Mushrooms as Bioindicators of Heavy Metals in Sites Affected by Industrial Activity in the Macatawa Watershed

Amber Bosch, Kathleen Fast, Brooke Mattson
Geology and Environmental Science, Hope College,
Holland, Michigan

Introduction

Biomonitoring is a method that is being developed as a way to quantify environmental conditions using natural organisms. This includes the tracking of contamination in the environment from the air, water, or soil. Organisms that have been used in other studies include nonvascular plants and oysters (Gatziolis et al., 2016; Fertig et al., 2009). In general, biomonitoring is a cost effective method of data collection because it allows for sampling a large area without the expense of deploying high-grade, and often costly, monitoring devices. The motivation for this study comes from the interest to investigate if the Lake Macatawa watershed contains regions of soils with elevated heavy metals. Biomonitoring was selected for this study because it would allow for a cost- and time-efficient way of evaluating heavy metals in the environment from several locations.

Mushrooms are widely available due to the various environments they inhabit, managed lawns to old-growth forests. The underground structures of mushrooms, known as mycelium, allow for bioaccumulation and depositing of metals into the above-ground structure of the mushroom, the fruiting body (Das, 2005). Mycelium can be found growing in the soil for many years, but it is not until the environmental conditions are just right that the fruiting body forms. While the fruiting body is forming it is acting as a sink for the compounds that the mycelium has accumulated. This mechanism is not yet fully understood, however it has been observed within many varieties of mushroom (Širić et al., 2016). Heavy metal uptake amounts may vary in different mushrooms species, but overall trends have indicated sites with industrial activity result in higher metal accumulation within the fruiting body of the mushroom (Širić et. al 2016; Mirończuk-Chodakowska et al., 2013).

The sites selected for sampling were either (a) connected to or exposed to industrial activity or (b) isolated from most forms of anthropogenic activity other than foot traffic and not impacted by industrial activity. The mushroom fruiting bodies were assessed chemically by flame atomic absorption spectroscopy (F-AAS) to see if the samples were accumulating heavy metals in a way that related to the known site history. This is a preliminary attempt to determine the effectiveness of using mushrooms as bioindicators as an accurate way to assess heavy metal concentrations in the soil of the Lake Macatawa Watershed.

Methods

Site Selection and Sample Collection

A total of five sites were selected, three of which were chosen based upon a history of industrial activity on and/or near the site or former dump activity, which raises the potential for contamination by heavy metals in the recent past. For this reason these three sites will be referred to as contaminated sites. In addition to these sites, two clean sites were selected, which were chosen because of their distance from road traffic and lack of an industrial history. These sites will be referred to as clean sites. The selected contaminated sites were Howard B. Dunton Park,

Riley Trails and the Dredge Material Placement Site. The selected clean sites were Upper Macatawa Natural Area and the Hope College Nature Preserve. Figure 1 depicts these locations by color.

At each site, mushrooms were searched for on the ground by lab members. When mushrooms were found, a photo was taken to document the location along with GPS coordinates. Mushrooms were bagged with a protected hand so as to not expose them to the other samples or surfaces. The pictures taken of the growing mushroom showed its unique characteristics to aid in identification. A surface level soil sample was also collected to correspond with each mushroom sample.

Sample Preparation and Digestion

Mushroom samples were cleaned with reverse osmosis (RO) water and cleaned with gloved hands to remove excess sediment, insects, and other particles from within the tissue. The clean mushrooms were placed into labeled beakers to dry at 60℃ in a drying oven for at least five days. Samples were then ground up into a fine powder using a mortar and pestle to breakdown the dried material into grains < 1.0 mm in diameter. The powder was then massed completely and approximately 0.5 g of samples was taken for digestion. The remaining sample was archived for future work.

The samples reserved for digestion were transferred into 5 mL Savillex teflon vessels with 3 mL of concentrated nitric acid (HNO_3) with a concentration of approximately 15.5 M. The closed vessel containing sample and acid was heated on a hot plate at 80℃ for at least eight hours before being cooled for one hour and being opened to dry down on the hot plate at the same temperature. This acid step was repeated a second time with a subsequent dry down before being repeated a third time with the addition of 120 µL of 3.0% hydrogen peroxide (H_2O_2) in a total volume of 5 mL with ~15.5 M HNO_3. Each concentrated acid heating took at least 8 hours to complete with a consequential dry down of between 1-3 hours. The total digestion process was completed for nine samples after one was removed due to contamination and excessive loss of material.

