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**Abstract**

**Introduction**

Exposure to heavy metals transported through the air can contribute to or worsen health issues including asthma, emphysema, and cancer (Kampa and Castanas 2007). Long term studies of air pollution are usually limited to stationary monitoring conducted by government bodies or large research institutions. These official monitoring sites can only measure a limited area, and the data they collect is then generalized. This leads to major gaps in knowledge and enforcement of pollution controls. Air pollution can vary significantly over small areas (see Kheirbeck et al. 2018, Ginzburg et al 2015). This spatial gap has spurred the interest of public scientists. The development of low-cost air quality monitors has enabled public scientists to examine their own exposure at a fine scale and become better informed on their health risks. However, while considered low cost compared to state-of-the-art government monitors, most of these sensors are prohibitively expensive for the majority of the population. Monitoring in the United States has been primarily in regions that are majority white and have high median incomes (deSouza and Kinney 2021). Further, no low-cost sensors yet exist for the measurement of heavy metals, so they have rarely been studied in public-led research (Commodore et al. 2017). Accessible, long-term air quality monitoring that can detect differences at fine-scale is needed.

Spider webs have been used before successfully to test air pollution, although never in the Americas and not yet at this fine spatial scale (Rybak 2015, Rybak et al. 2015, Rybak et al. 2012, Tahir et al. 2018, Hose et al. 2002, Xiao-li et al. 2006). We used webs made by the *Agelenidae* family (also known as funnel web spiders) to learn about air pollution in South West Baltimore, an area with a history of air pollution and known heavy metal releasing facilities (Kelly et al. 2016). We were guided by three central research questions, for which we created two experiments.Can spiderwebs detect fine scale differences in metal air pollution? Do those differences correlate with known sources of air pollution? Does the metal composition in spiderwebs relate to the composition of particulate matter (PM) measured by established monitors?

**Methods**

**Study site**

**Baltimore City**

The primary locations of this study were the Baltimore neighborhoods of Brooklyn and Curtis Bay. This area was chosen because it has high levels of air pollution and a history of environmental advocacy around the issue (Kelly et al. 2012). Figure one shows metal releasing facilities on the 2019 Toxic Release Inventory (TRI) list near the study area. The TRI is published by the US Environmental Protection Agency (EPA) and lists the size of known releases of certain chemicals dangerous to human health. The responsible facilities report releases which are then compiled by facility. Five metal-releasing TRI sites are within two miles of the study site. These facilities include a shipping terminal, a concrete plant, a gypsum production facility, a plastic production facility, and a chemical production facility. The Environmental Integrity Project operated stationary PM2.5 sensors at three sites in the Curtis Bay/Brooklyn neighborhood between 2013 and 2015. These sensors recorded consistently higher PM2.5 concentrations than the two nearest federal reference methods (FRM) monitors maintained by Maryland Department of the Environment (MDE) (Kelly et al. 2016). Using QGIS, we created a grid of 1000 x 1000-foot squares across the Curtis Bay/Brooklyn neighborhoods, using the neighborhood polygon file made available through MD iMAP. After removing the squares for which more than half of their area lay within a body of water, 39 squares remained.

**FRM Monitors**

We collected webs in October-November of 2020 at MDE’s two existing FRM monitoring stations which publish results on the elemental composition of the particulate matter they collect. One monitor is in a forested area- Howard University's Beltsville (henceforth referred to as HU Beltsville) campus in Prince George’s County, Maryland. The other is in the parking lot of Essex Senior Center (henceforth referred to as Essex), a suburb of Baltimore City. Webs were collected within a 200 meter buffer of these monitors in order to compare web results to a more established method of measuring elemental concentrations in particulate matter.

**Collection**

In September 2019, we cleared 10 funnel webs from each of the 11 Curtis Bay squares. Originally, there were 14 squares within the Curtis Bay area, but three of the squares were almost entirely contained within a privately owned, gated plot, so we were unable to collect from these three squares. We then returned two weeks later to the described locations to collect five webs from each square during the second round. Many spiders are known to have high site fidelity and quickly remake their webs after they are damaged or destroyed. The newly created webs were collected using clean glass vials and glass rods cleaned with acetone in between each collection and then frozen to kill any organisms that were inadvertently carried in the web.This two-step collection process was designed to create a time-series with a standard length of time in which webs were exposed to ambient PM. In October 2019, we performed the same two-part collection process within 23 squares in the Brooklyn neighborhood, this time over three weeks rather than two. 10 public scientists divided into five teams to collect in these squares. Volunteers including college students, scientists, and lay-people were trained in funnel web identification and collection. Volunteers collected more than 300 funnel webs over three collection days. We obtained second round webs for 31 of the original 39 squares, although some squares had fewer than the desired five webs. Webs collected in the first round were significantly, *t*(29.342)=2.0751, *p*= 0.04684, heavier than those collected in the same squares in the second round. This suggests that volunteers were successfully able to re-identify the locations of the webs they cleared in the first round. We used the same method when collecting webs near the two MDE monitors over two weeks in Fall of 2020, although no first round webs were processed.

