Winter 2019

Preservation Protocols for Maintaining Species Stability of Arsenic, Chromium, and Selenium in Water Samples

Contessa Lowery

Preservation Protocols for Maintaining Species Stability of Arsenic, Chromium, and Selenium in Water Samples

Final Report

Professional Project

Master Water Resources

University of New Mexico

Contessa Lowery

December 18, 2019

ABSTRACT

Water quality has become an increasing concern in recent years. The environmental challenges surrounding water quality include the presence of dissolved metals and their potential impact on human health and the environment. The challenge is exacerbated because many important metals have multiple oxidation states, which affect the health and environmental behavior of the metal. Many metals are known carcinogens and increased anthropogenic effects have inexorably made metals' accumulation an imposing threat.

Analytical instrumentation has advanced in many areas, making it now possible to measure very low concentrations of multiple elements in water. Current interest is determining the chemical speciation of metals such as arsenic (As), chromium (Cr) and selenium (Se) which have multiple oxidation states in environmental systems. Conventional protocols involve analysis of filtered and acid-preserved samples which measures the total soluble concentration. However acid preservation can affect metal speciation. The primary objective of this research was to investigate non-acid preservatives.

In this research, experiments were conducted on water samples subjected to different sample preservation methods and no preservation as a control. Metals studied were arsenic (III), arsenic (V), chromium (VI), chromium (III), selenium (IV), and selenium (VI). It was hypothesized that a non-acid preservation would limit redox reactions that would affect the speciation of these metals. Water samples were collected representing a range of different aqueous conditions and subjected to two alternate preservatives: EDTA+TBAOH and HEPES. Samples were then analyzed by a High-Pressure Liquid Chromatography (HPLC) instrument coupled to an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) to determine the concentration of species for each element. Data analysis followed appropriate data quality standards to validate the accuracy and precision of all results and analyzed data was evaluated to draw conclusions on the preservation techniques used. Data revealed that the HEPES and EDTA+TBAOH preservative were able to preserve most species for at least seven days.

INTRODUCTION

Environmental pollution from metals can occur in one of two ways; erosion of minerals from surface deposits or human activity (Nordberg et. al, 2014). Human activities cover a wide area of pollution and can range from the production of plastics, plating, the manufacture of lubricants, mining, smelting, fossil fuel combustion, semiconductors, superconductors, and nanotechnology (Nordberg et. al, 2014). More than 700 organic and inorganic pollutants have been identified in water in which metals have become a top priority (Ali, 2010). This is due to their inability to biodegrade as well as their carcinogenic and toxic properties (Ali, 2010).

As early as 370 B.C, Hippocrates documented one of the first known chronic health effects from metal poisoning; abdominal colic in a man who extracted metals (Goyer et. al, 1996). Metals can be introduced and recycled within the environment in one of three ways: naturally by geologic cycles, naturally by biological cycles, or artificially through human activity (Goyer et. al, 1996). In the case of natural introduction by geological cycles, rainwater dissolves metals from smaller pieces of rock and transports them to streams and/or lakes (Goyer et. al, 1996). In the case of natural introduction by biological cycles, metals are taken up by plants and animals and reintroduced into food chains (Goyer et. al, 1996).

Many metals can exist in the natural environment in multiple oxidation states that affects their physical and chemical behavior, but also their toxicity (Jain et. al, 2000). For this research a method of determining the oxidation states of arsenic, chromium, and selenium as well as methods of preserving aqueous samples was investigated. The oxidation states of arsenic are arsenic (III) and arsenic (V), the oxidation states of chromium are chromium (VI) and chromium (III) and the oxidation states of selenium are selenium (IV) and selenium (VI). For each of these single elements one of the two species is highly toxic at low levels while the other one is not (Heumann, 2004).

Arsenic (III) is the more toxic form of arsenic due to its ability to affect enzyme activity in the body; arsenic (V) has no effect on enzymes (Goyer et. al, 1996). Eighty to ninety percent of arsenic (III) is absorbed by the gastrointestinal tract where cellular mitochondria will begin to accumulate arsenic, impairing mitochondrial enzyme function (Goyer et. al, 1996). In large doses ingestion of arsenic will cause death; other acute symptoms include upper respiratory tract, gastrointestinal, and cardiovascular effects (Goyer et. al, 1996). Chronic exposure can lead to neurotoxicity of the peripheral and central nervous system, liver injury, cardiovascular disease, and cancer (Goyer et. al, 1996).

Chromium (VI) is the more toxic form of chromium due to its ability to cause cancer at low levels (Costa, 1997). Chromium (VI) has been introduced into water supplies as effluent from industrial process such as pigments manufacturing, chrome plating, leather tanning, and as an agent added to water-cooled machinery to prevent rusting. (Costa, 1997). Chromium (VI) can be absorbed by the gastrointestinal tract where ten percent of the total concentration ingested will remain in the body (Costa, 1997). Chromium (VI) can accumulate in all tissues, but it has been found that higher concentrations occur in the liver, kidneys, and bones (Costa, 1997). Long term exposure to Chromium (VI) has led to higher incidences of “urinary tract, bladder, testes, kidney,

prostate, brain, [and] stomach" cancers as well as "Lymphoma, Hodgkins, leukemia, and bone cancer" (Costa, 1997).

Selenium (IV) readily adsorbs into soil where selenium (VI) does not (Goldberg et. al, 2006). Selenium (IV) sometimes is present in agricultural waste, so that agricultural return flows may contaminate streams (Goldberg et. al, 2006). Selenium (IV) toxicity comes from an overabundance of selenium in the environment, which is taken up by plants, eaten by animals, and consumed by humans (Goyer et. al, 1996). Selenium toxicity is classified by alkali disease which is a condition where the amount of selenium in the body exceeds its ability to excrete it (Goyer et. al, 1996). Cases of this disease have been reported in several parts of the world, including the United States, and in parts of South Dakota and northern Nebraska (Goyer et. al, 1996). Some of the symptoms of alkali disease include poor teeth, jaundice, eruptions of the skin, and nail diseases of the fingers and toes (Goyer et. al, 1996).

Improved analytical technology, which includes the coupling of technologies, knowledge has been gained over the last twenty years with respect to metals species and speciation (Heumann, 2004). However, despite these advances it is more common to find studies on total metal concentration rather than effects of individual species. In addition to the difficulty of analyzing individual species, is the challenge of sample preservation techniques. Investigating the effects of metal speciation on an organism requires the ability to collect samples and preserve them for subsequent analysis while maintaining the original distribution of chemical species in the solution.

The United States Code of Federal Regulations, Title 40, Chapter 1, Subchapter D, Part 136 (40 CFR 136), corresponds to the protection of environmental water programs by the Environmental Protection Agency (EPA) which establishes guidelines and test procedures for the analysis of pollutants. Written within these guidelines are the procedures required for the collection and preservation of water samples for metals determination. The requirement of 40 CFR 136 is to preserve samples for metals analysis with nitric acid. Previous research has shown that the addition of nitric acid will affect the oxidation states of arsenic, chromium, and selenium making it impossible to determine the original speciation of a preserved sample (Hall, 1999).

Safe Drinking Water Regulations established by the USEPA are based on the total concentration of metals and most other constituents in solution. The standards do not consider the chemical form that the metal may have (40 CFR 136). Therefore, this provides very little data on a water sample's level of toxicity, as certain metal species are so toxic at low levels that a total concentration provides little to no information on the water body's actual toxicity (Heumann, 2004). Reporting the total concentration of metals limits our understanding of the health and environmental effects of individual species. Without knowing how much of a sample is toxic or non-toxic metal species, reported totals can be grossly inaccurate. Inaccurate data reduces the need to better understand how metals species react and are transported throughout the environment. Current methods for species determination are mostly done in the field (40 CFR 136). Review of these methods demonstrates a lot of slow and messy processes that are not necessarily representative, therefore, there is a need to advance preservation methods to coincide with advanced technology.

Redox reactions are highly prevalent with speciation chemistry (Barber, 2000). These constraints make analyzing these samples very difficult, leading to mistakes and inaccuracies that can highly bias data due to the multiple steps required for field analysis. This calls for a need to improve sampling preservation techniques that can stagnant redox reactions and improve sample precision and accuracy with respect to elemental species concentration.

Preservation capable of maintaining the stability and integrity of all metal species in a single sample could streamline the analytical process, allowing for the simultaneous analysis of multiple species. By taking a different approach to sampling preservation, in conjunction with analytical techniques, more can be understood about the role metal species play in the environment. Expanding preservation research to find solutions capable of stabilizing multiple metal species in water samples is a vital step towards the accuracy of results when evaluating water quality.

OBJECTIVES

The focus of this research study was to evaluate different methods of preserving natural water samples containing As, Cr, and Se and to use an advance analytical technique to determine the speciation of these metals. The goal is to work towards an efficient physio-chemical sampling protocol, using non-acid preservatives, capable of maintaining the integrity of arsenic, chromium, and selenium species in a single sample. A visual representation of each sample, subject to varying preservation conditions, will provide a medium for critical assessment.

BACKGROUND

Analytical chemistry emerged as a science in the early 19th century and within one hundred years analytical interest would become largely focused on inorganic chemistry (Heumann, 2004). In the early 20th Century the term 'trace elements' would become recognized as those elements whose concentrations were so low, they were at the threshold of instrument detection (Heumann, 2004). Over the next 60 years scientists worked on developing methods to increase sensitivity to better detect and determine trace elemental concentrations and by the 1960's concerns were raised about chemical forms of trace elements; technology has been developing and growing ever since (Heumann, 2004).

The International Union of Pure and Applied Chemistry (IUPAC) definition of chemical species states: "specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure (Heumann, 2004)." Throughout corresponding literature, metals species have commonly been defined as a difference in oxidation states of the same element. The IUPAC also defines speciation analysis as "analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample (Heumann, 2004)."

Preservation

Throughout preservation literature, a variety of techniques have been employed to preserve metal species for later laboratory analysis. The studies researched conducted multi-element and single element analysis. Nitric acid is the most common preservative used in the preservation of metals. Hence, metal analyses of acid preserved samples can only report their total concentration. Other studies chose to use a variety of dilute acids, while others used combinations of organic acids and EDTA, or just EDTA. Additionally, studies were done to separate arsenic species in the field using selective cartridges, as the EPA has a specific sampling method for the determination of chromium species (Telliard, 1996), and another study suggested that chloride be added to a sample to prevent selenium species conversion (Hill, 1997).

In a paper by Shafer et. al (2007) details are provided on field procedures used to determine arsenic (III) and arsenic (V) individually. The method uses ion exchange to extract anionic As(V) from a buffered solution leaving uncharged As(III) in solution. Once it was known how much arsenic (III) was in the sample, the concentration of arsenic (V) was determined by a simple difference calculation between the total concentration and the arsenic (III) concentration.

To determine chromium (VI) USEPA "Methods for chemical analysis of water and wastes (1979) states that no preservation should be added, the sample should be cooled and stored at four degrees centigrade and should be analyzed within 24 hours. Chromium samples have been treated this way since its induction and many sources have used this technique for both chromium (III) and chromium (VI) determination (Sun et. al, 2015). A more recent method, USEPA method 1669, chromium (III) and chromium (VI) are separated in the field and the samples preserved for subsequent analysis. The samples are pretreated with iron (III) hydroxide to remove Cr(III) as a co-precipitate and filtered with a 0.45 μm filter. The filter is then stored in

1% Ultrapure Nitric Acid. For chromium (VI) determination an additional sample is taken and treated with a 50% NaOH solution (Telliard, 1996).

The EPA Code of Federal Regulations 40 CFR 136 details the methods and sampling procedures required for the proper handling and preservation of water samples prior to sample analysis with respect to trace metals. These procedures require the use of nitric acid which has been shown in research studies to affect species stability in water samples. For example, arsenic (III) will oxidize to arsenic (V) (Hall, 1999), selenium (IV) will oxidize to selenium (VI) (Heninger et. al, 1997), and chromium (VI) will reduce to chromium (III) (Archundia et. al, 1993).

Past research studies commonly focused speciation analysis on individual elements and their corresponding metal species; (Hall, 1999) for As (III/V) and (Heninger et.al 1997) for Se (IV/VI). For chromium, preservation strategies have always treated chromium separately with respect to speciation analysis (USEPA 1669). However, in recent years, more studies have been conducted on multi-element species analysis in lieu of single element species analysis. In a study by Sun et. al (2015), information is provided on the preservation and analysis of multiple elements where the paper discusses the use of an HPLC-ICP-MS for the simultaneous analysis of arsenic, chromium, and cadmium. Samples for this study followed the preservation requirements of the most restrictive metal, which in this case was chromium, samples were collected and preserved by cooling to four degrees centigrade and analyzed within 24 hours (Sun et. al, 2015). A year later another paper by Wu et. al (2016) also takes on the task of multi-element analysis by experimenting with EDTA and acidification preservatives to simultaneously analyze arsenic, antimony, and selenium on an HPLC-ICP-MS. The purpose of using EDTA as a preservative for these species was to reduce the reduction potential of metal species to react with iron and manganese hydroxides. Using the EDTA as a complexing agent to react with iron and manganese compounds leads to the conclusion that this will keep additional metal species in solution without forming additional metal complexes (Wu et. al, 2016).

To visualize the different preservation approaches used in the literature, combined with how these preservations affected the stability of metal species, a summary of four papers is provided in Tables 1-4. **Table 1** looks at Wu et. al (2016) and the effects of EDTA and acidification on arsenic, antimony, and selenium species. **Table 2** focuses on selenium storage only and is the basis for a paper by Heninger et. al (1997). **Table 3** addresses the effect of acidification on arsenic species versus non-acidification in a paper by Hall (1999), and **Table 4** summarizes Sun et. al (2015), which utilizes refrigeration and analysis within twenty-four hours to test the stability of arsenic, chromium, and cadmium species.

Table 1: Summary of As and Se species preserved with EDTA and EDTA + Acid Samples Filtered (Wu et. al, 2016)

	Bottle	Initial pH	Preservation	Final pH	Temperature	Light	As(III)	As(V)	Se(VI)	Se(IV)
Groundwater	Polyethylene	6.2	None	6.2	Room	Yes	Lost	Lost	No change	Lost
River Water	Polyethylene	6.2	None	6.2	Room	Yes	Lost	Lost	No change	Lost
Lake Water	Polyethylene	6.2	None	6.2	Room	Yes	Lost	Lost	No change	Lost

	Bottle	Initial pH	Preservation	Final pH	Temperature	Light	As(III)	As(V)	Se(VI)	Se(IV)
Groundwater	Polyethylene	6.2	EDTA	6.2	$4^{o}C$	No	Reduced*	No change	No change	No change
River Water	Polyethylene	6.2	EDTA	6.2	$4^{o}C$	No	No change	No change	No change	No change
Lake Water	Polyethylene	6.2	EDTA	6.2	$4^{o}C$	No	No change	No change	No change	No change
*Reduction occurred after third week of storage										

	Bottle	Initial pH	Preservation	Final pH	Temperature	Light	As(III)	As(V)	Se(VI)	Se(IV)
Groundwater	Polyethylene	6.2	EDTA + Acid	3	$4^{o}C$	No	No change	No change	No change	No change
River Water	Polyethylene	6.2	EDTA + Acid	3	$4^{o}C$	No	No change	No change	No change	No change
Lake Water	Polyethylene	6.2	EDTA + Acid	3	$4^{o}C$	No	No change	No change	No change	No change

Wu, Debo, and Thomas Pichler. "Preservation of co-occurring As, Sb and Se species in water samples with EDTA and acidification." Geochemistry: Exploration, Environment, Analysis 16.2 (2016): 117-125.

Table 2: Summary of storage of Selenium solutions for speciation at trace level (Heninger et. al, 1997)

	Bottle	Initial pH	Preservation	Final pH	Temperature	Light	Results
DI Water	Teflon	No Data	0.4 mol/L HCl	No Data	$4^{o}C$	No	29% reduction of Se(IV) to Se(VI) in 27 days
DI Water	Teflon	No Data	NaCl	No Data	$4^{o}C$	No	No change in species after 35 days
DI Water	Teflon	No Data	Na_2SO_4	No Data	$4^{o}C$	No	No change in species after 15 days
DI Water	Teflon	No Data	0.4 mol/L H_2SO_4	No Data	$4^{o}C$	No	3.4% reduction of Se(IV) to Se(VI) in 37 days
All samples were spiked at 10 ppb in deionized water							

Heninger, Ingrid, et al. "Storage of aqueous solutions of selenium for speciation at trace level." *Fresenius' journal of analytical chemistry* 357.6 (1997): 600-610.

Table 3: Summary of Arsenic (III) and Arsenic (V) stability in water samples filtered and spiked at 0.5 ppb (Hall et. all, 1999)

Deionized Water						
Bottle	**Initial pH**	**Preservation**	**Final pH**	**Temperature**	**Light**	**Results**
Polyethylene	No Data	None	No Data	Room	No Data	As(V) valence conversion to As(III) in two days
Polyethylene	No Data	None	No Data	4°C	No Data	No changes in valence state or total concentration
Ottawa River						
Bottle	**Initial pH**	**Preservation**	**Final pH**	**Temperature**	**Light**	**Results**
Polyethylene	No Data	None	No Data	Room	No Data	As(III) reduction to As(V) within one week
Polyethylene	No Data	None	No Data	4°C	No Data	As(III) to As(V) is delayed by 6 days
Polyethylene	No Data	0.1% Acid	No Data	Room	No Data	As(III) reduction to As(V) within 2 days
Polyethylene	No Data	0.1% Acid	No Data	4°C	No Data	As(III) reduction to As(V) more slowly
Polyethylene	No Data	0.4% Acid	No Data	Room	No Data	Increased oxidation rate
Clyde Fork						
Bottle	**Initial pH**	**Preservation**	**Final pH**	**Temperature**	**Light**	**Results**
Polyethylene	7.2	None	No Data	Room	No Data	As(III) reduction As(V) within a few weeks
Polyethylene	7.2	None	No Data	4°C	No Data	Both species completely stable for 10 days
Polyethylene	7.2	0.1% HNO3	No Data	Room	No Data	As(III) reduction to As(V) within 15 days
Polyethylene	8.2	0.1 % HNO3	No Data	4°C	No Data	Stabilized
Polyethylene	9.2	0.1 % HCl	No Data	Room	No Data	As(III) reduction to As(V) within 30 days
Polyethylene	10.2	0.1 % HCl	No Data	4°C	No Data	Stabilized

Hall, GwendyáE M. "Stability of inorganic arsenic (III) and arsenic (V) in water samples." *Journal of Analytical Atomic Spectrometry* 14.2 (1999): 205-213

Table 4: Summary of Arsenic(III/V) and Chromium(VI/III) with refrigeration and analysis within 24 hours (Sun et. al, 2015)

	Bottle	**Preservation**	**Analysis**	**As(III)**	**As(V)**	**Cr(VI)**	**Cr(III)**
Drinking water	Polyethylene	4°C	24 hours	95-101(%)	96-99(%)	94-99(%)	92-98(%)
Surface water	Polyethylene	4°C	24 hours	93-108(%)	96-100(%)	93-102(%)	91-97(%)
Samples were filtered with a 0.45 μm filter and all samples were spiked with 5 ppb of analyte to assess recovery							

Sun, Jing, et al. "Simultaneous speciation and determination of arsenic, chromium and cadmium in water samples by high performance liquid chromatography with inductively coupled plasma mass spectrometry." *Analytical Methods* 7.6 (2015): 2653-2658

METHODOLOGY

Samples were collected in one-liter bottles from surface and ground water sources with varying types of water chemistry, preserved in the laboratory, and spiked with 100 µg/L of all six species to be studied. Samples with no preservation were spiked as a control. The non-acid experimental preservations used were Ethylenediaminetetraaceticacid (EDTA) combined with Tetrabutylammoniumhydroxide (TBAOH) (this is also referred to as eluent throughout the text) and N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES). Analysis was conducted on an HPLC coupled with an ICP-MS for arsenic, chromium, and selenium species simultaneously.

The goal was to collect samples, preserve them with a non-acid preservative capable of maintaining pH levels between 6 and 8, spike those samples with 100 µg/L of each elemental species, and analyze them over a period of two weeks. Samples containing no preservative were spiked with 100 µg/L of each species as a control. Once samples were analyzed the data was evaluated to determine how well each preservation performed. Looking at the individual concentrations of each species per analysis, the recovery of the 100 µg/L can be determined.

