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Detection and Quantification of Arsenic Pollution with a Moss Bio-Indicator and ICP-MS

by

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1: Abstract

Heavy metal pollution is a recognized concern with an established source of anthropogenic activity, which is ever-increasing. While work has been done to make the monitoring of some metals more accessible, resourceful, and efficient with the use of epiphytic moss as a bio-indicator, this method has not been optimized for the detection of arsenic. Previously used analytical instrumentation has not proven sensitive enough to reliably detect arsenic, creating a gap in the monitoring process. However, inductively coupled plasma mass spectrometry (ICP-MS) is proposed as the solution to fill this gap. Its potential for low limits of detection and quantification express a sensitivity that is optimal for trace heavy metal analysis. This instrument was used to investigate the same moss sampling sites around an area of interest where previous instruments were largely unable to even detect inorganic arsenic in the moss biomass. Analysis with ICP-MS resulted in the detection of arsenic in each moss sample, demonstrating its superiority over the previous instruments, but confirmation of accurate and precise quantification was still not achieved. The range of arsenic concentrations obtained, which averaged to 0.35 ± 0.054 mg/kg on a 95% CI, was above the limit of detection of but below the limit of quantitation. However, two distinct opportunities for further optimization of the calibration process and subsequent lowering of the quantitation limit were noticed and are recommended for future analysis.

Keywords: Arsenic, ICP-MS, heavy metal pollution, public health, urban air quality.

2: Introduction

Inorganic Arsenic (iAs) is an elemental metalloid that is toxic to human health and the environment even at low levels and especially with chronic exposure. Due to its high density relative to water and low-level toxicity, it has been classified as a heavy metal. Arsenic is naturally omnipresent in the environment at extremely low concentrations, but dangerous levels of arsenic may quickly accumulate due to anthropogenic activity. Especially in urban, industrial, and manufacturing areas, the threshold of safety of heavy metals can quickly be surpassed. At trace concentrations in soil, foods, groundwater, or atmospheric air, it can still impose a wide array of public health and ecological safety risks, disrupting the natural functioning of human organ systems and ecosystems alike. These risks have led several regulatory agencies and organizations to set safety limits on iAs concentrations but following and enforcing these regulations requires empirical data to be gathered on the actual arsenic concentrations in an area.

The need for consistent, reproducible monitoring of environmental heavy metal pollutants has not gone unrecognized. The capability to accurately and precisely determine concentrations of toxic metals in the environment is integral to investigating whether they are within established and accepted safety limits and ensuring that they continue to be. This has been achieved for other metals with the previously attempted use of inductively coupled plasma optical emission spectroscopy (ICP-OES), but arsenic could

not be reliably measured with this instrument due to its relatively low sensitivity. However, arsenic quantification is possible with a different instrumentation; inductively coupled plasma mass spectrometry (ICP-MS). The previous study's resourceful use of epiphytic moss as a bioindicator for atmospheric heavy metal pollution and its accessible sampling methods will be incorporated into this study on atmospheric arsenic concentrations in Portland, OR.

The goals are to illustrate the need for a method of atmospheric arsenic analysis and monitoring that is relatively simple, accessible, and optimized for this particular metalloid, and to prove that ICP-MS paired with the previous study's collection and sample preparation methods are the answer to that need. Even when using the same collection and preparation methods as would otherwise be used for ICP-OES analysis, ICP-MS offers the sensitivity required for accurate and precise monitoring of arsenic pollution.

2.1 iAs Regulations

The Occupational Safety and Health Administration (OSHA) has mandated that no more than ten micrograms of inorganic arsenic may be present per cubic meter of air, averaged over any eight-hour work period, as a permissible workplace exposure limit. This ten microgram of inorganic arsenic per cubic meter of air exposure standard has been repeated by the American Conference of Governmental Industrial Hygienists as a threshold limit value, while the National Institute for Occupational Safety and Health bases their limit of two micrograms per cubic meter of air on the classification of inorganic arsenic as a human carcinogen. The Environmental Protection Agency (EPA) has lowered the drinking water standard for maximum contaminant level of arsenic from 50 to 10 ppb (parts per billion by volume) as of 2001 and the World Health Organization (WHO) has echoed this 10 ppb guideline for drinking water as well. Furthermore, the levels of arsenic in some veterinary drug-treated food byproducts of animal agriculture have been set by the US Food and Drug Administration (FDA), such as 0.5 ppm for eggs and uncooked edible chicken and turkey tissue as well as 2 ppm for uncooked edible swine tissue (*Arsenic Toxicity...* 2009). The Oregon Department of Environmental Quality (DEQ) uses Oregon's ambient air toxic benchmarks to set consistent health-oriented goals in developing air toxic reduction strategies. The benchmarks are based on recommendations made by the 2014-2017 Air Toxics Science Advisory Committee. For arsenic in ambient air, this is marked at $0.0002 \mu\text{g}/\text{m}^3$ (*Ambient...* 2018). Although the severity of the toxic effects of iAs is dependent on species, pathway, and dose, public human health is imperiled in numerous ways if these guideline values are surpassed.

2.2 Physiological Effects of iAs

Arsenic may enter the human body through several pathways such as ingestion, inhalation, and absorption through the skin and mucous membranes, (ATSDR, 2007)(Tchounwou et al., 2012). Its toxic effects impact nearly all of the body's organ systems, such as the immune, nervous, endocrine, integumentary, respiratory, and reproductive systems, as well as others, leading to a vast array of

unfavorable health conditions and complications (Mohammed Abdul et al., 2015)(Guha Mazumder and Dasgupta, 2011). Because these organ systems are largely codependent, iAs toxicity to one may make the others more susceptible to damage as well.

As aforementioned, inorganic arsenic is a known human carcinogen, as classified by the EPA, that has shown association with lung, kidney, liver, breast, laryngeal, bladder, non-melanoma skin cancer, and other cancers. The risk of developing these cancers was also found to increase several-fold if exposure to arsenic occurred in utero or in early life stages, even if the exposure levels themselves were low-moderate. Furthermore, long-term, repeated exposure to iAs worsens the effects of cycling and build-up of the metalloid in the organ systems. Various other genotoxic and epigenetic effects that are harmful in nature may present, as iAs can cross the placental barrier and accumulate in the fetus with maternal exposure to the metalloid. Pregnancy adversities, infant mortality, and the impaired future health of infants are associated with arsenic poisoning (Fei et al, 2013)(Tchounwou et al., 2012). Major heritable alterations to gene expression are possible, and these epigenetic alterations are linked to a vast array of diseases and inherited gene mutations, which further raise the risk of the development of cancers. Gene methylation may help to mediate arsenic toxicity and carcinogenicity, yet arsenic can induce inhibiting regulatory changes to this process (Mohammed Abdul et al., 2015).

Neuropathic and neurotoxic effects on the body are another possibility. Diseases and disorders of the nervous system are a major risk, as the blood-brain barrier is easily passed by iAs, similarly to the placental barrier. The metal can then begin to accumulate in the tissues of all parts of the brain. This can quickly cause difficulties or deficits in learning, focus, memory, and concentration, as well as causing lethargy and an impairment to cognitive performance. In this way, the cognitive development, intelligence, and memory of children and adults are put at risk (Mohammed Abdul et al., 2015)(Tchounwou et al., 2012).

