Lead and Arsenic in *Morchella esculenta* Fruitbodies Collected in Lead Arsenate Contaminated Apple Orchards in the Northeastern United States: A Preliminary Study

by Elinoar Shavit[1] and Efrat Shavit[2]

Abstract

Twenty-nine paired samples of soil and *Morchella esculenta* fruitbodies were collected from abandoned apple orchards that were potentially contaminated with lead arsenate pesticides in the northeastern United States. Soil and fruitbody samples were tested for lead and arsenic content using Inductively Coupled Plasma–Mass Spectrometry (ICP-MS). Statistical analysis was performed on the results to determine summary statistics, correlation coefficients, and linear regression output. To determine the form of arsenic present, speciation analysis of arsenic was performed on one sample of *M. esculenta,* and 94% of the arsenic stored in the tissues of the mushroom was found to be in an inorganic form. The ranges of lead and arsenic found in the soil were 19.20–2450.00 mg/kg and 3.08–244.00 mg/kg, respectively. The ranges of lead and arsenic in the *M. esculenta* samples were 0.05–13.00 mg/kg and 0.15–2.85 mg/kg, respectively. Statistically significant positive correlations were found between the amount of lead in the mushrooms and the amount of lead in the soil (r=0.94), the amount of arsenic in the mushrooms and the amount of arsenic in the soil (r=0.57), and the amount of lead and arsenic in the soil (r=0.81). While the levels of arsenic in *M. esculenta* did not exceed regulatory safety standards for acute duration oral intake of inorganic arsenic, some did exceed standards for chronic duration oral intake of inorganic arsenic. The levels of lead in *M. esculenta* from contaminated apple orchards were sufficiently elevated to pose potential health risks.

Keywords: *Morchella esculenta*, apple orchard, lead arsenate, inorganic arsenic, topsoil, dripline.

Introduction

In 2007, Mr. Robert Peabody, an expert amateur mycologist and longtime member of the New Jersey Mycological Association (NJMA), was diagnosed with a severe case of heavy metal poisoning (Shavit, 2008). His case made headlines when the medical team that treated him attributed his condition to the consumption of *Morchella esculenta* (morels), which he had collected for decades in abandoned New Jersey apple orchards (Shavit, 2008).[A] Our study was conducted to provide morel collectors with answers to the following questions: do *M. esculenta* growing in lead and arsenic treated orchards in the northeastern United States concentrate lead and arsenic from their growing habitats, and if so, are these toxic elements found in the fruitbodies of *M. esculenta* at levels that could pose a threat to the health of consumers?

Morels are gourmet mushrooms that are popular worldwide. They are available fresh in the spring and can be found in dehydrated form in markets and specialty stores year-round. Since efforts to commercially cultivate them have not been exceptionally successful, almost all morels on the market today have been collected in the wild. Morels command a high price in the marketplace, and gathering them has become a lucrative venture in the U.S. and abroad (Boa, 2004). In the northeastern U.S. large numbers of *M. esculenta* are gathered around dying or dead apple and American elm trees (*Ulmus americana* and *U. rubra*), many of which grow in abandoned apple orchards (Bakaitis, 2008; 2009). Elm trees tend to grow in soils with pH levels similar to those regularly found in cultivated apple orchards (pH 6.5–8), and they are often among the first to grow in abandoned apple orchards. Such orchards slowly turn into woodlands, with their former identities discernable only during the spring when the remaining apple trees are in bloom. Morel hunters may collect in the same woodlands for years without ever noticing that they were once apple orchards (Bakaitis, 2008; 2009).

The problem of lead and arsenic contamination in apple orchards previously treated with lead arsenate pesticides was brought to public attention by the case of Barber Orchard (Hood, 2006). In 2001 the 500-acre former commercial orchard west of Waynesville, North Carolina, was declared a Superfund Site. Superfund is the common name for the Comprehensive Environmental Response,

1. Elinoar Shavit, contributing editor for Medicinal Mushrooms at FUNGI, can be reached at elinoarst@gmail.com. 2. Efrat Shavit is a medical student (MD 2010) at the Washington University School of Medicine in St. Louis, MO.

Compensation, and Liability Act of 1980 (CERCLA), the federal government's program to clean uncontrolled hazardous waste sites in the United States (EPA- Superfund). In 1999–2000, following reports of numerous birth defects among the population residing near this orchard, the federal Environmental Protection Agency (EPA) conducted a $4 million emergency removal of a foot of topsoil from the yards of 28 residents at Barber Orchard (Hood, 2006).

The urbanization process that is happening in the northeastern U.S., where former farmlands are being turned into residential and commercial areas, is affected by the historical contamination caused by the prolonged use of lead arsenate (Hood, 2006). Lead arsenate ($PbHAsO_4$) was the most extensively used arsenical insecticide from the 1920s to the 1950s (Peryea, 1998). It was introduced in Massachusetts in 1892 for use against the gypsy moth (*Lymantria dispar*), but due to its outstanding control of insect pests and low phytotoxicity compared to alternatives, lead arsenate quickly became the most used arsenical pesticide in the U.S. and internationally (Peryea, 1998). From 1900 to 1980 about 49 million pounds of lead arsenate and 18 million pounds of calcium arsenate were applied to soils, orchards, and other crops in the state of New Jersey alone (Murphy and Aucott, 1988). In New England states, the application of arsenical pesticides predates systematic record-keeping, but based on cultivation practices and history, it is estimated that the cumulative application rates of arsenic could have been 200 lbs of elemental arsenic per acre of cultivation (Robinson and Ayotte, 2007). Lead arsenate was replaced by DDT in the mid-1950s (Robinson and Ayotte, 2007), and its use was finally terminated in the U.S. in 1988. Millions of acres were sprayed with lead arsenate, representing the single largest anthropogenic input of arsenic into the environment (Wong et al., 2002).

