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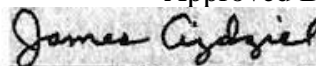
**Determination of Metals in Whey and Vegan Protein Supplements
using Inductively Coupled Plasma Mass Spectrometry**

By
Megan Lofaso

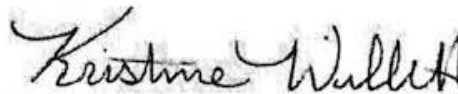
A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

Oxford, MS
May 2021

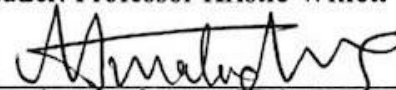
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DEDICATION

This thesis is dedicated to everyone who guided and encouraged me throughout the year. I would like to especially dedicate my thesis to my parents, who have always supported my dreams and allowed me to further my education at the University of Mississippi. Thank you.

ACKNOWLEDGEMENTS

I want to thank the Sally McDonnell Honors College for the generous funding to complete my thesis. I would also like to thank my research advisor, James Cizdziel, for mentoring me throughout this process, as well as Dr. Willett and Dr. Dass for agreeing to be my second and third readers. I would also like to thank a local smoothie shop for allowing me to borrow the protein samples. Finally, I would like to thank Byungwon Jeon for offering to help with my data analysis.

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ABSTRACT

Driven by a demand for health and wellness products worldwide, the dietary supplement industry continues to expand with an economic impact >\$100 billion in the USA alone. However, the industry is plagued by a lack of regulation and incidents of contamination, including with toxic heavy metals that can put consumers at potential risk. In this study, eight trace elements (Cd, Pb, Fe, Co, Mn, V, Cu, and Cr), including heavy metals (Cd and Pb), were determined in whey and vegan protein powder by inductively coupled plasma mass spectrometry (ICP-MS) after microwave-assisted digestion using nitric acid and hydrogen peroxide. Samples were run in triplicate along with blanks and a reference material. Mean concentrations ($\mu\text{g/g} \pm 1\text{SD}$) in the vegan protein powder were Fe (133 ± 1) > Mn (25.5 ± 10.3) > Cu (1.4 ± 5.4) > Cr (0.226 ± 0.0414) > V (0.17 ± 0.01) > Co (0.090 ± 0.001) > Pb (0.038 ± 0.013) > Cd (0.033 ± 0.003). This was higher than the whey protein powder: Fe (11.5 ± 4.3) > Cu (1.91 ± 2.2) > Mn (0.20 ± 0.01) > Cr (0.0491 ± 0.0505) > V (0.018 ± 0.005) > Pb (0.017 ± 0.005) > Co (0.012 ± 0.001) > Cd (0.010 ± 0.001). These levels correspond to amounts per serving that were below the US FDA recommended daily allowance for both the whey and vegan protein powders. However, the vegan protein powder had concentrations of Mn and Fe that could exceed the FDA criteria and be a risk to the consumer if they ingest more than the recommended daily serving (which is common for body builders) or attain these metals from other dietary sources. We intended analyze additional samples to confirm this finding and to determine how widespread the issue is, but unfortunately, the ICP-MS became inoperable and is in need of repair. Instead, on

suggestion from the Honor's College, I conducted a deep literature review on the subject of heavy metal contamination in the supplement industry and current guidelines. Herein I also provide my overview and recommendations on this subject.

LIST OF ABBREVIATIONS

ICP-MS: Inductively Coupled Plasma Mass Spectrometry

EAM: Elemental Analysis Manual for Food and Related Products

FDA: Food and Drug Administration

RDA: Recommended Daily Allowance

TDI: Total Daily Intake

CDI: Chronic Daily Intake

Cd: Cadmium

Pb: Lead

V: Vanadium

Co: Cobalt

Mn: Manganese

Fe: Iron

Cu: Copper

Al: Aluminum

Hg: Mercury

As: Arsenic

Cr: Chromium

Ni: Nickel

HNO₃: Nitric Acid

H₂O₂: Hydrogen Peroxide

SRM: Standard Reference Material

EPA: Environment Protection Agency

LOD: Limit of Detection

DSHEA: Dietary Supplements Health and Education Act

ICP-OES: Inductively Coupled Plasma Optical Emission Spectrometry

DSID: Dietary Supplement Industry Database

WHO: World Health Organization

INTRODUCTION

The Supplement Industry

The World Health Organization (WHO) and United States Dietary Supplements Health and Education Act (DSHEA) in 1994 defined dietary/health supplements as “vitamins, minerals, herbs, botanical products, and amino acids” (Abdulla, 2019). Dietary supplements include gel caps, pills, capsules, tablets, prenatal vitamins, diet pills, and protein powders (Roseland, 2008).

Dietary supplements are regulated as food products and additives rather than as drugs (Saldanha, 2007). The dietary supplement industry has been growing steadily since it started in the mid-1990s. The industry made \$4 billion in 1994 before the passage of DSHEA, but in 2007, the industry made \$21 billion (Saldanha, 2007).

Before the passage of DSHEA in October 1994, dietary supplements did not have to undergo Food and Drug Administration (FDA) review. The only regulations on supplements were created by the European Union in the “From the Farm to the Fork” initiative that analyzed risk and traceability, helping to guarantee food safety (Fernández-Segovia, 2016). However, the introduction of DSHEA prevented any progress the “From the Farm to the Fork” initiative made.

With DSHEA introduced, any supplements that market new ingredients that were not marketed before October 1994, must provide the FDA with evidence of safety (Cardellina, 2002). The DSHEA also has no required preapproval process for supplements. With the approval process, most plant-based supplements are accepted as safe if there are few minor side effects and when taken following the recommend safety guidelines (Cardellina, 2002). Since the DSHEA does not have strict guidelines, only 170 new supplement ingredients have been

registered with the FDA since 1994. There are an estimated 51,000 new supplements on the market, meaning only a small fraction of the new supplement ingredients have been registered (Cohen, 2012).

The DSHEA has caused a set-back in food safety. The European Union created the “From the Farm to the Fork” initiative to analyze risk and traceability, helping to guarantee food safety (Fernández-Segovia, 2016). However, the introduction of DSHEA prevented any progress the “From the Farm to the Fork” initiative made.

Since raw materials can take months to years to harvest, the industry suppliers have to anticipate certain trends and reach out to foreign networks. Wildcrafting became popular about 25 years ago, which entails harvesting raw materials for health supplements from natural habitats. Overharvesting has become a side effect of the increase in the supplement industry. Not only that, but adulteration of plant materials has become popular, leading to some health effects. Two women had digoxin poisoning as a result of taking a herbal product. The FDA tracked the cardiac glycosides diagnosis to a raw material that had been imported into the United States and sold to manufacturers and distributors in the herbal supplement industry (Cardellina, 2002).