There are also profuse dermatologic effects of arsenicosis, or chronic arsenic poisoning, such as dermal lesions, melanosis, and keratosis. These worsen with rising levels of iAs in the body and can act as a visible cue when diagnosing arsenicosis. The burning of mineralized coal which contained extraordinarily high levels of arsenic in Guizhou, China exposed the residents to extremely toxic levels of iAs in the range of 20–400 $\mu\text{g}/\text{m}^3$. Since 1976, over 3000 patients of this area have been diagnosed with arsenic poisoning, and one of the markers of this poisoning were the obvious skin lesions, which about 17% of all the Guizhou residents had developed. Skin ulceration, hand and foot keratosis, trunk pigmentation, and skin cancers were diagnostically associated with the arsenic poisoning (Liu et al., 2002)(Finkelman et al., 1999). There is a high correlation between endemic arsenic contamination and liver damage as well, especially from sources such as the burning of high-arsenic coal. Guizhou residents

were found to prevalently suffer with conditions such as hepatomegaly (or enlargement of the liver), liver damage, cirrhosis, ascites, and liver cancer (Liu et al., 2002)(Hu et al., 2021)(Yao et al., 2021).

The rivers of West Bengal India suffer some of the most extreme arsenic contamination recorded. This water is used for drinking, bathing, cooking, and cleaning, so the metalloid has several body entry pathways open to it. Occurrences of arsenicosis in seven districts of this area are common as a result. Six million people in West Bengal are estimated to be exposed to arsenic through groundwater and at least 560 villages and 200,000 people in this area have been found to be affected by arsenic toxicity in some way. This has produced thousands of cases of various chronic symptoms. Some of these are chronic lung diseases, bronchitis, bronchiectasis, and other pulmonary diseases, as well as liver diseases, polyneuropathy, weakness, and anemia. Skin lesions due to arsenic are common in this region as well, prevalently appearing as nonpitting edema of the feet and hands. When arsenic concentrations of 200 µg/L or greater occurred, there was found to be an associated sixfold increased risk of stillbirth (Guha Mazumder and Dasgupta, 2011)(Mandal et al., 1996).

Further toxicities to internal organs, including lung dysfunction, gastrointestinal and kidney damage, circulatory system disorders, respiratory illness, reproductive system disorders, and nephrotoxicity are clinically evident, as well as conditions such as Type 2 diabetes (Tchounwou et al., 2012)(Khan et al., 2020)(Paul et al., 2007). Public human health is threatened on many fronts by inorganic arsenic contamination. It is clear that the functional interdependence of the organ systems necessitate that inorganic arsenic toxicity, especially when chronic, creates self-perpetuating harm cycles within the human body.

Moreover, inorganic arsenic pollution puts the safety and functionality of the environment at risk as well. Protecting the health, safety, and functionality of ecosystems is one of the most effective ways humans can mitigate simple, complex, and wicked problems, anthropogenic or otherwise, that threaten the environment (Mulligan et al., 2017).

2.3 Environmental Effects of iAs

Although the main concern with arsenic pollution tends to be the possibility of adverse effects on human health, animals that live in arsenic-polluted areas may be at just as high of a risk as human populations. Animals in iAs-contaminated areas can be exposed to arsenic by contaminated water sources, as is the case in West Bengal, as well as by the grasses and leaves upon which they feed. There is evidence that arsenic is a carcinogen in animals as well as it is in humans. With acute toxicity, animals can experience uncomfortable to fatal symptoms like intense abdominal pain, tremors, paresis, vomiting and diarrhea, and circulatory collapse. Chronic arsenic toxicity in farm animals, who may have been distinctly exposed to arsenic via pesticide and herbicide application or contaminated feed, can present as fibrosis and ataxia (Mandal, 2017).

Furthermore, plants and small organisms are endangered by arsenic pollution. Seed germination has been found to be impaired as arsenic levels increase over 1 mg/kg (Li et al., 2007). If seeds do not germinate effectively, it may cause a cascading effect throughout the ecosystem in which it grows. Less biomass of wheat or other plants developing fully will reduce the overall food source available to primary consumers, and this is carried up the food chain. Similarly, the cycling of iAs in soil can impact the composition of soil microbial communities (Yu et al., 2020). Because the delicate health and natural functionality of soil can be key to an ecosystem thriving, arsenic contamination has the potential to threaten biodiversity starting from microbiota and moving up the food chain.

2.4 Sources of iAs in the Environment

High levels of arsenic in the environment are often caused by natural geological phenomena such as past volcanic activity and soil erosion. The soil of the Pacific Northwest region of the United States is naturally high in iAs due to its volcanic origins (Armitage, 2012). These natural arsenic levels in soil tend to range between 1 and 40 ppm, water concentrations of arsenic are usually less than 10 µg/L, and concentrations of arsenic in the air in remote locations are usually between 1 and 3 ng/m³ (*Arsenic Toxicity*, 2007). However, the other major source of arsenic contamination in the environment is through anthropogenic activities. These can raise the iAs levels in soil, water, and air to be much higher, reaching past toxic levels (Mohammed Abdul et al., 2015)(Tchounwou et al., 2012).

In urban areas, levels of arsenic in the air may increase to the hundreds of micrograms per cubic meter (Liu et al., 2002)(Tchounwou et al., 2012)(Guha Mazumder and Dasgupta, 2011)(Mandal et al., 1996). Arsenic contamination has been detected in a minimum of 1,149 out of the 1,684 sites present on the EPA's National Priority List, which identifies sites of US and US territory priority concerning active or threatened releases of hazardous pollutants, contaminants, or other substances (*Arsenic Toxicity*, 2007).

Arsenic occurs at high concentrations in soil near mining and waste disposal sites as well as areas where pesticide has been applied. This leads iAs to enter the food chain by being taken up by plant roots (Amist and Singh, 2021). In arsenic-containing coal processing and combustion, arsenic can be released directly into the air, as in Guizhou, China, and then deposited in water bodies and soil. Estimates have placed the total emitted arsenic from coal combustion in China alone at 1564.4 tons in 2005. The processing and consumption of fossil fuels is another source of arsenic entering the environment (Kang et al., 2011).

Various inorganic arsenic compounds can be emitted through anthropogenic activity, usually in the forms of arsenite and arsenate. Arsenic has been used in alloying, ore smelting, and veterinary medicine as well as the manufacture and processing of products such as textiles, paper, metal adhesives, ammunition, poison bait, and glass (Chung et al., 2014)(ATSDR, 2007). In this manufacturing, despite national (US) and international guidelines, regulations, and advisories, the inorganic arsenic compounds

can volatilize and surpass precautionary barriers, entering the environment. Once arsenic has been mobilized in the environment in this way, it can then contaminate ecosystems and be inhaled. The WHO asserts that poor air quality and air pollution contributes to one out of every eight global deaths per year, making air pollution the single largest environmental human health risk (*Arsenic...* 2018). The mitigation of this risk must begin with the development of reliable methods to identify sources of pollution so that environmental regulations can be effectively written and enforced. In Oregon, exposure to arsenic contamination through inhalation due to pesticide use and metal processing was observed to contribute to human lung cancer mortality (Armitage, 2012).

A manufacturing process of particular concern in this study is the production of stained glass, wherein various elements such as cadmium, lead, and arsenic are used to color the glass. The area in Portland under investigation in this study was nearby one such stained glass manufacturer.

