A novel approach to determine arsenic contamination in the environment

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This study examines a novel method to determine heavy metal contamination in the environment. This method will combine the established technique of atomic absorption spectroscopy with terrestrial isopods. The terrestrial isopods bioaccumulate heavy metals. The established forensic analysis by atomic absorption was used to quantify heavy metal poisoning.

Spectroscopy determined the presence of arsenic and lead in four study areas. Arsenic contamination was the focus of this study. Lead contamination was done in parallel to verify the method. As part of the controls, three types of lumber were offered the isopods to feed on. Those lumber samples were kiln dried lumber, ACQ preserved lumber, and CCA preserved lumber. Three samples of terrestrial isopods were collected from each control and test area. The test areas included a private residence, the Rochester Institute of Technology campus, a local park, and a local gun club. In addition, standing water at the gun club was included as a separate sample; it tested 0 ppm for arsenic, and 1.257 ppm for lead.

Although this method exhibited robustness and reliability, collection methods and weather conditions appeared to be the limiting factor. The impact of drought established that a minimum mass of sample is required.
The toxicity of heavy metals in the environment has been studied extensively. There are many methods that can be employed to estimate the level of metal contamination. In this study, the heavy metals to be tested for are arsenic and lead. The analytical technique utilized to detect and quantify arsenic (As) poisoning in a forensic setting is atomic absorption spectrophotometry. The samples to be collected in the area of interest are terrestrial isopods, whose metabolism sequesters heavy metals. This approach can establish if environmental metal contamination is present, and a reasonable estimation can be made of the level of contamination.

Throughout history, arsenic has been used for a variety of applications. Some are well intended, while other uses are not. The most familiar use of arsenic is that of a poison, which can lead to accidental injury or death, or more notoriously used as a murder weapon. The criminal aspect of arsenic’s use has lead to the development of methods to test for its presence in biological tissues.

Gerber and Saferstein\(^1\) discuss the use of arsenic as a weapon and the evolution of methodologies of detection. The use of arsenic for intentional harm extends back to the Borgia family in the 15\(^{th}\) and 16\(^{th}\) centuries. This family used poisonings to gain political power and wealth to such an extent that one member of the family was canonized as a saint, two became Pope, and many others reached high ranks in the church or royalty\(^2\). Another alleged example of arsenic’s use to gain power is by Catherine de Medici to kill Henry of Navarre (more widely known as Henry IV of France). In France, from 1832 – 1840, 141 of 194 reported deaths are associated with arsenic, and in England and Wales, from 1837 – 1838, 185 of 541 poisonings were performed with arsenic\(^1\). Even today, arsenic continues to be used as a poison.
The widespread use of arsenic for criminal acts fostered the development of sound analytical tests to confirm the presence of arsenic. The most common methods historically have been colorimetric methods and precipitation tests. Gerber and Saferstein\(^1\) give a summary of the methods and their development. During the trial of Mary Blandy in England in 1752, the form of arsenic used as poison was As\(_2\)O\(_3\) (white arsenic). Anthony Addington, Medical Examiner for the Crown, was one of the first to develop an analytical method for detection of arsenic. Addington placed the compound in a red-hot pan and noticed a distinct odor of garlic. In 1803, Joseph Black continued in developing methodologies for arsenic detection. His method involved reducing white arsenic powder to elemental arsenic after heating it with black flux (recently ignited charcoal) in a test tube, and thus depositing the arsenic on a bright copper plate. The resulting reactions are:

\[
\begin{align*}
2 \text{As}_2\text{O}_3 (s) + 6 \text{C} (s) &\rightarrow \text{As}_4 (s) + 6 \text{CO} (g) \\
4 \text{Cu} (s) + 4 \text{As}^{2+} (aq) &\rightarrow \text{As}_4 + 4\text{Cu}^{2+}
\end{align*}
\]

In 1806, Thomas Ewell developed a fairly quick method. The suspect powder was thrown on heater coals, and a positive result for arsenic resulted in a white flame and the odor of garlic, much the same as Addington’s analysis. At the same time, Benjamin Rush developed what is known as Green’s Test. This involved heating the arsenic powder between 2 copper plates. The resulting white powder on the plates, with an accompanying garlic odor, is washed with an alkaline copper sulfate solution. The result is a green precipitate. The reaction is:
Joseph Hume, in 1809, established the Hume’s test. The Hume’s test involves adding silver nitrate to arsenious acid or an arsenite solution in an alkaline environment. Immediately, silver arsenite precipitates out of solution (bright yellow in color). The reaction is:

$$3 \text{Cu}^{2+} + 2 \text{AsO}_4^{3-} \rightarrow \text{Cu}_3(\text{AsO}_4)_2(s)$$

In addition, the silver nitrate can be added to an arsenate solution to produce a reddish brown precipitate of silver arsenate, $\text{Ag}_3\text{AsO}_4$. The major detractor of the Hume’s test is that silver nitrate is ineffective in the presence of animal or vegetable matter. The result is an altered precipitate color or no precipitate at all.

To address the issue of arsenic composites in animal or vegetable matter, Sir Robert Christison advanced his method. A stream of sulfated hydrogen (hydrogen sulfide, $\text{H}_2\text{S}$) gas is passed over an arsenic solution acidified with hydrochloric acid. An amorphous bright yellow precipitate of arsenous sulfide ($\text{As}_2\text{S}_3$) is produced. This method is reported to have the ability to detect 1 part in 100,000. However, the test is not specific enough and unfortunately confuses sulfides of other metals – Cd, Sn, Se, and Sb.

