Determination of Trace Elements and Arsenic Species in Freshwater Fish

by

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A thesis submitted in partial fulfilment of the requirements for the degree of

Master of Science

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Abstract

Trace elements are naturally occurring in the environment and important to human health. Elevated concentrations of trace elements present in food could cause adverse health outcomes. Among trace elements, arsenic adversely affects about 200 million people around the world, whose drinking water contains arsenic higher than the World Health Organization guideline level of 10 μg/L. Currently, there is no meaningful guideline for arsenic in food, partly because of the complexity of various arsenic species. Arsenic species in seafood have been studied extensively, but not much research has been done on arsenic speciation in freshwater fish. Determination of arsenic species in freshwater fish is challenging because of lower concentrations of diverse arsenic species. This thesis focuses on the determination of trace elements and arsenic species in 266 freshwater fish collected from eight Alberta water bodies (seven lakes and a storm-water pond). For the determination of trace elements, fish samples were microwave-digested with nitric acid, and the concentrations of trace elements were determined using inductively coupled plasma mass spectrometry (ICP-MS). Fourteen elements, including Al, As, Ba, Cd, Co, Cr, Cu, Mn, Mo, Ni, Se, Tl, V, and Zn, were detectable in fish samples and their concentrations were compared among different fish species, eight water bodies, and multiple years of collection. Six elements, Ag, Be, Pb, Sb, Th, and U, were not detectable in any of the 266 fish samples. For the determination of arsenic species, a methanol-water mixture was used to extract arsenic species from fish filet. High-performance liquid chromatography (HPLC) coupled to the ICP-MS technique was used to achieve separation and quantification of arsenic species. A predominant arsenic species in the 266 fish samples is arsenobetaine (AsB), accounting for 34%-95% of all arsenic species detected in the fish. Dimethylarsinic

acid (DMA) and inorganic arsenate (As^V) are the second most commonly detectable arsenic species in freshwater fish samples from Alberta lakes. Monomethylarsonic acid (MMA) was detected in only two fish samples. Five arsenic species, whose chromatographic retention times did not match with any of the available arsenic standards, were also detected. The identity of these five arsenic species remained unknown. Comparisons among the major fish types, including northern pike, lake whitefish, and trout, showed differences in arsenic speciation. Comparisons between two years of fish sample collection from two lakes showed similar concentrations of arsenic species between two years. Comparisons of arsenic speciation results among fish from eight water bodies showed that fish from Cold Lake had approximately 6 times higher total arsenic concentration than fish from other seven water bodies. Most of this difference was attributed to AsB, which was approximately 8 times higher in fish from Cold Lake than fish from other seven water bodies. Future research is needed to understand the reasons for the higher concentrations of AsB and total arsenic in fish of Cold Lake. The results of this thesis research are useful for assessing human exposure to arsenic species and trace elements from fish consumption.

Preface

The introduction chapter, 1.5.1.2, Extraction of Fish, forms part of the collaboration of a review paper with Tetiana Davydiuk, Karen Hoy, Jordan Schofield, and professor X. Chris Le (arsenic speciation in fish, under editing). In Chapter 2 and 3, fish data from Cold Lake in 2016 and Sylvan Lake in 2012 are obtained by Ms. Xiufen Lu. No part of this thesis has been published previously.

Acknowledgements

I would like to express deep appreciation to my supervisor, Dr. X. Chris Le, for his patient guidance, suggestions, and support. His expertise and professional knowledge motivated me through my entire graduate research project. I have learned critical thinking and knowledge from him during group meeting discussions. It was a precious life experience to work with him.

I would like to thank my committee members, Dr. Michael Serpe and Dr. Sarah Styler for their critical comments and valuable ideas on my thesis.

I would like to specially thank Ms. Xiufen Lu for technical support and my research project discussion.

I would like to thank Dr. Anna Jordan for helping me edit the thesis.

I would like to express my great gratitude to the members of our group and other members of the Division of Analytical and Environmental toxicity for your support during my study. Thank you all for providing a friendly and comfortable environment.

I would like to thank my other friends I met in Edmonton for their support and for making my life easier. I'm blessed to have all the friendly people around me in my life.

I would like to acknowledge the Graduate Teaching Assistantship and Graduate Research Assistantship from the Department of Chemistry.

I would also like to thank my family for standing behind me and for giving me endless support whenever I encountered tough times.

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List of Abbreviations

As^{III}	arsenite
As^{V}	arsenate
AsB	arsenobetaine
BR	Beaver Lake
CL	Christina Lake
CO	Cold Lake
CX	Country Sportsplex pond
DMA^{V}/DMA	dimethylarsinic acid
FL	Fork Lake
HF	hydrofluoric acid
HNO_3	nitric acid
HPLC	high performance liquid chromatography
H_2SO_4	sulfuric acid
ICP-MS	inductively coupled plasma mass spectrometry
LD_{50}	median lethal dose
LKWH	lake whitefish
LKTR	Lake Trout
LOD	limit of detection
MMA^{V}/MMA	monomethylarsonic acid
NRPK	northern pike
RNTR	rainbow trout
SQ	Square Lake

SV	Sylvan Lake
UN	unknown
WALL	walleye
WF	Whitefish Lake

CHAPTER 1

Introduction

1.1 Trace elements

Natural and anthropogenic processes affect trace element concentrations in the environment. An excessive concentration of trace elements present in food could cause serious adverse health outcomes. Due to the increase in industrialization and globalization in recent years, some trace elements accumulate and pollute the water environment [1]. Humans may be exposed to trace elements through the food chain. Trace elements pollution have received attention in past decades [2]. Not all trace elements are toxic, they can be classified into three big groups, essential elements (e.g., copper, zinc, magnesium, selenium), non-essential elements, and toxic elements (e.g., chromium, nickel, cadmium, lead, arsenic). Health Canada provided a list of maximum levels of various contaminants in different food. Lead is limited to 0.5 mg/kg, and the total arsenic is limited to 3.5 mg/kg in fish protein [3]. Therefore, quantifying trace elements in the food is important to human health.

