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Arsenic Analysis: Comparative Arsenic Groundwater Concentration in Relation to Soil and Vegetation

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ARSENIC ANALYSIS:
COMPARATIVE ARSENIC GROUNDWATER CONCENTRATION
IN RELATION TO SOIL AND VEGETATION

A Project
Presented to the
Faculty of
California State University,
San Bernardino

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
in
Earth and Environmental Sciences

by
Romina Estefania Valentine Vecorena
March 2016
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ABSTRACT

Arsenic (As) is a toxic semi-metallic element found in groundwater, soils, and plants. Natural and anthropogenic sources contribute to the distribution of arsenic in the environment. Arsenic's toxic and mobile behavior is associated with its speciation ability. There are two types of arsenic available to the environment, inorganic and organic arsenic. Of the two, inorganic arsenic is more toxic to humans and more mobile in the environment. Two inorganic compounds responsible for arsenic contamination are trivalent arsenite, As (III), and pentavalent arsenate, As (V). Trivalent arsenate is considered to be more soluble, toxic, and mobile than pentavalent arsenate. Arsenic's absorptive properties in plant cells and ability to attach to minerals causing secondary contamination are due to environmental factors such as pH, redox potential, and solubility.

The current maximum contaminant level for arsenic in water is 10 µg/L (or ppb). Research on arsenic involving high concentrations already present in groundwater (>300ppb) are compared either with crops irrigated with such water or a human indicator (such as; hair, nails, blood, or urine) in order to determine exposure limits. In this current research, relationships between the area in the studies and the contaminated media (water, soil, vegetation) were tested to determine if arsenic in water was correlated with arsenic concentrations present in soil and vegetation. Commercially obtained ITS Quick Rapid Arsenic Test Kits were used to measure arsenic concentrations for the media tested. A method for
analysis of arsenic in vegetation was developed, with an estimated 80% recovery. The pH and conductivity were also taken for water and soil samples as a means of correlative comparison. The development of faster and portable methods for arsenic concentration may provide means for predicting the relationship between all contaminated media. The purpose of the study was to determine the correlation between arsenic water concentration and pH for water, soil, or vegetation and whether it plays an overall role in the amount of arsenic present. As a result, water and soil pH played a significant role in the presence of arsenic in the water and vegetation, respectively. A moderate negative correlation between arsenic in water and water pH was discovered to have a Spearman’s rho value of -0.708 with a $p \leq 0.05$. In addition, a significant negative correlation between soil pH and arsenic in vegetation was also discovered to have a Spearman’s rho of -0.628 at a $p \leq 0.05$. Even though, pH was significantly correlated with arsenic concentrations in different media, there is evidence that pH plays a role also in the amount of arsenic available in the soil and vegetation. Further studies are recommended.
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TABLE OF CONTENTS

ABSTRACT ......................................................................................................................... iii

ACKNOWLEDGEMENTS ................................................................................................. v

LIST OF TABLES ........................................................................................................ viii

LIST OF FIGURES ........................................................................................................ ix

CHAPTER ONE: INTRODUCTION

  Definition of Terms .................................................................................................. 1

  Background ........................................................................................................... 5

  Arsenic Speciation ............................................................................................. 5

  Arsenic Sources ................................................................................................. 6

  Arsenic Health Effects ....................................................................................... 8

  Purpose and Objective ..................................................................................... 9

  Scope and Significance ................................................................................... 10

CHAPTER TWO: LITERATURE REVIEW: COMPARISON AMONG
ANALYTICAL METHODS

  Arsenic in Humans ........................................................................................... 12

  Arsenic in Vegetation ...................................................................................... 13

  Arsenic in Soil .................................................................................................. 14

CHAPTER THREE: METHODOLOGY

  Site Description ................................................................................................. 16

  Sampling Method ............................................................................................. 18

      Water, Soil, and Vegetation Collection ...................................................... 18

  Laboratory Analysis ........................................................................................ 20

      Arsenic Water Analysis ........................................................................... 22
LIST OF TABLES

Table 1. Descriptive Statistics of Water Samples. .................................................. 33
Table 2. Descriptive Statistics for Soil Samples .................................................. 35
Table 3. Descriptive Statistics for Vegetation Samples ...................................... 37
Table 4. Percent Recovery for Water, Soil, and Vegetation of Arsenic Concentration as Compared to Arsenic Water Concentration Using a 20 ppb Spike ..................................................... 40
Table 5. Comparative Analysis for Significance Using Spearman’s rho Between Variables ............................................................................................................. 42
LIST OF FIGURES

Figure 1. Southern California Map of Thermal, CA and Surrounding Areas as Determined by Google Maps ................................................ 17

Figure 2. EPA Approved Wide Mouth Polyethylene Flip-top Bottle with Lock and Seal Lid Design Used for Sample Collection. ............... 19

Figure 3. ITS Quick Rapid Arsenic Test Kit. ............................................................ 22

Figure 4. Turret Cap Used for Analysis of Arsenic in Both Water and Vegetation Samples ............................................................ 23

Figure 5. Color Chart Used for Determination of Arsenic Concentration in Water and Vegetation ................................................. 24

Figure 6. Sample of Testing Pad Color and Corresponding Amount Based on the Color Chart Used .................................................. 24

Figure 7. Mortar and Pestle with 50.0mL of Ethyl Alcohol and 2.0g of Vegetation Sample. ................................................................. 26

Figure 8. Alcohol Extraction Apparatus with Ceramic Büchner Funnel Filter ................................................................................. 27

Figure 9. Turret Cap for Analysis in Soil Samples ..................................................... 28

Figure 10. Color Chart for Arsenic Concentration in Soil Samples. ...................... 29
CHAPTER ONE

INTRODUCTION

Definition of Terms

This section includes the definition of key concepts used and discussed throughout the project for better understanding of concepts in relation to the topic of arsenic. In addition, this section is also a reference for acronyms used throughout the text.

- Absorption is the process in which one object absorbs or is absorbed by another in terms of liquid or homogeneous substance containing uniform properties.

