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# Mobilization of Metals by Fungi in Historic Cemeteries

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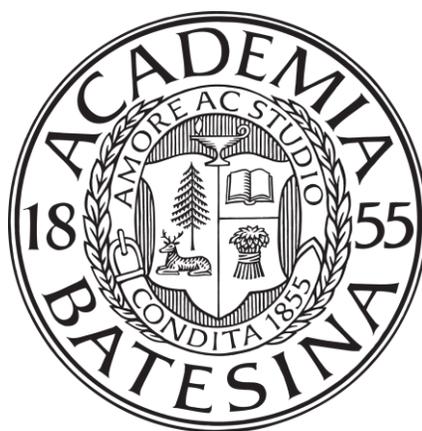
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# Mobilization of Metals by Fungi in Historic Cemeteries

A senior thesis

Presented to the faculty of the  
Departments of Geology and Chemistry

Bates College



In partial fulfillment  
of the requirement for the  
Degree of Bachelor of Science

By  
Eleanor D. Briggs

Lewiston, Maine 04240  
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## Abstract

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Interactions among fungi, soil, and metals are at the heart of nutrient cycling in terrestrial systems. Both major and trace elements are found in soils, but the degree to which they are biologically available is influenced by chemical weathering of soil minerals by fungi. In addition to contributing to weathering, mushrooms are known to bioaccumulate metals from soil, so edible mushrooms growing on soils contaminated with toxic metals can cause harm to those who eat them. This study focuses on metal content of mushrooms and soils from cemeteries that are suspected to be contaminated with arsenic as a result of late-19th Century embalming practices. Mushrooms and soil were collected from eight cemeteries and three control areas in Lewiston, Auburn, Sabattus, and Topsham, Maine and analyzed for metal content using acid digestion and ICP-OES. With particular focus on arsenic, concentrations of potassium, sodium, zinc, calcium, iron, magnesium, lead, and arsenic in mushrooms are compared to concentrations of those metals in the soil on which the mushrooms were growing.

## **Acknowledgements**

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## **Introduction**

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### ***Overview and Purpose***

The relationships among metal, soil, and fungi is an interface of geology, chemistry and biology that is at the heart of plant metabolism and vitality. Fungi break down mineral and organic matter in soil, thereby providing nutrients to the plants and animals feeding on the soil. Fungi are particularly effective in making metals biologically available and mediating the interactions between plants and metals. This mediation is particularly important in the presence of toxic metals or potentially lethal doses of biologically active metals. Due to their ability to interact with and take up metals, fungi can serve as an indicator of metal concentrations in soils.

Cemeteries are suspected to have elevated levels of arsenic as a result of late-19th Century embalming practices. Embalming became popular in the United States during the Civil War when demand for the service increased with an increased desire to preserve bodies of fallen soldiers for shipment home. Embalming fluid contained arsenic as the primary poison from the Civil War-Era until about 1910. This led to the suspicion that there would be elevated levels of arsenic in cemeteries. As a result, there is a rule of thumb for mycologists to avoid scavenging for edible mushrooms in cemeteries because they may contain arsenic. For this reason, this study focuses on arsenic concentrations of soil and fungi from cemeteries. Potassium, sodium, zinc, calcium, iron, magnesium, and lead are also analyzed in soil and fungi to understand more about the soil-fungi-metal triad.

<b>Name</b>	<b>Type</b>	<b>Town</b>	<b>Fungi Samples Collected</b>
Brookvale	Cemetery	Auburn, ME	5
Briggs	Cemetery	Auburn, ME	6
Fisher	Cemetery	Sabattus, ME	10
Golder	Cemetery	Sabattus, ME	4
Herrick	Cemetery	Lewiston, ME	4
Mountain View	Cemetery	Auburn, ME	5
North Auburn	Cemetery	Auburn, ME	4
Wrights	Cemetery	Lewiston, ME	7
Bates	Control	Lewiston, ME	2
Dostie	Control	Topsham, ME	11
Thorncrag	Control	Lewiston, ME	2

Table 1. Sample locations.

## ***Study Area***

### *Field Locations*

Fieldwork took place in Lewiston, Auburn, Sabattus, and Topsham, Maine. These cities and towns in Androscoggin and Sagadahoc Counties are adjacent, located at 070°W and 44°N. Thirteen cemeteries were visited for this study. Mushroom and soil samples were collected at eight cemeteries; no mushrooms were found at the remaining cemeteries. Fungal and soil samples were also collected in three non-cemetery control areas (Figure 1).

### *Local Geology*

The bedrock geology of the study area is primarily the rock units Ss and Sspm, with some sites on units Sov, Ocz, C1b(m,s) (Figure 2) (Maine, 2015). The study area is mostly covered by Pleistocene deposits. The main deposits are till, marine regressive

sand deposits, and marine nearshore deposits, including fine grained glaciomarine deposits (Figure 3) (Surficial, 1999).

### ***Metals in Soils***

Soils are mixtures of a few major constituents, the combinations of which result in immense variation in soil composition and texture. The major controls on soil composition are plants, parent material, climate, relief, and time (Buol et al., 1973). Typical topsoils are about 50% sediment, 20-25% soil water, 20-25% soil air, and 5% organic matter (Gerrard, 2000). This means that soils are typically about half solid mass and half pore space filled with water and air (Gerrard, 2000). Mineral content varies in abundance, mineralogy and grain size based on the parent material and weathering regime, while organic matter varies based on the environment of the soil (Wild, 1993). The chemistry and abundance of both soil water and soil air is primarily dictated by the mineralogy of the sediment and nature of the organic matter present (Wild, 1993). The larger sediment, such as sand and silt, defines physical properties of soil, but the clay fraction mostly affects the chemistry of soil air and soil water (Wild, 1993). Clay minerals result from chemical weathering and are distinct from their parent material, so they are actively engaged as both a result of and an affector in the chemical equilibria of soil systems (Gerrard, 2009). There are dozens of types of clay minerals that contain arsenic, whose breakdown may contribute to arsenic concentrations seen in this study (Drahota and Filippi).