1.1.1 Toxic Elements

Excessive toxic elements generally have negative effects on humans and other living organisms. According to their toxic levels, toxic elements can be divided into two major categories. The first group is elements that can reach toxic levels easily, including lead, nickel, beryllium, cadmium, and antimony, and the second group is an excessive concentration of elements that can become toxic, including arsenic and barium [4]. Many authorities determine the different maximum allowable limits (MALs) for different toxic elements.

Cadmium can cross the cell membrane easily; it has a high affinity to binding protein to form the Cd complex [5]. This makes cadmium a more stable form in organisms. Cadmium can be a neuron toxin and is carcinogenic [6].

Antimony is usually present in the Sb mining site, and the concentration can reach 6064–7502 μg/L [7]. The behaviour of antimony generally is considered the same as that of arsenic. Both arsenic and antimony are from the group V elements [8].

Lead is naturally occurring in the environment and is well known as one of the primary contaminants [6]. Lead commonly is used in industry and contributed a large amount to nature [9]. Organic lead is more toxic than inorganic lead. The main lead exposure route is through diet and poses a risk to human.

1.1.2 Essential Elements

Essential elements are important to human life and are involved in vital metabolism in the human body. Zinc, selenium, magnesium, and nickel are considered essential elements.

Zinc is vital to the physiological function, but excessive zinc intake might cause severe nervous system disorders [10]. Lack of zinc might cause behaviour changes [11]. Zinc is a prevalent metal in fish.

Selenium is involved in immune function [12]. It was found that selenium is essential for growth and development. Selenium can protect the cell from damage by free radicals and support the thyroid function and reproduction ability. It is reported that selenium can reduce the toxicity of arsenic. Arsenic and selenium are mutually detoxifying [13].

Nickel is a constituent part of organs. A lack of nickel might reduce growth and result in a low carbohydrate metabolism; it is providing a vital role in metabolism [14].

1.2 Arsenic Background

In all trace elements, arsenic is one of the well-known poisons in the world that affect people. Arsenic is a naturally occurring metalloid. Millions of people are exposed to arsenic from food and drinks in both anthropogenic sources and natural sources. Chronic exposure to arsenic may increase the risk of liver, skin, and bladder cancers, cardiovascular diseases, and diabetes.

1.2.1 Arsenic Chemistry

The atomic number of arsenic is 33, and its molecular mass is 74.921, placing it in group 15 of the Periodic Table. It is categorized as a semimetal or a metalloid in the nitrogen

family. The shell configuration determines arsenic showing four common valance states, –3, 0, +3, and +5 [15]. Different states of arsenic can combine with different elements to form different compounds. Arsenite (trivalent arsenic: +3) is predominant under reducing or anaerobic conditions, while arsenate (pentavalent arsenic: +5) exists mostly in an oxidizing environment. Arsenite can be oxidized to arsenate. Both arsenite and arsenate can bond methyl groups (CH_3) to form methylated organic arsenic species, such as monomethylarsonic acid (MMA^V) and dimethylarsinic acid (DMA^V) [16]. Since further reaction is needed to form organic arsenic, inorganic arsenic is more present in the natural environment. Pentavalent organic arsenic is excreted from the human body at a faster rate and is considered less toxic than inorganic arsenic [17]. Table 1.1 lists the arsenic species studied in this research project and their median lethal dose (LD_{50}). AsB has very little toxicity. The median lethal does for AsB is similar to that of sodium chloride (LD_{50} ~4,000 mg/kg). The toxicity of the arsenic species depends on its valence state and methylation condition. It is very important to determine individual arsenic species, not the total arsenic concentration.

Table 1.1. Acute Toxicity of Common Arsenic Species

Arsenic species	Abbreviation	Median lethal dose (LD_{50}) in rats
Arsenobetaine	AsB	>10, 000 mg/kg [18]
Dimethylarsinic acid	DMA^V	700–2,600 mg/kg [19]
Monomethylarsonic acid	MMA^V	700–1,600 mg/kg [19]
Arsenate	As^V	10–20 mg/kg [19]
Arsenite	As^{III}	10–20 mg/kg [19]

1.2.2 Arsenic Occurrence in the Environment

Arsenic naturally occurs in the air, water, soil, and rock, with an average concentration ranging from 2 to 3 mg/kg [20]. Uncontaminated soil contains arsenic ranging from 1 to 40 mg/kg. The arsenic concentration in sediments depends on the following factors: pH,

redox status, moisture, and microbes [21, 22]. There are over 300 arsenic minerals that can be found in nature, and it ranks as the 20th most occurring trace element in the Earth crust. Arsenosulfides, including arsenopyrite (FeAsS) and orpiment (As_2S_3), are the most common arsenic minerals that exist [23]. Arsenopyrite (FeAsS) can be found at contaminated sites, such as mining sites [24]. Orpiment commonly was used as a pigment in ancient times [25]. Arsenic exists either as a primary mineral-forming element or as an impurity in sulphide minerals. Nature releasing arsenic is a slow process, however, anthropogenic activity, including mining such as grinding, crushing, and heating, increases the release rate [26]. Arsenic minerals are converted readily to inorganic arsenic once in contact with water and oxygen.

