other members of its genus has implications for metal cycling dynamics in wetlands as *T. x glauca* achieves dominance.

Between muskrat livers and hybrid cattail roots, average BFs for both Cu (1.45) and Zn (8.03) exceeded 1, indicating that muskrats were accumulating more Cu and Zn in their livers than were present in the roots. Between muskrat livers and sediment, the average BF exceeded 1 for Zn (4.41), while the BF for Cu was also high (0.79). Together, these results provide some evidence that consumption of hybrid cattail and/or incidental ingestion of sediment may be sources of Cu and Zn, both essential elements, in muskrat liver tissue. That no other elements besides Cu and Zn were accumulating in notable quantities in muskrat livers likely indicates a greater bioavailability of essential elements (Cu, Zn) over non-essential (Cd, Hg, Pb) or comparatively more toxic elements (As, Cr, Ni).

SPATIAL ANALYSIS

In sediment, differences in concentrations between plots were found for all elements except Hg and Pb, while between sublots differences were not seen for Cd, Hg nor Pb (Table S5). In hybrid cattail roots, there were fewer significant differences between plots and subplots. Concentrations differed for As among subplots and Cd, Cr, and Ni between plots. Post-hoc tests revealed that among plots, it was always one of the two Main Pool sites that were higher than Radke or Teal, an expected result as Radke and Teal flow into Main Pool (Table S4). Overall, the high degree of patchiness in sediment between plots, and to a lesser degree between subplots was an expected result given the complexities of metal and metalloid mobility and bioavailability. Less variability in cattail roots indicates that the plants are potentially regulating the amount of metals and metalloids that they are taking up. For instance, plants are known to exert influence on the pH of the rhizosphere by 2–3 units, which could release essential metals for them to take up (Nason et al., 2018).

GIS analysis revealed that Cd, Cr, Cu, Ni, and Zn shared a hotspot in the sediment in the northernmost subplot in MPN with average concentrations within the subplot as follows in mg/kg dry weight: Cd 2.27, Cr 18.57, Cu 22.93, Ni 19.63, Zn 75.18 (Fig. 5). In *T. x glauca* roots, only Cr showed a hotspot in the same location, which aligns with the predicted effect of accumulation in plants based on sediment concentrations in this location exceeding the EPA's threshold of 1 mg/kg by about 20 times (sediment average of 18.6 mg/kg) (Fig. 5). Two additional hotspots were located. A hotspot for Cu in cattail in the middle subplot in MPS (average 36.6 mg/kg), but not in the sediment suggests that *T. x glauca* may be actively taking up the essential metal, Cu, at that location (Fig. 6). The other hotspot was As in sediment in the leftmost subplot in MPS (average 5.3 mg/kg) (Fig. 6). Although these hotspots represent locations where concentrations were higher than other sampling sites, concentrations still remained under EPA thresholds, besides Cd and Cr, which exceeded thresholds for mammals and plants, respectively, as previously discerned from the overall averages. Coldspots were found in sediment in Teal for As (average 1.65 mg/kg) and in Radke for Cr (average 4.2 mg/kg), and Ni (average 5.2 mg/kg) (Fig. 5, Fig. 6).

We suspect that the observed sediment hotspot for Cd, Cr, Cu, Ni, and Zn in MPN may be anthropogenically influenced from runoff from the agricultural fields that border it to the east as all of these elements are known components of sewage sludge, which is often used as an agricultural amendment (Canet et al., 1998). We are less inclined to believe it is naturally caused as the underlying bedrock primarily composed of dolostone was the same for the hotspot location as most other sampling sites that did not display elevated concentrations (Fig. 5). In addition, we would not expect environmental conditions such as pH, dissolved oxygen or soil composition, which can affect retention of heavy metals and metalloids in sediments, to be drastically different in the hotspot compared to the other nearby subplots within MPN. While the hotspots in MPS are less easily explained, anthropogenic factors may have influenced these concentrations as well given that these sampling locations are located along a road and receive input from the west branch of the Rock River that could carry in contaminants. The coldspots located at the north end of the refuge provide evidence that potential metal and metalloid inputs contributing to the hotspots are less likely to be coming from the north.