The supplement industry focuses on the quality of their products, which is related to the safety and benefits of the products. The quality of the products depends directly on the agricultural market since they provide the raw materials for health supplements (Cardellina, 2002). The U.S. EPA has required that agricultural companies must register the agrochemicals they use as well have tolerances on the pesticides used on crop plants. The raw materials used in plant-based health supplements are cultivated in small quantities, meaning that few pesticides are registered for plant-based health products. Another issue that the supplement industry has

encountered is that many countries do not have a registration/tolerance program to track the potential accumulation of heavy metals in health supplements (Cardellina, 2002).

In order for a new supplement ingredient to be approved, the safety requirements must prove “documented history of use (e.g., in foods or in supplements or herbal medicines sold outside the United States), formulation and proposed daily dose (e.g., more or less than was formerly consumed), and the recommended duration of use (e.g., intermittent or long term)” (Cohen, 2012). However, the DSHEA has ruled that companies can propose a new ingredient and be approved based on the documented history of use. However, long-term safety is not a guarantee when approving a new ingredient based on historical use alone (Cohen, 2012).

The Dietary Supplement Industry Database (DSID) helps to determine the amount of nutrients in dietary supplement products and reports analyzed nutrient levels on supplement labels. The DSHEA controls the DSID, meaning there is a lack of recommended methods reported used to analyze supplements (Roseland, 2008).

Botanical supplement use has stabilized since early 1999, but many Americans choose these whey and plant-based dietary supplements to follow a healthy diet and active lifestyle. They can also provide great health benefits, including natural antianxiety agents and sleep aids for stress. Many consumers believe that botanical health supplements help with preventive health care (Cardellina, 2002).

Different types of protein powder are analyzed in the study. Vegan protein is a plant-based protein powder supplement, whereas whey protein is a dairy byproduct protein powder supplement (Whey Protein, 2020/Mawson, 2003). Whey protein supplements are popular because of their nutritional benefits, subtle flavor, and digestibility (Shraddha, 2015).

Vegan based supplements are water-soluble extracts consisting of legumes, oilseeds, or cereals and made from plant-based products. Vegan based products also contain soy, rice, corn, nuts, almonds, and other plant-based substitutes. Vegan products have become more popular due to people's food intolerances and health diets (Silva, 2020).

Concerns about the safety of supplements including heavy metal contamination

Concerns over the safety of supplement products is an issue specifically related to heavy metal contamination of the products. One study found that 26 prenatal vitamin brands contained elevated concentrations of Pb in their samples (Schwalfenberg, 2018). It is known that toxic metals, such as Pb, Cd, As, and Hg are found in prenatal supplements and other supplements supplied by the industry (Schwalfenberg, 2018). Another study conducted in Lebanon found that out of the 33 dietary supplements tested, 24 % contained increased concentrations of Fe and 27 % contained increased concentrations of Mn. However, the samples did not pose a health risk because they were still below the chronic daily intake (CDI) (Korfali, 2013).

A Consumer Reports study analysis of 15 protein powder products stated that “the average amounts of heavy metals in three servings of protein powder per day exceeded the maximum limits in dietary supplements” (Bandara, 2020). The Clean Label Project had a subsequent report that stated that 40% of 133 protein powder products analyzed had high heavy metal concentrations (Bandara, 2020). The Clean Label project reported that 75 % of the plant-based samples alone tested positive for detectable trace amounts of Pb. They also reported plant-based products had a higher heavy metal concentration than the whey protein powder brands (Homepage, 2020).

To limit the amount of heavy metal consumption, the concentrations of heavy metals must be limited in the lower parts of the food chain. By preventing the number of heavy metals

in soil and water, then the concentration in health supplements should be lower, increasing the likelihood of good health effects rather than detrimental ones (Hapke, 1996). Food chain pollution plays an important role on toxic metal exposure in humans (Mudgal, n.d.).

Food is generally the main source of potentially toxic elements, including Cd, Hg, Ni, and Pb (Llorent-Martínez, 2012). One of the main concerns with health supplements is that they can be easily contaminated, mainly by pesticide residue (Abdulla, 2019). Heavy metal concentration has also increased in health supplements because of the fertilizers used for crop fields. Due to the increased demand of food production, more chemicals were introduced into fertilizers and pesticides, which can possibly contaminate the products resulting from farming (Khan, 2009).

Unsafe food supplements can cause acute and life-long diseases. The European Union created the “From the Farm to the Fork” initiative to analyze risk and traceability, helping to guarantee food safety (Fernández-Segovia, 2016). However, the introduction of DSHEA prevented any progress the “From the Farm to the Fork” initiative made.

A 2012 study in Spain analyzed 20 trace elements present in soy and dairy yogurts using microwave digestion and ICP-MS. Many of the plant-based soy yogurt samples were high in Ni concentration, a possible side effect of soil rich in heavy metal contamination. However, all the toxic trace elements analyzed, except for Al, was below the LOD (Llorent-Martínez, 2012).

A 2016 study conducted in Dubai analyzed the concentrations of Pb, Hg, Cd, Cr, and As intoxication (Abdulla, 2019). The study used ICP-OES and found that if following the daily intake as described on the nutritional labels, then the heavy metal concentration does not exceed the total daily intake (TDI). Heavy metal contamination can easily occur from industrial and agriculture contamination. Agriculture contamination can be determined by where a plant was

cultivated. This type of contamination is seen more commonly in vegan based protein powders where the plant-based products come from soil rich in heavy metal contamination.

Heavy metals released by industrial companies can contaminate soils surrounding the area and a larger radius due to run-off and erosion. The toxicity of these heavy metals is dependent upon its oxidation state, and when in an organism's system, they latch onto proteins, enzymes, and DNA to create stable bio-toxic compounds. These bio-toxic compounds disrupt the proteins, enzymes, and DNA in the body from working properly (Mishra, 2018).

Different heavy metal contamination can cause different adverse health effects in humans. According to the EPA, Pb is known to be a human carcinogenic risk factor that can lead to neurological deficits, weakness, an increase in anemia and blood pressure, serious brain and kidney damage, and death (Abdulla, 2019). Pb can cause mutagenic and genotoxic effects as well (Mishra, 2018).

One protein supplement brand, Herbalife, has been known to cause various side effects, including weight loss, heart health, and energy. There are reports in Spain, Israel, Latin America, Iceland, Switzerland, and United States of acute liver injury, but the ingredients that possibly cause liver damage have not been determined. There have been 50 cases of acute liver toxicity resulting from taking Herbalife products (LiverTox, 2019). Nutritional supplements can cause liver toxicity, which can be a side-effect of heavy metal contamination (LiverTox, 2019).

