**Overview**

Figure 1 depicts boxplots for all measured mercury concentrations in units of parts per million (ppm). The leftmost (gray) boxplot shows all measurements (without respect to region), with the minimum observation at 0.0628 and the maximum at 2.1750. The mean concentration is estimated at 0.5420 +/- 0.0454. (If you would prefer to just give the interval, you can remove the preceding figure and say something like the following: A 95% confidence interval for this mean is 0.4966ppm to 0.5874ppm.) The boxplot shows three outlying points, which consist of measurements from two caves in region 1 (Jerome’s Cave and Judge’s Cave) and one cave in region 3 (Sweet Gum). Can you say more about your intuition for why these values are higher than the others? Note: The three outliers do have a substantial effect on the upper bound of this interval. If they were removed, the 95% confidence interval would change to (0.4827, 0.5449).



**Figure 1: Measured mercury concentrations in ppm from all locations (left, gray box) and grouped by region (white boxes). Number of measurements shown in gray. (Amy – Your Table 1 has a total of 123 observations. I’m not sure what I’ve done differently to get 125.)**

The four white boxplots show mercury concentrations grouped by region. With only two measurements available from caves in region 4, it is inadvisable to draw conclusions about mercury concentrations in this particular area. Of the three remaining regions, region 2 typically has lower concentrations, with less variability than those in regions 1 and 3.

The samples in regions 1, 2, and 4 came exclusively from caves, in which the typical species present are predominantly of species MYAU, PESU, and/or MYGR. Region 3 differs in that it includes guano samples from caves, bat houses, and an interstate underpass. While the caves in region 3 are populated by MYAU bats, similar to the caves in the other regions, the bat houses and the interstate overpass are predominantly populated by the TABR and/or MFT species. For comparison with the previous study from Mammoth Caves, the estimated mean mercury concentration for caves (including all four regions) is 0.5619 +/- 0.0501 (95% confidence interval given by (0.5117, 0.6120)). Note: Again, the three outliers have a notable impact, as the confidence interval would be (0.4969, 0.5618) if they were excluded.

**Comparisons of mean mercury concentrations between regions 1, 2 and 3**

Given the geographical differences among the regions, the data were examined for evidence of differences in mean mercury concentrations among the caves in regions 1, 2, and 3. Region 4 was excluded, due to the sparsity of measurements from the region. Also, since no bat houses are present in regions 1 and 2, we limited this comparison to cave-based measurements.

The presence of the outlying observations and the different variability among the regions means that the traditional one-way analysis of variance analysis is likely not appropriate here. Instead, we utilize an exact permutation test, which is a non-parametric statistical testing procedure. (Do you think I should try to find a general reference about permutation tests that would be appropriate to put here?) In addition, the structure of the data is nested; for example, measurements within each cave may be correlated, and are nested within cave and then within region. To accommodate potential correlational structure, we utilize an approach which considers all possible permutations of caves among regions, rather than individual measurements among regions (Anderson and ter Braak, 2003). The resulting p-value is 0.0037, indicating a significant difference in mean mercury concentration in caves among regions 1-3. (This p-value includes all measurements. If we were worried about the correlation among core measurements, and only used the top layer, we would still have a p-value of about 0.02, which is still significant at the typically used 5% level.)

(I also tried a mixed-effects model to answer this question. It didn’t fit very well, however, due to the outliers and variability I mentioned before. However, it was in agreement with the conclusion reached on the basis of the permutation test.)

**Comparisons of mean mercury concentrations between caves and bat houses**

In region 3, three of the eight locations from which samples were drawn were manmade structures. These include 2 bat houses (at the University of Florida at Gainesville and Suwannee National Wildlife Refuge) and one interstate overpass (I-75 in Florida). The populations of bats inhabiting these areas are of different species than are found in the caves; they are predominantly of species TABR and MFT. For the purposes of this analysis, we group these structures together and, for simplification, refer to all three locations as “bat houses”. Figure 2 shows that in region 3, the mercury concentrations taken from the bat houses were generally somewhat lower than those taken from caves.



**Figure 2: Mercury concentrations in ppm in samples taken from region 3, grouped by location type.**

Another permutation test was used to investigate the question of whether the mercury concentrations differ between the bat house and cave-based populations in region 3. This test is considering all possible permutations of the 8 locations to the status of “cave” or “bat house”, comparing our actually results to those obtained with the various permutations. With no bat houses located outside region 3, we can only consider concentrations from the eight locations sampled in region 3. Even though we have 22 measurements in total, the permutation test accommodates the fact that the individual samples are nested within the sampling location (whether cave or bat house). This restriction limits the power of our testing procedure. The p-value for this test is 0.25, indicating that, based on this study, there is not enough evidence to indicate a significant difference in mean mercury concentration.

**References**

Anderson, Marti J. and Cajo J. F. ter Braak. (2003) “Permutation Tests for Multi-factorial Analysis of Variance”. *Journal of Statistical Computation and Simulation*, Vol 73 (2)., pp. 85-113.

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