COMPARISON OF SEDIMENT DATA 1990 TO 2021

Concentrations of elements in sediment were generally similar between 2021 and 1990 in both the northern and southern sampling sites with some exceptions (Warner, 2012). In the northern site, concentrations were significantly lower in 2021 on average for Cu (22.93 mg/kg in 2021, 31.33 mg/kg in 1990, $t = 4.3$, $p = 0.02$), Cr (18.57 mg/kg in 2021, 27.67 mg/kg in 1990, $t = 8.7179$, $p = 0.002$) and Zn (75.18 mg/kg in 2021, 90.33 mg/kg in 1990, $t = 3.21$, $p = 0.04$) (Fig. 7). No significant differences were found between years in the southern sampling site (Fig. 7). The observed decrease over three decades may be due to reduction in anthropogenic input or from natural changes in environmental conditions. Potential point source pollutants for Cu, Cr, and Zn include agricultural inputs, wastewater, or industry waste, which are all found in the vicinity of the refuge (Alloway, 2013). Therefore, reductions in agricultural applications or stronger regulations surrounding metal waste from wastewater treatment plants and/or industries over the last three decades may account for the observed changes. Reductions in inputs from non-point source pollutants including atmospheric deposition from mining, metal smelting and refining, manufacturing processes, transport, or waste incineration may also be a feasible explanation for the differences between years. The observed decrease may also be a natural phenomenon. Many environmental factors promote the release of metals from sediments, which would lead to lower detection in sediments. One such factor is lowered pH, which has an inverse relationship with dissolved oxygen and water temperature (Clark, 2017; Li et al., 2013). However, due to some known state regulatory changes regarding wastewater treatment plants as well as farming practice improvements, especially to the north of the refuge, we find more support for the anthropogenic improvement hypothesis.

It should be noted that the differences in sampling methods between 1990 and 2021 may have impacted these results. In 1990 samples were measured via ICP-OES (C. Dahman, personal communication, May 1, 2022), versus samples in the present study, which were measured with HR-ICP-MS. In addition, the 1990 samples were collected at a shallower depth of 0 -4 centimeters (K. James, personal communication, May 26, 2021), making them more susceptible to short term biological influences whether from human pollution or environmental such as changes in oxidation state. In addition, those samples were likely to contain more organic debris to which more metals and metalloids may have adhered. Nevertheless, while fine differences could be impacted by these method differences, both methods should give an accurate overall picture of total metal concentrations.

Conclusions

Taken together, our results show low concentrations of most of the studied elements, with concentrations in 2021 similar or less than levels measured in 1990 (Warner, 2012). In sediment, Cd and Cr concentrations in particular exceeded the EPA’s thresholds for the protection of mammals and plants respectively. However, weak accumulation patterns between sediment concentrations and hybrid cattail root and muskrat tissue levels hint at low bioavailability from the total metal concentrations, which suggests lower risk. *Typha x glauca* exhibited a definite ability to take up the studied elements in its roots like its parent species, but possibly for different elements, which has implications for metal and metalloid cycling dynamics in wetlands as *T. x glauca* achieves dominance. While accumulation of all the studied metals in the roots of *T. x glauca* indicates the possibility for biomagnification in muskrats, only Cu and Zn accumulated in significant quantities in muskrat livers. In contaminated wetlands where metal and metalloid concentrations are higher overall, this trophic transfer pattern may become more pronounced and apparent for other elements. Based on these data, no management action is currently recommended at Horicon National Wildlife Refuge beyond follow-up surveys in subsequent years. However, it would be valuable to also assess other potential trophic pathways, for instance, aquatic invertebrates to birds, as well as to collect data in other seasons and in unsampled areas to gain a fuller picture of the impact of metals and metalloids on the refuge. While not untouched by anthropogenic influences, we recommend that our results be taken as a rare “healthy” point of comparison regarding risk to plants and mammals for other researchers conducting heavy metal and metalloid surveys on other wetland complexes.

Declarations

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Data availability. Authors confirm that all relevant data are included in the article and would be ready to share the raw data upon request.

Conflict of interest. The authors declare that they have no conflict of interest/competing interests.

