

Spectrophotometric Analysis of Chromium (VI) Levels in Varying Arizona County Water Samples used to Determine which County Water Contains the Most Toxicity when Introduced to Yeast

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ABSTRACT

This study analyzed the Chromium (VI) levels present in three Arizona counties tap water, as represented by the major universities residing within them. Each sample was surveyed using spectrophotometry to determine the contaminant levels of Cr(VI) in the water. Introduction to the yeast strain *BY4742* was observed to determine whether the contaminated water dampened culture growth. When compared to the Maximum Contaminant Level (MCL) of 0.1 mg/L set by the EPA, Maricopa County contained 14x the amount allowable at 1.4 mg/L. The samples collected for Coconino County and Pima County also displayed contaminant levels higher than the set MCL at 0.3 mg/L and 0.5 mg/L, respectively. Data collected on yeast growth illustrated that Maricopa County was also the most hindering on yeast growth at an average cell count of 97. However, the chosen yeast strain also demonstrated an apparent resistance to Cr(VI) with an average cell count of 120 cells in the Cr(VI) plate. Due to this strains survival in Cr(VI) contaminated water, our initial hypothesis was rejected. Possible generational studies can be done to observe the toxic effects higher levels of Cr(VI) in drinking water have with regard to organismal growth hindrance and accumulation of the element leading to carcinogenic properties. Additional analysis on what other contaminants lay within Arizona counties can be performed, as well.

Introduction

Chromium Characteristics and Locations

Chromium is a tasteless metallic element found to naturally occur and is most abundant in rocks, plants, and soils. The two common forms in which chromium is most found in is its oxidation states of either trivalent chromium (Cr(III)) or hexavalent chromium (Cr(VI)) (EPA, 2017). While Cr(III) is found mostly in consumable plant materials and is an essential dietary element for humans, Cr(VI) is considered a contaminant due to the toxic effects it has on animals, microorganisms, and humans (Dakiky, 2002). Cr(VI) is produced through erosion of naturally occurring deposits or industrial processes that runoff into the surrounding environment (EPA, 2017). This industrial runoff of Cr(VI) is produced by major industries such as mining, pulp and paper mills, and metal-plating industries (Udy, 1956). The amount of wastewater discharged from these kinds of industries should be reduced and recycled when possible, however, there are instances where inadequate storage, leaks, or simply poor waste management practices are in effect, causing the Cr(VI) containing waste to leach into the surrounding environment and into the local water system.

The EPA is required to regulate the contaminant levels in drinking water via the Safe Drinking Water Act (SDWA), which determines the level at which no adverse health effects will likely occur when contaminated water is consumed (EPA, 2017). The set national maximum contaminant level (MCL) of chromium is 0.1 mg/L (EPA, 2017), which includes both oxidative forms of the element. This MCL standard was set in 1991 and the removal of such

contaminants have been improving since then with the use of processes such as ion exchange, solvent extraction, reverse osmosis, and so on (Dakiky, 2002).

This study focuses on the drinking water available in the three Arizona counties of Coconino, Maricopa, and Pima and their ability to maintain the levels of Cr(VI) contamination. Each county has housed an industry that is known to produce wastewater that possibly contains chromium and may still be present in the counties drinking water system. Coconino county was home to a timber, pulp, and paper manufacturer until its closure in mid-2017. The Bureau of Land Management recorded that Pima county contains 19,066 mining claims and 1,521 mineral deposits were recorded by the United States Geological Survey (USGS) (The Diggings, 2019). Maricopa county is home to several metal-plating and metal-working companies throughout the valley.

Toxic Effects of Chromium (VI)

There has long been concern revolving around human health risk and exposure assessment of Cr(VI). This is largely due to its reputation regarding the hazards and exposure in drinking water. Because inhalation of Cr(VI) can cause lung cancer in some persons exposed to a sufficient airborne concentration, questions have arose about the possible hazards associated with exposure to Cr(VI) in drinking water via ingestion, inhalation, and dermal contact (Finley et al., 2003). It is critical that the aforementioned counties be studied in order to see if there is a particular geographical location where chromium levels are high and need to be assessed. Pollution of water resources, both surface and underground, by spent wastes of chromium-based industries has become a serious global concern, for it has created an acute scarcity of safe drinking water in many countries (Chandra, Kulshreshtha, 2004).