Many studies conducted in agricultural lands have concluded that almost all of the lead and arsenic derived from the lead arsenate sprayed in orchards between 1929 and 1965 is contained in the top 25 cm of soil (Peryea, 1998; Wong et al., 2002). Handgun sprayers were used to apply aqueous slurries of lead arsenate to individual trees, resulting in extreme variability in the distribution of lead and arsenic in the topsoil (Peryea, 1998). The New Special Pesticide Task Force in New Jersey found that measurements of arsenic and lead between different orchards varied considerably, and even within the same orchard arsenic levels varied by a factor of 10 or more (NJHPTF, 1999; Merwin et al., 1994). This is because most insecticides are sprayed on the canopy of the trees, allowing the excess to drip to the ground. Most of the accumulations of arsenic and lead measured in orchard soils follow the tree driplines, forming doughnut-shaped areas of elevated contamination around the trees (Peryea, 1998; Veneman et al., 1983). Veneman et al. (1983) found concentrations of lead and arsenic reaching 1400 µg/g and 330 µg/g, respectively, around trees in Massachusetts apple orchards.

Morchella esculenta is traditionally collected year after year in the potentially toxic driplines around dying apple trees. This is perhaps because the dripline is typically the limit of the root extensions upon which *M. esculenta* are thought to depend (Harbin and Volk, 1999). There is evidence that *M. esculenta* is "facultatively mycorrhizal" and has both saprophytic and mycorrhizal properties (Pilz et al., 2007). Apart from forming associations with the apple trees, morel mycelium is also capable of decomposing organic matter on its own (Pilz et al., 2007; Volk and Leonard, 1989a; b). Living in orchard topsoil, particularly in the contaminated dripline area, places *M. esculenta* in an ideal position to accumulate lead and arsenic from the soil.

Regrettably, the implementation and effectiveness of cleaning programs for lead arsenate contaminated soils is limited by high costs and logistical problems (Belluck et al., 2003; Peryea, 1998). As a result, one provisional solution has become a favorite: do nothing to disturb the orchard soil (Veneman et al., 1983). By keeping lead arsenate contaminated apple orchards in ongoing production or by neglecting the orchards altogether, the likelihood that humans will be exposed to the lead and arsenic in the soil is brought to a minimum. In addition, the arsenic and lead in the topsoil are less prone to vertical movement into the lower soil levels and waterways when the soil is not disturbed (Renshaw et al., 2006). Some of the apple growers whom we interviewed opted to leave large parcels of lead arsenate treated apple orchards untended for this reason. However, in so doing they have created perfect habitats for *M. esculenta* to thrive.

Lead and arsenic are toxic elements that have the potential to cause devastating health effects. These effects occur both from acute exposure to large doses and from chronic exposure to smaller doses of these elements. Food is the main source of arsenic exposure; and although both organic and inorganic forms of arsenic are considered toxic to humans, there is evidence that inorganic forms of arsenic are far more toxic than organic forms (ATSDR ToxGuide™-As, 2007). Arsenic exposure can lead to devastating gastrointestinal, renal, cardiovascular, and neurological effects; and chronic exposure has been linked with skin, bladder, and lung cancers. When exposed to arsenic, children exhibit symptoms similar to those seen in adults, and there is evidence that long-term exposure to arsenic in children may result in lower IQ scores (ATSDR ToxFAQs™-As 2007). The Agency for Toxic Substances and Disease Registry (ATSDR) has set two Minimal Risk Levels (MRLs) for inorganic arsenic exposure, one for acute-duration oral exposure (0.005 mg As/kg/day) and one for chronic duration oral exposure (0.0003 mg As/kg/day) (ATSDR ToxGuide™-As 2007).

Lead exposure occurs through inhalation, food ingestion, and dermal exposure. Mushroom collectors should be aware that 95% of inorganic lead inhaled from lead contaminated soil is absorbed by the body (ATSDR ToxGuide™-Pb 2007). Gastrointestinal absorption of lead depends on age; children are far more susceptible to absorbing lead through food (40-50%) than are adults (3–10%). Once absorbed, lead remains in the body for a very long time, with a 30-day half-life in blood and a 27-year half-

life in bones. Lead exposure can have deleterious effects on nearly every body system, including the hematological, gastrointestinal, renal, cardiovascular, neurological, and reproductive systems. In children, exposure to lead may have devastating physical, neurological, and developmental consequences.[B] The U.S. Centers for Disease Control and Prevention (CDC) recommends that states test children at one and two years of age, and considers a blood lead level of 10 µg/dL to be a level of concern (ATSDR ToxGuide™-Pb 2007).

In 1993 the FDA set Provisional Tolerable Total Intake Levels (PTTIL) for lead of 6 µg/day for children 6 years of age and younger, 15 µg/day for children 7 and over, 25 µg/day for pregnant women, and 75 µg/day for adults (Fed. Reg., 1993). It is important to note that neither the FDA, the CDC, nor any official US government entity has set tolerable daily intakes (TDI), reference doses (RfD), or minimal risk levels (MRL) for lead ingestion because there is no threshold below which lead intake can be proven not to cause health effects in humans (ATSDR ToxGuide™-Pb 2007).