Preservatives

Ethylenediaminetetraaceticacid (EDTA) + Tetrabutylammonium Hydroxide (TBAOH)

Ethylenediaminetetraaceticacid (EDTA) is a chelating agent that forms complexes with cationic metals such as Ca^{2+}, Mg^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2+}, and others. Note that EDTA will not form complexes with anionic species such as As(III), As(V), Se(IV), Se(VI), and Cr(VI) which are all present as oxyanions. Chelating agents contain carboxylic groups connected to nitrogen, which allows for the formation of cation complexes (Kołodyńska, 2012). Once a metal complex is formed it becomes stable and limits reactions between the cation and other constituents in solution (Kołodyńska, 2012).

The chemical structure of EDTA using IUPAC Nomenclature is shown in **Figure 1.**

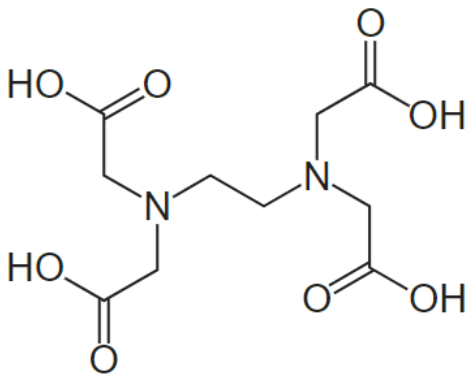
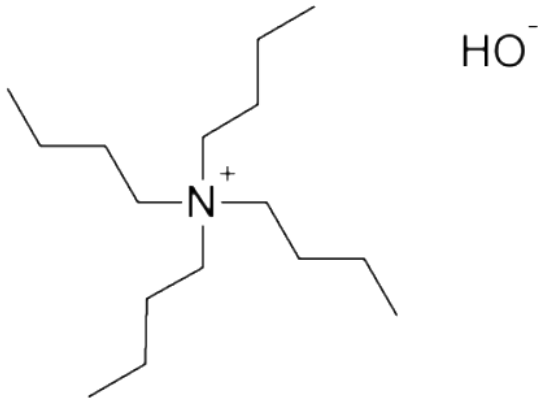


Figure 1: Structure of EDTA
Source: Favre, et. al, 2013

The main purpose of Tetrabutylammonium Hydroxide (TBAOH) is its use as an ion pair reagent for Cr(III) and Cr(VI) (Neubauer, 2004). The IUPAC Nomenclature for TBAOH is given in **Figure 2**. Once EDTA is added to the sample as preservative, Cr(III) will form a complex with EDTA resulting in a net negative charge for the species, whereas TBAOH exhibits a net positive charge (Neubauer, 2004). TBAOH is the mobile phase in HPLC chromatography that allows separation of Cr(III) and Cr(VI) by the chromatographic column followed by quantitation of Cr by ICP-MS. (Neubauer, 2004) It is hypothesized that as an ion pair reagent TBAOH will help aid in the stability of these species for long term storage. It is also hypothesized that adding the TBAOH to the EDTA together as a preservative, will initially form complexes with Cr(III), thus withholding it within solution. Once the sample reaches the analytical column, the Cr(III) complex should freely move through the column to achieve chromatographic separation. TBAOH is then serving as an intermediate step in stasis prior to analysis. These conclusions are based off information found in documentation by Perkin Elmer, the analytical instrument manufacturer (Neubauer et. al, 2003)



Fgure 2:Structure of TBAOH
Source: : Favre, et. al, 2013

The unique properties of EDTA and TBAOH combined was the basis for using this preservative as a part of this research study. It was hypothesized that EDTA will form complexes with the metal species studied. This will immobilize species from rapid chemistry conversion and allow for longer storage of speciation samples. In addition to this, it is also hypothesized that the inclusion of TBAOH as an ion pair reagent for chromium will aid in stabilization and separation of chromium species, ultimately leading to a reduction in redox potential.

N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPN-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) is widely used as a pH buffer in biological applications, because

its unique qualities make it a favorable choice for environmental applications when a pH 7 buffer is needed. Nomenclature for HEPES is given in **Figure 3**.

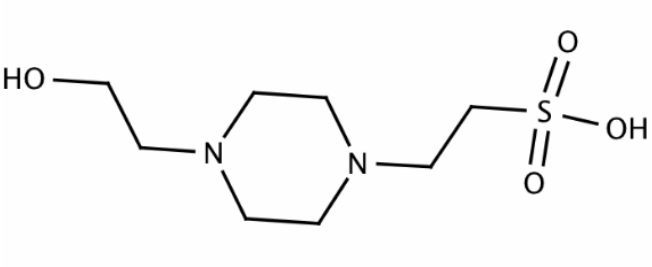


Figure 3: Structure of HEPES (created using IUPAC Rules)
Source: : Favre, et. al, 2013

The rationale behind choosing HEPES as a sample preservative is its ability to maintain neutral pH. It was hypothesized that this pH stability will prevent or retard species oxidation conversion as those reactions are pH dependent. In addition to pH stability the HEPES buffer is chemically stable and absorbs very little UV and visible light without adding bicarbonate ions (Good, et. al, 1966), making it an ideal physio-chemical choice for preservation.

The purpose of a pH buffer is to maintain the pH of samples. The very low pH and oxidizing conditions resulting from conventional sample preservation with HNO_3 may lead to dissociation of metal-ligand complexes and may also facilitate oxidation reactions, both of which affect the speciation of the metal. The addition of the HEPES buffer has the potential to maintain the pH of the solution, with respect to the many water chemistry changes taking place, and hypothetically disrupt changes in oxidation states due to shifting pH.

Good et. al, (1966), suggested basic criteria for buffers used in biological applications which are summarized below.

- The pK_a, otherwise known as the midpoint of the buffering range, should be between 6 and 8
- The buffer should have maximum water solubility, this will allow the use of higher concentrated stock solutions
- Produce a minimum of salt effects.
- There should be a minimum influence of buffer concentration, temperature, and ionic composition of the medium on the dissociation of the buffer.
- Complexes formed with cations should be soluble
- The buffers should be as stable as possible
- They should not absorb light in the visible or ultraviolet regions of the spectrum.
- Other properties include acid dissociation at and above neutrality, absence of ultraviolet absorption, and resistance to oxidation (Good et. al, 1966).

HEPES buffer can maintain a neutral pH, y is, water soluble, does not add any additional bicarbonate, is stable, and is a slowly dissociating weak organic acid and has been used a preservative in other supporting research., This led to the hypothesis that HEPES may be effective at preserving the speciation of As, Cr and Se in solution. No literature could be found in support of using a biological buffer as a natural water preservation for trace metal analysis.

Sample Collection and Preparation

Samples were collected in 1L polyethylene bottles at the four following sites: University of New Mexico ground water well, the Rio Grande River, Soda Dam in the Jemez Mountains upstream, and Soda Dam in the Jemez Mountains downstream. The pH of the samples ranged from 6.97 to 8.18s. Samples were stored on ice in Styrofoam coolers with a thermometer that read 0^{o} C until returning to the laboratory. All samples were collected in one day, 50 mL of each sample was filtered using a 0.45 µm filter, and all samples were refrigerated at 4^{o} C. Samples were preserved within 24 hours of collection. The non-acid preservatives used were EDTA/TBAOH made by combining 146 mg of Ethylenediaminetetraaceticacid (EDTA) at a concentration 0.15mM combined with 650 µLTetrabutylammoniumhydroxide (TBAOH) at a concentration of 0.1 mM in 50 mL of Methanol in 1 L of deionized water at a pH ranging from 6.9 to 7.0 and N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) commercially purchased. The pH of the EDTA/TBAOH preservative was adjusted using sodium hydroxide as needed.

Analytical Methods

All samples were prepared and analyzed on a PerkinElmer Series 200 HPLC and a PerkinElmer ELAN DRC II ICP-MS to test arsenic, chromium, and selenium species simultaneously. Instrument specifications are outlined in **Tables 5 and 6**.

Table 5: HPLC Conditions

HPLC SYSTEM	PerkinElmer Series 200 Binary Pump, Autosampler and Vacuum Degasser
COLUMN	Pecosphere C8; 3 µm particles; 3 cm
MOBILE PHASE	0.15 mM TBAOH + 0.1 mM EDTA (potassium salt) + 5% methanol
PH	6.9-7.0
PH ADJUSTMENT	Dilute HNO_3, NH_4OH
INJECTION VOLUME	100 µL
FLOW RATE	1.0 mL/min
AUTO SAMPLER FLUSH SOLVENT	5% methanol

Source: Neubauer, Kenneth R., et al. "Simultaneous arsenic and chromium speciation by HPLC/ICP-MS in environmental waters." *ICP-OES, ICP-MS* (2004): 614.

Table 6: ICP-MS Conditions

INSTRUMENT	ELAN DRC II (PERKINELMER SCIEX)
NEBULIZER	Meinhard
SPRAY CHAMBER	Quartz Cyclonic
RF POWER	1600 W
ANALYTES	Cr (m/z 52); AsO (m/z 91); Se(m/z 82)
REACTION GAS	NH_3 @ 0.6 mL min
RPQ	0.45
DWELL TIME	500 milliseconds per analytes
ANALYSIS TIME	240 seconds

Source: Neubauer, Kenneth R., et al. "Simultaneous arsenic and chromium speciation by HPLC/ICP-MS in environmental waters." *ICP-OES, ICP-MS* (2004): 614.

Laboratory Quality Control

Samples submitted for analysis were subjected to quality control measures to determine the accuracy and precision of the results. The quality control used for analysis followed the federal compliance regulations of the 40 CFR 136 and the National Environmental Laboratory Accreditation Conference (NELAC) Standard ISO Guideline 17025. **Table 7** is a reference guide to quality control acronyms.

Table 7: Reference Guide for the Identification of Quality Control Acronyms

ICBV	Initial Control Blank Verification
ICV	Initial Calibration Verification
CCV	Continuing Calibration Verification
CCB	Continuing Control Blank
LRB	Laboratory Reagent Blank

Table 8 provides an outline of how samples were analyzed with the corresponding quality control identified in **Table 7**.

Table 8: Analysis on HPLC-ICP-MS

Instrument Calibration	Sample
ICBV	Sample
ICV	Sample
CCV	Sample
LRB	Sample
Sample	Sample
Sample	Sample
Sample	Sample
Sample	Sample
Sample	Sample
Sample	Sample
Sample	CCB
Sample	CCV
Sample	End of Analysis

Experimental Procedures

Three experiments were conducted on the preserved samples. At the conclusion of the first experiment it was determined that the results had been compromised and a second experiment was redesigned . The second experiment was then conducted with the new experiment specifications. Consequentially, the data for experiment two had unverifiable results, and a third experiment was designed to verify unexpected behavior as well as address inconsistencies. The details of each experiment are outlined in the following experiment sections. Throughout the course of the experiments, there were several instances where the HPLC had to be disconnected from the ICP-MS to permit analyses by other users. This may have led to instrument instability. Manufacturer recommendations by Perkin Elmer are conclusive upon maintaining instrument stability under vacuum when being changed, and there were times where the instrument was not allowed a 24-hour stabilization time in between changes prior to analysis.

Experiment 1

For this experiment a volume of 20 mL, preservative and sample, were used for analysis. A combination of both EDTA+TBOH and HEPES together was experimented with briefly, however, it added more complexity that did not add anything additional to the experiments and no new information was learned, therefore it was concluded a combination of preservatives would not be tested. Samples were filtered with a 0.45 µm filter prior to being preserved. A mixed standard containing all tested species at 1 mg/L was prepared in EDTA/TBAOH solution. Preserved samples were spiked with the mixed standard containing all species to a final concentration of 100 ug/L; 2 mL of the 1 mg/L solution was added to all prepared samples. The larger spike amount lead to a change in final volume and a dilution factor was calculated for application to final data. **Table 9** summarizes how each sample from the four sites was prepared; the amount of preservative used, the amount of sample needed to come to a final

volume of 20 mL, the spike amount, and the dilution factor needed for each preservation. It was initially determined that a larger spike volume be used for sample spiking with the expectation that it would provide more species stability. However, dilution factor had to be used as the spike amount changed the final volume of the sample, thus affecting the concentration. It was determined later that this had been done in error and that a much smaller spike amount at a higher concentration would be more effective in determining final concentration.

Table 9: Sample preparation by preservative Experiment 1

	Preservation mL	Sample mL	Total mL	Spike mL	Dilution Factor
EDTA+TBAOH (Eluent)	10	10	20	2	1.1
HEPES	2	18	20	2	1.1
Eluent + HEPES	12	8	20	2	1.1
None	0	20	20	2	1.1

Once samples were preserved and spiked, instrument vials were prepared by adding 0.5 mL of sample along with an additional 0.5mL of EDTA/TBAOH (referred to as Eluent throughout the text). Samples were then immediately placed on the HPLC-ICP-MS for analysis. This designates time zero of the experiment. Samples were placed into the refrigerator until the next analysis. The process was repeated for day 1, 2, 7, and 14. It was intended to analyze samples at 30 days, but it was observed that throughout many stages of the analytical process the EDTA/TBAOH, which is used as mobile phase of the HPLC and is also being used as a preservative of samples, had been used to make the mixed spiking standard and as mentioned above, and was also used for preparing samples for sample analysis. In addition to this 2 mL of mixed standard was needed to bring the samples to a 100 ug/L concentration. Because the samples were spiked with a standard that contained one of the preservatives and because the samples were also prepared with the intended preservative for analysis, it was concluded that samples used for control purposes had been compromised and could not be validated for this experiment.

Additionally, another preservation error occurred in the amount of HEPES buffer used to preserve samples. The stock concentration of HEPES buffer is 1M and samples preserved for this experiment used 2 mL of HEPES buffer, thus making the concentration of HEPES in preserved samples 100mM. **Figures 4**, **5**, **6,** and **7** display chromatograms of As(III), As(V), Cr(III), Cr(VI), Se(IV), and Se(VI) with high levels of interference at the 100mM HEPES concentration. Because the HEPES buffer has never been used in this type of application and no literature could be found on what the concentration should be for preserving water samples it was concluded that the concentration of HEPES buffer should be reduced to 15 mM. This number was chosen arbitrarily based on a paper written by (Vasconcelos, 2002) for biological applications.

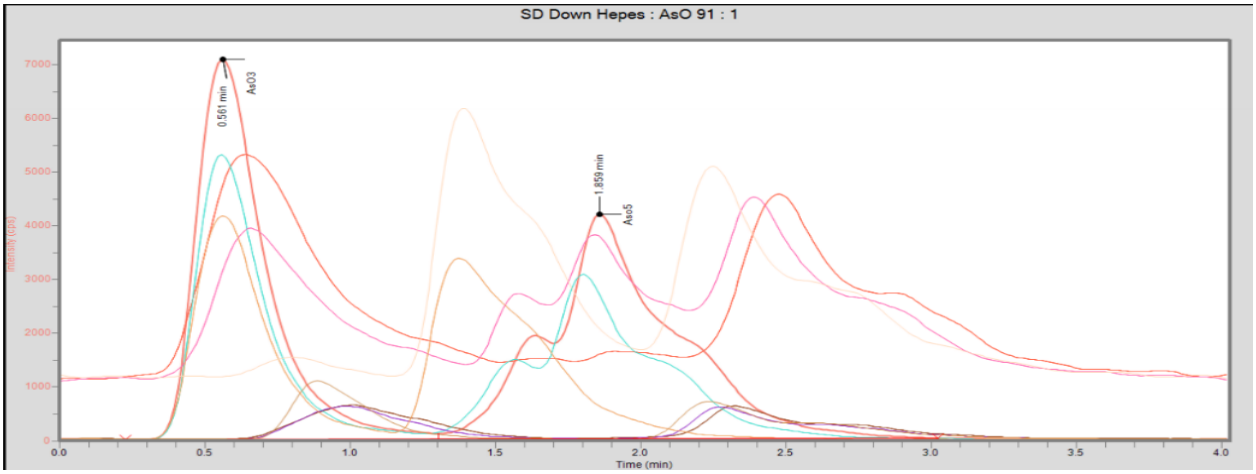


Figure 4: Soda Dam downstream sample with 100mM HEPES as preservative, spiked at a concentration of 100 µg/L. As(III) and As(V) are highlighted.

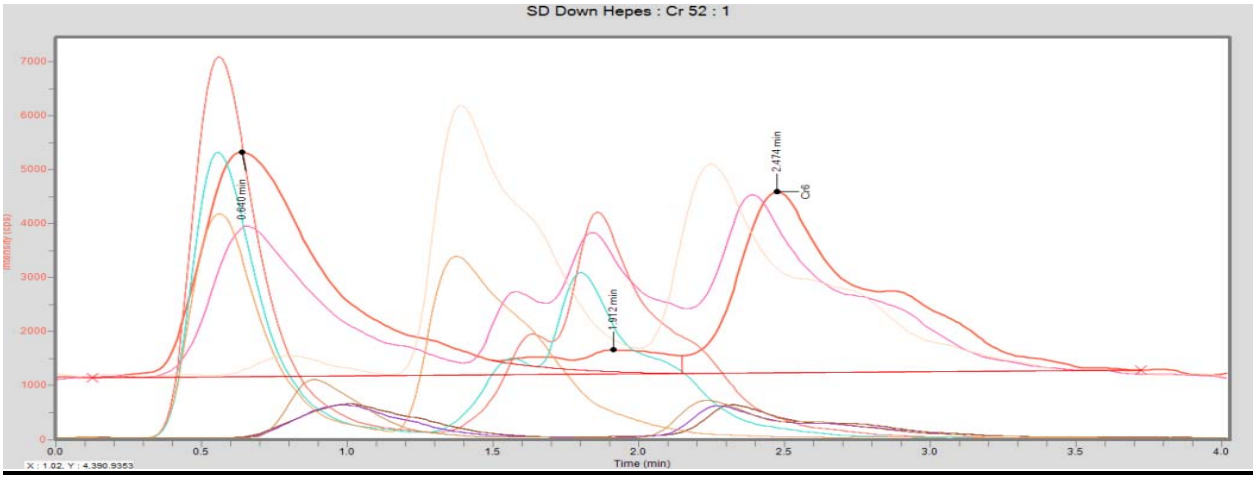


Figure 5: Soda Dam downstream sample with 100mM HEPES as preservative, spiked at a concentration of 100 µg/L. Cr(III) and Cr(VI) are highlighted.

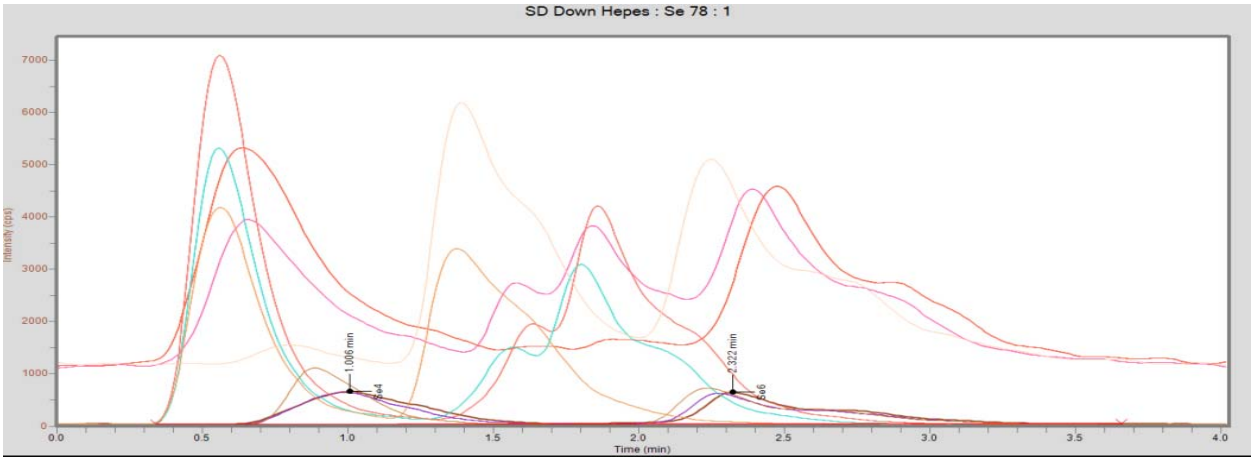


Figure 6: Soda Dam downstream sample with 100mM HEPES as preservative, spiked at a concentration of 100 µg/L. Se(IV) and Se(VI) are highlighted.