Sample Dilution and Standard Preparation

Dried digested samples were heated with 3 mL of RO water on a hotplate at 80℃ for one hour before 1 mL of this solution was taken for dilution. Each 1 mL aliquot was diluted to a total volume of 25 mL using 18.2 MΩ-cm RO water for analysis with F-AAS.

Heavy metal standards for Fe, Pb, Cd, and Ni were made using stock aqueous heavy metal solutions provided by the Watershed Lab Group of Hope College. From the concentrated solutions, sequential dilutions were done to prepare five standard solutions ranging from 0.5 - 50 parts per million (ppm) for Fe and from 0.05 - 5.0 ppm for Pb, Cd, and Ni. These standard concentrations are shown in Table 1.

Metal (ppm)	*Standard 1*	*Standard 2*	*Standard 3*	*Standard 4*	*Standard 5*	*Standard 6*
Iron (Fe)	0.5	1.0	2.5	5.0	10.0	50
Lead (Pb)	0.05	0.1	0.25	0.5	1.0	5.0
Cadmium (Cd)	0.05	0.1	0.25	0.5	1.0	5.0
Nickel (Ni)	0.05	0.1	0.25	0.5	1.0	5.0

Table 1. Standard concentrations in parts per million (ppm) for each heavy metal that was analyzed within the moss samples.

Flame Atomic Absorption Spectroscopy

Analysis of the digested mushroom solutions was done using lamps set to measure absorbance and transmission from each metal. The wavelengths that were used in the heavy metal lamps to assess concentration are shown in Table 2. The absorbance values were used in this study to record the proportion observed in comparison to the standards for each metal. All measurements were done using a Varian SpectraAA-50 Atomic Absorption Spectrophotometer.

Metal	*Wavelength Used in Analysis (nm)*
Iron (Fe)	248.3
Lead (Pb)	217.0
Cadmium (Cd)	326.1
Nickel (Ni)	341.5

Table 2. Wavelength settings used for analysis of each heavy metal.