In one case study, 11 patients had acute liver injury or hepatitis after a year of using Herbalife products. After these patients received treatment, three of them started consuming Herbalife products again and all three had hepatitis again (Elinav, 2007). In one case study, 12 cases of toxic hepatitis occurred in patients in Switzerland, another possible link between Herbalife supplements and acute liver toxicity (Schoepfer, 2007). Another case study shows that

liver injury was sustained by two patients from the consumption of Herbalife. However, further analysis showed that those Herbalife products consumed were contaminated with *Bacillus subtilis* (Stickel, 2009).

Heavy metal exposure side effects depend on the type of heavy metal ingested. Pb, V, and Cd are non-essential elements for the human body, whereas Fe, Cu, Co, Cr, and Mn are essential for the human body. The essential elements needed for the body have higher thresholds since they are required for bodily functions.

Cd ingestion is a serious concern for protein supplement users because bioaccumulation can occur, and increased levels of Cd can damage the kidneys and cause thyroid disruption (Bandara, 2020). Cd poisoning can also cause neurological diseases (Llorent-Martínez, 2012). Cr poisoning can cause severe liver damage, allergic reactions, and intestinal irritation (LiverTox, 2019). Too much Pb in the body can lead to neurotoxicity as well as kidney and bone marrow toxicity. Mn toxicity can cause neurologic disorders, such as hallucinations and anxiety (LiverTox, 2019). V exposure can cause neurologic, hematologic, renal, and hepatic toxicity (LiverTox, 2019). High amounts of Fe and Cu can also lead to liver toxicity (LiverTox, 2019).

Heavy metals can have a negative impact on human health, depending on the type of heavy metal (Karasakal, 2020). One of the more harmful effects of protein powder supplements has been acute liver toxicity (LiverTox, 2019). In one instance, eleven females were diagnosed after having consumed Herbalife whey protein for almost a year (Elinav, 2007). Another article stated that twelve cases of toxic hepatitis occurred after consumption of Herbalife supplements. The most prominent injury that can occur from protein powder supplements is liver toxicity, which can be fatal if not treated (LiverTox, 2019).

Current Regulations and Guidelines

The FDA's Elemental Analysis Manual for Food and Related Products (EAM) was created to regulate concentrations of Na, Mg, K, Ca, Sr, Ba, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, Cd, Hg, Ti, Pb, B, Al, P, and As in dietary supplements and other food products (Grembecka, 2016). The FDA's recommended daily allowance (RDA) of toxic metals is important for analysis so that the consumer knows the daily recommended intake of toxic metals. This is valuable information for those supplements that do not list the milligram and percentage of daily intake on their bottles (Center for Food Safety and Applied Nutrition, n.d./Recommended dietary allowances, 1989/Dietary reference, 2001).

**Table 1: FDA's Recommended Daily Allowance for Metals
(Center for Food Safety and Applied Nutrition).**

Element	RDA (mg)
Iron	18
Manganese	2.3
Vanadium	1.8
Copper	0.9
Chromium	0.035
Cadmium	0.025
Lead	0.0125
Cobalt	0.008

The CDI can be calculated from the metal concentration found in protein powder samples. This shows the consumer's risk to ingesting the protein powder supplements by using

the concentration of each metal found in the protein powder and the average daily intake rate of protein powder found on the protein powder's nutritional label (Bandara, 2020).

ICP-MS Background

Inductively coupled plasma mass spectrometry (ICP-MS) is a qualitative and quantitative trace element technique that uses mass spectrometry of ions created by an inductively coupled plasma (Figure 1). It has very low detection limits and high sensitivity (Llorent-Martínez, 2012/ Da Silva, 2016). The sample is introduced into an argon plasma through a sample introduction system, typically a nebulizer coupled to a spray chamber. The aerosol is carried by argon into the plasma where it is desolvated, dissociated, and ionized in the plasma at temperatures reaching 10,000 °K. The ions generated in the plasma then enter the mass spectrometer through the sample and skimmer cones. The ions enter the ion lens stack and are focused, accelerated, and steered through a slit and into the mass analyzer which consists of magnet and electrostatic sectors. Together the magnet and electric fields separate the ions based their mass-to-charge ratios with electric sector providing kinetic energy focusing. The separated ions pass through an exit slit where they are detected by an electron multiplier or faraday ion detector. The electron multiplier is used for low ion counts (< 2 million cps) and the faraday for higher ion counts. The instrument's principles are based on the separation of ions. It is used to measure trace elements and isotope ratios and works by having elements move through a plasma source, and in this instance, argon gas was used. The elements become ionized and then separate based on mass (Abou-Shakra, 2018).

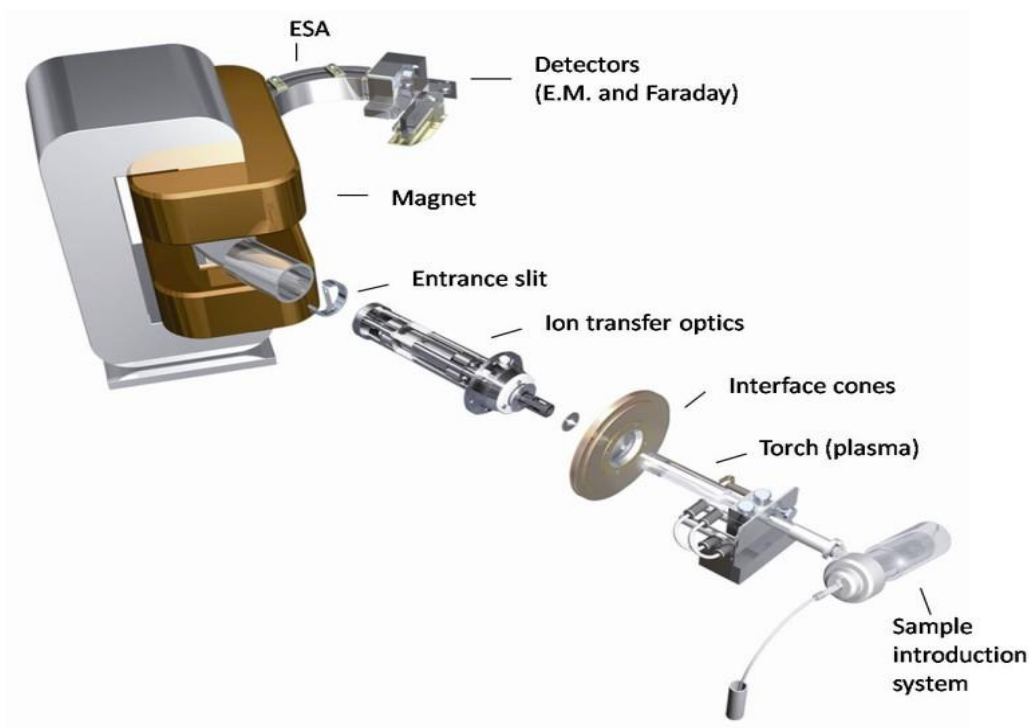


Figure 1: Schematic of the double-focusing ICP-MS used in this study, the Element-XR from Thermo Fisher.