A number of studies have shown that species of *Morchella,* including *M. esculenta,* can accumulate arsenic and lead, but the authors concluded that *M. esculenta* is not a strong accumulator of arsenic (Stijve and Bourqui, 1991; Cocchi et al., 2006; Gursoy et al., 2009). According to these studies, elevated levels of lead found in *M. esculenta* were not high enough to pose any health risks (Cocchi et al., 2006; Gursoy et al., 2009). However, a patent application submitted to the United States Patent and Trademark Office named *M. esculenta* hyphae among the high lead accumulators to be used in bioremediation efforts to remove excess lead from contaminated sites (Stamets, 2003). Multiple studies have examined the role of polluted habitats, such as busy roadsides, railroad tracks or smelting operations, in the accumulation of toxic elements in mushrooms (Kalač and Svoboda, 2000; Svoboda et al., 2006). This study examines the role of one contaminated micro-habitat on the levels of toxic elements found in the fruitbodies of a single species of a popular edible mushroom, the orchard morel.

Materials and Methods

2.1 Study sites

We identified commercial apple orchards that were active from the mid 1800s to the mid 1900s in the states of New Jersey, New York, Massachusetts, and Vermont. Some of the better collecting sites for *M. esculenta* in these states are former apple orchards that are overgrown with trees and vines and are often difficult to distinguish from their surrounding woodlands. Particular efforts were therefore made to identify orchards that were active during the decades when lead arsenate was in popular use, regardless of their current appearance. Only orchards known to produce considerable numbers of *M. esculenta* each year were considered. Soil pH ranged from 6.5 to 8. The identity and age of each sampled orchard was verified through the use of aerial maps, town-hall records, records of pesticide treatment, and interviews with property owners. The samples making up this study are representative of the typical range of apple orchard habitats favored by *M. esculenta* in the northeastern U.S.

2.2 Soil and mushroom sampling

Twenty-nine samples of American *M. esculenta* fruitbodies and 29 samples of soil were collected in the identified apple orchards in May of 2009. Soil and mushroom samples were collected as paired samples, such that each mushroom sample corresponded to a specific soil sample, and both were collected within 30 cm of each other. The samples were collected in accordance with the U. S. Environmental Protection Agency (EPA) Environmental Response Team, SOP 2012 (2/18/00) protocol for surface soil sampling (EPA/SOP, 2000), and the Recommended Procedures for Collecting, Processing, and Analyzing Soil Samples in CASMGS Research Plots 4/21/2003 (CASMGS, 2003).[C] Soil sampling at the orchards followed Oregon State Guidance for Evaluating Residential Pesticides on Lands Formerly used for Agricultural Production (Oregon Department of Environmental Quality, 2009). These protocols include specific details for collection, equipment, handling, preservation, storage and shipping in a manner suitable for the purpose of detecting metals in soil using Inductively Coupled Plasma– Mass Spectrometry (ICP-MS), which offers appropriately low detection levels for lead and arsenic in soil and organic matter.

Using a stainless steel planter, core samples (10 cm diameter) were collected from the first 15 cm of the topsoil, directly under the litter layer. Soil samples were collected in clearly marked, If You Care® parchment paper, which is 100% unbleached, no heavy metals added, quilon- and chrome-free. Soil samples were air-dried for 15 days and placed in clearly marked polyethylene bags. Each sample of five to six *M. esculenta* fruitbodies of similar size and maturity level was collected from an area within 30 cm of its paired soil sample. The sampled fruitbodies corresponded roughly to the average size and maturity of morel mushrooms collected by mushroom hunters. Mushroom fruitbodies were cut above ground with a stainless steel knife and included all of the edible parts of the mushroom, including the sporocarp and the portion of the stipe showing above ground. Tools were cleaned with demineralized water between each sampling according to protocol. Each fresh mushroom sample was collected in clearly marked, If You Care® parchment paper. Each fruitbody was lightly rinsed in demineralized water to remove adhering particles and soil dust and then dehydrated at 40° C for 18 hours. The dried mushroom samples were placed in clearly marked polyethylene bags and kept in a dark, cool place. The dehydrated soil and mushroom sample bags were kept in two separate dry, dark and cool locations until they were separately shipped to the laboratory for analysis.

2.3 Morchella identification

Even though the sampled mushroom fruitbodies were identified as *M. esculenta,* for verification purposes

10% of the mushroom fruitbody samples, selected from geographically diverse areas, were sent to the Applied Mycology and Microbiology Center at the Migal Galilee Technology Center in Kiryat Shmona, Israel, where they were analyzed for molecular characterization. DNA was extracted, and the sequences of the PCR products of the ITS region of the nuclear ribosomal internal transcribed spacer region (nrDNA) and partial LSU (28S rRNA gene) were determined, using the primers ITS1+4 and LROR+LR6. The ITS and LSU sequences of the analyzed *Morchella* fruitbodies were compared with GenBank (NCBI) sequences.

2.4 Laboratory analyses

The 29 paired samples of dry soil and mushroom tissues were sent to Brooks Rand Labs in Seattle, Washington, for total lead and arsenic analyses. This laboratory was chosen because it specializes in a variety of analytical services with a focus on ultra-trace level metals analysis and metals speciation. It is nationally accredited through NELAC, and has state accreditations in Washington, Oregon, California, Florida, Maine, New York, New Jersey, and Louisiana.

