**MERCURY CONCENTRATIONS IN BAT GUANO FROM CAVES AND BAT HOUSES IN FLORIDA, GEORGIA, AND SOUTH CAROLINA**

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**Abstract.-** Increasing concentrations of mercury in the environment from anthropogenic sources is a health threat for both humans and wildlife, and insectivorous bats are particularly vulnerable to this heavy metal via trophic transfer. A portion of the mercury bats ingest is excreted in their guano, which serves as a record of mercury contamination. Increased mercury levels are of particular concern in the southeastern U.S., especially Florida, a state with abundant waterbodies and high levels of mercury from local and global sources via atmospheric deposition. We measured total Mercury (THg) concentrations in bat guano from caves and bat houses in Florida, Georgia and South Carolina to quantify the amount of THg in the guano. Results show concentrations matching modern/fresh guano from a recent study in Mammoth Cave, Kentucky, in which dated guano was found to have much higher concentrations in modern/fresh guano than historical and ancient guano. The mean concentrations of Hg in this study for both caves (0.5666 ppm) and bat houses (0.4370 ppm) were higher than the ancient and historical guano and within or closer to the mean range of the modern/fresh guano (0.7 +/- 0.2 ppm) from the Mammoth Cave study, indicating that concentrations of THg in bats in Florida, Georgia and South Carolina are increasing with the increasing concentrations of mercury in the environment. Cave sediment was also analyzed to find the extent of THg in cave ecosystems, both within guano piles and in other sediments (mud, clays and sand). Bat guano had significantly higher concentrations than other sediment types, indicating that the main source of mercury contamination in caves is being brought in by the trogloxenic bats. This has implications for both the health of bats and fragile cave ecosystems. Bats with white-nose syndrome have been found with elevated levels of contaminants. Also, guano is an important food source for other cave species and can lead to increased Hg levels throughout the cave ecosystem..

Increasing concentrations of mercury (Hg) in the environment from anthropogenic activity is a health threat for both humans and wildlife. Elemental mercury can transform in aquatic systems by bacterial methylation to methylmercury, a neurotoxin which bio accumulates in the aquatic and terrestrial food webs (Selin, 2009). Methylmercury can then enter terrestrial food webs through consumption of fish or aquatic invertebrates. For example, Brasso and Cristol (2008) reported very high Hg concentrations in tree swallows (*Tachycienta bicolor*) which fed on insects that emerged from a Hg-polluted river in Virginia.

Due to several factors, insectivorous bats are particularly susceptible to Hg bio accumulation via trophic transfer through the food chain (Iskali and Zhang, 2015). Bats take up Hg when feeding on large quantities of insects (e.g. mosquitoes) that accumulate Hg during their aquatic larval stages in Hg-contaminated waterbodies, as well as when feeding on terrestrial insects that bio accumulate Hg (e.g. spiders) (Brack and Whitaker, 2001; Cristol et al. 2008). Bats have unquestionably been affected by this heavy metal, since several studies have found the presence of Hg in bat muscles, kidneys, livers, brains and fur (Miura et al., 1978; Powell, 1983; Hickey et al., 2001; O’Shea et al., 2001; Yates et al., 2008; Wada et al., 2010; Yates et al., 2012). Heavy metal contamination has been linked to bat population decreases (Mickleburgh et al, 2002), and studies have shown sub lethal biological effects of impaired reproduction, chronic health issues and death in bats exposed to high contaminant loads of heavy metals (Clark and Shore, 2001; Hickey et al., 2001).

One region in the United States where insectivorous bats are exposed to high levels of Hg in the environment is the southeastern U.S. coastal plain. This region includes the states of Florida, Georgia, and South Carolina. In particular, Florida receives high levels of mercury from local and global sources via atmospheric deposition (Prestbo and Gay 2009). The Florida Atmospheric Mercury Study (FAMS) of air monitoring stations during 1994-1995 found that Florida had a yearly average of 900 pounds of mercury coming from rainfall (Stephenson, 1997). The rainfall pollutes surface waters throughout the state and contributes to the trophic transfer of Hg from insects to bats. Once the bats ingest Hg, some of the metal is excreted in their feces (called guano), which primarily consists of bat hair, insect remains and bat mucus (Maher, 2006). Over time, guano deposits in sheltered environments, such as caves, may accumulate vertically to sizeable depths and offer a chronostratigraphic record in a range of hundreds to thousands of years (Mizutani et al., 1992; Maher, 2006). The southeastern U.S. has several cave regions which provide habitat for several insectivorous bat species, and guano is found in many of these caves.

