# Assessment of Metal Concentrations in Wild-caught Raccoons (*Procyon lotor*) in the Southeastern US

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**Abstract -** *Procyon lotor* (Raccoon) is a widespread and abundant omnivore that uses a diversity of habitats. Therefore, this species can be useful for biomonitoring the exposure and availability of metals to wildlife. We measured the concentrations of 5 metals (cadmium [Cd], copper [Cu], nickel [Ni], lead [Pb] and zinc [Zn]) in the liver tissue of 446 wild Raccoons that were collected at 2 sites in 2005 and 2006. We found that concentrations of Zn were positively correlated with those of Cu but negatively correlated with those of Ni. Liver concentrations of Cu and Zn exhibited strong negative relationships with body weight, whereas Cd had a positive relationship. Zn liver concentrations differed by sex, site captured, and year of sampling. Significant differences in Cd and Pb concentrations in liver tissue were observed due to sex and year, but no significant differences were found for the other 2 metals. Our results provide a large sample size of reference values for metal concentrations in livers of Raccoons collected from rural areas in the southeastern United States.

## Introduction

Increased anthropological activities in modern times have led to an increase in environmental contamination with metals. Common sources of metals in terrestrial ecosystems include, among others, erosion, development, industrial emissions, combustion of fossil fuels, and application of pesticides and fertilizers (Burger et al. 2002). Some metals are essential for physiological processes in animals (Gall et al. 2015). However, increased exposure to metals (essential or non-essential) can lead to accumulation above threshold levels, which can cause adverse effects (Guidotti et al. 1997, Mullally et al. 2004). Determining tissue metal concentrations can thus increase our understanding of the impact of metal contaminants on wildlife. Additionally, bioindicator species can provide an assessment of ecosystem health (Burger et al. 2002, Jarvis et al. 2013, Lockhart et al. 2016, Thomason et al. 2016).

*Procyon lotor* (L.) (Raccoon) has been used as an indicator species for environmental contamination (Burger et al. 2002; Eisler 1985, 1988, 1993, 1997, 1998; Gaines et al. 2002; Hernández et al. 2016; Shore and Rattner 2001) for several reasons. First, as a habitat generalist, this species is found in forested areas, wetlands, and urban and suburban areas throughout its broad range in North America, which extends from Canada south through parts of Mexico (Lotze and Anderson 1979, Seidensticker 1999). Quantifying metal concentrations in tissue from Raccoons can thus allow examination of large-scale contaminant levels across ecosystems

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(Burger 1999, Burger et al. 2002, Shore and Rattner 2001). Additionally, Raccoons are non-migratory mammals with restrictive home ranges and thus can provide an accurate assessment of localized metal contamination (Burger et al. 2002, Gaines et al. 2000, Hernández et al. 2016, Lord et al. 2002). Finally, Raccoons are medium-sized omnivores (Gehrt 2003, Gehrt and Fritzell 1999) that rely on both terrestrial and aquatic systems as a source for food (Burger et al. 2002, Gaines et al. 2002, Lord et al. 2002); therefore, they are likely exposed to metals in multiple ways, e.g., via consumption of contaminated vegetation and aquatic and terrestrial vertebrates and invertebrates as well as contact with polluted soils and water (Hernández et al. 2016, Lord et al. 2002).

The present study is a continuation of previous work on metal concentrations in the livers of terrestrial mammals from South Georgia and North Florida. To date, metal concentrations have been described in livers of 2 species that have direct contact with soils through diets and burrowing: *Didelphis virginiana* Kerr (Virginia opossum; Lockhart et al. 2016) and *Dasypus novemcinctus* L. (Nine-banded Armadillo; Jarvis et al. 2013). A third study examined metal concentrations in livers of *Lynx rufus* (Schreber) (Bobcat; Thomason et al. 2016). Because of their different feeding habits (insectivore [Armadillo], omnivore [Opossum], and carnivore [Bobcat]), these studies provided some insight into how trophic position affects metal accumulation. Here we provide an analysis of 5 commonly found metals—cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn)—in the livers of wild Raccoons. As in the earlier studies, we selected the liver as the target organ for testing because it regulates contaminant levels through detoxification and excretion in mammals; as such, the liver is one of the primary organs in which metals accumulate, often with adverse effects (Eisler 1985, 1988, 1993, 1997, 1998; Nwokocha et al. 2012; Roggeman et al. 2014). The results from this study provide reference values for metal concentrations in Raccoons that can be used in future comparative analyses, and further our understanding of how metal concentrations vary among members of a mammalian species assemblage.

## Methods

### Study sites

Raccoons were collected from the western portion of Pinebloom Plantation (referred to as Pinebloom West hereafter), located near Albany, GA, and Tall Timbers Research Station, near Tallahassee, FL. Both sites are relatively rural areas, focused on timber and wildlife management. Primary habitats at both locations consist of bottomland hardwood forests, upland pine, and open fields.

### Sample collection

Collection of animals at our study sites was part of a larger study, of which we were not part, that was designed to assess the impact of removing mesopredators on *Colinus virginianus* (L.) (Northern Bobwhite) populations (Jarvis et al. 2013, Lockhart et al. 2016, Thomason et al. 2016). Mesopredators, including Raccoons, were trapped or shot by United States Department of Agriculture-Wildlife Services technicians between 1 March and 30 September each year. The number and demographic composition of collected Raccoons per month each year at each site was similar (J.M. Lockhart, unpubl. data). For each animal, a liver sample was obtained and kept frozen at -20 °C in vials (with no preservative) until analyzed. Although mesopredators were collected at the sites from 2003 to 2006, due to a freezer failure the analyses reported here are limited to samples obtained in 2005 and 2006 ($n = 446$).

**Metal analysis**

We thawed and weighed liver samples to obtain wet weights (ww). We dried samples in an oven for 24 hours at 80 °C then reweighed them to determine dry weight (dw). Dried liver samples were digested with trace-metal–grade nitric acid (Fisher Scientific, Pittsburgh, PA) and heated for at least 24 hours in a 60 °C water bath until fully digested. Following digestion, we diluted liver samples with 18 mΩ Milli-Q® water prior to analysis for metals using atomic absorption spectrophotometry (AAS; Perkin Elmer AAnalysts 800, Norwalk, CT) with flame and graphite furnace detection (detection limit = 1–2 ppb). We used certified 1-g/mL metal standards dissolved in 2% HCl (Fisher Chemical, Fairlawn, NJ) for each metal and performed recalibration after every 40 samples. We analyzed standards and samples in duplicates. To determine metal extraction efficiencies, we used 2 types of Lobster hepatopancreas [LUTS-1 (Non defatted) and TORT-3] as reference materials (3 replicates each), treating each the same way as the samples. This method of digestion and metal analysis has proven reliable and effective in other studies in our laboratory (Jarvis et al. 2013, Lockhart et al. 2016, Main et al. 2010, Thomason et al. 2016). Data are reported as µg metal per g dw tissue.