Once at Brooks Rand Labs, the mushroom tissues were ground with mortar and pestle, and both soil and mushroom samples were homogenized by quartering and then mixing each quarter into the others. A 1g aliquot of the samples was dried at 105 °C ± 5 °C overnight, weighed again, and the percent of dried solid material was calculated according to standard operating procedure BR-1501 (modified standard method 2540G). The samples were analyzed by EPA Draft Method 1638 (modified) using Inductively Coupled Plasma–Mass Spectrometry (ICP-MS) for determining the total value of lead in the samples. To determine the total value of arsenic in the samples, ICP-MS with Dynamic Reaction Cell (DRC™) technology was employed to remove any polyatomic interference. Prior to analysis, 0.5 mg aliquots of the homogenized *Morchella* tissue samples were digested with 10 mL ultra-pure nitric acid and heated for a minimum of four hours at 100 °C. Sediment samples were digested with a closed-vessel reverse aqua regia (RAR) oven bomb digestion for total recoverable metals. The accuracy of the analyses for tissues was checked against the certified reference material, namely, Dogfish Liver CRM DOLT-4 for arsenic and lead and Peach Leaves, SRM NIST 1547 for lead. The accuracy of the analyses for soils was checked against the certified reference material MESS-3 (Marine Sediment) and NIST 2710 (Montana Soil) for both arsenic and lead. Calibration of the instrument was performed daily and a 5-point calibration curve was performed with a correlation coefficient ≥ 0.995, and 1st standard ≤ the method PQL. Internal standardization in standard mode for lead was accomplished using Li, Sc, Ge, In, Tm. Samples were analyzed for arsenic in the Dynamic Reaction Cell (DRC) mode of the instrument using Rh for internal standardization. To demonstrate accuracy and precision of sample preparation and to identify any matrix interference issues, matrix duplicates, matrix spikes, and spike duplicates were performed on three of the soil samples and three of the mushroom tissue samples. The total value of lead and arsenic in the soil and mushroom tissue samples were reported in milligram per kilogram (mg/kg) of wet weight as well as in mg/kg of dry weight (Brooks Rand Lab, personal communication).[D]

One sample of *M. esculenta* fruitbodies was analyzed for arsenic speciation by EPA Method 1632, Revision A (1/01): Chemical Speciation of Arsenic in Tissue by Hydride Generation Quartz Furnace Atomic Absorption Spectrometry. Prior to analysis, 100 mg aliquots of dried morel samples were digested with 2 M HCl and heated at 80 °C for 16 hours. An aliquot of digestate was placed in a specially designed reaction vessel and 6 M HCl was added. To this, 4% $NaBH_4$ solution was added to convert the inorganic arsenic to volatile arsines. Arsines were purged from the sample onto a cooled glass trap. The trapped arsines were thermally desorbed, in order of increasing boiling points, into an inert gas stream that carried them into the quartz furnace of an atomic absorption spectrophotometer for detection. The first arsine to be desorbed was AsH_3, which represents As (Inorg) in the sample (Brooks Rand Lab, personal communication).[D]

2.5 Statistical analyses

The total value (in dry weight) of lead and arsenic in the soil and mushroom tissues for the 29 paired samples was evaluated using the statistical package PASW (Predictive Analytics SoftWare), formerly known as SPSS (Statistical Package for the Social Sciences), version 17.0. Means, medians, standard deviations, 95% confidence intervals and ranges were calculated for the four variables: lead in the soil, arsenic in the soil, lead in the mushroom tissues, and arsenic in the mushroom tissues. Pearson correlations were calculated to examine the relationships between the four variables. Linear regressions were performed to predict the value of lead and arsenic in the mushrooms based on the value of these elements in the soil. Charts showing the scatter plot and best fit line demonstrating the relationships between arsenic in the soil and in the mushrooms and lead in the soil and in the mushrooms are presented.

2.6 Toxicity

We compared the arsenic levels in the fruitbodies of *M. esculenta* to the Minimal Risk Levels (MRL) published by the Agency for Toxic Substances and Disease Registry (ATSDR) of the Center for Disease Control and Prevention (CDC) in 2007 (ATSDR, ToxGuide™-As 2007), using 30 grams of dry *Morchella* as our one time daily serving, 70 kg as the standard adult weight, and 20 kg as the standard weight of a child.

We compared the lead levels in the fruitbodies of *M. esculenta* to the Provisional Tolerable Total Intake Levels (PTTIL) set by the Food and Drug Administration (FDA) in 1993 (Federal Registry, 1993), using 30 g of dry *Morchella* as our one time daily serving (Kalač and Svoboda, 2000; Gursoy et al., 2009).

Results

The ITS and LSU sequences of the *Morchella* fruitbodies selected for molecular characterization were compared with the GenBank (NCBI) sequences. The comparison showed them to be grouped in the *M. esculenta* clade. It should be noted that even though the results of the molecular characterization validated the expert identification of these fruitbodies as *M. esculenta,* species concepts and nomenclature of *Morchella* species are controversial (Kuo, 2008). However, since *M. esculenta* is the name commonly used for apple-orchard morels, we will continue to use it here.

Lead and arsenic levels in both the soil and *M. esculenta* fruitbodies for the 29 paired samples were used to calculate means, medians, standard deviations, 95% confidence intervals and ranges (Table 1). We calculated Pearson correlations between the four variables: lead in the soil, arsenic in the soil, lead in the mushroom tissues, and arsenic in the mushroom tissues (Table 2). Linear regression analysis was performed to predict the relationship between the arsenic found in the mushrooms and the arsenic found in the soil, as well as to predict the relationship between the lead found in the mushrooms and the lead found in the soil. Scatter plots, including a best-fit line and the regression results for both arsenic and lead in the soil and mushrooms, are provided (Figures 1 and 2). The sample of *M. esculenta* with the highest level of total arsenic (2.85 mg/kg) was sent for speciation analysis to determine what percentage of the total arsenic found in the tissues of the fruitbody was inorganic. We found that 94% of the total arsenic in the fruitbody was in a toxic, inorganic form (2.68 mg/kg).

Table 1. Levels of arsenic and lead in 29 paired samples of apple orchard *Morchella esculenta* and their associated soil. All values are reported in parts per million (mg/kg).

