

Analysis of Toxic Metals in *Lentinus edodes* by Atomic Absorption Spectroscopy

Introduction

For over 70 years manufacturing companies and the city of Chattanooga have been depositing waste into the Chattanooga Creek, which flows into the Tennessee River. In 1994, the EPA declared a tract of land, which includes an area of the Chattanooga Creek, a Superfund Site. The EPA gated the area of land because it is so severely contaminated by pollution.¹ Outside the gated area, along the banks of the creek, is an organic farm named Crabtree Farms. Covering over 22 acres, Crabtree Farms provides plots for community gardening and promotes research and education.² In May 2003, Chattanooga residents experienced heavy rainfall for nearly a week, resulting in severe flooding. The Chattanooga Creek overflowed its banks, covering most of Crabtree Farms with contaminated water.

Shiitake mushrooms (*Lentinus edodes*) are one of the main crops grown at Crabtree Farms. Near the back of their property, the *Lentinus edodes* are cultivated on a substrate of oak logs. The mycelium for *Lentinus edodes* is placed in drilled holes and left to grow.³ (See Figure 1)⁴

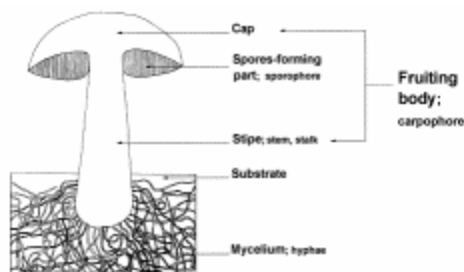


Figure 1: Schematic of a mushroom⁴

The logs can be used for up to 5 years, harvesting many crops of mushrooms. During the flood, the logs were submerged in the contaminated water for several days. Farm employees are concerned that metal contaminants in the creek may have concentrated within the logs and are being passed on to the mushrooms. Therefore, we will collect samples of the *Lentinus edodes* from Crabtree Farms to analyze the mushrooms for toxic metals.

Research Proposal

The goal of this proposal is to analyze *Lentinus edodes* for toxic metals using Atomic Absorption Spectroscopy (AAS). AAS is based on the principle that vaporized atoms will

¹ PCSD Briefing Book. *Superfund Remediation and Tennessee Products NPL Sites*. 29 Feb. 2004. www.chattanooga.net/membersites/sustain/pcsd_briefing_book/resources_chat_creek.html

² *Crabtree Farms*. 4 March 2004. <http://www.crabtreefarms.org/about/about.html>

³ Potts, Gretchen. Personal Interview. 25 Feb. 2004.

⁴ Kalacĭ, P and Svoboda, L. *Food Chemistry*. 69, 2000, 273 – 281.

absorb quantized light, indicative of the elements present.⁵ The analysis requires a homogeneous liquid sample. Therefore, the mushrooms will first be dried, ground and sieved so that the sample is homogeneous. The matter will then be digested in a microwave digestion bomb using oxidizing agents such as hydrochloric acid, nitric acid, sulfuric acid and hydrogen peroxide. Once the sample is digested, it will be analyzed by AAS. The mushrooms will be collected at Crabtree Farms and digested using EPA standard methods. Calibration curves will be made from standard reference samples to determine the concentration of metals in the unknown mushrooms. Additionally, standard reference materials (SRMs) from the National Institute of Standards and Technology (NIST) will be digested and analyzed. This will help us to determine any error in our digestion technique or analysis.

Mushrooms collection from forests is a popular pastime in Eastern Europe. Therefore, numerous studies have been performed to determine metal content in wild mushrooms grown in the soil. However, studies have yet to focus on cultivated mushrooms grown on tree logs. This area of research is untouched and needs to be explored. Therefore, we will determine if *Lentinus edodes* absorb large amounts of toxic metals from tree logs.

Research Plan:

1. **Atomic Absorption Theory:** I will learn about atomic absorption theory. I will also learn how to operate the AAS.
2. **Technique Preparation:** I will learn how to prepare standards from stock solutions and run calibration curves.
3. **Sample Collection:** I will collect samples from the logs at Crabtree Farms over the summer and into the 2004 – 2005 academic for comparison analysis. Multiple analyses will help me to determine if the metals persist in the mushrooms over a period of time.
4. **Sample Digestion:** I will learn several procedures for sample digestion and determine which method is suitable for the mushrooms.
5. **Sample Analysis:** I will analyze the samples on AAS and determine metal concentrations using calibration curves.

⁵ Harris, D.C. *Exploring Chemical Analysis*. New York: W. H. Freeman, 2001.

PSRA funding is requested so that research for this project can begin during the summer of 2004 and continue into the 2004-2005 school year. I will present my findings in a presentation at the regional meeting of the American Chemical Society, held in Charlotte, NC. This research will be valuable to my plans of attending graduate school for forensic science or analytical chemistry.

Budget and Budget Justification

After drying, the collected Shiitake mushrooms will be ground and sieved. Minor equipment funds are requested for the purchase of a 60-mesh sieve. This sieve will yield a homogenous sample with a fine particle size of 250 micrometers. Prior to analysis, the sample must be dissolved, requiring digestion acids and a microwave digestion bomb. The AAS instrument requires a constant flow of compressed air and gaseous acetylene. In addition, standard solutions must be prepared in calibrated glassware. Therefore, I have allocated \$300 for supplies including acids, AAS gases, standards and glassware. I plan to present the results of my research at the regional meeting of the American Chemical Society in the fall of 2004 and I propose \$375 of the award for travel.

Conclusion:

I propose to identify metals in the Tennessee River by (1) collecting Shiitake mushrooms (*Lentinus edodes*), (2) using AAS to analyze the samples, and (3) preparing calibration curves of standard samples and comparing them to the unknown samples. The detection of toxic metals in the Shiitake mushrooms can help design environmental measures to help lower the amount of toxic metals released into the environment by area industries.