**Statistical analyses**

As a first step, we used Pearson's product-moment correlations to determine if there were any significant relationships in concentrations in the liver between each pair of metals. Next, we wished to examine the effect of age of animals sampled on variation in the metal concentrations. Unfortunately, we did not have precise age estimates for the animals collected. Instead, we used body weight as a surrogate for age, with the assumption that older animals are heavier. We chose this approach for 2 reasons. First, our body-weight data were continuously distributed, from 0.45 kg to 6.67 kg. Thus, there was no obvious break point that allowed for designation of discrete age categories (e.g., juvenile and adult). Second, body weights of Raccoons vary substantially across their range in the United States, with animals from the southeastern US among the smallest (Lotze and Anderson 1979, Seidensticker 1999). Consequently, published weight data from populations elsewhere were of little help in identifying age categories. Given these issues we opted not to classify the animals in our sample into arbitrarily created discrete age categories.

We first examined the overall relationship between metal concentrations and body weight with linear regressions. To assess how body weight interacted with other possible influences on metal concentrations, we then performed a series of analyses of covariance (ANCOVA) in which a single dichotomous variable (sex, site, and year) was the factor and body weight was the covariate.

The ANCOVAs allowed us to assess the extent to which body weight interacted with each of the other possible influences on metal concentrations, but we could not examine interactions among the dichotomous variables. In addition, there were multiple instances where we had information on the sex of an individual but not its weight. In order to fully evaluate the effects of each dichotomous variable on variation in metal concentrations in the liver samples, and include data from as many animals as possible, we ran a series of 3-way analyses of variance (ANOVA) in which site, year, and sex were the 3 factors. Bonferroni-Dunn tests were used for post-hoc pair-wise comparisons.

## Results

### Correlations among metals

We found 3 significant correlations in metal concentrations from the liver samples (Table 1). Specifically, Cu concentrations were positively correlated with Ni and Zn concentrations, while Ni concentrations were negatively correlated with those of Zn (Table 1).

### Effects of body weight

Overall, there were strong negative relationships between body weight and Cu and Zn concentrations in the liver (Table 2). In contrast, Cd concentrations in the liver increased significantly with increased body weight (Table 2). The ANCOVA analyses echoed these results, once again revealing strong significant differences in Cd, Cu, and Zn concentrations that were due to body weight (Table 3). However, there was just a single significant interaction term between body weight and any of the 3 dichotomous variables (year x body weight for Cd concentration; see Table 3).

Table 1. Pearson product-moment correlations (*r* values) in concentrations of each pair-wise combination of metals in the livers of Raccoons sampled at 2 sites in 2005 and 2006. Sample sizes for each comparison are given parenthetically. * $P < 0.05$, ** $P < 0.005$.

| | Cu | Ni | Pb | Zn |
|---|---|---|---|---|
| Cd | -0.04 (352) | 0.03 (366) | -0.02 (351) | 0.01 (366) |
| Cu | | 0.10* (397) | 0.02 (430) | 0.19** (431) |
| Ni | | | 0.03 (396) | -0.17** (411) |
| Pb | | | | 0.09 (430) |

Table 2. Results of linear regressions of metal concentrations in the livers of Raccoons with body weight for individuals sampled at 2 sites in 2005 and 2006. Sample sizes (*n*), regression coefficients (*r*), test statistics, and their statistical significance (*F* and *P* values) are provided.

| | *n* | *r* | *F* | *P* |
|---|---|---|---|---|
| Cd | 342 | 0.12 | 4.71 | 0.03 |
| Cu | 403 | -0.32 | 44.38 | <0.0001 |
| Ni | 383 | -0.03 | 0.44 | 0.51 |
| Pb | 402 | -0.04 | 0.66 | 0.42 |
| Zn | 416 | -0.17 | 11.74 | 0.0007 |

**Effects of sex, site, and year**

Contrary to the ANCOVA results, which found only 2 significant main effects between sites for Pb and Zn concentrations in the liver, and none for sex and year (Table 3), the inclusion of additional animals for which we had information on sex but not body weight in the 3-way ANOVA analyses revealed additional differences (Table 4; Figs. 1–5). Results for Cd concentrations showed significant differences between males and females and between years (Table 4) but only the year effect was significant with a post-hoc Bonferroni-Dunn test (2005 > 2006, $P = 0.004$; see Fig. 1). Surprisingly, the significant difference between sites for Pb concentrations in the liver revealed by the ANCOVA analyses (Table 3) was not confirmed by the 3-way ANOVA results, which showed significant effects of sex and year but not site (Table 4, Fig. 4). These latter effects were weak though because neither post-hoc comparison of pair-wise differences was significant (Bonferroni-Dunn tests: both $P > 0.08$). Zn was the only metal to exhibit significant differences due to all 3 dichotomous variables (Table 4, Fig. 5). Specifically, Zn concentrations in the liver samples were greater at Tall Timbers than Pinebloom West (Bonferroni-Dunn test: $P < 0.0001$), for males than for females ($P = 0.03$), and in 2005 than in 2006 ($P < 0.0001$). There were no significant interaction terms in the ANOVA analyses for any metal (Table 4).