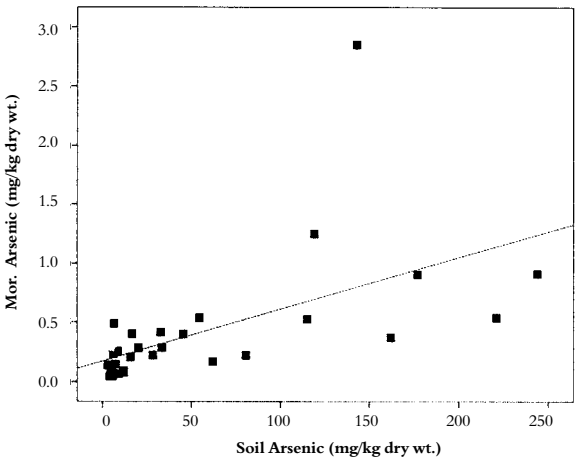
	Mean	Median	S.D.	Range	CI (95%)
Soil Arsenic	57.05	20.30	70.55	3.08–244.00	30.21–83.88
Soil Lead	430.45	126.00	590.22	19.20–2450.00	205.94–654.96
Morel Arsenic	0.42	0.26	0.55	0.15–2.85	0.22–0.63
Morel Lead	2.37	1.05	3.27	0.05–13.00	1.13–3.61

Table 2. Pearson correlations between lead in the soil, arsenic in the soil, lead in the tissues of *Morchella esculenta* and arsenic in the tissues of *Morchella esculenta* (n=29).

Factor	Soil Arsenic	Soil Lead	Mor. Arsenic	Mor. Lead
Soil Arsenic	–	0.84*	0.57*	0.81*
Soil Lead		–	0.29	0.94*
Mor. Arsenic			–	0.28
Mor. Lead				–

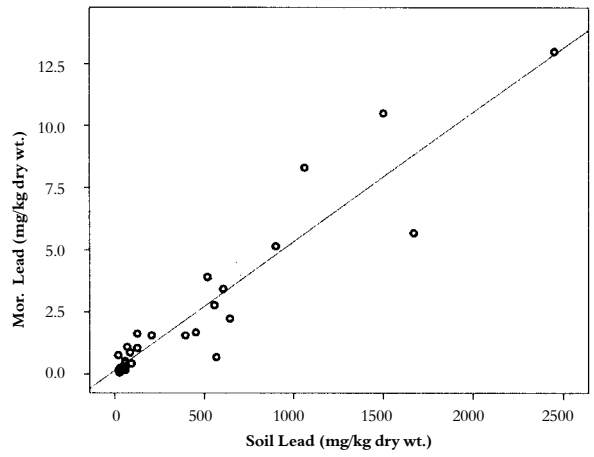
* P<0.001

Figure 1. Scatter plot and best-fit line showing the relationship between arsenic in the soil and arsenic in paired *Morchella esculenta* fruitbody tissues. (p<0.001)



Factor	β	SE β	P value	CI (95%)	R^2
Soil Arsenic	0.004	0.001	0.001	0.002–0.007	0.320

Figure 2. Scatter plot and line of best fit showing the relationship between lead in the soil and lead in their paired *Morchella esculenta* fruitbody tissues (p<0.001).



Factor	β	SE β	P value	CI (95%)	R^2
Soil Lead	0.005	0.000	0.000	0.004 - 0.006	0.880

Discussion and Conclusion

The results reported in Table 1 demonstrate a wide variance in the values of lead and arsenic in both the soil and *M. esculenta* samples. This can be explained by the inclusion of untreated orchards as well as lead arsenate treated orchards in our sample population. An illustration of this variance can be seen by comparing two orchards in our sample that are located approximately 200 yards apart in the state of New York. The first of these orchards has lead and arsenic levels comparable to those found in the background soil of the region, while the second orchard has substantially higher levels of both arsenic (27 times higher, 9.05 mg/kg vs. 244 mg/kg) and lead (16 times

higher, 56.7 mg/kg vs. 899 mg/kg). This increase is in a 1: 3.6 ratio, consistent with the ratio of arsenic to lead in lead arsenate pesticides (1:3.5) (FAO/WHO 1968; Renshaw et al., 2006), suggesting that the second orchard was treated with lead arsenate, while the first was not.

Judging by their low levels of lead and arsenic, which were similar to the levels of these elements in the soil of the area, we determined that approximately one-third of the orchards in our sample had not been treated with lead arsenate. The levels of lead and arsenic in the soil and mushroom samples from the three orchards where Mr. Peabody used to collect his morels were among the lowest in this study, suggesting that the orchards most likely predate lead arsenate treatment.

The correlations displayed in Table 2 provide a definitive answer to the core question posed by this study: do *M. esculenta* growing in orchards treated with lead arsenate pesticides absorb lead and arsenic from their habitats? There are statistically significant positive correlations between the lead in the soil and the lead in the mushrooms as well as between the arsenic in the soil and the arsenic in the mushrooms. In the case of lead, this correlation is extremely strong, nearly linear (r=0.94). This finding indicates that the higher the levels of lead in the soil, the higher the lead levels will be in the morels growing from that soil. It is difficult to be certain of previous treatment with pesticides, particularly when some of these treatments predate record keeping (Robinson and Ayotte, 2007). However, the strong correlation between the soil lead and the soil arsenic (r=0.84) validates our assumption that lead arsenate pesticides are responsible for the high levels of lead and arsenic in the soil. Given these results, we would expect to see a strong positive correlation between the amount of lead in the morels and the amount of arsenic in the soil. Our results confirm this expectation (r=0.81). The correlation between the arsenic in the mushrooms and the arsenic in the soil is weaker than that of lead, but is positive and statistically significant (r=0.57).