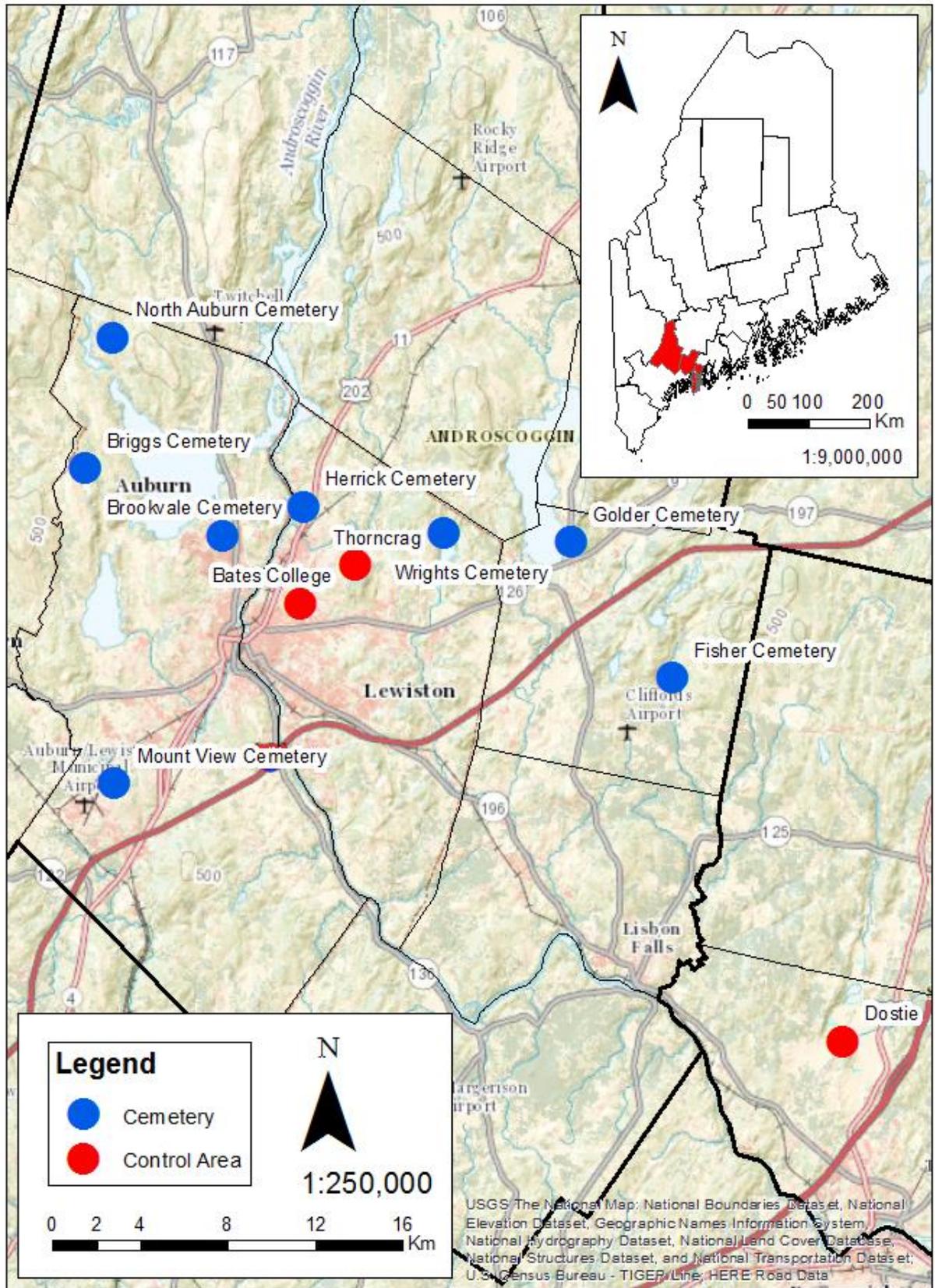


Figure 1. Locations of sampling.

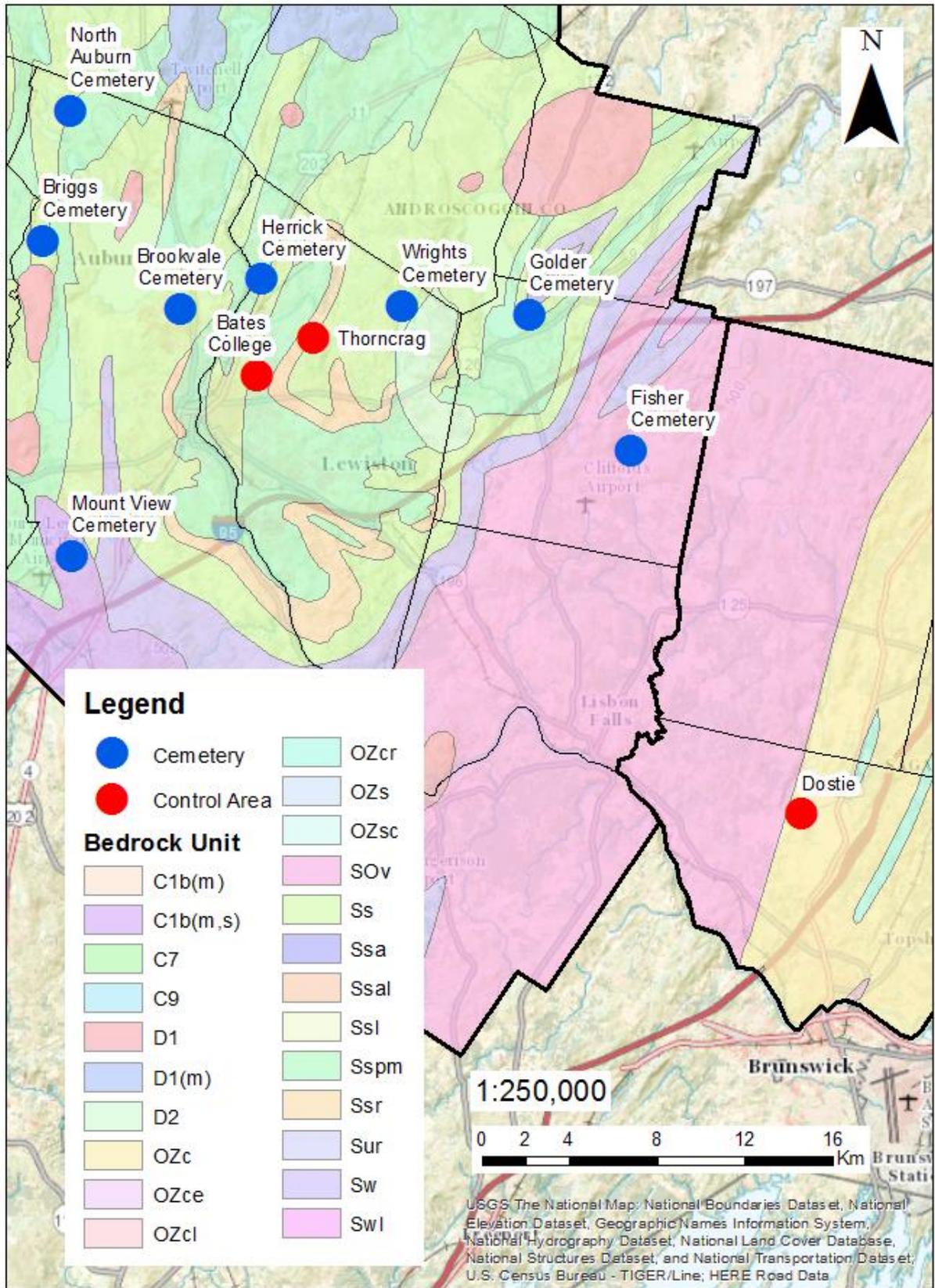


Figure 2. Bedrock underlying sampling locations.