Figure 7 also displays the effect of high concentration HEPES on the Rio Grande River sample with regards to Cr(III) and Cr(VI); which displays a more intense level of interference.

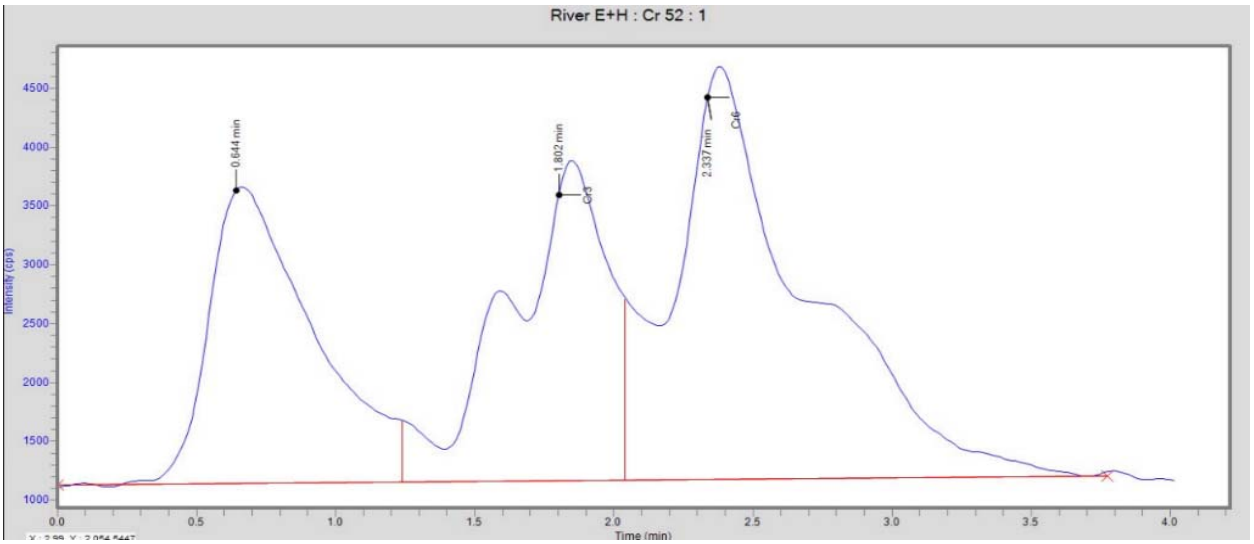


Figure 7: River sample with 100mM HEPES as preservative., spiked at a concentration of 100 µg/L. Cr(III) and Cr(VI) are highlighted.

Because of the issues with adding additional eluent to samples that contained no preservative and HEPES buffer and because of the high interference level with high concentration HEPES, this experiment was stopped after the 14 days. It was redesigned and then repeated as experiment 2.

Experiment 2

Experiment two included field samples as well as 18 mega ohm water and UNM tap water as controls. Out of the two samples collected from the Jemez Mountains, only Soda Dam upstream was used for the experiment. A sample volume of 20 mL was used for preservation and samples were filtered with a 0.45 µm filter prior to preservation.

Considering the lessons learned from experiment one, the amount of eluent and HEPES used to preserve the samples was reduced and the combination of HEPES and eluent was not used. The amount of eluent used to preserve samples was 2mL instead of the 10 mL used in the original experiment (see Table 9) and 300 μL of HEPES was used which yielded a final HEPES concentration of 15mM.

In addition to the changes made to sample preservation method, standards of individual species were not mixed into a single spiked standard. Stock standards of each species at 1000 mg/L were diluted to 100 mg/L in 18 MΩ water; 40 μL of each 100 mg/L standard was then spiked into all samples, yielding a final concentration of 200 ug/L of each individual species per sample to be tested. The reason for this deviation from the originally stated 100 ug/L is simply due to calculation error. Samples prepared for analysis on the HPLC-ICP-MS were poured directly into sample vials with no additional eluent added.

New samples were preserved, spiked, and immediately analyzed by HPLC-ICP-MS at time zero. Samples were then stored in a refrigerator at 4^{O} C and analyzed on day 1, day 4, day 7, day 14, and day 37 to complete the testing. Even though the test on day 37 deviates from the original guidelines of the experiment, the purpose of testing on this day was to determine whether an additional two weeks would dramatically affect the results. However, prior to the samples being analyzed at the 14-day mark, it was discovered that the samples had been left on a bench at room temperature exposed to light one week prior to the test. Samples were placed in the refrigerator for the remainder of the time, but the data revealed that the samples had indeed been compromised with unknown effects. It was concluded that this data was unreliable and inconsistent, thus, a third experiment was designed to address inconsistencies as well as address potential mistakes that had been made during spiking and preservation. The results and the discussion of this experiment are discussed in the Results section.

Experiment 3

With the goal of reducing error and producing more consistent results experiment 3 reduced the number of samples to be analyzed, while maintaining the desired matrix and chemistry effects, along with the proper samples needed for a control. In addition to sample optimization, precautions were taken when adding known concentration of species to samples to validate whether spiking errors had been made in experiment two. Several steps were also taken to ensure samples receive the absolute minimum light exposure during preservation and analysis.

Considering that it had been several months since the last samples were collected, it was decided that new samples should be collected prior to this experiment. Samples included 18 MΩ, UNM tap water, river water from the Rio Grande river, and surface water downstream from the Soda Dam in the Jemez mountains, which is known to have complex chemistry. Samples collected from the river and Soda Dam were stored on ice after collection and remained refrigerated until preservation which was completed within 24 hours after sample collection.

Samples were preserved and prepared using the sample protocol from experiment two. Calculation corrections were made to ensure that samples were spiked at the 100 μg/L as originally determined. Additional steps were taken to properly separate spiked samples from

non-spiked samples during sample preparation to eliminate potential data inconsistencies from human error.

Once all samples were properly prepared, they were analyzed immediately on the HPLC-ICP-MS and subsequently thereafter on day 2, day 7, and day 14. No further sample collection or analysis occurred after day 14. All data and relevant experiment information is presented in results.

RESULTS

The data and information presented in this section are exclusively the results of experiment two and three. Experiment two identified many of the challenges and inconsistencies produced in the data which led to a redesign and experiment 3. Despite efforts taken to eliminate error, more work is needed to refine the preservation method and analytical protocol.

The major ion chemistry was measured for each of the samples prior to preservation and is presented in **Table 10.**

Table 10: ICP results for calcium, potassium, magnesium, and sodium

	Ca mg/L	K mg/L	Mg mg/L	Na mg/L
18 MΩ water	1.316	0.807	0.058	1.11
Tap Water	34.4	4.637	5.5	33.2
Rio Grande	30.8	3	5.81	22.9
Soda Dam	30.5	12	3.5	69.6

Experiment 2 Data

It is important to note some anomalies and observations that occurred. After samples were prepared for analysis on day seven, they had been left on a prep bench and not returned to the refrigerator until one week later which constituted excessive light exposure. The sampling protocol dictates that samples be refrigerated when not in use. Additionally, between day fourteen and day 37 of analysis, the analysis column on the HPLC was changed, which produced unpredictable results for chromium, and it was observed that 18 MΩ and tap water samples did not contain some of the species required and it was inconclusive as to whether this had occurred due to spiking error.

In lieu of the challenges, the data shows that the HPLC-ICP-MS method and the preservation methods used have potential to preserve the speciation of As, Cr and Se and that the species can be subsequently determined by HPLC-ICP-MS. The data are summarized in Figures 8 through 12.

Arsenic (III) and Arsenic (V)

The graphical data for 18 MΩ water, tap water, UNM well water, Rio Grande River water, and Soda Dam water from the Jemez mountains is presented in **Figures 8**, **9**, **10**, **11**, and **12**.

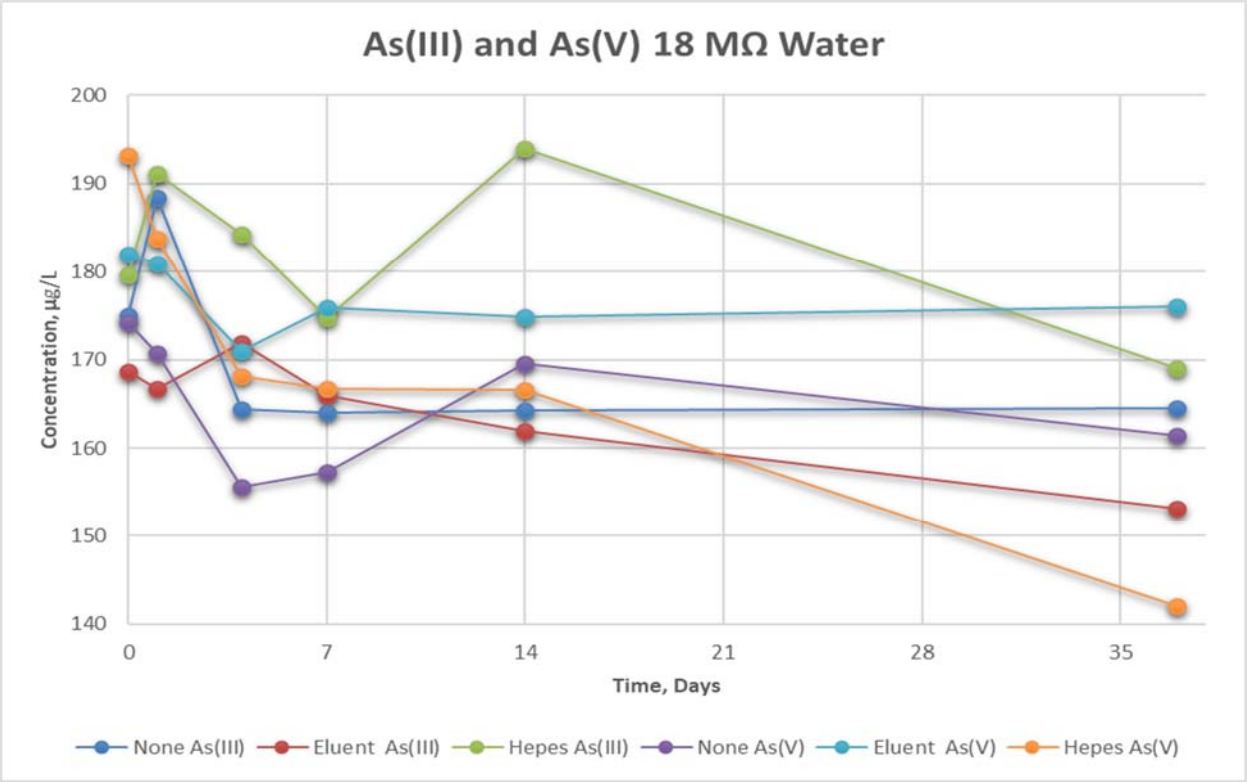


Figure 8: Graphical representation of As(III) and As(V) with preservatives in 18 MΩ water

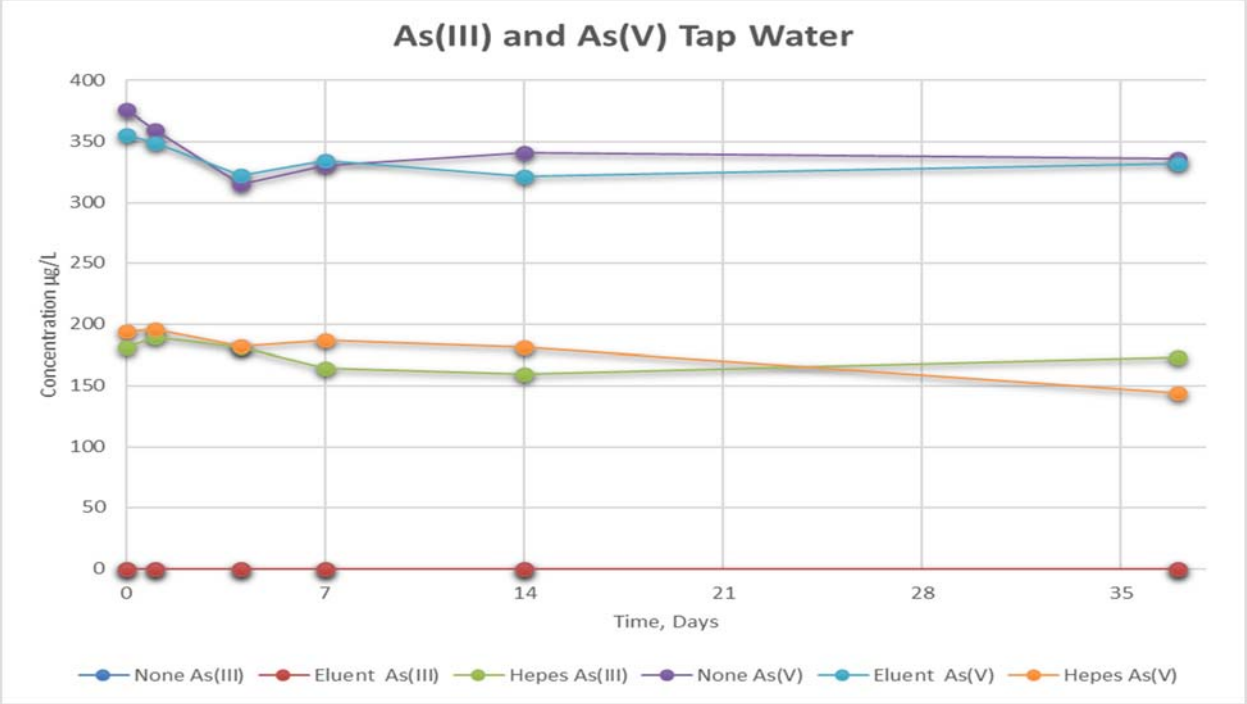


Figure 9: Graphical representation of As(III) and As(V) with preservatives in tap water

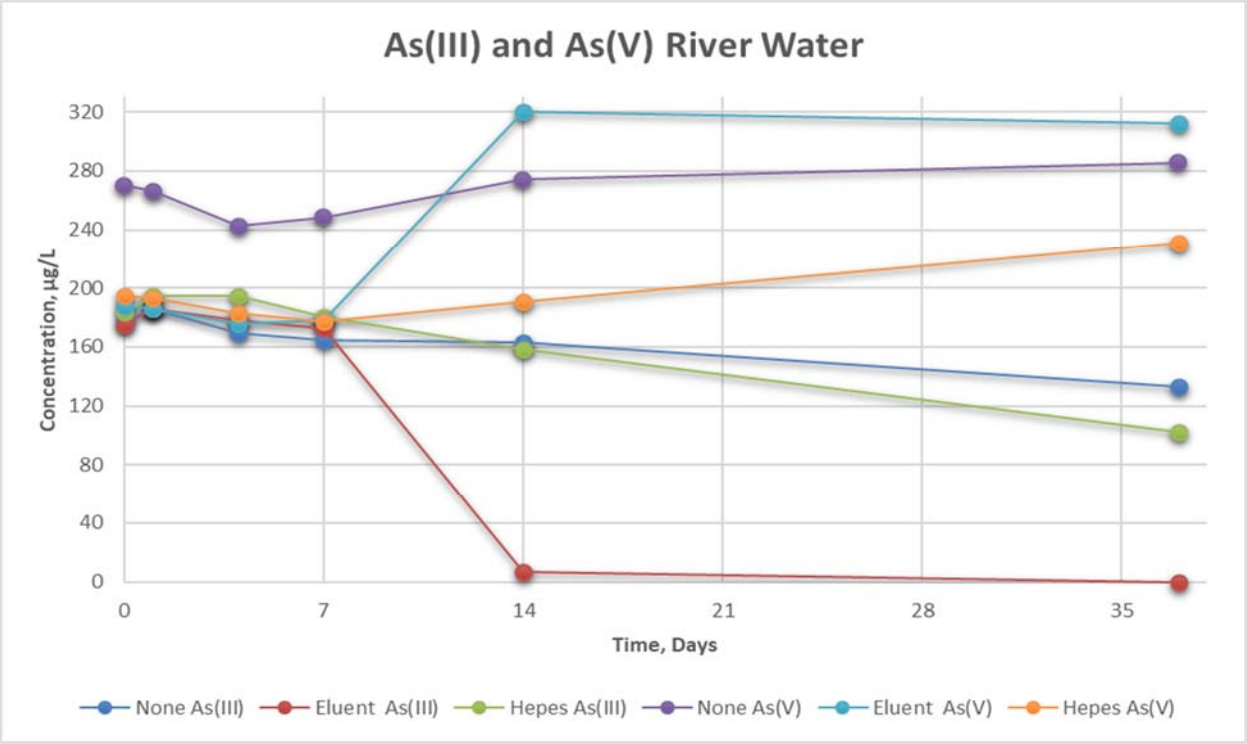


Figure 10: Graphical representation of As(III) and As(V) with preservatives in UNM Well water

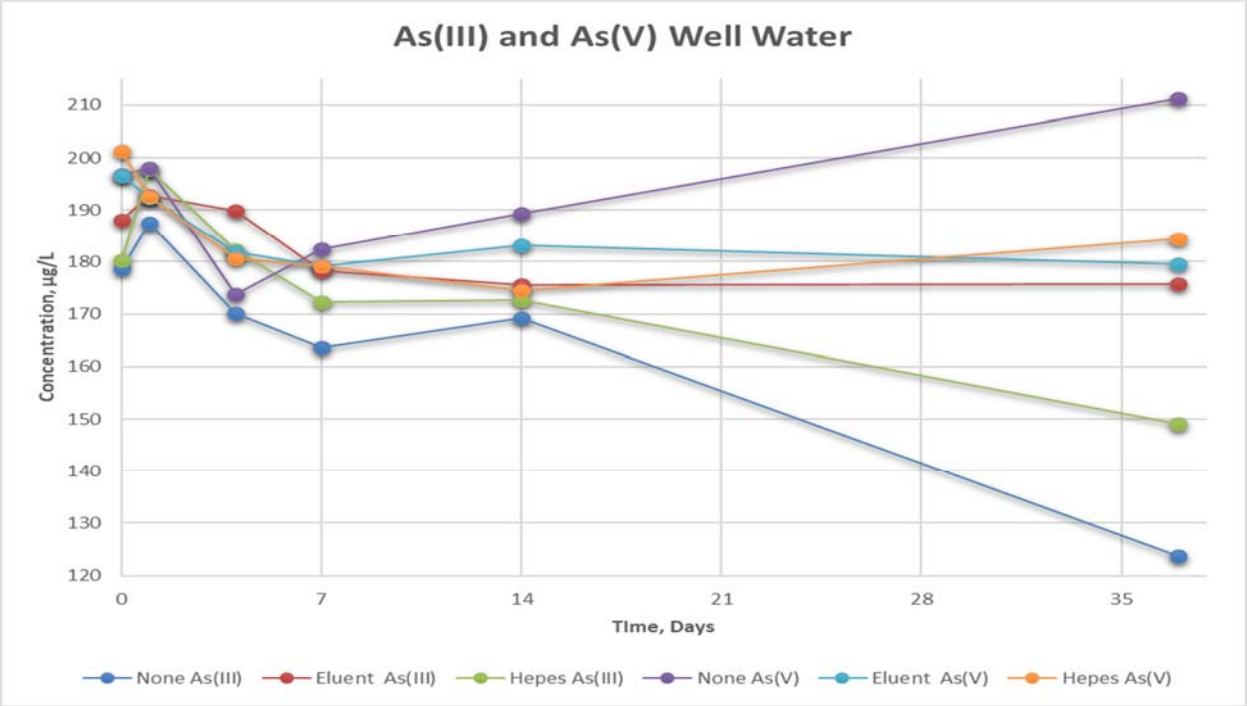


Figure 11: Graphical representation of As(III) and As(V) with preservatives in Rio Grande River water

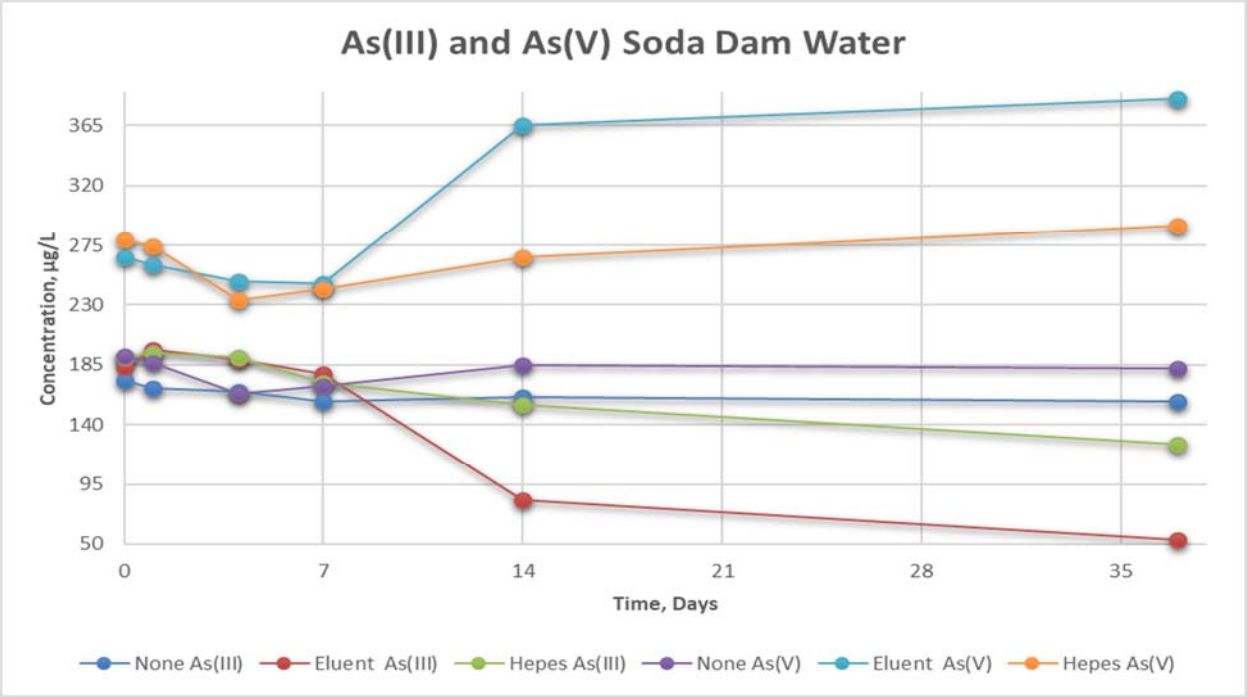


Figure 12: Graphical representation of As(III) and As(V) with preservatives in Soda Dam water of the Jemez Mountains

With the exception of 18 MΩ water and tap water, the surface and ground water samples demonstrated that interconversion of As(III) and As(V) did occur. **Table 11** outlines each of the samples with preservative and corresponds it to an observed behavior.