Table 3. Results of ANCOVAs comparing variation in metal concentrations in the livers of Raccoons sampled at two sites in 2005 and 2006 that was due to sex, site, and year, with body weight as the covariate. See Figures 1–5 for further information.

| | Cd | Cu | Ni | Pb | Zn |
|---|---|---|---|---|---|
| | df = 1, 338 | df = 1, 399 | df = 1, 379 | df = 1, 398 | df = 1, 412 |
| 1. Sex | $F = 0.23$, $P = 0.63$ | $F = 2.15$, $P = 0.14$ | $F = 0.02$, $P = 0.89$ | $F = 2.69$, $P = 0.10$ | $F = 0.01$, $P = 0.93$ |
| Body weight | $F = 9.67$, $P = 0.002$ | $F = 47.87$, $P < 0.0001$ | $F = 0.58$, $P = 0.45$ | $F = 1.34$, $P = 0.25$ | $F = 18.92$, $P = 0.0001$ |
| Sex x Body weight | $F = 1.49$, $P = 0.22$ | $F = 3.17$, $P = 0.08$ | $F = 0.06$, $P = 0.81$ | $F = 1.49$, $P = 0.22$ | $F = 0.55$, $P = 0.46$ |
| 2. Site | $F = 0.83$, $P = 0.36$ | $F = 1.94$, $P = 0.16$, | $F = 2.19$, $P = 0.14$ | $F = 4.02$, $P = 0.05$ | $F = 7.33$, $P = 0.007$ |
| Body weight | $F = 5.48$, $P = 0.02$ | $F = 42.24$, $P < 0.0001$ | $F = 0.13$, $P = 0.72$ | $F = 1.44$, $P = 0.23$ | $F = 13.83$, $P = 0.0002$ |
| Site x Body weight | $F = 1.02$, $P = 0.31$ | $F = 1.51$, $P = 0.22$ | $F = 1.32$, $P = 0.25$ | $F = 3.63$, $P = 0.06$ | $F = 3.23$, $P = 0.073$ |
| 3. Year | $F = 2.02$, $P = 0.16$ | $F = 1.65$, $P = 0.20$ | $F = 0.52$, $P = 0.47$ | $F = 0.22$, $P = 0.73$ | $F = 3.44$, $P = 0.064$ |
| Body weight | $F = 5.84$, $P = 0.016$ | $F = 35.67$, $P < 0.0001$ | $F = 0.26$, $P = 0.61$ | $F = 0.29$, $P = 0.59$ | $F = 14.18$, $P = 0.0002$ |
| Year x Body weight | $F = 4.81$, $P = 0.03$ | $F = 1.77$, $P = 0.18$ | $F = 0.15$, $P = 0.70$ | $F = 0.95$, $P = 0.33$ | $F = 0.66$, $P = 0.42$ |

## Discussion

Because the Raccoons in our study were originally collected as part of another project, our analyses are by necessity retrospective. As a result, we have little supporting information that would help us to explain some of the patterns we have discovered. For example, we know of no obvious reason why some metal

Table 4. Results of a three-way ANOVA comparing metal concentrations in the livers of Raccoons sampled at two sites in 2005 and 2006. See Figs. 1-5 for more information.

| | Cd | Cu | Ni | Pb | Zn |
|---|---|---|---|---|---|
| | df = 1, 358 | df = 1, 422 | df = 1, 403 | df = 1, 421 | df = 1, 437 |
| Sex | $F$ = 4.20, $P$ = 0.04 | $F$ = 2.10, $P$ = 0.15 | $F$ = 0.0004, $P$ = 0.98 | $F$ = 3.81, $P$ = 0.05 | $F$ = 4.74, $P$ = 0.03 |
| Year | $F$ = 5.50, $P$ = 0.02 | $F$ = 0.003, $P$ = 0.98 | $F$ = 1.83, $P$ = 0.18 | $F$ = 3.83, $P$ = 0.05 | $F$ = 20.22, $P$ = 0.0001 |
| Site | $F$ = 0.88, $P$ = 0.35 | $F$ = 1.98, $P$ = 0.16 | $F$ = 1.09, $P$ = 0.30 | $F$ = 0.04, $P$ = 0.84 | $F$ = 11.77, $P$ = 0.0007 |
| Sex x year | $F$ = 0.05, $P$ = 0.82 | $F$ = 0.04, $P$ = 0.84 | $F$ = 0.73, $P$ = 0.39 | $F$ = 0.81, $P$ = 0.37 | $F$ = 0.07, $P$ = 0.79 |
| Sex x site | $F$ = 2.84, $P$ = 0.09 | $F$ = 0.33, $P$ = 0.57 | $F$ = 0.46, $P$ = 0.50 | $F$ = 0.36, $P$ = 0.55 | $F$ = 0.31, $P$ = 0.58 |
| Year x site | $F$ = 0.01, $P$ = 0.91 | $F$ = 0.49, $P$ = 0.49 | $F$ = 0.32, $P$ = 0.57 | $F$ = 0.54, $P$ = 0.46 | $F$ = 0.43, $P$ = 0.51 |
| Sex x year x site | $F$ = 0.53, $P$ = 0.47 | $F$ = 0.14, $P$ = 0.71 | $F$ = 1.76, $P$ = 0.19 | $F$ = 0.12, $P$ = 0.73 | $F$ = 0.12, $P$ = 0.73 |

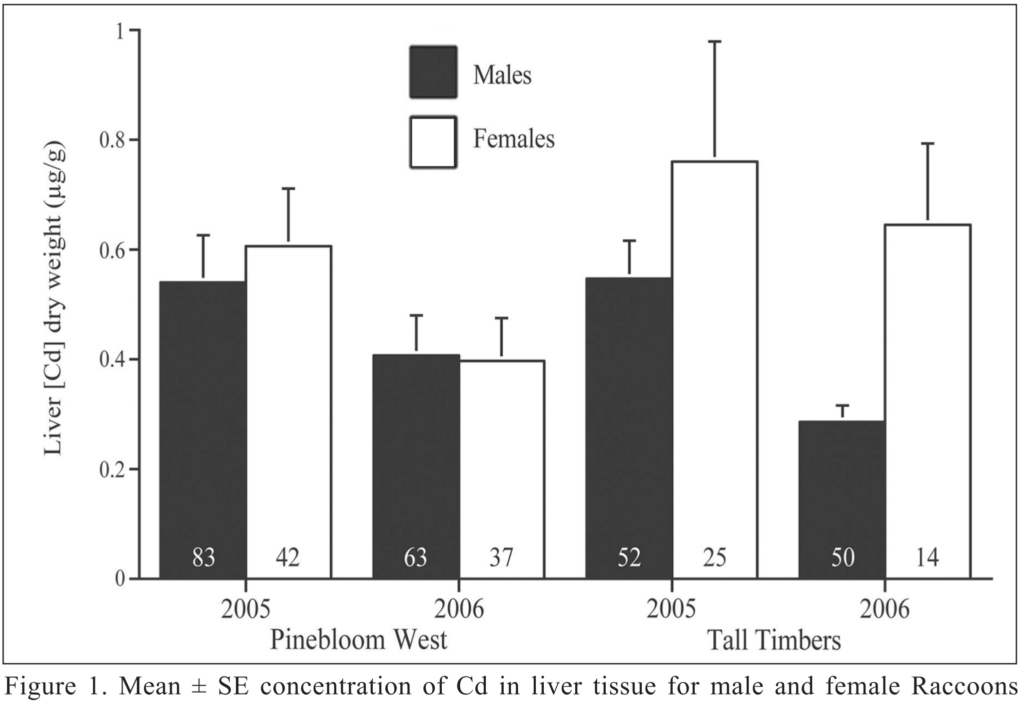


Figure 1. Mean ± SE concentration of Cd in liver tissue for male and female Raccoons sampled at 2 sites in 2005 and 2006. Sample size given within each bar. See Table 4 for results of statistical comparisons.

concentrations in the liver samples varied between years or sites. Even so, at a minimum, our results provide additional reference values for Cd, Cu, Ni, Pb, and Zn in Raccoons that can be used in future comparative studies, either of other populations of Raccoons or with other species of terrestrial mammals.