Author contributions. All authors contributed to the study conception and design. Field work was facilitated by Sadie O’Dell and Jon Krapfl and carried out by Sarah Woody. Lab work was conducted by Sarah Woody. Statistical analyses were completed by Sarah Woody with guidance from M. Elsbeth McPhee. The first draft of the manuscript was written by Sarah Woody, which was reviewed and edited by all authors. All authors read and approved the final manuscript.

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Figures

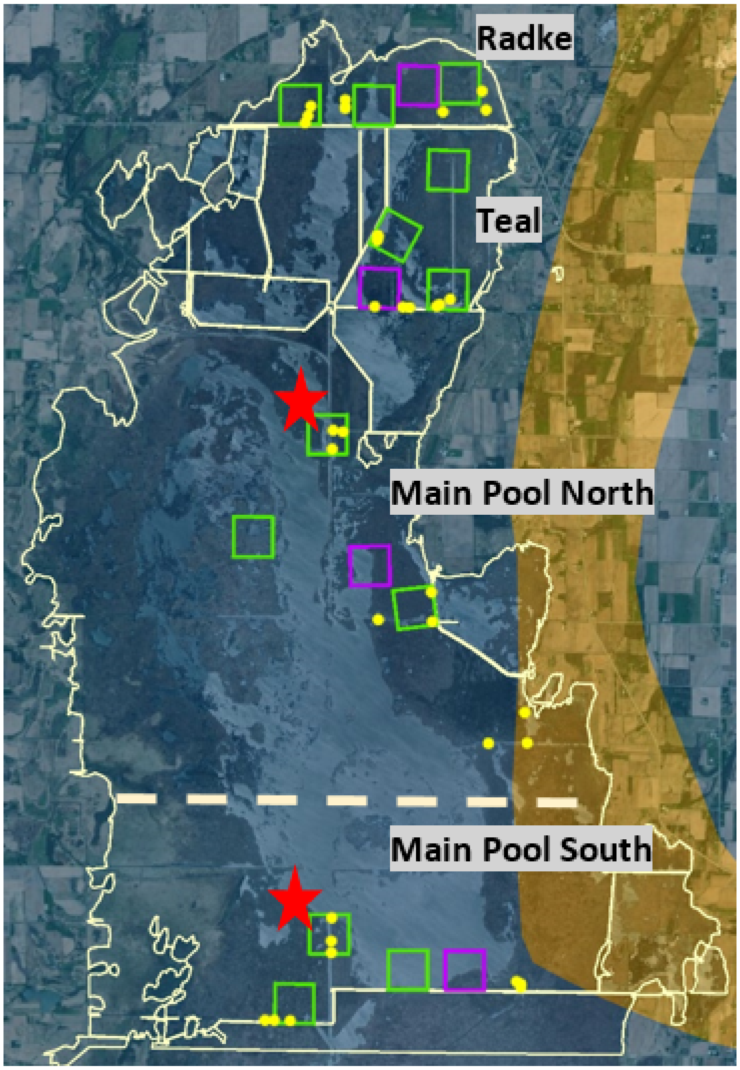

Figure 1

Map of the boundaries of the 18 impoundments at Horicon National Wildlife Refuge, with the four areas designated as plots labelled. Three 65-acre subplots (green squares) were designated within each plot with three sampling points in each (yellow dots). At each sampling point, sediment, hybrid cattail root, and a muskrat carcass were collected for heavy metal and metalloid analysis. The two plots marked with stars denote areas where sediment samples were also collected and analyzed for heavy metal and metalloid concentrations in 1990 (Warner, 2012). The majority of the refuge, shaded in blue, is underlaid by dolostone, with minor components of limestone and shale. A smaller portion in the southeast corner, shaded in orange, contains shale and dolomite with no minor components.

