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Mercury Concentrations in Bat Guano from Caves and Bat Houses in Florida and Georgia

Amy E. Edwards[1], Jenise L. Swall[2], and Charles H. Jagoe[3]

[1]Hanover County Government, 9015 Pole Green Park Lane, Mechanicsville, Virginia 23116; aeedwards@hanovercounty.gov
[2]Department of Statistical Sciences and Operations Research, Virginia Commonwealth University, 1015 Floyd Avenue, Richmond, Virginia 23284
[3]School of the Environment, Florida A&M University, 1515 S. Martin Luther King Jr. Blvd., Tallahassee, Florida 32307

ABSTRACT

Previous studies have indicated that several pollutants are bioaccumulating in insectivorous bats, including the heavy metal mercury. This has resulted in an increased presence of mercury in bat waste (guano). In this study, we collected bat guano from ten caves in Florida and Georgia and two bat houses in Florida and analyzed the samples for mercury concentrations (ppm). Since the predominant bat species using caves (*Myotis austroriparius)* versus bat houses (*Tadarida braziliensis*) were different, the objective of this study was to make statistical comparisons of the mercury concentrations among caves, between caves and bat houses, and between bat houses. We found no significant differences between caves and bat houses. The mean concentrations among caves were significantly different, as well as the concentrations between the two bat houses. These results show similar levels of mercury concentrations in bat guano in both predominant bat species that use these caves and bat houses in Florida and Georgia. But variability exists between all locations, which indicate that other variables (e.g., geographic hot spots for mercury exposure) also affect mercury concentrations in guano. This study provides baseline data for bat guano mercury levels, which is a barometer of bat health and potential bioaccumulation of mercury in guanitic food webs, such as those in cave ecosystems.

Key words: Bat guano, mercury, insectivorous bats, bioaccumulation

INTRODUCTION

Elemental mercury can transform in aquatic systems by bacterial methylation to methylmercury, a neurotoxin which bioaccumulates in aquatic and terrestrial food webs (Brasso and Cristol, 2008; Selin, 2009). Insectivorous bats are particularly susceptible to mercury bioaccumulation via trophic transfer (Iskali and Zhang, 2015; Syaripuddin et al., 2014). They take up mercury when consuming large quantities of insects that accumulate mercury during their aquatic larval stages in mercury-contaminated waterbodies, as well as when feeding on terrestrial insects that bioaccumulate mercury (Brack and Whitaker, 2001; Becker et al., 2017).

Mercury in the environment is unquestionably affecting bats, since several studies have linked mercury pollution and the presence of mercury in various body parts of bats (Powell, 1983; O'Shea et al., 2001; Wada et al., 2010; Syaripuddin et al., 2014). This heavy metal contamination is linked to bat population declines (Mickleburgh et al., 2002) and sub-lethal biological effects like impaired reproduction and chronic health issues, as well as death in bats exposed to high contaminant loads of heavy metals (Clark and Shore, 2001; Hickey et al., 2001). Bats with white-nose syndrome have been found with elevated levels of contaminants and mercury exposure that potentially predisposes bats to this disease (Kannan et al., 2010).

Part of the mercury load in bats is excreted in their fecal matter, called guano, which primarily consists of bat hair, insect remains and bat mucus (Maher, 2006). Guano can therefore be examined for the presence of mercury in a habitat or ecosystem that contain bats, such as a cave or bat house. Several studies have already shown the presence of mercury in bat guano from caves. Petit and Altenbach (1973) dated a guano core from a cave in Colorado and found levels of mercury throughout the core were related to production at a local copper smelter and open pit mine. O'Shea et al. (2001) found higher concentrations of environmental contaminants, including mercury, in bat guano near a superfund site than at a reference site in Colorado. Petit (1975) investigated mercury concentrations in a 1100 year-old guano core from an Arizona cave and suggested that mercury concentrations had been higher than expected in pre-industrial times, possibly due to geological processes such as volcanic activity. A recent study by Hagan (2014) analyzed three age-dated groups of bat guano from Mammoth Cave National Park in Kentucky and found that modern/fresh guano had higher concentrations of mercury than historical guano (~100-1100 years old), which in turn had higher concentrations than ancient guano (~30,000 years old).