2.5 Moss as a Bioindicator

An emerging approach to identifying heavy metal air pollution, which uses elemental analysis of epiphytic moss digests to detect and quantify the element in the air of a particular area, should be used. Epiphytic plants, or “air plants”, subsist without roots by growing on the surface of other plants, such as hardwood trees. They take in nutrients and water from the surrounding atmosphere, and therefore absorb and hold onto any heavy metal pollutants that may be present in the ambient air. For this reason, they are commonly used to monitor for airborne contaminants such as heavy metal pollutants like cadmium, lead, selenium, and arsenic. This technique is inexpensive, simple, and can be used to spatially analyze air quality. One study performed in Portland, Oregon with USFS made use of this method by collecting moss in a grid-based strategy to be used as a bioindicator for cadmium pollution. The levels of cadmium in the moss were determined with ICP-OES, allowing for spatial maps of cadmium contamination to be made across the city. Cadmium “hotspots”, which had gone undiscovered by environmental regulators, were found near two stained glass manufacturers this way, particularly one which was denoted stained glass manufacturer #1. Once these results were made public, the manufacturers voluntarily halted their use of cadmium and the cadmium levels in the air dropped significantly (Donovan et al., 2016). This USFS study, an impressive demonstration of the need for empirical data in bringing about positive changes of environmental policy and practice, is the basis of the current study.

Not only is the sampling process involved with this method simple, accessible, reliable, and cost-effective, but a particularly attractive aspect of the sampling process is that essentially anyone or any group of people can perform it. Providing access to a few fairly common household items such as a ladder, a sharpie, a notebook, Ziploc bags, and chemically protective gloves, ample moss samples can be easily collected to be analyzed for initial assessment of air quality. This accessibility allows the communities most affected by the trace metal pollution under investigation and the toxicity thereof can

become directly involved in the research process. The residents of these areas will then be meaningfully engaged and given a voice in studies which influence the health and safety of their community (Derrien et al., 2020). Producing more community researchers is instrumental in recontextualizing studies on pollution into a cultural and socioeconomic framework, from which they are often removed. Acknowledging and engaging the participation of those affected by environmental pollution reframes the issue under study to bring environmental justice into the foreground.

A direct quantification of elemental heavy metals in ambient air is difficult by use of this strategy, though, and it is the most useful for monitoring relative concentrations of metals in the air over a given area. This is because it has proven challenging to understand the relationship between levels of elemental metals in atmospheric air and the resulting levels of heavy metals accumulated in moss growth, especially over time. Concentrations of contaminants can vary drastically, even within short periods, due to the many physicochemical processes and environmental conditions upon which the uptake and storage abilities of the moss depend. This means that gathering temporally representative biomonitoring data from moss biomass is extremely challenging (Boquete et al., 2011). Nevertheless, though the correlation between the levels of contaminants accumulated in moss biomass and those present in atmospheric air is difficult to understand, it is assumed that when levels of a contaminant in the air increase, they increase in epiphytic moss tissue as well. This assumption allows initial measurements and identification of areas of particular concern in terms of heavy metal emissions to be made, which is an essential step in a comprehensive monitoring process.

However, ICP-OES analysis is not the most reliable tool when it comes to the quantification of other trace elements, including arsenic. While effective in the analysis of cadmium, the previous study's use of ICP-OES as the analytical instrument was not ideal for the detection or accurate and precise quantification of arsenic in the moss biomass. Due to its relatively high limits of detection (LOD) and limits of quantitation (LOQ), the ICP-OES is not sensitive enough, even under optimized conditions, to reliably detect or quantify trace amounts of this metalloid. Because of this, the materials and methods previously used could only produce mapped estimates via simple dot-maps of the arsenic in the moss, and many of the samples analyzed had resulted in non-detects (Donovan et al., 2016). These estimations suggested relatively low concentrations of arsenic compared to lead and cadmium, below what the ICP-OES instrumentation was capable of perceiving. Analysis was attempted for arsenic with ICP-OES again, as well as with Anodic Stripping Voltammetry (ASV), but neither of these methods were ideal for arsenic quantification. Both of these instruments were only capable of providing majority non-detect or unusably inaccurate and imprecise results.

2.6 ICP-MS

To account for this gap in methodology in quantifying arsenic, it is now suggested that ICP-MS be used to instrumentally analyze the moss samples instead. ICP-MS is a powerful tool in trace heavy metal analysis and, because it is more sensitive to certain metals like iAs, it is particularly suitable for this study. While even the lowest limits of detection (LOD) for ICP-OES are in the range of a few ppb, the LOD of ICP-MS can descend to the parts per trillion (ppt) (Olesik, 2020)(Comparison...). Strategically novel designs and extremely careful sample handling may lower this limit further into the parts per quadrillion (ppq) (Chemnitzer, 2019). Because the element of interest, iAs, is likely at a concentration within the moss that falls below what the ICP-OES may be able to quantify or even detect, ICP-MS is the optimal instrument in this case. A block diagram of the ICP-MS instrumentation is shown in Figure 3.

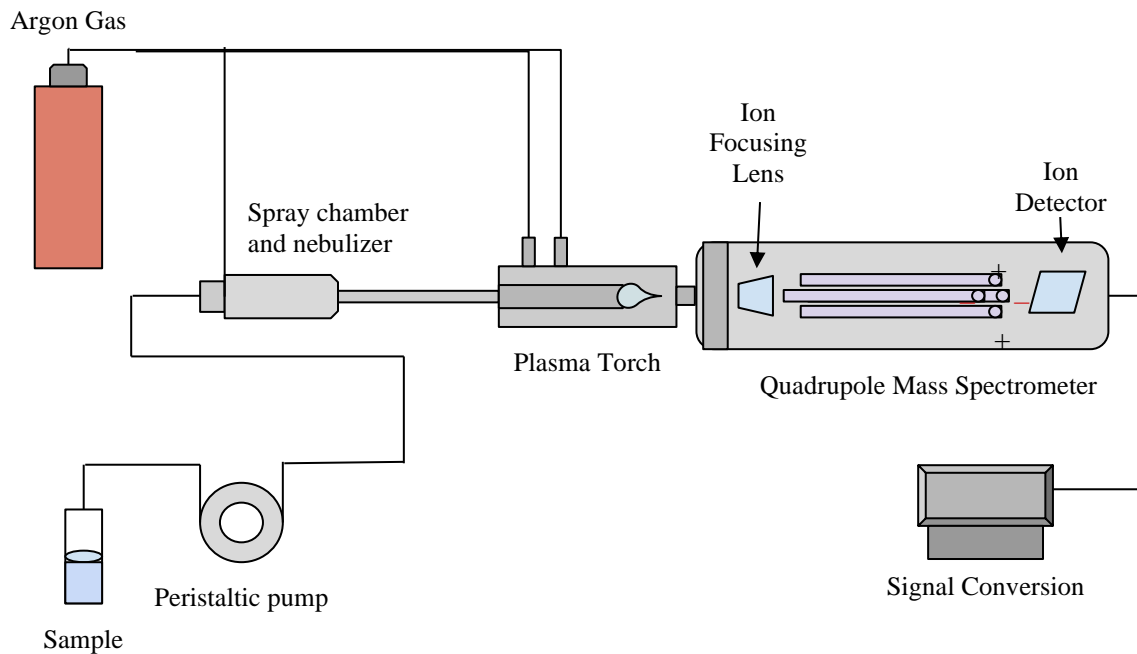


Figure 1: A block diagram of the ICP-MS instrumentation.

Both ICP-OES and ICP-MS feature an argon ICP torch as the sample ionization method. Therefore, the same samples may be collected and analyzed by either ICP-OES or ICP-MS depending on the metal of interest. Maintaining all other key procedural steps from the previous study would save time, effort, and money in sample collection and preparation. Analyzing the same sample rather than separately collected and prepared samples for different metals of interest may also reduce error. Error that could be introduced by sample inconsistencies—such as collecting moss from different patches, even on the same tree, or the contents of the respective matrix solutions—would not be present.