The following are tests that can be used at present. James Marsh (1794 – 1846) developed the Marsh test in 1836. Marsh received the gold medal of the Society of Arts of London for his work in arsenic detection. The Marsh test involves adding pure
metallic zinc to a sulfuric acid or hydrochloric acid solution until any suspect contamination is removed. The suspect arsenic solution is added to the flask so that arsine gas (AsH₃) is released with the hydrogen gas. The reactions are:

\[
\begin{align*}
\text{Zn(s)} + 2\text{HCl(aq)} & \rightarrow \text{ZnCl}_2\text{(aq)} + \text{H}_2 \text{(g)} \\
6\text{H}_2\text{(g)} + \text{As}_2\text{O}_3\text{(aq)} & \rightarrow 2\text{AsH}_3\text{(g)} + 3\text{H}_2\text{O}
\end{align*}
\]

Upon heating of the gas in a flame, a deposit of metallic arsenic is laid down on a white porcelain surface, or white arsenic (As₂O₃) if the dish is held above the flame.

![Figure 1. Representation of the Marsh apparatus from Gerber and Saferstein¹.](image)

Shortly after the Marsh test came into use, the Reinsch test was introduced as a simple, effective test that had the ability to detect approximately 0.00002 parts of arsenic in solution. A copper plate that has been previously treated with nitric acid is placed in an arsenic solution that has been previously acidified with HCl and heated to nearly boiling. Arsenic will appear as a gray, metallic-like, or black coat on the copper plate.
This method works well when halide ions are present along with enough $\text{H}^+$ ions present to accompany the copper. It has been reported that this test does not work well when chlorate ions are present. This leads to the question of which test to use. The Marsh test is the first analytical method used in toxicology to be presented in a criminal trial. Although the Marsh test is a preferred method, it does require some proficiency, whereas the Reinsch test is simple and effective.

These tests showed the presence of arsenic; however, quantitative values could not be determined. This led to the Gutzeit test. This test used the arsine gas produced by the Marsh test dissolved in a concentrated silver nitrate solution to yield a yellow color. This was exposed to a filter paper saturated with a reagent to produce a color change. Initially, the most sensitive reagent was found to be mercurous bromide ($\text{Hg}_2\text{Br}_2$). The saturated filter paper produced a color change depending on the level of arsine gas. Therefore, standardized stains could be prepared for quantitative analysis. This method is still regarded as a simple and rapid qualitative and quantitative method for arsenic detection.

Gerber and Saferstein\textsuperscript{1}, and many others describe more modern methods of detecting and measuring arsenic. The most common are Atomic Absorption Spectroscopy (AA) and Neutron Activation Analysis (NAA). The use of AA is well understood, and is the method of choice for this study. Atomic absorption has shown to be accurate and reliable, and is valued for its speed, sensitivity, precision, and specificity.
of measurement of many elements. These attributes are based on the Beers-Lambert law that states that the concentration of a solution can be determined by the amount of light it absorbs at its resonance line. The specificity of AA is due to the well-documented spectral resonance lines established for each element.

Loconto\textsuperscript{4} states that arsenic is usually bound to O\textsubscript{2} in the As(III) or As(V) state, and because of its extreme toxicity, the accepted limit for arsenic levels in drinking water is 50 ppb (0.050 ppm). Since arsenic has such a toxic effect, the majority of the work reviewed for this study deals with the effects of arsenic contaminated drinking water. Compounds containing arsenic, until recently, were common around the house. For centuries arsenic compounds were used as medicines and tonics to cure diseases, such as syphilis and amebic dysentery\textsuperscript{5}. Many sources report that it is found in paint, pesticides, food stuffs, medicinal solutions, Fowler’s solution, computer components, lumber preservatives, and smelting plants which have been shown to be a common source for occupational exposure. In addition, arsenic also occurs naturally in mineral deposits. These mineral deposits are the source of contamination in deep wells used as a supply of drinking water. The following table shows many of the common forms and uses of arsenic.

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|l|}
\hline
\textbf{Arsenic compound} & \textbf{Formula} & \textbf{Known as} & \textbf{Uses} \\
\hline
Arsenic & As & & Alloing additive  \
 & & & Electronic devices, i.e.  \
 & & & Transistors, etc.  \
 & & & Veterinary medicines  \\
\hline
\end{tabular}
\caption{Arsenic compounds used in industry, agriculture, and medicine\textsuperscript{6,7}.}
\end{table}
Table 1. Arsenic compounds used in industry, agriculture, and medicine\textsuperscript{6,7} (cont.).

<table>
<thead>
<tr>
<th>Arsenic compound</th>
<th>Formula</th>
<th>Known as</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic pentoxide</td>
<td>( \text{As}_2\text{O}_3 )</td>
<td>Arsenic oxide Boliden salts</td>
<td>Chemical intermediate Defoliant Wood preservative</td>
</tr>
<tr>
<td>Arsenic trioxide</td>
<td>( \text{As}_2\text{O}_3 )</td>
<td>Arsenolite White arsenic Arsenious oxide</td>
<td>Insecticides and Fungicides Glass Chemicals Anti-fouling paints Taxidermy Timber preservation</td>
</tr>
<tr>
<td>Arsenic trichloride</td>
<td>( \text{AsCl}_3 )</td>
<td>Butter of arsenic</td>
<td>Pharmaceuticals and Chemicals</td>
</tr>
<tr>
<td>Arsine</td>
<td>( \text{AsH}_3 )</td>
<td></td>
<td>Stabilizing selenium in transistors</td>
</tr>
<tr>
<td>Calcium arsenate</td>
<td>( \text{Ca}_3(\text{AsO}_4)_2 )</td>
<td></td>
<td>Insecticide, herbicide, and larvicide</td>
</tr>
<tr>
<td>Copper arsenite</td>
<td>( \text{CuHAsO}_3 )</td>
<td>Scheele’s green Paris Green Emerald Green</td>
<td>Larvicide</td>
</tr>
<tr>
<td>Copper aceto-arsenite</td>
<td>( 3\text{CuOAs}_2\text{O}_3\text{Cu(OOCCH}_3) )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orpiment</td>
<td>( \text{As}_2\text{S}_3 )</td>
<td></td>
<td>Depilatory— fireworks— pigment</td>
</tr>
<tr>
<td>Potassium arsenate</td>
<td>( \text{KH}_2\text{AsO}_4 )</td>
<td>Macquer’s salt</td>
<td>Preservation of hides Textile printing Flypapers</td>
</tr>
<tr>
<td>Potassium arsenite</td>
<td>( \text{KH(AsO}_2)_2 )</td>
<td>Fowler’s solution</td>
<td>Veterinary medicine Pigment Depilatory</td>
</tr>
<tr>
<td>Realgar</td>
<td>( \text{As}_2\text{S}_2 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lead arsenate</td>
<td>( \text{PbHAsO}_4 )</td>
<td></td>
<td>Insecticide, herbicide, and growth regulator</td>
</tr>
<tr>
<td>Sodium arsenate</td>
<td>( \text{Na}_2\text{HAsO}_4 )</td>
<td>Wolman salts</td>
<td>Wood preservative Calico printing Insecticide Weed killer</td>
</tr>
<tr>
<td></td>
<td>( \text{Na}_3\text{AsO}_4 )</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 1. Arsenic compounds used in industry, agriculture, and medicine\textsuperscript{6,7} (cont.).