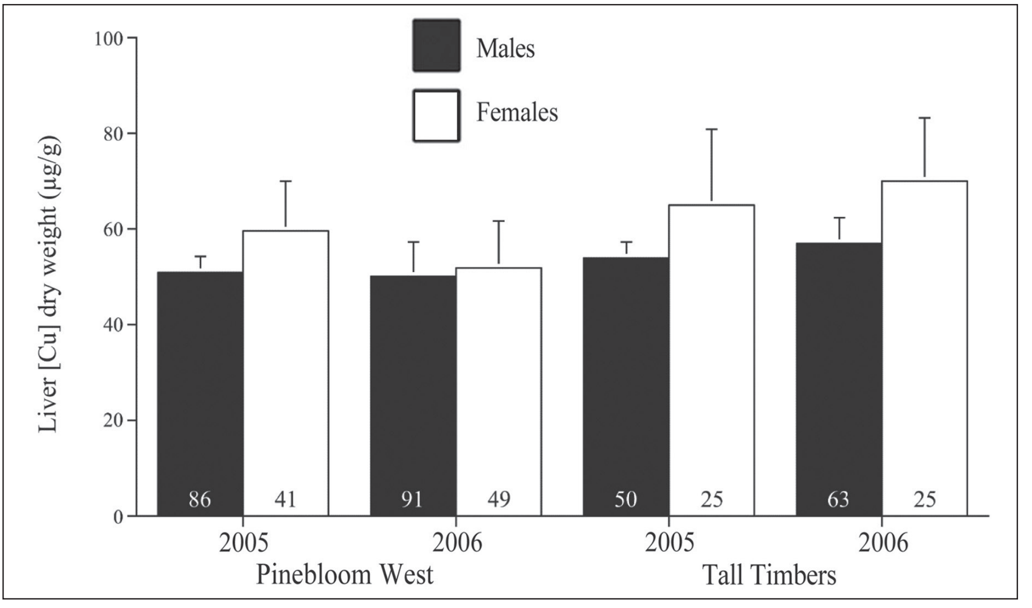


Figure 2. Mean ± SE concentration of Cu in liver tissue for male and female Raccoons sampled at 2 sites in 2005 and 2006. Sample size given within each bar. See Table 4 for results of statistical comparisons.

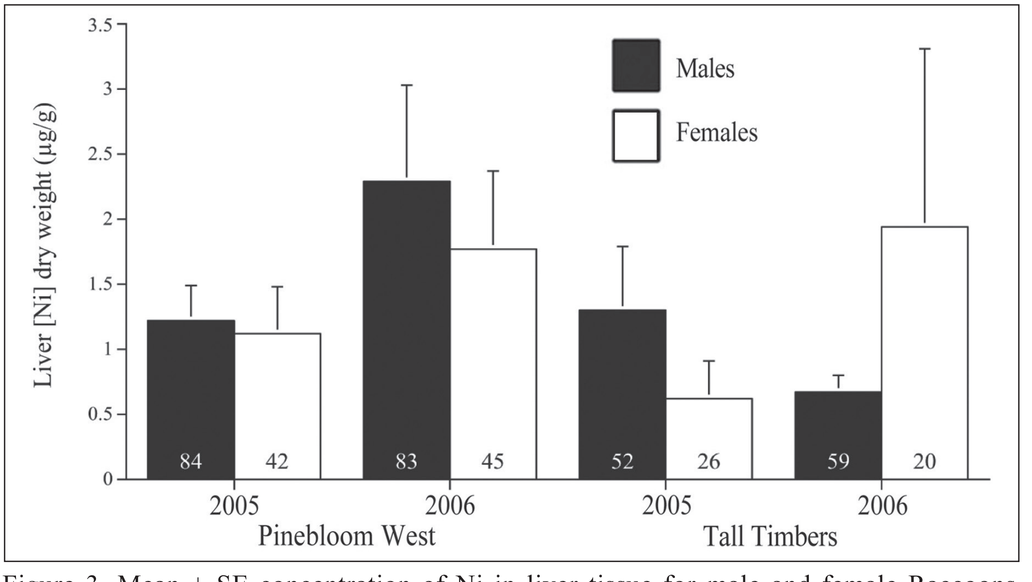


Figure 3. Mean ± SE concentration of Ni in liver tissue for male and female Raccoons sampled at 2 sites in 2005 and 2006. Sample size given within each bar. See Table 4 for results of statistical comparisons.