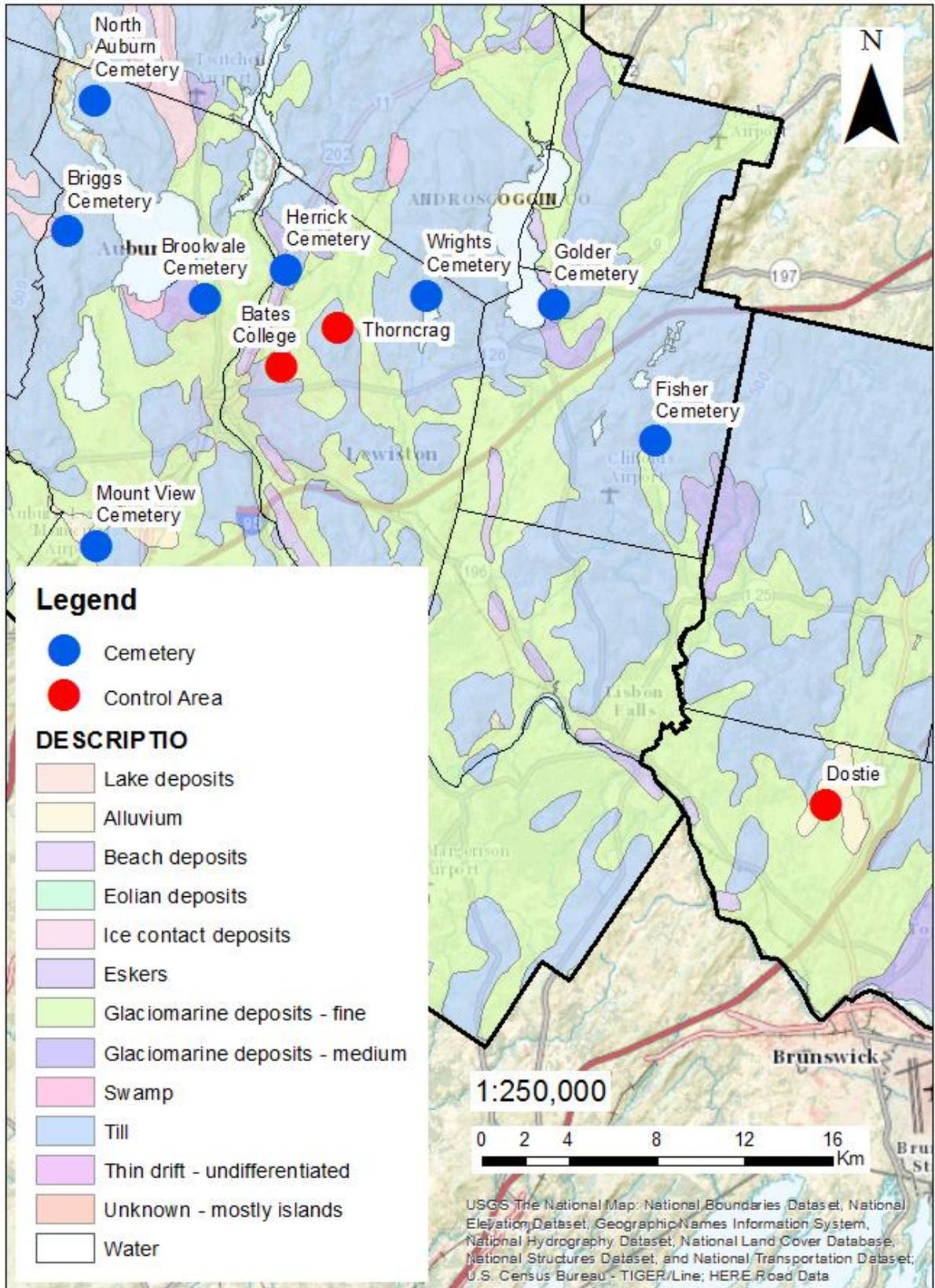


Figure 1. Surficial geology of sampling locations.

### *Major Elements*

Major elements in soils are defined as the elements whose concentrations exceed 100 mg/kg (Sposito, 2008). The major elements that are most common in the Earth's crust parallel those of soil, the primary difference being in the relative abundance of biologically critical elements. Oxygen, silicon, aluminum, iron, calcium, sodium, potassium, and magnesium, respectively, are the most common elements in the Earth's crust; oxygen, silicon, aluminum, iron/calcium/carbon, potassium, sodium, magnesium, titanium, nitrogen, and sulfur, respectively, are the most common elements in soil. The increase in relative abundance of carbon, potassium, titanium, nitrogen, sulfur in soil versus the crust is a direct result of the organic content of soil (Wild, 1993). Other major elements in soil and the Crust include phosphorus, chlorine, manganese, strontium, zirconium, and barium (Sposito, 2008). The major elements in soil and the crust are parallel due to the high mineral content of soil; the major elements in soil are those that play major roles in forming abundant minerals.

### *Trace Elements*

Trace elements in soils are defined as the elements whose concentrations do not exceed 100 mg/kg (Sposito, 2008). Common trace elements in soils are selenium, lithium, chromium, cobalt, nickel, copper, zinc, arsenic, and lead (Sposito, 2008). Trace elements are also found in minerals, but in much lower concentrations because the minerals in which they are found are less abundant and because they are less abundant within their minerals than other elements.

### *Metal Mobility in Soil*

Soil goes through various stages of aging, which most directly affects the mineral organic content, but results in changes in the chemistry of the soil air and soil water (Sposito, 2008). Soil age is reflected by the dominant mineral types, with clay minerals serving as an indicator of chemical weathering (Sposito, 2008). Organic matter decays from recognizable species, such as certain types of leaves, into a nondescript material termed humus, and then into readily available nutrients for plants and other organisms, such as fungi (Gobat, 2004; Wild, 1993). The chemical weathering of minerals into their clay descendants and the decay of organic material into humus and beyond provide the elemental nutrients that are found in soil air and soil water. Both the major elements and trace elements are made available in these aging processes. The resulting chemical environment is reflected in the organisms that feed on the nutrients in the soil.

### *Fungus*

The fungi in this study are basidiomycetes, specifically mushrooms and puffballs. Basidiomycetes generate spores and have club-like fruiting bodies (Minkoff, 1991). Mushrooms have two major parts: the fruiting body and the hyphae. Fruiting bodies are the part of mushrooms that people are used to seeing. They grow at the ground surface and range greatly in size, color, and form. Hyphae comprise an extensive network of thin, fibrous tubes that extend radially from the base of the fruiting body into the substrate and collect nutrients and water for the fruiting body (Cooke, 1979). The fruiting bodies of mushrooms contain an area, usually on the underside of the cap, of either gills or pores, which are delicate structures with high surface area that produce spores, the primary

reproductive mechanism for mushrooms. Spores are small particles that fall from the gills or pores and get carried by wind, spreading and allowing new mushrooms to grow where the spores land (Cooke, 1979).