<table>
<thead>
<tr>
<th>Arsenic compound</th>
<th>Formula</th>
<th>Known as</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium arsenite</td>
<td>NaAsO(_2)</td>
<td></td>
<td>Herbicides, Pesticides, Corrosive inhibitor, Chemical intermediate, Fluorescent lamps</td>
</tr>
<tr>
<td>Magnesium arsenate</td>
<td>Mg(_3)(AsO(_4))(_2)</td>
<td>Atoxyl</td>
<td>Trypanicide</td>
</tr>
<tr>
<td>Sodium arsenilate</td>
<td>NH(_2)C(_6)H(_4)AsO(_2)(OH)(ONa)</td>
<td></td>
<td>Pharmaceutical manufacture</td>
</tr>
</tbody>
</table>

Pesticides appear at the top of many lists of products containing arsenic compounds\textsuperscript{8}. Although arsenic compounds kill pests very well, they are just as toxic to higher life forms (i.e. people, and their pets). Since they are indiscriminate in their toxicity, most manufacturers have largely discontinued the use of arsenic as an active ingredient in pesticides. As of 2004, Grant’s Ant Control Ant Stakes is the only pesticide with an active arsenic ingredient – arsenic trioxide\textsuperscript{7,9}. The U.S. EPA has banned the use of arsenic in all pesticides other than the wood preservative CCA (chromated copper arsenate)\textsuperscript{10}. In addition, advances in science have developed pesticides that are more specific in target.

CCA is the most common use of arsenic; 90\% of industrial arsenic use is in CCA production\textsuperscript{10}, because it deters bacteria, fungal growth, and insect invasion. Pressure treated lumber is used to construct many outdoor wooden structures. Concern of leaching of the compounds out of the wood over time is well founded, and has led to a voluntary industry-wide discontinuation of the use of CCA by the end of 2003\textsuperscript{10,11}. 
Another source of arsenic is the addition of arsenic to lead in the casting of shot ammunition. Arsenic is added to lead shot intentionally to increase its surface tension, which helps make a more perfect spherical shape during manufacturing\textsuperscript{14}. Some work has also suggested that antimony and arsenic are added to lead shot, in small amounts, to increase the hardness of the shot.

One of the most common reasons that lead shot is outlawed for waterfowl hunting is not based on ballistic rationale. It was outlawed due to its toxic affects on the waterfowl feeding from the bottom of shallow waters, and resulting in lead poisoning from incidental ingestion. Arsenic is thought to be added to shot and added to primers of munitions, such as shotgun shells. This leads to the potential of not only lead toxicity, but arsenic toxicity as well, where shot can accumulate on firing ranges and in trap and skeet fields.

Another likely source of contamination in ballistics is the possibility of the copper having impurities, such as arsenic. Tseng\textsuperscript{13}, and Harte, Holdren, Schneider, and Shirley\textsuperscript{5} discuss occupational arsenic poisonings in workers at copper smelting plants poisoned by arsenic naturally occurring in the copper ore dust. The use of copper in ballistics is in bullet manufacturing; the lead core is “jacketed” with copper.

Upon absorption inorganic arsenic, arsenate, and arsenite will reach the liver. There, it is detoxified by methyltransferase. However, the enzyme S-adenosylmethionine (SAM) must be present in the liver for the methyltransferase to work. Toxicity is not achieved until the enzyme is overwhelmed, and metabolic saturation is reached\textsuperscript{14}. The SAM methylates arsenic compounds to two metabolites; these metabolites are methylarsonic acid (MAA) and dimethylarsinic acid (DMAA). The primary route of
excretion is through the kidney\textsuperscript{7}. Tam, Charbonneau, Bryce, Pomroy, and Sandi\textsuperscript{15} conducted a controlled study of As metabolism in humans and determined that the total excretion of arsenic metabolites in the urine of six subjects on successive days were very similar as shown by the small standard error. Their study showed that methylation of inorganic arsenic is remarkably consistent between individuals.

Arsenic causes toxicity by combining with sulfhydryl (-SH) enzymes and interfering with cellular metabolism\textsuperscript{8}. Berg, Tymoczko, and Stryer\textsuperscript{16} show that arsenite has a high affinity for neighboring sulfhydryl groups, such as those in the reduced dihydrolipoyl groups of the dihydrolipoyl dehydrogenase component of the pyruvate dehydrogenase complex (Figure 3).