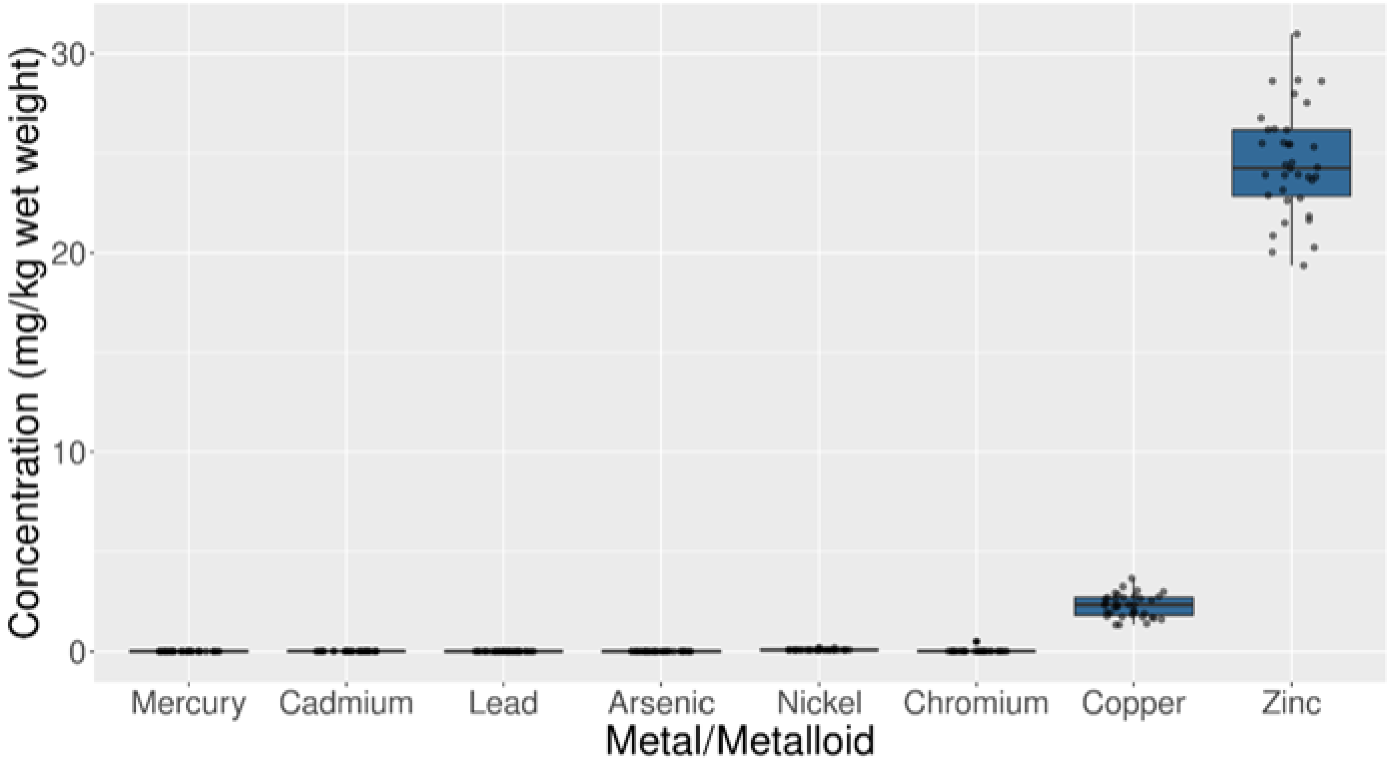

Figure 2

Concentrations of elements in muskrat livers analyzed via high resolution inductively coupled mass spectrometry (N = 36).