Arsenic is distributed extensively and is mobilized readily in the environment. Other than the weathering of rocks, biological activities, geothermal activities, hydrothermal activities, and anthropogenic activities are causing arsenic mobilization [27]. Normally, after arsenic release, arsenic will undergo reduction, methylating, precipitating, and immobilizing back into the soil. Industrial processes, such as coal burning and the usage of pesticides, greatly increase arsenic release in the environment and cause high arsenic concentrations in the aquatic environment. Arsenic is absorbed primarily by particulate matter, while volcanic activity can contribute to arsenic pollution in the atmosphere by the eruption; microbial activity also releases arsenic into the air [28, 29].

1.3 Dietary Exposure to Arsenic

1.3.1 Arsenic in Drinking Water

Arsenic can enter the food chain through many routes, including food and water. The major human exposure to arsenic is through food and water ingestion, while plants absorb arsenic from the soil. Natural contamination present in groundwater affects about 100 to 200 million people worldwide [30]. The current World Health Organization (WHO) guideline for drinking water is limited to 10 μg/L [31]. However, people in Bangladesh, west Bengal, and Vietnam are exposed to arsenic concentrations over 50 μg/L. In west Bengal, about six million people are exposed to arsenic concentrations from 50 to 3200 μg/L [18]. In Europe, arsenic concentrations present in groundwater in almost 400 towns in Hungary are several

times higher than the WHO guidelines [32]. Asia, Taiwan and northern China also are affected by high arsenic concentrations [33].

In Canada, most places show low arsenic concentrations (<10 μg/L). However, some locations in British Columbia, Alberta, Manitoba, New Foundland and Labrador, New Brunswick, Nova Scotia, Saskatchewan, and Québec appeared to have higher arsenic levels (defined as "hotspot", >10 μg/L) than the WHO guideline [34]. Cold lake, for example, located in northern Alberta, has naturally higher arsenic in groundwater [35]. Currently, the natural process caused known hotspots in Canada.

1.3.2 Arsenic in Seafood

The total global captured fish have been 86 to 93 million tonnes per year since the 1980s, while the highest level was recorded as 96.4 million tonnes (a 5.4% increase compared to the past three years). In 2018, marine captured fish were 84.4 million tonnes, an increase from 81.2 million tonnes in 2017. Anchoveta, Alaska pollock, and skipjack tuna are the most captured fish species, accounting for 19% of the total [36]. It is well known that high arsenic concentrations are present in marine fish [37, 38]. To date, arsenic in marine fish has been studied comprehensively. It is reported that more than 300 arsenicals are present in marine organisms [38]. Marine fish is an important food source for humans, and much research has been reported relating total arsenic and different arsenic species in marine fish [39-46].

Arsenicals present in marine organisms vary a lot and usually are found at between 5 and 100 μg/g dry weight [37]. Arsenobetaine accounts for 50% to more than 95% of arsenic species in marine fish [47]. The arsenic uptake may result from its similar chemical behaviour to phosphorous. Phosphate is an essential nutrient for microalgae. When phosphate is deprotonated at a certain pH, arsenate has a similar ionic radius to phosphate. Due to their structural similarity, arsenate could be uptaken by marine algae [48, 49]. Algae gradually convert arsenate to arsenosugars through multiple biomethylation steps and form arsenobetaine [50]. The pathway to biosynthesize arsenobetaine and its function is not clear yet. One possibility for the abundance arsenobetaine in marine fish is the salinity of the sea environment [51]. Arsenobetaine, including other organic arsenic species, is bioaccumulated readily in marine fish, and the arsenobetaine bioaccumulate efficiency is 100% [52]. Arsenobetaine present in marine fish at higher trophic levels is mainly due to the bioaccumulation of organisms at lower trophic levels through the food

web [53]. Other common organic arsenic species, including arsenolipds, thio/oxo-arsenosugar, and methylated arsenicals, are found in marine organisms [37, 38, 54]. Limited inorganic arsenic can accumulate through the food chain in marine fish. Inorganic arsenic species usually account for about 0.5 to 1% of the total arsenic in marine fish meat and maintains a low toxic to nontoxic level in marine fish [47].

The Health Canada guideline for arsenic in fish protein is 3.5 mg/kg**.** A summary of the total arsenic concentration in several marine fish that exceeded the maximum limit was reported [4]. The total arsenic concentration in flathead soles and rock soles in Aleutian Island is 19.5 ± 1 and 4.3 ± 0.7 mg/kg, respectively, while in Italy, red mullet, European hake, blue whiting, and Atlantic mackerel have a higher total arsenic concentration of 59.9 ± 9.5, 38.7 ± 7.7, 35.3 ± 2.8, and 30.8 ± 10 mg/kg, respectively. A review analyzed the arsenic in marine fish (demersal fish, pelagic fish, and molluscs) from the Mediterranean Sea and the European coast of the Atlantic ocean [55]. This was to evaluate the possible adverse health effects exposed to humans. The author collected 25 research papers, while only seven studies conducted an arsenic speciation analysis to specify the toxic inorganic arsenic for a better risk assessment. The average total arsenic concentration, including standard deviation in all demersal, pelagic, and molluscs, was 4.96 ± 5.28, 5.9 ± 6.87, and 3.56 ± 3.33 mg/kg (wet weight), respectively. The inorganic arsenic was 0.14%, 0.412%, 2.37% for demersal, pelagic, and molluscs, respectively. Frequent mollusc consumption in these two areas might cause inorganic arsenic exposure to humans.