Fungi play a vital role in the soil environment by facilitating the cycling of nutrients. They make nutrients biologically available by chemically weathering minerals and organic matter in soil. They work with bacteria to produce cell materials (Went and Stark, 1968). Fungi are uniquely able to interact with cells of many organisms, making them an excellent bridge between soil microbes and plants (Went and Stark, 1968). Breakdown of clay minerals frees inorganic ions, such as silicon, potassium, and others, while breakdown of organic matter first produces humus and later, organic carbon complexes, such as sugars, that can be taken up by biota (Sposito, 2008). In order for a metal to be biologically accessible, it must be soluble and fungi are particularly effective in changing the solubility of metals by employing three major reaction types that change speciation of metal complexes: reduction, methylation, and dealkylation (Morley et al., 1996). These reactions are specific between certain metals and certain fungal species, but the general ability of fungi to solubilize metals is ubiquitous (Morley et al., 1996). Fungal hyphae are intricately connected with root systems of plants, making the exchange of nutrients possible and, moreover, constant (Colpaert and Van Tichelen, 1996).

### *Bioaccumulation*

Mushrooms are known to take up metals, but they are also bioaccumulators of metals. Bioaccumulation factor (BAF) is the ratio of concentration of a compound in an organism to the concentration of that compound in the substrate. An organism with BAF greater

than one is considered hyperaccumulator, meaning that there is more of that compound in the organism than there is in the substrate. The purpose of fungi taking up metals is only partially known. Many fungi have a symbiotic relationship with plants, where the roots of the plant are heavily intermingled with hyphae of fungi. This allows the plant to exploit decomposition and take up the freed nutrients and the fungi to feed on the abundant carbon from the plant (Wild, 1993). Fungi not only take up the metals that they need for growth and pigmentation, but fungi also take up additional metals and in excessive quantities to protect plants from potentially fatal concentrations of metals in soil (Morley et al., 1996). The ability for fungi to take up metals suggests that there is potential for using fungi as a bioremediation technique, termed mycoremediation.

### *Metals in Fungi*

Certain metals are biologically active in mushrooms, including sodium, magnesium, potassium, calcium, manganese, iron, cobalt, nickel, copper and zinc. These metals are selectively bound to receptors in the membranes of fungi, following a physico-chemical model of many binding sites that differ in their binding affinities (Tobin et al., 1990). Toxic metals that are found in soils, often due to anthropogenic activities, can harm fungi by competing for binding with metals that fungi need (Hughes and Poole, 1989). Some organisms, particularly fungi, are able to adapt to their environment and metal-related conditions of the soil (Giller et al., 2009). Metals are heavily involved in central processes in fungi, including metabolism and reproduction, so changes in metal environment can affect almost any process in fungi (Gadd, 1993).

## Methods

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### *Field Methods*

#### *Field Area*

All samples were collected in Lewiston, Auburn, Sabattus and Topsham, Maine. Eight cemeteries were sampled, as well as three control areas. Cemeteries were chosen based on whether they had graves dated 1865-1910. From the cemeteries that fit this criteria in the study area, those for which access was granted were visited. Samples were collected whenever they were found.

#### *Sampling*

When a mushroom or puffball was located, it was first photographed *in situ*, along with the headstone to which it was closest. The information on the headstone and the GPS coordinate were recorded. Identification to the genus level was attempted with a basic field guide. The sample was then collected using a spade to dislodge the mushroom without damaging it. The sample was stored in a plastic bag. Any mushrooms growing as part of a cluster within inches of each other were collected as one sample and stored in one sample bag. Samples greater than 12 inches apart were considered separate samples. Soil was sampled from 6 inches below grade using a spade. Soil samples were taken directly below where the fungi were growing. Soil was also stored in plastic bags.

## ***Laboratory Methods***

### ***Fungal Acid Digest***

Fungal samples were dried for at least 12 hours on watch glasses at about 45°C. They were then crushed and up to 1g was loaded into a microwave XPress tube. Each of the mushrooms was then acidified with 10 mL of trace grade HNO<sub>3</sub> and 2 mL of trace grade HCl. They were then run in the microwave. Once digested, the samples were diluted with E-Pure water to 50 mL. The diluted samples were then run on an ICP-OES in triplicate to obtain metal concentrations.

### ***Soil Acid Digest***

Soil samples were dried for at least 12 hours in crucibles at about 45°C. Up to 1 g was then loaded into a microwave XPress tube. Each soil sample was then acidified with 10 mL of trace grade HNO<sub>3</sub> and 2 mL of trace grade HCl. They were then run in the microwave. Once digested, the samples were diluted with E-Pure water to 50 mL. The diluted samples were then run on an ICP-OES in triplicate to obtain metal concentrations.

## ***Analytical Methods***

### ***Wavelength Selection***

Numerous wavelengths were tested, but the ones that returned the best results were used for elemental comparison. Those that were run for arsenic were 189.0, 193.7, 197.2, and 200.3 nm and 200.3 nm was used for most analysis. The only wavelength run for calcium was 422.6 nm. Iron was tested at 259.9 nm. Potassium was tested 766.4 nm. Magnesium was run at 279.5 nm. Sodium was run at both 589.5 and 818.3 nm, and 589.5

was used for analysis. Lead was run at 182.2, 216.9, 220.3, 261.4, 283.3 and 220.3 nm was used for analysis. Zinc was analyzed at 213.8 nm.

### *Data Processing and Analysis*

Minima, maxima, and means were calculated using JMP. SPSS was used to perform a discriminant function cross-validation test. The four arsenic wavelengths were used together in two ways to determine what arsenic alone was showing. The first was with all of the values below the detection limit set to zero because they had such low levels, and the second was to eliminate those samples all together and perform analysis on the detectable values alone.

## Results

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### *Each Element (Table 1)*

#### *Potassium*

Potassium had range of 755-5980 mg/kg in soil and 9750-49600 mg/kg in fungi. The mean concentrations were 2300 mg/kg in soil and 30300 mg/kg in fungi, with higher concentrations in fungi and lower concentrations in soil. Potassium consistently had higher concentrations in soil in control areas than cemeteries. This element had slightly higher concentrations in control area fungi and slightly lower concentrations in cemetery fungi. Potassium was most concentrated in Dostie soil and least concentrated in Briggs, Brookvale, and Mount View Cemeteries, and Thorncrag soil. It was most concentrated in Brookvale and Fisher Cemeteries, Bates and Dostie fungi and least concentrated in Briggs Cemetery fungi.

#### *Sodium*

Sodium ranged from 53.1-464 mg/kg with a mean of 185 mg/kg in soil and 7.5-6,140 mg/kg with a mean of 690 mg/kg in fungi. There were higher concentrations in fungi than in soil, control area soil than cemetery soil, and cemetery fungi than control area fungi. Sodium was most concentrated in Bates and Dostie soil and least concentrated in Briggs and Mount View Cemeteries' soil. It was most concentrated in fungi from Fisher and Wrights Cemeteries and least concentrated in fungi from Mount View Cemetery.

<b>Element</b>	<b>Soil</b>			<b>Fungi</b>		
	<i>Min</i>	<i>Max</i>	<i>Mean</i>	<i>Min</i>	<i>Max</i>	<i>Mean</i>
Potassium	759	5980	2300	9750	2590	30300
Sodium	53.1	464	185	7.5	6140	690
Zinc	21.3	119	54	18.4	883	107
Calcium	BDL	11000	2850	BDL	3540	576
Iron	5650	39100	16800	33.5	9730	1210
Magnesium	936	5230	2670	522	2590	1210
Arsenic	BDL	19.2	BDL	BDL	7.5	BDL
Lead	4.4	193	29.4	BDL	86.7	3.6

Table 2. Results of this study in mg/kg. BDL = below detection limit.