- The Agency for Toxic Substances and Disease Registry (ATSDR) is a U.S. federal public health agency within the United States Department of Health and Human Services that emphasizes on minimizing human health risks related to exposure to hazardous substances.

- American Public Health Association (APHA) is a public health professional based organization in Washington, D.C. whose main concern is for the health issues of all people and all communities throughout the United States who can influence federal policy.

- American Water Works Association is an international non-profit educational and scientific association determined to improve water supply and quality for people throughout the United States.
- Atomic Absorption Spectroscopy (AAS) is an electroanalytical procedure used to determine qualitatively a concentration for a particular element.

- The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), is an act created in 1980 to provide liability compensation, cleanup, and emergency response for hazardous substances that are released into the environment as well as the cleanup of inactive hazardous waste disposal sites. Also associated with superfund definition.

- Desorption is the ability to release a substance or liquid from or through a surface. The opposite of adsorption.

- An Environmental Technology Verified (ETV) Program verifies the performance of newly discovered environmental technologies using qualified third parties with specified requirements and protocols.

- Inductively Coupled Plasma Atomic Absorption Spectroscopy (ICP-AES) is an analytical method developed for the determination of metals in water and wastewater samples.

- A Maximum Contaminant Level (MCL) is the maximum concentration of a chemical that is allowed in public drinking water system established by the U.S. EPA. For arsenic the MCL is 10 ppb.

- The National Institute for Occupational Safety and Health (NIOSH) is a U.S. federal agency responsible of making recommendations and
conducting research for the prevention of work-related illnesses and injuries.

- Natural Organic Matter (NOM) is matter composed of decomposed organic material from either animals, animal waste, or plants in the environment.

- The Occupational Safety and Health Administration (OSHA), is an agency of the United States Department of Labor established under the Occupational Safety and Health Act to ensure healthful and safe working conditions for both working men and women by providing training, education, assistance and outreach by enforcing and setting standards.

- Parts per billion (ppb) is used to describe the concentration of a substance in terms of one microgram per liter of water or one microgram per gram of soil or vegetation.

- Parts per million (ppm) is used to describe concentration of a substance in terms of one milligram per liter water or one milligram per gram of soil or vegetation.

- The Spearman’s Rank correlation coefficient (rho), is a statistical correlation between nonparametric variables, not normally distributed. A Spearman correlation greater than ±0.7 results in variable having a strong correlation. Values ranging from ±0.3 to ±0.7 are considered weak to moderate correlations. Any other value than less then ±0.3
(0.0 to ±0.3) is assumed to have a weak to no correlation between variables.

- Spike addition is a calibration technique used to quantify known amounts of a substance to an aliquot of an analyte.

- Software Package for Social Sciences (SPSS) is an IBM software package for statistical analysis.

- Standard Methods for the Examination of Water and Wastewater is a comprehensive reference that covers all aspects of water and wastewater analysis techniques for the determination of water quality, by water quality researchers who have been members of the Standard Method Committee (SMC) under organizations of the American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF).

- Turgor is the state of turgidity (or swollenness) and resulting rigidity of cell of plants in relation to the absorption of fluid.

- United States Environmental Protection Agency (U.S. EPA) is an independent federal agency responsible for setting policies, regulations as well as guidelines to protect national interests in environmental resources.

- Water Environment Federation (WEF) is an organization of different types of engineers and industry related to water, water use, and wastewater.
Background

Arsenic Speciation

Pure arsenic (As) is a grey, brittle, semi-metal element that is considered odorless as well as tasteless (1). When arsenic combines with hydrogen and carbon, it becomes organic, making it less harmful than its inorganic counterpart (14). Naturally occurring arsenic can exist in a wide variety of oxidation states at any given time. Organic forms of arsenic are found as arsenic acid (H₃AsO₄) and arsenous acid (H₃AsO₄⁻), plus other dissociative derivatives such as arsenites (H₂AsO₄⁻), arsenates (HAsO₄²⁻), monomethylarsenic acid (MMAA, H₂AsO₃⁻), and dimethylarsenic acid (DMMA, HAsO₃²⁻) (10). The more common oxidized states of arsenic in groundwater, which are especially toxic and mobile, are As (III) and As (V), which are known as trivalent arsenite (AsO₃³⁻) and pentavalent arsenate (AsO₄²⁻), respectfully (22). Of the two, trivalent arsenite, As (III), is regarded to be more soluble and toxic than pentavalent arsenate, As (V).

The geochemistry of arsenic determines the location and transport of arsenic in the environment. Arsenic as a pure element is insoluble in water, while the oxidized forms, are more soluble in water (1) and have the ability to mobilize (9). Because pentavalent arsenate and trivalent arsenite are the two main inorganic species of arsenic found, pH and redox conditions significantly influence arsenic speciation and concentration in the environment (22) in soils and natural waters. Other organoarsenic compounds present are generally negligible when compared to the inorganic compounds in soil and water (14).
As(III) is found in reducing conditions with pH closer to neutral, while As(V) is predominant in oxic conditions where the pH ranges from 5 to 8 (19). Both forms of arsenic exist in a higher ratio of one another, where the relation to oxidation of As(III) to As(V) is considered kinetically slow (22). The neutral pH range (6 to 7) of groundwater in a natural environment causes arsenate to have a negative charge whereas arsenite is neutral. Therefore, the adsorption reactions between varieties of aquifer contents, such as iron oxides, are stronger for arsenate than arsenite (16). However, as the pH of the water increases (or becomes more alkaline), desorption of arsenate as well as arsenite from materials such as iron oxides increases (22). Since adsorption or desorption is often pH dependent, changes in groundwater pH can result in changes in groundwater arsenic concentrations (22).