**Processing**

After being frozen, the webs were cleaned of organic debris such as leaves or insect parts. Webs were dried at 70 C for 48 hrs, and weighed to a hundredth of a milligram. Webs were processed using methods described by the EPA, originally intended for the analysis of metals in soil (EPA 2006). We combined .5 mL of water with 1 mL of HNO3 to digest the spiderwebs, which were placed in a dry bath at 90°C for two hours. After two hours, .2 mL of water and .3 mL of H2O2 were added, and the mixture was heated for two more hours. After cooling, 1 ml of HCL was added to each sample and then heated at 90°C for 15 minutes. Webs in solution were sent to University of Maryland, Baltimore County’s (UMBC) Molecular Characterization and Analysis Complex (MCAC) to be analyzed using an Inductively Coupled Mass Spectrometer (ICP-MS). Samples were analyzed for parts per billion (ppb) of four of the most common metals found in a six year study of particulate pollution in the Baltimore area: nickel, chromium, iron, and aluminum. (Orozco et. al. 2015). They were also analyzed for lead, which is far less common but of particular concern for human health.

**Statistics**

In order to compare results between webs of different weights and age, the ppb values reported by the MCAC were converted into a percentage of the dry weight of the webs by day. Four of the five metals tested (lead, iron, chromium, and nickel) in the second-round webs had values which were below the level of quantification (BLQ) of the ICP-MS. The majority of the chromium and nickel values were BLQ, so those metals were excluded from the round two analysis. To test whether spiderwebs could be used to detect fine-scale differences in pollution, metal values from webs were compared between sites using Welch's two-sample t-test with Spearman's due to the non-normal distribution of all data. The same tests were performed to compare speciated metal values for fall of 2020 (September to November) from the two MDE FRM monitors.

To test whether the metal content of webs could be spatially correlated with known metal sources, we had to tie each square to known sources.Using QGIS, we found the distance between the centroid of each study square and the TRI sites within a two mile radius of the study site. Maryland’s 2019 Average Annual Daily Traffic (AADT) is available as a line shapefile. Using this, we calculated the mean and maximum AADT for each square based on which roads they intersected. An ANOVA was performed comparing PM2.5 values for the same months from the HU Beltsville and Essex monitors along with values from a FRM monitor in downtown Baltimore which does not report the composition of PM 2.5. All values from FRM monitors were downloaded from the Environmental Protection Agency’s (EPA) Air Quality System Application Programming Index (AQS API). Statistical analysis was done in R.

**Results**

**Baltimore City**

To test whether spiderwebs could be used to detect fine-scale differences in pollution, we performed a Kruskal-Wallis test comparing metal percentages in webs by square. This test showed significant differences between squares in percent lead *H*(30)=64.326, *p*<.001 aluminum *H*(31)=68.253, *p*<.001, and iron, *H*(30)=58.699, *p*=0.001319. A t-test comparing the metal percentages by day between Brooklyn (*M*=0.0022337085, *SD*=0.0045349276) and Curtis Bay (*M*=0.0002960983, *SD*= 0.0001355332) showed that Brooklyn webs had significantly higher iron percentages, *t*(54.093) =3.1673, p=0.002529.

We tested whether the metal content of webs could be spatially correlated with known metal sources. A Spearman’s test of the correlation between the metal percentages in webs grouped by square and known metal sources (distance to TRI’s and AADT values) showed a positive correlation between percent iron and AADT maximum, rs(181341)=0.2254884, p=0.01683 and mean rs(185218)=0.02705, p=0.2089293.

**FRM Monitors**

We tested whether there was a relationship between the differences in monitor speciated metal values and the metals in nearby webs. There was no significant difference between the PM2.5 values in HU Beltsville, Essex, and Baltimore in 2020. Percent iron in PM2.5 in Howard University (*M*=1.467, *SD*=0.9113449) was significantly higher, *t*(40.97)= -3.4391, *p*=.001353, than Essex (*M*=.735, *SD*=0.4843993). We found no other significant differences for percentages or raw values of the relevant metals.

T-tests comparing the webs collected near the monitors showed that percent aluminum was significantly higher, *t*(29.222)=5.5349, *p* < .001, in Essex (*M*=0.0019627497, *SD*=0.0007360513) than in HU Beltsville (*M*=0.0008292952, *SD*=0.0004177085). Percent lead in Essex webs (*M*=0.0004874250, *SD*=0.0005687873) was also significantly higher, *t*(18.553)=2.4158, *p* =0.0262, than in HU Beltsville (*M*=0.0001401478, *SD*=0.0001143231).