### *Zinc*

For zinc in soil, the range and mean were 21.3-119 and 54.0 mg/kg. Zinc in fungi had a range and mean of 18.4-883 and 107 mg/kg. Zinc had higher concentrations in fungi and lower concentrations in soil. For soil, there was little difference between cemetery and control areas, but fungi showed slightly higher concentrations in control areas than in cemeteries. Zinc was most concentrated in Dostie and North Auburn Cemetery soil and least concentrated in Brookvale Cemetery soil. It was most concentrated in Briggs Cemetery fungi and least concentrated in Brookvale Cemetery fungi.

### *Calcium*

Maxima for calcium in fungi and soil were 3540 and 11000 mg/kg, respectively. Both had minima below the detection limit. Means for calcium in fungi and soil were 576 and 2850 mg/kg. This element was about the same in fungi from cemeteries and control areas. Calcium was most concentrated in the control area soils and least concentrated in Wrights Cemetery soil. It was most concentrated in Bates and Thorncrag fungi and least concentrated in Brookvale, Herricks, and North Auburn Cemetery fungi.

### *Iron*

The maximum value of iron in soil was 39,127.0 mg/kg and the minimum in soil was 5,654.4 mg/kg. The maximum value of iron in fungi was 9,727.0 mg/kg and the minimum in fungi was 33.5 mg/kg. The mean was 16,796.4 mg/kg in soil and 1211.4 mg/kg in fungi. This element generally had higher concentrations in soil and lower concentrations in fungi. This element was about the same in cemeteries and control areas. Iron was most concentrated in Dostie and Fisher Cemetery soil and least concentrated in Briggs, Brookvale, and Mount View Cemeteries soil. It was most concentrated in Bates fungi and least concentrated in Thorncrag fungi.

### *Magnesium*

Magnesium's range and mean were 936- 5,240 and 2670 mg/kg in soil and 522-2590 and 1200 mg/kg in fungi. Magnesium concentrations were higher in soil than fungi and control area soil than cemetery soil with little difference between cemeteries and control areas. In soil, magnesium was most concentrated at Dostie and least concentrated at Brookvale Cemetery. In fungi, it was most concentrated at Bates and least concentrated at Thorncrag.

### *Arsenic*

The maxima of arsenic were 19.2 and 7.5 mg/kg and in soil and fungi. Both minima as well as many other samples were below the detection limit, making both means unusable. Arsenic had higher concentrations in soil than in fungi, cemetery soil than

control area soil and control fungi than cemetery fungi. In soil, arsenic was most concentrated in Fisher Cemetery and Thorncrag soil and below detection level at Bates and Briggs, Brookvale, Mount View, North Auburn, and Wrights Cemetery. It was most concentrated in Dostie fungi and below detection limit in fungi from Brookvale, Herricks, Mount View, North Auburn, and Wrights Cemeteries and Thorncrag.

### *Lead*

In soil, lead ranged from 4.4-193 with a mean of 29.4 mg/kg. In fungi, lead had a maximum and mean of 86.7 and 3.6 mg/kg, with a minimum below the detection limit. Lead generally had higher concentrations in soil than fungi, cemetery soil than control area soil, and control area fungi than cemetery fungi. In soil, lead was most concentrated in Briggs Cemetery and Thorncrag soil and least concentrated in Fisher and Wrights Cemeteries. In fungi, it was most concentrated in Briggs Cemetery fungi and least concentrated in Fisher Cemetery.

### *Relationships between Fungi and Soil*

Based on these data, there are two distinct groups of metals in relation to fungi. The first group is comprised of the metals that have higher levels in soil than in fungi, and includes arsenic, calcium, iron, lead, and magnesium. The other group is the metals that are bioaccumulated by fungi, which includes potassium, sodium, and zinc.

There was no direct correlation between soil and fungus concentrations of any metal. That is to say, when plotted as  $[M]_{\text{fungi}}$  versus  $[M]_{\text{soil}}$ , the  $R^2$  value was well below 1 for all metals, in fact below 0.1 for all metals. This means that the amount of a certain metal

in the surface soil does not reflect the amount of that metal in a mushroom growing there. It is possible there would be a correlation between fungi and soil concentrations if the soil samples were taken deeper, where the fungi could be drawing in nutrients through their long-reaching hyphae network.

<b>Used in Comparison</b>	<b>Differentiated Parameters</b>	<b>Accuracy</b>
All Elements	Soil & Mushrooms	100%
	Cemeteries & Control Areas	88%
	Each location	57%
Arsenic only BDL=0	Soil & Mushrooms	69%
	Cemeteries & Control Areas	73%
	Each location	20%
Arsenic only BDL excluded	Soil & Mushrooms	97%
	Cemeteries & Control Areas	76%
	Each location	50%

Table 3. Results of discriminant function cross-validation test. BDL = below detection limit.

### ***Relationships among Cemeteries and Control Areas***

More interesting results emerge with more robust statistical analysis accounting for multiple wavelengths measured (Table 3). Using a discriminant function cross-validation test with all of the wavelengths chosen for analysis, it is possible to tell the difference between soil and fungi with 100% accuracy, which is no surprise based on the distinct ranges for any given element in soil versus the range in fungi. It is also possible to distinguish between cemeteries and control areas with 88% accuracy based on all of the elemental data. The difference between locations can be predicted with 57% accuracy based on these data.

When only arsenic is accounted for, it is also possible to make these distinctions (Table 3). When the values that are below the detection limit are set to zero and included,

the difference between soil and fungi is 69% accurate. This also leaves the difference between cemeteries and control areas 73% accurate and the difference between each location 20% accurate. Then the samples that were below detection limit for all four arsenic wavelengths were excluded, the difference between soil and fungus was 97% accurate. Without the samples with non-detectable arsenic, the difference between cemeteries and control areas was 76% accurate and the difference between each location was 50% accurate.

## Discussion and Conclusions

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### *Each Element*

Concentration values of each of the eight elements tested agrees with published values for both soil and fungi, which are shown here. This section also discusses the uses of each element in fungi, if applicable, which helps illuminate some of the concentrations measured. All of the published values and those of this study are listed in Table 4.

### *Potassium*

Karadeniz and Yaprak (2010) report potassium values of 22945-47023 mg/kg in fungi. Yoshida and Muramatsu (1998) found that potassium ranges from 12700-51700 mg/kg with a mean of 28700 mg/kg for fungi and a mean of 20100 mg/kg for soil. Tyler (1980) reports a range of potassium in fungi of 2200-135000 mg/kg.