Table 11: Observed Behavior of As(III) and As(V) in varying water samples and preservatives

Species	Preservative	Water	Observed Behavior
As(III) and As(V)	None	18 Ω Ohm	Conversion of As(III) to As(V) up until day 14. After day 14 As(III) shifts to As(V), however it is back to As(V) to As(III) after 37 days.
As(III) and As(V)	None	Tap	Did not contain As(III). Recovery of As(V) after 37 days is 84%.
As(III) and As(V)	None	Well	Steady conversion of As(III) to As(V) after day four.
As(III) and As(V)	None	River	Steady conversion of As(III) to As(V) after day one.
As(III) and As(V)	None	Soda Dam	Stable with minimal conversion after day four.
As(III) and As(V)	Eluent	18 Ω Ohm	Conversion of As(V) to As(III) until day four, after which As(III) begins to convert to As(V).
As(III) and As(V)	Eluent	Tap	Did not contain As(III). Recovery of As(V) after 37 days is 83%.
As(III) and As(V)	Eluent	Well	Highly stable. Minimal conversion between As(III) and As(V).
As(III) and As(V)	Eluent	River	Sharp conversion of As(III) to As(V) after day 14.
As(III) and As(V)	Eluent	Soda Dam	Sharp conversion of As(III) to As(V) after day 14.
As(III) and As(V)	Hepes	18 Ω Ohm	Steady conversion of As(V) to As(III) after day one.
As(III) and As(V)	Hepes	Tap	Conversion of As(III) to As(V) after day four, however, conversion shifts back to As(V) to As(III) after 37 days.
As(III) and As(V)	Hepes	Well	Initial conversion of As(V) to As(III) until day 14. After day 14 As(III) begins to convert to As(V).
As(III) and As(V)	Hepes	River	Initial conversion of As(V) to As(III) until day 7. After day 7 As(III) begins to convert to As(V).
As(III) and As(V)	Hepes	Soda Dam	Initial conversion of As(V) to As(III) until day 7. After day 7 As(III) begins to convert to As(V).

Observed arsenic behavior corresponds to hypothesized conclusions except for the HEPES buffer. It was expected that As(III) would oxidize to As(V) over time and for a majority of situations this did occur. However, in samples with HEPES buffer, arsenic behavior was unpredictable. In 18 Ω Ohm water, As(V) steadily converted to As(III) over time. In tap water, As(III) initially converted to As(V) but after four days that conversion shifted and As(V) began to shift to As(III). In well water, As(V) converts to As(III) and then after day 14 As(III) begins to convert to As(V). In river water, As(V) converted to As(III) initially, then shifted to As(III) to As(V) conversion after day seven, which is also the same behavior displayed for As in Soda Dam water. Considering that HEPES buffer has never been used for this type of application and that more extensive research on this behavior could not be conducted, the explanation for these differences are unknown.

After day 7 analyses of Rio Grande and Soda Dam waters preserved with EDTA/TBAOH showed an interesting phenomenon. After samples were analyzed on day seven, they were left on a prep bench for one week exposed to light before being returned to the refrigerator. When samples were analyzed on day fourteen, the graphs show that there was a drop in As(III) followed by a commensurate increase in As(V). In a publication by USGS it is noted that "arsenite can be oxidized to arsenate by photolytically produced free radicals; therefore, the exposure of the sample to light also should be minimized (Garbarino et. al, 2012)."

In a paper by Zhang et. al, 1999, exposure to light can mediate metal redox reactions in the presence of iron. The long exposure to light may have increased the potential for redox reactions to occur with iron and could be explored as a potential reason there is such a dramatic shift in

As(III) and As(V) concentration for these samples, although it doesn't look as if 18 Ω Ohm or tap water was affected (Zhang et. al, 1999). A quote from a publication from USGS regarding light exposure states:

> *"The laboratory arsenic speciation method can be affected by the precipitation of metal oxides. Many suboxic or anoxic… samples having arsenic concentrations greater than the USEPA 10-µg/L drinking-water standard also can contain substantial concentrations of reduced aluminum, iron, or manganese. Oxidation of these metal species during sample collection and processing produces metal-oxide precipitates that can sorb arsenic, resulting in negatively biased data (Garbarino et. al, 2012)."*

Note that tap water, 18 MΩ, and well water had no detectable concentrations of Al, Fe or Mn, this could explain why there wasn't a significant shift in As(III) and As(V) concentrations after light exposure. However, inadvertently exposing the samples to light may have revealed that the HEPES buffer is able to resist changes in these conditions. In a paper written by Zigler et. al, 1985, HEPES exposed to light will generate hydrogen peroxide. This powerful oxidant may explain this observed behavior.

Chromium (III) and Chromium (VI)

The graphical data for 18 MΩ water, tap water, UNM well water, Rio Grande River water, and Soda Dam water from the Jemez mountains is presented in **Figures 13**, **14**, **15**, **16**, and **17**.

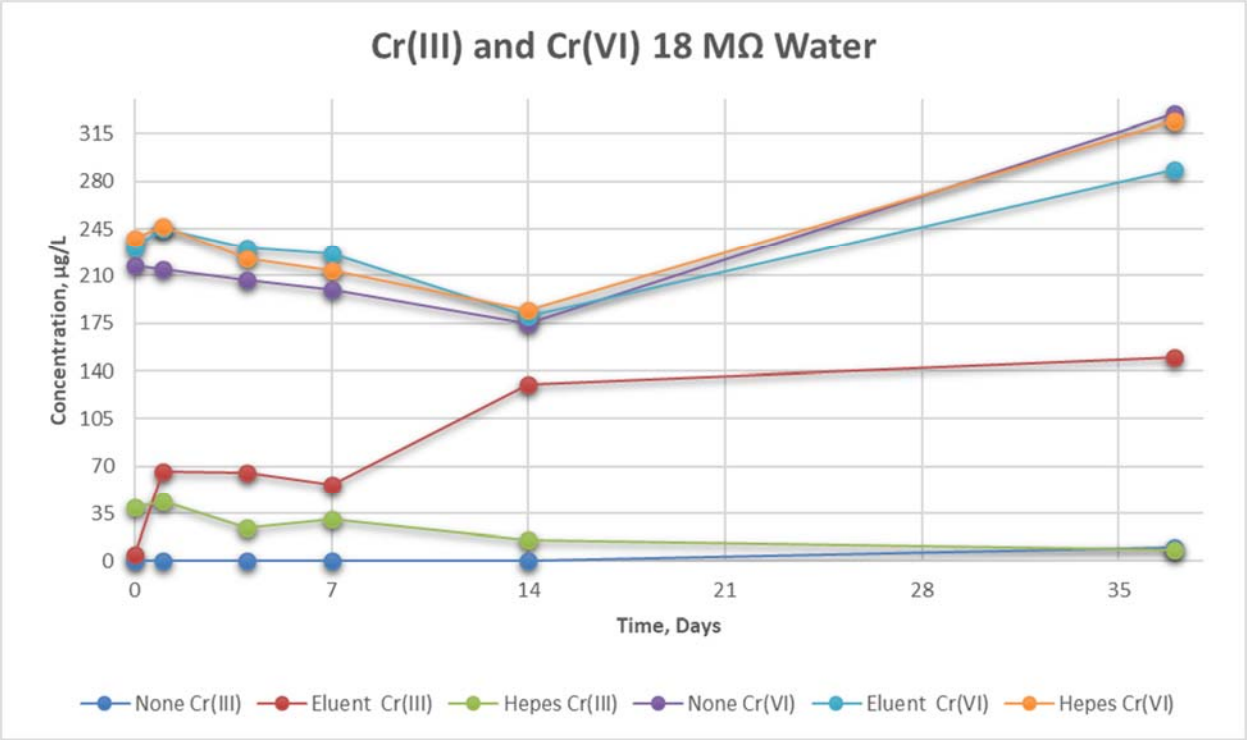


Figure 13: Graphical representation of Cr(III) and Cr(VI) with preservatives in 18 MΩ water

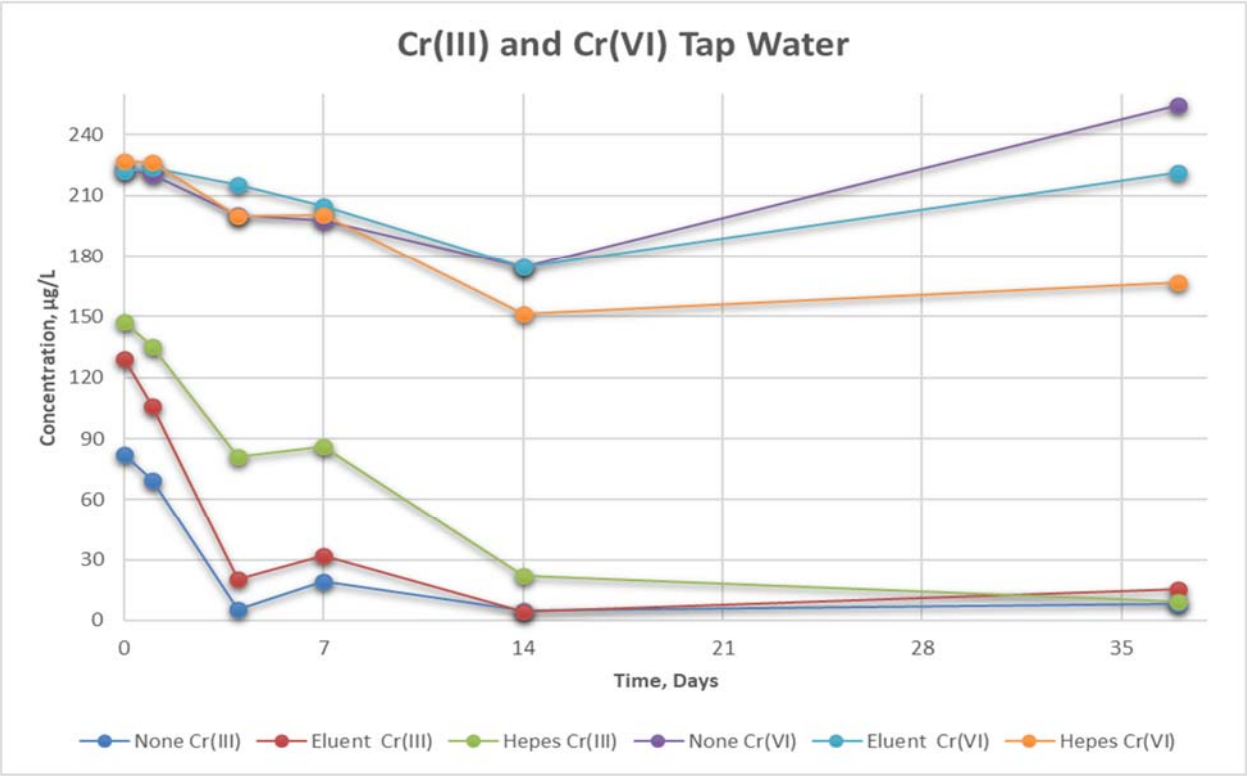


Figure 14: Graphical representation of Cr(III) and Cr(VI) with preservatives in tap water

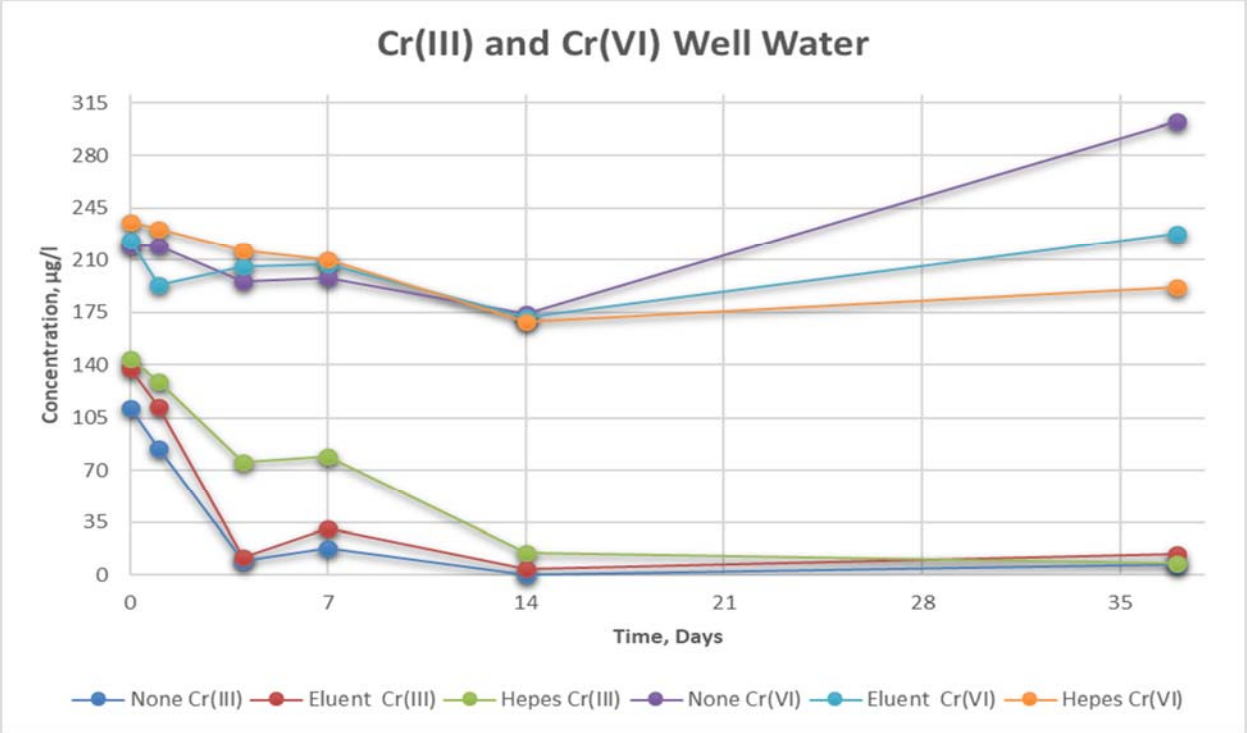


Figure 15: Graphical representation of Cr(III) and Cr(VI) with preservatives in UNM Well water

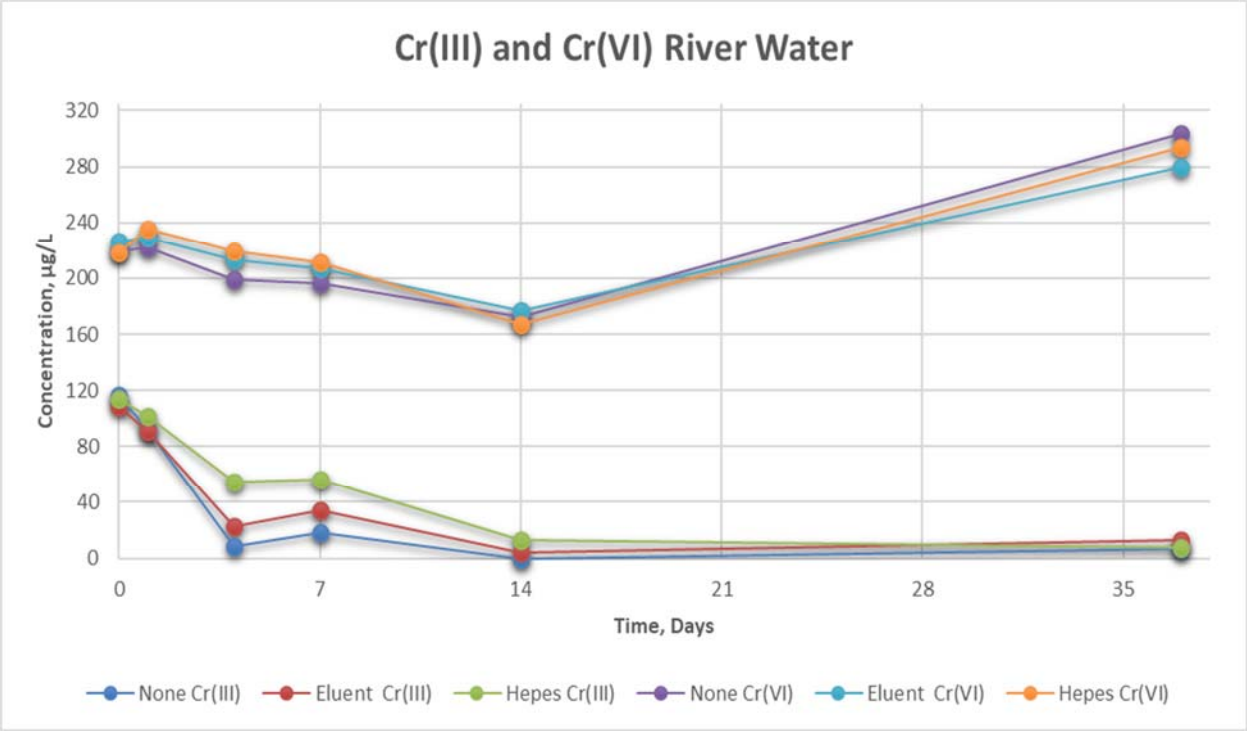


Figure 16: Graphical representation of Cr(III) and Cr(VI) with preservatives in Rio Grande River water

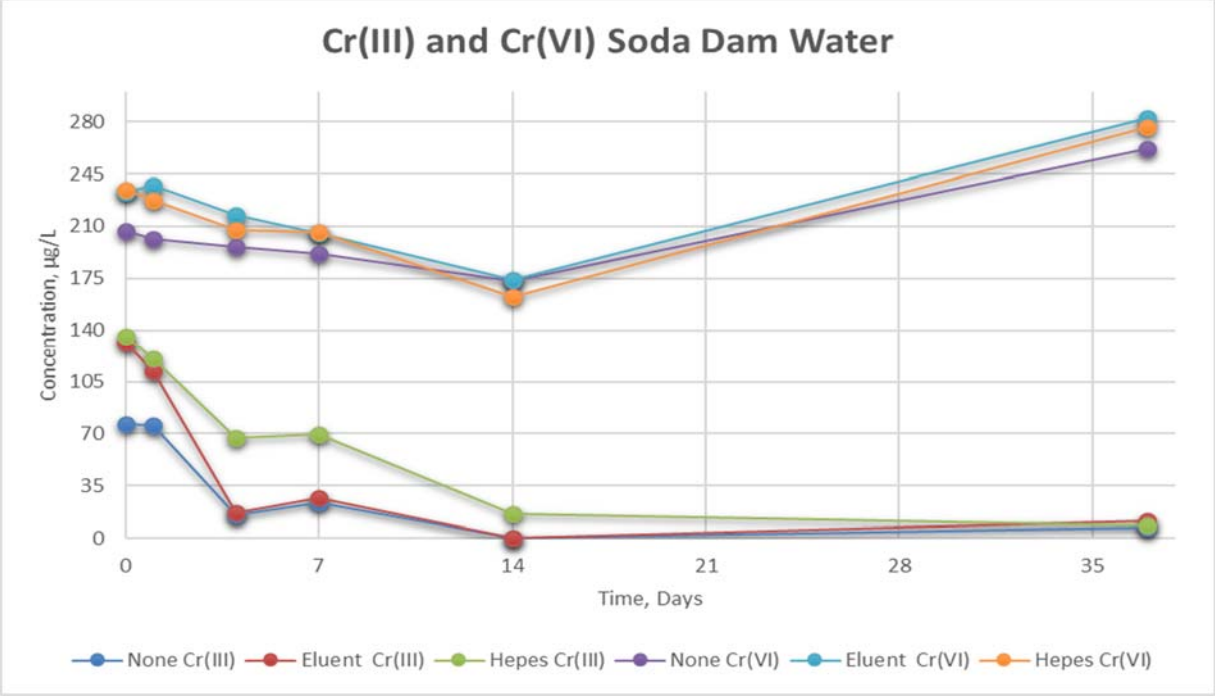


Figure 17: Graphical representation of Cr(III) and Cr(VI) with preservatives in Soda Dam water of the Jemez Mountains

Preservation of Cr species could not be achieved by any of the methods investigated in this study. For all samples tested and for all preservatives, Cr(III) converted to Cr(VI). This likely reflects the fact that Cr(VI) is the stable phase of Cr in oxygenated solutions. The observed behavior for each sample and each preservative is outlined in **Table 12**.