Few studies have focused on heavy metal concentrations in bat guano. 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 a reference site in Colorado. Petit (1975) investigated mercury concentrations in a 1100 year-old guano core from an Arizona cave and suggested that Hg concentrations had been higher than expected in pre-industrial times, possibly due to geological processes such as volcanic activity. Clark et al. (1986) found elevated concentrations of the metals cadmium, chromium and zinc in guano from a cave in the Florida panhandle, but did not analyze for mercury. Cuculić et al. (2011) found bat guano was responsible for high concentrations of metals, particularly cadmium, in anchialine caves in Croatia (Hg was not analyzed). A recent study by Hagan (2014) analyzed three dated groups of bat guano from Mammoth Cave National Park in Kentucky and found that modern/fresh guano had higher concentrations of Hg than historical guano (~100-1100 years old), which in turn had higher concentrations than ancient guano (~30,000 years old).

We analyzed total mercury concentrations (THg) in bat guano from caves and bat houses in Florida, Georgia and South Carolina and compared them to the dated guano results from Mammoth Cave (Hagan (2014). We hypothesized that the modern/fresh guano from bats in FL, GA, and SC would also have values similar to the modern/fresh and greater than the historical and ancient guano in Kentucky. Bat guano from bat houses were also analyzed to compare THg concentrations in guano from different bat species, as the dominant bat species in caves may differ from the species that utilizes bat houses. Other types of sediment besides guano (clay, mud, sand) were also analyzed for THg to find how extent of Hg concentrations throughout various caves away from the guano piles. Limestone rock from caves was also analyzed for THg to consider the lithogenic contribution of Hg from in-cave weathering. This data is valuable for monitoring potential mercury contamination in economically significant bat populations (Kasso and Balakrishnan, 2013) and fragile cave ecosystems.

METHODS

The guano from insectivorous bats in this study came from caves and bat houses in four cave regions of the southeastern U.S. Coastal Plain (Fig. 1). The guano collected was assumed modern/fresh since caves in this region are prone to flooding, which likely transports guano after deposition and prevents long-term accumulation of guano piles. The effect of flooding on guano piles in regards to mercury mobility is also unknown, and stratigraphically dated guano piles provide inconsistent results (Zukal et al., 2015). Therefore, the guano cores in this study were subsampled to find spatial, but not temporal correlations of Hg with depth.

The main bat species which use caves in this region are the Southeastern myotis (*Myotis austroriparius -* MYAU), tri-colored bat (*Perimyotis subflavus* - PESU) and Gray bat (*Myotis grisescens* - MYGR). The main bat species which use bat houses are the Brazilian free-tailed bat (*Tadarida braziliensis* – TABR), with some MYAU.

To protect the bats and caves, numbers from the state cave surveys are used instead of cave names for sampling locations (Fig. 1). Regions 1 and 3 are in Florida. Region 1 includes the caves Florida Cave Survey (FCS) 229, 338, 440, 535, 537, 555, 556, 557, 565, 872 and 925. The dominant bat species roosting in Florida caves is MYAU (Gore and Hovis 1998). The endangered MYGR was formerly abundant in some caves in region 1, 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). Region 3 includes the caves FCS 3, 84, 188, 213, 265, 1373, 1390, and 1630. Region 2 includes the caves GGR 56, GSS 36, GSS 250, and two caves without a survey numbers, with MYAU and PESU as the dominant species identified during bat surveys (Morris, per. Comm., 2013). Cave region 4 includes the cave SC 1. This cave has a maternity colony of MYAU (Reeves, 2001).

Two bat houses, one at University of Florida at Gainesville and one at the Lower Suwanee National Wildlife Reserve (both Region 3) were sampled. TABR is the predominant species at these bat houses, with MYAU also present. Both bat houses are in Region 3 (Fig. 1). Both bat houses are in Region 3.



Figure 1.

Core guano samples in caves and bat houses were collected with a Russian sampler to avoid compaction of guano (Maher 2006, Johnston et al. 2010). Cores were divided into 1 inch subsamples starting from the top of the core. Sediment and guano from cave surfaces were collected with non-metal utensils in ~5 g amounts and put into plastic bags. All samples were stored in a freezer until freeze dried. Sediment samples from caves were generally taken at the entrance, along stream paths through the cave (whether active stream or dry stream) and in areas that could possibly represent natural background and unpolluted sediment. All samples were collected between January 12, 2013 and February 13, 2014.