Yeast Strain BY4742

Yeasts are eukaryotic and single cell fungi. The scientific name for yeast is *Saccharomyces cerevisiae*, which is sugar-eating. Yeast divide by a process called budding; this process occurs when a new yeast cell develops from an old grown bud. Yeast cells can produce by asexual and sexual reproduction, which can go through mitosis and meiosis. All yeast cells have DNA, RNA, proteins, lipids, and carbohydrates. Like any other living organism, yeast cells require oxygen to grow, which is by aerobic respiration; however, in the absence of oxygen, yeast ferment their own sugar and carbohydrates to produce carbon dioxide and ethanol (Hall 1993).

Yeast cannot be grown in everything. For this study, a growth assay was prepared to test if yeast growth is affected by Cr(VI). The Yeast Strain *BY4742* “is part of a set of deletion strains derived from S288C in which commonly used selectable marker genes were deleted by design in order to minimize or eliminate homology to the corresponding marker genes in commonly used vectors without significantly affecting adjacent gene expression” (Brachmann CB, et al. 1998). *BY4742* is a parent strain, which was used for international systematic *Saccharomyces cerevisiae* gene disruption project.

The chromium levels in each county sample will be surveyed using spectrophotometry and its toxic effects will be studied further by introducing the yeast strain *BY4742* to the contaminated waters. Since the industry’s most suspect in producing Cr(VI) runoff were – and still are – present in each county, this study will focus on if there are any possible dangers still present in each county drinking water by observing whether the contaminated waters dampen or completely hinder yeast growth.

Materials and Methods

Preparation of 1,5-Diphenylcarbazide Solution

In order to detect the Chromium (VI) levels, a solution of 1,5-Diphenylcarbazide was prepared by dissolving 0.5 g of 1,5-Diphenylcarbazide powder into 100 mL of acetone. This was diluted by adding 100 mL of distilled water and then transferred into a foiled bottle. This solution was refrigerated and set aside for later use. When introduced to Chromium, a 1,5-Diphenylcarbazide Solution will turn a royal purple color.

2.2 Chromium (VI) Standard Curve Preparation

In this experiment, the standard curve was determined by making test samples containing various concentrations of Cr(VI), Sulfuric Acid, and Distilled Water. This allowed the tubes to display different color changes, thus exhibiting different absorbance. Three sets of six test tubes had to be used in order to reach a more diluted and appropriate absorbance range for this specific experiment. The following table displays what was placed in tube set #1:

	1 (<i>blank</i>)	2	3	4	5	6
Cr(VI)	0	100 μ L	200 μ L	300 μ L	400 μ L	500 μ L
Sulfuric Acid (3M)	2 mL (2000 μ L)	2 mL (2000 μ L)	2 mL (2000 μ L)	2 mL (2000 μ L)	2 mL (2000 μ L)	2 mL (2000 μ L)
Water	5 mL (5000 μ L)	4.9 mL (4900 μ L)	4.8 mL (4800 μ L)	4.7 mL (4700 μ L)	4.6 mL (4600 μ L)	4.5 mL (4500 μ L)

0.5 mL of the solution was taken from tube set #1 and transferred into each respective tube of tube set #2. Then, 1 mL of the previously prepared 1,5- Diphenylcarbazide Solution was also added into each tube of tube set #2. Tube set #3 was then prepared by adding 4.5 mL of distilled water to each tube using a serological pipette. 0.5 mL of the solution from set #2 was then transferred into tube set #3. The test samples were mixed and then kept for five minutes before the absorbance of each sample from tube set #3 was read.

Chromium (VI) Detection in Water Samples

Stock solutions of unknown water samples were prepared by placing 10.0 mL of the water samples in a large centrifuge tube. 600 μ L of 3 M sulfuric acid was added to each tube, then 500 μ L of the previously prepared 1,5-diphenylcarbazide solution was pipetted into the samples. The solution was mixed gently and sat at room temperature for at least 5 minutes in order to allow color formation. 3 mL of the stock solution was pipetted into 3 smaller test tubes and the absorbance was then measured at 540 nm. Using the equation derived from chromium (VI) standard curve, the average amount of Cr(VI) present in each unknown sample group was calculated.