**Arsenic Sources**

**Natural Sources.** Like most elements, arsenic naturally exists throughout the earth’s crust in the form of arsenic sulfide and metal arsenates or arsenites, all of which are inorganic (1) and are widely dispersed throughout natural ecosystems. Worldwide traces of total arsenic (more than 99%) in the environment are reported to be present in rocks (15). Natural sources include naturally existing minerals or ores (such as pyrites), and soils which are melting pots for the weathered forms of arsenic compounds introduction into the environment and mineral-rich geothermal waters (7). Natural waters, including surface water, typically have a lower concentration of arsenic as compared to
groundwater concentrations of arsenic. Causes of arsenic groundwater contamination are known to arise from several geochemical, biological, and geophysical processes. These processes include reductive dissolution of arsenic containing hydroxides, oxidation of arsenical sulfides, leaching of arsenic from sulfides by carbonates, evaporative concentration of arsenic, release of arsenic through geothermal waters, and desorption of arsenic from hydroxides (16, 21-22).

**Anthropogenic Sources.** Increased human activity releases higher concentrations of arsenic into the environment by allowing consistent use of arsenic-containing products (like wood preservatives and pesticides) to accumulate in ecological and geological systems. Unlike other heavy metals, arsenic cannot bio-accumulate in the environment and therefore can change to other forms of arsenic accumulating as inaccessible compounds (1). Man-made sources of arsenic are usually industrial effluents, which may result from direct discharge of arsenic compounds into soil, water, and air (22). As a result, reports of contaminated agricultural areas that have been irrigated with arsenic rich groundwater are becoming more common (6). Additional sources of arsenic are due to mining activities, where arsenic-containing wastes are produced as a by-product of extraction methods for metals like tin, nickel, copper, and gold (3). Mine site locations are notoriously known for high arsenic contamination in the groundwater and soil surrounding the area. Other sources of arsenic in the environment include coal combustion and processing, where high temperatures
release arsenic from minerals and rocks in the form of fly ash, which settles in the environment (22).

**Arsenic Health Effects**

The U.S. Environmental Protection Agency (U.S. EPA) maximum contaminant level for arsenic in water is not to exceed 10 μg/L (or ppb) (1). The U.S. EPA, Occupational Safety and Health Administration (OSHA), and Food and Drug Administration (FDA) are federal agencies that help develop regulations for known toxic substances, such as arsenic, with the help of the National Institute for Occupational Safety and Health (NIOSH) and Agency for Toxic Substance and Disease Registry (ATSDR). Arsenic toxicity in humans is more often due to inorganic arsenic exposure over long periods of time. Arsenic toxicity due to oral exposure or inhalation is associated with cancer and/or cancer risk (1). Inhalation exposure results in primary tumors in the respiratory system, while oral exposure causes various types of skin tumors, but both with secondary tumors in vital organs, such as the liver, bladder, kidneys, and prostate; depending on the exposure (1). Typical severe dermal effects associated with arsenic poisoning (called arsenosis) are formation of hyperkeratinized corns or warts and hyperpigmentation of skin around foot soles and palms of hand when people are exposed to chronic concentrations of arsenic, over 300μg/L in some studies (4;11). The exposure time and concentration of arsenic present ultimately determines the severity of the health effects. More importantly, children rather than adults are at a higher risk for arsenic exposure and toxicity regardless of the
amount present. Children unlike adults are still undergoing developmental changes that can be significantly hindered by the effects of arsenic poisoning (1). In addition, children tend to weigh less than adults until puberty and with less mass associated with their bodies have a chance of higher exposure rate per square footage than an adult over the same period of time (9). Lower concentrations of arsenic can have similar negative outcomes if exposures occur over a longer period of time (chronic exposure). Along with different cancers arsenic is associated with irreparable DNA damage in children who were chronically exposed to arsenic in drinking water (11). Attributed health effects are not only are caused by water consumption but also from contact with arsenic-containing materials, soil, and some foods. As a result, arsenic was the highest priority chemical listed on the 2005 Priority List of Hazardous Substance for the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) (11).

Purpose and Objective

The purpose of this project is to compare results of arsenic concentration in the water samples collected, to those of the soil and vegetation from the same location. The arsenic concentration of samples taken will reflect the amount arsenic that is readily available in the soil and in the vegetation to use
based on local water irrigation. The project will also test an approved test kit for rapid detection of arsenic that can be used on the field in case of catastrophic emergency or in areas where scientific research is not readily available. The test method used in this project has been approved by the EPA as an approved method for arsenic detection, the availability of the product and ease of use will give scientists the ability to determine result in a timely manner. Approved methods for both water and soil exist using the test kits, but a method for vegetation samples was developed. The development for faster and portable methods for arsenic concentration may provide means for predicting the relationship between all contaminated media (water, soil, vegetation).

Scope and Significance

A comprehensive comparison between arsenic concentration in the soil and vegetation to that of the water source has not been performed prior to this study. Since the 1980’s researchers have compared instruments and methods to determine arsenic concentration in water and human exposure indicators, such as nails, hair, blood or urine. They used methods such as atomic absorption spectroscopy (AAS) or inductively coupled plasma atomic emission spectroscopy (ICP-AES) to analyze the arsenic in samples (3; 12; 20; 24). They helped to determine where arsenic originated from and how arsenic travels throughout the ecosystem, as well as the difference between inorganic and organic arsenic.
Pioneering studies on potential arsenic containing foods and soils have helped to further determine that arsenic contamination is in part related to contaminated groundwater. These previous studies have established a foundation for future research. The main disadvantage with these methods is that large quantities of samples must be collected and then concentrated for analysis. Faster and more efficient methods to determine the amount of arsenic present in water, soil, and vegetation are still in demand.
CHAPTER TWO

