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ABSTRACT

Title of Document: CHIROPTERA AS BIOMONITORS OF HEAVY METAL DISTRIBUTION IN BALTIMORE CITY

Christopher Blume M.S 2023

Directed by: Dr. Dawn Biehler, Dr. Ela-Sita Carpenter, Dr. Chris Hawn, Dr Tamra Mendelson
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Heavy metal contaminants such as arsenic, cadmium, chromium, lead and mercury are often found in urban zones dominated by industry, construction and traffic. However, due to historic trends of racism and inequality, not all communities experience pollution in the same way. Low socioeconomic and communities of color have often borne the brunt of this ecological injustice due to the disenfranchisement and lack of investment in these communities. This burden impacts both the human communities near sources of contamination but also the wildlife species exposed. However, due to their exposure and adjacency to human communities, certain wildlife species can be used as biomonitors providing information about the health of communities and ecosystems. Bats are one such species due to their high trophic level, closeness to human populations, diet and bioaccumulation of contaminants. By sampling their guano from roosting sites, data on the distribution of contaminants and their concentrations can be determined.

Chiroptera as Biomonitors of Heavy Metal Distribution in Baltimore City

By

Christopher Blume

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Table of Contents

Chapter One: Literature Review

Abstract

Heavy metal pollution is a plight that communities in urban areas, like Baltimore City are often forced to deal with. Heavy metal contaminants such as arsenic, cadmium, chromium, lead and mercury are often found in urban zones dominated by industry, construction and traffic. However, due to historic trends of racism and inequality, not all communities experience this issue in the same way. Low socioeconomic and communities of color have often borne the brunt of this ecological injustice due to the disenfranchisement and lack of investment in these communities. This burden impacts both the human communities near sources of contamination but also the wildlife species exposed. However, due to their exposure and adjacency to human communities, certain wildlife species can be used as biomonitors to provide information about the health of our communities and ecosystems. Bats are one such species due to their high trophic level, closeness to human populations, diet and bioaccumulation of contaminants. By sampling their guano from roosting sites, data on the distribution of contaminants and their concentrations this literature review provides the basis and justification for this research project and the observed results.

Introduction

Heavy metal pollution in urban areas can have strong adverse effects on communities near contamination zones (Rodríguez et al 2015). In urban areas like Baltimore City, heavy metal contaminants are continuing to spread due to the increase in anthropogenic changes (Aguilera et al 2022). The demolition of older buildings into vacant lots along with the burning of fossil fuels and trash in industrial areas all result in an amplified spread of these pollutants (Yesilonis et al 2008). However, this contamination burden is not felt the same among different urban communities, with lower socioeconomic and marginalized neighborhoods disproportionately affected by the risks of pollution (Corburn 2017). Urban wildlife populations have a close connection to human communities and are exposed to these risks as well (Somers et al 2004). Therefore, finding ways to track these contaminant movements throughout urban ecosystems is incredibly important to understanding not only who they impact but also the manner in which they are affected.

Certain wildlife species in Baltimore such as native bats can be considered biomonitors and provide researchers with important information about the health of air, soil and water in urban ecosystems. Bats have adapted well to living in close contact with humans in urban settlements and their high trophic level and long lifespan make them a good species of interest as a biomonitor. Heavy metals will bioaccumulate in the tissue of these bats as they encounter pollutants and may provide vital information about the impact of contamination on human and wildlife populations (Chételat et al 2018).

The objective of this review is to provide background on heavy metal pollution in urban environments and how they impact the communities located nearest to these contaminants. Especially noteworthy is the disproportionate influence of contamination felt by communities with certain socioeconomic predictors. I will also justify the use of native wildlife species as biomonitors of urban pollution because of how they are built into the complex dynamic of wildlife-human interactions. These interactions show the close connection that humans and nature have even in human-dominated urban areas, and give information about how anthropogenic changes impact the ecosystem.

Other organisms have previously been investigated for their ability to inform us about the environment. Avian, small mammal and lichen species are all common sources of biomonitoring water, soil, and air quality (Biag 2019). The articles chosen for this review are based on the variety of quantitative methods used to answer similar questions with biomonitor species. This review of literature surrounding the topic of heavy metal contamination provides a vast collection of knowledge that spans various locations globally. Although many of these studies occur in locations or use methods that are not entirely comparable to the parameters of this project, the framing of their questions and how they approached this topic is notable and should be considered.

The sources used for this literature review encompass a wide perspective to investigate the historical framework of environmental injustice in Baltimore City. By examining the literature about the different contributing factors that have developed this system of injustice, a more comprehensive understanding of why heavy metal contaminants have become widespread as well as the specific communities who are most impacted can be created. Changes in city infrastructure, the development and growth of industrial zones and movement of traffic were all investigated as sources of this ecological change and were utilized for this analysis. This largely included examining prior literature on the passage of these contaminants through urban ecosystems and the potential health effects on humans and wildlife species.

The connection between urban wildlife and human communities is an important subject for my research; therefore, I sought information on the biology, ecology, anatomy and physiology of my target wildlife species. For the purpose of this analysis, these topics are interdependent, thus making the connection between them provides a better understanding of the final conclusions.

Urban Pollution and Environmental Racism

Urban areas are defined by the complex relationships between biological, physical and social structures. The uneven distribution of greenspaces, amenities, and wealth in urban areas have left communities divided by structural inequalities (Schell et al 2020). These inequalities manifest themselves in various environmental issues such as the disproportionately high levels of pollution by industrial heavy metals. Urban environments like Baltimore must handle heavy metal pollution from various sources largely due to industrial center growth and from environmental changes such as persistent organic pollutants from transportation corridors, fossil fuel combustion and incineration emissions (Hall et al, 2021).

Furthermore, Baltimore is considered a shrinking city, losing around 35,000 residents from 2010-2020, nearly a 6% decline. This decline stems from systemic disinvestment that began in the 1930s and has continued onwards in majority black neighborhoods based on the recommendations of the Home Owners Loan Corporation (HOLC). As neighborhoods in Baltimore were mapped, locations described as dangerous and therefore not worth investing in were outlined in red, leading to the coining of the term redlining (Huang and Sehgal, 2022). This exodus from communities has left many buildings abandoned and their lots vacant. The commonly used and often cheaper methods of complete demolition of these homes leads to further pollution (Hall et al 2021). For example, when buildings containing lead hazards are demolished, lead dust is released into the surroundings posing a threat to community health (Yesilonis et al 2008). Furthermore, this means of handling older buildings is usually found in lower socioeconomic communities and can expose the community to a larger quantity of harmful contaminants. Prior research has shown that houses that once contained lead paint can serve as environmental health hazards if the lead paint was simply covered with regular paint. This is due to the fact that lead paint can deteriorate as the house ages, elevating the threat of lead-related hazards (Hall et al, 2021).

The importance of this investigation is highlighted by the various health risks associated with prolonged exposure to heavy metal contaminants. Even trace amounts of these metals can be hazardous to humans, making heavy metal pollution a serious environmental problem (Engwa et al 2019). Irreversible damage to cellular organelles, structures and DNA have all been documented as result of contact with these contaminants. These changes in cellular composition can contribute to diseases such as Alzheimer's, cancer as well as neurological, respiratory and kidney diseases (Yin et al 2019). Therefore, finding ways to track these metals and methods of remediation should be a critical field of interest.

The various pathways of contaminant release mean that direct contact is not necessary for these toxins to accumulate and magnify in many organisms. The health consequences seen in wildlife communities affected by mercury and heavy metal contamination was noted as being an "early warning sign of the health risks to humans" (Colborn and Clement 1992). For example, mercury can enter the tissues of organisms through ingestion, inhalation or direct contact with skin. Similarly, there is evidence to support reproductive health effects in animals that consume fish from contaminated waters. In Wisconsin, blood samples obtained from the common loon (*Gavia immer)* found that Mercury levels correlated with a decrease in the upper limits of loon productivity (Burgess & Meyer, 2007).

Investigating the documented accounts of adverse health impacts relating to heavy metal pollution is important for contextualizing the research behind this thesis. For example, studies on the effects of heavy metals released from the mining industry on children's health in Peru show how detrimental these contaminants can be to communities (Piñeiro et al 2021). Health effects from heavy metals like arsenic, copper, lead and zinc were reviewed. The children studied showed a substantial risk of colic, nosebleeds, mood changes and change in skin color, among other symptoms. In some instances, the physical manifestations of children exposed to metal contaminants were increased by 15-fold when compared to unexposed children (Piñeiro et al 2021). These metals are common industrial contaminants often found in urban areas such as Baltimore therefore, investigating potential sources and distribution of these pollutants is critical to the health and safety of Baltimore's communities.

Heavy metal contaminants are only some of the pollutants that the citizens of Baltimore City must constantly contend with. Exposure to atmospheric pollution such as ozone, sulfur oxides and particulate matter are other common hazards that are associated with increased rates of respiratory disease, most specifically exacerbating asthma symptoms in those who have it. In fact, Baltimore City has already been shown to have much higher rates of asthma when compared to the other counties in Maryland, and recent surveys have shown that rates of respiratory illness are highest in Baltimore's poorest counties. In 2010, Baltimore City's asthma-related hospitalizations were 2.2% higher than the state average and three times higher than the national average. Furthermore, when measures of poverty such as median household income were factored into the surveys, it was found that these areas strongly correlated with emergency room visits and asthma hospitalizations. Poverty and poor housing in Baltimore City are considered to be major factors in the impact on asthma for the communities living in these conditions. It is even more unfortunate that atmospheric monitors used to collect and analyze air samples are not located near the source of pollutants or the communities that are most impacted. In some cases, they are purposefully sited away from poorer areas to avoid pollution hotspots (Grainger et al 2019). Some of the poorest communities in south Baltimore, Curtis Bay and Brooklyn, were able to directly link their rates of asthma hospitalization with the emissions from two nearby coal plants. Between 2009-2013 these plants began to use emission-reducing technology to decrease their sulfur oxide by over 37,000 tons and particulate matter pollution by 546 tons. Asthma hospitalizations were in turn reduced by 57% over the same period (Asthma and Air Pollution in Baltimore City, 2017).

A major concern highlighted by this research is that of environmental justice, due to the history of environmental racism and its effect on poor and minority communities. Much of the current population distribution in Baltimore City has been shaped by the historical segregation of minority communities which designated them as hazardous by Homeowner Loan Corporations (Carpenter 2019). The city of Baltimore is an urban area with a long history of environmental injustice and contamination due to legacy pollution from heavy metals and industrial hazards that are disproportionately placed in the communities of people of color (Hall et al 2021).

Examining both historical environmental injustice and differences in socioeconomic groups provides unique information on the impact of not only pollution but also biodiversity, amongst other environmental issues. In Baltimore, studies have explored both ecological legacy effects and more recent socioeconomic factors as drivers of biodiversity and species abundance. A major socioeconomic variable is the abundance and distribution of vacant lots which poses a potential environmental justice issue (Carpenter 2019). For these reasons, Baltimore is an ideal location to investigate how the historical economic and racial unevenness still lingers to this day as well as the long-term impact it has on both the health of the communities and the ecosystem within the city.

Biomonitor Species

Wildlife communities in urban areas can be vital resources for monitoring the movements of heavy metals through ecosystems and have been utilized in this way for decades (Biag 2019) Understanding the complex connections and relationships within the natural world and then using these relationships to monitor community and ecosystem changes due to chemical input can be a powerful tool. As damages from human expansion increase and climate change persists, it becomes essential to find a broad scale network to understand and forecast potential ecological damages on both a local and regional scale (Frontasyeva et al, 2020). A biological monitor is an organism that can provide researchers information about the quality of the surrounding environment. This information can then be used to identify the distribution and intensity of pollution as well as to highlight areas most at risk for potential exposures.

There are certain ecological and biological traits used to determine if a species is an appropriate biomonitor. An ideal choice would be a species that has reliable contact with a contamination source while also having the ability to withstand those environmental changes, which is an important gauge of the general endurance and response of other taxa. For this reason, it is beneficial to find a network of wildlife taxa that respond to changes in their environment and habitat loss stressors in a way that reflects the larger-scale adaptation of local biodiversity. Changes in individual biological processes as a response to environmental factors is also a bioindicator and should be acknowledged. Temperature sensitivity, changes in growth and development and bioaccumulation of pollutants can all vary in individuals based on natural and human based changes in their environment. For example, mosses and lichen species have been considered as important atmospheric biomonitor species since the 1970s because of their ability to uptake trace elements directly from precipitation instead of the soil. Since moss greatly retains precipitation, it is a much cheaper alternative to the traditional collection methods and offers a high density of precipitation data. They are ideal candidates as biomonitors given their widespread abundance and affordable management and may be used for measuring changes in levels of contaminants over time (Frontasyeva et al, 2020).

Insects are often commonly considered biomonitors, used to monitor atmospheric and water pollution. The caddisfly (*Trichoptera, Insecta*) is one well known example of this, as it is often used as a monitor of water quality. Caddisflies are sensitive to changes in levels of dissolved oxygen as well as increases in nutrients in the freshwater in which they live. These changes are often connected to increases in pollution or other environmental stressors. Caddisflies are also very abundant in many aquatic ecosystems and ecologically diverse, thereby making caddisflies strong candidates for bioindicators of freshwater ecosystem health (Thamsenanupap et al 2021).

Mammal species have also become useful biomonitors of soil and groundwater contamination. In fact, a recent study in New Mexico examined the population density of Peromyscus spp. (deer mice) in contaminated zones of former nuclear reactors (Gaukler et al., 2020). The mice in this study were chosen as biomonitors because of their high population density, easy capture rate and high exposure rate to contaminants. This exposure was found to cause growth of abnormal livers and spleens, as well as reduced survival and reproductive rates in mammals. It was therefore hypothesized that lower population densities of deer mice would be seen in polluted areas (Gaukler et al 2020). This project's data concluded that deer mice density was lower in zones contaminated with mercury when compared to non-affected zones. This could in turn lead to lower biodiversity and species abundance in these polluted areas.

Another example highlighting the use of mammals as bioindicators of contamination utilized six populations of short-tailed shrews (*Blarina brevicauda*) along the Housatonic River in Massachusetts as detectors for soil contaminated with Polychlorinated biphenyls (PCBs). These PCBs can have a negative effect on the physiology of mammal species, impacting growth and reproductive rates. These chemicals can bioaccumulate in food chains and can cause biomagnification, so that the levels of toxicity are intensified by the time they reach the species at the top of those chains. The short-tailed shrew was chosen for this study because of its high metabolic rate, high food consumption along with its life history and diet which make this species an ideal bioindicator. Previous studies with Dichlorodiphenyltrichloroethane (DDT) had shown chemical bioaccumulation in *B. brevicauda* had the longest duration and highest levels relative to other small mammals that were tested. The target samples were live captured and marked with sex, size and age measured of the specimen captured. Using capture-recapture techniques and population estimates, researchers were able to estimate the size of the population of shrews and measure the survivability of individuals in the population. Soil samples were analyzed for levels of PCBs and compared over the six population sites. Overall, this study showed that survivability and reproductive rates were good and the population density of *B. brevicauda* were high. Therefore, there was no correlation between the parameters in *B. brevicauda* populations or the contamination of the soil with PCB (Boonstra & Bowman, 2002).