Preparation of YPD Medium

YPD medium was prepared by measuring out 2 g of peptone, 2 g of D-glucose, 1 g of yeast extract, and 2 g of agar for a total five separate YPD media to accommodate plating for the conditions of Control, Cr(VI), Arizona State University (ASU), University of Arizona (U of A), and Northern Arizona University (NAU) samples. The measurements were suspended in 100 mL of each school's respective water. Distilled water was used for Control and Cr(VI) sample. The Cr(VI) water contained a 2% w/v dilution of distilled water and the Cr(VI) standard. All of the samples were mixed to allow the components to dissolve, then heated to boiling while stirring for 30 minutes at 121°C to ensure complete homogenization. After the YPD solutions cooled down, each was poured into 15 separate petri dishes labeled for triplicate analysis of the Control, Cr(VI), ASU, U of A, and NAU. The dishes were then left at room temperature for a week to completely dry prior to plating the yeast sample.

Preparation of Yeast Sample

Yeast strain *BY4742* was prepared by diluting 3 mL of water with 330 μ m of yeast into a test tube. The absorbance was measured at 0.610 A to calculate the amount of yeast used for each petri dish sample. A serial dilution of the prepared yeast sample was done to ensure that each plate contained only 1 cell/ μ L of yeast. This was done by adding 10 μ L of the prepared yeast sample into 10 mL of water then vortexed. A final dilution was performed by adding 37 μ L of the first dilution into 10 mL and vortexed once more. 100 μ L, 200 μ L, and 500 μ L of the final diluted yeast

sample was added to separate YPD plates, totaling 3 sample volumes for each sample condition. These plates were then left to incubate for 2 days. A viable cell count was performed to count the total number of yeast colonies present on each plate.

Results and Discussion

A Standard curve was created with Cr(VI) standard obtained from Fisher Scientific containing a Cr(VI) concentration of 1000 mg/L. The absorbance was measured at 540 nm to derive the measurements of the known sample (**Table 1**) and the equation of $y=2.1924x$ was calculated when the μL used was divided by the known concentration of Cr(VI) (**Figure 1**). This equation was then used to determine the concentration of Cr(VI) present in the unknown water samples.

When preparing the standard curve for Cr(VI) detection, tube 2 was prepared to contain the lowest amount of Cr(VI) and was found to have an absorbance at 0.404 nm (**Table 1**). In contrast, tube 6 was prepared to contain the most Cr(VI) level and yielded a 1.012 nm absorbance, which also produced a deep purple color. These results illustrated that the more Cr(VI) present in water, the darker the solutions color would be when diphenylcarbazide is used to detect contaminant levels.

Table 1. Absorbance Values of the known Cr(VI) Sample at 540 nm: The tube containing the highest absorbance at 1.012 contained the most Cr(VI), indicating the absorbance increases with the concentration level of Cr(VI).