The heightened sensitivity of ICP-MS to heavy metals, even if they are present at trace amounts, comes largely from the fundamental difference of its analytical component, the quadrupole mass spectrometer or “mass filter”. In the place of the ICP-OES’s optical emission spectrometer which uses diffraction gratings or prisms to split light into characteristic wavelengths, the mass spectrometer filters by particle mass to charge ratio (m/z). The set of two pairs of oppositely charged parallel rods selectively analyzes ions of a particular m/z ratio at a time by electrically stabilizing their trajectory towards the detector. The electrical oscillations between the pairs of rods will not allow ions of any other m/z ratio through, forcing them to collide with the rods and go undetected. Because of this selectivity, the efficiency of ion transmission to the detector is increased, leading to higher sensitivity in quantification.

One potential downside to favoring ICP-MS is that this instrumentation is inherently more susceptible to matrix effects than ICP-OES and there are generally a few possible polyatomic mass interferences for any analyte. The most prominent of these for arsenic would be $^{40}\text{Ar}^{35}\text{Cl}^+$, whose mass of 75 amu could present as elemental arsenic (May and Wiedmeyer, 1998). However, this interference complex will not be formed in this study, as the digestion process in the laboratory sample preparation of this study uses HNO_3 rather than HCl . Other possible matrix effects of polyatomic spectral interferences will also be reduced to negligible levels by the instrument’s helium collision cell to further optimize the analysis. Therefore, this potential downside is mitigated to the point of nullification by way of sample and instrument optimization.

As Portland and other major cities grow, anthropogenic and industrial activity inherently increases, and heavy metal pollution may increase with it if it goes unmonitored and unregulated. The use of epiphytic moss as a bio-indicator is a viable, reliable, and highly accessible method of environmental heavy metal pollution monitoring. Once the optimal instrumentation to analyze a particular metal of interest is identified and applied, this method will contribute to fulfilling the need for simple, accurate, and cost-effective atmospheric pollution measurement. In turn, the empirical data that results will allow sources of pollution to be identified, leading to informed environmental decisions and policy changes to be made, effectively developing a healthier and safer urban ecosystem.

The use of the ICP-MS instrumentation in place of ICP-OES enables the same accessible field sample collection process and cost-effective laboratory preparation techniques to be used, while also obtaining more accurate and precise data on the levels of iAs. Therefore, the attractive aspects from the original study of civic science and environmental justice are maintained and additional informative data can be gathered that could not have been without the use of ICP-MS. It is expected that the industrial areas which were found in the adjoint study to be contaminated with relatively high levels of lead and cadmium will also present relatively high concentrations of arsenic. This is in part because of the tendency for arsenic to be co-emitted with cadmium in some industrial processes such as glass

manufacturing (Donovan et al., 2016). Similarly, results showing higher concentrations of arsenic near busy roads or near the railyard of Portland are expected. Ultimately, it is expected that the LOD and LOQ provided by ICP-MS will be sufficiently low to provide informative data on the concentrations of iAs in the epiphytic moss samples, filling a gap in the methodological approach of using epiphytic moss as a bioindicator for heavy metal pollution.

3: Materials and Methods

Because this study was designed to replicate the USFS study on quantifying cadmium in moss in regard to sampling design and laboratory analysis methods, the same materials and methods were generally maintained. These include the grid-based sampling strategy and general methods of laboratory analysis. All sampling and sample preparation equipment and chemical reagents used were obtained from the Chemistry Department and Chemistry Stockroom at Portland State University (PSU). The PSU Geology Department's Agilent Model 7900 ICP-MS instrumentation was used to perform the sample analysis.

3.1 Field Sample Collection

The sampling process first involves selecting the trees in the area from which the moss samples are to be obtained for an effective map of arsenic pollution. This study used the same sampling sites as the study on cadmium and lead pollution by USFS. All of the selected sampling locations were given an identifying natural number 1-X.

There was no distinction made between the species of hardwood tree to be the sample source, but the species of epiphytic moss was preferentially chosen to be *Orthotrichum Iyellii*. This moss grows abundantly on hardwood trees across Portland, including areas hypothesized to be polluted with trace metals. Also, because it is an acrocarpous moss its loose, cushioned, and tuft-like structure allows for easy separation of the healthy primary stems from the anchoring rhizoids of the plant. An image of one of the trees that moss was collected from for this study is shown in Figure 2.



Figure 2. An example of the hardwood trees with *Orthotrichum Iyellii* growth that was sampled from. This tree was from location no. 2.

This collection was done by hand using nitrile gloves and the samples were individually stored in Zip-Loc baggies that were sealed and labeled with the correlated identifying number of the moss's

location. Sampling was done on moss that was a minimum of 1 m high up the tree to prevent non-informative variability in results caused by potential vehicle spray or dog urine. A ladder was used in some locations to obtain moss from taller trees where moss growth was centralized on the high branches. The amount of moss collected from each tree aimed to be enough such that, once dried, a minimum of 0.50 g of the moss sample would be available. The exact latitude and longitude of each sampled hardwood tree were recorded with its identifying sample point number for future reference and bi-annually (twice per year at 6-month intervals) continued sampling. This can be done for any number of sample points, although this study on arsenic only analyzed moss samples from 8 sites in SouthEast Portland out of the 20+ sampling sites.

3.2 Laboratory Sample Preparation

Throughout the entire process of laboratory sample preparation, the Zip-Loc bags, beakers, centrifuge tubes, or other storage or analysis containers that the moss was held in were kept labeled with the particular sample's location-specific identifying number and the date it was sampled.

The moss was cleaned with sterile forceps to remove all dirt, rhizoid hairs and moss base, pieces of leaves, and other debris that were not part of the primary, healthy moss tufts. The samples were not washed with water in any way so that any particulate matter on the moss's surface would be retained.

Each sample was dried in a Bluebird Laboratory oven at 40 °C for a minimum of 24 hours. Once dry, the moss was ground into a fine powder with a clean ceramic mortar and pestle. Then, a 0.5 g portion of this powder was massed and transferred into a clean 50.0 mL centrifuge tube. The excess moss powder following the 0.50 g subtraction was transferred into a second centrifuge tube, labeled with the same location number and date with an "excess" specification in the label. Because the initial 0.50 g taken from each powdered sample was used in the initial ICP-OES analysis for other metals, this study for ICP-MS analysis obtained the powdered moss to be analyzed from the remaining excess powdered samples. Another 0.50 g amount of powdered moss sample was taken from each of eight of the "excess" centrifuge tubes and placed into a new, clean centrifuge tube that was labeled accordingly.

In the chemical digestion process, all eight of the tubes were placed into an AI water-filled digestion block with a heating and shaking water bath in a fume hood. Aliquots of 10.0 mL reagent-grade concentrated nitric acid were added to each tube and the caps were placed loosely back on their samples for the initial oxidation so that the resulting gasses could easily begin to escape at ambient temperature. The digestion block was turned on and allowed to come to a temperature of 94°C, beginning the continuous swirling and heating of the samples. After 1 hour, brownish yellow gas was evolving from the samples and a Pasteur pipette was used to add 1 mL aliquots of 30% hydrogen peroxide. This process of adding H₂O₂ was repeated sixteen times (approximately every hour) over the course of one day. At the end of this, each sample solution was very pale yellow or almost entirely clear and had no visible gas

evolving. The bath was turned off and the samples were allowed to cool in the block overnight. The following day, each cooled sample solution was filtered by gravity filtration directly into yet another clean and labeled centrifuge tube to remove undigested particulate matter—mostly silicate materials not dissolved by the combination of nitric acid and hydrogen peroxide—that was filled to 20.0 mL volume with DI water.