Similarly, studies in Switzerland used red fox (*Vulpes vulpes*) to track differences in heavy metal contaminants between urban, suburban, and rural areas. Foxes were chosen for this study because of their widespread distribution, the sudden increase in their population sizes and their interactions with heavy metals that reflect similar experiences of humans living near the study area (Dip et al, 2000). Red foxes are also well adapted to environmental changes and living in urbanized areas, and they occupy small home ranges, making their capture for the study more convenient. Samples were collected from foxes that were killed from either population control programs or from motor vehicle road accidents. Kidney and liver samples were compared between foxes from each area for their levels of cadmium, lead, copper, and zinc. The results of this research project show that urban fox populations accumulate heavy metal pollutants in their organ tissues that were not detected in the populations of rural and suburban foxes, thereby meaning urban foxes could serve as biomonitors of certain toxins when compared to their rural counterparts (Dip et al, 2000).

The studies described above are examples of how different wildlife species can be used as biomonitors to track the presence and movement of heavy metal contaminants through different types of ecosystems. Biomonitors themselves are indicators of changes in the health of various ecosystems and are beneficial when creating a more comprehensive knowledge base of the various relationships that create ecosystems.

Bat Biology

Bats are mammals of the order Chiroptera, which is the second largest order of mammals, behind only Rodentia*,* with over 1200 different species. Their name Chiroptera in Latin means "hand-wing" referring to their adaptation of forelimbs as wings – making bats the only mammal species capable of true flight. Nearly 70% of bat species are insectivores, feeding at night on insects such as moths, mosquitos and beetles. Many of the other non-insectivore bats are frugivores, feeding on fruits, nectar and flowers. In this way, bats are fundamental to many ecosystems and provide important services like pest control and pollination. More specifically, bats are a vital part of the agricultural industry due to their diet. In fact, it is estimated that because they are such voracious predators of insects, they save the commercial agriculture industry nearly $1 billion per year in cost from pesticide and crop damages(Boyles et al 2011).

Bats can be separated into the categories Yangochiroptera and Yinpterochiroptera. Their size, diet, and means of finding food determine this classification, with Yinpterochiroptera being much larger in size. These bats are mostly fruit-eaters and because their prey is stationary, they no longer have the ability to echolocate. Yangochiroptera species are relatively small and are mostly insectivores. Since their prey are small and mobile, they rely on echolocation, the ability to locate objects based on ultrasonic sounds, transmitted from their mouth or nose and reflected back to their ears, as their means of hunting prey. This ability is important because although bats are not blind as commonly misrepresented, they are nocturnal. Echolocation provides the unique advantage of being able to hunt in complete darkness. By using echolocation, bats can better interpret the distance and size of nearby objects while flying with limited visibility. These ultrasonic calls are usually beyond the hearing range of humans, requiring specialized monitoring devices to interpret them. These calls can be used to identify the different species and behavior of bats as the frequency, pitch and duration of calls vary between species and their activity (Mifsud & Vella, 2019).

Due to their diversity, there is much variation in the life cycle and reproductive habits of bats. However, most species will mate in the fall season, while delaying egg fertilization into early spring. During the winter months, with cooler temperatures and a decrease in insects to feed on, bats will either migrate or hibernate. Hibernating bats will enter a physiological state called torpor, where their body temperature, metabolism and water levels are all regulated.

During this period, bats will use their stored fat reserves as necessary until early spring when they begin to re-emerge. Bats will use a variety of natural structures for hibernation such as caves and the hollow of a tree. Bats in human dominated locations such as cities are known to use vacant buildings and the attics of homes as a hibernaculum as well. These roosting locations serve a variety of purposes for the bats. Roost protects bats from predation, changes in weather conditions, and is a place that bats can use to socialize, reduce parasite loads, digest food and rest between foraging trips (Carter & Feldhamer 2005).

In early spring, usually around March, bats will begin to re-emerge from their winter roosting site and look for these new seasonal roosting sites with ample supplies of insects to feed on. In some cases, female bats will begin roosting with non-reproducing bats and create what is known as a maternity colony. In these colonies, all individuals have a role in the rearing of newly born young. The females give birth to their young in the early to midsummer months depending on their location. Bats reproduce at a slower rate when compared to other mammal species, having fewer reproductive cycles in their lifetimes. Female bats usually produce one or two offspring at a time called pups. Pups will stay in the roost and are fed milk for the first six weeks of their lives. Most pups are usually ready to fly on their own between 4-6 weeks. At this point, the young bats will leave the roost they shared with their parents and attempt to establish their own roosting site. A bat is considered fully mature when they reach somewhere around 1-2 years of age.

Bats as Biomonitors

For this study, local chiropteran species were ideal species to study due to both their biology and their widespread relationships with humans regardless of usual predictors of socioeconomic status such as luxury effects, which hypothesizes a positive relation between wealth and biodiversity (Leong et al., 2018). Except Antarctica, bats are present in every continent, Bats comprise about 20% of mammal species, with the greatest diversity existing in the tropics (Mansour et al 2016). They provide a range of essential ecosystem services including pollination, seed dispersal and insect moderation. Bats are well adapted to living in urban environments, specifically in areas of high human occupation, so many of the disruptions that may negatively affect other species would not have the same impact. This makes studying them less difficult. Where lack of proper habitat and traditional food and water sources can be detrimental to other species, to the point that urban space livability is impossible, different bat species have adapted their behavior to survive in urban areas.

The absolute definition of what comprises a species a biomonitor is still somewhat ambiguous; however, there are some criteria that help define which species make better monitors than others. Having a high trophic level within the ecosystem allows researchers to examine the effects of contaminants as they accumulate within the organisms. Bats, like humans, are at the top of their food chain. These metals bioaccumulate; becoming increasingly concentrated as trophic levels increase (de Souza et al, 2020). The metabolic rate of insectivorous bats is incredibly high and bats need to constantly hunt and prey on insects. This high intake of food increases the bats' exposure and consumption of more heavy metals which provides more information about their bioaccumulation in bats (Zukal et al. 2015).

Another criterion for a suitable monitor species is life span. A long life span allows longer duration studies so that the impacts of the adverse impacts of the pollutants can be followed over time. This also allows multiple generations to be studied and see what the effects of heavy metal contamination are on offspring and how they are passed down (Ferrante et al, 2018). Their long life-span along with their small body size and high food intake means that they can be particularly prone to heavy metal exposure and are, therefore, useful bioindicators to study.

Reliable contact with contaminant sources is necessary to establish a link between the contaminants and the changes in the affected individuals. The insects that make up the main food source of bats emerge from water sources in urban areas. In polluted areas, these toxins will not only spread into local water sources but will also bioaccumulate in these invertebrate communities that will eventually become the food for bats (Zukal et al 2015). Prior research has also concluded that the presence and levels for heavy metals found in the guano in bats is proportional to the presence of heavy metals in the environment (Milan, 1990).

Finally, these heavy metals will accumulate in the adipose tissue of bats. Once the bats have depleted these fat reserves through migration, nursing or hibernation, the adverse effects will begin to manifest in the development of health conditions including reproductive declines, liver and kidney damage. (Zukal et al 2015). This information is interesting to explore further as it could give insight into the timing and duration of bioaccumulation in organisms and their impacts on organisms during different life stages.

However, there are also factors that might limit bat species in their role as bioindicators. Some bat species will travel longer distances for food foraging, which hinders accuracy of geographic distribution. Also, their elusive, nocturnal nature can make steady observations difficult to manage. This can also make recognition of individual die off due to heavy metal toxicity difficult to definitively assess. In urban areas such as Baltimore City, little prior research exists about the diversity of bat species as well as where roosting sites might be located. In addition, the risk of disease from contact with bats such as rabies, although mostly overstated, should still be considered when working around these species (Zukal et al, 2015). Proper precautions such as rabies vaccinations and use of personal protection equipment should be used if considering using bats as a target research species. However overall, wildlife species such as bats can be an extremely useful tool for urban ecotoxicological studies.

Bats have been previously used as biomonitors, making them reliable sources of ecosystem information. Previous studies conducted have been able to find elevated levels of metals in the environments as well as identify a correlation between the concentrations of metal concentrations found in the tissue of bats sampled (Houchens 2020). Bats have been used as biomonitors in studies conducted around the globe, particularly highlighting their versatility and usefulness. One example, a study in Italy from 2018, looked at biomagnification in fur and tissues of bats that were roosting in the Palombara cave system in eastern Sicily. This area is known to be polluted with arsenic, cadmium, lead and mercury due to its proximity to petrochemical plants. Bat populations had declined in the area and changes in population survivability were examined, in large part due to toxic heavy metal contamination. The toxic levels of these bats were compared to bats that lived in another cave system, the Pipistrelli caves, which were unaffected by heavy metal contamination stressors. Data from these bats gave evidence that affected bats (those roosting in these contaminated caves) had higher levels of toxic metal contamination than the bats that lived in unpolluted caves. However, the levels of contamination found in these bat tissues was lower than the overall toxic threshold suggested for small mammals, so these bats cannot be considered contaminated. Also, other potential chemical stressors could not be ruled as having an impact on the decline of these bat populations (Ferrante et al, 2018). Still the methods used for this paper show the value of bats as bioindicators and their use as such so far.

In Egypt, Egyptian tomb bats (*Taphozous perforates*) and lesser mouse tailed bats (*Rhinopoma cystops*) were used to investigate Persistent Organic Pollutants (POPs) and PCBs and their changes in the environment over time (Mansour et al, 2017). PCBs were widely used for the production of electrical equipment as well as for making fluids, like oils, paints and lubricants (*Polychlorinated Biphenyls (PCBs)*, n.d). POPs are used as Organochlorine pesticides for agricultural purposes and can have a high toxicity, easily contaminating the environment and food sources. Both of these contaminants can also result in chronic damage to organisms and humans as they undergo trophic transfer. In addition, for bats, POPs and PCBs pose one of the largest threats to the species and have already been seen as one of the biggest reasons for their population decline (Bayat et al 2014). Their resistance to natural breakdown processes have resulted in traces of POPS and PCB still being found in the tissue of bats years after they were banned. The use of these chemicals is largely banned globally; however, their residues remain in the environment from previous and accidental releases. For this study, two caves were selected for their large population of bats roosting there and their proximity to agricultural fields and sewerage water treatment plants. These water and foraging sources were considered to be in contaminated zones. Since sources such as these are important to the bat populations in the area, it was believed that bats would use these areas and therefore provide useful information on contamination biodistribution. The bats from these caves were collected by mist nets at the entrance of the caves and their livers and kidneys were harvested for contaminant monitoring. The research concluded that POPS and PCBs were still able to be detected in the environment, however the levels of detection were in low concentrations and much lower than the fatality threshold for these chemicals (Mansour et al, 2017). This study once again demonstrates the usefulness of bats as bioindicator species for other studies.

Finally, a study out of the central portion of the United States studied 10 different species of bats to monitor mercury pollution in their foraging home range (Korstian et al, 2017). Mercury was chosen as the subject of this study because of its large distribution, high toxicity and influence on terrestrial and aquatic wildlife as well as human populations. Bats were chosen as the bioindicator because of their high trophic level, high metabolic rate and high population density in the research sites chosen. The locations studied were across the Central United States as well as three sites in Minnesota and two sites in Texas. These were specifically chosen because of their high deposition of mercury, high density of foraging bats and the collection of wind farms sited there. Wind farms are sites of high mortality for foraging bats which causes many wind farms to have bat fatality monitoring programs in place. This allowed the research team to salvage the corpses from the wind farm facilities, giving them an opportunity to collect bat samples without actively capturing or disturbing their roosting sites.

Over the course of the study, five hundred and seventy-six corpses of ten different bat species were collected from these wind facilities. The corpses collected were presumed to be from collision with the wind turbines and the bodies were checked for signs of scavenge from other wildlife before being considered for the study. The exception to this was that many of the bats were scavenged by ants, but the scavenging was contained mostly to the eyes of the corpse, so they were still used for the study. Hair samples of the bats were cleaned and tested for levels of heavy metal contamination. In the two most abundant species, the Eastern red bat (*Lasiurus borealis*) and the Hoary bat (*Lasiurus cinereus*), breast muscle samples were collected as well for mercury analysis. For this study, all the fur samples collected showed ranges of mercury contamination that had been previously seen in bats respective of their species. Furthermore, fur samples showed a higher contamination of mercury when compared to the muscle samples that were collected from the two species. These contamination levels however were under the limits known to have lethal effects in mammal species (Korstian et al, 2017). This is another example of how bat species can be used as bioindicators in future studies. This study also featured the importance of less invasive data collection and in fact, the fur collection method might be shown to provide more complete data than muscle sources. This would need to be tested against other tissue and organ samples for a more comprehensive comparison (Korstian et al, 2017).

Using Bat Guano to Collect Ecotoxicological Data

From their biology, ecological niche, and success of the previous research conducted using bats, I believe that utilizing bats native to Baltimore as biomonitor species is a useful tool in studying heavy metal contamination. They have an established occupancy in urban areas and those with high-density human populations, thus they are known to be exposed to the same contaminants. They also complete the other prerequisites as indicator species based on their diet, longevity, and trophic rank. This research involving toxicological studies with bats is novel to the city of Baltimore. In general, little research into Chiroptera species has been done in Baltimore, and studies that have utilized this species were more interested in species richness, abundance and socioeconomic indicators of bat populations in urban environments. However, in other parts of the world, bats have long been used as bioindicators of toxins – and the Big Brown Bat(*Eptesicus fuscus*), one target species for this research, is one of the most studied species for this purpose. Creating a map utilizing various socioeconomic indicators could be beneficial to identifying potential roosting sites and comparing possible patterns associated with the distribution of contaminants at these locations.

Traditionally, samples from organs such as liver, kidney and brain have been harvested for ecotoxicology research with bats. However, with the decline of bat populations, calls for changes in sampling methods have been made (Zukal et al. 2015). These declines have been attributed to habitat loss, collisions with buildings and wind turbines, rapid urbanization, and significantly from the spread of the fungus, *Pseudogymnoascus destructans*, which causes the deadly and highly contagious white nose syndrome (Ford et al 2011). For this reason, guano sampling is an emerging source of ecotoxicological information because it provides a much less invasive method of collecting contaminant data and is comparable to tissue samples. It is also readily available for collection from roosting sites even without the presence of bats, overall decreasing the stress involved (Mulec, Covington, and Walochnik, 2013). Guano sampling also allows researchers to look at the health of a collective colony in a desired location. Although in some cases it may be more appropriate to connect guano samples in an individual, for this type of study it is more useful to look at the colony (Houchens, 2020). It also provides researchers an easy method of tracking changes in levels of contamination over time by collecting colony samples as opposed to focusing on sampling from an individual.