Table 12: Observed Behavior of Cr(III) and Cr(VI) in varying water samples and preservatives

Species	Preservative	Water	Observed Behavior
Cr(III) and Cr(VI)	None	18 Ω Ohm	Slow decrese with absence of Cr(III) until column change. After column change, Cr(VI) doubled while Cr(III) was detected when it was previously thought to be not spiked in error.
Cr(III) and Cr(VI)	None	Tap	Initial Cr(III) recovery low with a steady decline. Gradual decrease of Cr(VI) until column change which shows an increase in Cr(VI).
Cr(III) and Cr(VI)	None	Well	Initial Cr(III) recovery low with a steady decline. Gradual decrease of Cr(VI) until column change which shows approximately half of Cr(III) converted to Cr(VI).
Cr(III) and Cr(VI)	None	River	Initial Cr(III) recovery low with a steady decline. Gradual decrease of Cr(VI) until column change which shows an increase in Cr(VI).
Cr(III) and Cr(VI)	None	Soda Dam	Initial Cr(III) recovery low with a steady decline. Gradual decrease of Cr(VI) until column change which shows an increase in Cr(VI).
Cr(III) and Cr(VI)	Eluent	18 Ω Ohm	Cr(III) began to slowly increase over time, while Cr(VI) slighly decreased. Cr(VI) increased again after column replacement.
Cr(III) and Cr(VI)	Eluent	Tap	Initial Cr(III) recovery low with a steady decline. Gradual decrease of Cr(VI) until column change which shows an increase in Cr(VI).
Cr(III) and Cr(VI)	Eluent	Well	Initial Cr(III) recovery low with a steady decline. Gradual decrease of Cr(VI) until column change which shows approximately half of Cr(III) converted to Cr(VI).
Cr(III) and Cr(VI)	Eluent	River	Initial Cr(III) recovery low with a steady decline. Gradual decrease of Cr(VI) until column change which shows an increase in Cr(VI).
Cr(III) and Cr(VI)	Eluent	Soda Dam	Initial Cr(III) recovery low with a steady decline. Gradual decrease of Cr(VI) until column change which shows an increase in Cr(VI).
Cr(III) and Cr(VI)	Hepes	18 Ω Ohm	Initial Cr(III) recovery low with a steady decline. Gradual increase of Cr(VI), with a significant increase after column change.
Cr(III) and Cr(VI)	Hepes	Tap	Initial low recovery of Cr(III) at time zero but highest of the three preservatives with a gradual decline over time. Cr(VI) gradual decline with increase after column change.
Cr(III) and Cr(VI)	Hepes	Well	Initial Cr(III) recovery low with a steady decline. Gradual decrease of Cr(VI) until column change which shows approximately half of Cr(III) converted to Cr(VI).
Cr(III) and Cr(VI)	Hepes	River	Initial Cr(III) recovery low with a steady decline. Gradual decrease of Cr(VI) until column change which shows an increase in Cr(VI).
Cr(III) and Cr(VI)	Hepes	Soda Dam	Initial Cr(III) recovery low with a steady decline. Gradual decrease of Cr(VI) until column change which shows an increase in Cr(VI).

The initial recovery of Cr(III), at time zero, in many samples was 75%. This indicates that Cr(III) may not have been fully recovered at the time of instrument analysis. In a publication by Perkin Elmer, the instrument manufacturer, it is recommended that mobile phase used for optimum Cr(III) and Cr(VI) separation be comprised of 0.6 mM EDTA and 1mM TBAOH (Neubauer et. al, 2003). The mobile phase used for this analysis only consisted of 0.15 mM EDTA and 0.1 mM TBAOH which is 4 times less than the optimal EDTA recommended and 10 times less than the optimal TBAOH recommended. The reason the concentration of the mobile phase is so important is because Cr(III) creates a complex with the EDTA which remains on the ion exchange column used for this analysis (Neubauer et. al, 2003). This complex pairs with the dichromate complex of Cr(VI), where the positive hydrocarbons of the TBAOH interact with the

chromium species and separate them during analysis (Neubauer et. al, 2003). It can be theorized that if there is not enough EDTA to form complexes with the amount of Cr(III) in the sample and not enough TBAOH in the eluent mix to separate all negatively charged species, then a full separation of Cr(III) and Cr(VI) may not occur as there is not enough reagent to fully compensate. This may explain why Cr(III) recovery was lower than expected and continued to be low throughout the analysis.

The redox chemistry of Cr can be summarized in a pe-pH diagram (also known as an Eh-pH diagram). This shows that under oxidizing conditions Cr(VI) is stable whereas under reducing conditions Cr(III) is the stable oxidation state. Further, above pH 5, Cr(III) forms an insoluble precipitate as $Cr_2O_{3(s)}$.

Consequently, research into the solubility of Cr(III) species in water at a pH of seven revealed that Cr(III) is soluble and the low recovery of Cr(III) at this pH may have been due to dissolved $Cr(OH)_3(s)$ species. Converting the concentration of Cr(III) at 100 µg/L to a molar concentration, calculates to a molar concentration of 1x10-5. In **Figure 18** an Eh-pH diagram is provided for chromium.

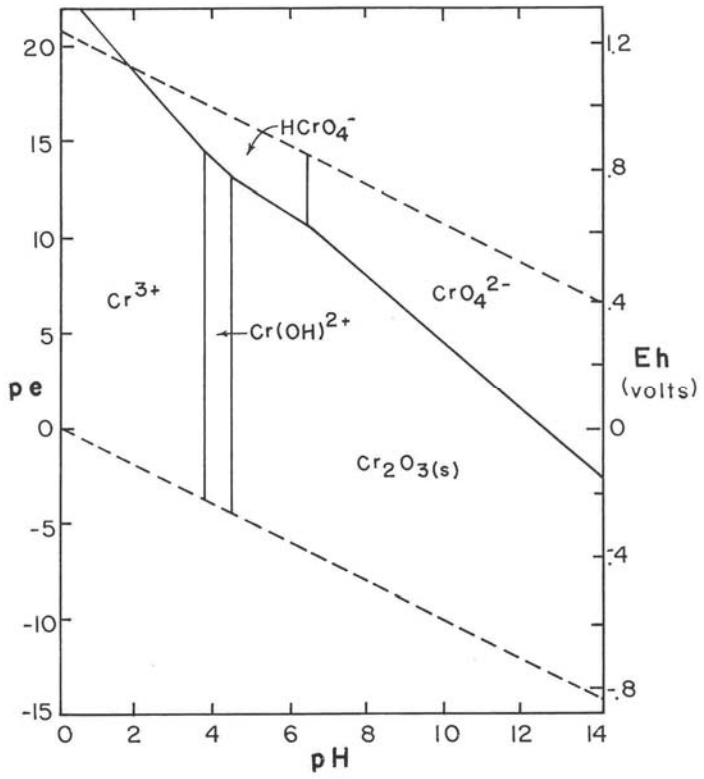


Figure 18: Eh-pH diagram for Chromium at varying pH and concentration. (Source, Thomson, 2019)

Inadequate chromium separation and the precipitation of $Cr(OH)_3(s)$ could account for the loss of Cr(III) over the analysis period. This can be supported by the fact that a few samples, initially thought to contain no Cr(III) at all due to spiking errors, suddenly had small recoveries of Cr(III) after the column was changed. In all samples that contained no preservative Cr(III) was either

not detected or had very low concentrations by day 14. However, at day 37, when the analytical column was replaced, Cr(III) concentrations became detectable.

In addition to these observed phenomena, it is speculated that for the well, river, and soda dam samples conversion of Cr(III) to Cr(VI) occurred. This is consistent with the redox chemistry summarized in **Figure 18**. However, Cr(VI) to Cr(III) readily occurs under acidic conditions and all the samples tested were not acidic. In neutral pH situations the proportions of Cr(III) and Cr(VI) are dependent on the level of oxygen present in the sample and there are several oxidants that can push Cr(III) to oxidize to Cr(VI), although MnO_2 is the only one at sufficient levels to do so (Barałkiewicz et. al, 2013). Manganese was not detected in samples, however, a test for total manganese was not conducted. Therefore, manganese cannot be completely ruled out as a means of explaining why conversion of Cr(III) to Cr(VI) was observed.

Taking into consideration all observations, it is more feasible that Cr(III) was precipitated as $Cr(OH)_3(s)$ and dissolved. This would account for the absence of Cr(III) recovery in preserved samples.

Selenium (IV) and Selenium (VI)

The graphical data for 18 MΩ water, tap water, UNM well water, Rio Grande River water, and Soda Dam water from the Jemez mountains is presented in **Figures 19**, **20**, **21**, **22**, and **23**.

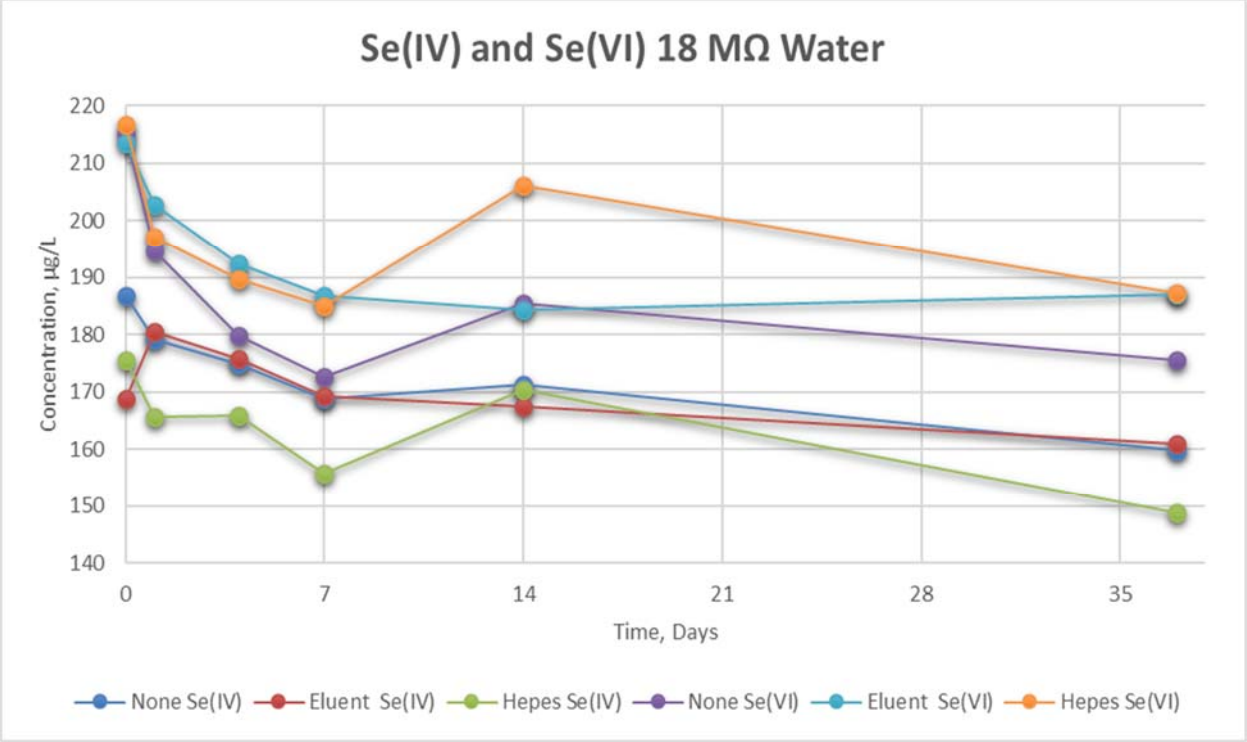


Figure 19: Graphical representation of Se(IV) and Se(VI) with preservatives in 18 Ω Ohm water

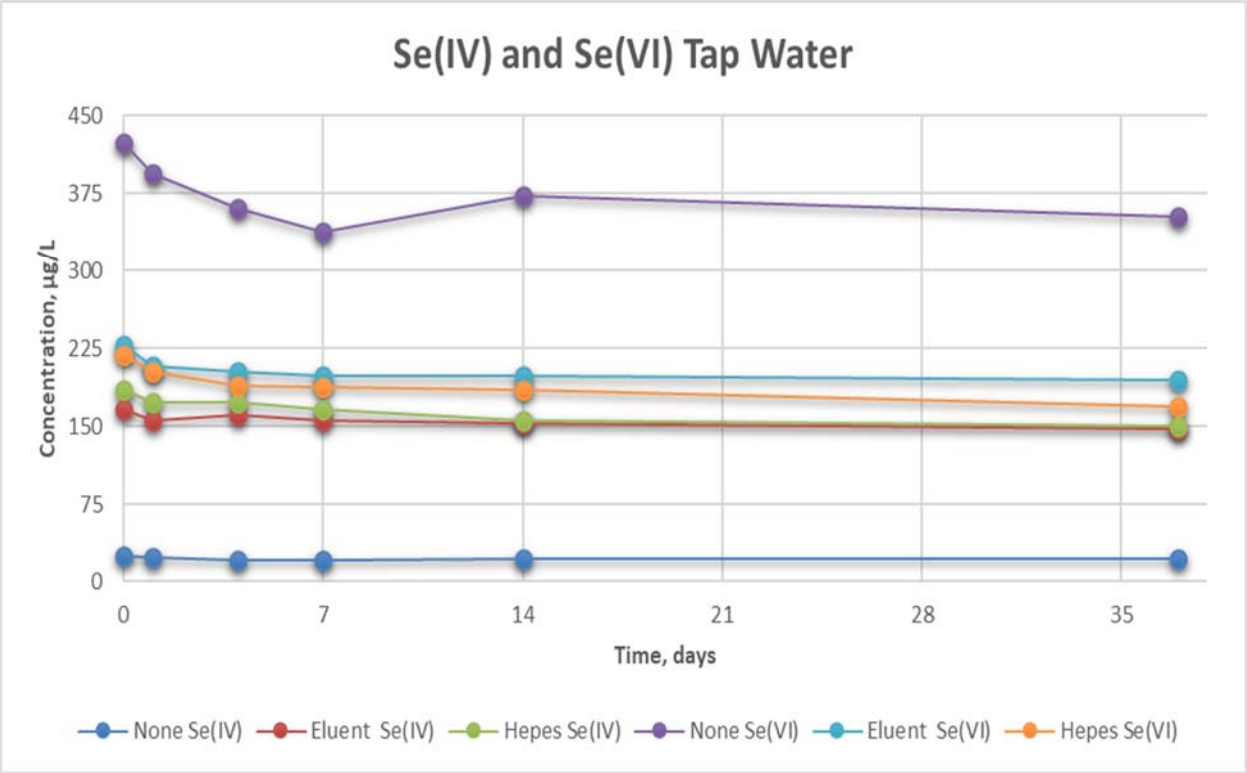


Figure 20: Graphical representation of Se(IV) and Se(VI) with preservatives in tap water

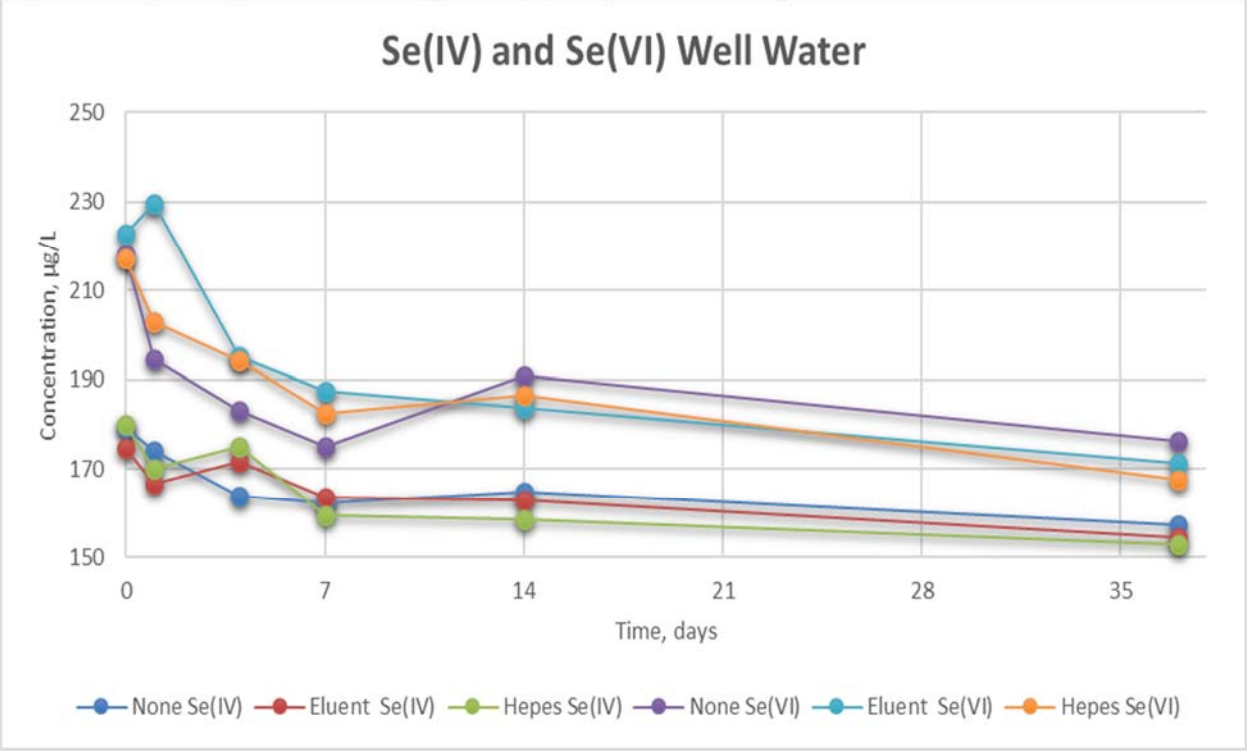


Figure 21: Graphical representation of Se(IV) and Se(VI) with preservatives in UNM Well water