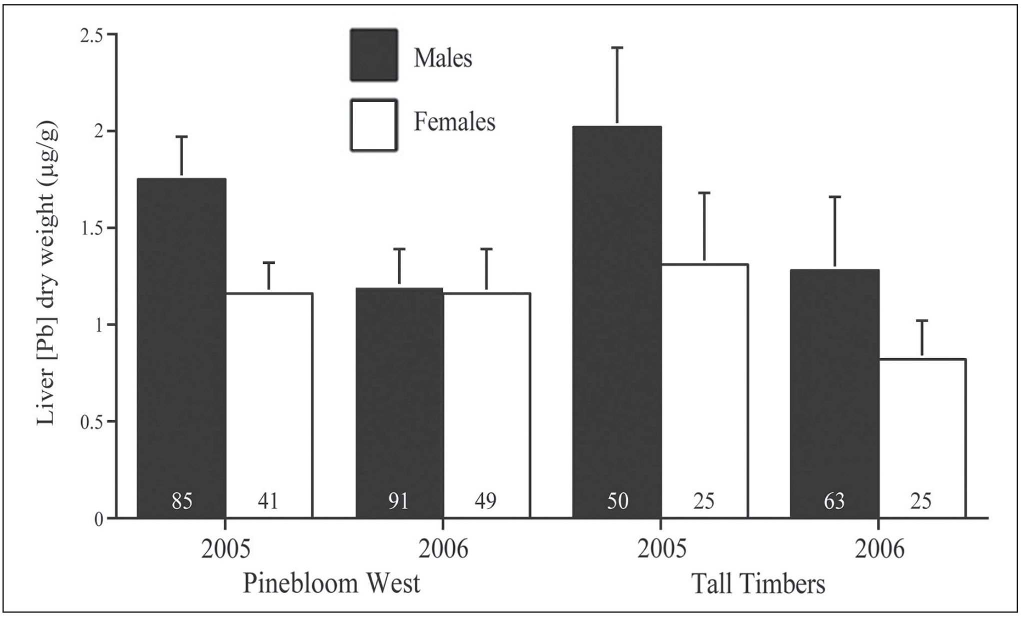


Figure 4. Mean ± SE concentration of Pb in liver tissue for male and female Raccoons sampled at 2 sites in 2005 and 2006. Sample size given within each bar. See Table 4 for results of statistical comparisons.

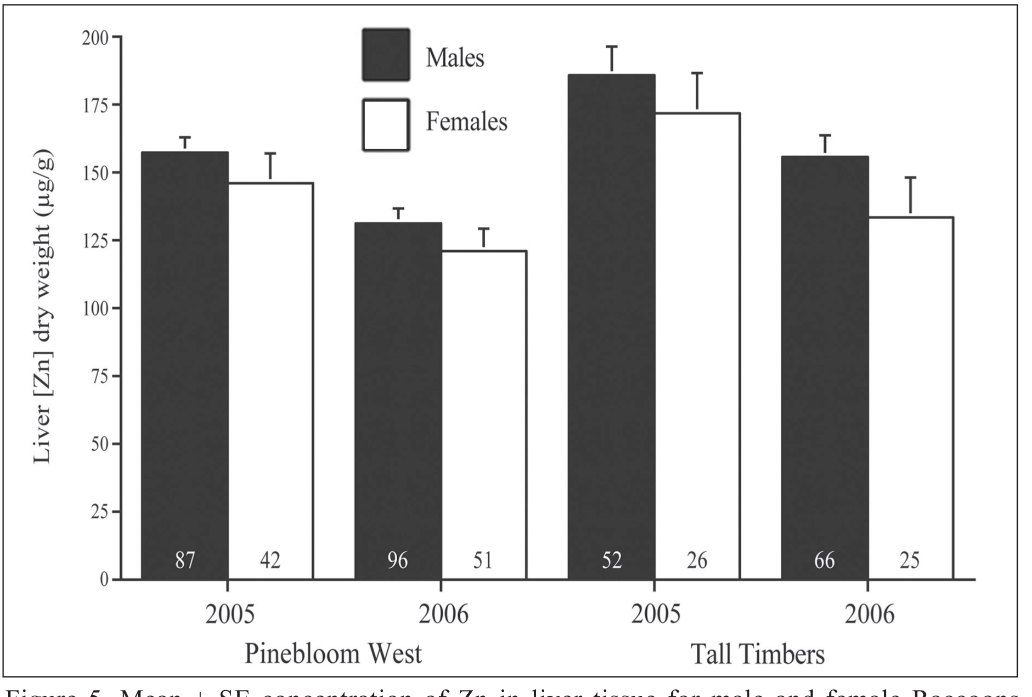


Figure 5. Mean ± SE concentration of Zn in liver tissue for male and female Raccoons sampled at 2 sites in 2005 and 2006. Sample size given within each bar. See Table 4 for results of statistical comparisons.

There are a number of studies that have documented the concentrations of metals in liver and other tissues of Raccoons (Table 5). Of particular interest are data collected from the Department of Energy's Savannah River Site (SRS), which is located on the Georgia/South Carolina border and contains habitats similar to those found at the sites we sampled. Studies at SRS reported onsite metal concentrations for Cd, Cu, and Pb (see the South Carolina sites in Table 5). The values we obtained at our sites were somewhat higher for Cu and Pb, but comparable for Cd (Table 5). Differences in concentrations of Cu and Pb in Racoon livers between our sites and SRS might reflect biological differences between the populations or differences in the environmental concentrations of the metals. The latter seems plausible given that we found at least a few differences in metal concentrations between our Tall Timbers and Pinebloom sites, even though they are located within 100 km of each other, and SRS is substantially farther away. It is worth noting that, although our values for Cu and Pb were higher than those obtained at SRS, they are similar to values reported from sites elsewhere (Table 5).

Our values for liver Ni concentrations are also comparable to those from other studies. Wren (1984) reported no detectable levels of Ni in the livers of Raccoons caught in Canada, but did obtain values similar to ours when muscle tissues from the same specimens were tested (Table 5). Hernandez et al. (2016) reported from 0.51 to 0.89 µg Ni/g ww, which is similar to the values in our study when normalized for dw (Table 5).

The values we obtained for liver Zn concentrations are slightly higher than those published previously (Table 5). However, they are lower than the levels Eisler (1993) concluded to be indicators of Zn poisoning in mammals (465 mg Zn/kg dw in liver). Despite the somewhat higher liver Zn levels we found, the overall conclusion from comparisons of our data with those that have been published previously is that it seems unlikely that any of our values indicate abnormally high exposure of the Raccoons in our study to the metals we tested.

In general, one might not expect to detect sex differences in metal concentrations in Raccoons because there are few differences in the physiology and behavior of males and females (Gehrt and Fritzell 1999, Lotze and Anderson 1979). Thus, it would seem likely both sexes are equally exposed to metal contaminants within an ecosystem. Consistent with this expectation, previous studies of Zn concentrations in Raccoons did not detect any sex differences (Herbert and Peterle 1990, Levengood and Hubert 2001), though these studies did not examine liver tissues. In contrast, our analyses revealed a significant sex difference in concentrations of Zn in the liver tissue, and possible sex differences for Cd and Pb. A case study by Burger (2007) that reviewed 43 studies of sex differences in metal concentrations in a variety of vertebrates found little evidence of them. The only study that reported males with higher concentrations of Zn than females was for *Anas rubripes* Brewster (Black Duck; Gochfeld and Burger 1987). Thus, our study provides the first evidence of such sex difference in any species of mammal.