The published values for potassium closely mimic those found in this study. The maximum value of potassium in fungi in this study was about 50 mg/kg, which is remarkably close to the published maxima of 47 and 52 mg/kg (Karadeniz and Yaprak, 2010; Yoshida and Muramatsu, 1998). The maxima of 135 mg/kg is well above that of this study, but is in the same order of magnitude (Tyler, 1980). The minimum concentration of potassium in fungus in this study of 9750 mg/kg falls in the middle of the set of published minima of 22945, 12700, and 2200 mg/kg (Karadeniz and Yaprak, 2010; Yoshida and Muramatsu, 1998; Tyler, 1980). The literature also supports the finding that potassium levels tend to be higher in fungi than in soil.

Element	Soil			Fungi			Source
	Min	Max	Mean	Min	Max	Mean	
Potassium	754.8	5977.3	2295.3	9752.2	2590.2	30305.5	<i>This Study</i>
	-	-	-	22945	47023	-	Karadeniz and Yaprak, 2010
	-	-	-	2200	135000	-	Tyler, 1980
	-	-	20100	12700	51700	28700	Yoshida and Muramatsu, 1998
Sodium	53.1	464.2	185.2	7.5	6142.7	690.2	<i>This Study</i>
	-	-	-	80	240	-	Karadeniz and Yaprak, 2010
	-	-	-	10	3970	-	Tyler, 1980
	-	-	15900	75	1970	957	Yoshida and Muramatsu, 1998
Zinc	21.3	119.2	54	18.4	882.9	107.1	<i>This Study</i>
	-	-	-	44	381	-	Byrne and Ravnik, 1976
	61	141	-	43	350	-	Giannacci et al., 2012
	-	-	-	297	4325	-	Karadeniz and Yaprak, 2010
	-	-	-	-	162	-	Mendil et al., 2005
	-	-	-	34.43	98.99	-	Ouzouni et al., 2009
	-	-	-	-	99.3	-	Ozcan et al., 2013
	-	-	-	43.5	205	-	Sesli et al., 2008
	-	-	-	33.5	89.5	-	Soylak et al., 2005
	-	-	-	-	-	122	Turkekul et al., 2004
-	-	-	9	1025	-	Tyler, 1980	
Calcium	BDL	11025.2	2846.3	BDL	3543.4	575.8	<i>This Study</i>
	-	-	-	20	200	-	Karadeniz and Yaprak, 2010
	-	-	-	118	472	-	Stijve et al., 2002
	-	-	11900	63	1990	535	Yoshida and Muramatsu, 1998
Iron	564.4	39127	1676.	33.5	9727	11211.4	<i>This Study</i>
	-	-	-	340	4850	-	Karadeniz and Yaprak, 2010
	-	-	-	-	628	-	Mendil et al., 2005
	-	-	-	38.9	499.0	-	Ouzouni et al., 2009
	-	-	-	150	1741	-	Sesli et al., 2008
	-	-	-	102	1580	-	Soylak et al., 2005
	-	-	-	51	2080	-	Stijve et al., 2002
	-	-	-	-	-	3904	Turkekul et al., 2004
	-	-	-	8	480	-	Tyler, 1980

Element	Soil			Fungi			Source
	Min	Max	Mean	Min	Max	Mean	
Magnesium	935.7	5235.3	2669.7	522.3	2590.2	1205.4	<i>This Study</i>
	-	-	-	540	6210	-	Karadeniz and Yaprak, 2010
	-	-	-	688.7	1150.7	-	Ouzouni et al., 2009
	-	-	-	440	4180	-	Tyler, 1980
	-	-	6290	683	1580	1090	Yoshida and Muramatsu, 1998
Arsenic	BDL	19.2	BDL	BDL	7.5	BDL	<i>This Study</i>
	-	-	-	0.065	6.8	-	Byrne and Ravnik, 1976
	-	-	-	0.44	1.48	-	Chen et al., 2009
	4.2	18.8	-	0.02	2.7	-	Giannacci et al., 2012
	-	-	-	BDL	<0.02	-	Ouzouni et al., 2009
	6.5	65	-	BDL	125	-	Slekovek and Irgolic, 1996
	-	-	-	-	-	0.5	Soeroes et al., 2004
	-	-	-	BDL	149.6	-	Vetter, 2004
Lead	4.4	192.9	29.4	BDL	86.7	3.6	<i>This Study</i>
	-	-	-	1.9	10.8	-	Chen et al., 2009
	22	51	-	0.4	15.5	-	Giannacci et al., 2012
	-	-	-	0.60	10.9	-	Karadeniz and Yaprak, 2010
	-	-	-	BDL	11.4	-	Mendil et al., 2005
	-	-	-	BDL	1.16	-	Ouzouni et al., 2009
	-	-	-	0.9	2.6	-	Sesli et al., 2008
	-	-	-	0.75	1.99	-	Soylak et al., 2005
	-	-	-	0.51	2.4	-	Stijve et al., 2002
	-	-	-	-	-	2.7	Turkekul et al., 2004
	-	-	-	0.4	36	-	Tyler, 1980

Table 4. Reported concentrations from this study and published studies in mg/kg. BDL = below detection limit.

### *Sodium*

Karadeniz and Yaprak (2010) found that fungi had concentrations of sodium ranging from 80-240 mg/kg. Yoshida and Muramatsu (1998) found that sodium ranges from 75-1970 mg/kg with a mean of 957 mg/kg for fungi and a mean of 15900 mg/kg for soil. Tyler (1980) reports a range of sodium in fungi of 10-3970 mg/kg.

The published concentrations for sodium are similar to those found here, but the ranges fall within the range found here. This study found a range of 7.5-6140 mg/kg, which has a lower minimum and higher maximum than all three studies. The reported mean for soil was much higher than that found here, which was 185 mg/kg. This contradicts the finding of this study that sodium concentrations tend to be higher in fungi than in soil.

### *Zinc*

Sesli et al. (2008) found that zinc had concentrations ranging from 43.5-205 mg/kg. Giannaccini et al. (2012) found the range for zinc was 43-350 mg/kg in fungi and 61-141 mg/kg in soil. Ozcan et al. (2013) found fungi concentrations of zinc up to 99.3 ppm on substrates enriched with zinc. Byrne and Ravnik (1976) found similar zinc concentrations, ranging from 44-381 ppm. Karadeniz and Yaprak (2010) show a range zinc concentrations in fungi of 297-4325 mg/kg. Ouzouni et al. (2009) reported zinc concentrations in fungi in the range 34.43-98.99 mg/kg. Turkekul et al. (2004) found a mean zinc concentration in fungi of 122 mg/kg. Mendil et al. (2005) reported the zinc concentration maximum in fungi at 162 mg/kg. Soylak et al. (2005) showed a zinc range

of 33.5-89.5 mg/kg in fungi. Tyler (1980) reports a range of zinc in fungi of 9-1025 mg/kg.

The values for zinc in fungi found in this study ranged from 18.4-883 mg/kg with a mean of 107 mg/kg. These values are supported by the published values, except that of Karadeniz and Yaprak (2010), which is distinctly higher than the other published values. The range of soil concentrations of zinc given by Giannaccini et al. (2012), 61-141 mg/kg, closely mimics that of this study, 21.3-119 mg/kg. The reported ranges for zinc in fungi are higher than those of zinc in soil, which supports the findings of this study.