Our expectation at the onset of this study was to find high, potentially toxic levels of arsenic in *M. esculenta* fruitbodies growing in heavily lead arsenate treated apple orchards. However, the highest levels of arsenic that we found were substantially lower than the MRL set by the ATSDR (0.005 mg/kg/day As) for acute duration oral exposure, and they were consistent with those reported in previous studies. (Stijve et al., 1990; Cocchi, 2006; Gursoy et al., 2009). Although there is no published data on the species of arsenic found in *M. esculenta* fruitbodies (T. Stijve, personal communication), we were surprised that 94% of the arsenic in the tissues of the fruitbody analyzed for arsenic speciation was found to be in a toxic, inorganic form. While we cannot draw general conclusions based on the result of one speciation analysis, this result clearly demonstrates that *M. esculenta* are capable of concentrating inorganic arsenic from arsenic contaminated habitats.

The level of inorganic arsenic found in the sample of *M. esculenta* that was sent for arsenic speciation (2.68 mg/kg) significantly exceeded the MRL for chronic duration oral exposure to inorganic arsenic (0.08 mg/day vs. 0.02 mg/day for a 70 kg person, or 0.006 mg/day for a 20 kg child), assuming a 30g (in dry weight) daily intake of morels over the course of a year. While few people are fortunate enough to be able to consume morels every day of the year, morel enthusiasts do so regularly over the course of the season and also preserve mushrooms for later use. Since there is no MRL set for intermediate duration oral exposure to inorganic arsenic, the health risks that this poses are not known. However, this result certainly raises questions about the safety of such consumption. Furthermore, since we found a significant, positive correlation between the levels of arsenic in the soil and the levels of arsenic in the morels (Table 2), and since 94% of the arsenic that we found in the morel sample was in a toxic, inorganic form, we cannot rule out that in areas where the soil values of arsenic are significantly higher than those that we measured, the levels of inorganic arsenic found in the morels could be of concern.

The most striking results to come out of this study are the levels of lead found in the morel fruitbodies and the magnitude of the correlation between the lead in the soil and the lead in the fruitbodies of *M. esculenta.* Based on the R^2 value of 0.88, displayed under Figure 2, we can determine that the amount of lead in the soil is a good predictor of the amount of lead in the mushroom fruitbodies. This R^2 value indicates that 88% of the variation in the amount of lead found in the tissues of the mushrooms can be explained by the amount of lead found in the soil from which they grew.

These results are disturbing. We considered the Provisional Tolerable Total Intake Levels (PTTIL) for lead set by the FDA in 1993, taking into account that Tolerable Daily Intake levels have not been set because no quantity of lead is considered safe for consumption. The lead content of 90% of our samples of *M. esculenta* exceeded the PTTIL for children six years old and younger (6 µg/day), 69% exceeded the PTTIL for children seven years and older (15 µg/day), 59% exceeded the PTTIL for pregnant women (25 µg/day), and 28% exceeded the PTTIL for all other adults (75 µg/day). The fact that so many of our samples exceed even the provisional tolerable intake levels is particularly concerning (Federal Registry, 1993). Our findings differ from previous studies that found relatively lower levels of lead in the mushrooms they sampled. This difference is explained by the fact that while we sought to sample potentially contaminated habitats, previous studies either sampled sites at random or sought out pristine habitats (Cocchi et al., 2006; Gursoy et al., 2009; Campos et al., 2009).

We would feel uncomfortable consuming morels from those orchards in our study that were heavily sprayed, and would not serve them to children. Compounding this problem is the fact that *M. esculenta* has recently started emerging in many younger apple orchards that were planted and heavily treated with lead arsenate in the heyday of lead arsenate. We sampled a number of younger apple orchards that have been included in

town recreation areas in the states of New York and Massachusetts, and found them to have high values of both arsenic and lead. The lead values were particularly high, since lead was also used in fungicides (Wong et al., 2002). Such younger contaminated apple orchards in recreation areas and among the dirt roads of apple growing areas in New York and New England states are just beginning to emerge as potentially prolific morel collecting sites. Given that some northeastern apple growers are opting to leave contaminated orchard plots untilled, uncut, and untended in order to minimize the amount of arsenic and lead that leaches into the waterways, we anticipate that high numbers of morels will be collected from these orchards in the years to come. In light of our conclusions regarding lead and arsenic levels in *M. esculenta*, we find this prospect quite worrisome.

The high variance in the lead and arsenic levels among the soil samples used in this study is a good indication that there may be areas with even higher levels of both metals. It is entirely possible that people are collecting morels in areas with dangerously high levels of lead and arsenic, and it is therefore recommended that people test the soil of their frequently visited morel collecting spots. Fortunately, testing facilities for heavy metals in soil are available at several research centers in the Northeast; and determining the heavy metal levels in the soil of mushroom collecting spots can be both simple and affordable.

This study has taught us that we cannot take the safety of what seem like pristine habitats for granted. The highest levels of both arsenic and lead in the soil and in the fruitbodies of *M. esculenta* came from an apple orchard adjacent to a picturesque neighborhood in Vermont and from a large orchard in the middle of a residential neighborhood in a New York town. Considering these realities, the key conclusion of this study is that in order to be responsible consumers of wild mushrooms, we must be more vigilant about ascertaining the safety of the mushrooms that we pick and eat.