The arsenic standards for analysis were prepared from Ricca VeriSpec 100 ppm As in 2% HNO₃ for ICP-MS. A 100 ppm As stock solution was made, then external standards of 0, 10, 25, 50, and 100 ppb arsenic were prepared from the stock with 0.1 M HNO₃ as the diluent matrix. The 0 ppb standard, or the blank, was prepared with only this 0.1 M HNO₃ matrix solution and no arsenic. A greater volume of 0.1 M was also prepared as a between-sample rinse. The digests were placed into the autosampler and analyzed using quadrupole ICP-MS.

3.3 ICP-MS Analysis

An Agilent 7900 model quadrupole ICP-MS was used with Mass Hunter 4.4 Workstation Software to analyze the samples. The argon flow to the plasma torch was set to 1.7-1.2 L/min at 90 PSI. The helium flow to the collision cell was 4.3 mL/min and functionality of the cell was observed. In the software, it was confirmed that the iAs selectivity was to an abundance of 100%. A blank offset origin and linear curve fit was selected. A 1 ppb tuning solution was used for the autosampler before the calibration standards were analyzed. Three replicate measurements were taken of each solution, which were automatically averaged. The sample uptake and stabilization parameters were originally set to 25 and 20 seconds respectively and all of the calibration standards and samples were analyzed at this setting, but this was changed once it was observed that the % RSD values obtained for the samples were unacceptably high. Both the sample uptake and stabilization parameters were raised to 30 seconds and the analysis of the moss samples was reinitiated. Following this change, more consistent replicate data for iAs concentrations with sufficiently low RSD values were obtained.

4: Results and Discussion

The data collected from the analysis was evaluated to determine whether the hypothesized ability and optimal sensitivity of ICP-MS to reliably detect and quantify arsenic in the digested moss samples was empirically supported. The data for each sample was collected as signal in counts per second (CPS) vs. concentration of arsenic in ppb for each of three replicate measurements. The percent relative standard deviation (RSD) of these CPS and concentration values were also automatically calculated for each standard and sample.

4.1 Calibration Results

As noted previously, the sample uptake and stabilization parameters in the Mass Hunter 4.4 Workstation Software were initially set too low for proper stabilization, resulting in unacceptably high

RSD values for a majority of the standards. The analysis was re-initiated and performed on the standards and samples without changing any parameters, but the mean RSD value obtained was higher than in the first run. Finally, the two parameters in question were both updated to 30 seconds, but the analysis was re-initiated from the beginning of the samples rather than from the beginning of the calibration standards. This resulted in much lower RSD values for the sample data but left the calibration standard data and the resulting calibration curve the same. A table of the obtained concentration and RSD values of the standards and the sample solutions can be seen in Table 1.

Table 1: The mean arsenic concentration and RSD values obtained for the both runs of the calibration standards and the stabilized run of the digest samples with ICP-MS analysis. The pink cells indicate rejected calibration values.

Std/Sample Name	[As] (ppb)	Conc. RSD (%)
0 ppb std (1st run)	2.78434938	0.2774121
10 ppb std (1st run)	21.0310359	2.8852059
25 ppb std (1st run)	20.4639223	7.3164744
50 ppb std (1st run)	81.1465495	17.226225
100 ppb std (1st run)	167.037419	10.337982
0 ppb std (2nd run)	2.8018851	0.1712027
10 ppb std (2nd run)	16.0506429	32.630296
25 ppb std (2nd run)	20.1309267	37.741771
50 ppb std (2nd run)	40.8086826	0.1812618
100 ppb std (2nd run)	105.207863	75.629928
19L	15.5222982	1.0583201
8	18.7189878	0.1265695
13	18.5753332	0.5620251
7	16.1732897	0.6504855
3	18.2434804	0.5845488
2	21.6393026	0.6894995
11	18.1287452	1.0676339
20	15.8228115	0.5209103

Two runs of measurements of the calibration standards were performed before the issue with the sample uptake and stabilization parameters was discovered. The set of data from the first run was selected to construct the calibration curve due to its lower RSD values. The replicate measurements taken of the 25 ppb standard showed illogical disagreement between the signal and concentration in both runs, so this standard was excluded from the final calibration curve and data analysis.

From Table 1, it can also be seen that the calibration standards did not return values in agreement with their prepared concentration values. While some slight deviation was expected, differences between the intended and obtained concentration values over 30 ppb—as in the case of the 50 and 100 ppb standards of the first run—indicate low accuracy of the calibration curve points. It was similarly determined that the RSD values associated with a majority of the calibration standards—the 10, 25, and 100 ppbs standards in particular—are disagreeably high and further indicate low precision. The maximum RSD value within the first run of the calibration standards is 17.23%. Comparing this to the samples' maximum RSD value of 1.07%, it is clear that raising the sample uptake and stabilization parameters allowed the replicate measurements to be taken under more stable and precise conditions.

This also indicates that the calibration curve has some avoidable error associated with its constituent points. If the calibration standards had been measured with the optimized parameters as well, it is likely that the resulting concentration data that would be used to build the calibration curve would be in better agreement with the intended concentration values of 0, 10, 25, 50, and 100 ppb. Their RSD values would likely be agreeably low, around 1 ppb or less, as is the case for the samples' data resulting from the parametric time extension.

A calibration curve was constructed from the averaged measurements taken of the first run of the external standard solutions that had been prepared with arsenic concentrations of 0, 10, 50, and 100 ppb. Again, the 25 ppb standard was excluded from the curve due to discrepancy between its signal and its signal-derived concentration value. The averaged concentration values for the eight samples were then recalculated in accordance with the rejection of the 25 ppb standard and overlaid on the calibration curve. Only the data from the stabilized and unrejected measurements were considered and included. This data is plotted as a linear CPS vs. arsenic concentration curve in Figure 3, which shows the calibration and sample data points as well as the linear regression.

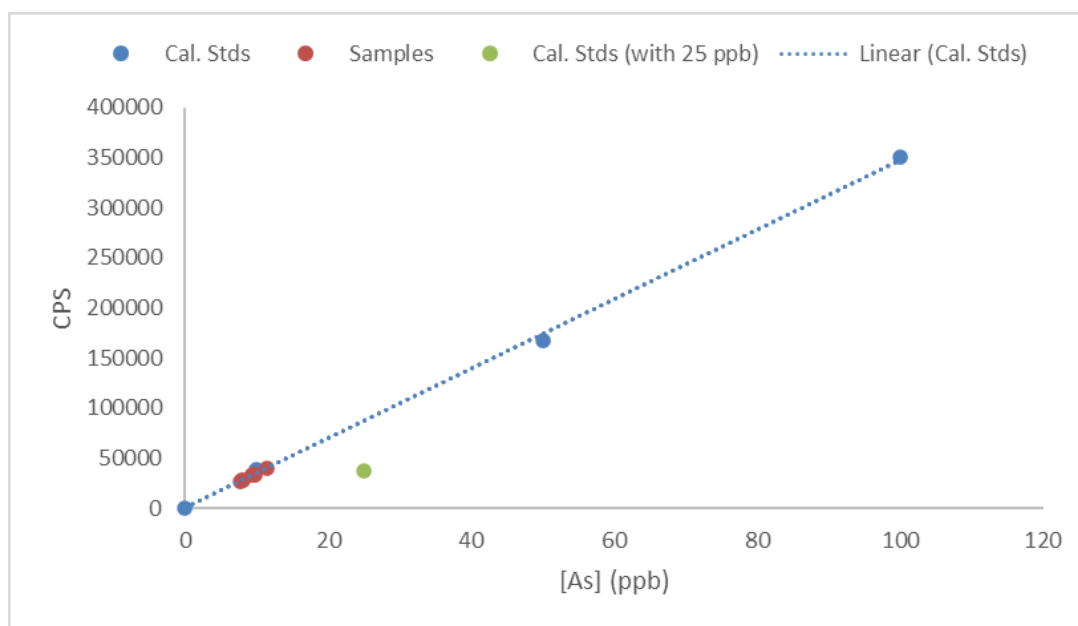


Figure 3: External standard calibration curve, along with the unknown samples (red points). The blue points show the calibration standards, and the dotted blue line is the linear fit.