Other than Europe and Alaska (US), other places in the world also have been studied. A regional study was conducted in Salvador, Bahia, and northeastern Brazil [56]. The average total arsenic concentration in amberjack, catfish, tuna, lookdown, acoupa weakfish, dolphinfish, grouper, whitemouth croaker, snook, mullet king mackerel, snapper, flounder, and mullet was 0.72 ± 0.39 mg/kg. Lookdown contained the highest arsenic concentration (1.85 mg/kg), while amberjack contained the lowest arsenic concentration (0.12 mg/kg). Yang et al. compared the arsenic concentration in Japanese Spanish mackerel, yellow croaker, sardine, barracuda, Japanese seaperch, and largehead hairtail from northeast China [57]. The average arsenic concentration in the fish studied was 1.37 mg/kg (range from 0.17 to 5.04 mg/kg, wet weight).

1.3.3 Arsenic in Freshwater Fish

A record of 96.4 million tonnes of global captured fish was reached in 2018, out of which 12 million tons of freshwater fish was from inland fisheries [36]. Africa accounted for 25%, America for 9%, and Asia for 57% of inland fisheries in 2018. China, India, Bangladesh, Myanmar, and Cambodia are the top five countries that produce the most inland water captures. The four main fish groups constitute 85% of inland fisheries. The first fish group is carps, barbels, and other cyprinids, which increased from 0.6 million tonnes in the mid-2000s to 1.8 million tonnes in 2018. The second fish group, tilapia and other cichlids captured fish every year ranged from 0.7 million tonnes to 0.85 million tonnes. The rest of the two groups, freshwater crustaceans and freshwater molluscs, remain stable at between 0.4 million tonnes and 0.45 million tonnes per year.

Currently, arsenic species in marine fish have been studied extensively, but not much research has been done on arsenic in freshwater fish. It is analytically challenging to determine arsenic species in freshwater fish. The freshwater environment generally contains lower total arsenic than seawater. The average total arsenic concentration in seawater is 1.7 μg/L, while the average arsenic concentration in freshwater is 0.8 μg/L [58, 59]. Yang et al., who were mentioned in the marine fish part also analyzed freshwater fish and compared the total arsenic concentration between freshwater fish and marine fish [57]. Four freshwater fish species, including grass carp, crucian carp, carp, and bighead carp, were investigated. The arsenic concentration in freshwater ranges from 0.007 to 0.49 mg/kg, with an average concentration of 0.075 mg/kg, while the average marine fish arsenic concentration is 1.37 mg/kg (0.17–5.04 mg/kg). Arsenic present in freshwater fish is much less than in marine fish. A US market basket study also shows a similar trend from Yang [60]; the average arsenic concentration in freshwater fish is 0.16 mg/kg, while in marine fish it is 2.36 mg/kg. This trend also matched a study that was performed in the Belgian market [61]. The huge difference in arsenic concentration in freshwater fish and marine fish might be due to the sampling places, the fish trophic level, the fish species captured, and other factors [62].

The arsenic concentration in freshwater fish also might correlate with seasonal changes [63]. Arsenic in water reservoirs of Chihuahua County (Mexico) varies depending on the water sampling, season, and location of reservoirs. Another survey found that the

average arsenic was highest in February (0.11 mg/L) in the Conchos river (Mexico), while it was lowest in October (0.01 mg/L) [64].

The arsenic speciation pattern in freshwater fish varies, and the dominant arsenic species may vary depending on the type of fish and the freshwater environment. One paper analyzed the arsenic extracted from salmonids, nase, barbel, Danube roach, burbot, and catfish [65]. AsB was present and predominant in catfish, barbel, Danube roach, and all the *Salmonidae* family. DMA was present as the dominant arsenic species in all three nase samples, with TMAO and unknown arsenic species detected. Another paper collected northern pike, largemouth bass, yellow perch, and pumpkinseed in Moira lake, Canada [66]. Tetramethylarsonium ion (TETRA) was the dominant arsenic species in both largemouth bass and pumpkinseed, and DMA is predominant in northern pike. As^V is present as the main arsenic species in yellow perch. Except for common arsenic species, oxo-arsenosugar-glycerol (AsSugar-OH), thio-arsenosugar-phosphate (Thio-AsSugar-PO_4), oxo-arsenosugar-phosphate (AsSugar-PO_4), oxo-arsenosugar-sulfate (AsSugar-SO_3), arsenolipids, and unknown species also were detected [54, 67-69].

There are many freshwater lakes in Alberta, which hold various freshwater fish and serve as a food source for local people. Knowing the concentrations of the arsenic species in freshwater fish is significant to human health.

1.4 Arsenic in Humans

Chronic exposure to arsenic is associated with an increased risk of lung, skin, bladder, and liver cancers, and non-cancer diseases, including cardiovascular diseases and diabetes [70]. Excessive arsenic is causing severe problems. In India and Bangladesh, millions of people are suffering from skin lesions that are caused by high arsenic contaminated water [71]. A peripheral vascular disease, named “Blackfoot disease, (BFD)”, found in Taiwan [72, 73] and turned out to be related to chronic exposure to arsenic in drinking water. Chronic arsenic exposure also may damage respiratory systems and cause reno-vascular diseases, as well as impair cognitive capacity and cause lower intelligence in infants [74, 75].

The major route of human arsenic exposure is through drinking water. Arsenic tends to accumulate in keratin-rich tissues, including skin, hair, and nails [76]. The average arsenic concentration in hair ranges from 0.08 to 0.25 μg/g. Exceeding this value indicates

over-exposure to arsenic [77]. Arsenic concentration in hair and nails generally indicates the arsenic exposure level [78].