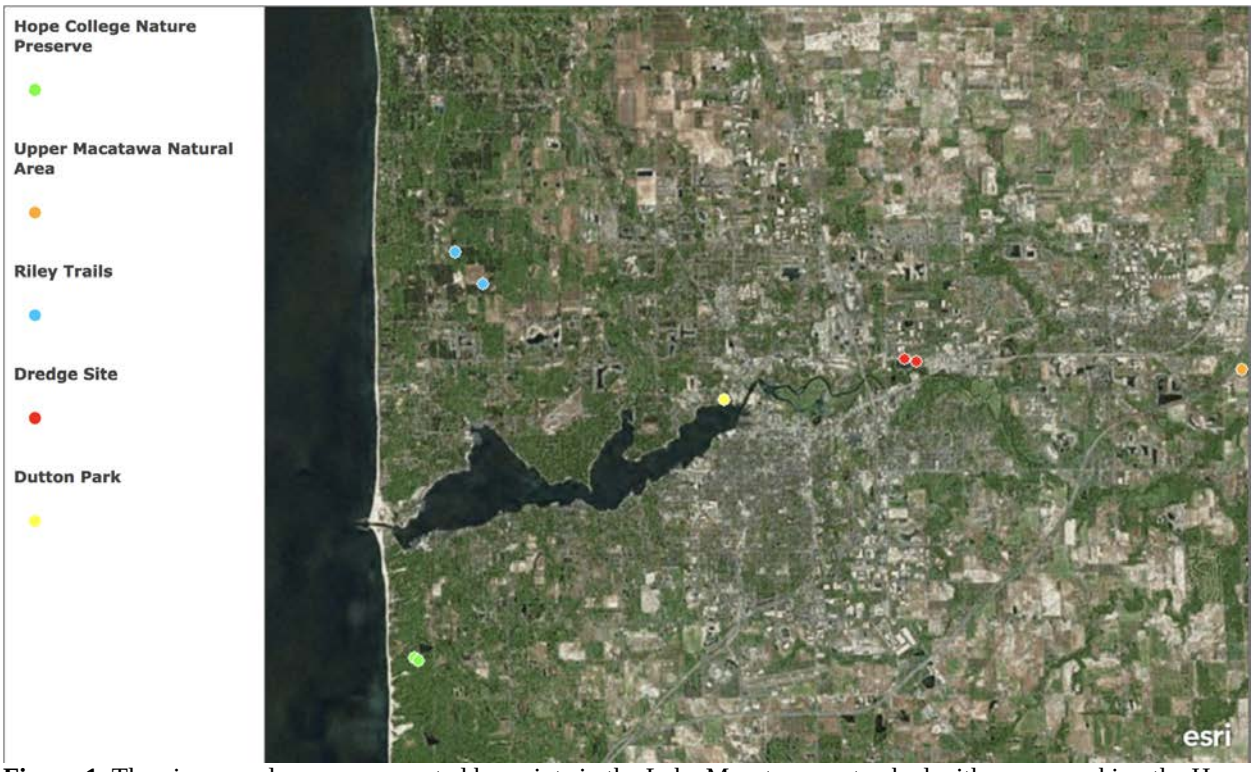

Figure 1. The nine samples are represented by points in the Lake Macatawa watershed with green marking the Hope College Nature Preserve, orange marking the Upper Macatawa Natural Area, blue marking Riley Trails, red marking the Dredge Site, and yellow marking Dutton Park. Lake Macatawa can be seen in the western portion of the map, connected to Lake Michigan to the far west.

Results

The concentrations of each of the four metals (iron, lead, cadmium, and nickel) in all mushrooms samples are shown in Table 3 in parts per million (ppm, mg/kg). This represents the mass of metal relative to the dry mass of the sample. Looking down the columns of metals, the variance in concentrations between samples can be observed. Concentrations of iron varied from 6ppm to 87ppm, cadmium 1ppm to 5ppm, nickel 2ppm to 6ppm, and all samples had a lead concentration of 1ppm (Table 3). Mushroom genus is also given in this table along with collection site and whether the site was predicted to be contaminated or clean. R^2 values are also given in Table 4 and vary between 0.37682 and 0.99885. These represent the accuracy of the calibration curves created with standards of known concentrations which were used to calculate the concentrations of metals in mushroom samples. Average concentrations for each metal are given in Table 5. These are presented with average concentrations from 5 different papers of similar studies on heavy metals in mushrooms. Also given here are the average concentrations for the four metals in Michigan soils, and the EPA criteria of metal concentrations for direct contact with contaminated soils in residential areas.

Type of Site	***Site Name***	***Mushroom Genus***	***Heavy Metal Concentration in Fruiting Body***			
			Fe	***Pb***	***Cd***	***Ni***
Contaminated Site	Howard B. Dunton Park 1	*Scleroderma* spp.	87 ppm*	1 ppm	2 ppm	4 ppm
Contaminated Site	Howard B. Dunton Park 2	*Scleroderma* spp.	10 ppm	1 ppm	2 ppm	5 ppm
Contaminated Site	Riley Trails	*Lycoperdon* spp.	8 ppm	1ppm	2 ppm	4 ppm
Contaminated Site	Riley Trails	*Lycoperdon* spp.	9 ppm	1 ppm	2 ppm	5 ppm
Contaminated Site	Dredge Material Placement Site	*Cortinarius* spp.	7 ppm**	1 ppm	2 ppm	6 ppm
Contaminated Site	Dredge Material Placement Site	*Leucoagaricus* spp.	6 ppm	1 ppm	1 ppm	3 ppm
Clean Site	Upper Macatawa Natural Area	*Leucoagaricus* spp.	9 ppm	1 ppm	5 ppm	2 ppm
Clean Site	Hope College Nature Preserve	*Mycena* spp.	9 ppm	1 ppm	5 ppm	2 ppm
Clean Site	Hope College Nature Preserve	*Clitocybe* spp.	9 ppm	1 ppm	5 ppm	2 ppm

Table 3. Heavy metal concentrations of Fe, Pb, Cd and Ni in mushroom fruiting body samples at three contaminated sites and two clean sites. Contaminated sites are defined as having industrial influence near or on the site. Clean sites are defined as having no known history of industrial activity. Iron $R^2 = 0.99829$, $*R^2 = 0.92536$, and $**R^2 = 0.99885$. Cadmium $R^2 = 0.37682$; Nickel $R^2 = 0.94166$; Lead $R^2 = 0.99744$

Metal	***R^2 Value***
Iron (0-50 ppm)	0.92536
Iron 2 (0-10 ppm)	0.99885
Iron (0-10 ppm)	0.99829
Cadmium	0.37682
Nickel	0.94166
Lead	0.99744

Table 4. R^2 values for the standard curves of each metal. An R^2 value close to 1.000 indicates a more linear fit for the calibration curve generated from the known concentrations of standards relative to the measured absorbances from the flame atomic absorption spectrometer.