Microwave Digestion Background

Microwave-assisted acid digestion is a common technique used to decompose solid samples so that they can be analyzed by ICP-MS and is useful in trace metal analysis in food products (Figure 2) (Llorent-Martínez, 2012). Briefly, samples are typically weighed into Teflon vessels, acids are added, and the vessels are sealed and placed into the specially designed microwave. A combination of nitric acid and hydrogen peroxide is commonly used to dissolve organic materials for microwave digestion (Aldabe, 2013/Bizzi, 2011/Muller, 2016). These same chemicals were used to dissolve the protein powders in this experiment. A temperature program is set, and the samples are heated. The advantages over open-vessel heating are lower blanks (less contamination) and faster digestions because as higher pressures are reached in the sealed vessels, the boiling point of the solution is increased.



Figure 2: Picture of the Milestone Ethos microwave digestion system used in the study

Purpose of this research

The purpose of this research was to determine the concentration of trace metals in protein powders bought from a local store and to assess the risk associated with consumption of potentially toxic levels of metals. Since the supplement industry is not heavily regulated, trace amounts of toxic metals are in the supplements, which can lead to harmful side effects. In addition, an extensive literature search was performed to assess the current state of the industry, with emphasis on heavy metal contamination.

EXPERIMENTAL

Protein powder samples

The protein powder samples used in this project were sampled from a local smoothie and juice store in Oxford, MS. Vanilla whey protein (Whey brand) and vanilla vegan protein (Arbonne brand) were analyzed in triplicate. Listed on the back of the Arbonne vanilla flavor protein shake mix was Fe (45 μg), Cu (0.69 mg), and Mn (10.5 μg). According to the manufacturer's label, a single serving of Arbonne powder is two scoops, accounting for 17 %, 30 %, and 30 % of the daily recommended intake value of metals, respectively. A single serving of the whey protein powder is one scoop but had no labels listing the amounts of metals present in a single serving.

Sample preparation by microwave-assisted acid digestion

The whey and vegan protein samples were run in triplicate. Table 2 shows the weights of the protein powder samples, ~0.15 g, that were weighed into acid-washed Teflon PFA vessels on an analytical balance (Table 2). For microwave digestion, 1.5 mL HNO_3 and 1.5 mL H_2O_2 (both trace metal grade, Fisher Chemical) were added to each vessel. The vessels were sealed and placed into a Milestone Ethos microwave digestion system. The microwave operates at 1200 W and the temperature program had a 20 min ramp to 180 °C, after which the temperature was held for an additional 30 min. This liberated metals from the protein samples, and since the samples are primarily organic matter, there was little residue after digestion. Each sample and standard were spiked with 10 μg with an internal standard, yielding ~1 ng g⁻¹ Rh in 2% HNO_3 . Two blank samples were run for the control. Once the samples were completely digested, the samples were

transferred into 50 mL polyethylene vials. The resulting samples were diluted to 50 mL with 18.2 MΩ deionized water.

Table 2: Weights of protein powder used for microwave digestion

Sample	Weight (g)
Whey 1	0.1454
Whey 2	0.1435
Whey 3	0.1520
Vegan 1	0.1413
Vegan 2	0.1471
Vegan 3	0.1447
Spiked 1	0.1518
Spiked 2	0.1433
Spiked 3	0.1410

Table 3: Milestone Ethos microwave digestion parameters

Voltage	1200 W
Temperature Program	20 min to 180 °C, held at 180 °C for 20 min.

Determination of metals by ICP-MS

Concentrations of metals (Cd, Pb, V, Mn, Fe, Co, Cu) were determined by sector field ICP-MS using a Thermo Fisher Element-XR. The instrument has resolving power ($m/\Delta m$) settings of low (~300), medium (~3000), and high (~10,000). This allows for certain isobaric interferences to be removed. Samples were introduced using a pfa microflow nebulizer and a pfa cyclonic spray chamber. Table 3-4 shows the data acquisition parameters. The instrument was tuned prior to analysis for sensitivity and stability, yielding ~1 million counts-per-second for 1 ppb of ^{115}In in low resolution mode with <4% relative standard deviation (RSD). The elements were quantified using external calibration. Appendix 1 shows the calibration curve for each element determined.

For each element, a calibration curve was created using the average intensity for the calibration blank and standards at 0.05, 0.1, 0.2, 0.5, 1, 2, and 5 ppb (Appendix 1). The isotopes used for quantitation were: Cd111, Pb208, V51, Mn55, Fe56, Co59, and Cu63. Rh103 was used as an internal standard and incorporated into the results. Pb and Cd were run in low-resolution mode, whereas V, Mn, Fe, Co and Cu were run in medium resolution because of isobaric interferences. Next, each element's test runs of blanks 1 and 2 were averaged. This was then subtracted from the average intensity for each sample for the particular element analyzed.

The y-value in the equation was the intensity calculated by subtracting the average intensities of the blank samples. The x-value in the equation was the concentration determined by ICP-MS. This method was used for each sample of whey and vegan protein powders. However, the final concentration of each respective sample had to be calculated using the dilution factor. This was found by multiplying the concentration found by the final weight of the sample (50 g) and divided by the respective microwave digestion weight listed in Table 2. Once the final concentration was found for each element, the average final concentration for the three whey samples and three vegan samples used were found (Table 2). Error bar percentages were also added to each calibration curve to assess the precision of the experiment.

Table 4: ICP-MS Operating Parameters

ICP-MS	Thermo Fisher Element-XR
RF Power	10,000 W
Nebulizer	PFA Microflow
Spray Chamber	PFA Cyclonic
Replicate	3

RESULTS AND DISCUSSION

Concentrations of eight trace elements (Cd, Fe, Mn, V, Co, Cu, Pb, Cr) in the whey and vegan protein samples are given in Table 5. Accuracy was assessed by co-analysis of NIST 1640 standard reference material (Table 6) along with samples spiked with a known amount of the elements (Table 7). Recoveries for both the reference material and spiked ranged between 78% and 112%, except for Fe, which fell outside this range. This could be due to potential isobaric interferences present in the samples.

Table 5 show the element concentrations present in both the whey and vegan samples. Figure 3 displays the elemental concentration in ppm for Mn, Fe, and Cu. Figure 4 shows the elemental concentrations in ppb for Cd, Pb, V, Co, and Cr.