### *Calcium*

Karadeniz and Yaprak (2010) found a range of 20-200 mg/kg for calcium in fungi. Yoshida and Muramatsu (1998) found that calcium ranges from 63-1990 mg/kg with a mean of 535 mg/kg for fungi and a mean of 11900 mg/kg for soil. Stijve et al. (2002) found a range of calcium in fungi of 118-472 mg/kg.

The published values for calcium are similar to those found from this work. The maximum in fungus from this study was 3540 mg/kg, which is much more than any of the published maxima, but the mean, 576 mg/kg, is close to the mean reported by Yoshida and Muramatsu (1998) of 535 mg/kg. The mean reported for soil of 11900 mg/kg is remarkably close to that of this study, 11000 mg/kg. Yoshida and Muramatsu (1998) found that calcium levels are higher in soil than in fungi, which is supported by this study. Tyler (1980) reports a range of calcium in fungi of 11-6720 mg/kg.

### *Iron*

Sesli et al. (2008) found that iron had concentrations ranging from 150-1741 mg/kg. Karadeniz and Yaprak (2010) report concentrations of iron in fungi ranging from 340-4850 mg/kg. Ouzouni et al. (2009) showed that iron ranges from 38.9-499.0 mg/kg in fungi. Turkekul et al. (2004) found a mean iron concentration in fungi of 3904 mg/kg. Mendil et al. (2005) reported the iron concentration maximum in fungi at 628 mg/kg. Soylak et al. (2005) showed a iron range of 102-1580 mg/kg in fungi. Stijve et al. (2002) found a range of iron in fungi of 51-2080 mg/kg. Tyler (1980) reports a range of iron in fungi of 8-480 mg/kg.

The literature shows similar values for iron concentrations in fungi as those found in this study. The minimum reported by Ouzouni et al. (2009), 38.9 mg/kg, was very close to 33.5 mg/kg, which was found in this study. The mean of this study, 1210 mg/kg, falls within four of the ranges reported (Sesli et al., 2008; Yaprak, 2010; Soylak et al., 2005; Stijve et al., 2002).

### *Magnesium*

Karadeniz and Yaprak (2010) found that magnesium ranges from 540-6210 mg/kg in fungi. Yoshida and Muramatsu (1998) found that magnesium ranges from 683-1580 mg/kg with a mean of 1090 mg/kg for fungi and a mean of 6290 mg/kg for soil. Ouzouni et al. (2009) found that magnesium ranges from 688.7-1150.7 mg/kg in fungi. Tyler (1980) reports a range of magnesium in fungi of 440-4180 mg/kg.

The values found in this study fit in nicely with the reported values. The minima are all similar, as are the mean from this study, 1200 mg/kg, and that reported by Yoshida

and Muramatsu (1998), 1090 mg/kg. The published maxima show some variation (1150.7, 1580, 4180, 6210 mg/kg), but the maximum for this study falls directly in the center of the values at 2590 mg/kg (Ouzouni et al., 2009; Yoshida and Muramatsu, 1998; Karadeniz and Yaprak, 2010; Tyler, 1980). The reported mean concentration of magnesium in soil of 6290 mg/kg is higher than that of this study, 2670 mg/kg (Yoshida and Muramatsu, 1998). Their findings also show that magnesium levels are higher in soil than in fungi, which supports this study (Yoshida and Muramatsu, 1998).

### *Arsenic*

Giannaccini et al. (2012) found a range of arsenic concentrations from 0.02-2.7 mg/kg in fungi and 4.2-18.8 mg/kg in soil. Chen et al. (2009) found arsenic ranging from 0.44-1.48 mg/kg in mushrooms. Byrne and Ravnik (1976) found arsenic concentrations ranging from 0.065-6.8 ppm in fungus. Slekovec and Irgolic (1996) found fungal arsenic concentrations up to 125 mg/kg in one species and 33 mg/kg in others, as well as a soil range of 6.5-65 mg/kg. Ouzouni et al. (2009) found that arsenic concentrations in fungi were all below their detection limit of 0.02 mg/kg. Vetter (2004) found an arsenic concentration range for fungi of less than 0.05 mg/kg to 146.9 mg/kg. Soeroes et al. (2004) found a mean of 0.50 mg/kg in fungi.

The published values for arsenic concentrations in both fungi and soil are very similar to those found in this study. All reported fungal minima were below the detection limit of this procedure, as were most of the samples analyzed in this study. The minima for arsenic in soil in this study were somewhat lower than the published values, but the published minima were already near the detection limit of this study. Giannaccini et al.

(2012) found that arsenic levels are higher in soil than in fungi, which is supported by this study.

### *Lead*

Giannaccini et al. (2012) found a range of lead concentrations from 0.4-15.5 mg/kg in fungi and 22-51 mg/kg in soil. Sesli et al. (2008) found that lead had fungal concentrations ranging from 0.9-2.6 mg/kg. Ozcan et al. (2013) found fungi concentrations of lead up to 62.8 ppm on substrates enriched with lead. Chen et al. (2009) found arsenic ranging from 1.9-10.8 mg/kg in mushrooms. Karadeniz and Yaprak (2010) found lead concentrations in fungi to be in the range of 0.60-10.9 mg/kg. Ouzouni et al. (2009) found that lead ranges from below their detection limit to 1.16 mg/kg for fungi. Turkekul et al. (2004) found a mean lead concentration in fungi of 2.7 mg/kg. Mendil et al. (2005) reported the lead concentration maximum in fungi at 11.4 mg/kg. Soylak et al. (2005) showed a lead range of 0.75-1.99 mg/kg in fungi. Stijve et al. (2002) found a range of lead in fungi of 0.51-2.4 mg/kg. Tyler (1980) reports a range of lead in fungi of 0.4-36 mg/kg.

The data reported in the literature closely resemble those found in this study. All of the reported minima were below the detection limit of this study, as were some of the fungal samples tested for lead. While the maxima for this study was above those that were reported, the mean was within the ranges reported for almost all of the mushroom studies. The only published soil lead range falls within the range of this study. The mean from this study falls within the reported range, which is much tighter than the range of

this study. Giannaccini et al. (2012) also found that lead levels are higher in soil than in fungi, which supports this study.

### ***Fungi versus Soil***

There is a distinct difference between fungi and soil samples, based on all of the elements or based on arsenic alone. The most marked distinction between soil and fungi was in iron, with fungi ranging from 33.5-9730 mg/kg and soil ranging from 5650-39100 mg/kg. It is no surprise that soil and fungi would have different concentrations of metals. The elements that are abundant in minerals are not necessarily those that would be used by fungi, so there is often no benefit for fungi to collect them. Some elements, however, are important for fungi and for the plants with which fungi share their nutrients. For those elements, it would be expected that there are higher concentrations in fungi than in the substrate, meaning that they are being bioaccumulated. Potassium, sodium and zinc are the three elements in this study that are more concentrated in fungi than in soil.