A previous study in Western Kentucky collected guano samples to examine the contamination of Mercury and other trace metals. The mining of phosphate rocks and minerals for agricultural purposes can expose heavy metals to terrestrial and aquatic systems and impact the wildlife ecosystems in those areas. Gray bat colonies in Crumps cave western Kentucky (*Myotis grisescens*) were the targeted species. Gray bats are endangered species due to factors like urbanization and the rapid spread of white nose syndrome in their roosting sites. For this reason, guano sampling was chosen as the method of collection as it is much more noninvasive than organ collection and less stressful than fur samples. Taking cores from guano mounds in these caves allowed for high quality data while being minimally invasive. Cores were taken from fresh samples on the top because older samples at the bottom of the mounds could be confounded by migrating bats and would reflect samples of heavy metal contaminants from elsewhere. The conclusion of this research shows that heavy metals such as mercury, lead and cadmium can bioaccumulate in wildlife such as bats and those species can be used as monitors and their exposure measured. This study also demonstrated that guano sampling is a viable method of measuring contaminants in bat populations. The bat guano analysis consistently showed a positive correlation between mercury, lead and cadmium in the population of bats sampled and reflected the findings of other mercury and trace metal analyses that had previously been conducted. Research such as this is a positive step for finding less invasive methods of using bats as bioindicator species and useful for the scope of this project as guano sampling will be the primary analysis process (Houchens, 2020).

Even though it is a less invasive method, much of the literature reviewed here discusses some considerations that should be taken when collecting guano from roosting sites. Guano may carry Histoplasmosis or Cryptococcosis spores, which can have negative health implications. Proper protection equipment like gloves and safety glasses should be worn while handling samples. Further, although not directly contacting bats when focusing on guano sample collection, spreading fungal spores of white nose syndrome between roosts is still possible. Mindful handling of samples and properly discarding or cleaning of research material is necessary to prevent accidental exposure of multiple roosting sites to this disease (Houchens, 2020). Finally, when entering the roosting sites of hibernating bats, it is important to understand how vital hibernation is to the life cycle of those species (Boyles et al 2020). Waking bats from hibernation can be disastrous as disturbance during this period causes them to quickly use up their fat stores. With little-to-no food sources available during this time, bats can run out of fat storage and quickly starve. Therefore, taking care to not disturb bats during this time when collecting samples is very important to the health of the roost.

Conclusion

Heavy metal pollution has a real impact on both the communities and urban ecosystems that are exposed to them. In cities like Baltimore, industrial pollution zones and environmental contaminants areas are inherently connected to the race and socio-economic status of nearby communities. Based on many of the environmentally racist practices that plague Baltimore and other cities, many of the communities that are exposed to these contaminants are from minority and lower socioeconomic groups (King et al., 2019). Heavy metal contaminants come from various sources such as agricultural pollution, industrial zones or car emissions from heavy trafficked areas. Atmospheric pollution is also a major problem in cities like Baltimore and disproportionately affects communities that deal with poverty, poor housing and low-income conditions. These contaminants come with numerous detrimental health concerns that make living in these marginalized communities even more difficult.

Wildlife species that live adjacent to humans can provide an ecosystem service and contain information about the detrimental effects of these heavy metals. Finding common wildlife in areas to create a broad-scale image of contaminants in ecosystems is vital to predicting affected communities and protecting those communities against their impacts. Native bat species are common and abundant in urban areas. Their ecological niche can be used to help researchers understand the movement and impact of contaminants in urban settings. Previous research demonstrates that bats are useful tools in gathering data about heavy metal movement throughout urban areas as well as examining the adverse health risk that they may pose to the public. While using species like bats for toxicological studies, consideration should continue to be given to non-invasive methods that provide the necessary data without harming the experimental species population.

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Chapter 2: Acoustic Monitoring for Bat Species Richness in Baltimore City

Abstract

Bats provide many critical ecosystem services that are often overlooked. Although different bat spaces occupy many urban areas, the species richness of urban bats for research is often overlooked. Using acoustic monitoring equipment in Baltimore City, the purpose of this project was to investigate which species are in Baltimore as well as their occupancy and detection probability rate is at different locations in the city. Six species of bats were identified by acoustic monitoring. These six species are the big brown bat *(Eptesicus fuscus),* eastern red bat, *(Lasiurus borealis),* hoary bat *(Lasiurus cinereus*), tri-colored bat (*Perimyotis subflavus*), evening bat (*Nycticeius humeralis*) and the silver haired bat (*Lasionycterus noctivagans).* The big brown bat had the highest occupancy and detection rate of all species at 86.4% and 55.2% respectively. The tricolored bat had the lowest occupancy rate at 4.5% and a occupancy rate of 15.8% The Eastern red bat had an occupancy rate of 40.4% and a Detection rate of 38.5% The Hoary bat had a occupancy rate of 32.5% and a detection rate of 14.9%. The Evening bat had a occupancy rate of 27.6% and a detection rate of 16.5. Finally the silver-haired bat had and occupancy of 27.3 and a detection probability rate of 15.8%

Introduction

Bats play an important role in the health of their ecosystems by providing a variety of ecological services, including pest control and pollination, as well as functioning as biomonitors (Ramírez-Fráncel et al., 2022). These services have aided them with adapting to various environments, in turn, increasing their viable range and species diversity. However, some aspects of bat behavior and biology are not fully understood and, largely due to their nocturnal behavior, observations can be difficult to make. Therefore, it is essential to find methods of tracking and monitoring bats to better understand their behavior, social interactions and population distribution.

Acoustic monitoring is an informative, yet non-invasive way of bat detection and is especially effective due to the variety of behavioral interactions detected through vocalizations. Bats emit pulses of high frequency sounds, known as echolocation. They accomplish this through specialized mouth and nose structures that serve different purposes depending on the situation (Taray et al., 2021). Most often these calls are associated with how bats hunt – sending and receiving the sound pulses to identify nearby insects. However, echolocation can serve other purposes, and can be dependent on the presence or absence of vocalizations as well as the frequency and duration. These all provide different information about bat behavior.

Acoustic surveying of bats is a technique of increasing popularity for identifying bats by species. This method provides a flexible means of sampling since the collection parameters are set prior to installation and do not require the presence of the researcher to collect data over time. The absence of constant human presence and longer duration of data collection allows acoustic monitoring to capture data that would be difficult to obtain otherwise. Acoustic monitoring also creates a standardized way that bats species are surveyed (Hughes et al, 2010). Less commonly employed, but still useful are active sampling monitors, which provide species identifying information at the moment. Both passive and active monitoring are non-invasive methods that do not interfere with the bats behavior, removing interference variation that might be seen otherwise (Voigt et al., n.d.).

The diversity of bat species and their location is often understudied and overlooked in urban areas. Prior research shows that urbanization can prove both beneficial and harmful to the bats with some bat species favoring these locations for foraging and roosting, while others struggle under the burden of habitat loss and pollution (Ford et al 2011, Russo and Ancilotto 2015). Although the impact of urbanization on bats is not fully understood, it is well-documented that bats have vital ecosystem importance. Providing ecosystem services such as pest control and nutrient recycling are necessary to the overall health of the environment in which they are found (Kunz et al., 2011). Therefore, investigating which species of bats are found in urban areas is vital to bridging the gap in the knowledge of urban ecosystems.

In the state of Maryland, ten species of bats have been identified. All ten species existing in Maryland are considered a "Species of Greatest Conservation Need" by the Maryland Department of Natural Resources (*Guide to Maryland's Bats*, n.d.). In the city limits of Baltimore, six of those ten species reside. These are the big brown bat *(Eptesicus fuscus),* eastern red bat, *(Lasiurus borealis),* hoary bat *(Lasiurus cinereus*), tri-colored bat (*Perimyotis subflavus*), evening bat (*Nycticeius humeralis*) and the silver haired bat (*Lasionycterus noctivagans).* The paucity of information on urban bat populations combined with their need for conservation reinforces the necessity of investigating species richness and distribution areas.

The primary objective of this chapter is to define which species are present in Baltimore City and the probability of detecting bats acoustically in an urban environment. With this data, a more comprehensive understanding of bat species richness in urban Baltimore, can then be used to extrapolate information about how bat populations are currently adapting to the pressures of increasing urbanization.

Study Site

Acoustic monitoring was conducted in Baltimore at 41 different sites. These sites varied in the amount of urbanization they represented. Urban farms, greenspaces and parks were sampled along with apartment complexes, business centers and college campuses. Locations were not chosen randomly but instead were chosen based on anecdotal bat sighting by members of the community as well as their ecological potential to provide roosting opportunities. Information from citizen science platforms such as iNaturalist were also used to confirm previous bat sightings in the studied locations.

Materials and Methods

The research was conducted at various sites in Baltimore City and Baltimore County, Maryland. Twenty locations were identified with seventeen of these being within city limits and the remaining five located in the county. These sites were identified based on the habitat that potentially could support bat colonies, the presence of bats that had previously been noticed by members of the community or by first-hand identification by members of the research team.

Bat presence, activity and species identification were further confirmed by installation of acoustic monitoring devices to capture the echolocation calls of bats that were foraging or roosting in these areas. Three Titley Scientific Anabat express passive monitors containing omni-directional ultrasonic microphone US-O V3 and one Titley Scientific Anabat Chorus with a directional ultrasonic microphone US-D were placed at these locations and left for approximately four to ten days to monitor activity. Monitoring began in late March 2022 to capture acoustics from the first bats emerging from hibernation. Monitoring continued throughout the spring and summer and was concluded in the second week of October 2022. Bat activity began to decline at this time as most species began to prepare for hibernation or migration. Three sites were monitored at a time using passive acoustic monitors with a fourth and fifth site monitored using active monitors. Passive devices were set to record in the evenings from 2000-0700 and installed on a nearby tree facing out towards a clearing or an open area to obtain the most precise calls possible and reduce interference noise. These monitors were set at a 30-45 degree angle and locked into position using a steel lockbox to prevent damage or theft while not interfering with the data collection, and a cable lock strap to wrap around the diameter of the tree. After the collection period, the monitors were taken down and moved to the next location to be surveyed. This cycle allowed each location to be surveyed once a month throughout the monitoring period.

Active monitoring was also performed at these locations with Titley Anabat walkabout detectors as well as Wildlife Acoustic Echometer Touch Pro 2 monitoring devices. Echometer Touch Pro 2 requires users to mark their geographic location and the species most likely to be encountered to more accurately identify calls by species. Monitoring either happened alone or with others in a group for approximately four hours from 2000-0000. Call data were logged on a SD card utilized for all of the passive monitoring equipment as well as the Anabat walkabout monitor.

Analysis

Once collected and downloaded from the SD card, each set of echolocation data was analyzed to identify the species and the type of activity (foraging vs. social), if possible. Identifying characteristics of the call such as call duration, minimum frequency, maximum frequency and shape were all used to classify into the species detected . Anabat insight and Anabat tools software was utilized to analyze calls collected by each acoustic monitor. Parameters were set based on known bat vocalizations and extraneous sounds were automatically removed and placed in a separate folder called “Noise”. Therefore, only audio files that were within the range of bat vocalization were analyzed.

Bat vocalizations were manually identified by species using echolocation characteristics that are unique to each species. Characteristics such as minimum and maximum frequency, call duration and shape of call were primarily utilized in determining the species identification (see figure 1). Calls were then added to an excel spreadsheet categorized by location. Acoustic data in the Excel spreadsheet was converted to occupancy data with “1s” denoting presence and “0s” denoting absence. This spreadsheet was then placed in Rstudio where species occupancy and detection probability was determined. The Rstudio package “unmarked” was installed and used for the occupancy and detection modeling. Occupancy and detection probability models were created for each species in total to determine the probability of each species occupying each studied location. Detection probability was used to more accurately determine species occupancy by considering how effectively each species was detected by the monitors. A bayesian detection probability was calculated as an unbiased predictor of the probability of species occupancy as a proportion of monitor detection. Confidence intervals for each species occupancy and detection probability were calculated.

Results

Over 1,200 calls were collected over the 6-month monitoring period at 20 sites around Baltimore City. Six species were detected across each of the 20 sites. *Eptesicus fuscus* was the most prevalent bat species found and was present at all but one of the sites monitored, while *Perimyotis subflavus* was the least common, occurring at only three studied sites. *Lasiurus borealis, Lasiurus cinereus, Lasionycteris noctivagans and P. subflavus* were the other species identified . *E. fuscus* had the highest occupancy probability of 86.4% for the locations that were monitored and a detection probability of 55.2%. Bayesian probability for the overall proportion of sites occupied by *E.fuscus* was 75%. *P. subflavus* was the species that was least detected, having only an occupancy probability of 4.5%, a detection probability of 15.8% and a bayesian probability of 5.1%. *L. borealis* occupancy percentage was 41% with a bayesian probability of 45% and a detection probability of 38.5%. *L. cinereus* occupancy probability was 32.5% with a detection probability rate of 14.9% and a bayesian occupancy probability of 35.8%. *L.noctivagans* had a occupancy probability of 27.3%, a detection probability of 31.1% and a bayesian occupancy probability of 31%. *N. humeralis* occupancy rate was 27.6%, detection probability was 16.5% and bayesian occupancy rate was 30.4%.

The sites monitored varied in terms of their geographic location around the city, type of space (greenspace, high density housing, city center) and type of green spaces (farms, parks and gardens). These categories were then utilized to investigate how different urban environments might impact species richness, detection and occupancy probability. Cylburn Arboretum, a large city park made up of 200 acres of trails and vegetation residing in the northern part of Baltimore City had the highest occupancy rate of 71.4% and detection rate of 49.3%. This location also housed the largest number of identified bat species including: *E.fuscus, L. borealis, L.noctivagans and L. cinereus*, all detected over the monitoring time frame. Rash Field, an urban park situated closest to the urban center west of the Baltimore harbor and surrounded by city shopping centers and apartment complexes. This location had the lowest occupancy at 17.5% and a detection rate of 6.6%. Bliss meadows, an urban community garden in Northeast Baltimore is a mix of greenspace surrounded by residential buildings. This site had a mixed occupancy probability of 57.1% and a detection probability of 52.5% and detected three out of the six Baltimore bat species.

Discussion

The purpose of this study was to investigate the function of acoustic monitoring of bat species in an urban environment. A secondary objective was to investigate the species diversity of bats in Baltimore City, the urban occupancy probability and likelihood of detection using acoustic software. Although bat populations do persevere in urban locations, their diversity and abundance are relatively low. Noise, roost availability and distribution are the major culprits for this lack of diversity (Moretto & Francis, 2017). Thus, investigating the species occupancy and detection rates is essential to understanding their distribution. Identifying bats to the species level by using echolocation takes considerable time and thorough examination. This is especially so as there is some overlap between different species' vocalizations based on factors such as activity and distance to monitor. Fortunately, the six species detected in this study have distinct variation in the metrics of their calls, so it was therefore possible to make accurate identifications based on patterns in their vocalizations.

This study further coincides with previous research – that of the ten species of bats that have been recorded in the state of Maryland, six reside in urban Baltimore City (Carpenter, 2019). Careful consideration was taken to investigate the potential presence of the remaining four species – the Eastern small-footed bat (*Myotis leibii*), little brown bat (*Myotis lucifugus*), Northern long-eared bat (*Myotis septentrionalis*), and Indiana bat (*Myotis sodalis*). However, none of these species were detected at any of the locations during the monitoring period. Of the species that were detected, *E. Fuscus* was the most prevalent and was present at seventeen of the twenty sites monitored. The presence of *E.fuscus* was not limited by type of location, and was detected in both heavily vegetated greenspaces as well as urbanized zones. This finding is consistent with *E. Fuscus* being an urban generalist species that has adapted well to urban environments (Clare et al 2014).