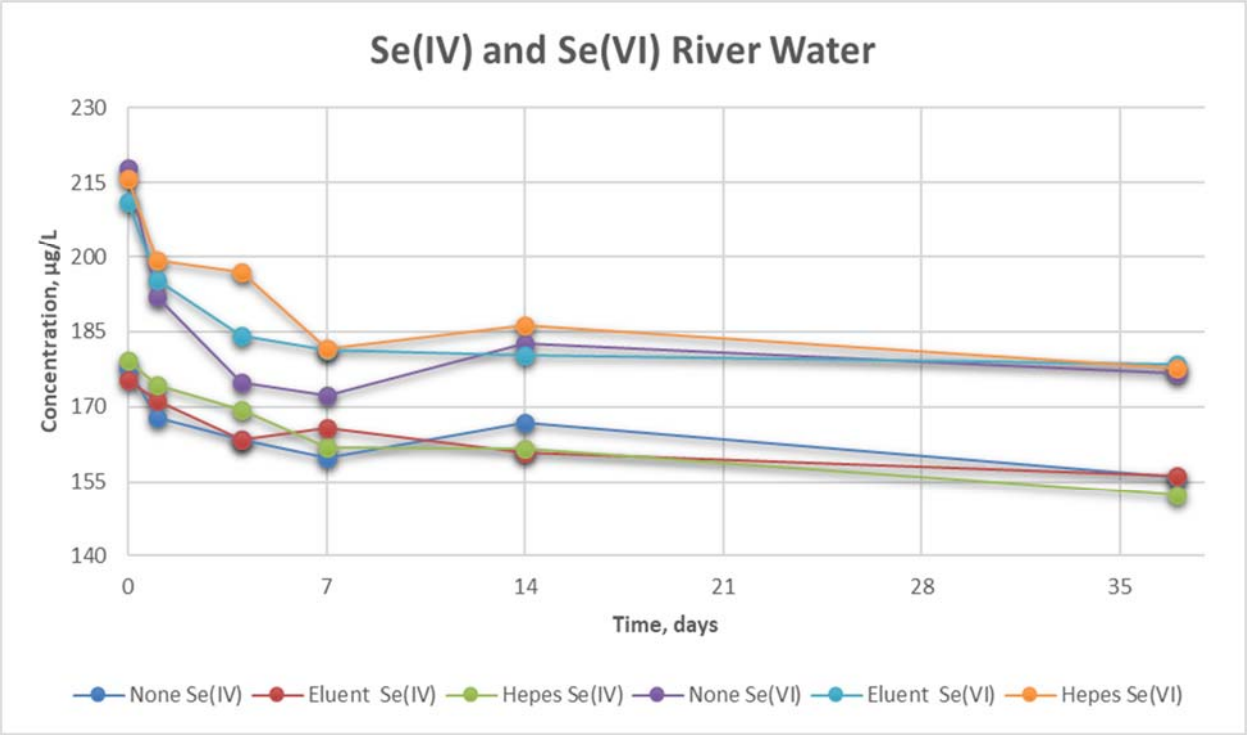


Figure 22: Graphical representation of Se(IV) and Se(VI) with preservatives in Rio Grande River water

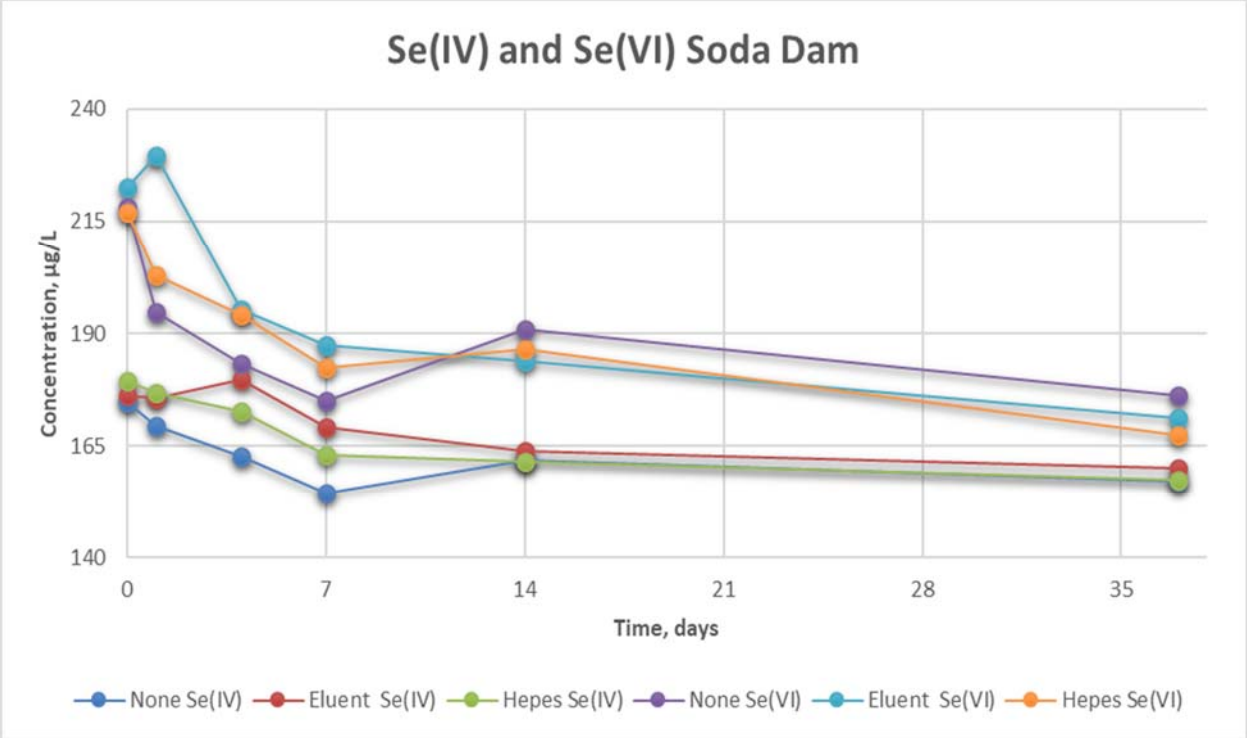


Figure 23: Graphical representation of Se(IV) and Se(VI) with preservatives in Soda Dam water of the Jemez Mountains

Selenium was the most stable of all the species and did not exhibit evidence of any redox reactions affecting the concentrations of Se(IV) or Se(VI). Both Se(IV) and Se(VI) displayed a slow gradual decline over the course of the examination period. Observed behavior for each of the samples and their preservatives are described in **Table 13**.

Table 13: Observed Behavior of Se(IV) and Se(VI) in varying water samples and preservatives

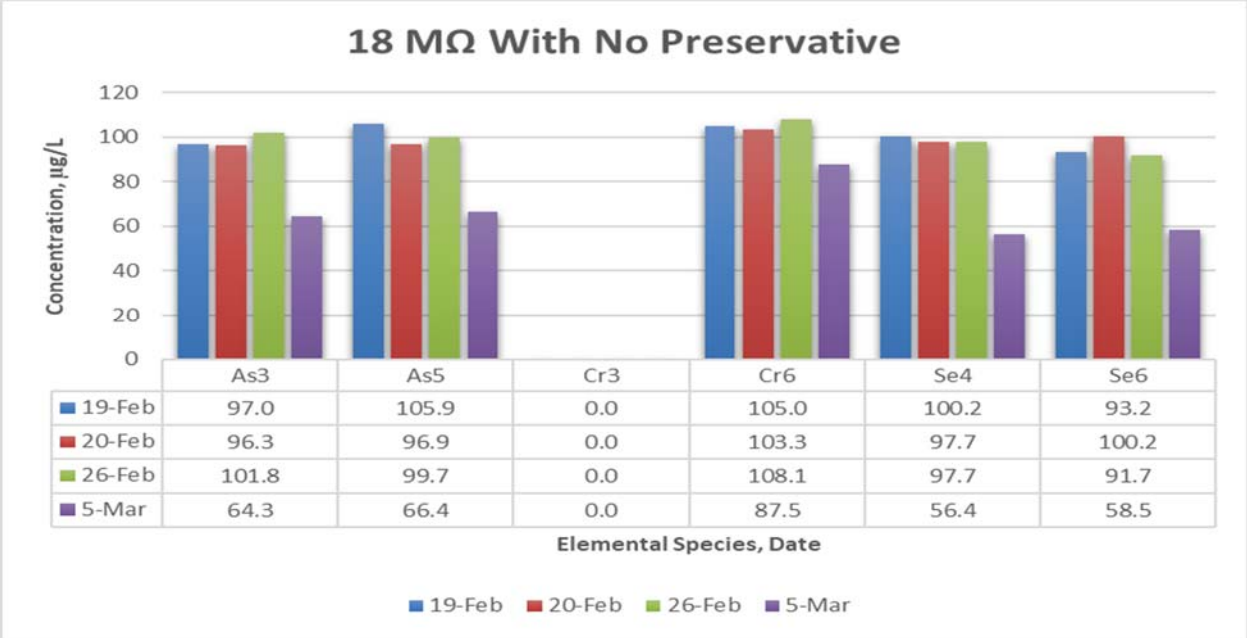
Species	Preservative	Water	Observed Behavior
Se(IV) and Se(VI)	None	18 Ω Ohm	Gradual decrease in both species with a small increase at day 14, followed by a consistent decline.
Se(IV) and Se(VI)	None	Tap	Did not contain spike for Se(IV). Se(VI) saw an increase at day fourteen, but declined at day 37.
Se(IV) and Se(VI)	None	Well	Gradual decrease in both species with a small increase at day 14, followed by a consistent decline.
Se(IV) and Se(VI)	None	River	Slow steady decline for both elements with no dramatic shifting.
Se(IV) and Se(VI)	None	Soda Dam	Gradual decrease in both species with a small increase at day 14, followed by a consistent decline.
Se(IV) and Se(VI)	Eluent	18 Ω Ohm	Slow steady decline for both elements with no dramatic shifting.
Se(IV) and Se(VI)	Eluent	Tap	Slow steady decline for both elements with no dramatic shifting.
Se(IV) and Se(VI)	Eluent	Well	Gradual decline for both elements with no dramatic shifting.
Se(IV) and Se(VI)	Eluent	River	Gradual decline for both elements with no dramatic shifting.
Se(IV) and Se(VI)	Eluent	Soda Dam	Gradual decline for both elements with no dramatic shifting.
Se(IV) and Se(VI)	Hepes	18 Ω Ohm	Gradual decrease in both species with a small increase at day 14, followed by a consistent decline.
Se(IV) and Se(VI)	Hepes	Tap	Slow steady decline for both elements with no dramatic shifting.
Se(IV) and Se(VI)	Hepes	Well	Slow steady decline for both elements with no dramatic shifting.
Se(IV) and Se(VI)	Hepes	River	Slow steady decline for both elements with no dramatic shifting.
Se(IV) and Se(VI)	Hepes	Soda Dam	Slow steady decline for both elements with no dramatic shifting.

Experiment 3 Data

Incorporating the lessons learned from experiment two, the data from experiment three is much more consistent. Observed behavior in experiment two revealed that what was initially thought to be spiking errors was not an error at all, as Cr(III) was not detected in 18 MΩ water without preservative and As(III) was not detected in tap water without preservative. Subsequently, spiked and preserved samples did show some recovery in 18 MΩ water and tap water. This observation is important as it reveals that even though the chemistry may not be completely understood, the preservation method prevented redox reactions from occurring. Data is presented in this section per preservation type to better understand how the preservation itself affected species recovery and analysis.

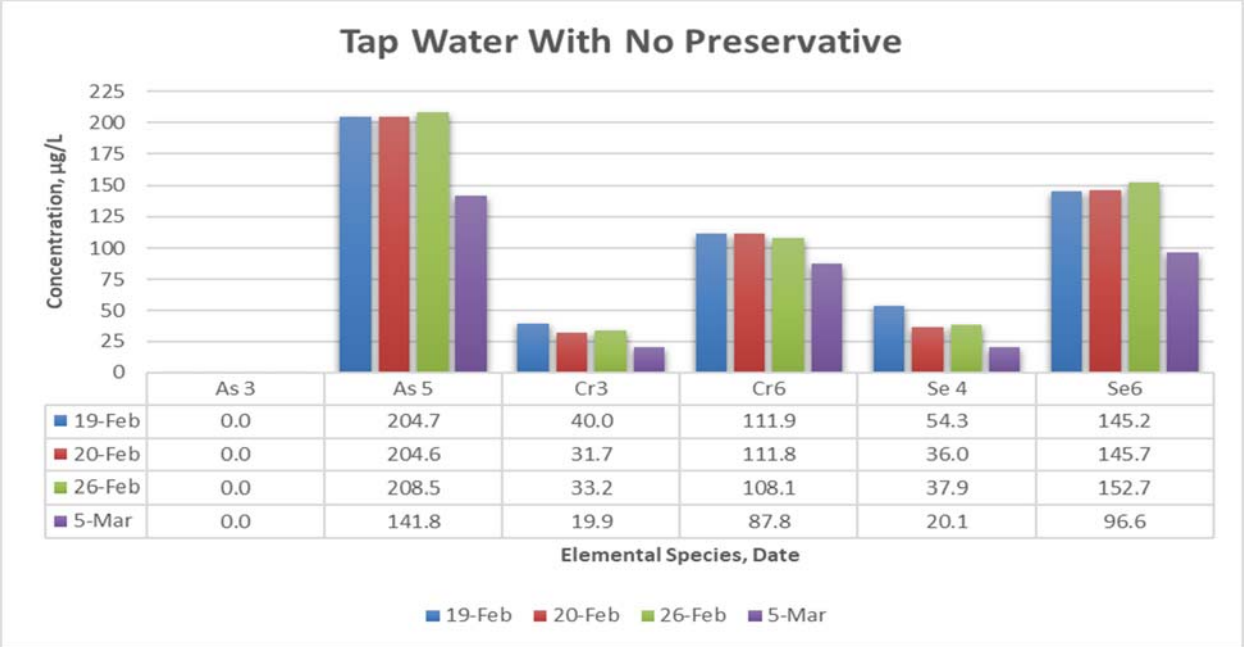
Samples with No Preservative

Data presented in this section operates as a control as these are graphs that demonstrate the recovery of each elemental species per sample with no preservation used. This is the recovery of all species spiked at the 100 ug/L over the course of 14 days as outlined in the sample protocol and serves as a baseline to draw conclusions. Preservation data for each of the four samples is presented in **Figures 24, 25, 26, and 27**.



	As3	As5	Cr3	Cr6	Se4	Se6
19-Feb	97.0	105.9	0.0	105.0	100.2	93.2
20-Feb	96.3	96.9	0.0	103.3	97.7	100.2
26-Feb	101.8	99.7	0.0	108.1	97.7	91.7
5-Mar	64.3	66.4	0.0	87.5	56.4	58.5

Figure 24: Graphical representation of species recovery in 18 MΩ water with no preservative.



	As 3	As 5	Cr3	Cr6	Se 4	Se6
19-Feb	0.0	204.7	40.0	111.9	54.3	145.2
20-Feb	0.0	204.6	31.7	111.8	36.0	145.7
26-Feb	0.0	208.5	33.2	108.1	37.9	152.7
5-Mar	0.0	141.8	19.9	87.8	20.1	96.6

Figure 25: Graphical representation of species recovery in tap water with no preservative.

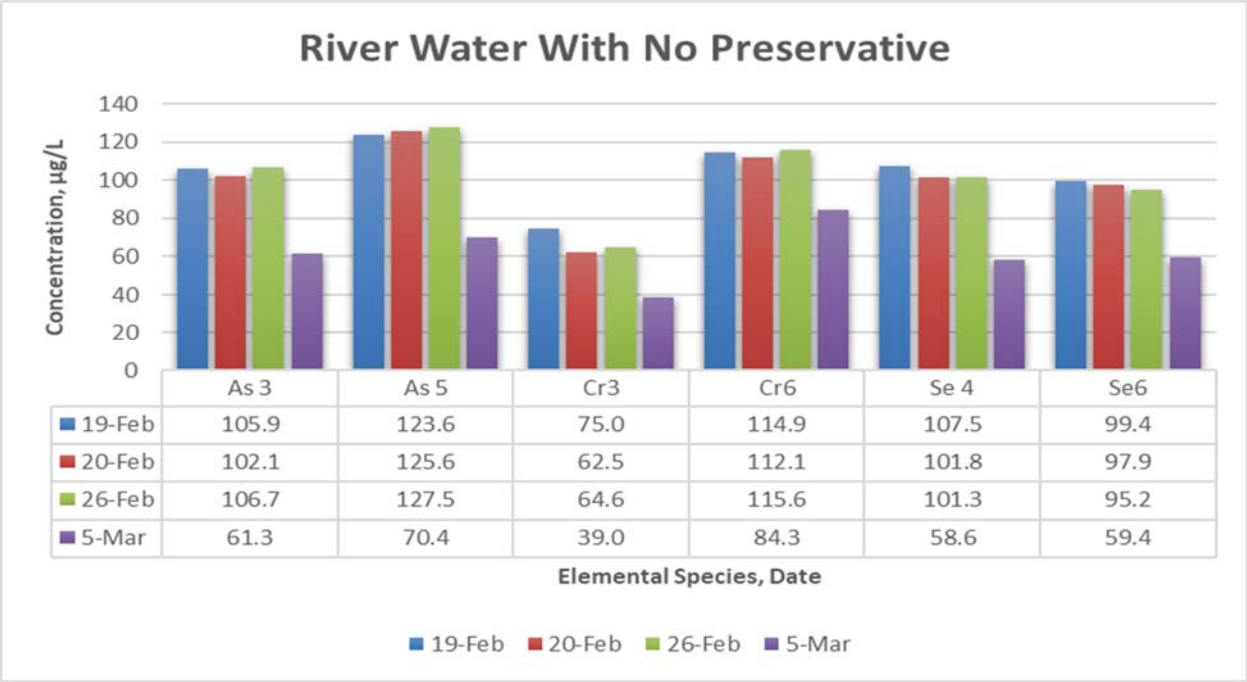


Figure 26: Graphical representation of species recovery in river water with no preservative.

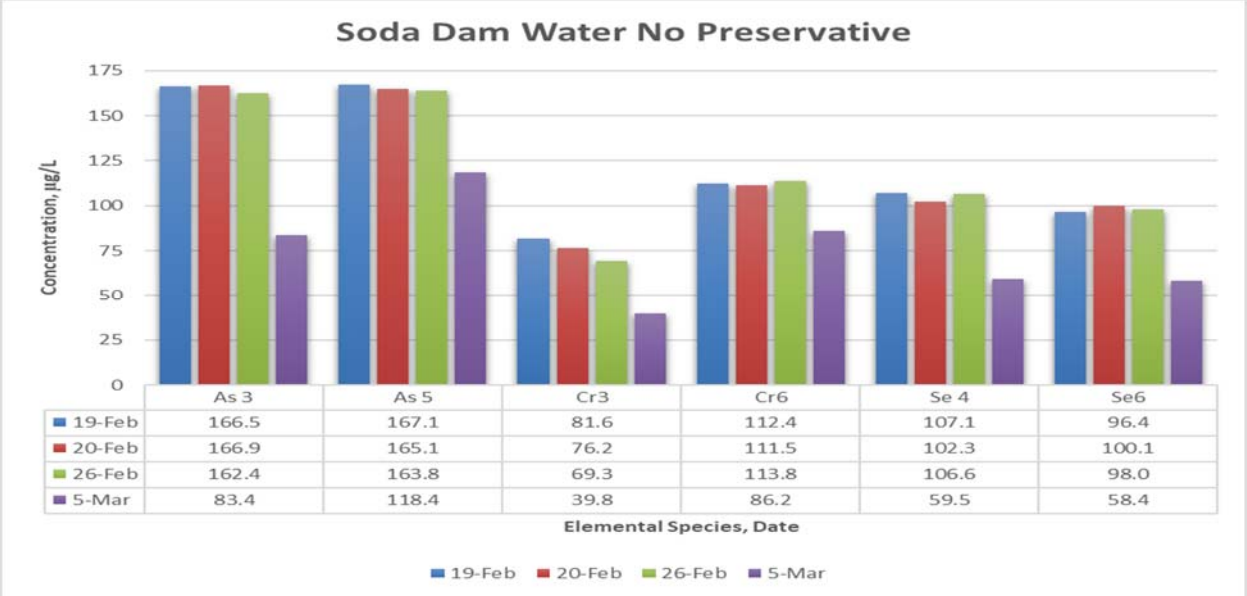


Figure 27: Graphical representation of species recovery in Soda Dam water with no preservative.