As seen in Figure 3, the 25 ppb standard, shown as the green point, was not in agreement with the linearity of the other calibration points, further justifying its rejection. Because of this rejection, a better linearity was obtained for the regression. It can also be seen from Figure 3 that the samples' concentration values derived from their signal fall within a narrow range of 7-12 ppb that is completely encompassed by the calibration data extending from 0-100 ppb. This shows that the calibration standards were prepared with a wide enough range of arsenic concentrations to encapsulate the concentration values of the moss samples upon analysis.

When a linear regression analysis was performed on the calibration curve, it was found that the standard error of the regression, or S_y value, was about 5860 CPS. This represents the average distance that the calibration points fall from their regression line. Although the S_y is above the signal measured for

the lowest of the calibration standards—that of the 0 ppb which had a signal of about 169 CPS—it was below even the lowest sample signal of around 27300 CPS. It was determined that even though the calibration points were not aligned with their intended values and not perfectly in line with the linear regression, the regression fit itself is still valid. Therefore, even if the obtained calibration could have been more precise, it is not likely that this affected the validity of any of the eight sample concentration results. Further data analysis was performed to determine whether the sensitivity of the analytical method was high enough to validate the recalculated sample concentration values.

4.2 Sensitivity

There is empirical evidence of the improved sensitivity of ICP-MS compared to ICP-OES in the limit of detection, which describes the minimum analyte concentration value that is consistently detectable on a certain confidence interval (CI) by the analytical method. The limit of quantitation, however, which describes the minimum analyte concentration that can be accurately and precisely quantified on a certain CI, was not low enough to be beneath the range of sample concentrations. The LOD and LOQ values were calculated with the data obtained from the calibration curve's regression analysis, and these are displayed in Table 2 along with key statistics regarding the measured sample concentrations.

Table 2: Statistical analysis and the sensitivity parameters derived from the sample's obtained concentration values.

Sample Statistic Values (ppb)	
Minimum	7.76
Maximum	11.52
Mean	9.19
Std. Dev.	1.23
Sensitivity Parameters (ppb)	
LOD	3.52
LOQ	11.96

The LOD value is less than the minimum sample concentration and is consequently confirmed to be sufficiently low. In fact, the minimum sample concentration of 7.76 ppb is greater than twice the value of the LOD. The entire range of the obtained sample concentration values, 7.76-11.52 ppb, is above the detection limits, so none of the sample values fall below the arsenic levels that can be detected by ICP-MS. Because of this, it is affirmed that the sensitivity of the ICP-MS is exceptional in the detection of

trace levels of arsenic, especially compared with the previously employed instruments, ICP-OES and ASV. The values shown in Table 2 also indicate that each analyzed sample contains an amount of arsenic that is at least above the LOD and is therefore concentrated with a minimum of 3.52 ppb arsenic. However, the LOQ value of 11.96 is not far enough below the sample concentrations to give credence to the superior sensitivity of the ICP-MS in regard to quantitation. Not only this, but the entire range of sample concentrations lies between the LOD and LOQ. This suggests that this method can be used to successfully and reliably detect arsenic present in moss samples but cannot accurately and precisely quantify it.

The need to reject the 25 ppb calibration standard is supposed to have contributed to the unsatisfactorily high LOQ value. Moreover, the previously described initial setting of the sample uptake and stabilization parameters to 20 and 25 seconds respectively, rather than a longer and more adequate setting of 30 seconds each, caused high RSD values for the calibration standards. This likely led to much of the increase to the LOD and LOQ values, and both of these may have been below the range of sample concentrations had the equilibration parameters originally been set long enough. In the future, it is recommended that a full set of five or more acceptable external calibration standards be prepared and used. In addition, it is recommended that these standards be analyzed with sufficiently high equilibration parameters so that the calibration standards' resulting RSD values are as low as possible. With these corrections and optimizations, the ICP-MS should be able to not only detect, but also accurately and precisely quantify arsenic concentrations with expressible and defensible confidence.

Nevertheless, evidence of the superior sensitivity of ICP-MS compared to the ICP-OES can also be recognized by the fact that none of the samples analyzed with the current methods returned non-detect results for arsenic concentrations. This study analyzed moss samples from eight different collection sites around South East Portland and obtained arsenic concentration values for each sample. A previous study by USFS analyzed moss samples from a total of 346 different sites, but 177 of those sites returned non-detect (n.d.) data for arsenic with ICP-OES. Although it is true that there were far more sites analyzed previously, and it is possible that the eight samples analyzed in this study may have all been "lucky hits" wherein arsenic concentrations were high enough to be detected even with ICP-OES, lucky sampling is not likely to be the cause for the success of ICP-MS analysis of arsenic.

This study collected moss samples from eight of the exact same trees that were sampled in the previous study by USFS, many of which had previously resulted in non-detects by ICP-OES. A map of the arsenic levels at the sampling sites made with the results of the USFS study is shown in Figure 4.

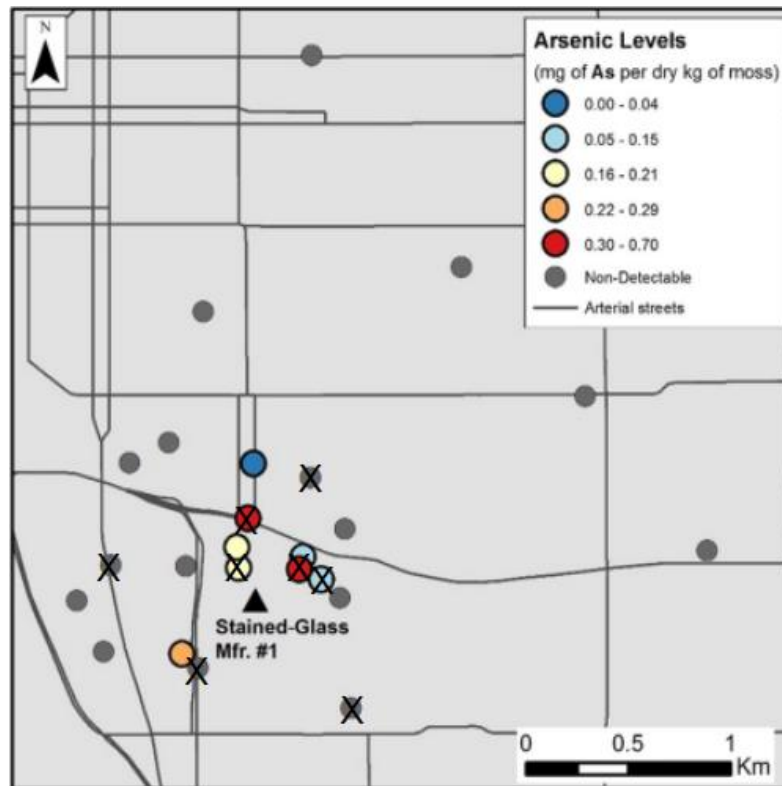


Figure 4: A simple dot map of the area under investigation. The multi-colored dots represent the previously sampled sites analyzed with ICP-OES and the black X-marks represent the resampled sites for ICP-MS analysis.