All samples 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. This method was rapid and inexpensive, and provided detection limits in the sub-parts per billion range. QA/QC included blanks, replicates and matrix spikes. All duplicates had <10 percent difference and were averaged. The DMA80 was calibrated with NIST-traceable standards, and the calibration was verified using standards purchased from NIST and the National Research Council of Canada.

Organic matter (OM) was found for each sample using ASTM D 2974 Standard Test Methods for Moisture, Ash and Organic Matter of Peat and Organic Soils (ASTM, 2013), with the exception of using 430°C instead of 550°C. A temperature below the dissociation temperature of calcium carbonate was used as by Hettwer et al., (2003), since the cave sediments and guano cores came from caves formed in carbonates.

RESULTS

**Mercury concentrations in bat guano from caves and bat houses**

Results for THg concentrations in bat guano from caves and bat houses are shown in Table 1. The main bat species in caves are MYAU, PESU, and MYGR. The minimum concentrations ((ppm (mg/kg)) from caves from all regions was 0.1183, the maximum was 2.1754, and the mean was 0.5666 (n=106). The main bat species using bat houses are TABR/MFT. Guano from bat houses had minimum concentrations ((ppm (mg/kg)) of 0.2730, the maximum was 0.8249, and the mean was 0.4370 (n=17).

**Table 1. THg concentrations (ppm mg/kg) in guano cores from caves and bat**

**Houses**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Main Bat Species | Hg Concentrations (ppm) | | |  |
| Min | Max | Mean | n |
| Caves (all regions) | MYAU, PESU, MYGR | 0.1183 | 2.1754 | 0.5666 | 106 |
| Bat Houses | TABR/MFT | 0.2730 | 0.8249 | 0.4370 | 17 |

**Mercury concentrations in bat guano from caves**

Results for the THg concentration in bat guano from caves are shown in Table 2. The same data set from Table 1 is used, broken down by region. Region 1 had a minimum of 0.1354, a maximum of 1.6793, and a mean of 0.6016 (n=68). Region 2 had a minimum of 0.1868, a maximum of 0.4883, and a mean of 0.3919 (n=22). Region 3 had a minimum of 0.1183, a maximum of 2.1754, and a mean of 0.6657 (n=14). Region 4 had a minimum of 0.5897, a maximum of 0.6179, and a mean of 0.6038 (n=2).

**Table 2. Results for THg concentrations for bat guano from caves by region.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Region | n | Hg Concentrations (ppm) | | |
| Min | Max | Mean |
| 1 | 68 | 0.1354 | 1.6793 | 0.6016 |
| 2 | 22 | 0.1868 | 0.4883 | 0.3919 |
| 3 | 14 | 0.1183 | 2.1754 | 0.6657 |
| 4 | 2 | 0.5897 | 0.6179 | 0.6038 |

**Significance of Mercury concentrations in cores by depth**

Results for the THg concentrations in cores and their significance with depth are shown in

Table 3.

**Table 3. Significance for THg concentrations in core depths p>0.05?**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Depth from top of core (1 inch intervals) | GSS 36 | FCS 872 | FCS 555 | FCS 555 | FCS 556 | FCS 556 | UF  Bat House | UF  Bat House |
| 1 | 0.4414 | 0.3957 | 0.7654 | 0.5877 | 0.4264 | 0.4263 | 0.3163 | 0.3403 |
| 2 | 0.3769 | 0.4075 | 0.7253 | 0.6650 | 0.5020 | 0.5037 | 0.2890 | 0.4086 |
| 3 | 0.4338 | 0.6868 | 0.5240 | 0.5404 | 0.5011 | 0.6324 | 0.2786 | 0.3655 |
| 4 | 0.4481 | 0.6699 | 0.5303 | 0.4627 | 0.5443 | 0.6391 | 0.2730 | 0.4058 |
| 5 | 0.4516 | 0.5450 | 0.6110 | 0.5593 | 0.6149 | 0.7229 | 0.3081 | 0.3871 |
| 6 | 0.4246 | 0.6668 | 0.6987 | 0.5093 | 0.6656 | 0.6063 | 0.2921 | 0.2899 |
| 7 | 0.3984 | - | 0.7171 | 0.4569 | 0.7270 | 0.8284 | - | - |
| 8 | 0.4106 | - | 0.6895 | 0.7082 | 0.7624 | 0.3184 | - | - |
| 9 | 0.4287 | - | - | - | 0.6728 | - | - | - |
| 10 | 0.4883 | - | - | - | 0.6692 | - | - | - |
| 11 | - | - | - | - | 0.7305 | - | - | - |
| Significant? |  |  |  |  |  |  |  |  |

* No data

**Mercury concentrations in Limestone samples from caves**

Results for THg concentrations from limestone samples from caves is shown in Table 5.