The concentration of mercury in bat guano has implications not just for the health of bats, but also cave ecosystems. The presence of mercury in guano allows potential for mercury to bioaccumulate in guanitic food webs for trogloxenes, troglophiles and troglobites. Coprophagy of guano is observed in cave-adapted salamanders (Fenolio et al., 2006), dermestid cave beetles (Mizutani et al., 1992), and even meat ants who enter caves to collect and transport guano back outside to their mounds (Moulds, 2006). Macroinvertebrate communities in caves increase after fresh guano is deposited (Poulson and Lavoie, 2000), and the nutrient quality of guano influences biodiversity of macroinvertebrates in caves (Iskali and Zhang, 2015).

This study had two objectives: one, to collect data on total mercury concentrations (ppm) in bat guano from two major bat ecosystems (ten caves and two bat houses) in a geographic region with documented inorganic mercury loading via atmospheric deposition from local and global sources (Stephenson, 1997; Prestbo and Gay, 2009), and abundant waterbodies to methylate the mercury; and two, to use the data to make statistical comparisons between caves and bat houses. Our first hypothesis predicted that since similar species roost in all caves in the study, then the concentrations of mercury in guano from all caves would be similar. Our second hypothesis predicated that the since the dominant species of bats differs between caves (*Myotis austroriparius*) and bat houses (*Tadarida braziliensis*), then concentrations of mercury in guano would be significantly different between these types of dwellings. Data resulting from this study are a valuable baseline for future studies to monitor the potential mercury contamination in bats and cave ecosystems in Florida and Georgia.

MATERIALS AND METHODS

The guano from insectivorous bats in this study was collected from eight caves and two bat houses in Florida and two caves in southwestern Georgia (Figure 1), depicted using the R package ggmap (Kahle and Wickham, 2013). The total number of guano samples we collected from caves was 95, and 17 for bat houses. All samples were collected between January 12, 2013 and February 13, 2014. Due to the heterogeneous nature of the quantity and depth of guano available in the caves sampled, the number of samples collected from each cave were not the same.

Both core and surface samples were collected for the study. Core samples were collected with a Russian sampler to avoid compaction of guano (Maher, 2006, Johnston et al., 2010). Cores were divided into 1 inch (25.4 mm) subsamples starting from the top of the core. Guano samples from cave surfaces were collected with plastic spoons and put into clear, reclosable plastic bags, with a new spoon and bag for each sample. Detailed information on guano samples is presented in Table 1. Sample locations were estimated on cave survey maps and may be requested from the corresponding author.

The dominant bat species roosting in Florida and Georgia caves are the maternity/wintering colonies of the Southeastern myotis (*Myotis austroriparius*) (Gore and Hovis, 1998), with lesser contributions from Tri-colored bats (*Perimyotis subflavus* - formerly known as eastern pipistrelle, *Pipistrellus subflavus*). The endangered Gray bat (*Myotis grisescens*) was formerly abundant in some caves in the Florida, but the Florida population has decreased in the last few decades and the species may no longer be present in the state (Gore et al., 2012). The caves in Georgia have *Myotis austroriparius* and *Perimyotis subflavus* as the dominant species (pers. comm. K. Morris, Georgia Department of Natural Resources, April 24, 2013). Thus, we assumed that *Myotis austroriparius* is the dominant species contributing to the guano piles in caves in this study. M. *austroripariu* forage near water (Barbour and Davis, 1969) and consume arthropods, (primarily Coleptera, Lepidoptera and Culicidae (Zinn, 1977). *Perimyotis subflavus* also forage near water (Fujita and Kunz, 1984; Whitaker and Hamilton, 1998) and consume insects in the orders of Trichoptera, Homoptera, Coleoptera, Hymenoptera and Lepidoptera (Sherman, 1939; Ross, 1961; Whitaker, 1972; Carter et al., 1999).

Guano samples were taken below two bat houses in central Florida, detailed in Table 1. The dominant species roosting in bat houses in Florida is the Brazilian free-tailed bat (*Tadarida braziliensis*), with *Myotis austroriparius* present to a lesser degree. In the southeastern United States, the *Tadarida braziliensis* diet includes insects in the order of Coleoptera, Diptera, Lepidoptera and Hymenoptera (Sherman, 1939).