After exposure to arsenic, the human body goes through an arsenic biomethylation to form different arsenic species (Figure 1.1). Methylation reactions and oxidation-reduction reactions can transform toxic inorganic arsenic into pentavalent and trivalent arsenic species [79]. Until the detection of MMA^{III} and $DMA^{III,}$, arsenic biomethylation was considered a detoxification process since MMA^{V} and DMA^{V} are less toxic than inorganic arsenic. Many studies revealed that MMA^{III} and DMA^{III} are more toxic than inorganic arsenic [80, 81]. This contradicted the idea that the biomethylation process is a detoxication process.

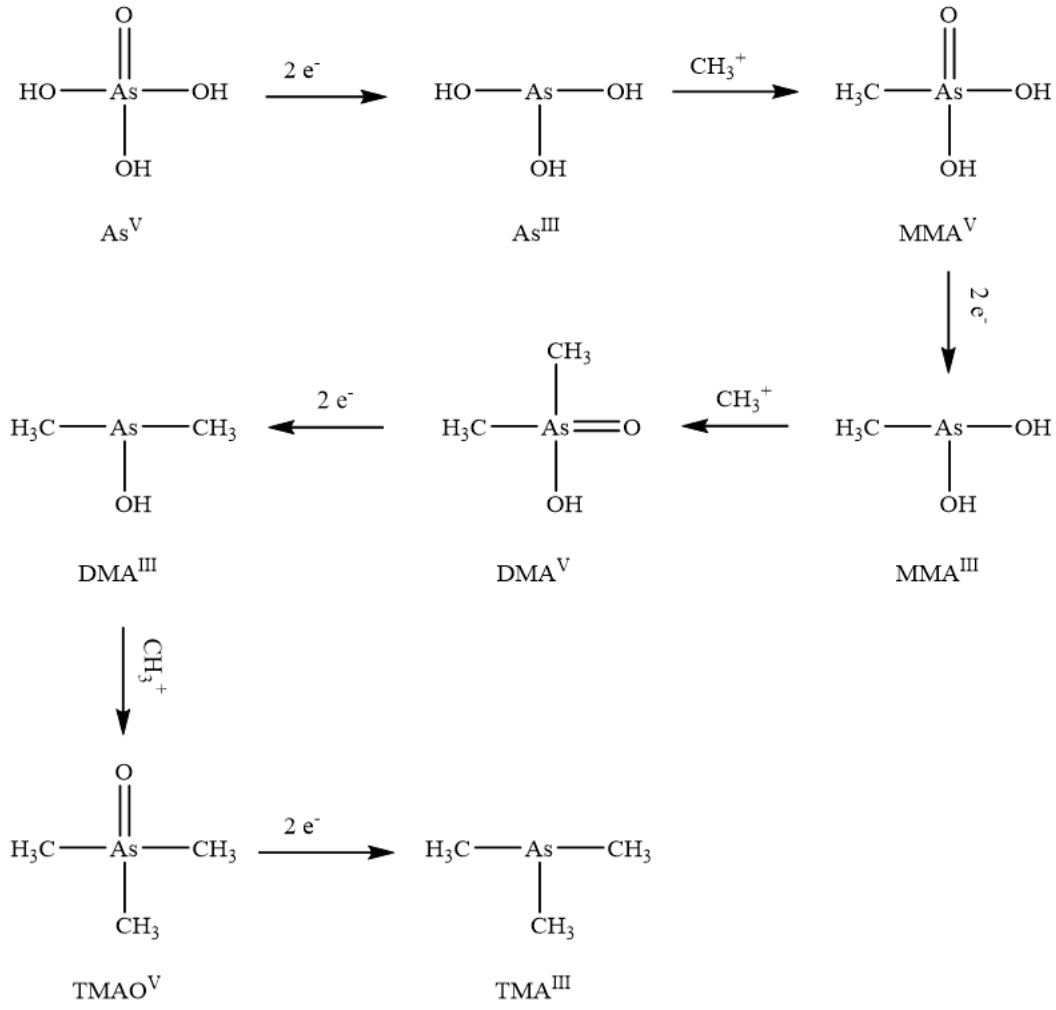

Figure 1.1. Arsenic biomethylation pathway in the human body [79].

1.5 Technology for Metal Analysis and Arsenic Speciation

1.5.1 Sampling and Pretreatment of Samples

Many factors need to be considered for sampling fish. Contamination and sample loss might occur during sample collection. A portable instrument might be used onsite after sampling. However, a portable detecting technique is not suitable for this research project since the objects we are studying are solid. It also is important to consider weather conditions and transportation approaches. Dry ice (solid carbon dioxide) usually is used in the transportation of meat [82]. In addition, the arsenic species transformation should be considered. Inorganic arsenic, As^{III} and As^{V}, may interconvert during the storage time [83]. Light, moisture, temperature, oxygen activity, and microbial creatures might change the

arsenic species. To preserve the original arsenic species distribution, fish should be stored at low temperatures. Temperature as low as –20 °C will maximize the effect to prevent microbial activities in fish [84]. Vacuum packing eliminates oxygen and minimizes oxidative activity [85]. Freeze-drying meat to a powder also can preserve samples. Sample homogenization is necessary prior to the extraction process. Small fish particles can maximize the contact area with the extracting solvent and obtain optimum extraction efficiency.