Table 5 and 8 can be used to compare the elemental concentrations present in the samples to its respective RDA. The amount (mg) / serving for each element was found by multiplying the concentration found by the number of grams for each serving and dividing by 1000. A standard whey sample's amount per serving was one scoop or 40 g, whereas a vegan sample's amount per serving was two scoops or 40 g. All elements for vegan and whey protein powder samples had a lower concentration than the FDA's RDA. The concentrations found in the samples do not appear to pose a risk alone; however, if the consumer has multiple servings per day, which is fairly common among body builders, or if they ingest the trace amounts of metals from other dietary sources during the day, there may indeed be a risk to the individual. This is especially

plausible for the vegan based protein powder for Mn and Fe since the concentration amount (mg) / serving was fairly close to the FDA's RDA per day.

While the single serving concentrations of Mn and Fe were lower than the FDA's RDA, the bioaccumulation of metals in the body can pose a threat. It is common for protein shake users to have more than one protein shake a day, meaning that the serving amount can range from one serving to three servings in a single day. With the increased serving size, the amount of trace metals in the protein powder supplement would increase (Liu, 2013). This could pose a threat to the consumer's health, possibly resulting in health deficits. Some health deficits include neurotoxicity, hepatotoxicity, and an increased risk of cancer (discussed in Introduction).

The percent recovery and error of NIST 1640a (Table 6) can be calculated using the dilution factor and the certified reference values given by NIST 1640a. The sample's intensity was subtracted from the blanks used in the experiment. Each element's calibration curve equation was used and then divided by the dilution factor. The dilution factor was then divided by certified reference value and multiplied by 100 to get the percent recovery. Percent error was found by subtracting the certified reference concentration value by the concentration found and dividing by the certified reference concentration value. This was then multiplied by 100 to get percent error.

The percent recovery for the spiked samples (Table 7) was calculated as well. The sample and spike concentration were averaged and the average concentration in the sample was calculated. The difference was taken between the two and the percent recovery for each element could be calculated. Cu's percent recovery in the spiked samples could not be calculated, possibly a result of interference.

One way the study can be expanded is to test other brands of protein powder supplements. Herbalife, Equate, and Gold Standard are some brands that have different nutritional labels and would add a different component to the study. Also, testing different flavors of protein powder supplements can be done in future analysis. Protein powder supplements generally come in vanilla and chocolate flavoring; and thus, would possibly produce different results than the samples tested due to the different flavoring.

Another way the study could be expanded is to increase sample size. Due to the ICP-MS instrument breaking down during my study, my sample size was limited to the three replicate trials already analyzed. The study can also be expanded by testing different supplement types. Since the supplement industry is not regulated harshly, other supplements could possibly have heavy metal contamination. By expanding my sample types to include diet pills, prenatal vitamins, and other vitamin supplements to see if heavy metal contamination for other supplements is an issue that needs to be regulated.

Table 5: Concentrations ($\pm 1SD$) of Elements in Protein Powders

Element	Whey	Vegan
Cd	9.66 ± 0.646 ppb	33.1 ± 2.38 ppb
Pb	17.0 ± 4.55 ppb	38.0 ± 13.0 ppb
V	18.0 ± 4.85 ppb	167 ± 0.981 ppb
Co	12.2 ± 0.652 ppb	90.4 ± 1.03 ppb
Cr	49.1 ± 50.5 ppb	226 ± 41.4 ppb
Mn	0.203 ± 0.010 ppm	25.5 ± 10.3 ppm
Fe	11.5 ± 4.32 ppm	133 ± 0.814 ppm
Cu	1.91 ± 2.18 ppm	1.39 ± 5.43 ppm

Table 6: Percent Recovery/Error for NIST 1640a

Element	Certified (ppb)	Found (ppb)	Recovery (%)	Error (%)
Cd	3.96	4.44	112	12.1
Pb	12.0	12.4	103	3.33
V	14.9	16.8	113	12.8
Mn	40.1	42.4	106	5.74
Fe	36.5	17.3	48	52.6
Cr	40.2	42.0	104	4.48
Co	20.1	22.2	111	10.4
Cu	85.1	65.9	78	22.6

Table 7: Percent Recovery for Spiked Samples

Element	Recovery (%)
Cd	100
Pb	98
V	112
Mn	112
Cr	95.1
Fe	280
Co	109
Cu	NA

Table 8: Concentration of trace elements in whey and vegan powders along with amounts per serving and FDA criterion (Center for Food Safety and Applied Nutrition). Values in red are at risk of exceeding the FDA’s RDA if multiple servings are consumed.

Element	Concentration \pm 1 SD		Amount (mg) / serving		FDA (mg)
	Whey	Vegan	Whey	Vegan	
Cd	9.66 \pm 0.646 ppb	33.1 \pm 2.38 ppb	0.000386	0.00132	0.025
Pb	17.0 \pm 4.55 ppb	38.0 \pm 13.0 ppb	0.00068	0.00152	0.0125
V	18.0 \pm 4.85 ppb	167 \pm 0.981 ppb	0.000720	0.00668	1.8
Co	12.2 \pm 0.652 ppb	90.4 \pm 1.03 ppb	0.000488	0.00362	0.008
Cr	49.1 \pm 50.5 ppb	226 \pm 41.4 ppb	0.00196	0.00904	0.035
Mn	0.203 \pm 0.010 ppm	25.5 \pm 10.3 ppm	0.00792	1.02	2.3
Fe	11.5 \pm 4.32 ppm	133 \pm 0.814 ppm	0.449	5.32	18
Cu	1.91 \pm 2.18 ppm	1.39 \pm 5.43 ppm	0.0745	0.0556	0.9

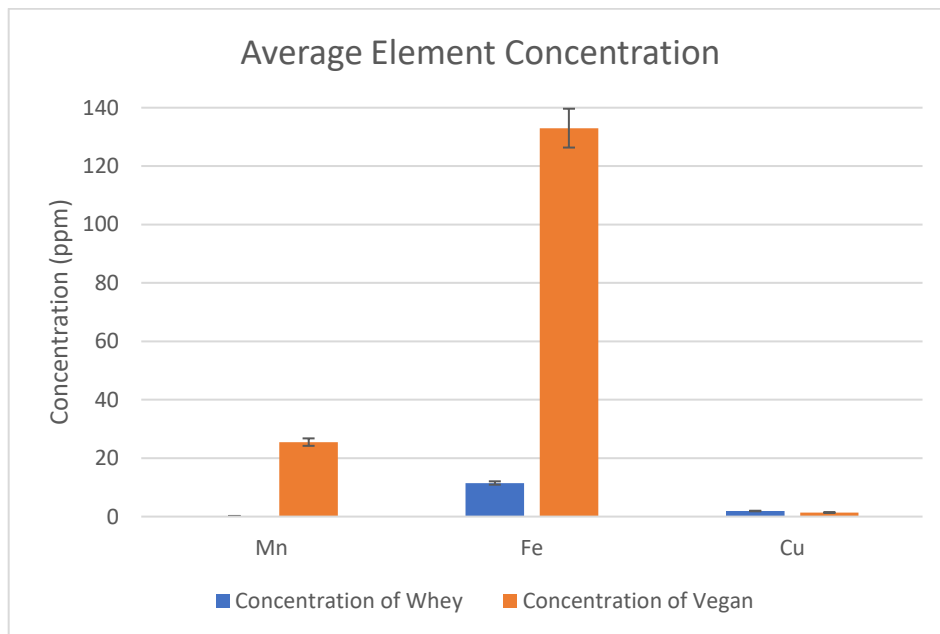


Figure 3: Average concentration of Mn, Fe, and Cu in whey and vegan protein powder