\textbf{Figure 3}. Mechanism of As inhibition of pyruvate metabolism and the detoxification of As poisoning by 2,3-Dimercaptopropanol\textsuperscript{16}. 
The binding of arsenite to the dihydrolipoyl groups inhibit the complex and lead to central nervous system pathologies. This disruption in pyruvate metabolism is significant due to the pathologies of the central nervous system that may develop, since glucose is the preferred energy source for the highly differentiated nervous system cells. If pyruvate metabolism is disrupted, the energy needs of any cell that utilizes glycolysis, gluconeogenesis, or the Kreb’s cycle, will not be met. Berg et al.\textsuperscript{16} state this concept very concisely; an energy-converting pathway that is stopped at pyruvate will not proceed for long because the redox balance has not been maintained.

In addition to the disruption of pyruvate metabolism, many mechanisms of electron transport may be disrupted, as well. Zubay\textsuperscript{17} discusses the role of heme groups and iron sulfur clusters in cytochromes involved in electron transport. He states that the iron of the heme is bound on one side to the sulfur atom of a methionine residue (see Figure 4). This residue also has a sulfylhydryl group that is subject to attack by inorganic arsenic. Also, he shows (see Figure 5) that NADH dehydrogenase, succinate dehydrogenase, and the electron-transfer flavoprotein ubiquinone oxidoreductase all contain iron atoms that are bound by the sulfur atoms of cysteine residues of the proteins (iron sulfur clusters). Cotton,
Wilkinson, and Gaus\textsuperscript{18} propose a hypothesis that oxidation and reduction of the [4Fe-4S]\textsuperscript{2+} cluster lead to different sorts of structural deformations (see Figure 6). In a 2001 study, Xie, Kondo, Koga, Miyamoto, and Chiba\textsuperscript{19} conclude that arsenic poisoning may influence porphyrin biosynthesis. They state porphyrins and \(-\)aminolevulinic acid (ALA) are intermediates in the heme biosynthetic pathway, which is highly susceptible to environmental factors. Disorders in this pathway may cause changes in urinary porphyrins and ALA. The effects of arsenic on the heme biosynthetic pathway and urinary excretion of porphyrins have been reported in humans and animals. These studies conclude that there is a correlation between arsenic exposure and an increase in urinary excretions of porphyrins, as well as an influence on porphyrin biosynthesis from arsenic exposure. Lu and Kacew\textsuperscript{20} state that acutely large amounts of arsenic exposure may interfere with heme formation as evidenced by the increase in urinary excretion of porphyrins. Therefore, from the disruption of porphyrin biosynthesis and the subsequent interference in heme biosynthesis from the toxic effects of arsenic, it can logically be deduced that the energy pathways, electron transport, oxygen transport, and cellular respiratory waste transport mechanisms that involve the use of heme-containing proteins can be, in effect, shut down.
Li and Chou propose that the toxicity of arsenite results from its affinity for sulfhydryls. A number of sulfhydryl-containing proteins and enzymes are altered by exposure to arsenites. Although arsenic affects mitochondrial enzymes and impairs tissue respiration, which appears relevant to cellular toxicity manifestation, the precise mechanism and intracellular events leading to cell injury by this heavy metal ion are still not well defined. These findings on the impact of arsenic exposure on the Krebs cycle, glycolysis, gluconeogenesis, cytochrome metabolism, and porphyrin and heme production all indicate profound perturbations in energy production within the cell, and throughout the organism as a whole.

Li and Chou’s study focused on injury to the architecture of the cell when the cells are exposed to arsenic. They state that the cytoskeleton is important in maintaining cellular architecture, internal organization and regulating cell shape, motility, cell division, and other cell processes. Cytoskeletal proteins (e.g. tubulin) contain many cysteine residues, and certain tubulin –SH groups are crucial for microtubule polymerization. The abundant distribution of cytoskeletal elements suggests that the cytoskeletal system may be an ideal target for As$^{3+}$ insult. The implication of their study
suggests that a cell that is exposed to arsenic poisoning may not be able to maintain cellular structural integrity. Lu and Kacew\(^{20}\) also propose a theory of damage to the cell structure since they state that intracellular structural proteins, such as cytoskeleton, may be damaged by toxicants, e.g. arsenic and paraquat. Since the cell cannot maintain proper structure to continue with normal metabolic functions, eventually cell death is inevitable.

Another aspect of the unilateral impact of arsenic poisoning is shown in a study conducted by Vahter, Marafante, Lindgren, and Dencker\(^{22}\). Their study showed that arsenic binding to microsomes seemed to be considerably stronger than in other portions of cells and tissues, possibly interfering with normal protein production.

In one of the many articles and studies that describe the signs and symptoms of arsenic poisoning, Hutton and Christian\(^{23}\) discuss symptoms that include multi-system disease (a disease that imitates many other diseases). Burning and dryness of the oral cavity, esophagus, and stomach, nausea, protracted vomiting, abdominal pain, and diarrhea occur in acute arsenical poisoning. Chronic arsenic intoxication is manifested by malaise and fatigue. Skin effects include increased pigmentation and hyperkaretosis of the palms and soles. Transverse white lines in the nail beds (Mee’s lines) appear approximately six weeks following intoxication. Peripheral neuropathy with prominent muscle atrophy is also a late finding in severe arsenic poisoning. Severe hematological abnormalities including pancytopenia are associated with arsenic intoxications. A neurological finding that occurs earlier but has received very little attention is an unusual posturing of the hands. This may be explained as neural disturbances due to the altered energy pathways and cellular structural damage. The paradox of this is found in early
arsenic intoxication, where the neural abnormalities are not homogenous across the entire nervous system, or the localized injury. This implies a partial neuropathic effect from the arsenic insult. The literature researched did not address the manifestation of hyperkeratosis and increased pigmentation of the palms and soles; however, it is addressed in the study done by Chowdhury et al\textsuperscript{24} stating that those suffering from severe keratosis may develop skin cancer in the long run. This is also presented by Stelljes\textsuperscript{14}, who claims that ingestion of high enough levels of arsenic, usually from contaminated drinking water, can lead to skin cancer. This is different from skin cancer caused by ultraviolet light, as the cancer develops in areas not typically exposed to UV light.