Metal	*Experimental Concentration Average (ppm)*	*Literature Concentration Average (ppm)*	*Michigan Soils Concentration Average (ppm)*	*EPA Soil Contaminant Direct Contact Criteria (ppm)*
Iron	17	70-4,000	10,520	160,000
Cadmium	2	0.3-25	0.9	550
Nickel	5	0.6-18	12	40,000
Lead	1	1.0-8	9.2	400

Table 5. A comparison of heavy metal concentration averages from this study to published studies, Michigan soil concentrations, and EPA soil contaminant direct contact criteria. The literature concentration averages used similar methods to evaluate heavy metal concentrations in mushrooms. The Michigan soil concentration averages are for heavy metal concentration in the entire state of Michigan. The EPA soil contaminant direct contact criteria show the safety limit for these heavy metals for direct contact. EPA (2013), Khodabakhshi et al. (2016), Chen et al. (2009), Zhu et al. (2011), Zhang et al. (2008), Siric et al. (2016)

Discussions

Concentrations of each heavy metal were relatively consistent from site to site. This meant that there is no proven trend that shows that heavy metal uptake by mushrooms is impacted by the predicted level of heavy metal pollution due to anthropogenic activity. However, there was one outlier in the set of concentrations of iron that may show the need for further experimentation to determine the validity of this conclusion. This was the Dunton Park 1 sample (Du1) which contained a concentration of 87 ppm of iron while the average concentration of the other 8 samples averaged around 8 ppm. This high concentration may be the true concentration of iron in this sample or it may be irregularly high due to an unknown event of contamination either during processing or predating collection.

Furthermore, of the four heavy metals investigated in mushrooms, analysis of iron was best suited for the sensitivity of the instrumentation used. The concentrations of iron standards were an order of magnitude higher than those of cadmium, nickel, and lead. This placed the range of iron concentrations being analyzed in a better range within the detection limits of the flame absorption spectrometer. Conversely, because the concentrations of the standards of cadmium, nickel, and lead were so much lower they were closer to the lower detection limit of the instrument causing the generated calibration curve to either follow a less linear trend or to increase only slightly in absorbance relative to known concentration. This indicates that the calibration curves may not give accurate concentrations of the metals relative to the absorbances measured by the instrument. More accurate results may be obtainable with an instrument that has a lower detection limit. The level to which the calibration curve can be used to calculate accurate experimental concentrations based off the absorbances measured by the instrument is indicated

by the R^2 value. The R^2 values for each calibration curve are shown in Table 4. An R^2 value of 1.000 indicates perfectly linear points on a concentration/absorbance curve and the less linear the points, the lower the R^2 value and, therefore, the lower the accuracy of the calculated concentrations.

Relative to the concentrations of other studies presented in Table 5 (A.M et al. (2016); Chen et al. (2009); Zhu et al. (2011); Zhang et al. (2008); Siric et al. (2016)) the concentration of iron found in these samples is low. This may be due to biological limits of iron uptake by mushrooms or a truly low concentration of iron in local soils. It is much lower than the average concentration of iron in Michigan soils and well below the direct contact EPA limit for iron in soil. The concentration of cadmium in mushroom samples was within the range of the literature values, higher than that of average michigan soils, and much lower than EPA soil criteria. The average concentration of nickel amongst mushrooms was within the range of literature values, slightly lower than the average for michigan soils, and much lower than EPA soil criteria. Finally, the concentration of all lead samples was at the lower cusp of literature values, below the average concentration of Michigan soils, and, again, much lower than EPA soil criteria. While the literature data show that these average values are likely reasonable, it's not surprising that the values of this study tend to be lower than other literature values as other studies tend to concentrate on areas with known high levels of heavy metal pollution and may show concentrations skewed higher than a normal range.