Although woodland and forested habitats are more beneficial environments for *E. fuscus,* it has adapted well to a variety of other habitat types (Gehrt & Chelsvig, 2004). Prior research has shown its ability to successfully roost in buildings in both urban and residential areas, establish multiple roosting sites,move across large urban spaces into foraging habitats and forage for longer periods of time when compared to species like *N. humeralis* (Duchamp 2004). *E. fuscus* has also been shown to thrive in locations where man-made structures are readily available for roosting, which offers more locations to establish day and night roosts, and larger foraging ranges when compared to other species living in cities (Pearce & O'Shea, 2007).

Tree roosting species of bats like *L. cinereus* and *N. humeralis* were detected far less during monitoring sessions likely attributed to their roosting limitations. These species tend to fly shorter distances between their foraging sites and urban greenspaces offer opportunity to set up successful roosting habitats at areas with ample food sources nearby (Carpenter, 2019). Their smaller range of foraging and roosting present less opportunities for them to be monitored acoustically which could be a likely explanation for the reduced species occupancy and detection probability rates, significantly lower when compared to a more generalist species like *E. fuscus.* Locations with the highest species richness were those containing both urban greenspace and residential buildings or structures.

The eastern red bat, *L. borealis,* is a tree roosting migratory species. Although detected less than *E. fuscus*, it was one species that seemed to do well in locations mixed with both greenspaces and residential buildings. *L. borealis* has been observed using streetlamps as foraging locations where it catches small moths as prey (Walters et al 2007). This adaptation in foraging behavior could be one of the main factors influencing its higher occupancy and detection in residential areas, more than some of the other bat species monitored. Landscape patterns have also been shown to have an impact on the activity levels of *L. borealis;* most specifically demonstrated as decreased activity with increasing levels of urbanization (Furlonger et al. 1987).

The hoary bat, *L. cinereus,* was a relatively uncommon species that was acoustically captured at less than half of the sites examined. *L. cinereus* is a solitary roosting species that prefers tree roosts rather than the use of man-made structures (Gehrt & Chelsvig, 2004). *L. cinereus* seems to be sensitive to anthropogenic changes as their populations have also been shown to decline with increasing urbanization, most likely from the negative impact on resources vital to its migratory lifestyle (Kunz et al., 2007). Although less common than other species, hoary bats have been observed using streetlamps as foraging grounds, and have a preference for larger moth species (Hickey et al 1996). Acoustically, the hoary bat has a lower frequency call than the other monitored species which allows for longer distance foraging. Thus, an increased potential for detection of these species should correlate with open spaces and foraging locations with high moth populations(Carpenter 2019).

The tricolored bat, *P. subflavus* was detected least of all the species monitored. This is largely allocated to their preferred habitat which is generally upland and in riparian forested areas (Veilleux et al 2003). Additionally, *P. subflavus* populations have seen significant declines in recent years due to the spread of the fungal disease white nose syndrome (Loeb & Winters, 2022). In Maryland, the *P. subflavus* is listed as a species of “Greatest Conservation Need” and ranked as “Highly State Rare” by the Maryland Department of Natural Resources. As of September 2022, the U.S fish and Wildlife announced their proposal to list the tri-colored bats as endangered under the Endangered Species Act (Tricolored Bat (*Perimyotis Subflavus*) | U.S. Fish & Wildlife Service, 2019). Their preferred habitat that is uncommon in Baltimore combined with their drastic population decline from disease are probable factors for their lack of detection.

The species identified using acoustic monitoring are identical to those identified in prior research studying species richness in Baltimore City (Carpenter 2019). Similarly, none of the *Myotis* species that have been detected in Maryland were captured during the duration of this study. *Myotis* species have been successfully detected in other urban areas due to their highly adaptable nature. However, these species do not roost in man-made structures nor do they forage in small greenspaces like parks and gardens. Instead, these *Myotis* species seem to only be active mainly in the interior portion of forested areas. This offers an explanation for the lack of detection of these species since monitors were placed on habitat edges or in open areas for best audio quality. However, Lights Out Baltimore, a nonprofit project of the Baltimore Birding Club whose goal is to make cities safe for migratory species, found a little brown bat during their recent surveys in downtown Baltimore (USFWS biologist Ela-Sita Carpenter, pers. comm). Future research is needed to confer the possibility of *Myotis* species detection in Baltimore's green spaces particularly focusing on the interior portion of these forested areas.

Further, *Myotis septentrionalis* (Northern long eared bat) and *Myotis lucifugus* (little brown bat) have suffered high mortality due to white nose syndrome in recent years and likely factors heavily into their decline and lack of identification. Finally, *Myotis lucifugus* also suffer mortalities due to car collisions as they attempt to move between greenspaces across trafficked areas (Furlonger et al., 1987). Percent of available canopy cover also contributes to how these species move between patches. Particularly, low canopy cover causes *M. lucifugus* to fly low and closer to traffic as it moves between spaces, which also increases mortality levels. In Baltimore

City, it is unlikely that *Myotis* would use available greenspaces or lots for roosting sites as many of these locations are near high-traffic zones.

Other factors influencing detection rates are the location and method of placement of the monitors at each research site. They were placed between 7-10 ft off the ground, on a man-made structure or tree. When placed on a tree, it was positioned on the edge of forested habitat facing out toward an open field or clearing in the vegetation. This was to allow the omnidirectional microphones the clearest reception of calls without any interference or distortion from calls echoing off nearby rocks and vegetation. Interference has been shown to be limited by the angle with which the monitor is placed with the most successful being a ~ 30–45 degree angle over a location that is unobstructed by foliage or debris (Ford et al, 2011). Using the edge of the habitat for monitor installation has previously been shown to be a predictor of successful monitoring of bat activity. The further a monitor is placed from the forest edge, the less likely bat calls would be captured on the monitors (Gehrt & Chelsvig, 2004).

This study was an opportunity to follow up on previous research investigating which species of bats are occupying Baltimore City and to better understand how acoustic monitoring can be utilized in urban areas. A secondary goal was to gain a more extensive understanding of bat species richness in Baltimore and how these species are adapting to the pressures of urbanization. When used correctly, acoustic monitoring can be a powerful tool to identify local species even in areas dominated by human presence (Macey, 2020, Mifsud & Vella, 2019). The results of this particular study correlated with previous studies, identifying identical bat species, without detecting any novel species. Greenspaces seem to be critical to maintaining species richness as the most consistent bat observations were made in large parks or urban gardens. Generalist species like *E. fuscus* were found to occupy nearly all of the observed locations due to their strong behavioral adaptations in favor of man-made change. For the other species observed, there seems to be some negative impact of urbanization that affects occupancy and detection probability in the green spaces that are available. Future research may prove successful to the detection of these species, focusing on the interior portion of forested areas as well as a longer study duration.

Conclusion

The purpose of this study was to determine the species richness of bats in Baltimore City and their occupancy and detection rates. Six species of bats have been identified to occupy Baltimore City. These six species, the Big Brown, Eastern red, Silver-haired, Tri-colored, Hoary, and Evening bat. The Big Brown bat was the most common species encountered during acoustic monitoring surveys, while the tri-colored bat was observed the least. Although bats are considered urban adaptor species, their populations are still declining. Local farms, gardens and green spaces provide foraging habitat and roosting sites for these bats and increased urbanization and anthropogenic activity are a constant threat to these species. Monitoring and identifying which species are active in Baltimore City and what areas they use for foraging and roosting important to the conservation of these important creatures in our cities.

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Species	Call Duration	Average Fmin	Average Fmax	Visual Appearance
Big Brown Bat	>6ms	26.5kHz	49.4kHz	Steep slope with slight upturn in hook at end
Eastern Red Bat	>7ms	40.2kHz	67.6kHz	Calls at lower frequency flat, almost u-shaped. Sharp upturn at end of call. Bounce in frequency between burst
Evening Bat	>6ms	36.1kHz	63.0kHz	Distinct alternating in frequency range. Mix of high frequency calls and moderate frequency calls with reverse J-shape
Hoary bat	>10ms	19.7kHz	26.0kHz	Lower frequency call with bounce between frequencies. Subtle or pronounced upturn at end of call. Lowest calls in NE United States
Silver Haired bat	<6ms	25.4kHz	41.7kHz	Long uniform sequences. Higher frequency calls are steep with pronounced hooks on end. Lower frequency calls tend to be very flat
Tri-Colored Bat	>7ms	41.3kHz	57.6kHz	Produce long sequences of

	similar calls. Calls are low frequency and flat or steep, high frequency burst

Table 1. Identifying Characteristics for Eastern Bat Species

Figure 1. Acoustic Monitoring Locations in Baltimore City and Baltimore County

Species	**Occupancy**	**Detection**
E. Fuscus	0.864	0.552
L. borealis	0.409	0.385
L. noctivagans	0.273	0.311
P. subflavus	0.045	0.158
L.cinereus	0.325	0.149

N. humeralis	0.276	0.165

Table 2. Overall Species Occupancy and Detection Probability

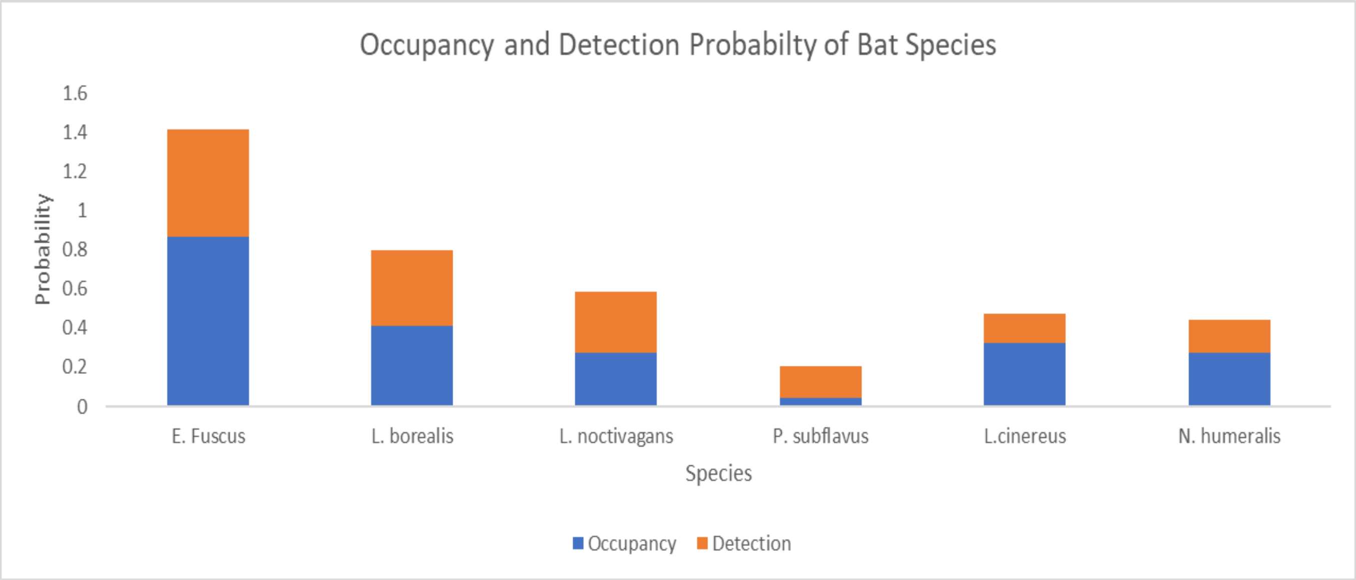


Figure 2. Occupancy and Detection Probability of Bat Species in Baltimore City

Research Sites	Occupancy Probability	Detection Probability
Bliss Meadows	0.571	0.525
Leakin Park	0.286	0.364
Chinquapin Run	0.286	0.357
Clifton Park	0.429	0.292
Cylburn	0.714	0.493
Desoto Park	0.143	0.346
Druid Hill Park	0.429	0.364
Filbert Street	0.429	0.386

Green Mount West	0.143	0.654
Green Street Academy	0.286	0.458
Hidden Harvest	0.143	0.192
Hopkins JHU	0.574	0.281
Irvin Luckman Memorial park	0.286	0.251
Patapsco Park (Avalon)	0.286	0.729
Patuxent	0.714	0.452
Peace Park Irvington	0.143	0.396
Pierce's Park	0.284	0.236
Old York Community Farm	0.143	0.371
Rash Field	0.175	0.065
Stillmeadow	0.571	0.193