As can be seen by the graphs, each of the four samples provides information about its matrix chemistry and how it affects species stability. In 18 MΩ water all species exhibited expected behavior except for Cr(III). Hexavalent Cr recovery was not double the initial spike amount, therefore, it has been concluded that no inter species conversion occurred, however, referencing the solubility table for Cr(III) at a pH of 7 reveals that Cr(III) is soluble at this pH, which verifies why Cr(III) recovery did not occur, and the expected behavior of Cr(VI) was not observed

(Barber, 2000). Not including a preservative allowed wide variation in pH, as the pH of the 18 MΩ water was measured at 5.07, the pH of tap water was 7.73, the pH of Soda Dam was 6.88, and the pH of the river was 8.33. An additional unexpected phenomenon was the complete conversion of As(III) to As(V) which was shown by the recovery of As(V) in the sample. Data presented in the graph shows that As(V) concentrations were double that of what it should have been and As(III) was not recovered.

Samples Preserved with HEPES

The following graphs in **Figures 28, 29, 30,** and **31** display the recovery of species with samples that are preserved with the HEPES preservative. The pH of the samples after the HEPES preservative was added is as follows: 18 MΩ water pH was 7.20, tap water pH was 7.24, river water pH was 7.23, and Soda Dam water pH was 7.19.

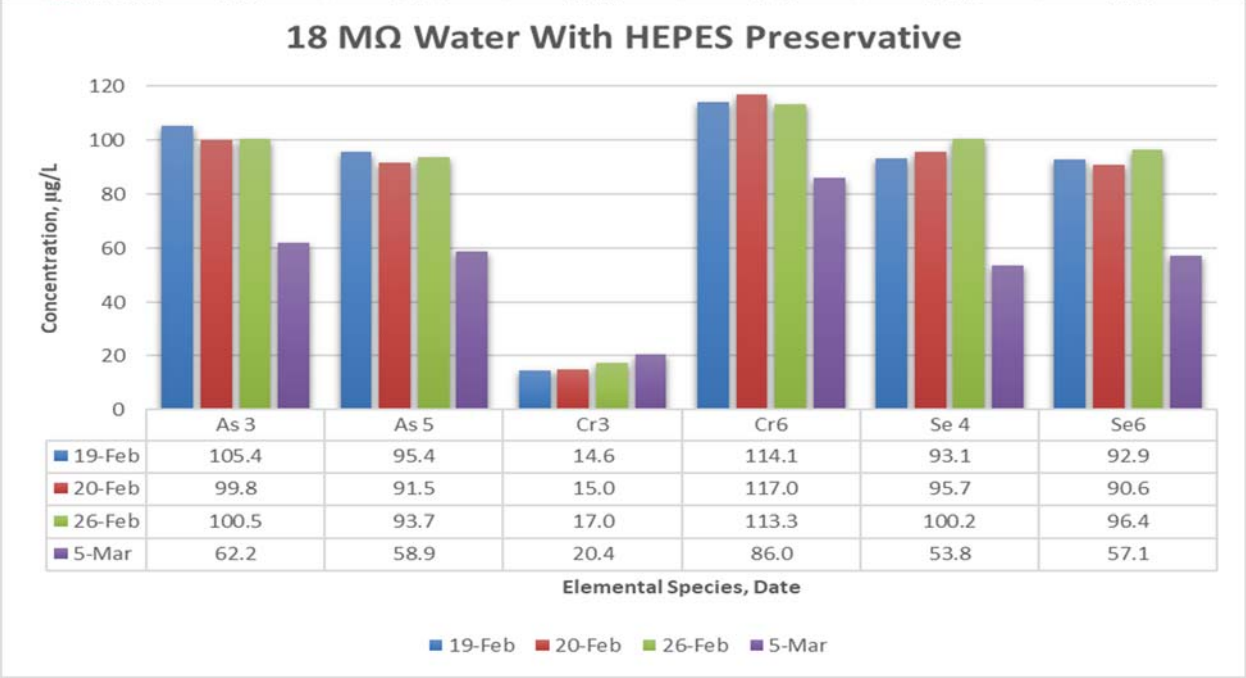


Figure 28: Graphical representation of species recovery in 18 MΩ water with HEPES preservative.

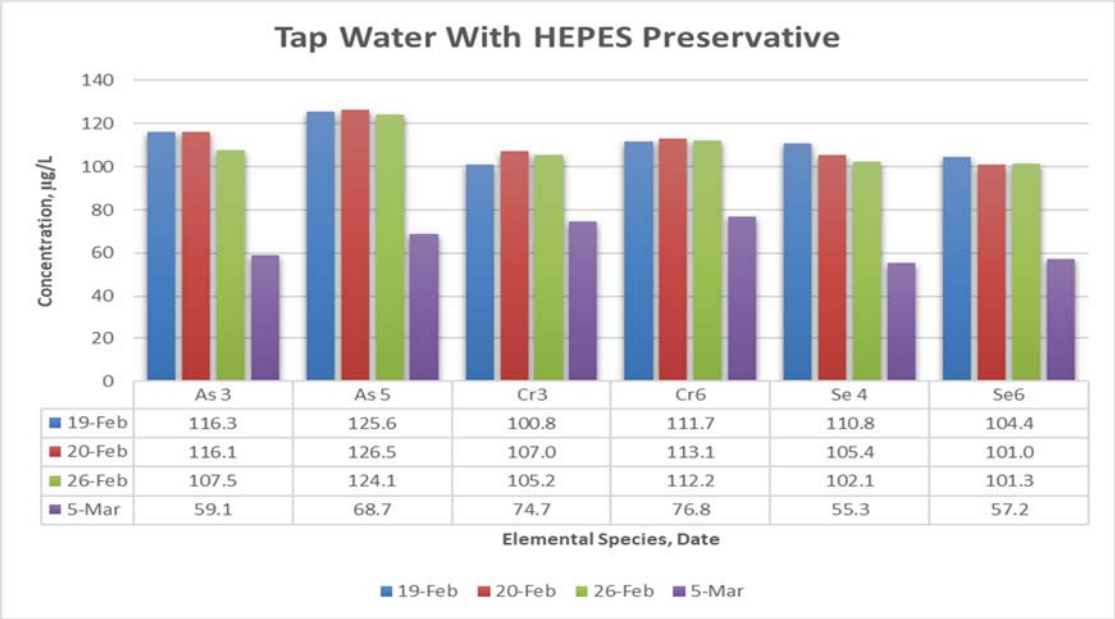


Figure 29: Graphical representation of species recovery in tap water with HEPES preservative.

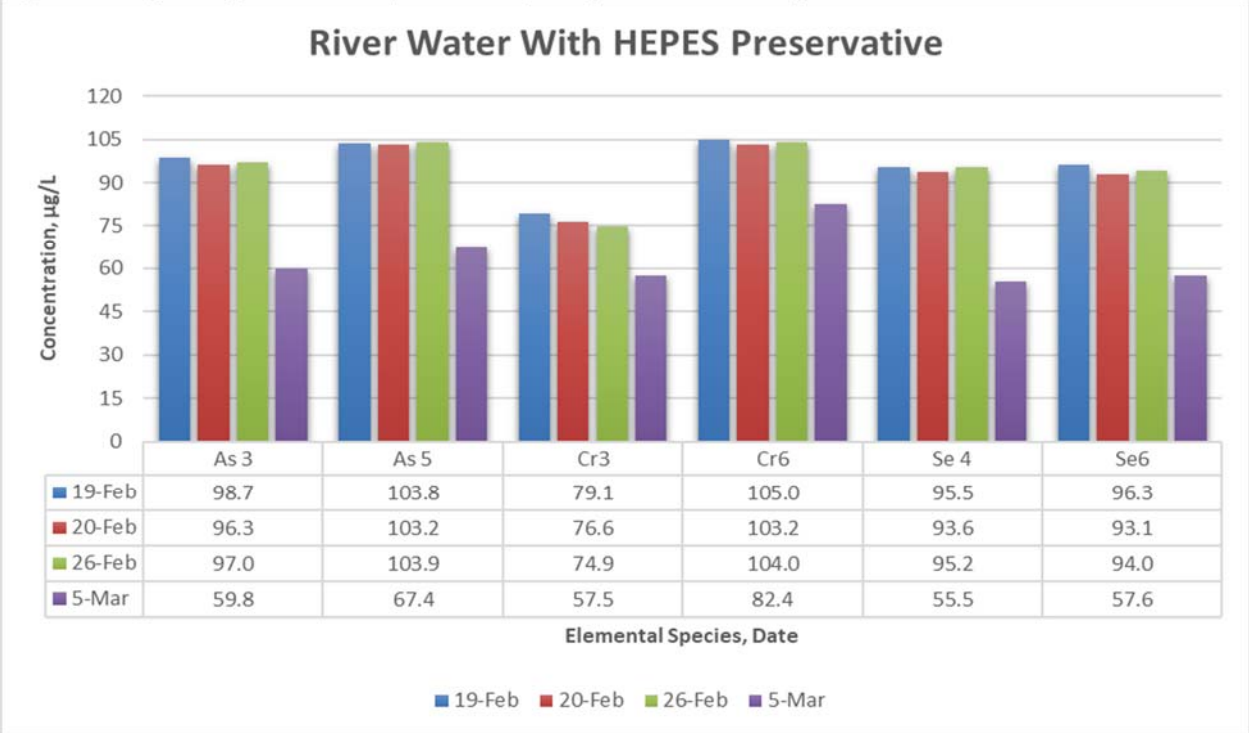


Figure 30: Graphical representation of species recovery in river water with HEPES preservative.

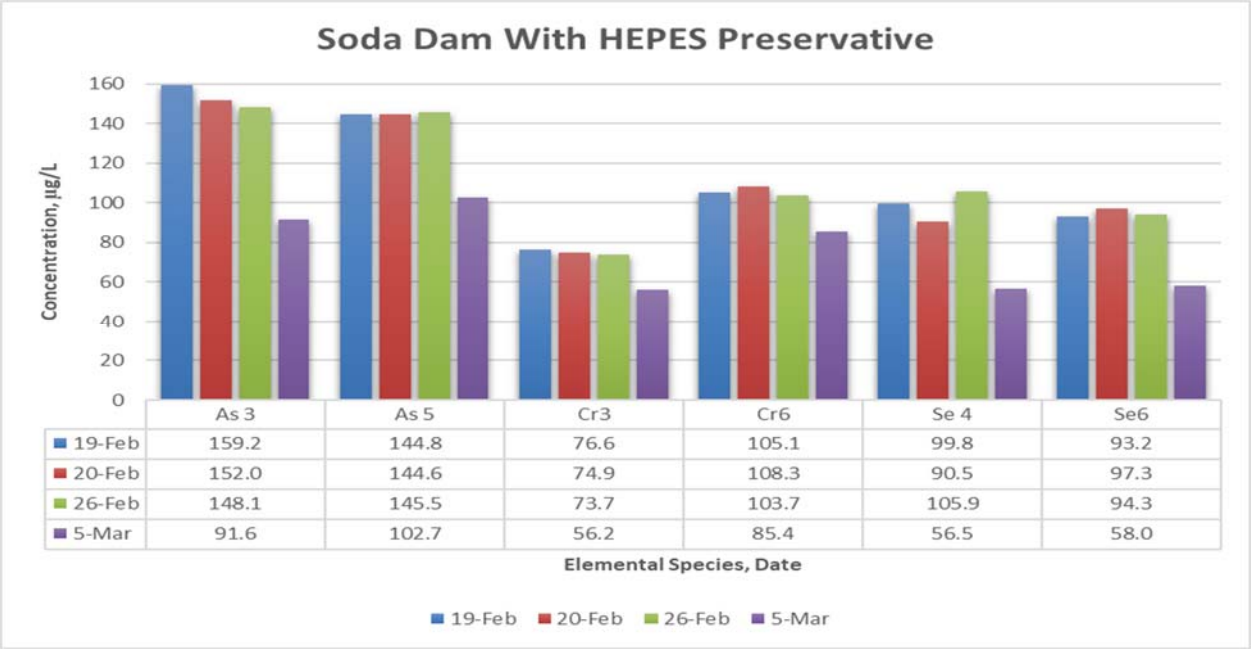


Figure 31: Graphical representation of species recovery in Soda Dam water with HEPES preservative.

Deionized water spiked with Cr(III) in 18 MΩ water, increased over time as was shown in the previous experiments. At the end of the two-week analysis the pH was tested on each of the samples with the 18 MΩ water being at 7.07. The pH for tap water was 7.24, the pH for river water was 7.15 and the pH for Soda Dam water was 7.15. Although observationally small, the pH of the mega ohm water shifted from 7.24 initially to 7.07 which reflects the absence of buffering capacity (i.e. alkalinity) in deionized water. Tap water, river water, and Soda Dam water did not show this significant of a pH change where the difference between the three is 0, 0.08, and 0.03 respectively. This suggests that although Cr(III) was not detected on the instrument where no preservative was used, Cr(III) was indeed present as some other elemental species. The addition of the HEPES buffer and a change in pH over time may have been the driving factor for elemental species change, thus allowing it to be detected on the HPLC-ICP-MS. As was also seen in tap water, the addition of the HEPES buffer prevented the conversion of As(III) to As(V) and produced acceptable recovery. The chemical explanation is unknown.

Samples Preserved with EDTA/TBAOH (Eluent)

The graphs presented in **Figures 32, 33, 34,** and **35** represent the data for samples preserved with the EDTA/TBAOH preservative. The pH of samples after the addition of HEPES preservative is as follows: 18 MΩ water 6.18, tap water 7.57, river water 7.72 and Soda Dam water 6.65. At the end of the two-week cycle the pH of 18 MΩ water was 4.79, tap water was 7.47, river water was 7.72, and Soda Dam water was 7.36.

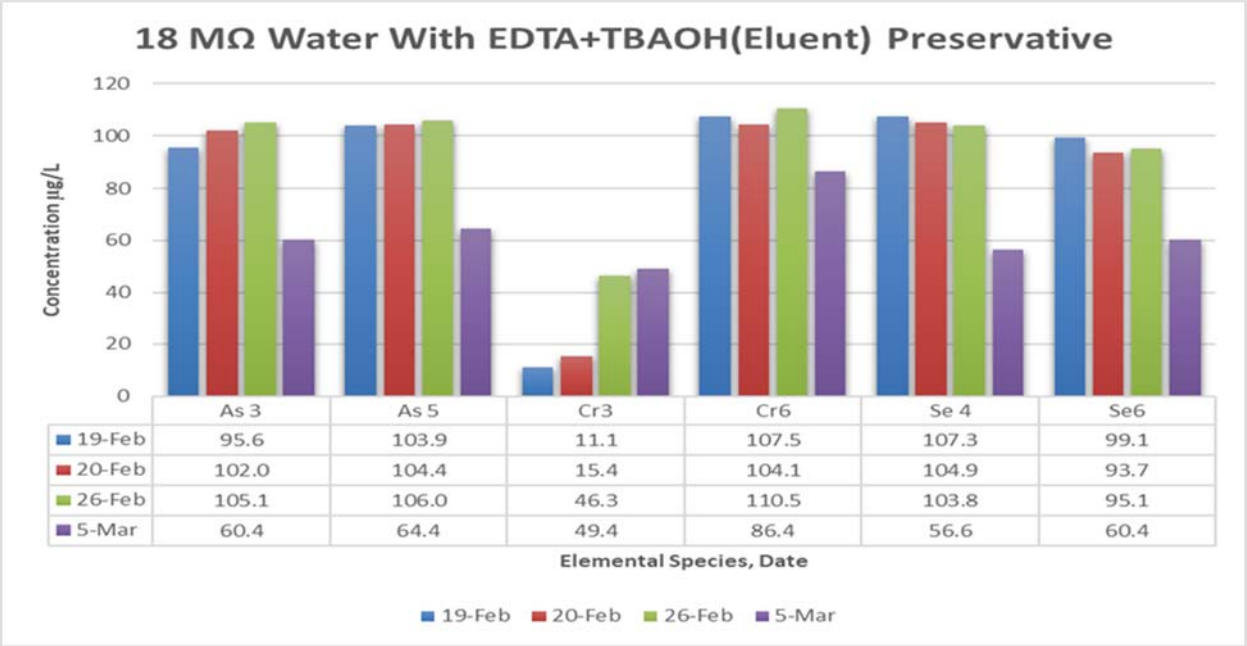


Figure 32: Graphical representation of species recovery in 18 MΩ water with EDTA/TBAOH preservative.

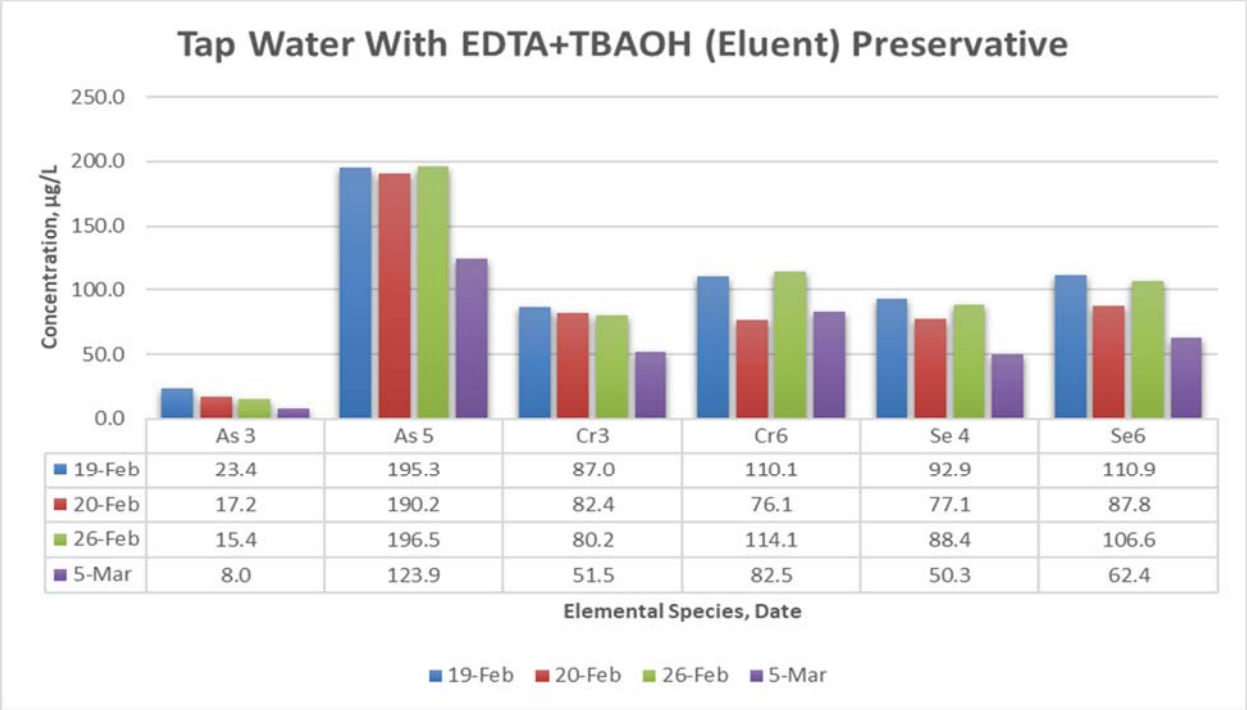


Figure 33: Graphical representation of species recovery in tap water with EDTA/TBAOH preservative.