1.5.1.1 Digestion of Fish

To analyze the trace elements present in fish, liquidizing the fish meat is necessary. Dry ashing and chemical digestion are two methods that are used extensively. Dry ashing requires a high temperature (about 550 °C) for a long time and eventually discomposes to a solid [86]. Chemical digestion usually is performed by different acids, such as HNO_3-H_2SO_4, HNO_3-HCl, and HNO_3-HF [87]. A lower temperature can be applied to acid digestion, and H_2O_2 also was used to decompose the organic proportion in the solid.

Microwave-assisted digestion became popular in the late 80s. It can digest solid and release elements into the acid solution safely, efficiently, and rapidly [88]. Closed vessels were used in sample preparation and were heated by microwave. The instrument also provides on-time temperature and pressure change monitoring during digestion. Less reagent is required, fewer solid residues are formed, free of matrix interference to the instrument, and no sample loss are the highlight of microwave digestion [89]. Microwave-assisted digestion can reach a high digestion efficiency, and it is used for different samples for trace element analysis, namely, plant, soil, and meat [90-92].

1.5.1.2 Extraction of Fish

In arsenic speciation analysis procedures, an important step is the sample preparation. During its preparation, each step must maintain the original arsenic speciation concentration and chemical forms without contamination, losses, and interconversion, while obtaining maximum extraction efficiency of arsenic species and good reproducibility [93]. Compared with the total arsenic concentration in marine fish, arsenic concentrations in freshwater fish are much lower and more variable in arsenic species composition [61,

94, 95]. When a small amount of total arsenic is distributed as several arsenic species, it is more challenging to extract and quantify each arsenic species from freshwater fish.

Arsenic species must be extracted from solid fish samples into a solution. Extraction efficiencies of arsenic species use a variety of extraction methods. The conventional sonication bath is a classic method of extracting arsenic species from freshwater fish [96-98]. Water or methanol-water is the most common solvent for extracting arsenic species [67, 69, 99]. A mixture of methanol and water in different ratios or pure water commonly have been used as the extraction solvents. However, methanol/water extraction can extract only water-soluble arsenic species, which results in a low extraction efficiency. For extraction of nonpolar arsenic species, acetone or hexane extraction was performed, followed by methanol-water extraction, and then the supernatants were combined for evaporation [100, 101]. Organic solvents, such as methanol, a mixture of dichloromethane (DCM) and methanol, and chloroform-methanol-pure water, were helpful to extract nonpolar arsenolipids from fish samples [100, 102].

It has been reported that the low extraction efficiency of inorganic arsenic from biological matrices could be due to the inability of the water-methanol mixture to break the bonds between As^{III} and thiol groups in proteins [103]. Various enzymes, such as trypsin, pancreatin, pepsin, pronase E/lipase, protease XIV/α-amylase, and a combination of enzymes, have been used to assist the extraction of arsenic species from fish and plant samples [103-106]. The functions of enzymes are to help digest fats, break cell walls, and digest proteins by hydrolysis of peptide bonds. Enzyme assisted extraction typically was conducted in a buffer solution, e.g., tris-HCl or phosphate buffer, which are compatible with the selected enzymes. However, enzyme-assisted enzymes require a long incubation time for hydrolysis. An inorganic arsenic background in an enzyme buffer solution was reported in the literature [107], and purification of the enzyme solution before extraction was performed [104]. Freshwater fish has relatively low arsenic concentration, and enzyme-assisted extraction may not be suitable for this project.

Microwave-assisted extraction (MAE) has been used successfully for speciation analysis. Compared with conventional extraction techniques that rely on mechanical sonication or shaking, MAE uses smaller amounts of solvents and a shorter extraction time

[103]. MAE, sonication probe, and ultrasound water bath help extract arsenic species in a shorter time (less than one hour). This is particularly useful for speeding up the enzyme-assisted extraction because enzymatic hydrolysis often requires an extensive incubation time (several hours) [108]. Chen and Jiang et al. optimized various extraction solutions and selected a mixture of 1% (v/v) HCl and 0.1% (m/v) protease XIV for MAE. They detected AsB, As^{III}, As^{V}, MMA, and DMA in freshwater tilapia and bass, with an extraction efficiency of more than 95% [109]. By combining enzyme-assisted extraction and MAE, the extraction efficiency is higher compared to methanol-water sonication extraction. MAE commonly uses acid to extract arsenic species, which might cause sample loss. The extraction efficiency of methanol-water is lower, but this method can maintain the integrity of the arsenic species extracted from fish. We used the traditional methanol-water extraction.

1.5.2 Determination of Arsenic Species Using High-Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) is a commonly used analytical method for efficiently separating multiple components in a mixture. Many chromatographic columns, such as anion exchange, cation exchange, reverse phase exchange, and ion pairing column, were used in HPLC.

An anion exchange column is used widely in separating arsenic species, including AsC, AsB, As^{III}, MMA, DMA, and As^{V} [110]. The anion exchange column was packed with polymer and positively charged groups were attached. The proton association-dissociation equilibrium (pK_a) and the pH in the HPLC mobile phase play an essential role in the elution time [111]. Arsenic species mostly have a pK_a smaller than 8.0 [112]. Different arsenic species exchanged ions to the column and eluted at different times. Arsenic species, which were charged with more negative ions stay longer in the column. A commercially available strong anion exchange column, Hamilton PRP-X100, can achieve an efficient separation for common arsenic species. However, AsC is a cationic arsenic species, which elutes at void volume. Other cationic arsenic species will coelute with AsC before AsB.