All samples were stored in a freezer until they were freeze dried to constant weight and analyzed for Total Mercury (THg) by thermal decomposition, gold amalgamation and atomic absorption spectroscopy (EPA method 7473) using a Milestone DMA80 mercury analyzer (Milestone, Inc., Shelton CT). This analyzer has a detection limit as low as 0.001 nanograms of mercury and as high as 300 ppm (mg/kg). The QA/QC included blanks, replicates and matrix spikes. All replicates had <10 percent difference and were averaged. The DMA80 was calibrated with NIST-traceable standards. Quality assurance included standard reference materials with similar total Hg concentrations purchased from NIST and the National Research Council of Canada. Results are reported as total mercury per gram dry weight of guano. The open-source statistical computing package R (R Core Team, 2018) was used to make graphics and conduct statistical analyses.

RESULTS

A total of ten caves were surveyed during the data collection phase of this project. However, in four of these caves, we were able to collect fewer than three samples. This is too little information to reliably ascertain the mean of mercury concentrations in these four caves, so we excluded these caves from our comparison of mean mercury concentrations at the various locations. The left panel of Figure 2 shows the concentrations from the remaining six caves, along with the number of observations available for each.

For the samples which came from cross-sections of guano cores, we investigated whether there was evidence indicating whether mercury concentration tended to increase or decrease with depth (distance in inches from the top of the core). We also checked for serial correlation between the layers of each core. Only in the case of one core, taken from Judge's Cave, was there any evidence of statistically significant association between mercury concentration and depth or statistically significant autocorrelation. However, the subsamples from the other core taken within Judge's Cave did not conform to this pattern. We suspect that the seeming significance of this result may have been due to type I error, given the lack of corroboration with the other core taken from the same cave. With only eight guano cores of varying sizes, containing 6-11 subsamples each, we are limited in our ability to detect meaningful relationships. In addition, we note that the caves are subject to frequent flooding events, which may affect the mercury concentrations within the guano cores.

The Fligner-Killeen test of homogeneity of variances (Fligner and Killeen, 1976) indicated that variances in mercury concentrations among the six caves cannot be assumed to be equal ($p < 0.001$). Therefore, we cannot compare the mean concentrations among the caves using the traditional one-way analysis of variance (ANOVA) approach, since ANOVA assumes homogeneity of variances. Instead, we use generalized least squares (e.g. Sen and Srivastava, 1990, Chp. 6) to estimate a mean concentration for each cave, while also accommodating the differing variability among the caves.

To facilitate the comparison of all pairs of caves, we used the emmeans package in R (Lenth, 2018) and applied Tukey's adjustment for multiple comparisons. We found significant differences in mean mercury concentrations between Climax Cave and Florida Caverns Old Indian Cave ($p < 0.001$) and between Climax Cave and Judge's Cave ($p < 0.001$). The mean mercury concentration in Florida Caverns Old Indian Cave is estimated to be 0.20 +/- 0.08 ppm higher than that in Climax Cave, while the mean in Judge's Cave is estimated to be 0.21 +/- 0.10 ppm higher. Using the estimated cave mean concentrations and variances, we can also derive a confidence interval for the overall mean concentration of mercury across the caves, which is 0.55 +/- 0.05 ppm.

The right panel of Figure 2 depicts the mercury concentrations in the samples obtained from the two bat houses. Given the small number of samples taken from the Lower Suwanee National Wildlife Reserve (NWR) Bat House, it is difficult to assess the variability of mercury concentrations, and we cannot assume that the population variances of the two groups are equal. Using the generalized least squares technique, as we did for the caves, the mean concentrations in the two bat houses were found to be significantly different ($p < 0.001$). The mean concentration at the bat house at the Lower Suwanee NWR was estimated as 0.37 +/- 0.14 ppm higher than the concentration at the bat house at the University of Florida at Gainesville.

In the sample sizes provided in Figure 2, we note that the samples available from the two bat houses were limited, both in sample size and in the number of bat houses observed. Given the smaller sample sizes for the bat houses, especially for the one at the Lower Suwanee NWR, the validity of the normal distribution assumed by our generalized least squares model can be reasonably be questioned. However, the Wilcoxon-Mann-Whitney non-parametric test procedure, which does not depend on the normality assumption, provides additional evidence that the concentrations are significantly different between the two bat houses ($p < 0.001$).

Using the estimated means and variances from the generalized least squares model, as we did for the caves, we estimate the average mercury concentration for the two bat houses as 0.51 +/- 0.05. Since this confidence interval overlaps the confidence interval we developed for mercury concentrations in caves, we do not have enough evidence to say that the mean concentration levels are significantly different between the cave-dwelling and bat house-dwelling bats we observed. We note that this aspect of the analysis is limited by the number of bat houses that could feasibly be sampled; the addition of observations from other bat house locations would improve our estimates.