A more disturbing effect of chronic arsenic poisoning is a condition known as “black foot disease.” Lu and Kacew\textsuperscript{20} describe black foot disease as gangrene of the lower extremities resulting from peripheral endarteritis. Tseng\textsuperscript{13} previously gave a more precise description of black foot disease, as the gangrene appears to result from arteriosclerosis and thromboangitis obliterans. Tseng\textsuperscript{13} concluded that the degree of impairment of patients with black foot disease might be directly related to duration of intake of arsenical water. This is in agreement with Harte, Holdren, Schneider, and Shirley\textsuperscript{5} as they report that peripheral vascular changes as well as general vascular collapse occur that may lead up to gangrene. There is no need to discuss the deleterious systemic affects of gangrene. The long-term investigations by Lu and Kacew\textsuperscript{20}, and Chowdhury et al\textsuperscript{24} into the affects of arsenic poisoning both involve arsenic contaminated ground water. The study by Xie, Kondo, Koga, Miyamoto, and Chiba\textsuperscript{19} examines the effects of arsenic poisoning as precipitated from burning arsenic contaminated coal.
All the stated evidence indicates that arsenic poisoning has ubiquitous consequences in terms of systemic damage. The cells have their redox balance disrupted, which leads to impaired energy production, disrupted functioning of any protein with an exposed –SH group, and distorted or destroyed cell structure, which allows for vascular failure, at which time, gangrene is a distinct possibility.

With a better understanding of arsenic’s impacts at the biochemical level, the role of the forensic analysis can be applied to the detection of arsenic poisoning. The methods of detection illustrated at the start of this article are both useful. The analytical method of choice for this study is Atomic Absorption Spectroscopy (AA). This method is suitable for detection of nearly all metals. It is widely known that forensic examiners use this method in evaluating chronic heavy metal poisoning. The distinctive piece of evidence that prompts forensic examiners to test for heavy metals is the appearance of Mee’s lines. Since Mee’s lines are a characteristic found in nails, the testing of nails and similar structures, i.e. hair, are the tissue of interest for analysis. Hopps\textsuperscript{25} explains the preference for hair and nails, as stating that body tissues are in a state of dynamic flux. Hair and nails are exceptions. They are formed in a relatively short time, after which the finished structure is expelled from the skin surface to become isolated from the body’s continuing metabolic activities. Hopps\textsuperscript{25} contends, moreover, that the hard, relatively impermeable keratinous outer structure seals in the hair’s constituents, holding them in place for a much longer time than is comparable in other tissues. Nail is comparable to hair in these respects, but the time of its formation is longer. Interpretation of Hopps\textsuperscript{25} study reveals that elements bound in the structure of hair and nails are sequestered until the nail or hair is shed. Hopps\textsuperscript{25} states, definitively, that an impressive body of literature supports the
view that trace element content of hair and nail reflects body intake from which one can conclude that hair and nail are suitable samples for evaluating body stores. Therefore, the use of hair and nail is a good historical record of the body’s metabolism, with respect to trace elements. In Hopps' article there is a point that is of significant importance. He describes keratin as not a specific chemical substance, but a category of highly stable, fibrous protein which contains disulphide bonds and which is remarkably resistant to enzymatic digestion and all but the strongest chemicals. The most important of the several types of bonds that stabilize the keratin molecule is the sulfur-to-sulfur bond. It is this characteristic –S-S- linkage (formed by two cysteine residues in the adjacent polypeptide chains) that is unique to keratin among the fibrous proteins found in the skin. The preponderance of arsenic’s affinity for the sulfur groups of cysteine is already established. So, it is to be expected that arsenic will be found in proteins with high cysteine content such as hair and nail. It is only logical to examine them. The advantage of this is shown in Shapiro’s article. Deposition of arsenic begins within a matter of minutes after absorption and this explains the high concentration observed in the proximal bulb-containing half inch of hair. The conclusion in Hopps' article is that hair and nail samples are easy to get and easy to store. Trace element analysis of these materials is precise, accurate, and reproducible. An interesting example of the ability of keratin proteins to sequester trace elements over time is the study conducted by Weider and Fournier where they conclude Napoleon was assassinated using arsenic. They reached this conclusion by an examination of a hair sample taken from Napoleon’s body.

The goal of this study is to use these methods in a novel manner. The impact of arsenic in the environment is well established as a negative one. Terrestrial isopods are
the sample of interest. Terrestrial isopods are common, and can be easily collected if fair weather exists. Common species that can be collected in this study area are *Oniscus asellis, Porcelio scaber,* and *Armadillium vulgare.* The unique aspect of these organisms is that they are detritivores, and the surrounding state of the environment can appear as a function of the isopods general health, or more precisely, a function of their metabolic state. Jones and Hopkin\textsuperscript{28} state this very well. Woodlice (terrestrial isopods) are important detritovores in many habitats and are suitable for assessing the ecotoxicological effects of metals. In addition, Kulzer\textsuperscript{29} states that sow bugs have the amazing ability to store very high concentrations of metals in the hepatopancreas. This is supported by Hopkin’s\textsuperscript{30} earlier study in which he states that hepatopancreas of terrestrial isopods from sites contaminated with metal can accumulate zinc, cadmium, lead, and copper to concentrations which are among the highest recorded in the soft tissue of any animal. Hopkin\textsuperscript{30} concludes that woodlice provide excellent models for studying the ecophysiological factors controlling species-specific differences in metal pollutant assimilation, storage, and excretion, which may be applicable to a much wider range of terrestrial arthropods.