Tube #	Absorbance
1 (<i>Blank</i>)	0
2	0.404
3	0.434
4	0.670
5	0.929
6	1.012

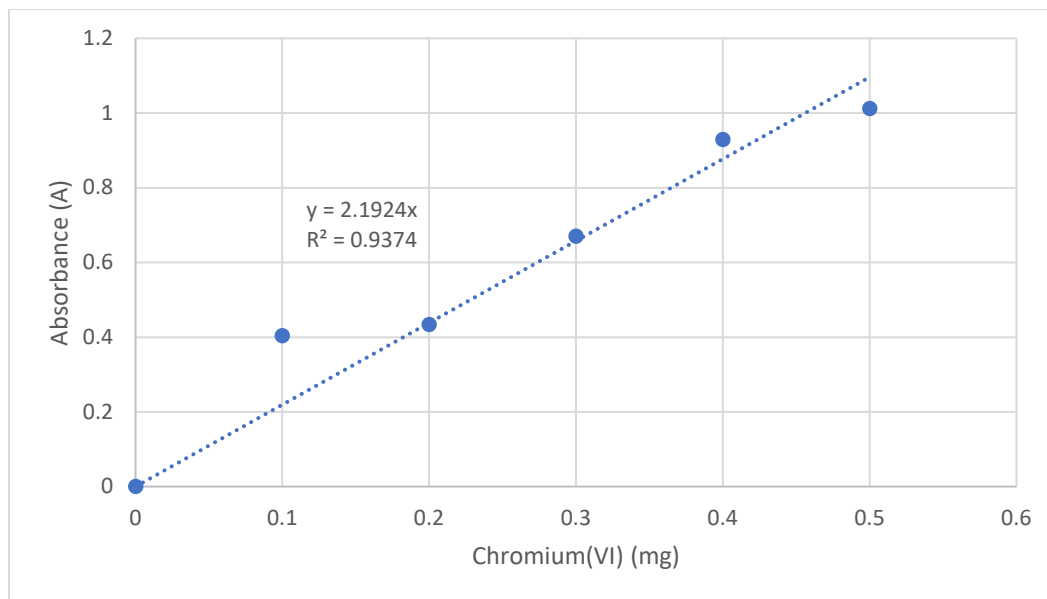


Figure. 1 Cr(VI) Standard Curve: The completed Chromium (VI) Standard Curve done at 540 nm.

A triplicate analysis was performed on the collected water samples by placing 3 mL of the diphenylcarbazide treated water solution in a spectrophotometer set at 540 nm. Each county was represented by the major universities that reside within them, and the absorbance for each universities water sample was recorded to determine their average absorbance value. Using the equation derived from the standard curve, the amount of Cr(VI) was calculated and the concentration was then derived by dividing the amount of water assayed. The concentration was then converted from mg/mL to mg/L by multiplying the initial concentration level by 1000. The calculated results are given in **Table 2** and are further illustrated in **Figure 2**.

Table 2. Absorbance Values and Calculated Concentration Levels of Cr(VI) Found in Each County

Sample: The absorbance of the three different universities' water were measured at 540 nm and the Cr(VI) concentration of each unknown was calculated to compare which county contained the highest level of Cr(VI), found at ASU with a concentration of 1.4 mg/L.

School	Concentration of Cr(VI) (mg/L)
ASU	1.4
NAU	0.3
U of A	0.5

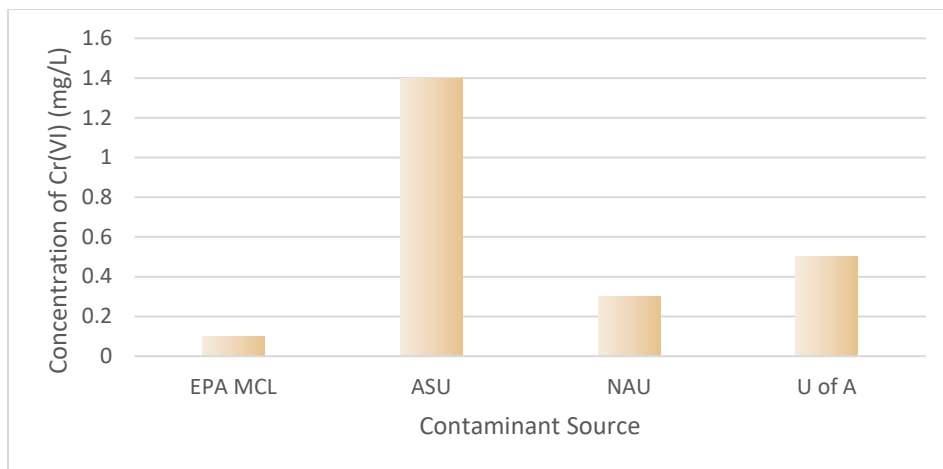


Figure 2. Cr(VI) Concentration Comparison of Unknown Water Samples Against the MCL Set by the EPA: Further analysis of the collected county water to determine the highest level of Cr(VI) found in ASU water as well as illustrate each county sample containing higher than allowable levels of Cr(VI).