LITERATURE REVIEW: COMPARISON AMONG ANALYTICAL METHODS

Arsenic in Humans

In many studies, indicators such as hair, nails, urine, and blood have been used to determine if an increase in arsenic levels in the body would aid in explaining health effects observed with arsenic-associated diseases (4; 9; 12; 24). Such an attempt allowed researchers to reassess the effect of increasing arsenic concentrations in contaminated groundwater based on water consumption. Although many of the studies were successful in making a connection between high arsenic concentration in a water sample and a human indicator (usually hair follicle), none attempted to correlate the water concentration present in the area to the potential arsenic ingested by the person from other sources, such as local vegetation. Samples using nails or hair allowed for the determination of arsenic ingested for up to a period of 6 months, while urine samples gave scientists an actual amount ingested and excreted in a period of a day (3; 11; 12; 24). There are known areas that are in fact high-risk for arsenic contamination, and studies testing these areas have affirmed the theory that arsenic produces disease or cancer in the people living in those areas.
From the literature review, various methods for the collection of sample data were apparent. The most effective method came from an older study (24) where hair follicles were treated with chemicals, then “ashed” using high heat to remove any organic molecules, and finally analyzed using an atomic absorption spectrophotometer (AAS) to determine the amount of arsenic present. Samples were burned until only ash material remained because organic matter influences the adsorption behavior by interacting with mineral surfaces present and with arsenic itself. Absorption may play a substantial role in the release of arsenic from soils and sediments into the groundwater. Previous studies investigated areas of India where there were extreme issues with arsenic water contamination (greater than 300µg/L) that caused widespread devastation (4; 3; 2; 13). The important facts were that arsenic sampling from hair needed to be at least a gram worth and that only a small concentration could be determined from these samples (24). Other studies that included samples of soil and produce in congruency had a better understanding of the amount of arsenic present and therefore provide an extra support for the hair follicle tests in determining the potential arsenic exposure for a given area (20).

Arsenic in Vegetation

Since arsenic occurs in all soils and natural waters; plants have had to thrive in the presence of arsenic ions. The transfer between soil to crop, to food, and to direct ingestion of drinking water is considered a large contributor of the
arsenic transfer exposure pathway (2). Essential elements such as phosphorus, which allow plants to flourish and grow, is known to be chemically similar to arsenic and in some cases arsenic may be substituted for plant nutrition (8). When arsenic is in a solution (e.g., water) it penetrates the outer cuticles of the plant where other enzymes of the plant are stored and also affects the plant’s turgor. In addition, aresenate is associated with rapid loss of turgor to a plant by uncoupling phosphorylation of adenosine diphosphate (ADP) and deactivating the energy available of converting ADP to adenosine triphosphate (ATP) (8). Arsenic’s ability to replace phosphate in a chemical reaction is one of the important ways that arsenic can act as toxicant on a cellular level. According to Commission on Life Sciences and the Division of Earth and Life Studies (8), arsenic not only substitutes for phosphorus in several ways, but arsenic components (both As(III) and As(V)) are also absorbed and translocated similarly as much as phosphates in the plant. Arsenic in plants has only further confirmed that secondary exposure due to ingestion of contaminated vegetation can occur as a result of contaminated water used for irrigation.

Arsenic in Soil

Arsenic’s ability to travel in soils is governed mainly by desorption and adsorption on mineral surfaces. The ability to attach to mineral surfaces depends on a competing anions’ ability to create stronger bonds, and on the pH of the environment (6). Know nutrients associated with plant growth and development
have verified that plants receive a majority of their nutrients from either the atmosphere or the soil they are grown in. The primary elemental nutrients from the atmosphere include carbon, hydrogen, and oxygen, while elemental nutrients from the soil include calcium, copper, boron, iron, magnesium, manganese, molybdenum, nitrogen, phosphorus, potassium, and zinc (8). Areas rich in natural organic matter (NOM) which in turn, are rich in essential elements are needed for plants to grow and develop, are highly reactive towards both metal hydroxide surfaces and soluble metals. This helps arsenic’s ability for speciation, mobility, absorptivity, and bioavailability of both inorganic and organic arsenic components (22). In addition, studies indicate that locations of high groundwater arsenic concentrations correlate with arsenic concentrations in soil (6; 13; 20), which then in turn is associated with plants that have notable amounts of arsenic present (2).
CHAPTER THREE

METHODOLOGY

Site Description

Thermal California in East Riverside County was selected as a study site because of its known high concentration of arsenic in groundwater (17). The city of Thermal is located southeast of the city of Palm Springs within the Coachella Valley community and northeast of the Salton Sea (Figure 1). The city is 138 feet below sea level and has a total area of 9.45 square miles all surrounded by desert landscapes with local highs of 121˚C (15). The total population consist of 2,865 people with the majority of the population classified as of Latino or Hispanic decent (US Census, 2010). The area is also commercially used as an agricultural farmlands by local farmers growing seasonal crops for exportation and consumption. Local irrigation includes using municipal city water and groundwater during growing seasons.
Figure 1. Southern California Map of Thermal, CA and Surrounding Areas as Determined by Google Maps.

Google Maps shown above (Figure 1) demonstrates the area surrounding Thermal, California is desert lands where little to no water is found (Google Maps, https://www.google.com/maps/place/Thermal,+CA/@33.6276816,-116.1690471,9838m/data=!3m1!1e3!4m2!3m1!1s0x80da5ead1390ab4f:0xcc9676f67418ab). Water scarcity in the area demands that people living within the surrounding area use local groundwater available for daily necessities. The Salton Sea located south of Thermal is the only visible water outside of the coastal ocean and is unfit for human consumption. Map generation of the current arsenic concentration for the given area was accessed and based on the amount of arsenic present, which was then used to select sampling points. Samples
taken from local residents within the area were coded and cross-referenced with the amount of water arsenic concentration of an area (21). Water, soil and vegetation samples were obtained under the permission of the local residents. A sample of the soil used to grow edible vegetation was collected near the plant root system. Soil samples were collected around the base of the plant, because this is where the plant was more likely to be regularly watered. The focus for collection was to collect any edible produce or herbs used as a part of the dietary intake for the local residents.

Sampling Method

Water, Soil, and Vegetation Collection

A total of 15 water isolates as well as 12 corresponding soil and vegetation samples were examined throughout the East Riverside County of Thermal California, in areas known to have high arsenic in water concentrations. Sample areas tested were chosen based on previous results from a similar evaluation of arsenic contamination within the area (17). Samples were not only tested on basis of water contamination but also collected for any vegetation grown in the area. A soil sample from the base of the vegetation was collected congruently for comparative analysis. Each sample collected, (water, vegetation, and soil) was stored in a 120mL research quality and EPA approved wide mouth polyethylene flip-top bottle with lock and seal lid design (Figure 2). All sample
bottles were acid-soaked and acid-washed with 10% nitric acid in an effort to remove any organic material that may have adhered to the plastic 24 hours prior to sampling. After the 24 hour period, all sample containers received a triplicate rinse using super-distilled water. Samples collected were labelled according to location, with each sample receiving the same number for a location and different letter for each type of sample. For example, water was coded with a “W”, soil an “S”, and vegetation a “V” followed by the assigned location number. The location, time, and description of the area were recorded for future reference.