Due to the detrimental effects of arsenic poisoning, whether it be accidental or otherwise, arsenic has to be monitored in the environment. The focus of this study is to show that terrestrial isopods have an ability to sequester arsenic taken from the environment, and established methods already exist to analyze for the presence of arsenic. The trusted method, i.e. Atomic Absorption Spectrophotometry, can quantify the presence of the arsenic. Additionally, the toxic effects of lead are well established, and lead analysis will be conducted in parallel with arsenic.
Methods and Materials

Controls

The controls consist of three groups: one container with a piece of untreated dimensional kiln dried lumber (negative control), one container with ACQ treated lumber (intermediate control), and one container with a piece of CCA treated lumber (positive control). Each container has ~45 terrestrial isopods of mixed species (Ward’s Natural Science Cat. No. 87w5525). In each container, a food source of 50g of leaf litter from the same source (Northampton Park, Brockport, NY) is placed in the container on top of and around the piece of lumber. The media for the control containers will be sterilized potting soil (Ward’s Cat. No.20w8306) at a depth of 2-3 inches. The container is covered with clear perforated plastic wrap in a controlled environment to allow sunlight in and to maintain humidity. This is to maintain a natural photoperiod. All 3 groups were maintained for 4 weeks. A fourth group of terrestrial isopods will be digested without being maintained in a container. This group is an untreated control group.

To establish the control values, 3 equal groups of isopods were removed from each container, and placed in a freezer (~ -20°C). Each group was weighed on an analytical scale just prior to digestion with concentrated nitric acid, which is heated as necessary. The digest will be diluted in an equal volume of either distilled or deionized water to prevent any metal contamination that can be introduced by tap water. The dilution is two-fold. The digest will be analyzed using the Atomic Absorption (AA) spectrometer. The AA will be calibrated using standard solutions of known arsenic
concentrations. This result will be used to determine a ratio of arsenic concentration (ppm)/weight of isopods (g).

The lumber will have 2 small pieces removed prior to the start of the experiment. The first piece for each type of lumber will be weighed, digested and analyzed to determine the starting concentration of arsenic. The second small piece will be in along side the lumber in a manner as if it is still attached. At the end of the control period, the second small piece will be weighed, digested and analyzed. The difference of the initial and end of study results will be compared.

For all analysis, a lead analysis will be done as a parallel study for each digest. This is to allow for the examination of possible inconsistencies.

Test Sample collection

The test samples will be collected in a fashion similar to that described by Jones and Hopkin\textsuperscript{28}. Each collection will be done in a 15-foot by 15-foot square; logs, stones, and other obvious suitable habitats that may contain terrestrial isopods will be overturned for a period of 30 minutes. Any specimens found will be collected; each sample set will be labeled with the location, date, and sample number. Three samples will be collected from each location. The locations will be a private residence (Spencerport, NY), Four Point Rod & Gun Club (Chili, NY), a local park (Black Creek Park, North Chili, NY) and the Rochester Institute of Technology campus (Rochester, NY). Each sample will be placed in a freezer for a minimum of overnight. At the time of analysis, each frozen sample will be weighed on an analytical balance, digested with concentrated nitric acid,
and analyzed. Every reasonable effort is to be taken to ensure the samples remain frozen immediately prior to digestion.

The exception to the collection areas is Four Point Rod & Gun Club. In addition to samples being collected as above, three water samples will be collected, as there is standing water on the property. The water samples will be digested with nitric acid to a final concentration of 50% nitric acid. If the samples are off scale, they will be diluted appropriately to achieve a value on scale, while maintaining a final concentration of 50% nitric acid.

**Instrument Calibration**

The atomic absorption spectrometer used is a Perkin-Elmer Model AAnalyst 100. The spectrometer standard solutions used are for arsenic, 1000 g/mL solution (Perkin-Elmer Cat. No. N9300180, 100mL), and for lead, 1000 g/mL solution (Perkin-Elmer Cat. No. N9300175, 100mL). The standard solution is diluted to 10, 20, 30, 40, 50, 60, 70, 80, and 90 ppm for arsenic, and 5, 10, and 15 ppm for lead. The blank solution for each analysis is 50% nitric acid. Hollow cathode lamps were used in the instrument, arsenic lamp – Perkin-Elmer Cat. No. N305-0105, set at 18 mAmp; and lead lamp-Perkin-Elmer Cat. No. N305-0157, set at 12 mAmp. The instrument had wavelengths set for arsenic, 193.7 nm, and lead, 283.3 nm, as listed in Varma’s work. The slit opening for both metals is 0.7 nm. The instrument is interfaced to AA WinLab software.
Sample Preparation

Each set of control samples and each set of test samples are prepared in the same manner. The frozen samples are weighed on an analytical balance, the weights recorded, and then digested in 10mL of concentrated nitric acid (HNO$_3$). Additional HNO$_3$ may be added to dissolve any remaining sample material, as needed. The volume of acid required is recorded along with the weight of each sample. Each sample is diluted two-fold, as done previously with the controls. The amount of metal (ppm)/g of sample is calculated. Each sample is analyzed 3 times. Each mean value is then compared.

Results

Instrument calibration

The Perkin-Elmer A Analyst 100 was used to establish a standard curve that maintained linearity for arsenic and lead. Each sample was dissolved in 50% nitric acid, as described in the methods and materials section. Each standard curve is shown below.

![Standard curve for Arsenic](image)

**Figure 7.** Standard curve showing the absorbance values of standard As solutions in 50% nitric acid.
The standard curve and all test samples for arsenic used background correction to reduce any interference. The lead samples did not require background correction. Since linearity is established, the results within the range are trusted, and considered accurate. In the event that a negative value occurred, it was adjusted to have a value of zero.