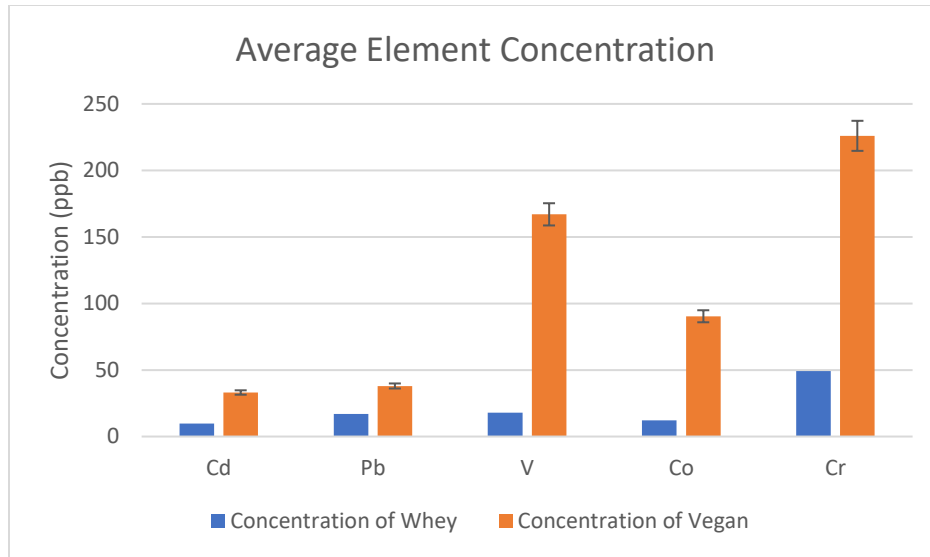


Figure 4: Average concentration of Cd, Pb, V, Co, and Cr in whey and vegan protein powder

Study Limitations

The ICP-MS became inoperable during the course of this study, which prevented us from analyzing more samples. The coronavirus pandemic also slowed down testing and limited the number of trials run. This led to a small sample size in the experiment, which further limited the results of the study. Without more analysis being performed, the results should be considered as preliminary and viewed with caution.

Another study limitation was funding. Without proper funding for the experiment, the sample types were limited. Only two types of protein powders were tested, instead of expanding into other brands and types of protein powder supplements.

The supplement industry is not adequately regulated, restricting the information forthcoming from manufacturers. Without complete nutritional labels on the products analyzed, the CDI cannot be computed.

CONCLUSIONS

The protein powder analysis showed that vegan protein powders had a higher concentration of potentially toxic elements (Cd, Pb, V, Co, Cr, Mn, and Fe) than the whey protein powder samples. The only elemental concentration for amount per serving that was lower in vegan protein powders than whey protein samples was Cu.

The whey samples tested found that all elements had a lower concentration than the RDA set by the FDA as the safe amount that can be ingested (Table 8). The vegan protein powder samples had lower elemental concentrations than the RDA as well. However, the concentrations of Mn and Fe were close to the FDA's RDA. If the consumer chooses to consume more than the average serving, their intake of possibly toxic metals would increase and with the ingestion of heavy metals from other food sources, the amount can possibly exceed the FDA's RDA. This can possibly lead to health issues like neurotoxicity. These higher heavy metal concentrations may be attributed to the plant-based ingredients used. If the soil is rich in heavy metals, then the product produced from the soil might have high heavy metal concentrations as well (Hapke, 1996). Other studies have shown that some vegan farms have used soil for plant-based products that contained elevated levels of metals, which cause the plant-based products to have a higher concentration of metals present (Hapke, 1996). For the whey protein powder samples, the higher concentrations may be attributed to industrial contamination when producing the supplements. Due to the DSHEA, the regulations are more lenient, and bioaccumulation of heavy metals can occur, potentially threatening the lives of consumers (Liu, 2013).

Empirical data, like that determined in this study, is needed to inform consumers and guide policy makers. This research can help better regulate dietary supplements and can help limit the amount of health issues that can arise from heavy metal contamination from protein powder supplements. The FDA's EAM reference has set into place the safe concentration level of possibly toxic metals that can be ingested from food sources. However, dietary supplements are not as regulated as other products on the market. Further research needs to be done to see if other dietary supplements have higher concentrations than the RDA set by the FDA and if there is a possible trend of protein powder supplements having higher metal concentrations than what the FDA says is safe.