Figure 2. EPA Approved Wide Mouth Polyethylene Flip-top Bottle with Lock and Seal Lid Design Used for Sample Collection.
Duplicate water samples were gathered in order to accurately determine the amount of arsenic present in the area of question. First draw water samples were collected to ensure more probable scenario of water received by the plants being watered. All water samples were collected using a low-flow setting from the outside tap-water hoses used to irrigate vegetation. The vegetation collected at each location was selected based on what local residents were able to grow and consume. The amount retrieved ranged from 5.0 grams to 8.0 grams of newly sprouting leaves visibly available, under the assumption that new growth is more likely to represent recent conditions associated with the current water sample, as opposed past conditions that may not reflect the water sample collected. Similarly, soil sampled was collected using a small garden shovel (Home Depot, California) to remove the top layer of topsoil that is readily watered. Between soil samples, the shovel was rinsed using super-distilled water and a standard triplicate rinse method using 1 Liter buckets to reduce any potential for cross contamination. After each rinse the rinse water was discarded and rinsed additionally with super-distilled water before refilling for another rinse cycle.

Laboratory Analysis

Upon collection, all samples were then separated into corresponding categories before laboratory analysis; all the vegetation samples were promptly refrigerated at 4.0 ± 0.5°C in order to reduce decomposition prior to analysis.
(using Fisher-Scientific Fridge, model number 97-960-1). In order to determine the quality of the water as well as its potential for arsenic contamination, water and soil samples were tested for conductivity and pH using a Hach Senslons 5 and Accumet AP72 pH meter at 24.0 ± 1.0˚C. The conductivity of the water is affected by the presence of inorganic dissolved solids that contain positively charged ions, such as total arsenic. The range of conductivity should be less than 500 µS/cm (or ppm) to be in compliance with U.S. EPA standards for drinking water (11). For both pH and conductivity the probes were thoroughly rinsed with super-distilled using a triplicate rinse method to ensure no cross-contamination from sample to sample occurred. Triplicate rinse container water was also changed after two uses or after significant debris was visible. Both water and vegetation samples were tested using an Industrial Test Systems (ITS) Econo II Quick Rapid Arsenic Test Kit (Model number 481304) approved by Environmental Technology Verification Program (ETV) and the U.S. EPA standards for arsenic determination. Soil samples were measured using the ITS Quick Rapid Arsenic Test Kit (Model number 481396-5), designed for soil testing, in accordance with the manufacturer’s instructions.
Figure 3. ITS Quick Rapid Arsenic Test Kit. Model number 481304 used for arsenic analysis of water and vegetation (left). Model number 481396-5 used for arsenic analysis of soil (right). Both test kits are EPA and ETV approved.

Arsenic Water Analysis

For arsenic water analysis, 50.0 mL of the sample water was placed into the reaction vessel. To the 50.0mL of water the first reagent (main component, L-tartaric acid) was added and the vessel was shaken vigorously for 15 seconds. Next, the second reagent (main component, potassium peroxymonosulfate) was added, then the vessel was recapped and vigorously shaken another 15 seconds. Immediately after, the water sample was left to incubate for 2 minutes. During this time a special turret cap was prepared by attaching a colorimetric indicator to the inner cap for exposure to arsine gas to be released in the final stage (Figure 3). The third reagent (main component, zinc) was then added and the vessel was shaken for 5 seconds before replacing the original cap with the special turret cap, previously prepped. Results were recorded after 10 minutes
elapsed by comparing the colorimetric indicator pad color to a provided color chart (Figure 6). The color matched represents the amount of arsenic present ranging from less than 2 ppb to greater than 100 ppb (Figure 5).

Figure 4. Turret Cap Used for Analysis of Arsenic in Both Water and Vegetation Samples.
Figure 5. Color Chart Used for Determination of Arsenic Concentration in Water and Vegetation. Color match testing strip to color chart determines the amount of arsenic in sample.

Figure 6. Sample of Testing Pad Color and Corresponding Amount Based on the Color Chart Used.
Arsenic Vegetation Analysis

Unlike the water analysis, the vegetation samples required a serial alcohol extraction method to release arsenic from the plant tissues, whereas each alcohol extraction was measured individually and cumulatively totaled for the amount of arsenic. The alcohol extraction method was chosen instead of the well know acid digestion method (Standard Method, 2012), because spiked recovery of arsenic could not be validated. It can only be assumed that the pH of the sample was too acidic and allowed for a change in the type of arsenic compound present, which could not be quantified by the test kit. Approximately 2.0 g of vegetation was measured for each sample and pulverized using 50.0mL of ethyl alcohol (Fisher Scientific A401) in a ceramic mortar and pestle (Figure 7). The liquid was then decanted into a vacuum apparatus and filtered through a ceramic Büchner funnel filter (CoorsTek 60239, 30mL) (Figure 8). The mortar and pestle was further rinsed with 5.0mL ethanol and decanted again into the apparatus (Figure 8). The filtered liquid was evaporated until only a small volume of concentrated ethanol extract remained. The concentrated extract was then rehydrated with 100.0mL of super-distilled water and mixed thoroughly. Next, 50.0mL of the rehydrated extract was placed in the reaction bottle and tested in the same matter as the water sample using the Econo II Quick Rapid Arsenic Test Kit. If any amount of arsenic was present, then another extraction and analysis was performed. Serial extractions and analyses were performed until arsenic was no longer detected in the extracts, which helped ensure that arsenic
was efficiently extracted from the plant tissues. Average extractions for vegetation samples collected were between three to five. Arsenic concentrations for serial extractions were then totaled and reported as the actual amount present in the vegetation.