**Table 5. Results for THg concentrations from Limestone samples**

|  |  |
| --- | --- |
| Cave Survey No. | Region |
| Hg Concentration ppm |
| FCS 537 LS | 1 | 0.0563 |
| FCS 537 Stalagmite | 1 | 0.0023 |
| FCS 556 | 1 | 0.0147 |
| FCS 557 | 1 | 0.0127 |
| GSS 36 | 2 |  |
| SC 1 | 4 | \*0.0152 |

\*Edwards et al, 2016

DISSCUSSION

The highest and lowest concentrations of THg were found in bat guano from caves. This could represent a difference in diet between dominant bat species using caves and bat houses, or sampling bias since a greater number of samples came from caves (n=106) than bat houses (n=17). Both bat houses had shallower guano piles than the caves due to the periodic practice of guano removal (guano is offered free to the public for fertilizer), resulting in fewer subsamples. The guano piles under the bat houses are also not in sheltered environments, leaving the guano affected more by weather, such as rain, wind, and temperature extremes not encountered in caves. This could lead to possible leaching of Hg into the underlying soil, runoff and transport of Hg away from the piles, or evaporation of mercury during hot summer months. The mean concentrations of Hg for both caves (0.5666 ppm) and bat houses (0.4370 ppm) are higher than the ancient and historical guano and within or closer to the mean range of the modern/fresh guano (0.7 +/- 0.2 ppm) from the Mammoth Cave study (Hagan, 2015). Results indicate that the modern/fresh guano from Florida, Georgia and South Carolina have similar Hg concentrations to the modern/fresh guano in Kentucky. Kentucky is geographically not far from the southeastern coastal U.S., and is likely experiencing the same phenomenon of increasing deposition and trophic transfer of Hg in the environment.

The min and max THg values from insectivorous bat guano in this study were 0.1183-2.1754 ppm for all guano, with a mean of 0.5666 ppm from caves and 0.4370 ppm from bat houses. Zukal et al. (2015) summarized values of Hg in insectivorous bat guano from several studies and found a range of 0.07 and 4.2 and a mean of 0.54 ppm. The results from this study were above the min but below the max, with a mean of guano from caves very similar to the mean from the summarized studies.

Region 3 had the highest THg (2.1754 ppm) in bat guano from all the cave regions. This could indicate a Hg deposition hot spot in Region 3, or perhaps a higher population of bats that are bio accumulating Hg and contributing to greater concentrations of guano (and therefore Hg).

When concentrations from non-guano cave sediment are included with the guano, the min and mean concentrations decrease for all regions (except region 4 – which only had 2 guano samples and no cave sediment). This indicates that guano, acting as a biogenic sediment in caves, has much higher concentrations of THg than non-guano sediment (e.g. mud, clays and sand). The lower concentrations of THg in limestone from the caves indicates that the mercury is likely not coming from in-cave weathering of limestone, but brought in by the bats and possibly spread throughout the cave from the guano piles during flooding events. This has implications for cave ecosystems, as other cave species feed on this contaminated guano, and flooding prevents the sequestering of contamination to only some areas of the cave (i.e. guano piles). This has implications for both the health of bats and fragile cave ecosystems. Bats with white-nose syndrome have been found with elevated levels of contaminants and could potentially predispose bats to this disease (Kannan et al., 2010).

The presence of Hg in guano effects not only the health of bats, but contaminated guano may allow Hg to bio accumulate in cave ecosystems and food webs for trogloxenes, troglophiles and troglobites. Coprophagy of guano has been 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 have been found to increase after fresh guano is deposited (Poulson and Lavoie, 2000), and the nutrient quality of guano has been found to influence biodiversity of macroinvertebrates in caves (Iskali and Zhang, 2015).

Future studies could look at the methylmercury (MeHg) concentrations in both fresh guano and the hair from the bats roosting above the guano to correlate concentrations between the bats and the bat waste. It would also be beneficial to know if the bacteria that converts inorganic forms of mercury to methylmercury (MeHg) existed in caves, as the MeHg is the bioavailable form.

**Significance of Mercury concentrations in cores by depth?**

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