A cation exchange column is useful for separating cationic arsenic species, such as TMAO, TETRA, AsC, and AsB that do not retain in an anion exchange column [113]. Instead of positively charged groups, negatively charged groups are attached to the polymer in the cation exchange column. The two toxic arsenic species, As^{III} and As^{V}, may not retain in the cation exchange column. Many studies used both the anion exchange column and the cation exchange column [65, 66, 69]. An anion exchange column was used primarily to separate common arsenic species. If there are any arsenic species coeluted at void volume, a cation exchange column may be used for the secondary separation to determine the rest of the arsenicals.

1.5.3 Detection of Trace elements Using Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

There are many detecting techniques available to determine metal elements. These techniques include atomic absorption spectrometry (AAS) [114], atomic fluorescence spectrometry (AFS) [115], inductively coupled plasma atomic emission spectrometry (ICP-AES) [116], and inductively coupled plasma mass spectrometry (ICP-MS) [117]. ICP-MS is a commonly used technique to date [118] that allows multiple element detection, high sensitivity, low limit of detection, wide dynamic range, and low sample volume [119]. Hydride generation can be coupled to ICP-MS to decrease the instrument detection limit further [120]. However, this technique can be used only for arsenic species, which can form volatile hydrides. Many organic arsenic species cannot be converted and detected.

First, the liquid sample solution is introduced into the nebulizer by a peristaltic pump and aerosolized. A spray chamber filters large aerosol droplets, leaving 1–2% of the sample exiting to the torch. The instrument uses argon gas ionization to form argon plasma, which causes temperatures up to 10,000 K. The sample is vaporized and ionized by inductively coupled plasma. Sample ions are passed through vacuum to electrostatic lenses and reach the mass analyzer. The quadrupole is a widely used detector.

Arsenic ion has an m/z of 75 so that if the sample contains chloride, argon chloride ion ($^{40}Ar^{35}Cl^{+}$) can form and interfere with the arsenic detection. Argon chloride isobaric ion ($^{40}Ar^{37}Cl^{+}$) has the same m/z as ^{77}Se, which interferes with the selenium detection; selenium (m/z 82) also is monitored [68]. To avoid common polyatomic interferences, a

collision or reaction gas is introduced to the collision cell before the analyte enters the quadrupole. A reaction gas forms ions and the ions collide with the gas molecules to reduce the polyatomic interference to a large extent. Analyte ions also might be affected but to a lesser extent than polyatomic ions. Lower polyatomic interference results in lower detection limits through this process, so-called kinetic energy discrimination (KED).

The sample ions are multiplied by an electron multiplier and detected. Mass spectra are generated for the elements to be detected. Through a calibration curve, the peak intensity of a sample mass spectrum can be converted to a concentration (parts per billion). With ICPMS, we can detect multiple elements in 1 to 3 min.

1.6 Study Hypothesis and Objectives

This study is part of a survey of trace elements and arsenic species in Alberta freshwater fish for over a decade. Throughout the study, the Alberta Government will know the baseline concentration for trace elements in Alberta freshwater fish. Our results will help us understand the nature of arsenic species in different fish species in different Alberta lakes. The results will be provided to Alberta Health and Health Canada for provincial and national environmental surveillance and for considering future dietary guidelines.

I hypothesize that different freshwater fish species have different arsenic speciation patterns and trace elements concentration profiles, while some fish species have non-toxic arsenobetaine (AsB) as the predominant species, other fish species contain mainly toxic forms of arsenic (inorganic and methylated). This information is important for assessing the dietary intake of toxic arsenic species from freshwater fish.

First, 20 trace elements were analyzed in Alberta freshwater fish. The ICP-MS method will be validated and applied to the total element analysis in freshwater fish due to its high sensitivity and multi-element detection. The trace elements profiles will be evaluated in different fish species, different lakes, and different years to better understand Alberta freshwater fish.

Second, the arsenic speciation pattern will be investigated massively in Alberta freshwater fish. HPLC coupled with ICP-MS will be validated and applied to an arsenic speciation analysis in freshwater fish. I will focus on the arsenic speciation pattern and the

arsenic concentration in different fish species, the same fish species in different lakes, regardless of years, the different fish species in the same lake, and the same fish species in the same lakes between two years to provide an Alberta arsenic profile in freshwater fish.

I will compare the trace elements profile and arsenic speciation profile in Alberta freshwater fish with Health Canada and WHO guidelines and evaluate whether they are safe for human consumption.

1.7 References

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CHAPTER 2

Determination of Trace Elements in Freshwater Fish

2.1 Introduction

Trace elements are spread widely worldwide. They are associated closely with the environment and the health of living creatures. Trace elements may be divided into three groups, essential elements, non-essential elements, and toxic elements [1]. Both natural and anthropogenic sources contribute to trace elements detected in the environment. Natural sources include bedrock weathering, soil, and volcano eruptions. Anthropogenic sources are mainly from industrial processes, including mining, combustion, and disposal of waste [2].

Human exposure to trace elements is mainly through food and water [3]. Fish is one of the food sources for humans. Alberta holds various lakes, and fish are commonly consumed by local people. Knowing the concentration of trace elements is essential for assessing daily intake from fish and for the government to make future food consumption guidelines. In my study, 20 elements, including ^{9}Be, ^{27}Al, ^{51}V, ^{52}Cr, ^{53}Cr, ^{55}Mn, ^{56}Ba, ^{59}Co, ^{63}Cu, ^{65}Cu, ^{66}Zn, ^{75}As, ^{77}Se, ^{82}Se, ^{95}Mo, ^{105}Ag, ^{111}Cd, ^{114}Cd, ^{121}Sb, ^{137}Ba, ^{205}Tl, ^{208}Pb, ^{232}Th, and ^{238}U were considered for analysis. A long-term goal was to determine these elements in freshwater fish from Alberta lakes and gain information on their background levels and any temporal and special changes.