We are not aware of any previous studies that have examined sex differences in Cd or Pb concentrations in Raccoons. However, in some other mammals, females

Table 5. Results from previous studies of metal accumulation in Raccoons. ND = not determined. [Table continued on following page.]

| Metal | Concentration (min–max) | Site (year) | Tissue | Sample size | Reference |
|---|---|---|---|---|---|
| Cd | 2.48 ± 1.66[A] | Florida (not provided) | Kidney | 14 | Hoff et al. (1977) |
| | ND | Canada (not provided) | Muscle | 4 | Wren et al. (1984) |
| | 0.23 ± 0.09[C] | Canada (not provided) | Liver | 4 | Wren et al. (1984) |
| | 0.79 ± 0.50[B] | Michigan (1984,1986) | Liver | 25 | Herbert and Peterle (1990) |
| | 0.51 ± 0.05[A] | South Carolina (1996–1997) | Liver | 47 | Burger et al. (2000) |
| | 0.78 ± 0.12[A] (0.60–1.09) | Illinois (1983–1985) | Liver | 62 | Levengood and Hubert (2001) |
| | 0.54 ± 0.05[A] (0.03–1.90) | South Carolina (1996–1997) | Liver | 46 (onsite) | Burger et al. (2002) |
| | 0.45 ± 0.09[A] (0.15–1.51) | South Carolina (1996–1997) | Liver | 25 (offsite) | Burger et al. (2002) |
| | 1.63 ± 0.38[A] | South Carolina (2013) | Liver | 15 (contaminated) | Hernandez et al. (2016) |
| | 1.61 ± 0.45[A] | South Carolina (2013) | Liver | 11 (reference) | Hernandez et al. (2016) |
| | 0.47 ± 0.03[D] (0.004–4.87) | Georgia/Florida (2005–2006) | Liver | 410 | Present study |
| Cu | 2.5 ± 0.1[C] | Canada (not provided) | Muscle | 4 | Wren et al. (1984) |
| | 4.8 ± 1.1[C] | Canada (not provided) | Liver | 4 | Wren et al. (1984) |
| | 12.86 ± 0.77[A] | South Carolina (1996–1997) | Liver | 47 | Burger et al. (2000) |
| | 4.32 ± 0.54[A] (1.27–26.70) | Illinois (1983–1985) | Liver | 62 | Levengood and Hubert (2001) |
| | 12.40 ± 0.71[A] (5.83–29.1) | South Carolina (1996–1997) | Liver | 46 (onsite) | Burger et al. (2002) |
| | 14.20 ± 1.16[A] (4.93–25.7) | South Carolina (1996–1997) | Liver | 25 (offsite) | Burger et al. (2002) |
| | 9.35[E] | Tennessee (2009) | Liver | 10 (contaminated) | Souza et al. (2013) |
| | 7.8[E] | Tennessee (2009) | Liver | 10 (reference) | Souza et al. (2013) |
| | 7.75[E] | Tennessee (2010) | Liver | 10 (contaminated) | Souza et al. (2013) |
| | 34.53 ± 4.24[A] | South Carolina (2013) | Liver | 15 (contaminated) | Hernandez et al. (2016) |
| | 29.24 ± 3.78[A] | South Carolina (2013) | Liver | 11 (reference) | Hernandez et al. (2016) |
| | 56.67 ± 2.95[D] (7.43–663.32) | Georgia/Florida (2005–2006) | Liver | 456 | Present study |
| Ni | 1.0 ± 1.2[C] | Canada (not provided) | Muscle | 4 | Wren et al. (1984) |
| | ND | Canada (not provided) | Liver | 4 | Wren et al. (1984) |
| | 0.51 ± 0.06[A] | South Carolina (2013) | Liver | 15 (contaminated) | Hernandez et al. (2016) |
| | 0.89 ± 0.17[A] | South Carolina (2013) | Liver | 11 reference | Hernandez et al. (2016) |
| | 1.40 ± 0.20[D] (0.01–43.32) | Georgia/Florida (2005–2006) | Liver | 421 | Present study |

Table 5, continued.

| Metal | Concentration (min–max) | Site (year) | Tissue | Sample size | Reference |
|---|---|---|---|---|---|
| Pb | 0.47 ± 0.22[A] | Florida (not provided) | Kidney | 14 | Hoff et al. (1977) |
| | 6.8 ± 1.8[A] | Illinois (1958–1959) | Liver | 101 | Sanderson and Thomas (1961) |
| | 6.2 ± 5.4[B] (<1–35) | Connecticut (not provided) | Liver | 14 | Ditters and Nielson (1978) |
| | 0.24 ± 0.17[B] | Michigan (1984,1986) | Liver | 25 | Herbert and Peterle (1990) |
| | 3.24 ± 1.07[A] | Alabama (1992–1993) | Liver | 15 | Khan et al. (1995) |
| | 0.48 ± 0.06[A] | South Carolina (1996–1997) | Liver | 47 | Burger et al. (2000) |
| | 1.43 ± 0.36[A] (0.81–3.96) | Illinois (1983–1985) | Liver | 62 | Levengood and Hubert (2001) |
| | 0.34 ± 0.04[A] (0.01–2.18) | South Carolina (1996–1997) | Liver | 46 (onsite) | Burger et al. (2002) |
| | 0.54 ± 0.11[A] (0.03–1.12) | South Carolina (1996–1997) | Liver | 25 (offsite) | Burger et al. (2002). |
| | 0.54 ± 0.29[A] | South Carolina (2013) | Liver | 15 (contaminated) | Hernandez et al. (2016) |
| | 0.24 ± 0.04[A] | South Carolina (2013) | Liver | 11 (reference) | Hernandez et al. (2016) |
| | 1.38 ± 0.10[D] (0.01–21.10) | Georgia/Florida (2005–2006) | Liver | 440 | Present study |
| Zn | 75.88 ± 16.54[A] | Florida (not provided) | Kidney | 14 | Hoff et al. (1977) |
| | 63.6 ± 46.0[C] | Canada (not provided) | Muscle | 4 | Wren et al. (1984) |
| | 34.4 ± 2.0[C] | Canada (not provided) | Liver | 4 | Wren et al. (1984) |
| | 44.4 ± 10.6[B] | Michigan (1984,1986) | Liver | 25 | Herbert and Peterle (1990) |
| | 39.85[E] | Tennessee (2009) | Liver | 10 (contaminated) | Souza et al. (2013) |
| | 44.9[E] | Tennessee (2009) | Liver | 10 (reference) | Souza et al. (2013) |
| | 40.9[E] | Tennessee (2010) | Liver | 10 (contaminated) | Souza et al. (2013) |
| | 112.98 ± 5.83[A] | South Carolina (2013) | Liver | 15 (contaminated) | Hernandez et al. (2016) |
| | 106.02 ± 6.39[A] | South Carolina (2013) | Liver | 11 (reference) | Hernandez et al. (2016) |
| | 148.69 ± 3.05[D] (0.83–516.28) | Georgia/Florida (2005–2006) | Liver | 456 | Present study |

[A]Mean (± SE) µg/g or mg/kg ww
[B]Mean (± SD) mg/kg ww
[C]Mean (± SD) µg/g dw
[D]Mean (± SE) µg/g or mg/kg dw
[E]Median mg/kg ww

had higher Cd concentrations than males (Millán et al. 2008, Wijnhoven et al. 2007), just as we found in our study. Additional sampling at other sites will be needed to clarify whether sex differences in Cd, Pb and Zn concentrations in Raccoons are common and, if so, to determine the reason(s) for their occurrence.