**Conclusion**

While we cannot yet correlate any metal other than iron to our known pollution sources, we were able to detect significant differences across squares, and detect many metals in webs that were only two weeks old at most. We were also able to use funnel webs to detect a significant neighborhood level difference between two small neighborhoods right next to one another. The reason for this difference is not yet clear, but it may be linked to the higher traffic in the Brooklyn area and its higher population density, as of the last census.

The Curtis Bay and Brooklyn area has a history of advocacy against environmental injustice, particularly against air pollution. Perhaps most famously, neighborhood advocates succeeded in preventing placement of a new trash incinerator in their neighborhood in 2016 (Fears 2016). As of 2019, Maryland is the state with the highest median income in the country (not including Washington D.C.) (Guzman 2020), but Baltimore is the poorest city and county in the state, with a median income well below both the state and national average, and a poverty rate twice that of the rest of the country (U.S. Census Bureau QuickFacts, 2019). A 2014 study estimated that there are 191 pounds of released toxins per Baltimore city resident, as compared to 47 pounds for the average county resident (Boone et al. 2014). The Curtis Bay/Brooklyn area is an area with many industrial sites that used to have even more heavy industry. The neighborhood has a poverty rate more than 10% above the city’s average, and although Curtis Bay/Brooklyn overall has a higher percentage of white residents than the city average, it also has a higher percentage of latino residents than the city average. The proportion of Black and Latino residents has grown steadily over the last few years (Ahmann 2018) (BCHD 2017). It has often been called the most polluted neighborhood in Baltimore (Fears 2016). The Baltimore City Health Department found that between 2005-2009, the rates of mortality from lung cancer, lower respiratory infections and heart disease were some of the highest in all Baltimore City neighborhoods. In 2017, Curtis Bay/Brooklyn had the third highest rate of death from chronic respiratory infection among 54 Baltimore neighborhoods (BCHD 2017). Curtis Bay and Brooklyn are marginalized communities with a disproportionately high vulnerability to pollution in a city that is already overexposed compared to the rest of the state. Public health data indicates that this community has a higher risk of diseases related to and exacerbated by air pollution, but the risks faced by this community are not well represented in the spatially generalized data available.

It is unclear how the values in webs are related to the values recorded by nearby FRM monitors. Although the generally higher values of metal in Essex webs seems to line up with the higher PM2.5 values, they do not line up well with the metal composition of thatPM2.5. Previous researchers have theorized that metals in webs may be better attributed to PM10 than PM2.5 , as the heavier PM10 falls faster, varies over space more, and is more likely to fall directly on webs, rather than being deposited through precipitation as is much of PM2.5 (Rybak 2015). Unfortunately, PM10 values are only available for Baltimore City and Essex so they cannot be compared to our data. There may be a disconnect in the scale measured by these methods as well. Most FRM monitors intend to measure pollution over an entire county or neighborhood, while webs may be collecting highly local pollutants that are drowned out in the spatially generalized FRM data.

Spiderwebs as biomonitors have potential applications to public science. A group of volunteers of varying backgrounds were able to successfully identify funnel webs after a brief training. Bioindicators have been effectively used to monitor metals at a fine-scale for years. Mosses have been used across Europe to monitor trans-boundary air pollution (Harmens et al. 2004, Harmens et al. 2010). Many of the plants commonly used as bioindicators are only found in certain ecosystems, limiting their applicability for air quality monitoring worldwide (Boquete et al. 2017). Funnel webs are abundant across many environments, and are particularly easy to identify in urban areas that may have little plant life.

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CAN HAVE TWO MORE REFERENCES- Cooper 2021 and Hawn 2018

Figures:  
  
**Figure one**: (a) shows the three study sites in Maryland. HU Beltsville in red, Baltimore City in green, and Essex in peach. (b) shows a zoomed in map of the Baltimore site. Red diamonds mark metal-releasing TRI sites and yellow lines indicate highways. The original 39 squares are in green (Brooklyn) or blue (Curtis Bay) and the 31 included in the final analysis have bold outlines. The TRI information is from the EPA, the highways and Maryland outline from Baltimore Open Data, and the basemap is Stamen Toner Lite.

**Figure two:** A comparison by neighborhood of the mean percent lead (a), aluminum (b), and iron (c) in second round webs. Asterisks indicate a significant difference in means. All percentages are normalized by the number of days between destruction of the first round webs and collection of the second round webs.

**Figure three:** The top bar graph (a) compares the mean PM2.5 reported by FRM air monitors in Essex, HU Beltsville, and Baltimore City. The three leftmost bar graphs compare the mean lead (b), aluminum (c) and iron (d) percent in PM2.5 between FRM monitors in HU Beltsville and Essex. The three rightmost bar graphs compare the mean lead (e), aluminum (f) and iron (g) percent in second round webs collected around those FRM monitors. Asterisks indicate a significant difference in means. All percentages are normalized by the number of days between destruction of the first round webs and collection of the second round webs.