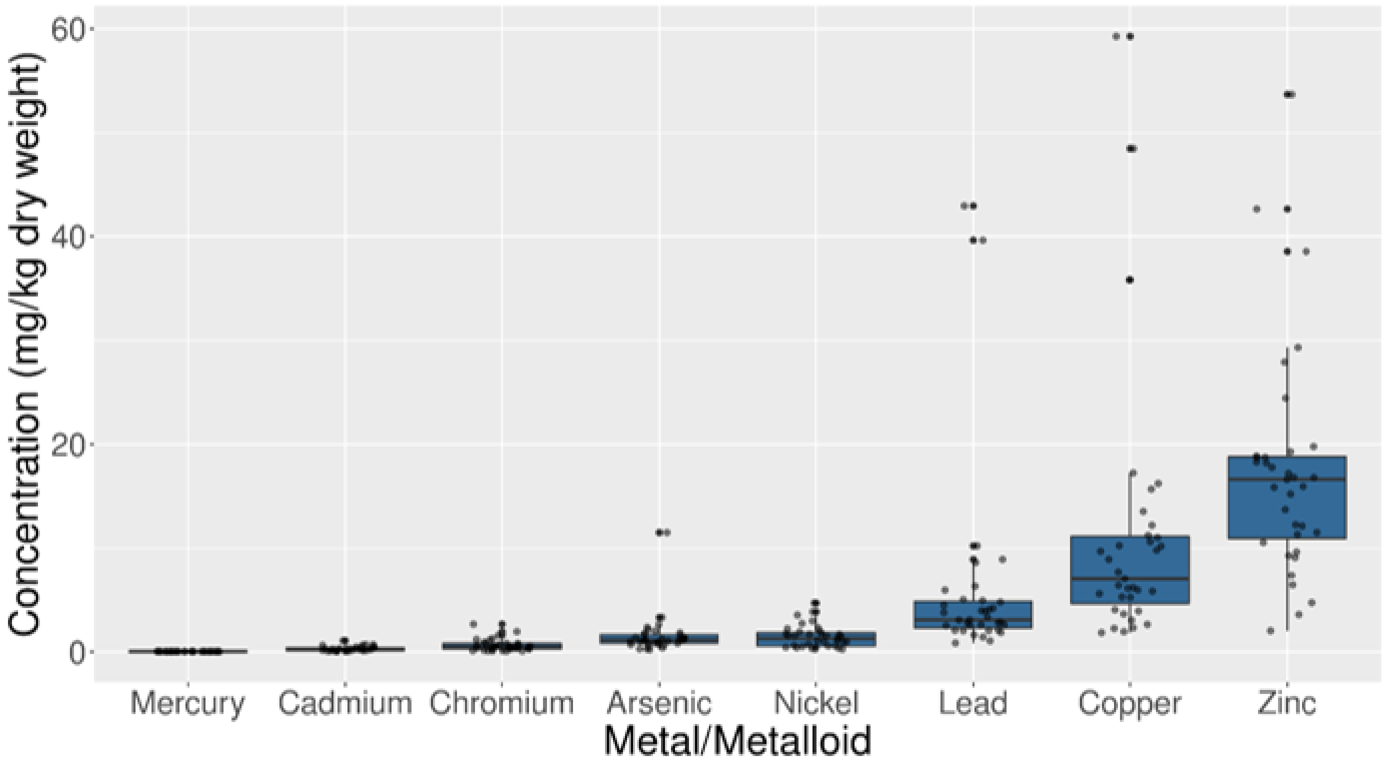

Figure 3

Concentrations of elements in hybrid cattail roots analyzed via high resolution inductively coupled mass spectrometry (N = 35).