One potential source of error for this experiment is the potential for contamination of heavy metals during the processing of samples due to the lack of a metal free lab space. In addition, the varying detection limits of the instrumentation used may have limited the accuracy of the concentrations calculated with generated calibration curves. Furthermore, due to trial and error in methodology, the sample size of the experiment was not large and varied in species. A larger sample size might answer more questions such as the validity of the iron concentration of the Du1 sample and whether or not species variation impacts the uptake of heavy metals by mushrooms.

Further investigation of this study could include analysis of mushroom samples with ICP-OES to obtain results from instrumentation with a lower detection limit. Analysis of soil samples taken from the sites of mushroom collection could reveal the level to which the concentrations of mushrooms parallels the true concentrations of heavy metals in soils. Additionally, collecting mushrooms throughout the year may allow for greater investigation of the relationship between mushroom species and heavy metal uptake as well as how regional precipitation patterns and water availability impact this uptake.

Conclusions

The trend that was predicted for clean versus contaminated sites was not observed within the samples analyzed from those sites, however the similar trend between all samples suggests that the presumed industrial history was either not sufficient to cause contamination or the mushrooms served as a poor indicator of contamination. The high Fe concentration at one of the Howard B. Dunton Park samples can be attributed to possible contamination within the lab, or a unique source of iron where the mushroom was collected from, such as debris washed ashore. Of the four metals, Fe was best suited for analysis with instrumentation of this sensitivity, so therefore the other metals of interest could not be used in determining the effectiveness of mushrooms as bioindicators. Observing other heavy metals at this level would require the use of more technical instrumentation with lower detections limits, like the ICP-OES.

Without the analysis of soil samples, the accuracy of biomonitoring as a method of quantification could not be fully determined. In order to conclude the feasibility of biomonitoring in the Macatawa watershed, further work needs to be done. As of right now, this study showed that the mushrooms from all sites had heavy metal concentrations well under the EPA regulations for heavy metal concentrations in soil. This indicates that the recent and current industrial activity that were presumed to effect heavy metal concentrations in the environment could be less detectable than what was thought before this study.

Acknowledgements

We would like to thank Dr. Bodenbender for his support and guidance when trouble arose. We would also like to thank Kelly Goward and Judy Visscher for access to sampling sites during the fieldwork.

References

Khodabakhshi, A., Sedehi, M., & Shakeri, K. (2016). Investigation of heavy metals in edible mushrooms consumed in Shahrekord. *Journal of Shahrekord University of Medical Sciences*, *18*(1), 54-62.

Chen, X., Zhou, H., & Qiu, G. (2009). Analysis of several heavy metals in wild edible mushrooms from regions of china. Bulletin of Environmental Contamination and Toxicology, 83(2), 280-285.

Das, N. (2005). Heavy metals biosorption by mushrooms.

EPA. (2013). Risk-Based Cleanup Criteria. *Cleanup Criteria for Contaminated Soil and Groundwater*, 11-20

Fertig, B., Carruthers, T.J.B., Dennison, W.C. et al. Estuaries and Coasts (2009) 32: 773. https://doi.org/10.1007/s12237-009-9148-x

Gatziolis, D., Jovan, S., Donovan, G., Amacher, M., & Monleon, V. (2016). Elemental atmospheric pollution assessment via moss-based measurements in Portland, Oregon.

Mirończuk-Chodakowska, I., Socha, K., Witkowska, A. M., Zujko, M. E., & Borawska, M. H. (2013). Cadmium and lead in wild edible mushrooms from the Eastern region of Poland's 'Green Lungs'. Pol J Environ Stud, 22, 1759-1765.

Širić, I., Humar, M., Kasap, A., Kos, I., Mioč, B., & Pohleven, F. (2016). Heavy metal bioaccumulation by wild edible saprophytic and ectomycorrhizal mushrooms. Environmental Science and Pollution Research, 23(18), 18239-18252.

Zhang, D., Gao, T., Ma, P., Luo, Y., & Su, P. (2008). Bioaccumulation of heavy metal in wild growing mushrooms from Liangshan Yi nationality autonomous prefecture, China. *Wuhan University Journal of Natural Sciences*, *13*(3), 267-272.

Zhu, F., Qu, L., Fan, W., Qiao, M., Hao, H., & Wang, X. (2011). Assessment of heavy metals in some wild edible mushrooms collected from yunnan province, china. Environmental Monitoring and Assessment, 179(1), 191-199. doi:10.1007/s10661-010-1728-5