Figure 7. Mortar and Pestle with 50.0mL of Ethyl Alcohol and 2.0g of Vegetation Sample.
Arsenic Soil Analysis

All soil samples were initially dried before analysis. A 5.0g soil sample was weighed and placed into a sterile Petri Dishes to dry in the oven at 60.0 ±1.0°C for 12 hours as recommended by the manufacturer of the arsenic test kit. The dried soil was pulverized using a mortar and pestle for consistency, any large pebbles and stones were removed. About 0.5g of the crushed soil was measured and placed in the specialized reaction bottle (Figure 3). The soil was then rehydrated with 50.0mL of super-distilled water and tested for pH and conductivity at 24.0 ±1.0°C before testing for arsenic concentration. The first reagent (main component, L-tartaric acid) was added and shaken vigorously for 15 seconds. Next, the second reagent (main component, potassium peroxymonosulfate) was added, the vessel recapped and vigorously shaken another 15 seconds. Immediately after, the rehydrated soil sample was left to
incubate for 2 minutes. During the incubation period the specialized turret cap was prepped with the testing strip (Figure 9). The last reagent, reagent 3 was added following the incubation period and the vessel shaken thoroughly for 5 seconds before letting stand for 10 minutes for results with the newly place turret cap. The colorimetric indicator pad was then compared to the color chart provided by the test kit for the amount of arsenic present (Figure 10).

Figure 9. Turret Cap for Analysis in Soil Samples.
Figure 10. Color Chart for Arsenic Concentration in Soil Samples. Color match testing strip to color chart determines the amount of arsenic in sample.

Statistical Analysis of the Data

Data were analyzed using IBM’s Software Package for Social Sciences (SPSS) version 13. Spearman’s rho correlation was performed to determine the degree of correlation between different arsenic concentration methods. The correlation coefficient (rho) provide observed differences between arsenic concentration in water, soil, and vegetation. Spearman’s rho analysis, a non-parametric method, was used because not all variables were normally or log-normally distributed. Other methods such as descriptive statistics were determined using SPSS to define the mean, standard deviation, skewness, and kurtosis to evaluate the distribution of data. The correlation analysis can further demonstrate whether moderate to strong correlations exist and deemed significant enough to conclude the congruent similarities with arsenic as a
common factor. The data analysis was further used to investigate the relationship between groundwater arsenic concentration to that of soil and vegetation arsenic concentrations.

Quality Assurance and Quality Control

For the assurance of the quality of the collected data, several measures were initiated. All sample containers that were in physical contact with the sample materials (either the water, soil, or vegetation) were properly acid washed and received a super-distilled triplicate water rinse prior to analysis in accordance with standard methods (18) instructions (Method 3030E) to minimize any other organic or arsenic-containing compounds that may contribute to the actual amount of arsenic present. In order to preserve and maintain the integrity of the samples, all samples were properly labeled and accounted for prior to analysis. The shovel used for soil collection also received a triplicate super-distilled water rinse using a three bucket method between different sample collections.

Upon analysis all equipment used throughout the experimentation for arsenic concentration was washed and triplicate rinsed with super-distilled water. Further refrigeration and separation of the vegetation samples from the soil and water samples was required to prevent decomposition while analysis took place. In addition, samples collected and sorted were done so in a period of one day and analysis of samples occurred on the following day until completion. It is also
important to note that all reagents used for the test kits used premeasured spoons (water and vegetation test kit) or pre-measured packets (soil test kit).
CHAPTER FOUR
RESULTS AND DISCUSSION

The results and discussion portion of this project are organized similarly to the previous sections of the paper. The first portion is an examination of the arsenic concentration in relation to the water samples collected. The second section will examine the results of arsenic concentration in comparison to soil samples. The third portion of the results will examine the results of arsenic concentration as compared to vegetation samples collected. The final section of the discussion includes a comparative analysis of arsenic concentration of water, soil, and vegetation to that of the corresponding pH and conductivity.

Water Concentration

Table 1 represents the data obtained from the water samples collected. The minimum concentration of arsenic was 5 ppb and the maximum concentration was 25 ppb. There was an asymmetric distribution with the longer end of the tail pointed towards lower concentrations and a mean value of 17 ppb. The median value for the asymmetric data was 20 ppb with a majority of the data were distributed between the 25% and 75% quartiles at 16 and 20 ppb, respectively. Water conductivity ranged from 11.2 to 540 μS/cm and close to normal but flatter distribution of the data with a standard deviation of 170 μS/cm.
The water pH of the data shows distribution of the data close to the neutral range (6.35 to 7.15) with flatter but left skewed data with standard deviation 0.2 values away from the mean.

Table 1. Descriptive Statistics of Water Samples.

<table>
<thead>
<tr>
<th>Water Statistics</th>
<th>As Water Conc (ppb)</th>
<th>Water Conductivity (µS/cm)</th>
<th>Water pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Valid</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Missing</td>
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<td>0</td>
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<tr>
<td>Mean</td>
<td>17.1</td>
<td>263</td>
<td>6.79</td>
</tr>
<tr>
<td>Median</td>
<td>16.0</td>
<td>250</td>
<td>6.81</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>5.7</td>
<td>170</td>
<td>0.23</td>
</tr>
<tr>
<td>Skewness</td>
<td>-0.64</td>
<td>0.01</td>
<td>-0.43</td>
</tr>
<tr>
<td>Std. Error of Skewness</td>
<td>0.64</td>
<td>0.64</td>
<td>0.64</td>
</tr>
<tr>
<td>Kurtosis</td>
<td>0.85</td>
<td>-0.78</td>
<td>-0.18</td>
</tr>
<tr>
<td>Std. Error of Kurtosis</td>
<td>1.23</td>
<td>1.23</td>
<td>1.23</td>
</tr>
<tr>
<td>Range</td>
<td>20</td>
<td>529</td>
<td>80</td>
</tr>
<tr>
<td>Minimum</td>
<td>5</td>
<td>11.3</td>
<td>6.35</td>
</tr>
<tr>
<td>Maximum</td>
<td>25</td>
<td>540</td>
<td>7.15</td>
</tr>
<tr>
<td>Percentiles</td>
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<td>50</td>
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<tr>
<td></td>
<td>75</td>
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<td>404</td>
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</table>
Soil samples showed high concentrations of arsenic present ranging between 1500 ppb and 30000 ppb with a mean value of 7625 ppb. Compared to the mean value the concentration has a significant skew to the right with a large peak much like that for soil conductivity. The soil samples had a mean conductivity of 175 μS/cm with a range of values between 21.7 and 1332 μS/cm. In addition, the soil conductivity data showed a strong skew to the right and a significant large peak present. The soil pH ranged in the alkaline spectrum (7.63 to 9.83) with a mean of 9.83. The skewness is to the right of the mean value and has a much flatter distribution than the soil conductivity and concentration (Table 2).
Table 2. Descriptive Statistics for Soil Samples.