Acknowledgments

We owe a debt of gratitude to Mr. Robert Peabody of the New Jersey Mycological Association, whose case of heavy metal poisoning has brought to our attention the potential health hazards associated with consuming edible mushrooms collected in contaminated habitats. His support, encouragement, and courage followed us through this long process. We wish to extend our gratitude to Thomas J. Volk, Professor of Biology at the University of Wisconsin–La Crosse, for his invaluable advice and support throughout this project. This study would not have been possible without the financial contributions of the following mycology clubs, members of the North East Mycological Federation, who demonstrated their commitment to providing their members and amateur mycologists everywhere with important information regarding the safety of the mushrooms they collect: New Jersey Mycological Association (NJMA), New York Mycological Society (NYMS), Mid-Hudson Mycological Association (MHMA), Boston Mycological Club (BMC), and Monadnock Mushroomers Unlimited of Keene, NH (MMU). Special thanks to Julia Barringer of the USGS–NJ Water Science Center for supplying valuable information regarding the locations of arsenic-treated areas in New Jersey. We owe special thanks to Misty Kennard-Mayer at Brooks Rand Laboratories, whose help during the process of preparing and analyzing the study samples was invaluable. We wish to thank Segula Masaphy, PhD, at the Applied Mycology and Microbiology Center at the Migal Galilee Technology Center in Kiryat Shmona, Israel, for performing the molecular characterization of the study's *M. esculenta,* and Adi Sharabi-Nov, lecturer at the Academic Collage Tel-Hai and statistician at ZIV Medical Center, Zefat, Israel, for her assistance with statistical analyses. Our thanks to Donald H. Pfister, curator of the Farlow Library and Herbarium at Harvard University, for his constant encouragement. Last but not least—we extend our deepest gratitude to the people who allowed us to sample apple-orchard *M. esculenta* in their favorite collecting spots all over the northeastern U.S. We are particularly grateful to Sharon Kitagawa, Dennis Aita, Pamela Kray, and Paul Sadowski (NYMS), Bill Bakaitis (MHMA), and Noah Siegel (MMU). Thank you all.

The photo of an apple orchard being sprayed with lead arsenate, which appears on the first page of this article, is printed courtesy of Cornell University, Department of Plant Pathology.

Notes

A. For the full story about Mr. Peabody's medical ordeal, a discussion of some of the studies dealing with the accumulation of heavy metals in mushrooms, and facts pertaining to heavy metal poisoning, please refer to "Arsenic in Morels collected in New Jersey apple orchards blamed for arsenic poisoning" (Shavit, 2008, *FUNGI* 1(4): 2–10, online at www.fungimag.com/winter-08-articles/Rev_Medicinal.pdf).

B. For detailed information about the medical conditions caused by exposure to arsenic and lead please refer to the U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry (ATSDR). Online at http://www.atsdr.cdc.gov/toxguides/(ATSDR-ToxGuides™).

C. (CASMGS) is the Consortium for Agricultural Soils Mitigation of Greenhouse Gases.

D. For detailed description of the methods used refer to the Brooks Rand Labs website at www.brooksrand.com.

References

1. Agency for Toxic Substances and Disease Registry (ATSDR). 2007. ToxGuide™ for Arsenic, CAS#7440-38-2 online at www.atsdr.cdc.gov/toxguides/toxguide-2.pdf [accessed Jan. 2010].

2. Agency for Toxic Substances and Disease Registry (ATSDR). 2007. ToxGuide™ for Lead, CAS#7439-92-1 online at www.atsdr.cdc.gov/toxguides/toxguide-13.pdf [accessed Jan. 2010].

3. Agency for Toxic Substances and Disease Registry (ATSDR). 2007.ToxFAQs™ for Arsenic. Online at www.atsdr.cdc.gov/tfacts2.html [accessed Jan. 2010].

4. Agency for Toxic Substances and Disease Registry (ATSDR). 2007.ToxFAQs™ for Lead. online at http://www.atsdr.cdc.gov/tfacts13.html [accessed Jan. 2010].

5. Agency for Toxic Substances and Disease Registry (ATSDR), Quick Reference pocket guide for toxicological profiles. Online at www.atsdr.cdc.gov/toxguides [accessed Jan. 2010].

6. Bakaitis, B. 2008. Collecting wild mushrooms, part 1 (Morels), Lesley Land, online at http://leslieland.com/2008/07/collecting-wild-mushrooms-part-1-morels [accessed Jan. 2010].

7. Bakaitis, B. 2009. Morels from the apple orchards: a look at some toxins, some risks, and need for specific research. North East Mycological Federation, online at www.nemf.org/files/bakaitis/MORELS_FROM_APPLE_ORCHARDS.pdf [accessed Jan. 2010].

8. Belluck, D.A., S. L. Benjamin, P. Baveye, J. Sampson, and B. Johnson. 2003. Widespread arsenic contamination of soils in residential areas and public spaces: an emerging regulatory of medical crisis? *International Journal of Toxicology* 22(2): 109–28.

9. Boa, E. 2004. Wild edible fungi a global overview of their use and importance to people, FAO/Forestry Department, Rome, Italy, online at www.fao.org/docrep/007/y5489e/y5489e00.htm#TopOfPage [accessed Jan. 2010].

10. Campos, A. J., N. A. Tejera, and C. J. Sanchez. 2009. Substrate role in the accumulation of heavy metals in sporocarps of wild fungi. *Biometals* 22: 835–41.

11. CASMGS Executive Committee. 2003. Recommended procedures for collecting, processing, and analyzing soil samples in CASMGS research plots, submitted to CASMGS Executive Committee 4/21/2003. Online at www.casmgs.colostate.edu/vignette/448_8___recommended%20procedures.pdf [accessed Jan. 2010].

12. Cocchi, L., L. Vescovi, L. E. Petrini, and O. Petrini. 2006. Heavy metals in edible mushrooms in Italy. *Food Chemistry* 98: 277–84.

13. EPA Environmental Response Team Standard Operating Procedures, SOP: 2012 PAGE: 1 of 13 REV: 0.0 DATE: 02/18/00, for Soil Sampling. 2000. Online at http://www.scribd.com/doc/2015076/Environmental-Protection-Agency-EPAERTSOILSOP2012__[accessed Jan. 2010].