**Controls**

To establish that the control samples were indeed exposed to arsenic compounds, the test lumber was analyzed in the same manner as the control and test samples. The lumber samples included: kiln dried dimensional lumber (negative control lumber); ACQ pressure treated lumber (intermediate control lumber); and CCA pressure treated lumber (positive control lumber). The terms negative control, intermediate control, and positive control are in reference to the presence of arsenic throughout this study, even though the same samples were tested for lead. The values of each lumber sample as a function of the mean value for the lumber initially and finally are shown below.
The results show that, in fact, arsenic is present in the positive control lumber (PCL). The PCL had a value of 42.11 ppm/gram of lumber initially, and 40.90 ppm/gram of lumber after the control period. In aS also indicates that no arsenic is present in the other control lumber samples (NCL and ICL). These results are as expected, as the NCL is just kiln dried dimensional lumber, and the ICL is, as stated previously, contains a quaternary ammoniacal copper compound.

In contrast to a very distinctive presence of arsenic in the PCL, the presence of lead appeared in both the treated lumber samples. Although research into the process of pressure treating lumber yielded no mention of the use of lead in the process, these may be proprietary processes. Although the presence of lead in such minute quantities may also be of little consequence in the overall presence of metals as a whole in pressure treated lumber; it is of value for this study.
After the 4-week control period, the control isopods were collected. The negative control group showed evidence of active feeding, as approximately 50% of the leaf litter had been consumed. The lumber had a slight amount of mold on the bottom (<~10%) where it was in contact with the soil. The isopods were very physically active at the time of collection, and fecal matter was observed on top of the lumber. This indicates favorable conditions. The intermediate control group showed much less favorable conditions. The culture had a distinct odor of ammonia. The bottom side of the lumber showed coverage (>~90%) by a white mold. There was the largest amount of leaf litter remaining of all control groups, a lack of evident fecal matter, and the amount of dead remains indicates that the ACQ lumber had a toxic affect. The positive control lumber showed similar results as the negative control lumber with respect to the condition of the isopods. The leaf litter showed signs of feeding, the presence of fecal matter, and the active movement demonstrated favorable conditions. There was no visible mold anywhere on the lumber. The control samples were digested as described in the method and materials section. The results are shown in figure 11.

**Figure 11.** The mean As concentrations for each control group. The abbreviations are as follows; NC, Negative control; IC, Intermediate control; PC, Positive control; UC, untreated control. The error bars represent the standard error of the mean.
**Figure 12.** The mean concentrations of Pb for each sample group. The abbreviations are as follows; NC, Negative control; IC, Intermediate control; PC, Positive control; UC, untreated control. The error bars represent the standard error of the mean.

![Graph showing mean concentrations of Pb for each sample group.]

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean Mass (grams)</th>
<th>Mean As conc (ppm/gram)</th>
<th>Mean Pb conc (ppm/gram)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>0.175</td>
<td>0.000</td>
<td>2.372</td>
</tr>
<tr>
<td>IC</td>
<td>0.206</td>
<td>11.167</td>
<td>3.010</td>
</tr>
<tr>
<td>PC</td>
<td>0.322</td>
<td>10.679</td>
<td>3.191</td>
</tr>
<tr>
<td>UC</td>
<td>0.513</td>
<td>20.907</td>
<td>2.556</td>
</tr>
</tbody>
</table>

*Table 2.* The mean mass, As concentration, and Pb concentrations of the control groups

**Test samples**

The test samples were collected in a 15-foot by 15-foot square for 30 minutes, searching under logs, stones, and any other obvious, suitable environment for terrestrial isopods. This method is similar to the method described by Jones and Hopkin. The above digestion and analysis was done as described in the methods and materials. The digest was then analyzed. The values for each sample are shown in figures 13 and 14.
**Figure 13.** The mean As concentration of each test sample. The abbreviations are as follows; H, samples collected from a private residence; R, samples from The Rochester Institute of Technology campus; B, samples from Black Creek Park; F, samples from Four Points Rod & Gun Club; samples 1, 2, 3. The error bars represent the standard error of the mean.

**Figure 14.** The mean Pb concentration of each test sample. The abbreviations are as follows; H, samples collected from a private residence; R, samples from The Rochester Institute of Technology campus; B, samples from Black Creek Park; F, samples from Four Points Rod & Gun Club; samples 1, 2, 3. The error bars represent the standard error of the mean.
Table 3. The mean mass, As concentration, and Pb concentration for each of the test samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mass (grams)</th>
<th>Mean As conc (ppm/gram)</th>
<th>Mean Pb conc (ppm/gram)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-1</td>
<td>2.106</td>
<td>3.632</td>
<td>1.430</td>
</tr>
<tr>
<td>H-2</td>
<td>0.363</td>
<td>25.014</td>
<td>7.730</td>
</tr>
<tr>
<td>H-3</td>
<td>0.825</td>
<td>11.078</td>
<td>2.568</td>
</tr>
<tr>
<td>R-1</td>
<td>0.233</td>
<td>48.146</td>
<td>5.176</td>
</tr>
<tr>
<td>R-2</td>
<td>0.834</td>
<td>12.338</td>
<td>1.670</td>
</tr>
<tr>
<td>R-3</td>
<td>0.037*</td>
<td>258.937</td>
<td>27.118</td>
</tr>
<tr>
<td>B-1</td>
<td>0.046*</td>
<td>146.797</td>
<td>21.217</td>
</tr>
<tr>
<td>B-2</td>
<td>0.234</td>
<td>23.667</td>
<td>5.399</td>
</tr>
<tr>
<td>B-3</td>
<td>0.010*</td>
<td>466.600</td>
<td>102.933</td>
</tr>
<tr>
<td>F-1</td>
<td>0.044*</td>
<td>0.000</td>
<td>26.561</td>
</tr>
<tr>
<td>F-2</td>
<td>0.080</td>
<td>0.000</td>
<td>14.458</td>
</tr>
<tr>
<td>F-3</td>
<td>0.042*</td>
<td>0.000</td>
<td>30.000</td>
</tr>
</tbody>
</table>

* Mass < 0.050 grams may cause variability. Drought suspected cause of low mass.