Table 3. Occupancy and Detection Probability for Each Research Location

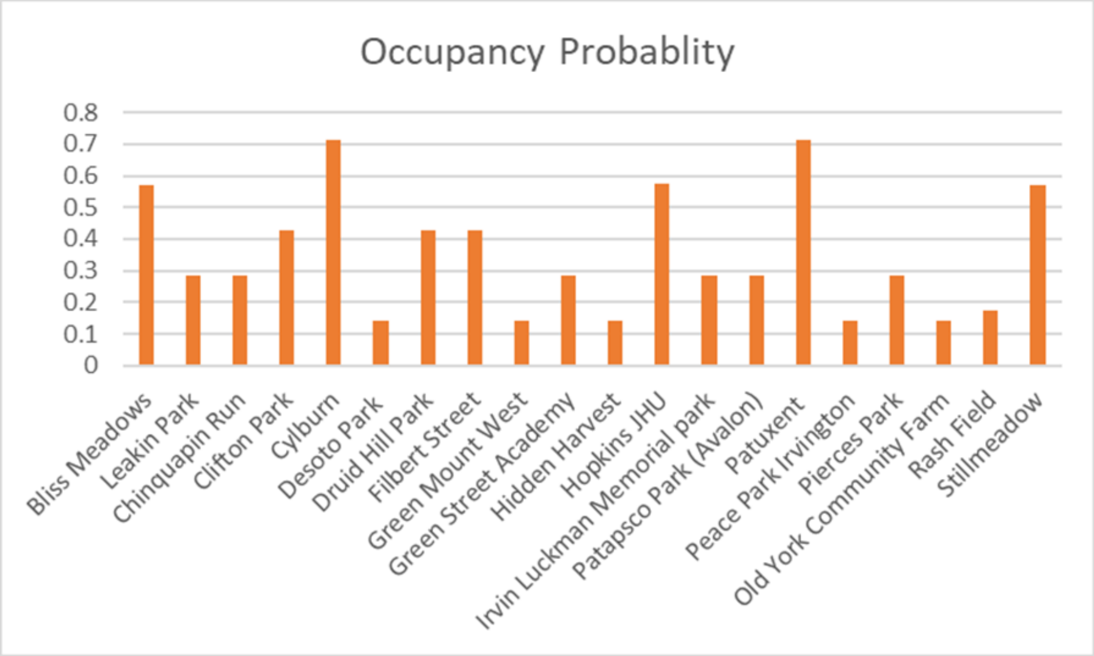


Figure 3. Occupancy Probability of Bat Species per Location Monitored

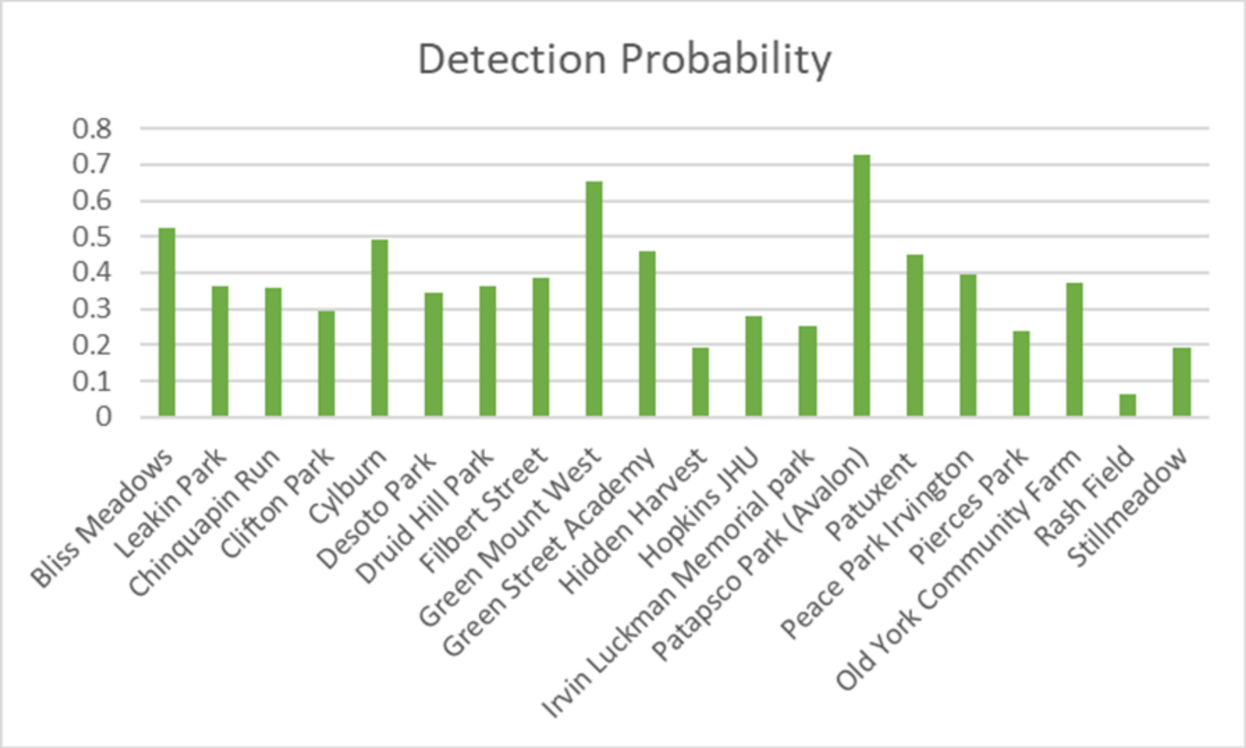


Figure 4. Detection Probability of Bat Species per Location Monitored

Species	March	April	May	June	July	August	September
Big Brown	2.4	2.636364	3.25	3.214286	3.111111	3.05	2.25
Eastern Red	0	0.909091	1.583333	2.071429	1.944444	1.7	1.1
Evening Bat	0	0	0.416667	0.428571	0.833333	0.9	0.7
Hoary Bat	0	0.454545	0.916667	1.785714	1.277778	0.65	0.15
Silver Haired	0	0	0.5	0.642857	0.388889	0.35	0.1
Tricolored	0	0	0	0.071429	0.105263	0	0.05

Table 4. Days of Detection Per Month

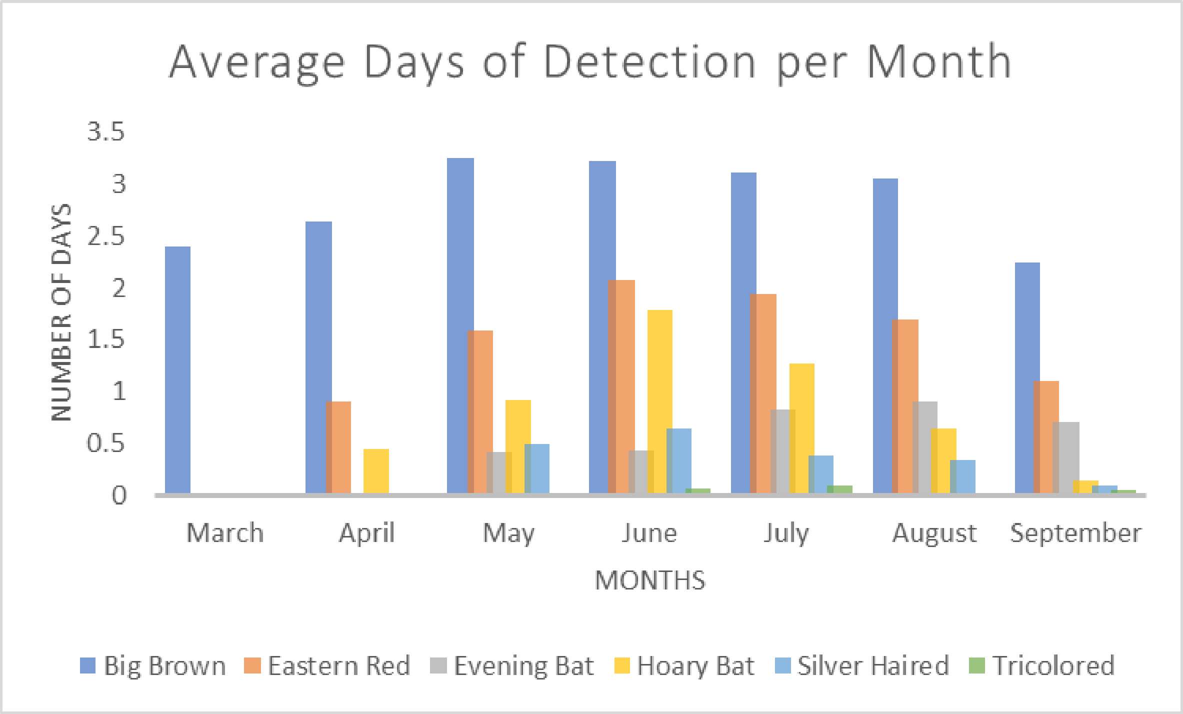


Figure 5. Average Number of Acoustic Detections per Month per Species.

Chapter 3: Using Chiroptera Species as Biomonitors of Heavy Metal Pollution

Abstract

Pollution has an impact on the health and livelihood of communities in urban areas. Heavy metal pollutants such as arsenic, cadmium, chromium lead and mercury are common in urban areas due to increases in traffic, construction and industrial activities. These contaminants not only impact human communities but also can be a burden on wildlife populations. However, certain species such as bats can be biomonitors; organisms used to investigate the overall environmental health. By collecting samples of their guano, bats can inform researchers which contaminants are in the environment and their distribution patterns. Guano samples were taken from 15 roosting sites around Baltimore City to observe if this method of sampling yields toxicological information about these contaminants. Further, concentrations from these samples were compared with census tract data to see if any relationship exists between contaminants and demographics. All five heavy metal contaminants were observed in the guano samples collected, many of them at a concentration above the EPA guidelines of exposure. One of the five metals, cadmium, was shown to have a negative correlation with the income of the communities exposed.

Introduction

Heavy metal pollution is a subject of growing concern in many urban areas. Heavy metals are chemicals toxic to humans and animals that can have adverse effects even in small doses due to their relatively high density. These contaminants can be introduced through a variety of different methods such as increases in industrial, transportation, mining and agricultural activities. Over time, increases in pollutants can reach urban communities and have a direct affect on the health and livelihood of the people exposed. Some heavy metals such as Arsenic, chromium, cadmium, lead and mercury are noted in particular because of the dangers they pose. Human communities are not the only ones at risk to these contaminants as wildlife populations in and around urban areas are often also exposed to them. Therefore, finding the distribution of these contaminants and exposed populations is vital to understanding and improving both the health of our communities and our environment.

Arsenic is a nearly odorless and tasteless metallic element that is a threat to the general public through contaminated water, soil and most often food products. Seafood, rice, mushrooms and poultry are the most common food sources of arsenic contamination. In the environment, arsenic occurs from weathering processes, mining and volcanic activity (Kuivenhoven et al 2022). From an industrial perspective, arsenic is used to make paints, fungicides, insecticides, pesticides, herbicides and wood preservatives (Ratnaike, 2003). Contact and absorption of arsenic has been linked with potential carcinogenesis such as kidney, bladder and lung cancer. Further, arsenic has been associated with cardiomyopathy, hypotension and peripheral neuropathy – a weakness and pain in the hands and feet (Cohen et al., 2006).

Cadmium is a non-essential, naturally occurring pollutant that comes from agricultural and industrial sources that causes adverse health effects in both humans and animals (Aguilera et al 2022). The burning of fossil fuels, use of fertilizers and inhalation of dust from contaminated soils are all routes of exposure. Cadmium absorption typically occurs through inhalation but can also be absorbed through the gastrointestinal tract and in rare cases, through skin contact. Environmental cadmium exposure has been connected to various cancers such as breast, lung, prostate and kidney (Tinkov et al., 2018). Further, in humans, cadmium has been shown to be excreted from the body over time in saliva, urine and milk during lactation (Satarug, 2018).

Chromium is a metallic element that is considered an important macronutrient for bodily function, but becomes carcinogenic at higher concentrations (Pavesi & Moreira, 2020). Exposure to naturally occurring chromium rarely poses a threat to human health as it occurs in levels under the hazardous threshold. However, the danger to the general population occurs when chromium is derived from the combustion of fossil fuels, industrial wastewater, the incorrect disposal of industrial waste and the manufacturing of concrete (Welling et al 2015). The most common methods of exposure to humans are through inhalation and oral ingestion with contaminated water sources. Therefore, chromium toxicity is especially a threat to workers in construction and manufacturing occupations (IARC 1990).

Lead is considered one of the oldest occupational toxins; in fact, lead poisoning dates as far back as 370 BC (Gidlow, 2015). Human exposures typically occur in occupational environments from leaded gasoline, smelting lead materials and the use of leaded paint and tools. Although in many countries the use of lead has been discontinued, it is still employed in industries such as car repair and recycling (Wani et al., 2015). Lead is often found in the environment from the mismanagement of discarded industrial waste and the burning of waste products and combustion of fossil fuels. Ingestion, inhalation and direct contact to these lead contaminants can all result in various levels of toxicity, which has been linked with many pathologies. Reproductive toxicity can include infertility, low libido and miscarriage. Neurotoxic effects from lead exposure include irritability, agitation, headaches, confusion, ataxia, drowsiness, and at higher levels – convulsions and coma (Sachdeva et al 2018). There is also evidence of lead as a carcinogen specifically with renal and lung cancers (Wynant et al 2013).

Mercury is among the heaviest of metals and often considered to be one of the most toxic. Environmental mercury is rare, as it is often found in areas with volcanic or geothermal activity. However, in industrial settings it is more common, often found in the combustion of fossil fuels, incineration of waste materials and the use of batteries and incandescent light bulbs. The mismanagement of waste has also resulted in mercury contamination in aquatic ecosystems (Guzzi et al 2006). Once introduced, mercury is often absorbed by fish and shellfish and creates a health hazard to people whose diet relies heavily on those food sources. Other methods of exposure, and subsequent toxicity, are through skin contact and inhalation. Exposures to mercury can lead to acute or chronic symptoms of coughing, fever, tremors, insomnia and neurocognitive disorders and have been found to trigger autoimmune processes (Guzzi and La Porta 2008, Schiraldi and Monestier 2009).

Utilizing biomonitor species, information about toxic exposures can be gathered to determine the overall health of the ecosystem. Bats are especially valuable bioindicators of heavy metal contamination due to their large geographic distribution, high metabolic intake of insects and their high trophic levels. Concentrations of heavy metals in bats have been shown to increase linearly in the presence of heavy traffic and industrial zones (Aguilera et al 2022). Additionally, insectivore species tend to have higher levels of heavy metal accumulation when compared to herbivores in the same environments, since insects often breed in contaminated water and soil (Houchens, 2020, Zukal et al., 2015). These metals are known to bioaccumulate over time, increasing in concentration in the tissues and organs of bats. Previously, toxicology research has utilized samples of tissue from organs such as the brain, liver and kidneys; however, bat guano is a non-invasive form of collection and provides much of the same information. Bat guano can reveal contaminant types, concentrations and potential sources of exposure (Zukal et al 2015). With bat populations declining due to disease and habitat loss, it is vital to utilize non-invasive methods that provide data comparable to previously used destructive sampling (Russo and Ancilotto 2015).

Although some studies have investigated the effects of heavy metals on bat health and population change, there is limited information on this topic. Moreso, there is even less evidence about the connection between these contaminants and the health of humans, bats and other wildlife. Because bats and humans can often live adjacent to one another in urban communities, the results of this study can be extrapolated to human exposure; however, it should be noted that the differences in bat and human health may make comparisons limited. Further research on this topic may be useful in determining exactly how the exposures compare; however, the information gathered from this study remains an effective tool in exploring the levels of heavy metal contamination in the population of Baltimore City.

Study Site

Monitoring for bat activity was done across 40 different locations in Baltimore City and County, with 22 of those sites successfully confirming the presence of bats. Further, 15 out of these 22 sites provided guano samples for testing. These roosting sites varied in their foundation, from those with less human interactions, such as abandoned or unmaintained man-made structures and lots, to others within the populated community. These roosting sites consisted of bat boxes hung at parks, gardens and farms, placed on the side of buildings and poles. 10 of the 15 locations were specifically within the Baltimore city boundaries while the other 5 sites were at wildlife refuges and parks outside of city limits. Samples from these locations were collected to be used as standards to compare heavy metal data between urban and rural zones.

Materials and Methods

The locations utilized for this study were specifically chosen based on bat activity. Prior to placing bat boxes or selecting a site, the area was scouted for bat activity using acoustic monitors to identify species and amount of activity. Once a location had been identified as having bat activity, possible roosting sites were searched for the presence of guano. If a roost was found, then guano samples were collected using a pair of stainless steel tweezers into a 2 mL glass vial. Each sample was marked with the corresponding site , the date of collection and local species presence, if any were identified in the area. The samples were refrigerated in the laboratory until utilized for further analysis.

To prepare the samples for analysis, each sample was removed from its glass vial and weighed.. Approximately 100 mg of each sample was used for chemical digestion, while the rest of the sample remained refrigerated. Guano was then placed in a 2 mL centrifuge tube along with 500 µL of nitric Acid (HNO_3) and 500 µL of DI water, and covered with a watch glass. Samples were then placed in a dry bath set to 95 degrees celsius for a total of 15 minutes. After this time, the samples were given 5 minutes to cool down, at which time an additional 500µL of Nitric Acid was added to the centrifuge tube and the samples were placed in the dry bath at 95 degrees celsius for 2 ½ hours.

After that time, samples were removed and placed on a vial stand for cooling. After samples were cooled, 200 µL trace select water was added, followed by 300 µL hydrogen peroxide (H_2O_2). The samples were then placed back in the dry bath, set to 95 degrees celsius for 2 hours. While the samples digested in the dry bath, a mixture of 700 µL of deionized water, 1 mL of Hydrochloric Acid (HCL), 300 µL of hydrogen peroxide and 1 mL of Nitric Acid were combined to create a diluted aqua regia mixture. After 2 hours, the diluted aqua regia was added to each sample, to fill the 2 mL centrifuge tubes. After each 2 mL vial was filled, they were divided in half between an identical tube. 1 mL of digestate was removed from the original centrifuge tube and placed in the second tube and 500 µL of HCL was added into both. The samples were then placed back in the dry bath for 15 minutes at 95 degrees celsius. After this time, the samples were removed, allowed to cool and combined into a 15 mL tube. Samples were marked with identification information and sent to the University of Maryland Baltimore County's Molecular Characterization and Analysis Complex (MCAC) for MS-ICP analysis of heavy metals.