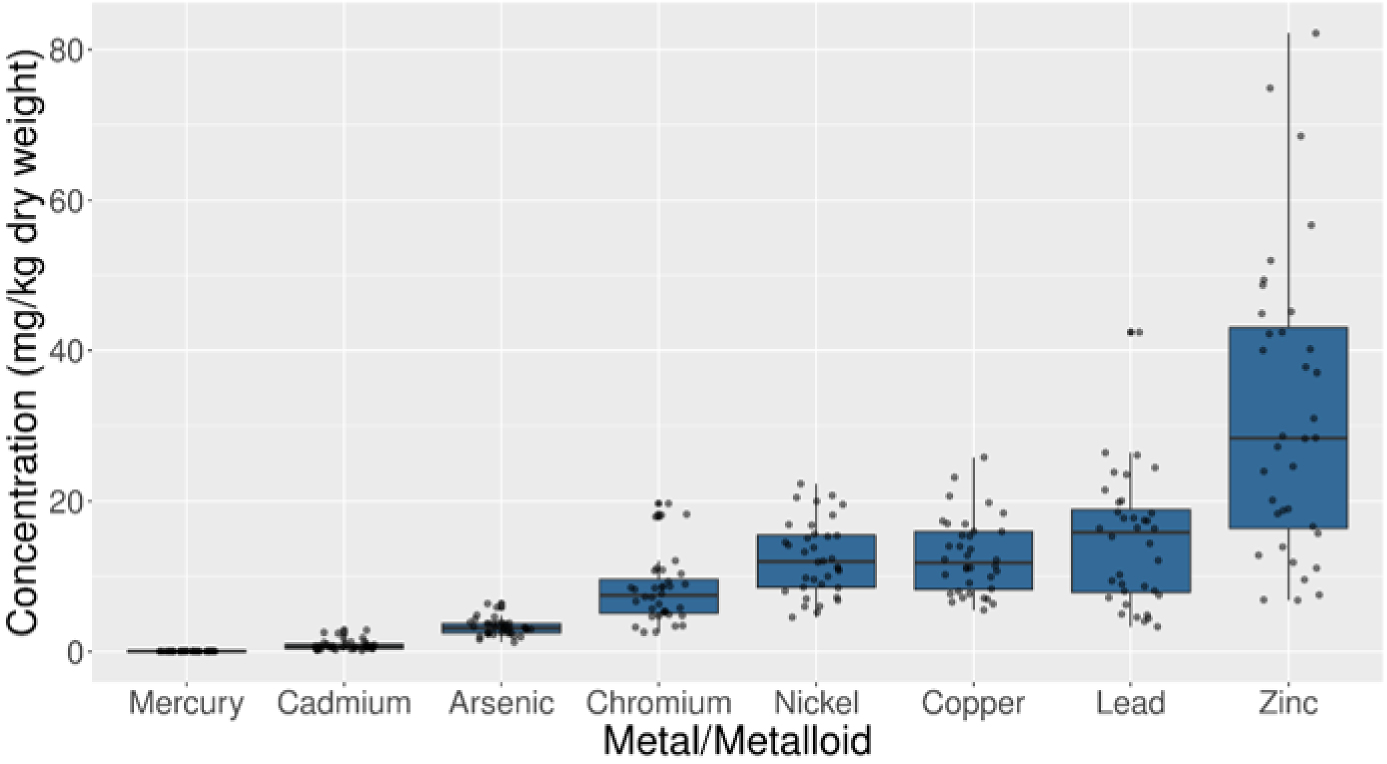

Figure 4

Concentrations of elements in fine sediment fractions collected from depths of 10-20 centimeters analyzed via high resolution inductively coupled mass spectrometry (N = 36).