APPENDIX 1

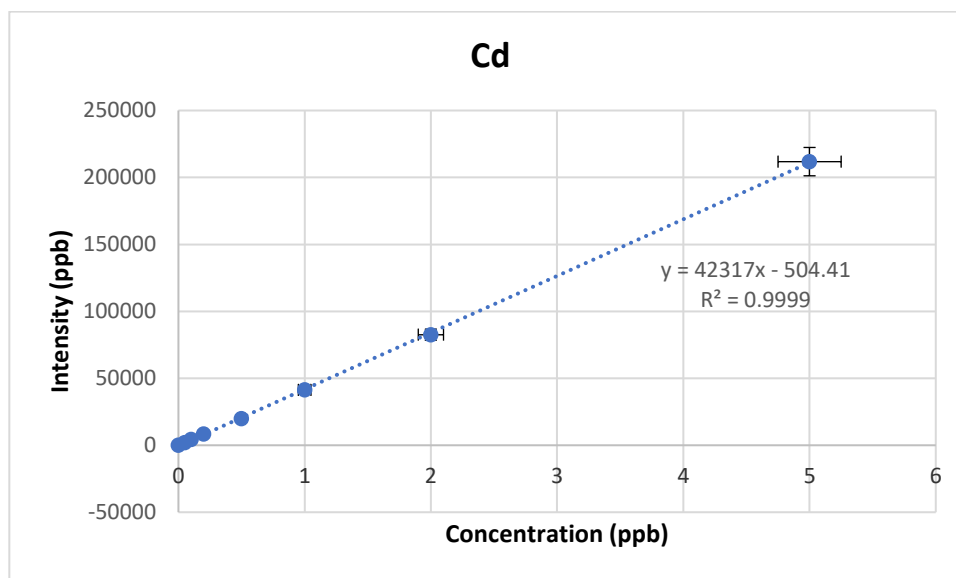


Figure 5: Cadmium Calibration Curve

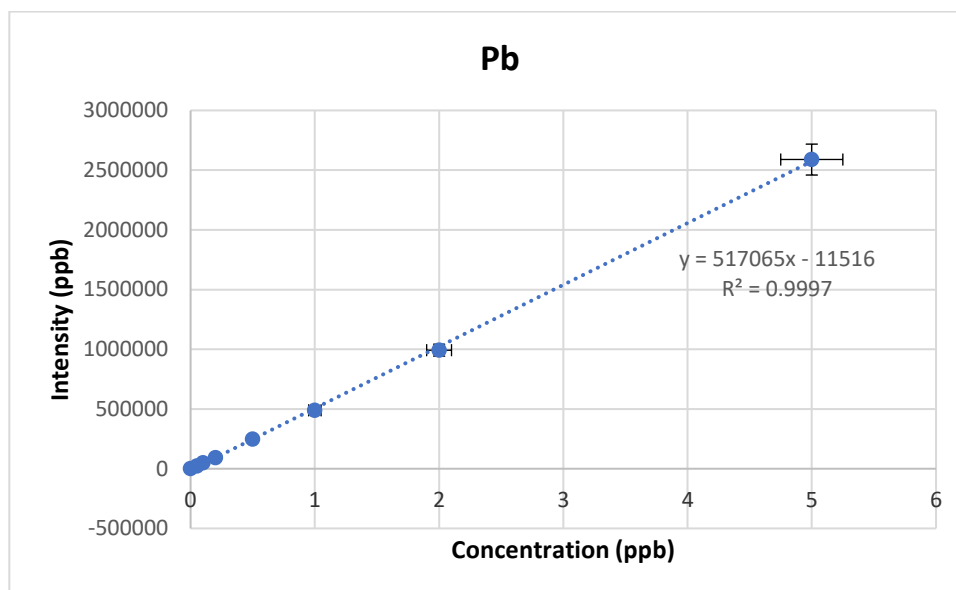


Figure 6: Lead Calibration Curve

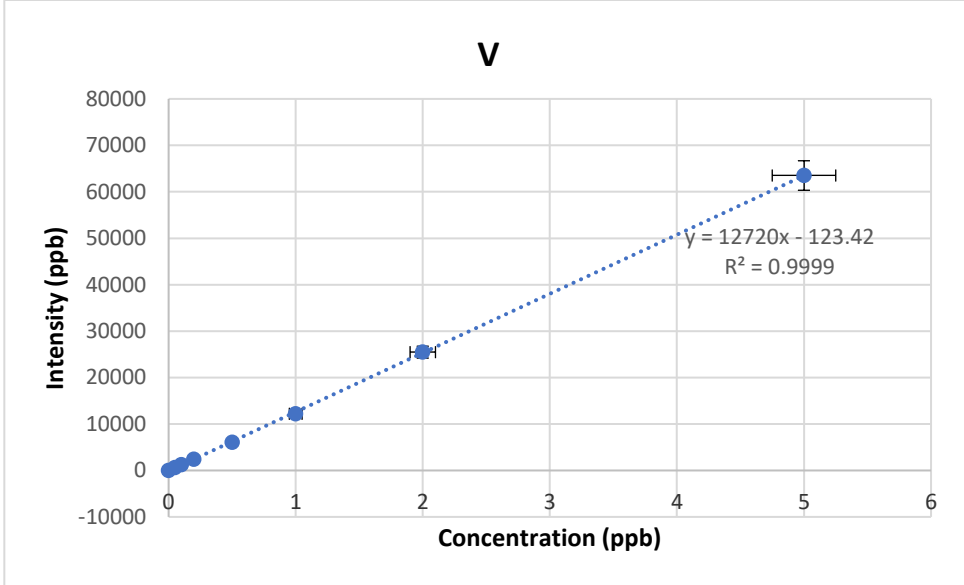


Figure 7: Vanadium Calibration Curve

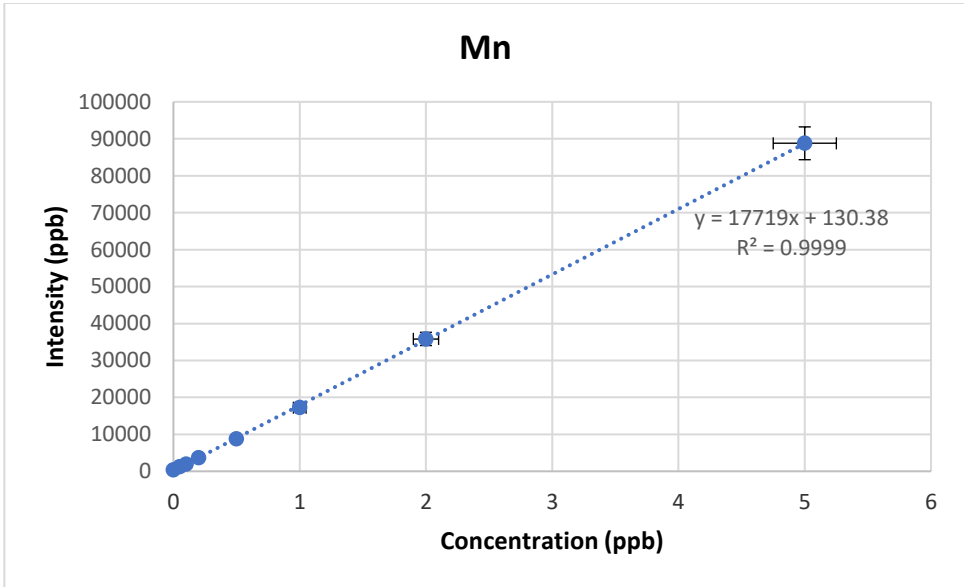


Figure 8: Manganese Calibration Curve