An external calibration curve was created using Mercury Atomic Spectroscopy Standard and Multi-Element Calibration Standard 3. For this, calibration points of 1, 5, 10, 50, 100, 250, 500, 750 and 1000 ppb were created by serial dilution using 6% aqua regia as the diluent. Analysis for the elements was conducted using the PerkinElmer NexION300D ICP mass spectrometer, equipped with a PerkinElmer S10 autosampler. Calibration standards were run immediately prior to client samples, with two blank runs of 6% aqua regia spiked with 200 parts per billion (ppb) Au between standards and client samples. Analysis method included 40 sweeps per reading with 1 reading per replicate. All sample analysis was conducted in triplicate and averaged. This data was then placed in an Excel spreadsheet where concentration in ppb was converted to parts per million (ppm) for the mass of guano measured and mass of heavy metals was calculated for each total sample. An T test was run between the different contaminants to determine if any relationship exists between the heavy metals at each location. Additionally, demographic information like mean household income was collected from the United States Census Bureau to investigate connections between levels of contaminants observed and socioeconomic status of communities in near guano collection sites. . An Anova test was run in

Rstudio with this data to compare relationships between concentration levels of heavy metals observed with the mean household income (RStudio Team, 2023).

Results

A total of 32 guano samples were taken from 15 locations between Baltimore City and Baltimore County. Concentrations were received in ppb (Table 1) and converted to ppm n (Table 2) for comparison with published data in ppm. . Levels of arsenic, cadmium, chromium, lead and mercury were compared to the permissible exposure limits in place by the United States Environmental Protection Agency (EPA). Guano samples from 6 out of the 15 sites had arsenic levels greater than the 10 ppb quantitation threshold defined by the MCAC protocols. The EPA's exposure limit for arsenic is 0.5 ppm for food sources such as poultry and 2 ppm for other food sources. Arsenic exposure levels in water sources are anything greater than 0.01 ppm (*Arsenic Toxicity*, 2021). Three sites at Patapsco State Park had arsenic levels of 8.8 ppm, 7.2 ppm and 6.9 ppm. Irvin Luckman Memorial Park, a small public green space in Northern Baltimore that was sampled in June, July and August 2023, had arsenic levels of 6.4 ppm, 6.6 ppm and 10.0 ppm respectively. Green Street Academy, a public charter school in West Baltimore that was sampled in July 2023, had arsenic values of 10.5 ppm. Samples collected at the Patuxent Wildlife Refuge in August tested for arsenic at a level of 10.0 ppm.

For cadmium exposure, the EPA set a guideline of 0.1 ppm for vegetables and cereals, 0.2 ppm for poultry and animal meat, 2.0 ppm for seafood and 0.005 ppm for water (*Cadmium Toxicity*, 2021). Guano from 13 of the 15 sites tested for cadmium levels above the 1.0 ppm level of quantitation. Guano was collected at 3 different roosting sites at Patapsco State Park on two separate occasions. Guano from the two of the roosting sites was collected in July and September

2023 and produced levels of 1.2 ppm and 1.6 ppm, respectively. Cadmium levels for the other 10 sites ranged between 0.3 ppm and 0.9 ppm.

Chromium is a trace metal that is often found in many food sources such as meats, grains, fruits and vegetables. However, the amount of chromium in these food products varies greatly depending on soil, water conditions along with the agricultural and manufacturing processes (*Office of Dietary Supplements - Chromium*, n.d.). The EPA sets the exposure limits of chromium at 0.1 ppm for drinking water and 1.5 ppm for food sources. The level of quantitation for the MCAC's ICP-MS used to analyze the guano samples is 1.0 ppb. Guano from 14 out of 15 sampled sites had chromium levels that were above the ICP-MS levels of quantitation. Guano samples from Leakin Park, the largest woodland park on the east coast consisting of over 1,200 acres, had the highest concentration of over 11.2 ppm. One of the lowest Chromium levels found was at Stillmeadow Peace park, which had chromium levels at 2.0 ppm of chromium. The only site where chromium levels were below the range of quantitation was in guano samples taken in July 2023 from Druid Hill Park, a 740 acre greenspace in Northern Baltimore.

Exposures to lead from air, soil and water often occur due to natural occurrence or from anthropogenic activities; therefore the EPA sets a standard of 5 ppb for bottled water, 15 ppb for tap water and a maximum threshold of 0.1 ppm for food sources. The level of quantitation for lead analyzed by UMBC's MCAC ICP-MS is 0.1 ppb. Of the 15 sites used for guano collection, five sites had lead samples above the levels of quantitation. Each of those five sites, which were Stillmeadow Peace Park, Leakin Park, Filbert Street Community Garden, Old York Community Farm, and 1 of the 3 roosting sites at Patapsco State Park, all had Lead concentrations of greater than 0.1 ppm. The concentrations of the other eleven sites used for guano sampling were below the level of quantitation.

Mercury is a naturally occurring metal that is present in many environments. Mercury, in its inorganic form is called Methylmercury and can be found in air, water and soil where it binds to proteins of organisms and then accumulates. Therefore, EPA guidelines for mercury exposures is 1.0 ppm for food such as seafood, and 0.002 ppm for water. For UMBCs's ICP-MS used to analyze the guano samples collected, the level of quantitation was 0.5 ppb. Of the 15 sites sampled, two sites contained samples above the level of quantitation. One site sampled from Patapsco State Park had a level of 0.4 ppm. The second site, Green Street Academy, had mercury readings at 1.1 ppm. Samples collected from the other 13 locations were below the 0.5 ppb level of quantitation of the ICP-MS.

Two sample sites, Green Street Academy and Patapsco: Avalon had guano that contained all five of the tested heavy metals. The mean concentration for all five heavy metals at each location was taken for comparison (Table 3). The overall median concentration for each contaminant (Table 4) was used to mitigate the amount of skewed data from the outliers in the data set.. This median concentration was then compared to the EPA thresholds provided. Median concentrations of arsenic and chromium exceeded both the thresholds for food and water sources. Cadmium was above the thresholds for most food sources and water but below the guidelines for seafood. Median concentrations for mercury were below the EPA threshold for seafood but above the guidelines for water. Lead concentration was below all EPA guidelines provided.

Data from the U.S Census Bureau was used to collect information on population demographics for comparison with heavy metal concentrations found in different census tracts. Mean annual income was utilized as a means of comparison between the community's socioeconomic status and the contaminants found. A one-way Anova test was performed in

Rstudio to compare the mean concentrations of contaminants found with mean income for all the sampled sites (Table 5). Cadmium was the only heavy metal with a significant ($p = 0.031$) relationship between its concentration and the community's mean annual income. This relationship was negative with cadmium concentration levels increasing as mean annual household income decreased.

Next, heavy metal concentrations were compared with one another to see if there was any relationship between contaminants at locations. A one-way T test was completed between two metals at a time to evaluate for any relationships. For each pair of heavy metals compared, there was no significant relationship found ($p > 0.05$); therefore, the null hypothesis (no relationship between heavy metals) was not rejected (Table 6).

Discussion

The purpose of this study was to explore the possibility of using guano samples from bat species to monitor the distribution of heavy metals contaminants in an urban area. Analyzing the diets of the bats in Baltimore City as well as their behavior, roosting sites and the surrounding environmental factors all provide insight into the observed results. No current measurement of heavy metal concentrations in bat guano and comparing of observed values with environmental concentrations of these contaminants has been created. This information would provide valuable comparative data for assessing contaminants not only in the ecosystem but also its effects on wildlife that are exposed. My aim is that the data provided could be used to provide a foundation for this type of research.

Arsenic itself has many industrial utilizations including the processing of pressurized and preserved wood. Of the five sites that had arsenic levels found in the guano sampled, three of these sites were at Patapsco State Park and one site at Patuxent Research Refuge. Four of the roosting sites sampled were found in old, picnic pavilions and structures built with pressurized wood. Further, these structures were also the breeding and roosting sites for various insects such as crane flies (*Tipula paludosa*) and mosquitos (Culicidae) which make up a large part of the diet of bats(Schnitzler et al., 1983). These specific roosting site factors as well as nearby prey likely account for the arsenic levels found in these guano samples.

Guano samples from the Green Street Academy had an arsenic concentration of 10.5 ppm. Guano from this location was collected from a bat box made of untreated pine wood, which is unlikely to be a source of contamination. Running directly to the East of the Academy is the Gwynns Falls Stream, a 25-mile long body of water that passes through Baltimore City and Baltimore County before emptying into the middle branch of the Patapsco River. Trash, sewerage and pollution in the Gwynns Falls has been documented by previous studies, detecting heavy metals such as chromium, nickel, cadmium, lead and zinc (Kaushal et al., 2011). Given the nearby location, the bats at these study sites are likely using this nearby stream for drinking as well as a foraging site for insects. These insects, such as mosquitoes, additionally use this contaminated stream as a breeding site – typically utilizing the pools of water trapped within the trash along the stream. It may be beneficial to obtain samples of the water and soil from these sites as well as from the wooden structures used as roosting sites in future research. This could provide useful context for how significantly these environmental factors contribute to the results observed.

Cadmium was one of the most common contaminants found in guano samples, appearing in 13 of the 15 locations used. All of the samples were higher than the 0.1-0.2 ppm EPA regulation for food and the 0.005 ppm regulation for water. However, all samples were also below the EPA regulation of 2.0 ppm used to monitor cadmium concentrations in seafood. The highest concentration of cadmium was 1.6 ppm found in samples obtained in July 2022 at Patapsco State Park. Chromium was the most prevalent contaminant found during the sampling period, occurring at 14 of the 15 sampled sites. All specimens collected were beyond the 0.1 ppm threshold for water and 1.5ppm for food. The lowest concentration of chromium in guano was 2.0 ppm found at Stillmeadow Peace Park in West Baltimore. The highest concentration was 11.7 ppm found at Leakin Park, which is a location situated between both the Gwynns Falls Stream and several local roads, all acting as potential entryways for contaminating the local environment.

Mercury was the least prevalent heavy metal found, only occurring at two locations. Both sites, Green Street Academy and Patapsco State Park had mercury concentrations above the 0.002 ppm EPA limit for water but were either below or at the 1.0 ppm threshold for food sources. These concentrations could provide insight into the environmental source of contamination and whether exposure is coming from a water source or one of the bats' food sources. Concentrations found at Green Street Academy was 1.1 ppm and concentrations at Patapsco was at 0.4 ppm. Samples from the other sites were all below the 0.5 ppb level of quantitation for the ICP-MS used.

Collecting soil and water samples from each field site would provide valuable data for comparison to the guano obtained from bat roosting sites. Further, sampling insects from these locations could provide information on the diversity of prey available to nearby bats and could provide more information about the process of contaminants moving through trophic levels. It could also give insight about the potential sources of contamination based on the breeding habits of the insects commonly found in the diet of bats.

Specific identification of the bat species encountered at each field site could also yield information on the rate and impact of heavy metal bioaccumulation within each unique species. Furthermore, temporal information could also provide insight about the impacts of seasonality on the accumulation of heavy metal contaminants. Big Brown, Tri-colored and Eastern Red bats, in most cases, all hibernate during the winter months, re-emerging in the early spring (Dunbar & Tomasi, 2006, Perry & Jordan, 2020, Sullivan et al., 2022). Collecting guano samples starting at their reemergence and continuing through their active season until hibernation would yield valuable data on trends of heavy metal exposure and bioaccumulation in bats. Increases in heavy metal levels over time could be used to investigate bioaccumulation and rate of biomagnification as exposure occurs over time. Decreases during seasonal migration and hibernation would inform researchers about how these contaminants are expelled over time and how they store in the fat reserves of mammals. However, the timeline for that investigation was outside the realm of this project.

The other three bat species found – Hoary, Silver-haired and Evening bats – all migrate to warmer climates during the colder months (Fraser et al., 2017, Geluso et al., 2008, Valdez & Cryan, 2009). Prior to migration, these species will begin to create fat stores for their trip ahead. Heavy metals tend to accumulate in adipose tissue of organisms, thus an increase in fat stores likely results in a respective increase in the amount of metal concentration within the individual bat. (Speakman and Thomas, 2003, Zukal et al 2015). Future research is needed to collect guano samples prior to migration and early in the spring, when bats make their return trip to Baltimore, as a valuable tool in investigating toxicological trends related to migration.

Another possible avenue of future research is to explore the impact of pregnancy on the accumulation of heavy metals in bat species. Breeding for bats typically occurs in the early fall months, and females store the sperm for delayed fertilization that takes place in the spring (Willis et al., 2006). As with migration, females will begin to increase their food intake and build fat stores for their pregnancy. Birth takes place in the late spring or early summer months. Not only can this increase in food intake affect heavy metal accumulation in exposed bats, but may have devastating consequences for their offspring. This is especially critical as large concentrated doses of lipophilic contaminants are transferred to offspring in milk (Kurta et al., 1989, Zukal et al, 2015). Investigating changes in heavy metal accumulation in bat species due to pregnancy was outside the scope of the project present but can be a worthwhile endeavor for future projects.

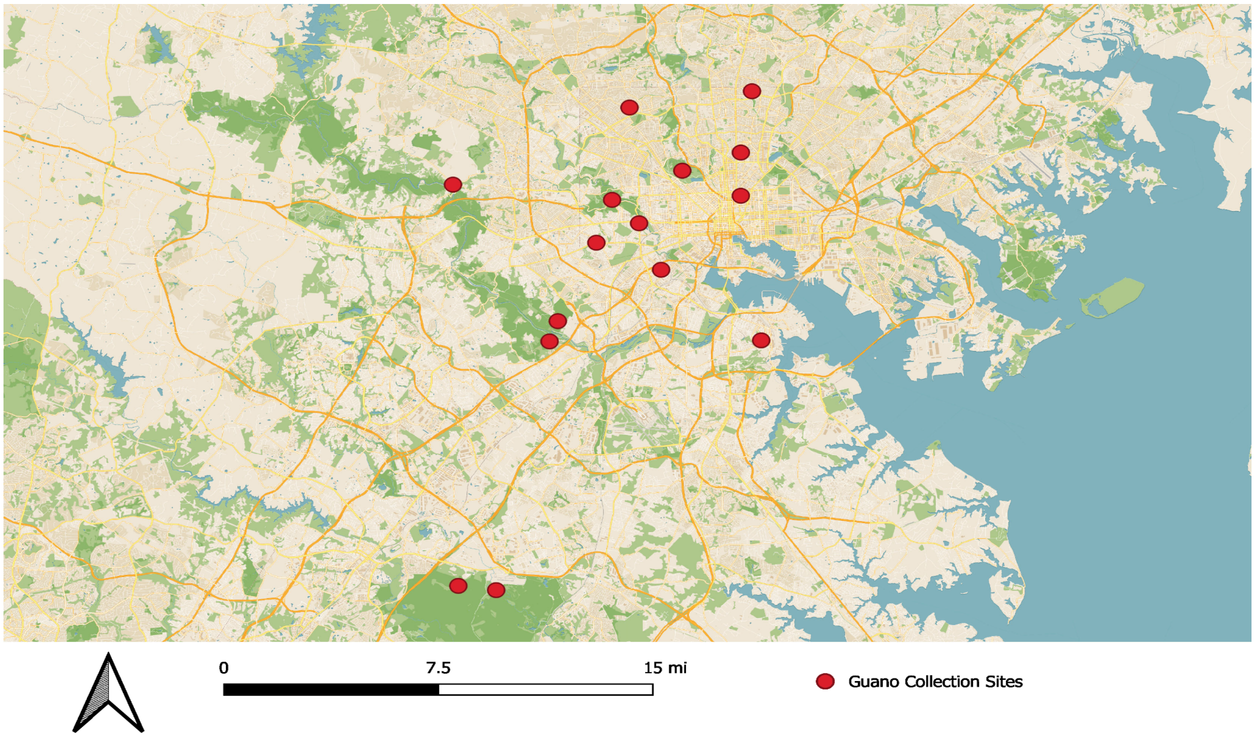
For the purpose of this study, all six species of bats in Baltimore were considered to have similar rates of heavy metal accumulation as well as similar rates of exertion of contaminants in their guano. However, the metabolism of these contaminants may vary from species-to-species, which may impact the concentrations found in their guano samples. This is another topic that may be investigated in further research. Further, 14 of the 15 roosting sites used for this study were confirmed occupied by brown brown bats. One of my research locations, Patapsco: Hollofield, was occupied by silver-haired bats. Each location was examined and the species roosting within was identified multiple times. For these reasons, I feel certain that my guano was coming from the roosting species that was identified.