The X-marks in Figure 4 represent the sites that were resampled and analyzed with ICP-MS in this current study. The gray dots represent sites that had previously resulted in n.d. data. With 117 out of the 346 previously sampled sites returning non-detects, there was roughly a 2.71 in 8 chance of arsenic not being detectable. Even in this small sampling area, half of the eight sites that were resampled had previously resulted in n.d. data. If the chance of a non-detect for arsenic was the same with ICP-MS as it was with ICP-OES, the newly obtained data may have included two to four non-detect results out of the eight analyzed samples, reflecting that similarity. This was not the case, however, and none of the eight samples analyzed with ICP-MS returned n.d. data.

Furthermore, the resampled area was in the vicinity of stained glass manufacturer #1, which is represented by the black triangle in Figure 4. This is a facility that was previously found to be at the center of high cadmium contamination in Portland. Even in an area of interest that was hypothesized to be

contaminated with relatively high levels of arsenic based on the high level of cadmium, the ICP-OES could not adequately detect this arsenic, indicated by all of the gray n.d. dots. Half of the moss samples of this study were collected from the same trees which had previously resulted in n.d. data, but arsenic was detected by ICP-MS for each of these sites.

4.3 Arsenic Levels in Natural Moss

It can also be seen from Table 2 that the range of iAs concentrations in the prepared samples is between 7.76-11.52 ppb. This range is relatively small in comparison to the allowance of the calibration curve and indicates that arsenic levels do not vary by more than 5 ppb across the eight samples. This also suggests that the arsenic concentrations in the natural moss growth would be within a relatively narrow range across the eight sampling sites. To better represent the concentrations of arsenic that were present within the moss samples as they existed naturally before collection, the concentrations in ppb were converted to units of milligrams of arsenic per kg of moss (mg/kg). This was done with respect to the true mass of the dehydrated moss body which was prepared and used in the analysis. These concentration values as well as the uncertainty values for each concentration, or the S_c values, which were calculated in relation to the external calibration and by using the regression analysis data, are displayed in Table 3.

Table 3: Information regarding the arsenic concentrations at each collection site.

Sample #	Latitude	Longitude	Location Description	[As] in moss (mg/kg)	S_c (mg/kg)
19L	45.49954	-122.64507	Adjacent to a major road	0.307	0.0581
8	45.49692	-122.6405	Dumpster near large Grocery Store	0.391	0.0582
13	45.49755	-122.65365	Street by a Cafe	0.359	0.0540
7	45.4961	-122.63934	Edge of Parking Lot by Powell Park	0.294	0.0527
3	45.49315	-122.64825	Residential Street	0.364	0.0559
2	45.49138	-122.63866	Street by railyard	0.378	0.0469
11	45.49742	-122.64568	Powell Park	0.396	0.0613
20	45.50127	-122.64115	Residential Street	0.321	0.0591

The calculated S_c values seen in Table 3 are similarly inconsequential to the regression curve's S_y value gathered directly from the linear regression analysis. The S_c values of the calibrated arsenic concentration results range between 0.0469-0.0613 mg/kg. A 95% confidence interval was constructed

from these values for each milligram per kilogram result to illustrate the precision of the mg/kg results, yielding an average concentration and associated error of 0.35 ± 0.054 mg/kg. This also shows that the previous estimations from the study using ICP-OES suggested lower arsenic concentrations than those that were found with ICP-MS. All of the arsenic concentrations in mg/kg seen in Table 3—with the exception of sample #7—are within the highest range that was previously expected and displayed in the key of Figure 4. Although it is true that the obtained concentrations cannot be confirmed to be totally accurate due to the relatively high LOQ, they suggest the possibility of higher levels of arsenic in this area than were previously estimated.

Statistics calculated from these mg/kg results are displayed in Table 4. The standard deviation was calculated in accordance with the fact that the data collected represents a sample of the total population of moss samples to be found in Portland.

Table 4: Statistics of the concentration values of arsenic that were calculated to be present in the natural moss growth.

Statistic	Value (mg/kg)
Minimum	0.294
Maximum	0.396
Mean	0.351
Standard Deviation	0.0391

The USFS study estimated that the range of arsenic concentrations across their 346 sampled sites across Portland was likely 0.240–0.945 mg/kg, and 0–0.70 mg/kg in the area of interest near stained glass manufacturer #1. As seen in Table 4, the current study found a range of 0.294–0.396 mg/kg in the subset of eight samples from this area of interest. This narrow range is within both the past ranges of estimation for the greater Portland area and the area around stained glass manufacturer #1. Not only does this current study’s use of ICP-MS therefore corroborate the previous study’s estimations, but it was able to more successfully and reliably detect arsenic. Although arsenic could still not be reliably quantified, the similarity between the previous and current concentration estimations helps to validate the accuracy of the newly obtained data as well as further crediting the previous study’s estimation methods. Furthermore, the current findings suggest that arsenic may be present at higher levels in this area of particular interest than were previously expected.

The low variability, or relatively narrow range, between the concentrations of arsenic in moss can be recognized by the standard deviation of 0.0391 mg/kg seen in Table 4. In contrast, the standard

deviation of the estimated arsenic concentrations in the previous USFS study was 0.124 mg/kg. The difference in variability is almost certainly due to the fact that the samples of the current study were gathered from the smaller region of the area of interest near stained glass manufacturer #1. This was all within a 1.63 km² area within the city of Portland. This small area is shown in Figure 5 with the sampled sites marked.