As the comparison of each of the county waters was performed, the highest Cr(VI) concentration level of 1.4 mg/L was observed in the water sample collected at ASU (Table 2). When compared to the Maximum Contaminant Level (MCL) of 0.1 mg/L provided by the EPA (EPA, 2017), this representative sample collected for Maricopa County contained 14x the amount allowable as determined by the EPA, which is further illustrated in Figure 2. Figure 2 also illustrates how each county’s representative sample contains higher levels than the allowable MCL. The water collected from NAU (Coconino County) contains 3x the MCL at 0.3 mg/L and the water collected from U of A (Pima County) contained 5x the MCL at 0.5 mg/L (Table 2).

Triplicate analysis of yeast growth was performed on the collected water samples by placing a diluted yeast solution in prepared plates containing the contaminated county waters. Once incubation was complete, the cells present in each plate were counted and an average cell count was determined for each sample tested (Table 3).

Table 3. Yeast Assay Count of the Known and Unknown Water Samples: The average cell count of each tested sample was done, and the lowest yeast growth was found to be 97 for the ASU sample.

Sample	Average Cell Count
Control	112
Cr(VI)	120
ASU	97
NAU	110
U of A	105

Analysis the yeast assay provided further insight on which county hindered the cell growth of the chosen yeast strain. The water collected at ASU contained a count of 97 cells, which had the lower average of the other water samples (**Table 3**). Since ASU is the representative sample for Maricopa county, the lower count of cells further deems this county to contained the most contaminated water of the three counties being represented. In contrast, U of A yielded 105 cells, and NAU yielded 110 cells (**Table 3**). This indicates that the yeast growth survived more in Coconino County (NAU) water when considering both cell count and the calculated Cr(VI) concentration of 0.3 mg/L. However, the most yeast cells grew in Cr(VI) which yielded an average of 120 cells (**Table 3**). This may be indicative of the yeast strains resistance to Cr(VI) levels, raising the question of whether there may be another potential contaminant present in the county waters that may be hindering growth. Past studies have shown that Cr(VI) did not affect the growth of *Candida* yeast with 1.7 mM Cr(VI) concentration. In contrast, when 3.3 mM concentration of Cr(VI) was added, the *Candida* cells started to lysis which caused yeast growth to decrease (Guillen-Jimenez. Et al, 2008).

Conclusion

While the focus of this study was to determine if Chromium (VI) is among the toxic elements to prevent yeast cell growth, the yeast strain of *BY4742* studied was found to be resistant to Cr(VI). Due to this strains survival in Cr(VI) contaminated water, our initial hypothesis was rejected. While this was found, the alarming amount of Cr(VI) present in Arizona county waters poses questions. According to the American Water Works Association, Cr(VI) may not pose any present health risks, however a concern still lies on whether this element may cause health problems at a specific level in water (Crow, 2011).

Future Work

With the apparent resistance our chosen yeast strain displayed against the pure Cr(VI) contaminated water, further research may be warranted. Regarding the adverse effects of Cr(VI) on cell growth, an analysis on higher contamination levels of Cr(VI) present in drinking water could be done as a generational study to study possible effects this contaminant has on the growth of eukaryotic microorganisms. According to the EPA's IRIS database on health hazards, Cr(VI) has a reference dose (RfD) of 3×10^{-3} mg/kg per day with regard to oral exposure (EPA, 1998), so an accumulation study on yeast or other eukaryotic microorganisms could be done to see if continued exposure to contaminated water poses a potential carcinogenic threat.

Since this study mainly focused on the drinking water available through the tap, further spectrophotometric investigation on different sources of drinking water, such as filtered or well water, could be done to compare the contaminant level of each sample source. Additional studies, like the Ames Test, can be performed on the collected water samples to identify whether Cr(VI) present in drinking water poses a carcinogenic threat to Arizona residents. This can assist in determining if Cr(VI) has more prevalence in certain water sources and if further containment or removal should be done. Doing additional analysis of different water sources may assist in understanding other drinking water contaminants - since the yeast effectively grew in the purely contaminated Cr(VI) plate but was dampened in the unknown samples, additional analysis on what other contaminants lie within Arizona counties can also be done.

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