Finally, guano collection is a non-invasive method of collecting toxicological data about the environment. Often tissue samples are used for this purpose (Zukal et al 2015). Comparing the concentrations of heavy metal contaminants collected to tissue samples, such as wing punctures from roosting bats can provide information about how similar accumulation and magnification data is between the two mediums.

Conclusion

In conclusion, the purpose of this research was to see if heavy metal contaminants could be analyzed to investigate the distribution and concentration of heavy metal in urban areas. Prior research into heavy metal contaminants has yielded that guano can be used to detect concentrations of cadmium, chromium, lead nickel and mercury (Houchens, 2020, Zukal et al., 2015). Arsenic concentrations have not previously been investigated using guano, making this study unique in that way Further, other studies were not conducted on bat populations occupying heavily urbanized areas where exposures to these contaminants would be much higher. I further hypothesized that lower socioeconomic communities deal with the burden of contamination more than affluent communities. Guano samples collected from bat roosting locations around Baltimore City showed evidence of heavy metal contamination for the five contaminants being analyzed for. Additionally, data reflected a negative relationship between the concentration of levels of cadmium and mean household income for sampled sites. For the other four metals, the analysis showed no relationship between income level and the concentrations of heavy metals found. When comparing concentrations of heavy metals to one another, cadmium showed a positive correlation with levels of arsenic and chromium. However, this interaction was not shared with the other metals in the samples collected. Overall, this project confirms the ability of bat guano to be an non-invasive viable source of collecting information about heavy metals contamination and its distribution in urban environments.

Tables and Figures



Map 1: Guano Collection Sites

	Concentration (ppb)				
Location ID	**As**	**Cd**	**Cr**	**Hg**	**Pb**
Chinquapin Run Park (June)	BLQ	BLQ	190.7	BLQ	1.3
Chinquapin Run Park (July)	BLQ	11.5	146.3	BLQ	BLQ
Chinquapin Run Park (August)	BLQ	8.8	150.1	BLQ	BLQ
Desoto Park (July)	BLQ	BLQ	122.7	BLQ	0.8
Desoto Park (August)	BLQ	BLQ	161.1	BLQ	1.3
Druid Hill Park (July)	BLQ	12.2	BLQ	BLQ	BLQ
Druid Hill Park (August)	BLQ	15.2	101.9	BLQ	BLQ
Filbert Street Community Garden (May)	BLQ	BLQ	132.4	BLQ	1.2
Filbert Street Community Garden (June)	BLQ	BLQ	139.6	BLQ	1.3
Filbert Street Community Garden (July)	BLQ	13.1	134.7	BLQ	1.2
Filbert Street Community Garden (August)	BLQ	17.7	136.4	BLQ	1.5
Green Mount Park (July)	BLQ	BLQ	131.2	BLQ	1.1
Green Street Academy (June)	BLQ	BLQ	125.3	BLQ	1.4

Green Street Academy (July)	335.8	27.1	126.0	34.5	1.5
Irvin Luckman Memorial Park (June)	174.1	7.9	124.6	BLQ	1.4
Irvin Luckman Memorial Park (July)	188.4	9.7	138.2	BLQ	BLQ
Irvin Luckman Memorial Park (August)	240.7	12.4	155.0	BLQ	BLQ
Leakin Park (July)	BLQ	25.4	131.5	BLQ	1.4
Leakin Park (August)	BLQ	11.9	334.4	BLQ	1.5
Old York Community Farm (May)	BLQ	BLQ	139.3	BLQ	1.6
Old York Community Farm (June)	BLQ	10.2	264.6	BLQ	BLQ
Patapsco: Avalon Entrance (September)	BLQ	27.0	128.9	12.3	1.5
Patapsco: Avalon Entrance (October)	251.6	10.1	181.3	BLQ	BLQ
Patapsco: Hollofield (August)	BLQ	20.5	187.4	BLQ	1.9
Patapsco: Hollofield (Sept)	190.3	45.4	187.1	BLQ	BLQ
Patapsco: Soapstone Trail (July)	248.6	32.4	208.7	BLQ	BLQ
Patuxent Wildlife Refuge Wildlife Loop (July)	BLQ	10.0	136.6	BLQ	1.4
Patuxent Wildlife Refuge: Wildlife Loop (August)	300.2	12.1	199.1	BLQ	BLQ
Patuxent Wildlife Refuge: Wild Turkey Trail (August)	BLQ	BLQ	177.1	BLQ	BLQ
Stillmeadow Peace Park (June)	BLQ	BLQ	173.0	BLQ	BLQ
Stillmeadow Peace Park (July)	BLQ	13.0	53.0	BLQ	BLQ

Table 1: Concentration of heavy metals (ppb) at sites. BLQ= Below Levels of Quantitation As=Arsenic, Cd= Cadmium, Cr=Chromium, Hg= Mercury, Pb= Lead

	Concentration (ppm)				
Location ID	**As**	**Cd**	**Cr**	**Hg**	**Pb**
Chinquapin Run Park (June)	-	-	6.8	-	0.0
Chinquapin Run Park (July)	-	0.5	6.5	-	-
Chinquapin Run Park (August)	-	0.3	4.9	-	-
Desoto Park (July)	-	-	4.6	-	0.0
Desoto Park (August)	-	-	4.9	-	0.0
Druid Hill Park (July)	-	0.4	-	-	-
Druid Hill Park (August)	-	0.4	2.9	-	-
Filbert Street Community Garden (May)	-	-	5.2	-	0.0
Filbert Street Community Garden (June)	-	-	5.3	-	0.1
Filbert Street Community Garden (July)	-	0.5	4.7	-	0.0
Filbert Street Community Garden (August)	-	0.5	4.2	-	0.0
Green Mount Park (July)	-	-	4.7	-	0.0

Green Street Academy (June)	-	-	5.4	-	0.1
Green Street Academy (July)	10.5	0.9	4.0	1.1	0.0
Irvin Luckman Memorial Park (June)	6.4	0.3	4.6	-	0.1
Irvin Luckman Memorial Park (July)	6.6	0.3	4.8	-	-
Irvin Luckman Memorial Park (August)	10.4	0.5	6.7	-	-
Leakin Park (July)	-	1.0	5.1	-	0.1
Leakin Park (August)	-	0.4	11.2	-	0.1
Old York Community Farm (May)	-	-	4.1	-	0.0
Old York Community Farm (June)	-	0.3	8.8	-	-
Patapsco: Avalon Entrance (September)	-	0.9	4.2	0.4	0.0
Patapsco: Avalon Entrance (October)	7.2	0.3	5.2	-	-
Patapsco: Hollofield (August)	-	0.8	7.5	-	0.1
Patapsco: Hollofield (Sept)	6.9	1.6	6.8	-	-
Patapsco: Soapstone Trail (July)	8.8	1.2	7.4	-	-
Patuxent Wildlife Refuge Wildlife Loop (July)	-	0.3	3.7	-	0.0
Patuxent Wildlife Refuge: Wildlife Loop (August)	10.0	0.4	6.7	-	-
Patuxent Wildlife Refuge: Wild Turkey Trail (August)	-	-	5.8	-	-
Stillmeadow Peace Park (June)	-	-	6.2	-	-
Stillmeadow Peace Park (July)	-	0.5	2.0	-	-

Table 2: Concentration of heavy metals (ppm) at sites. As=Arsenic, Cd= Cadmium, Cr=Chromium, Hg= Mercury, Pb= Lead. Red Coloring Denotes about EPA Threshold

	Mean Concentration (ppm)				
Location ID	**As**	**Cd**	**Cr**	**Hg**	**Pb**
Chinquapin Run	-	-	6.1	-	0.0
Desoto Park	-	0.2	4.8	-	0.0
Druid Hill Park	-	0.4	1.8	-	-
Filbert Street Community Garden	-	0.25	4.85	-	0.025
Green Mount Park	-	-	4.7	-	0.0
Green Street Academy	5.25	0.45	4.7	0.6	0.05
Irvin Luckman Memorial Park	3.5	3.7	5.37	-	0.03
Leakin Park	-	0.7	8.15	-	0.05
Old York Community Farm	-	0.15	6.45	-	0.0
Patapsco: Avalon	3.6	0.6	4.7	0.2	0.0
Patapsco: Hollofield	3.45	1.2	7.15	-	0.05
Patapsco: Soapstone Trail	8.8	1.2	7.4	-	-
Patuxent Wildlife Refuge: Wildlife Loop	5	0.35	5.2	-	0.0

Patuxent Wildlife Refuge: Wild Turkey Trail	-	-	5.8	-	-
Stillmeadow Peace Park	-	0.25	4.1	-	-

Table 3: Mean Concentrations of Heavy Metal (ppm) at Each Location
As=Arsenic, Cd= Cadmium, Cr=Chromium, Hg= Mercury, Pb= Lead. Red Coloring Denotes about EPA Threshold

Heavy Metal	**Median Concentration (ppm)**	**Minimum**	**Maximum**	**St.Dev**
Arsenic (As)	8.03	6.4	10.5	1.79
Cadmium (Cd)	0.46	0.3	1.2	0.35
Chromium (Cr)	5.13	2	11.2	1.79
Mercury (Hg)	0.74	0.4	1.1	0.48
Lead (Pb)	0.05	0.0	0.1	0.01

Table 4: Basic Statistics for each heavy metal sampled

Heavy Metal	**Sum Squares**	**P Value**
Arsenic	68.9	0.844
Cadmium	11.878	**0.0308**
Chromium	29.191	0.312
Mercury	0.1573	0.975
Lead	0.0049	0.538

Table 5: Heavy metal concentrations compared to mean income of sample sites

Heavy Metals	**Sum Sq**	**P Value**
Arsenic ~ Cadmium	2.146	0.119
Arsenic ~ Chromium	2.298	0.343
Arsenic ~ Lead	2.95	0.557
Arsenic ~ Mercury	0.0483	0.178
Cadmium ~ Chromium	0.364	0.537
Cadmium ~ Lead	1.792	0.157
Cadmium ~ Mercury	0.0364	0.846
Chromium ~ Lead	4.735	0.165
Chromium ~ Mercury	0.9233	0.552
Mercury ~ Lead	0.0584	0.135

Table 6: Relationships of Heavy Metal concentrations in Guano Sample

Metal Concentration and Mean Income	**Sum of Squares**	**Mean Square**	**P -Value**
Cadmium ~ Chromium + Mean income			
Chromium	0.364	0.3635	0.1285
Mean Income	11.66	1.06	0.05
Cadmium ~ Arsenic + Mean income			
Arsenic	2.1463	2.146	0.00944
Mean Income	9.952	0.9047	0.02242
Cadmium ~ Mercury + Mean Income			
Mercury	0.036	0.0364	0.65
Mean Income	11.841	1.0765	0.113
Cadmium ~ Lead + Mean Income			
Lead	1.792	1.7919	0.0612
Mean Income	10.106	0.9187	0.1217
Arsenic ~ Chromium + Mean Income			
Chromium	7.52	7.525	0.535
Mean Income	73.95	6.722	0.822
Arsenic ~ Lead + Mean Income			
Lead	2.95	2.949	0.681
Mean Income	79.746	7.244	0.788
Arsenic ~ Mercury + Mean Income			
Mercury	14.69	14.695	0.452
Mean Income	59.78	5.434	0.917
Chromium ~ Lead + Mean Income			
Lead	4.735	4.735	0.256
Mean Income	24.636	2.24	0.548
Chromium ~ Mercury + Mean Income			
Mercury	0.923	0.9233	0.565
Mean Income	28.308	2.5735	0.514
Mercury ~ Lead + Mean Income			

Lead	0.05842	0.03842	0.137
Mean Income	0.27982	0.02536	0.317

Table 7: Heavy Metal Concentrations compared with Mean Income of Collection Sites

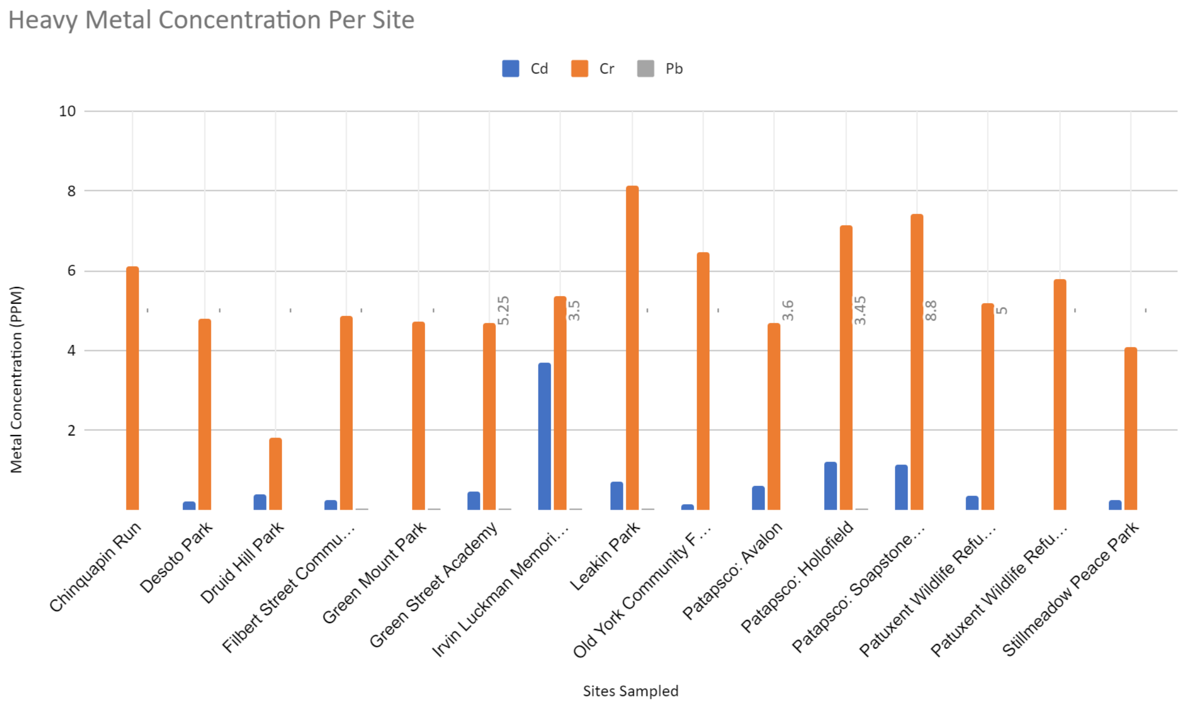


Figure 1: Heavy Metal Concentrations Per Site, Cd= Cadmium, Cr=Chromium, Pb= Lead