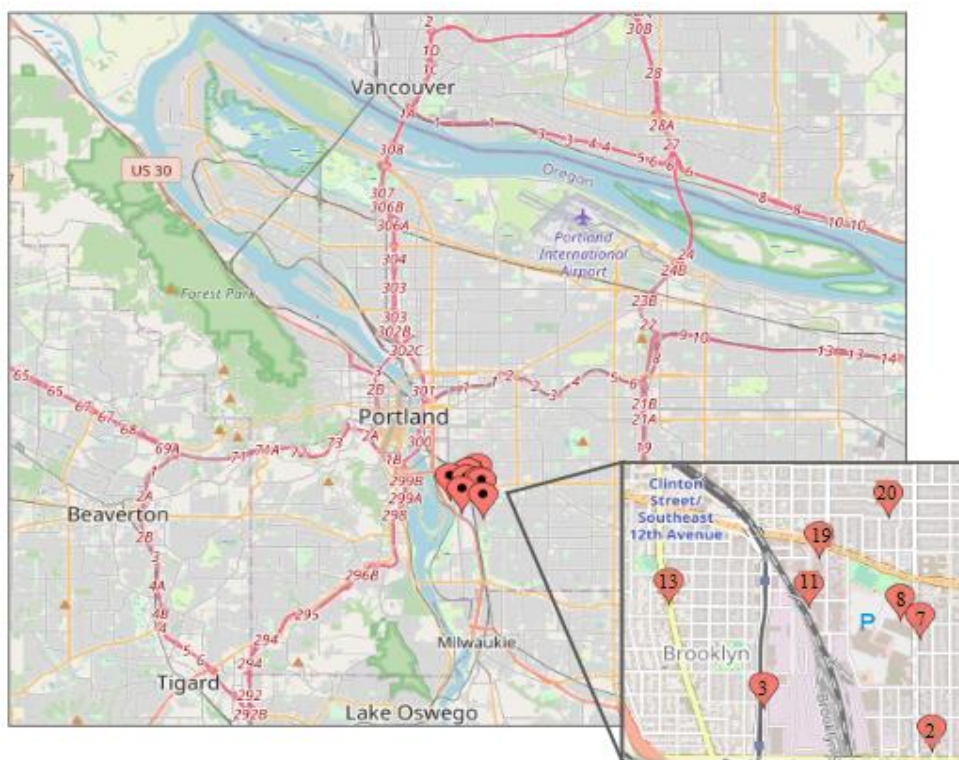


Figure 5: A map encompassing the area that was sampled from in the previous study with the locations that the arsenic samples in this study were collected from being plotted at the red bubbles. A magnification of the area of the current study's arsenic samples is shown at the bottom right, where the red bubbles are labeled with the sample numbers.

The larger portion of the map in Figure 5 shows the breadth of the area that was collected from in the previous study. Because the current study sampled from a smaller area within Portland, focused on less than one square mile in South East Portland, the sampled trees are in much more of a chemically similar environment to each other. If the sites that were sampled had been spaced out over as large of an area as the previous study's sites were, the concentration results would likely show a larger range between their maximum and minimum and a higher standard deviation. However, by collecting and analyzing only a few samples from a smaller area, especially an area that had been of particular interest previously, this study more effectively concentrated its focus to re-investigate this area with a more sensitive instrument.

Not only is the sampled area of this study much smaller than the sampled area of the USFS study by several-fold, but the arsenic samples for this study were all gathered from an area which was originally identified as a cadmium hotspot. The low variability in the arsenic concentrations could be attributed to a relatively high level of arsenic that is blanketing this area due to arsenic emission from the glass manufacturer. The general range of arsenic concentrations obtained with ICP-MS analysis being on the higher end of the previous study's estimates for this area may be attributed to this as well, even if the measurements aren't confirmed to be fully accurate. This means that a substantial argument that one of the eight sampled locations—such as the railyard or a busy road—having a meaningfully higher concentration of arsenic than the others cannot be confidently made. However, this connection also gives credit to the logic of the obtained concentration values and key statistics seen in Tables 3 and 4 respectively, which show an average arsenic concentration of 0.351. This is higher than the average cadmium concentration found across Portland by the previous study, which was 0.308 mg/kg (Donovan et al., 2016). If the assumption is held that toxic heavy metals tend to be co-emitted and if the estimations of arsenic concentrations made by the previous study—which suggest that this area is a heavy metal hotspot because of the stained glass manufacturer—are accurate, this logic is validated.

4.4 Arsenic in Atmospheric Air in Portland

All of the concentrations of iAs in moss shown in Table 3 may exceed the DEQ limit on arsenic in ambient air of $0.0002 \mu\text{g}/\text{m}^3$, but the accumulation of arsenic in epiphytic moss over time must be taken into account. The results in Table 3 are not measurements made directly from a water source, food, or ambient air, but from a patch of moss biomass which has presumably been taking in and storing arsenic throughout its life. As previously mentioned, concentrations of elemental metals have been found to change significantly even within short periods of time due to the various environmental and biological factors upon which they depend. The uptake rate and storage capacity of elemental metals by moss have a notably high associated error, which makes obtaining temporally representative biomonitoring data extremely challenging (Boquete et al., 2011). All of this also makes it difficult to compare the obtained concentrations directly to the set benchmark concentrations.

However, although the relationship between the levels of arsenic accumulated in moss tissue and the arsenic present in atmospheric air is not well understood, it is assumed that when arsenic levels in the air increase, they increase in moss tissue as well. Therefore, as was done previously, analyses of varying arsenic levels across a large area can be performed to identify locations of high emission. In addition, this means that changes in arsenic levels in the air might be monitored over long periods of time at locations of interest by determining whether the concentrations in its moss growth are increasing, decreasing, or stagnating from year to year. This can be made possible for arsenic levels in particular by the sensitivity of ICP-MS, especially with the aforementioned corrections to the calibration process.

4.5 Future Directions

In the future, aside from the recommended refinement to the calibration process with acceptable standards and instrumental equilibration parameters for re-analysis of arsenic levels in the area of interest, the sensitivity of ICP-MS may be used in various other ways to investigate heavy metal pollution. This type of instrumentation may be the best option in the study of heavy metal pollutant speciation or isotopic variation in discovered pollution sites. Although the latter would be fruitless for inorganic arsenic due to the 100% natural abundance of the ^{75}As isotope, heavy metals such as lead and cadmium, which are similarly toxic to arsenic, do have disparately abundant isotopes that the ICP-MS can separate and quantify individually. The particular polyatomic mass interferences of the prospective elemental isotopes under investigation would have to be accounted for, and it is recommended that a collision cell continue to be used to filter by particle volume. This route of investigation would take advantage of the fact that there is virtually no possibility of isobaric interference with the ICP-MS instrument.

The quantification of other elements that have proven difficult to quantify with ICP-OES would be worth pursuing with ICP-MS analysis as well. Selenium (Se) concentrations, for example, could only be estimated in the previous study along with arsenic. Se is an essential nutrient, yet extremely high levels can cause dangerous and even fatal side effects such as endocrine system disruption, breathing difficulties, and kidney and heart failure. With a safe upper limit in adults between about 300 $\mu\text{g/day}$, the monitoring of Se emissions could be just as critical as of arsenic (Vinceti et al., 2001). Selenium also has disparately abundant isotopes that may be explored in the same selective way as mentioned.

Future studies should also investigate arsenic levels in Portland using more sampled location points. More sites across Portland should be sampled outside of the area near the stained glass manufacturers in order to rule out the possibility of similar arsenic levels blanketed across the whole city. This will also help to back the claim that arsenic is likely co-emitted with other heavy metals in industrial activities such as glass manufacturing.

5: Conclusions

As had been expected, the data analysis performed with the obtained data and linear regression analysis support that the arsenic concentrations gathered were successfully detected with ICP-MS. The LOD was sufficiently low that the range of arsenic concentrations obtained were at no risk of going undetected by this instrumentation. ICP-MS is demonstrated to be the ideal choice for arsenic detection in moss biomass. The accurate and precise quantification of arsenic was not accomplished with the methods used, as the entire range of concentrations obtained was below the LOQ. However, opportunities for further optimization of the methods and instrumentation were noticed and are recommended for future analysis. These include the preparation of five or more proper external calibration standards and the initial setting of the sample uptake and stabilization parameters of the ICP-MS to 30 seconds or greater to

achieve sufficiently low RSD values. With these corrections, it is presumed that the LOQ will decrease enough to validate the accuracy and precision of the arsenic concentrations that can be obtained in the future. Due to the small size of the sampled area and its relatively homogenous chemical environment, it could not be confidently confirmed with the results obtained whether any particular location from the eight sites had higher arsenic levels in their moss. However, this study demonstrates that further arsenic pollution analysis, whether across a larger area of Portland or in other cities entirely, is possible with moss bioindicators and ICP-MS.

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