We found a strong negative relationship between Cu and Zn concentrations and body weight, and a significant positive relationship for Cd. Assuming Raccoons with low body weights are younger, our result for Cd is consistent with previous studies that reported higher liver Cd concentrations in adult Raccoons when compared to juveniles (Herbert and Peterle 1990, Levengood and Hubert 2001). Again, assuming body weight is a reliable surrogate for age, our results suggest higher exposure and/or uptake of Cu and Zn in young animals, which then declines as they age. A negative relationship between Cu and body weight is consistent with other studies that reported increased Cu concentrations in young mammals (Eisler 1997, Hillis and Parker 1993). Hillis and Parker (1993) suggested that younger mammals accumulate higher levels of Cu for various physiological processes and developmental growth and, as they age, the need for excess Cu decreases. A related idea is that the higher metabolic rates of young mammals compared to adults generates a greater rate of metal accumulation in the liver, and as metabolism decreases with age, so does the accumulation of heavy metals (Blagojević et al. 2012). Similar reasoning may also apply to the relationship of Zn concentrations with body weight, although this relationship has not been reported previously. However, it is important to bear in mind these arguments hinge on the assumption that body weight reliably indicates age. An alternative hypothesis is that low body weights are indicative of poor condition, and thus potentially associated with increased contaminant loads. Unfortunately, our data do not allow us to discriminate between these 2 possibilities.

Our results showed that Zn concentrations in Raccoon livers were positively correlated with those of Cu but negatively with Ni. The findings for Cu and Zn are consistent with prior studies in our lab that found the same relationship in Nine-banded Armadillos and Opossums, albeit not in Bobcats (Jarvis et al. 2013, Lockhart et al. 2016, Thomason et al. 2016). We also found a negative relationship between Ni and Zn concentrations in Nine-banded Armadillos (Jarvis et al. 2013), but in Opossums the relationship was positive (Lockhart et al. 2016); Thomason et al. (2016) did not test Bobcats for Ni. The consistency of the relationship between Cu and Zn across species might indicate similar levels of exposure to these metals in the environment or similar physiological needs (Eisler 1997, Jarvis et al. 2013).

Based on the information in Table 5, we believe ours is the largest study assessing liver metal concentrations in Raccoons that has been conducted to date, both in terms of the number of individuals sampled and the number of metals tested per animal. As such, our study is valuable in providing an extensive set of reference values for metals in the liver of Raccoons. We have also documented important sources of variation in metal concentrations that should be explored in more detail in future studies.

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**Literature Cited**

Blagojević, J., V. Jovanović, G. Stamenković, V. Jojić, V. Bugarski-Stanojević, T. Adnađević, and M. Vujošević. 2012. Age differences in bioaccumulation of heavy metals in populations of the Black-striped Field Mouse, *Apodemus agrarius* Rodentia, Mammalia. International Journal of Environmental Research 64:1045–1052.

Burger, J. 1999. Animals in Towns and Cities. Kendall-Hunt, Dubuque, IA. 679 pp.

Burger, J. 2007. A framework and methods for incorporating gender-related issues in wildlife risk assessment: Gender-related differences in metal levels and other contaminants as a case study. Environmental Research 104:153–162.

Burger, J., C.G. Lord, E.J. Yurkow, L. McGrath, and K.F. Gaines. 2000. Metals and metallothionein in the liver of Raccoons: Utility for environmental assessment and monitoring. Journal of Toxicology and Environmental Health A 60:243–261.

Burger, J., K.F. Gaines, C.G. Lord, I.L. Brisbin Jr., S. Shukla, and M. Gochfeld. 2002. Metal levels in Raccoon tissues: Differences on and off the Department of Energy's Savannah River site in South Carolina. Environmental Monitoring and Assessment 74:67–84.

Ditters, R., and S.W. Nielsen. 1978. Lead poisoning of Raccoons in Connecticut. Journal of Wildlife Diseases 142:187–192.

Eisler, R. 1985. Cadmium hazards to fish, wildlife, and invertebrates: A synoptic review. Biological Report 85, Contaminant Hazards Review No. 2. US Fish and Wildlife Service, Laurel, MD. 30 pp.

Eisler, R. 1988. Lead hazards to fish, wildlife, and invertebrates: A synoptic review. Biological Report 85, Contaminant Hazards Review No. 14. US Fish and Wildlife Service, Laurel, MD 94 pp.

Eisler, R. 1993. Zinc hazards to fish, wildlife, and invertebrates: A synoptic review. Biological Report 85, Contaminant Hazards Review No. 26. US Fish and Wildlife Service, Laurel, MD. 106 pp.

Eisler, R. 1997. Copper hazards to fish, wildlife, and invertebrates: A synoptic review. US Geological Survey, Biological Resources Division, Biological Science Report USGS/BRD/BSR, Contaminant Hazards Review No. 33. Pawtuxent Wildlife Research Center, Laurel, MD. 98 pp.

Eisler, R. 1998. Nickel hazards to fish, wildlife, and invertebrates: A synoptic review. US Geological Survey, Biological Resources Division, Biological Science Report USGS/BRD/BSR, Contaminant Hazards Review No. 34. Pawtuxent Wildlife Research Center, Laurel, MD. 76 pp.

Gaines, K.F., C.G. Lord, C.S. Boring, I.L. Brisbin Jr., M. Gochfeld, and J. Burger. 2000. Raccoons as potential vectors of radionuclide contamination to human food chains from a nuclear industrial site. Journal of Wildlife Management 64:199–208.