DISCUSSION

In the first hypothesis, we predicted that, since similar species roost in all the caves sampled in this study, the concentrations of mercury in guano among caves would be similar. Data failed to support this hypothesis, as there was a significant difference of mean concentrations between caves. Given that the caves play host to the same predominating species of bat, any significant differences in mean mercury concentrations among locations is a possible indicator of differing pollutant levels in the surrounding environment. Variations could be due to geographic hot spots of mercury pollution, since a major source of mercury pollution in Florida and Georgia is atmospheric deposition. There could also be other factors that potentially affect mercury bioaccumulation in bats including local differences in available prey types, and possible differences in colony sex ratios, animal sizes or population age structure, which would affect the bat colonies and their guano in the caves.

Our second hypothesis was also not supported by the data comparing cave and bat houses with different predominant species, as the mercury concentrations in guano were not significantly different between caves and bat houses. This would indicate that mercury concentrations in guano from both predominant species in caves (*Myotis austroriparius*) and bat houses (*Tadarida braziliensis*) were similar.

It is interesting to note that the estimated mean concentrations of mercury in bat guano in both caves and bat houses in this study area of Florida and Georgia lies within the mean range of the modern/fresh guano (0.7 +/- 0.2 ppm) from the Hagan 2014 study in Kentucky. The guano collected for this study was assumed modern (<100 years old, as in the Hagan, 2014 study), since none of the guano produced cores over 11 inches (279.4 millimeters), indicating a lack of long-term accumulation of guano piles. The guano piles from the bat houses were also known to be disturbed. The guano at the University of Florida, Gainesville bat houses are collected in 55 gallon drums and given away in 5 gallon buckets to gardeners several times per week (Kenneth Glover, per comm, 4/26/2013).

Analysis would be improved by increasing the number of samples from bat houses. This would include increasing the sample size from each bat house, particularly as we had only 5 samples from the Lower Suwanee NWR bat house, or increasing the number of bat houses from which samples were obtained. The Lower Suwanee bat house is a much smaller bat house than the bat house at University of Florida at Gainesville, which has the largest occupied bat house in the world (https://www.floridamuseum.ufl.edu/bats/) and two structures for bat houses instead of the one structure at Lower Suwanee.

Other weaknesses of the study include the low sample size and the differences in the age of the guano among locations, possibly skewing the results. The two bat houses in this study are known to give away guano on a routine basis to gardeners, thereby removing the older guano. Although guano in cave environments are disturbed by cavers and cave scientists, caves are a more protected environment from the elements than bat houses, so the guano from caves in this study are likely older and less disturbed than the guano under the bat houses. The effect of flooding on guano piles in regards to mercury mobility is also unknown, as is bioturbation from fauna.

Future studies should evaluate methylmercury concentrations in both fresh guano and hair from individual bats to correlate concentrations between the bats and the bat waste. It would also be beneficial to know if the bacteria that convert inorganic forms of mercury to methylmercury existed in caves, as the methylmercury is the bioavailable form. A study of mercury mobility in guano would also be interesting, as our study found a lack of significant difference in variances among the core and surface samples, as well as samples from the same core. Data resulting from this study are a valuable baseline for future studies to monitor the potential mercury contamination in bats and cave ecosystems in Florida and Georgia.