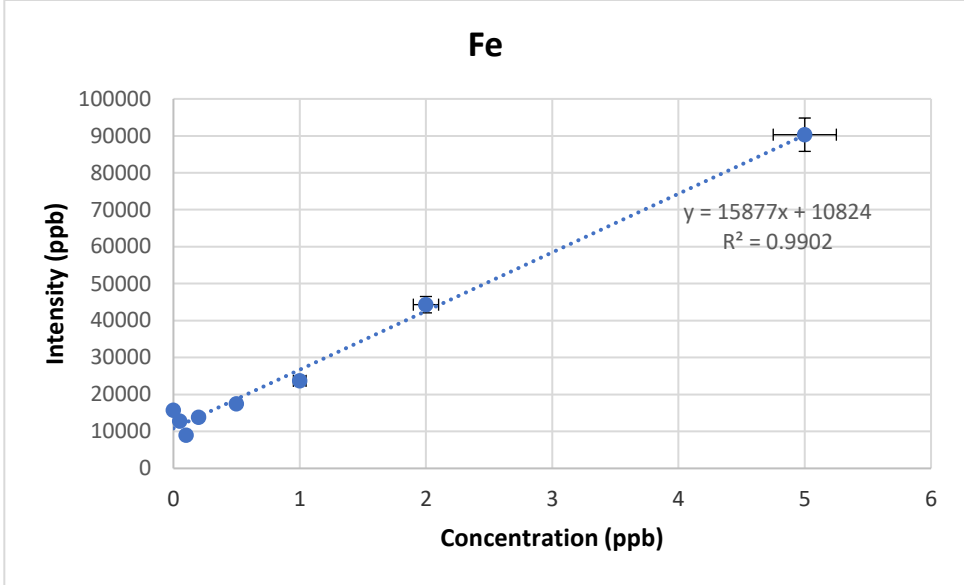


Figure 9: Iron Calibration Curve

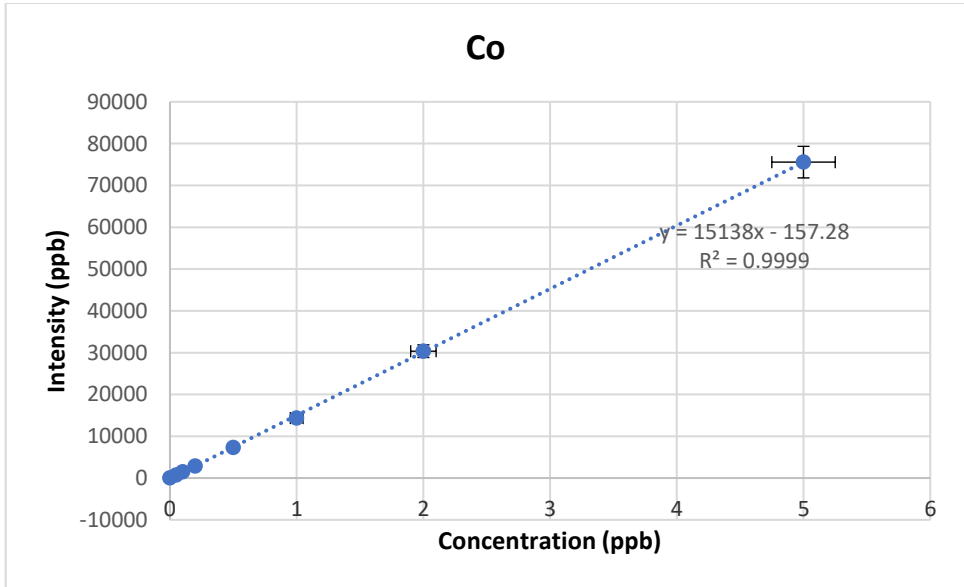


Figure 10: Cobalt Calibration Curve

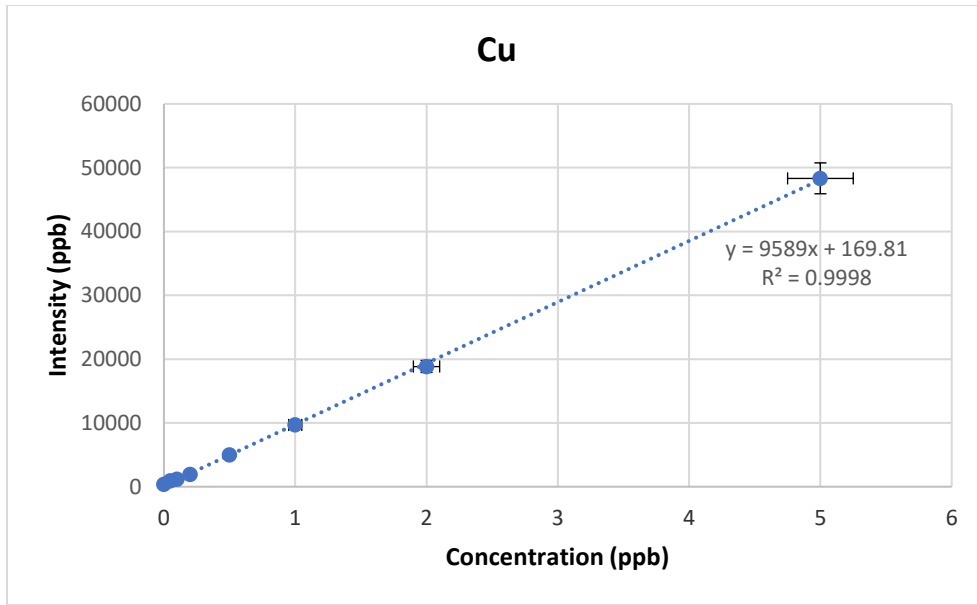


Figure 11: Copper Calibration Curve

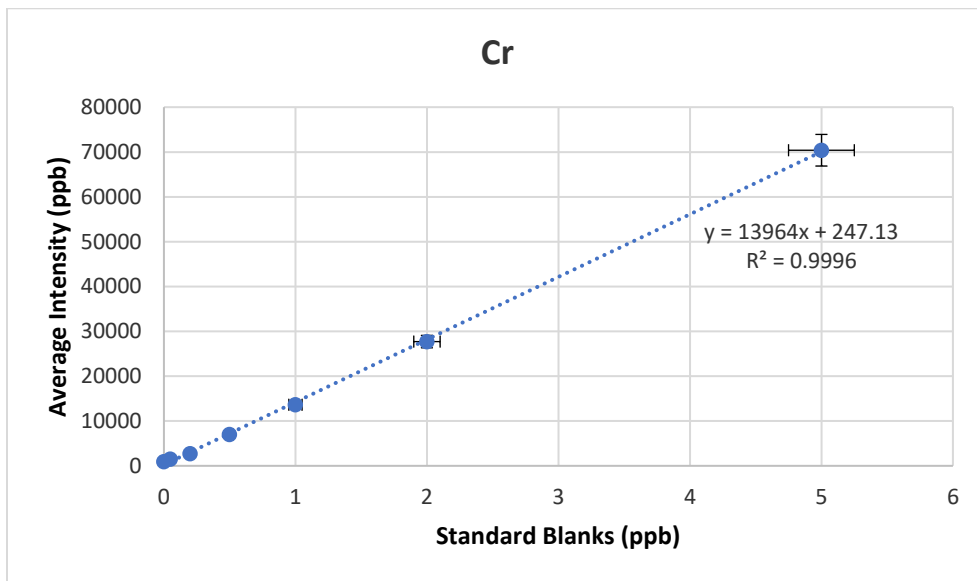


Figure 12: Chromium Calibration Curve

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