14. Environmental Protection Agency (EPA). Superfund—Cleaning up the Nation's Hazardous Wastes Sites. Online at www.epa.gov/superfund [accessed Jan. 2010].

15. FAO/WHO/FOOD ADD. 1968. FAO/PL: 1968/M/9/1 online at www.inchem.org/documents/jmpr/jmpmono/v068pr23.htm [accessed Jan. 2010].

16. Federal Registry. Vol. 58, pg. 33860, June 21, 1993. www.fda.gov/ohrms/dockets/dailys/03/Sept03/091503/77N-0094I-c000005-06-tab4-vol8.pdf [accessed Jan. 2010].

17. Gursoy, N., C. Sarikurkcu, M. Cengiz, and M. H. Solak. 2009. Antioxidant activities, metal content, total phenolics and flavonoids of seven *Morchella* species. *Food and Chemical Toxicology* 47: 2381–88.

18. Harbin, M., and T. J. Volk. 1999. The association of *Morchella* with plant roots. XVI International Botanical Congress Abs. #1601 p. 559.

19. Hood, E. 2006. The apple bites back: claiming old orchards for residential development. *Environmental Health Perspectives* 114(8): A470–76.

20. Kalač, P. C., and L. Svoboda. 2000. A review of trace element concentrations in edible mushrooms. *Food Chemistry* 69(3): 273–81.

21. Kuo, M. 2008. *Morchella tomentosa*, a new species from western North America, and notes on *M. rufobrunnea. Mycotaxon* 105: 441–46.

22. Merwin, I., P .T. Pruyne, J. G. Ebel, K. L. Manzell, and D. J. Lisk. 1994. Persistence, phytotoxicity, and management of arsenic, lead and mercury residues in old orchard soils of New York State. *Chemosphere* 29(6): 1361–67.

23. Murphy, E. A., and M. Aucott. 1988. An assessment of the amounts of arsenical pesticides used historically in a geographical area. *The Science of the Total Environment* 218(2–3): 89–101.

24. New Jersey Historic Pesticide Task Force (NJHPTF). 1999. Findings and recommendations for the remediation of historic pesticide contamination. New Jersey Department of Environmental Protection, Trenton, New Jersey, online at www.state.nj.us/dep/special/hpctf/final/hpctf99.pdf [accessed Jan. 2010].

25. Oregon Department of Environmental Quality. 2009. Guidance for evaluating residential pesticides on lands formerly used for agricultural production (# DEQ-06-LQ-011-Oregon). Online at www.deq.state.or.us/lq/pubs/docs/cu/GuidanceEvalResidualPesticides.pdf [accessed Jan. 2010].

26. Peryea, F. J. 1998. Historical use of lead arsenate insecticides, resulting soil contamination and implications for soil remediation. Proceedings of the 16th World Congress of Soil Science (CD Rom), Montpellier, France. 20–26 Aug 1998. Online at http://soils.tfrec.wsu.edu/leadhistory.htm [accessed Jan. 2010].

27. Pilz, D., R. McLain, S. Alexander, L. Villarreal-Ruiz, S. Berch, T. L. Wurtz, C. G. Parks, E. McFarlane, B. Baker, R. Molina, and J. E. Smith. 2007. Ecology and management of morels harvested from the forests of western North America. Report PNW-GTR-710. United States Department of Agriculture/Forest Service/Pacific Northwest Research Station. Online at www.fs.fed.us/pnw/publications/gtr710/pnw_gtr710a.pdf [accessed Jan. 2010].

28. Renshaw, C. E., B. C. Bostick, X. Feng, C. K. Wong, E. S. Winston, R. Karimi, C. L. Folt, and C. Chen. 2006. Impact of land disturbance on the fate of arsenical pesticides. *Journal of Environmental Quality* 35: 61–67.

29. Robinson, G. R. Jr., and J. D. Ayotte. 2007. Rock-bound arsenic influences ground water and sediment chemistry throughout New England. U.S. Geological Survey, Open-File Report 2007-1119 16: 1–18.

30. Shavit, E. 2008. Arsenic in morels collected in New Jersey apple orchards blamed for arsenic poisoning. *Fungi* 1(4): 2–10, online at www.fungimag.com/winter-08-articles/Rev_Medicinal.pdf.

31. Stamets, P. E. 2003. Delivery systems for mycotechnologies, mycofilteration, and mycoremediation, online at www.faqs.org/patents/app/20080264858 [accessed Jan. 2010].

32. Stijve, T., E.C. Vellinga, and A. Herrmann. 1990. Arsenic accumulation in some higher fungi. *Persoonia* 14(2): 161–66.

33. Stijve, T., and B. Bourqui. 1991. Arsenic in edible mushrooms. *Deutsche Lebensmittel-Rundschau* 87(10): 307–10.

34. Svoboda, L., B. Havlickova, and P. Kalač. 2006. Contents of cadmium, mercury, and lead in edible mushrooms growing in a historical silver-mining area. *Food Chemistry* 96: 580–85.

35. Veneman, P. L. M, J. R. Murray, and J. H. Baker. 1983. Spatial distribution of pesticide residues in a former apple orchard. *Journal of Environmental Quality* 12: 101–4.

36. Volk, T. J., and T. J. Leonard. 1989. Experimental studies on the morel. I. Heterokaryon formation between monoascosporous strains of *Morchella. Mycologia* 81: 523–31.

37. Volk, T. J., and T. J. Leonard. 1989. Physiological and environmental studies of sclerotium formation and maturation in *Morchella. Applied and Environmental Microbiology* 55: 3095–3100.

38. Wong, C. K., C. E. Renshaw, X. Feng, and S. Sturup. 2002. New Hampshire apple orchards as a source of arsenic contamination. American Geophysical Union, Spring Meeting 2002, abstract # H42A-05.