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Figure 1: Location Map

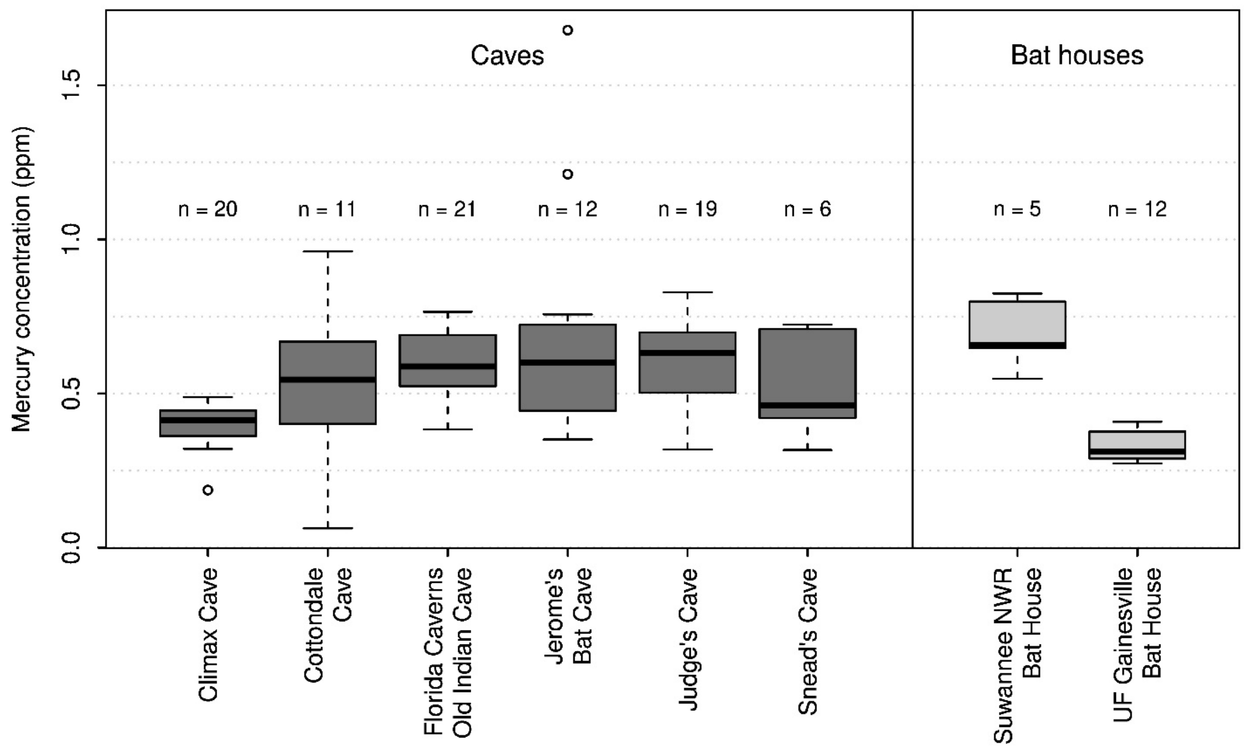

Figure 2: Boxplots of mercury concentrations, caves versus bat houses

Table 1: Guano samples from caves and bat houses

Location	State	Date collected	Sample details
Big Mouth Cave	Florida	7/13/2013	bat pellets collected throughout cave and compiled into 1 sample
Cottondale Cave	Florida	10/30/2013	5 surface samples taken throughout the cave and a core that was 6 in (152.4 mm) deep and subsampled at 1 in (25.4 mm) intervals
Florida Caverns Old Indian Cave	Florida	2/13/2014	two 8 in (203.2 millimeters) cores subsampled at 1 in (25.4 mm) intervals in the Rotunda Room, 5 surface samples taken throughout cave
Jerome's Bat Cave	Florida	10/30/2013	12 surface samples taken throughout cave
Judge's Cave	Florida	10/30/2013	one 11 in (279.4 mm) core subsampled at 1 in (25.4 mm) intervals, and one 8 (203.2 mm) core at 1 in (25.4 mm) intervals
Newberry Bat Cave	Florida	9/15/2013	1 sample of bat pellets
Snead's, also known as Pope's Bat Cave	Florida	10/30/2013	6 surface samples
Thornton's Cave, also known as Sumter Bat Cave	Florida	7/13/2017	1 sample at one of the entrances
Climax Cave	Georgia	2/28/2013	3 surface samples from the Barrel Room
Climax Cave	Georgia	7/6/2013	10 in (254 mm) core subsampled at 1 in (25.4 mm) intervals from Barrel Room, 4 surface samples, 2 composites of 2 separate 11 in (279.4 mm) cores, two separate samples that were the bottom inches of two separate 11 in (279.4 mm) cores
Waterfall Cave	Georgia	8/17/2013	2 surface samples
University of Florida bat house	Florida	9/13/2013	One core had 5 intervals of 1 in (25.4 mm) each. The other core had 7 intervals, with the first six intervals at 1 in (25.4 mm) and the 7^{th} interval comprising the last 1.5 inches of the core.
Lower Suwanee National Wildlife Reserve	Florida	1/26/2014	The guano piles were not deep enough to use the corer, so samples were taken as compilations within

			different locations under the bat house. Depths were measured in inches with a ruler from the top of the pile to the concrete bottom. A 4 in (101.6 mm) sample was compiled from the middle under the bat house, a 3 in (76.2 mm) sample was compiled from the back left corner, a top inch (25.4 mm) was taken from the back middle, and the bottom inch (25.4 mm) was taken from the back middle.