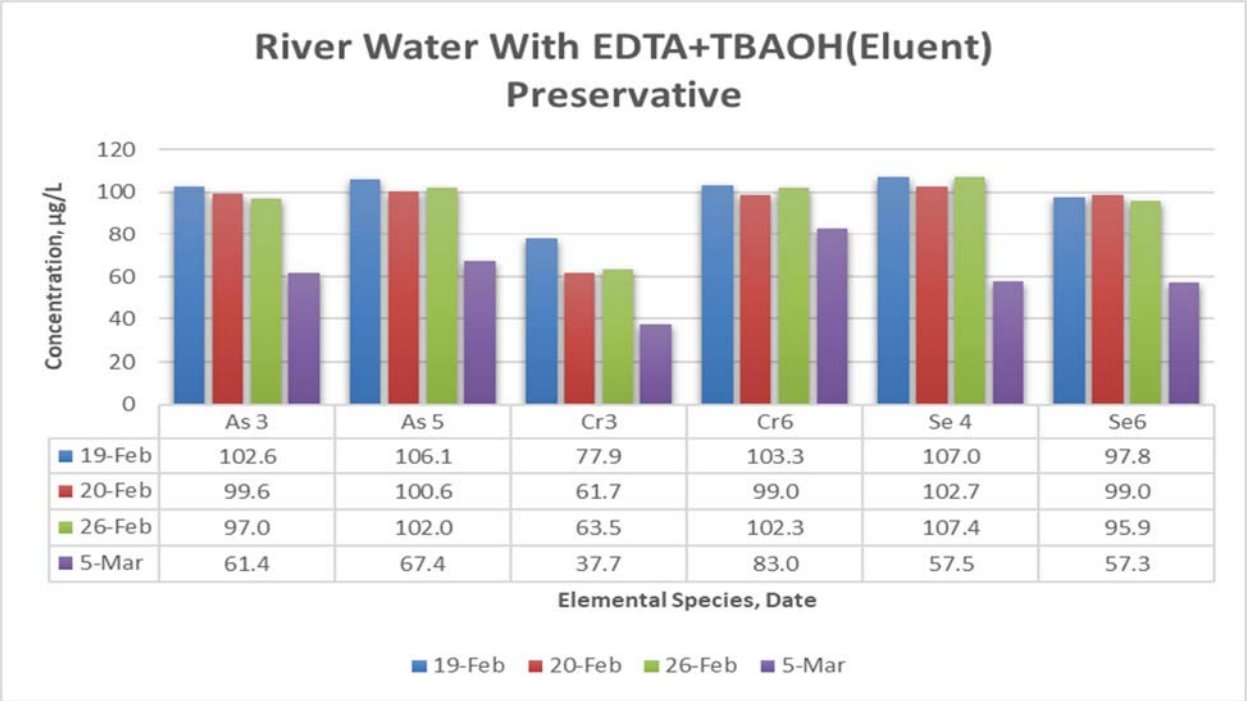


Figure 34: Graphical representation of species recovery in river water with EDTA/TBAOH preservative.

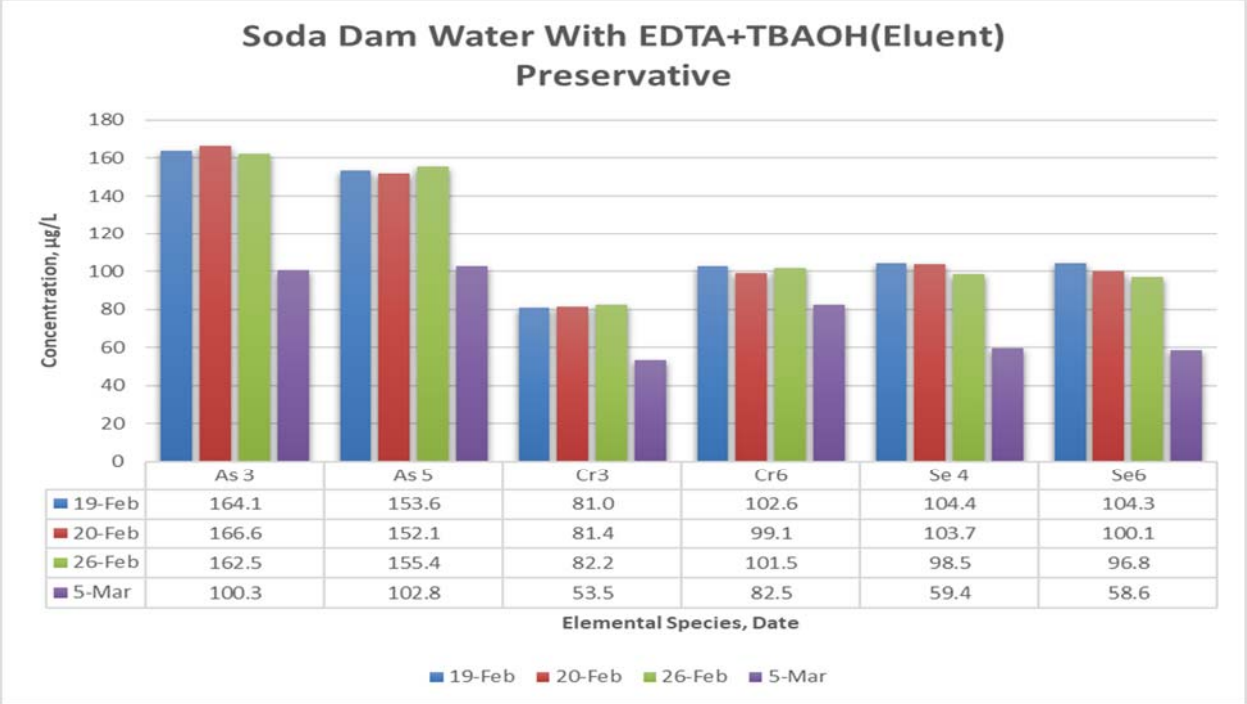


Figure 35: Graphical representation of species recovery in Soda Dam water with EDTA/TBAOH preservative.

The behavior of Cr(III) and As(III) with this preservative in 18 MΩ water and tap water are just as puzzling as no preservative. Arsenic (III) in tap water was completely converted as can be

seen by the recovery amount of Arsenic (V) in the sample. Although this data is not what was expected, it does suggest confirmation that As(III) was converted to As(V) in tap water with no preservative. In lieu of this information the explanation is unclear.

Chromium (III) in 18 MΩ water also produced puzzling results. The recovery of Cr(III) over time is increasing instead of decreasing while Cr(VI) is not changing. It is known from the literature review that the TBAOH used as part of the preservative forms dichromate from Cr(III) to stabilize and aid in the separation of species during analysis. The increase in Cr(III) concentration over time suggests that chromate concentration drops and Cr(III) concentration increases as the pH begins to change. The final pH for 18 MΩ water at the end of the two-weeks was dramatically different than when it began. The initial pH after preservation was 6.18 but dropped to 4.79 after the two weeks.

DISCUSSION AND CONCLUSIONS

To validate this data and determine its efficacy, it is important to decide whether either of the preservations worked better than no preservation at all. A comparison table that shows the final concentration of species on day seven and day fourteen reveals that species remained relatively stable until after day seven, where the concentration of species drops approximately 50%. This suggests that the preservatives were able to maintain some level of species stability in comparison to no preservation at all for the first seven days. However, the drop in concentration on day fourteen does not have a mass balance, suggesting that day fourteen data was skewed.

Throughout the course of the experiments, the HPLC was disconnected from the ICP-MS several times. It is speculated that skewed data from day fourteen may have been due to instrument bias as it had previously been disconnected prior to this analysis. It is also hypothesized that inconsistent instrument analysis was the cause of skewed data and that further experimentation may reveal that the preservatives used were still stable by day fourteen. **Table 14** shows how the concentrations of analytes changed over the course of the two weeks. Initial concentration for all species was 100 μg/L.

Both preservatives demonstrated the ability to stabilize certain species in certain solutions better than no preservative. Not all species remained equally stable throughout this process, and there were certain species that did not show improvement depending on the water source, however, each of the preservatives demonstrated potential to stabilize arsenic, chromium, and selenium species in multiple medias. Considering that biological buffer has not been used prior for water preservation, further research could lead to a better understanding of how biological buffers assist in the stabilization of metal species.

Regarding further research, the TBAOH as part of the EDTA preservative, may need further evaluation, and inherently may have caused issues with the chromium species recovery. In the literature, TBAOH is meant to aid in the separation of chromium species during liquid chromatographic analysis (Neubauer et. al, 2003), although it is not known exactly how this affected species as a preservative. It is possible that an inadequate amount of TBAOH during analysis would inhibit the separation of chromium species, thus leading to under reported concentrations, however, it is unknown how or if the TBAOH effects the recovery of other species. Further experimentation is needed to determine whether TBAOH is effective as part of the preservative.

The scope of this research does not consider other factors when it comes to species stabilization regarding water chemistry and composition. Any additional research pertaining to the preservation techniques of these experiments will need to consider some of these factors to determine the viability of either of these preservations for potential use. In lieu of future challenges the data presented in this report offers a segue into a better understanding of how the water chemistry of metal species work and can provide insight on how pH buffers can be used for preservative applications in the future.

Table 14: Comparison table of species recovery by preservative.

18 MΩ Water with No Preservative						
	As(III)	**As(V)**	**Cr(III)**	**Cr(VI)**	**Se(IV)**	**Se(VI)**
Day 7	101.8	99.7	0	108.1	97.7	91.7
Day 14	64.3	66.4	0	87.5	56.4	58.5
Tap Water with No Preservative						
	As(III)	**As(V)**	**Cr(III)**	**Cr(VI)**	**Se(IV)**	**Se(VI)**
Day 7	0.0	208.5	33.2	108.1	37.9	152.7
Day 14	0.0	141.8	19.9	87.8	20.1	96.6
River Water with No Preservative						
	As(III)	**As(V)**	**Cr(III)**	**Cr(VI)**	**Se(IV)**	**Se(VI)**
Day 7	106.7	127.5	64.6	115.6	101.3	95.2
Day 14	61.3	70.4	39.0	84.3	58.6	59.4
Soda Dam with No Preservative*						
	As(III)	As(V)	Cr(III)	Cr(VI)	Se(IV)	Se(VI)
Day 7	162.4	163.8	69.3	113.8	106.6	98.0
Day 14	83.4	118.4	39.8	86.2	59.5	58.4
18 MΩ Water with HEPES Preservative						
	As(III)	**As(V)**	**Cr(III)**	**Cr(VI)**	**Se(IV)**	**Se(VI)**
Day 7	100.5	93.7	17.0	113.3	100.2	96.4
Day 14	62.2	58.9	20.4	86.0	53.8	57.1
Tap Water with HEPES Preservative						
	As(III)	**As(V)**	**Cr(III)**	**Cr(VI)**	**Se(IV)**	**Se(VI)**
Day 7	107.5	124.1	105.2	112.2	102.1	101.3
Day 14	59.1	68.7	74.7	76.8	55.3	57.2
River Water with HEPES Preservative						
	As(III)	**As(V)**	**Cr(III)**	**Cr(VI)**	**Se(IV)**	**Se(VI)**
Day 7	97.0	103.9	74.9	104.0	95.2	94.0
Day 14	59.8	67.4	57.5	82.4	55.5	57.6
Soda Dam with HEPES Preservative						
	As(III)	As(V)	Cr(III)	Cr(VI)	Se(IV)	Se(VI)
Day 7	148.1	145.5	73.7	103.7	105.9	94.3
Day 14	91.6	102.7	56.2	85.4	56.5	58.0
18 MΩ with EDTA+TBAOH						
	As(III)	**As(V)**	**Cr(III)**	**Cr(VI)**	**Se(IV)**	**Se(VI)**
Day 7	105.1	106.0	46.3	110.5	103.8	95.1
Day 14	60.4	64.4	49.4	86.4	56.6	60.4
Tap Water with EDTA+TBAOH Preservative						
	As(III)	**As(V)**	**Cr(III)**	**Cr(VI)**	**Se(IV)**	**Se(VI)**
Day 7	15.4	196.5	80.2	114.1	88.4	106.6
Day 14	8.0	123.9	51.5	82.5	50.3	62.4
River Water with EDTA+TBAOH Preservative						
	As(III)	**As(V)**	**Cr(III)**	**Cr(VI)**	**Se(IV)**	**Se(VI)**
Day 7	97.0	102.0	63.5	102.3	107.4	95.9
Day 14	61.4	67.4	37.7	83.0	57.5	57.3
Soda Dam Water with EDTA+TBAOH Preservative						
	As(III)	**As(V)**	**Cr(III)**	**Cr(VI)**	**Se(IV)**	**Se(VI)**
Day 7	162.5	155.4	82.2	101.5	98.5	96.8
Day 14	100.3	102.8	53.5	82.5	59.4	58.6

REFERENCES

40 CFR 136. Title 40 Chapter 1 Subchapter D Part 136. Federal Code of Regulations. Protection of Environment. Environmental Protection Agency. Water Programs. Guidelines for Establishing Test Procedures for the Analysis of Pollutants. Current as of 2/16/2017.

Ali, Imran. "The quest for active carbon adsorbent substitutes: inexpensive adsorbents for toxic metal ions removal from wastewater." *Separation & Purification Reviews* 39.3-4 (2010): 95-171.

Archundia, C., et al. "Reduction of low concentration Cr (VI) in acid solutions." *Science of the total environment* 130 (1993): 231-236.

Barałkiewicz, Danuta, et al. "Speciation analysis of chromium in drinking water samples by ion-pair reversed-phase HPLC–ICP-MS: validation of the analytical method and evaluation of the uncertainty budget." *Accreditation and Quality Assurance* 18.5 (2013): 391-401.

Barber, W. P., & Stuckey, D. C. (2000). Metal bioavailability and trivalent chromium removal in ABR. *Journal of environmental engineering*, *126*(7), 649-656.

Bissen, Monique, and Fritz H. Frimmel. "Arsenic—a review. Part I: occurrence, toxicity, speciation, mobility." *CLEAN–Soil, Air, Water* 31.1 (2003): 9-18.

Costa, Max. "Toxicity and carcinogenicity of Cr (VI) in animal models and humans." *Critical reviews in toxicology* 27.5 (1997): 431-442.

Duruibe, J. O., M. O. C. Ogwuegbu, and J. N. Egwurugwu. "Heavy metal pollution and human biotoxic effects." *International Journal of Physical Sciences* 2.5 (2007): 112-118.

Edmunds, W. M., and P. L. Smedley. "Groundwater geochemistry and health: an overview." *Geological Society, London, Special Publications* 113.1 (1996): 91-105.

Favre, H. A., & Powell, W. H. (2013). *Nomenclature of organic chemistry: IUPAC recommendations and preferred names 2013*. Royal Society of Chemistry.

Garbarino, J. R., M. J. Lewis, and A. J. Bednar. "CONSTITUENT SPECIES 5.6. 4." U.S. Geological Survey TWRI Book 9 Chapter A5. Arsenic Speciation 5.6.4.A, version 2.1, 10/2012.

Goldberg, Sabine, et al. "Speciation of selenium (IV) and selenium (VI) using coupled ion chromatography—hydride generation atomic absorption spectrometry." *Soil Science Society of America Journal* 70.1 (2006): 41-47.

Good, Norman E., et al. "Hydrogen ion buffers for biological research." *Biochemistry* 5.2 (1966): 467-477.

Goyer, Robert A., and Thomas W. Clarkson. "Toxic effects of metals." *Casarett & Doull's Toxicology. The Basic Science of Poisons, Fifth Edition, Klaassen, CD [Ed]. McGraw-Hill Health Professions Division, ISBN* 71054766 (1996).

Hall, GwendyáE M. "Stability of inorganic arsenic (III) and arsenic (V) in water samples." *Journal of Analytical Atomic Spectrometry* 14.2 (1999): 205-213

Heninger, Ingrid, et al. "Storage of aqueous solutions of selenium for speciation at trace level." *Fresenius' journal of analytical chemistry* 357.6 (1997): 600-610.

Heumann, Klaus G. *Handbook of elemental speciation: techniques and methodology*. John Wiley & Sons, 2004.

Hill, Steve J. "Speciation of trace metals in the environment." *Chemical Society Reviews* 26.4 (1997): 291-298.

Howard, J. Hatten. "Geochemistry of selenium: formation of ferroselite and selenium behavior in the vicinity of oxidizing sulfide and uranium deposits." *Geochimica et Cosmochimica Acta* 41.11 (1977): 1665-1678.

Jain, C. K., and I. Ali. "Arsenic: occurrence, toxicity and speciation techniques." *Water Research* 34.17 (2000): 4304-4312.

Kołodyńska, Dorota. "Adsorption characteristics of chitosan modified by chelating agents of a new generation." *Chemical engineering journal* 179 (2012): 33-43.

Kumar, A. Ramesh, and P. Riyazuddin. "Preservation of inorganic arsenic species in environmental water samples for reliable speciation analysis." *TrAC Trends in Analytical Chemistry* 29.10 (2010): 1212-1223.

Lazorchak, James M., Donald J. Klemm, and David V. Peck, eds. "Environmental monitoring and assessment program surface waters: field operations and methods for measuring the ecological condition of wadeable streams." (1998).

Markiewicz, Barbara, et al. "Chromium and its speciation in water samples by HPLC/ICP-MS–technique establishing metrological traceability: a review since 2000." *Talanta* 132 (2015): 814-828.

Martinez-Bravo, Y., et al. "Multielemental Determination of Arsenic, Selenium and Chromium (VI) Species in Water by HPLC ICP MS." *Journal of Chromatography A* 926.2 (2001): 265-274.

McCleskey, R. Blaine, D. Kirk Nordstrom, and Ann S. Maest. "Preservation of water samples for arsenic (III/V) determinations: an evaluation of the literature and new analytical results." *Applied Geochemistry* 19.7 (2004): 995-1009.

Mohan, Dinesh, and Charles U. Pittman. "Arsenic removal from water/wastewater using adsorbents—a critical review." *Journal of hazardous materials* 142.1 (2007): 1-53.

Neubauer, Kenneth, Wilhad Reuter, and Pamela Perrone. "Chromium speciation in water by HPLC/ICP-MS." *PerkinElmer Appl. Note* (2003).

Neubauer, Kenneth R., et al. "Simultaneous arsenic and chromium speciation by HPLC/ICP-MS in environmental waters." *ICP-OES, ICP-MS* (2004): 614.

Nordberg, Gunnar F., Bruce A. Fowler, and Monica Nordberg, eds. *Handbook on the Toxicology of Metals*. Academic Press, 2014.

Shafer, M. M., Joel T. Overdier, and S. C. Kerr. "Arsenic Species (III, V) Distribution in Wisconsin Groundwaters: Field Measurements and Prediction Using Multivariate Analysis of Geochemical Data." *Water Resources Institute, University of Wisconsin, Madison. 21p. Final_WR05R001. pdf* (2007).

Sun, Jing, et al. "Simultaneous speciation and determination of arsenic, chromium and cadmium in water samples by high performance liquid chromatography with inductively coupled plasma mass spectrometry." *Analytical Methods* 7.6 (2015): 2653-2658.

Telliard, W. A. "Sampling ambient waters for trace metals at EPA water quality criteria levels, US EPA method 1669." *Washington, DC* (1996).

Thomson, B.M. “pe-pH diagram for chromium species,” Environmental Engineering Chemistry course notes, University of New Mexico.

Thorn, Conde R., Douglas P. McAda, and John Michael Kernodle. *Geohydrologic framework and hydrologic conditions in the Albuquerque Basin, central New Mexico*. US Department of the Interior, US Geological Survey, 1993.

Thornton, Iain. "Sources and pathways of arsenic in the geochemical environment: health implications." *Geological Society, London, Special Publications* 113.1 (1996): 153-161.

Unceta, N., et al. "Chromium speciation in solid matrices and regulation: a review." *Analytical and bioanalytical chemistry* 397.3 (2010): 1097-1111.

US EPA, March. "Methods for chemical analysis of water and wastes." *Cincinnati, Ohio, USA* (1979).

Vail, Jonathan. Groundwater Sampling Operating Procedure. U.S. Environmental Protection Agency Science and Ecosystem Support Division. *Athens, Georgia, USA*, 2013.

Vasconcelos, M. T. S. D., and Maria Fernanda C. Leal. "Influence of N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid pH buffer on the biological response of marine algae." *Environmental toxicology and chemistry* 21.2 (2002): 404-412.

Wu, Debo, and Thomas Pichler. "Preservation of co-occurring As, Sb and Se species in water samples with EDTA and acidification." Geochemistry: Exploration, Environment, Analysis 16.2 (2016): 117-125.

Yeskis, Douglas, and Bernard Zavala. "Ground-water sampling guidelines for superfund and RCRA project managers." *Ground Water Forum Issue Paper EPA*. 2002.

Zhang, H., & Bartlett, R. J. (1999). Light-induced oxidation of aqueous chromium (III) in the presence of iron (III). *Environmental science & technology*, *33*(4), 588-594.

Zigler, J. S., et al. "Analysis of the cytotoxic effects of light-exposed HEPES-containing culture medium." *In Vitro Cellular & Developmental Biology* 21.5 (1985): 282-287.