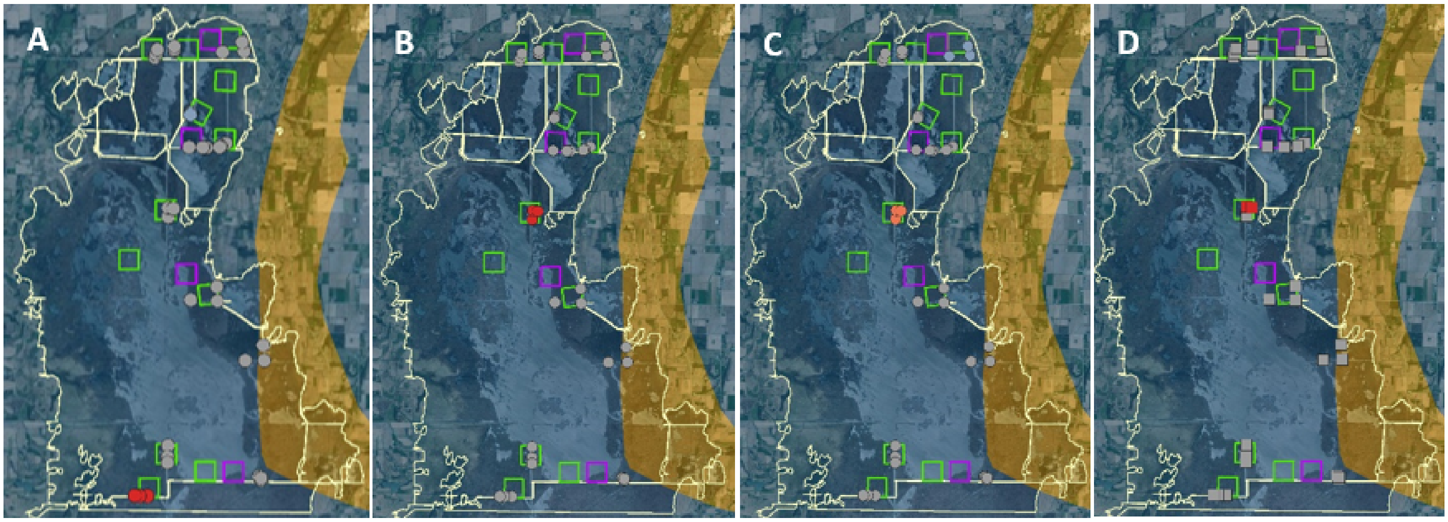

Figure 5

Concentrations were high for several elements in one particular subplot in Main Pool North compared to other subplots. (A) hotspot for Cr in sediment with 99% confidence in Main Pool North, coldspot for Cr in Radke with 90% confidence, (B) hotspot for Cd, Cu, and Zn in sediment with 99% confidence in Main Pool North, (C) hotspot for Ni in sediment with 95% confidence in Main Pool North, coldspot with 95% confidence in Radke, (D) hotspot for Cr in hybrid cattail root with 99% confidence in Main Pool North.

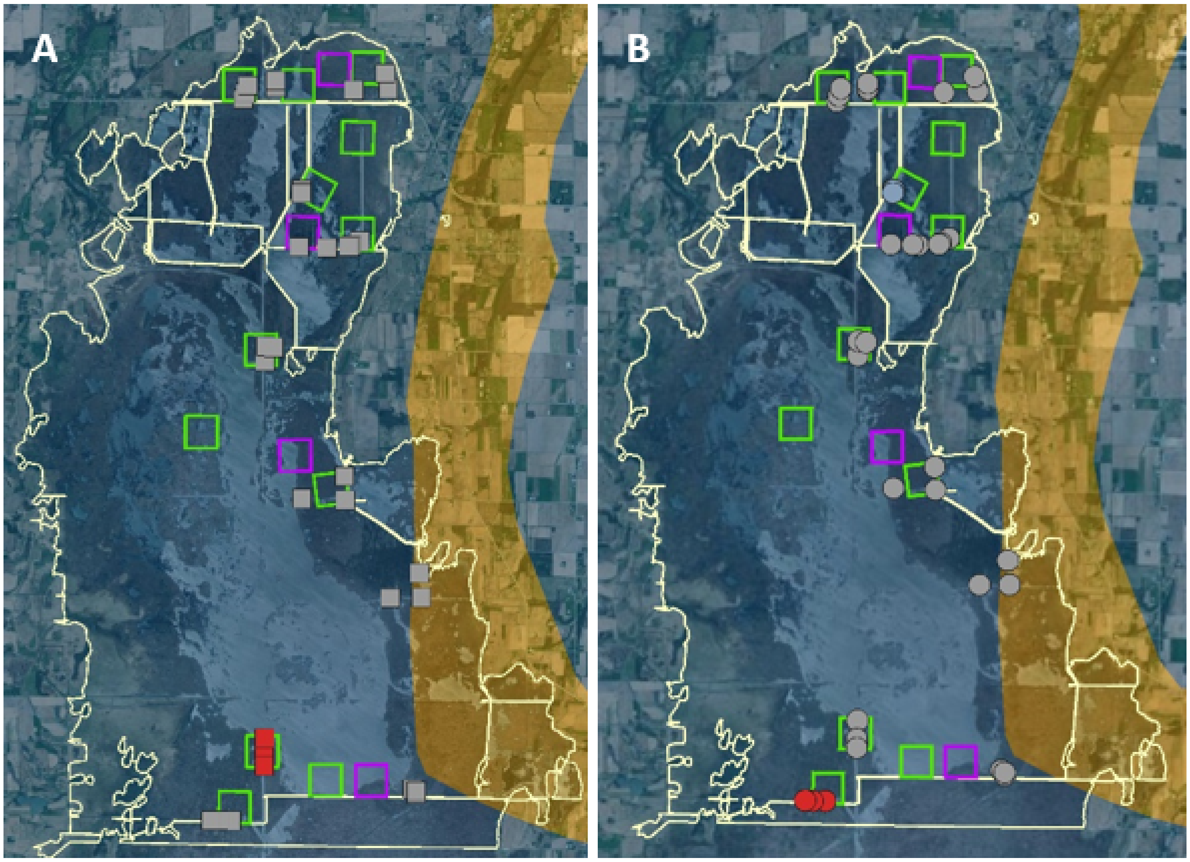

Figure 6

Additional hotspots and coldspots where concentrations were high or low relative to other samples. (A) hotspot for Cu in hybrid cattail root with 99% confidence in Main Pool South, (B) hotspot for As in sediment with 99% confidence in Main Pool South, coldspot with 95% confidence in Radke.