Soil Statistics

<table>
<thead>
<tr>
<th></th>
<th>As Soil Conc (ppb)</th>
<th>Soil Conductivity (µS/cm)</th>
<th>Soil pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Valid</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Missing</td>
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</tr>
<tr>
<td>Mean</td>
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<td>7625</td>
<td>175</td>
</tr>
<tr>
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<td>3000</td>
<td>45.8</td>
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<tr>
<td>Skewness</td>
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<td>3.23</td>
</tr>
<tr>
<td>Std. Error of Skewness</td>
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<td>0.64</td>
</tr>
<tr>
<td>Kurtosis</td>
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<td>Std. Error of Kurtosis</td>
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</tr>
<tr>
<td>Range</td>
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<td>1500</td>
<td>21.7</td>
</tr>
<tr>
<td>Maximum</td>
<td></td>
<td>30000</td>
<td>1332</td>
</tr>
<tr>
<td>Percentiles</td>
<td>25</td>
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<td>30.5</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>3000</td>
<td>45.8</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>15000</td>
<td>90.1</td>
</tr>
</tbody>
</table>

Vegetation Concentration

Results observed for vegetation samples only include concentrations in ppb. The range of concentration of arsenic in the vegetation samples was
between 0 and 19 ppb with a calculated mean value of 5 ppb (Table 3). The standard deviation was calculated at 7.1 ppb and was considered very far away from the mean value. Skewness of the data was right-handed from the mean value and has a flatter distribution, which is explained by the skewness and kurtosis results. The median for the data was 1.50 ppb while the interquartiles for the data were 0.0 ppb and 11.8 ppb (25-75% distribution). In addition, pH for the vegetation samples was not collected due to the addition of alcohol used for serial extractions. Conductivity for the data was also not collected due to the filtration of the samples prior to the analysis for arsenic concentration.
Table 3. Descriptive Statistics for Vegetation Samples.

<table>
<thead>
<tr>
<th>Vegetation Statistics</th>
<th>As Veg Conc (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>Valid</td>
</tr>
<tr>
<td></td>
<td>Missing</td>
</tr>
<tr>
<td>Mean</td>
<td>5.0</td>
</tr>
<tr>
<td>Median</td>
<td>1.5</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>7.1</td>
</tr>
<tr>
<td>Skewness</td>
<td>1.23</td>
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<tr>
<td>Std. Error of Skewness</td>
<td>0.637</td>
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<tr>
<td>Kurtosis</td>
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<tr>
<td>Std. Error of Kurtosis</td>
<td>1.23</td>
</tr>
<tr>
<td>Range</td>
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<tr>
<td>Minimum</td>
<td>0</td>
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<tr>
<td>Maximum</td>
<td>19</td>
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<tr>
<td>Percentiles</td>
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</tr>
<tr>
<td>25</td>
<td>0.0</td>
</tr>
<tr>
<td>50</td>
<td>1.5</td>
</tr>
<tr>
<td>75</td>
<td>11.8</td>
</tr>
</tbody>
</table>

Multiple linear regression analysis controlling for pH and using water, soil, and vegetation arsenic concentrations was attempted and showed insignificant results ($p > 0.05$). All attempts to determine the correlation between pH and arsenic concentration (for water, soil, and vegetation) proved to have similar values as those of Spearman’s rho correlation if not less. Those considered moderate correlations using Spearman’s rho were deemed insignificant under
linear regression correlation interpretations. Contributing factors that may have led to the outcome of arsenic distribution in plants may be a result of the adaptation changes in the roots of plants (5). Different species of plants have unique abilities to deal with environmental stress, such as arsenic in water and can compensate for it. Because only leaves of several edible plants were taken for sample analysis nothing could be said of the root system develop in terms of the arsenic ability to travel throughout the plant (6). Although leaves give a fair representation of arsenic that may have gone unfiltered by the roots and represent the human exposure route, no significant correlation could be determined. Future studies may include not only collecting edible leaves species of plants but also roots that are in direct relation to water consumption for survival of the plant.

Studies on arsenic in soil only verify that soil’s ability to filter through chemical exposure is dependent on several external factors that play a major role in the process (2; 7; 8). Soil arsenic not only depends on the type of soil but the layers that are predominant in the area based on groundwater surveys and geological mapping of the area in question (5). Water’s ability to mitigate through several surfaces, such as vegetation and soil, should provide easy measuring techniques for finding equal concentration of already contaminated water to the media in question (leaves of vegetation and soil). The data examined by this project did not have any correlation of the concentration of groundwater when compared to both vegetation and soil concentration. We were unable to obtain
consistent results; however, the data did provide a cohesive moderate correlation between water, vegetation, and soil concentration verses pH.