The test areas for each metal show significant differences (p < 0.05) as to their concentration/gram of sample. In addition, the mass of each sample showed a significant difference (p < 0.05) between each sample group. The matter of mass will be discussed in a later section. The mean mass, arsenic concentration, and lead concentration are shown in table 3.

Water samples

As stated earlier, the test samples from Four Points Rod & Gun Club included samples from a water source on the club property. The water samples were collected from three separate areas of the property. Sample 1 was collected from an area, which sees little use. Samples 2 and 3 were collected from an area down range of the trap.
fields. All samples were collected from the same body of water. The mean concentration for arsenic was 0 ppm, and the mean concentration for lead was 1.257 ppm. The results are shown in Table 4.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (ppm)</th>
<th>Sample</th>
<th>Mean (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FW-1-As</td>
<td>0</td>
<td>FW-1-Pb</td>
<td>1.179</td>
</tr>
<tr>
<td>FW-2-As</td>
<td>0</td>
<td>FW-2-Pb</td>
<td>1.294</td>
</tr>
<tr>
<td>FW-3-As</td>
<td>0</td>
<td>FW-3-Pb</td>
<td>1.277</td>
</tr>
</tbody>
</table>

Table 4. Mean metal concentrations of water samples taken from Four Points Rod & Gun Club.

The analysis of the water samples indicates that it is an effective method to assess and quantify the level of metal contamination. In this study, the evidence shows that there is no arsenic detected in the water on this property. In conjunction, the evidence shows a mean value of 1.257 ppm of lead in the water samples taken.

Conclusions

The environmental assessment of heavy metal contamination is of concern. In fact, there are entire industries based on environmental assessment. The scientific community is also making advances in determining the impacts of heavy metal contamination. As previously discussed, this includes the possible contamination of the food we eat, common products used around the house, and occupational exposure, to name just a few. The assessment done in this study used two established techniques, the ability of terrestrial isopods to survive relatively large amounts of heavy metal poisoning, and the established forensic technique for quantifying the amount of metal present.
These two methods used in tandem give a fairly straightforward and reliable method of determining contamination.

This method shows promise based on the fact that in figures 9–14, many of the error bars (representing the standard error of the mean) are very tight. This is indicative of the results being reliable. Those samples that show larger error bars are samples with very little or no metal content, or are of a very small mass. The low mass of some samples suggests that a minimum mass should be achieved as a caveat to this method. The outliers appeared in those groups with a mass of less than 0.050 grams. The low mass is suspected to be a result of the severe drought in the areas of study. The drought may have impacted the ability to collect terrestrial isopods due to possible mortality from the dry conditions, or aestivation; a hibernation like state during severe conditions, such as drought. There was locally < 2 inches of rain during the 8 week period that test samples were collected.

The study demonstrated significant differences between the test groups (p < 0.05). This suggests that different locations can be tested with this method. Differences between the test groups suggest that the method is reliable in its validity, and versatile in its application. The versatility stems from the analytical technique used; the atomic absorption spectrophotometer.

The fact that the method shows reliability in the analysis of each group, that it can detect differences between test areas, if they are present, and it’s versatility for most metal contaminants provides strong support for the robustness of this method.

The lumber used in this study contradicts the point of view reported in the media. The public outcry for the removal of CCA lumber from the market appears to be based on the fear of the toxic affects of arsenic. In comparing the isopods in each control group,
the lumber that showed the most toxicity was the ACQ group. The odor of ammonia and
the large amount of dead loss in the culture suggests that the ACQ lumber exhibits more
toxic affects than the CCA lumber. The conjecture is that the ammonia in the ACQ is the
suspected toxin. The CCA lumber showed similar effects as the untreated lumber. There
was more active feeding and a lower amount of dead loss. Another interesting
observation was the large amount of mold present on the ACQ lumber. Pressure treating
lumber is a method of preservation against insect and microbial attack. The ACQ
appeared to kill some of the isopods, so it does protect against insect invasion. However,
the mold growth after only 4 weeks shows little affect against microbial attack.
Concomitantly, the decaying insects promote microbial proliferation. Since the evidence
in this study shows that the ACQ preservative is ineffective as a deterrent to microbes, it
appears that ACQ is not a suitable alternative to CCA preservation.

This study was based on the work by Jones and Hopkins\textsuperscript{31}. In their study, the
length of time that the isopods were in culture was up to 20 weeks. In this study, the
control period was 4 weeks. The shorter time period perhaps is the reason for so little
arsenic absorption. It is obvious that the isopods would take advantage of a preferred
food source before feeding on any food source that presents itself. If the leaf litter had
been exhausted as a food source, it is speculated that the lumber samples would be fed
upon more readily. Thus, more absorption of metal would occur. The smaller time frame
indicates that this may not have happened. The resulting values for the control values
may be ambient levels. Further study would address this.

In addition, several other factors may have to be studied to create more precision
in this method. These include, but are not limited to, rate of absorption, mobility of
isopods from one area to another, the impact of weather conditions, and long-term survival in culture.

The model displayed in this study shows many advantages. The foremost is the robustness of the method (keeping in mind that large enough samples need to be available) and its simplicity. Both lend themselves to cost effectiveness. The materials used are all commercially available at relatively low cost. This leads to the conclusion that it is a method that can be used for on-going monitoring of suspected areas. The method’s limitations as a monitoring tool include weather conditions. The conditions must be suitable for terrestrial isopods to be present and active, implying that in this area collections cannot be made in winter. Even though collections were made during drought conditions, some collections of effective mass were collected. This method shows reasonable estimates of the level of arsenic and lead contamination in the test areas.

References


Additional references