Gaines, K.F., C.S. Rommanek, C.S. Boring, C.G. Lord, M. Gochfeld, and J. Burger. 2002. Using Raccoons as an indicator species for metal accumulation across trophic levels: A stable isotope approach. Journal of Wildlife Management 66:811–821.

Gall, J.E., R.S. Boyd, and N. Rajakaruna. 2015. Transfer of heavy metals through terrestrial food webs: A review. Environmental Monitoring and Assessment 187:201.

Gehrt, S.D. 2003. Raccoon, *Procyon lotor*, and allies. Pp. 611–634, *In*, G.A. Feldhamer, B.C. Thompson, and J.A. Chapman (Eds.) Wild Mammals of North America: Biology, Management ,and Conservation. The Johns Hopkins University Press, Baltimore, MD. 1232 pp.

Gehrt, S.D., and E.K. Fritzell. 1999. Growth rates and intraspecific variation in body weights of Raccoons, *Procyon lotor*, in Southern Texas. American Midland Naturalist 141:19–27.

Gochfeld, M., and J. Burger. 1987. Heavy metal concentrations in the liver of three duck species: Influence of species and sex. Environmental Pollution 45:1–15.

Guidotti, T.L., R.J. Audette, and C.J., Martin. 1997. Interpretation of the trace metal analysis profile for patients occupationally exposed to metals. Occupational Medicine 47:497–503.

Herbert, G.B., and T.J. Peterle. 1990. Heavy metal and organochlorine compound concentrations in tissues of Raccoons from East Michigan. Bulletin of Environmental Contamination and Toxicology 44:331–338.

Hernández, F., R.E. Oldenkamp, S. Webster, J.C. Beasley, L.L. Farina, and S.M. Wisely. 2016. Raccoons *Procyon lotor* as sentinels of trace element contamination and physiological effects of exposure to coal fly ash. Archives of Environmental Contamination and Toxicology 72:235–246.

Hillis, T.L., and G.H. Parker. 1993. Age and proximity to local ore-smelters as determinants of tissue metal levels in Beaver, *Castor Canadensis*, of the Sudbury Ontario area. Environmental Pollution 80:67–72.

Hoff, G.L., W.J. Bigler, and J.G. McKinnon. 1977. Heavy metal concentrations in kidneys of estuarine Raccoons from Florida. Journal of Wildlife Diseases 13:101–102.

Jarvis, T. A., J.M. Lockhart, W.J. Loughry, and G.K. Bielmyer. 2013. Metal accumulation in wild Nine-banded Armadillos. Ecotoxicology 22:1053–1062.

Khan, A.T., S.J. Thompson, and H.W. Mielke. 1995. Lead and mercury levels in Raccoons from Macon County, Alabama. Bulletin of Environmental Contamination and Toxicology 54:812–816.

Levengood, J.M., and G.F. Hubert Jr. 2001. Concentrations of selected elements in Illinois Raccoons. Transactions of the Illinois State Academy of Science 94:89–99.

Lockhart, J.M., S. Siddiqui, W.J. Loughry, and G.K. Bielmyer-Fraser. 2016. Metal accumulation in wild-caught Opossum. Environmental Monitoring and Assessment 188:317.

Lord, C.G., K.F. Gaines, C.S. Boring, I.L. Brisbin Jr, M. Gochfeld, and J. Burger. 2002. Raccoon, *Procyon lotor*, as a bioindicator of mercury contamination at the US Department of Energy's Savannah River Site. Archives of Environmental Contamination and Toxicology 43:356–363.

Lotze, J.H., and S. Anderson. 1979. *Procyon lotor*. Mammalian Species 119:1–8.

Main W.P.L, C. Ross, and G.K. Bielmyer. 2010. Copper accumulation and oxidative stress in the sea anemone, *Aiptasia pallida*, after waterborne copper exposure. Comparative Biochemistry and Physiology C 151:216–221.

Millán, J., R. Mateo, M.A. Taggart, J.V. López-Bao, M. Viota, L. Monsalve, P.R. Camarero, E. Blázquez, and B. Jiménezc. 2008. Levels of heavy metals and metalloids in critically endangered Iberian Lynx and other wild carnivores from Southern Spain. Science of the Total Environment 399:193–201.

Mullally, A.M., G.B. Vogelsang, and A.R. Moliterno. 2004. Wasted Sheep and premature infants: The role of trace metals in hematopoiesis. Blood Reviews 18:227–234.

Nwokocha, C.R., D.U. Owu, M.I. Nwokocha, C.S. Ufearo, and M.O.E. Iwuala. 2012. Comparative study on the efficacy of *Allium sativum,* Garlic, in reducing some heavy metal accumulation in liver of Wistar rats. Food and Chemical Toxicology 50:222–256.

Roggeman, S., G. de Boeck, H. De Cock, R. Blust, and L. Bervoets. 2014. Accumulation and detoxification of metals and arsenic in tissues of Cattle, *Bos taurus*, and the risks for human consumption. Science of the Total Environment 466-467:175–184.

Sanderson, G.C., and R.M. Thomas. 1961. Incidence of lead in livers of Illinois Raccoons. Journal of Wildlife Management 25:160–168.

Seidensticker, J. 1999. Northern Raccoon, *Procyon lotor*. Pp. 221–223 *In* D.E. Wilson and S. Ruff (Eds.). The Smithsonian Book of North American Mammals. Smithsonian Institution Press, Washington, DC. 750 pp.

Shore, R.F., and B.A. Rattner (Eds.). 2001. Ecotoxicology of Wild Mammals. John Wiley and Sons, Chichester, UK. 752 pp.

Souza, M.J., E.C. Ramsay, and R.L. Donnell. 2013. Metal accumulation and health effects in Raccoons, *Procyon lotor*, associated with coal fly-ash exposure. Archives of Environmental Contamination and Toxicology 64:529-539.

Thomason, R.K., J.M. Lockhart, W.J. Loughry, and G.K. Bielmyer-Fraser. 2016. Metal accumulation in Bobcats in the southeastern USA. Environmental Monitoring and Assessment 188:565.

Wijnhoven, S., R.S.E.W. Leuven, G. van der Velde, G. Jungheim, E.I. Koelemij, F.T. de Vries, H.J.P. Eijsackers, and A.J.M. Smits. 2007. Heavy-metal concentrations in small mammals from a diffusely polluted floodplain: Importance of species- and location-specific characteristics. Archives of Environmental Contamination and Toxicology 52:603–613.

Wren, C.D. 1984. Distribution of metals in tissues of Beaver, Raccoon, and Otter from Ontario, Canada. Science of the Total Environment 34:177–184.