### ***Concentrated Elements: Potassium, Sodium and Zinc***

Potassium, sodium and zinc are elements that are necessary for plant and fungal growth (Baldrian, 2010; Ozcan et al., 2013; Sesli et al., 2008; Soylak et al., 2005). For this reason, many fungi are able to solubilize numerous species of insoluble potassium, sodium, and zinc and collect them (Baldrian, 2010). Potassium is a macronutrient in cells and plays a large role in growth of fungal cells. It regulates cellular osmotic potential and transport processes, as well as binding to proteins and activating critical enzymes. Potassium deficiency inhibits glycolysis and respiration, two processes that are central to

fungal metabolism and growth (Garraway and Evans, 1984). Sodium plays key roles in growth, respiration, and fermentation in fungal cells. In addition, it has been shown to stimulate phosphate uptake, which in turn promotes production of DNA, RNA, and other critical molecules (Garraway and Evans, 1984). Zinc is a major participant in metabolism; hormone, nucleic acid, and protein synthesis; and muscle and brain function (Giannaccini et al., 2012; Garraway and Evans, 1984).

As such, it is critical for fungi, plants, and animals to consume potassium, sodium, and zinc, and fungi are integral in making this happen. Fungi and plants form a symbiotic relationship in which fungi help plants acquire trace nutrients in exchange for carbon (Martino and Perotto, 2010). About 80% of plant roots participate in this type of relationship, meaning that almost all nutrients taken up by plants pass through fungi first (Martino and Perotto, 2010). The job of fungi, then, is to accumulate essential elements for themselves, plants, and the animals that might consume the plants. This means that fungi specialize in concentrating these elements and passing them along to plants, so their fruiting bodies should have higher concentrations than their soil substrate.

#### *Biologically Active Non-concentrated Elements: Calcium, Iron and Magnesium*

Calcium, iron, and magnesium all have lower concentrations in fungi than in soil, but are known to be biologically active. Calcium and magnesium are both macronutrients, while iron is a micronutrient, which shows up when the soil numbers are compared to the fungi numbers from this study (Garraway and Evans, 1984). The means for calcium and magnesium in fungi are roughly 20% and 45% of the soil means, respectively. The mean of iron in fungi is only 7% of the mean of iron in soil. This suggests that while there is

plenty of all three elements available to the fungi, the fungi is accumulating a greater fraction of the two macronutrients, calcium and magnesium.

Calcium is known to enhance growth and reproduction and play critical roles in cell membranes and structural components of fungi, as well as serving as an integral regulator of cellular processes (Garraway and Evans, 1984; Pitt and Ugalde, 1984). Magnesium has a more extensive and diverse set of roles in fungal cells, including having regulatory roles, binding ATP and ADP, facilitating phosphate transfer, playing membrane and structural roles, participating in cell division and nucleic acid synthesis, and serving as a cofactor for a diverse set of enzymes (Garraway and Evans, 1984). While it is considered a micronutrient, iron is also an important element in fungi. Iron is an enzyme activator, participates in electron transfer in hemelike porphyrins, and complexes with genetic material, playing a role in gene expression. Iron deficiency has particularly marked repercussions, including inhibition of DNA synthesis and less ATP production. The most impressive part of the relationship between fungi and iron, however, is the ability of fungi to secrete a chelator that can very effectively bind iron, solubilizing it and allowing for it to be brought into the cell (Garraway and Evans, 1984). Calcium, iron, and magnesium are all elements that are critical to biological processes, but only in small amounts relative to the amount of each that is available in soil.

#### *Non-biological Elements: Arsenic and Lead*

Arsenic and lead, the two remaining elements that were analyzed in this study, are both not biologically active and very toxic at high levels. These two elements, not surprisingly, were found at very low levels in fungi, both with minima below the

detection limit of the method. Neither of these elements is very common in soil, as shown by the mean concentrations of arsenic and lead in soil being 3.0 and 29.4 mg/kg, respectively, much lower than the other elements. It has also been shown that the arsenic concentrations in soil in this study are elevated in cemeteries, which means that this value of 3.0 mg/kg for soil is higher than the mean of typical, non-cemetery soil.

### *Cemeteries versus Control Areas*

Another central result that emerges from the data is that there is a distinction between cemeteries and control areas. This distinction is 88% accurate when all elements are compared and 76% accurate when only detectable arsenic values are compared.

The data show that there is more arsenic in soil in cemeteries than in soil outside of cemeteries. There are two known sources of arsenic in soil in Maine: arsenic coming from bedrock, or former use of arsenic pesticides, which were predecessors of DDT. In addition to those, there is the possibility of elevated arsenic in cemeteries from historic embalming practices that used arsenic-based embalming fluid. This study shows that there is elevated arsenic in cemetery soil, but all three of these histories are possibly contributing to the arsenic values measured.

The first idea, that bedrock is contributing to the concentrations of arsenic in soil, is unlikely to be the cause of elevated arsenic because the rock units are not correlated with the cemeteries that are showing arsenic in soil (Maine, 2015). The second possible source for elevated arsenic is pesticide use, mostly in apple orchards. This is also highly unlikely as a source of arsenic in cemeteries because the time frames during which arsenic was used for embalming fluid and when arsenic was used as a pesticide coincide. In fact,

embalming fluid moved to alternative poisons before pesticides, which mostly changed to DDT in the 1940s. This means that land occupied as apple orchards cannot also be land that is a historic cemetery today, which means that arsenic was not used as a pesticide in the cemeteries sampled in this study. Since bedrock and pesticides are both unlikely sources of arsenic in cemeteries, it is most likely the historic embalming practices that are bringing arsenic to the soil.

The data show that there are not higher arsenic levels in fungi from cemeteries than from control areas. This can be attributed to the lack of need for arsenic by fungi. Arsenic is a toxic metal, and fungi have no need for it in their biological processes, so they do not have the mechanisms to solubilize it and accumulate it.

### ***Location versus Location***

The data also show that there is some degree of difference between locations, but this is only 57% accurate when all elements are compared, and even less accurate when only arsenic is compared. There are two major contributors to this effect. The first is that in some cases, a single location has only one type of mushroom. The second is that each location has slightly different soil characteristics based on different conditions, such as underlying bedrock, vegetation, groundwater chemistry, and anthropogenic influences. These controls on soil are probably a major deciding factor on which species exist at each site, along with the fact that fieldwork was conducted in the fall, a time when only a few robust species still had fruiting bodies.

### ***Final Remarks on Arsenic in Cemeteries***

There were several other small findings of this study that are worth noting. The EPA and FDA give action levels for drinking water and apple juice, respectively, and both are set at 0.01 ppm, which is well below the detection limit of this study (EPA, 2014; FDA, 2013). This number, however, is for soluble forms of arsenic that would easily enter body tissue if consumed. While some of the values in this study are way above this limit, the values here are for soil and fungi, which contain arsenic that is much less readily available. The FDA is in the process of setting an arsenic action level for rice after recent concern, and preliminary data show levels up to 0.7 mg/kg, which is still below the detection limit of this study (FDA, 2012). In addition, only one cemetery, Mount View, had edible mushrooms (*Boletinus*). All of the samples from that site were below the detection limit for arsenic. In addition, there was no difference between arsenic levels in fungi from cemeteries than fungi from control areas, so, according to this data, a historic cemetery is of no concern to a hungry mycologist.

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