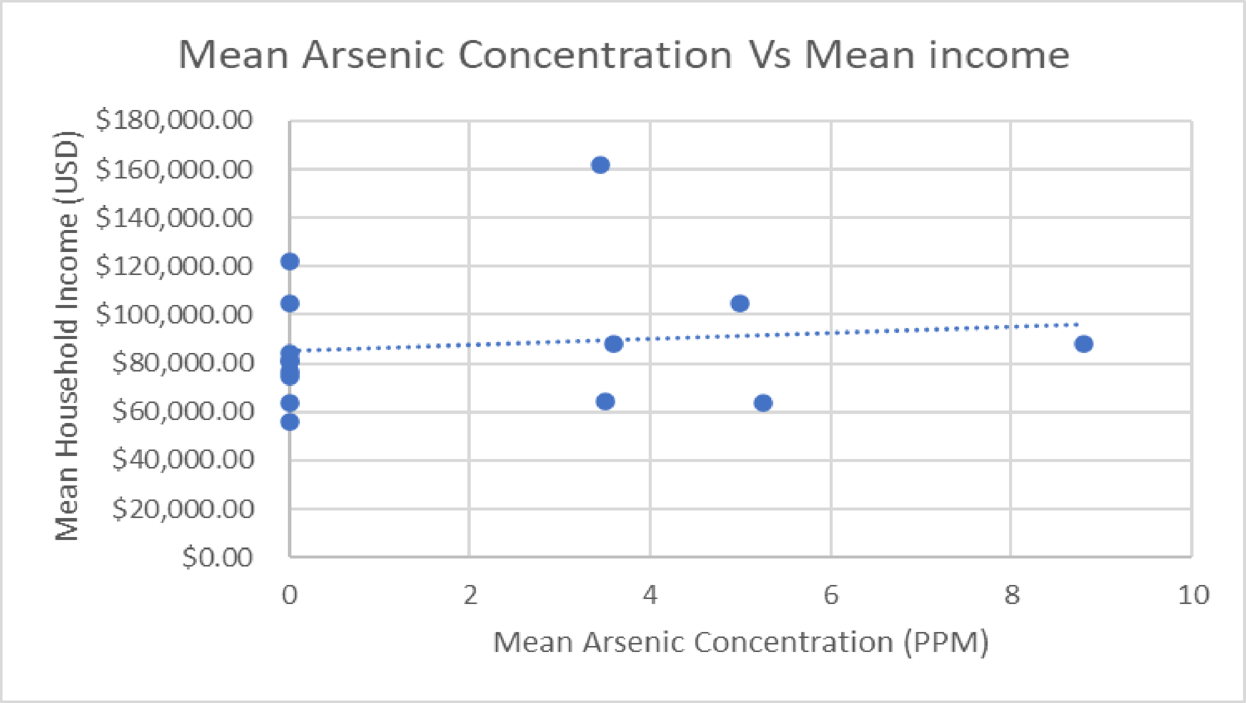


Figure 2: Mean Arsenic Concentration Vs Mean Income

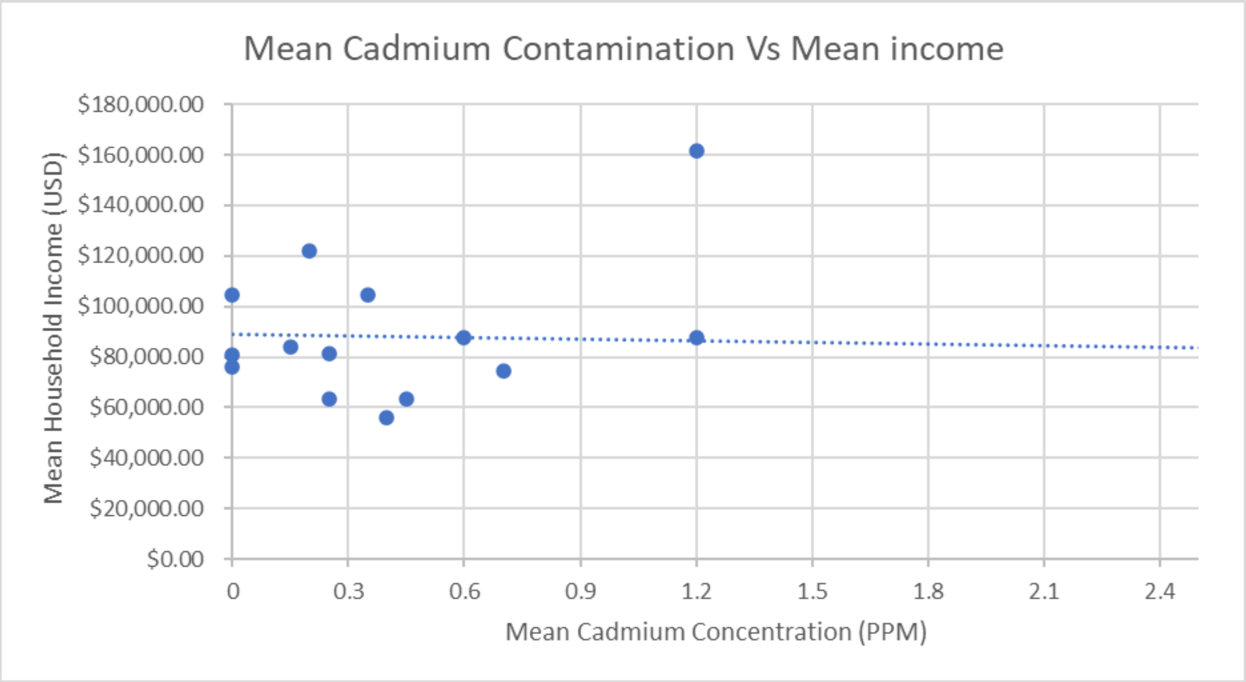


Figure 3: Cadmium Concentration Vs Mean Household Income

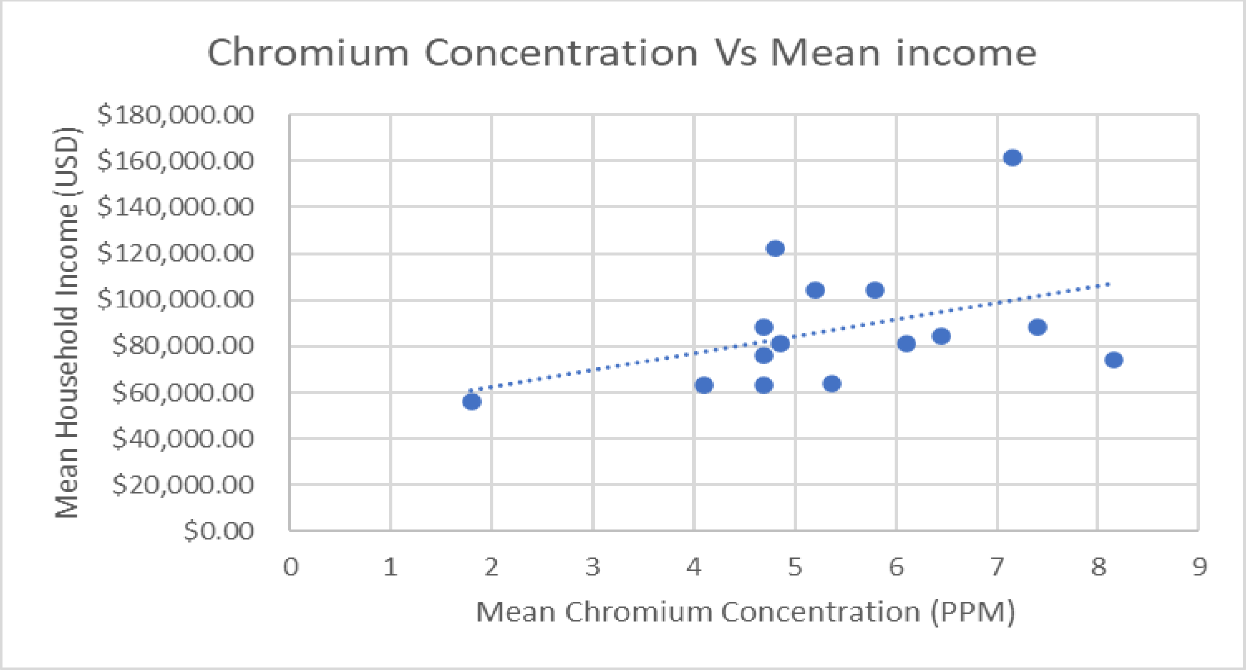


Figure 4: Chromium Concentration Vs Mean Household Income

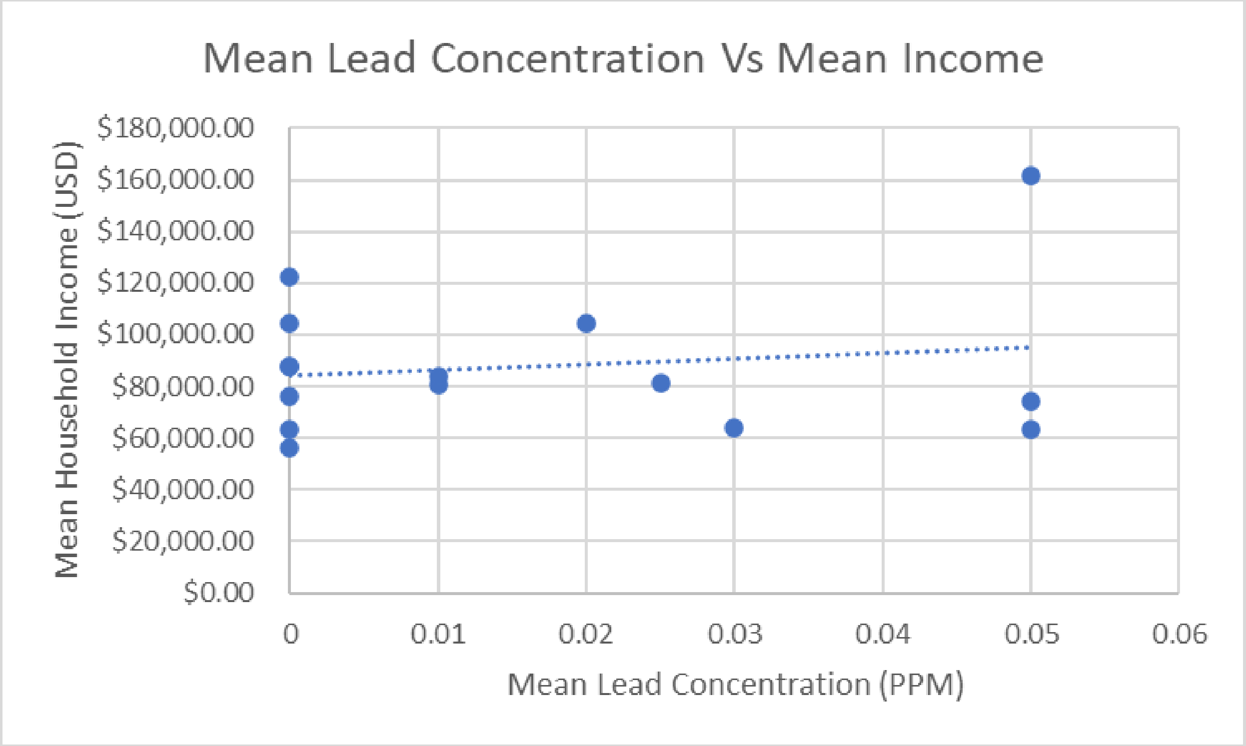


Figure 5: Mean Lead Concentration Vs Mean Income

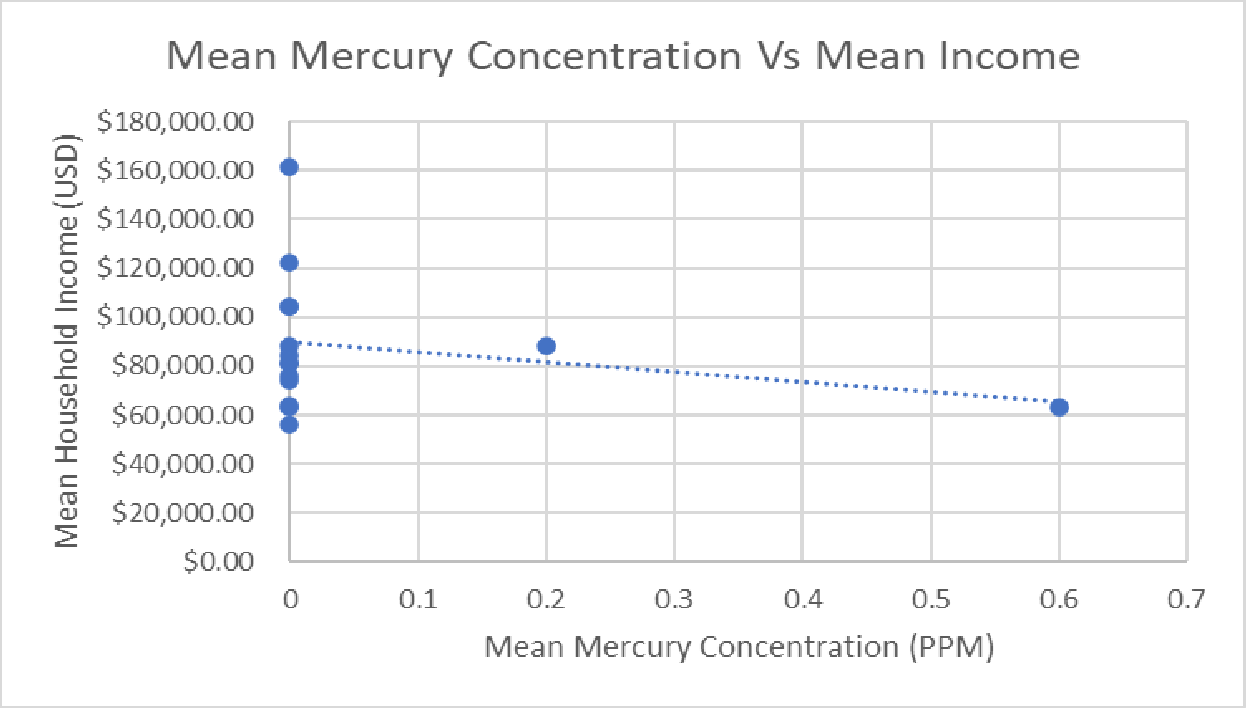


Figure 6: Mean Mercury Concentration Vs Mean Income

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