Percent Recovery

Percent recovery of the amount of arsenic for each variable (water, soil, and vegetation) was compared to the amount of arsenic concentration used to spike each sample (Table 4). Because the test kit is specific for water concentration of arsenic or soil arsenic concentration, arsenic water, and vegetation concentration were done using the arsenic water kit while arsenic soil concentration was done using the arsenic soil kit. Much like an actual sample collection samples were treated under sterile conditions in order to assume no external sources of arsenic attributed to the results. The percent recovery can then determine the accuracy of each test kit to achieve the amount of arsenic that was originally spiked for each sample. Each sample originally started with a 20 ppb arsenic spike added prior to testing with the specified arsenic test kit. A 100% recovery signified that 100% of the 20 ppb arsenic was properly measured using the test kit in question while any percentage lower is assumed to have lost or gone unmeasured by the test kit in question. For purposes of significance anything below 70% was considered a non-viable testing method for any of the samples used. As expected, both the validated water arsenic and soil arsenic methods provided 100% recovery, which means that the entire 20 ppb spike was
detected by the test kit method. The method for arsenic in vegetation was developed and validated with an 80% recovery. Even though this is considered a viable method, there may have been other underlying factors that contributed to a 4 ppb loss undetected by the test kit. One explanation could be attributed to the different types of vegetation obtained and their ability to retain and filter out nutrients. Another possible and more likely explanation is that the losses in arsenic occurred with the serial extraction method. In addition, acid digestion method resulted in 0% recovery, thus alcohol extraction was used for all analyses.

Table 4. Percent Recovery for Water, Soil, and Vegetation of Arsenic Concentration as Compared to Arsenic Water Concentration Using a 20 ppb Spike.

<table>
<thead>
<tr>
<th>Water Arsenic Conc (ppb)</th>
<th>% Recovery Water</th>
<th>Soil Arsenic Conc (ppb)</th>
<th>% Recovery Soil</th>
<th>Vegetation Arsenic Conc (ppb)</th>
<th>% Recovery Vegetation</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>100.0%</td>
<td>20</td>
<td>100.0%</td>
<td>16</td>
<td>80.0%</td>
</tr>
</tbody>
</table>

Arsenic Water, Soil, and Vegetation Concentration In Association with pH

In order to better compare values that were not consistent with parametric methods, a comparative analysis between variables using a Spearman’s rho
correlation was used. Significant correlation between pH and arsenic concentration can be observed between different variables. Moderate correlations included the arsenic water concentration (in ppb) and water pH with a rho value = -0.708, \( p < 0.05 \), indicating that as pH affects arsenic concentration in water causing it to decrease as pH increases. Another significant moderate negative correlation was between the vegetation concentrations of arsenic in relation to soil pH (rho = -0.628, \( p < 0.05 \)). This demonstrated that as soil pH decreases the vegetation concentration increases. Moderate correlation are considered to be results with positive or negative Spearman’s rho values between 0.3 and 0.6 with a significance level less than 0.05. Spearman’s rho values beyond ±0.7 represent stronger correlation. Spearman’s rho values lower than ±0.3 were considered not have any significant correlations between variables tested.
Table 5. Comparative Analysis for Significance Using Spearman's rho Between Variables.

*Spearman’s Rho Correlation for Arsenic*

<table>
<thead>
<tr>
<th></th>
<th>Water Conc (ppb)</th>
<th>Water Conductivity</th>
<th>Water pH</th>
<th>Soil Conc (ppb)</th>
<th>Soil Conductivity</th>
<th>Soil pH</th>
<th>Veg Conc (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Conc (ppb)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation Coefficient</td>
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<td>-.708*</td>
<td>.040</td>
<td>.135</td>
<td>-.099</td>
<td>.206</td>
<td></td>
</tr>
<tr>
<td>Sig. (2 tailed)</td>
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<td>.830</td>
<td>.010</td>
<td>.902</td>
<td>.675</td>
<td>.760</td>
<td>.522</td>
</tr>
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<td>.329</td>
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<td>.297</td>
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<tr>
<td>Soil Conc (ppb)</td>
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<td>Veg Conc (ppb)</td>
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</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 level (2-tailed), in bold.
Conclusion

The data provided the means to evaluate the potential and probable correlation between water, soil, and vegetation concentrations of arsenic present for the given study area. Even though strong correlations between arsenic concentrations for water as compared to soil and vegetation concentration were expected, it was not the case for this project. There were no significant correlations between arsenic concentration in water when compared to soil and vegetation concentrations of arsenic. Only moderate to strong correlations were discovered against water and soil pH compared to water and soil arsenic concentrations. Correlations between pH and arsenic concentration indicate that pH is a contributing factor for any observed variability. This study was an experimental project in testing new methods for rapid testing for arsenic concentration in soil and vegetation, which proved useful. In support of these findings, other studies indicate the pH may also affect plant uptake of nutrients and environmental contaminants. Because pH plays a major role in arsenic concentration in terms of travel and contribution to contamination, a further evaluation of pH is required. Thus further studies should include analysis of water and soil pH, as well as the root system in vegetation studied. Additional analysis of relationship between pH and arsenic uptake can provide a potentially viable option for field testing using the vegetation method as support.
APPENDIX

ARSENIC ANALYSIS DATA
Table 6: Arsenic Analysis Data

<table>
<thead>
<tr>
<th>Sample:</th>
<th>Water Conc (ppb)</th>
<th>Water Conductivity (μS/cm)</th>
<th>Water pH at 21.5˚C</th>
<th>Soil Conc (ppb)</th>
<th>Soil Conductivity (μS/cm)</th>
<th>Soil pH at 24.0˚C</th>
<th>Veg Conc (ppb)</th>
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<tr>
<td>1</td>
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<td>11.2</td>
<td>6.7</td>
<td>1500</td>
<td>1332.0</td>
<td>9.2</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>238.0</td>
<td>6.8</td>
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<td>16</td>
</tr>
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<td>N/A</td>
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<td>N/A</td>
<td>N/A</td>
</tr>
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<td>1500</td>
<td>21.7</td>
<td>8.4</td>
<td>19</td>
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<tr>
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*Samples 3 and 6 are water samples of filtration system on site of study area and are checked for the absence of arsenic using the ITS Quick Rapid Arsenic Test Kit.

**Sample 15 is a water sample from the bucket used for the triple-rinse method for the shovel; water was checked for absence of arsenic to confirm no cross-contamination of arsenic from one sample to another.
REFERENCES