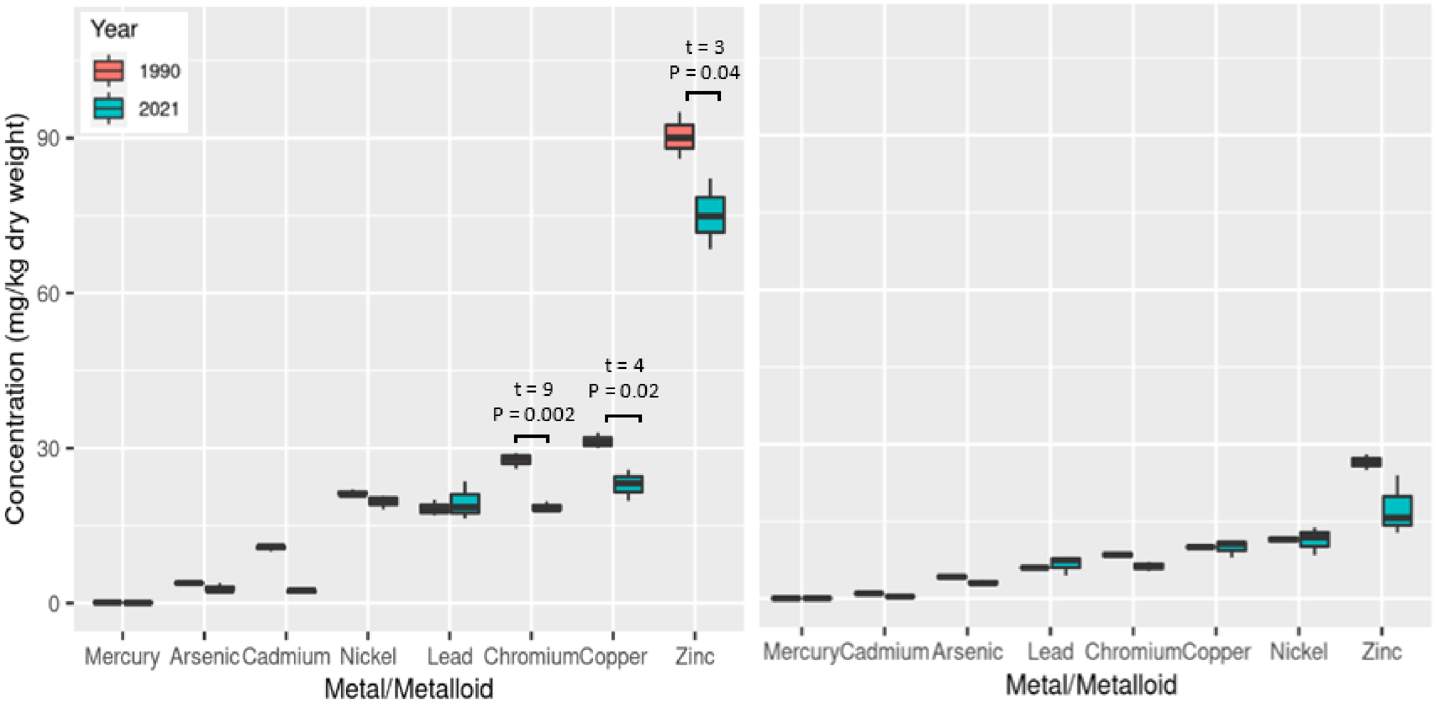

Figure 7

Comparison of concentrations of the elements of interest in sediment between the years 1990 (N = 2-3) and 2021 (N = 36) in (A) the northern sampling site, Main Pool North and (B) the southern sampling site, Main Pool South. T-tests revealed significant differences between years for Cr, Cu, and Zn in the northern sampling site (α < 0.05).

Supplementary Files

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