A number of techniques can be used for the determination of trace elements. ICP-MS has the following advantages for trace analysis: multi-element detection, high sensitivity, low detection limit, wide dynamic range, and high sample throughput [5]. The object of this chapter was to determine trace element concentrations in freshwater fish using ICP-MS. Samples of common local fish were collected from a variety of Alberta lakes in multiple years. The results should allow for preliminary anlaysis of any possible trends with respect to fish species, lakes, and the year of sample collection.

2.2 Materials and Methods

2.2.1 Reagents and Standards

An environment standard for ICP-MS (Agilent Technologies, US) was used as a calibration standard solution and diluted for daily analysis. Nitric acid (certified ACS plus) (Fisher Scientific, US) was used for fish acid digestion. A Millipore Milli-Q integral system (18.2 MΩ cm, Millipore (Sigma, Fisher Scientific, US) was used to purify tap water and produce deionized water. A standard reference material (SRM) 1643f of trace elements in water, from the National Institute of Standards and Technology (NIST, Gaithersburg, US) was used for quality control of trace element analysis. SRM 1566b oyster tissue and DORM-4 fish protein were used for acid digestion method quality control.

All vessels, beakers, 15 mL polypropylene centrifuge tubes (Fisher, US), and 10 mL volumetric flasks for trace element analysis were washed with tap water, rinsed with deionized water, and then soaked overnight in a 5% HNO_3 tank. The next day, all equipment was rinsed thoroughly three times with deionized water and dried before use.

An environmental standard solution (10 mg/L) (Fisher Scientific, US) was stored at 4 °C before the time of analysis.

2.2.2 Freshwater Fish Samples

Fish samples were collected from seven Alberta lakes and a storm-water catchment pond by Alberta Health (AH). These eight water bodies were Beaver Lake (BR), Christina Lake (CL), Cold Lake (CO), Fork Lake (FL), Square Lake (SQ), Sylvan Lake (SV), Whitefish Lake (WF), and County Sportsplex Pond (CX). County Sportsplex Pond (CX) is a storm-water catchment pond, and rainbow trout was stocked. The fish fillets were cut and sealed by the Alberta Centre for Toxicology (University of Calgary, AB). The fish fillet samples were shipped on dry ice to the University of Alberta, AB and stored at –80 °C until analysis.

2.2.3 Microwave-assisted Acid Digestion

The fish fillet samples were thawed first at room temperature overnight. After they thawed, they were ground in a blender (Kitchen Aid) till the meat texture is uniform. A 1 ± 0.1 g portion of the ground fish sample was weight into a vessel and the weight was recorded precisely. In the fume hood, 5 mL optimum (68.0%–70.0%) HNO_3 was added slowly to the fish meat, covered with Kimwipes (Fisher Scientific, US), and digested overnight. To

the vessels were added 5 mL of deionized water after overnight digestion. A plug and cap were put on the vessels, and the cap was closed tightly with a plastic block to avoid any acid escape during digestion. Then, the vessels were microwave-digested by MARS 6 (CEM, US) for complete fish dissolution. The fish mixture was heated to 200 °C for 20 min and stayed at 200 °C for 25 min. The next day, the digested solution was transferred to a 50 mL beaker, and the vessel was rinsed with 10 mL 2% HNO_3 three times in a fume hood. The beaker was heated on a hotplate at 200 °C until the acid solution had evaporated to less than 3 mL, while maintaining trace elements dissolved in acid. The entire digest was transferred to a 10 mL volumetric flask and diluted with 2% HNO_3. Then, the solution was poured into a 15 mL centrifuge tube. Each fish sample and blank were prepared in triplicate.

A 0.25 g portion of certified material SRM 1566b oyster tissue and DORM-4 fish protein were weighed, and the weight was recorded. The certified materials were digested by the same approach as the fish samples. They were used to ensure the accuracy of the acid digestion method. The fish supernatant and fish residue for mass balance calculation (Chapter 3) also were digested in the same manner as the fish samples.

2.2.4 Determination of Trace Elements Using ICP-MS

We used Agilent 7500cs ICP-MS (Agilent Technologies, Japan) to analyze 20 elements. Samples were introduced to the instrument by an ASX-510 autosampler (CETAC, Omaha, US). The optimum ICP-MS operation conditions are shown in Table 2.1. ^{9}Be, ^{27}Al, ^{51}V, ^{52}Cr, ^{53}Cr, ^{55}Mn, ^{56}Ba, ^{59}Co, ^{63}Cu, ^{65}Cu, ^{66}Zn, ^{75}As, ^{77}Se, ^{82}Se, ^{95}Mo, ^{105}Ag, ^{111}Cd, ^{114}Cd, ^{121}Sb, ^{137}Ba, ^{205}Tl, ^{208}Pb, ^{232}Th, and ^{238}U were monitored by ICP-MS under helium collision mode. The helium collision mode can remove argon chloride ion ($^{40}Ar^{35}Cl^{+}$) effectively and prevent it from reaching the mass analyzer [6].

A trace element standard solution was diluted from the environmental standard (10 mg/L) with 2% HNO_3. Two sets of calibration curves were prepared, as different elements naturally are present in higher or lower concentrations in fish samples. The calibration curve with lower concentrations is 0.1 µg/L, and 0.2 µg/L, 0.3 µg/L, 0.4 µg/L, 0.5 µg/L, 1 µg/L, 5 µg/L, 10 µg/L, and 20 µg/L were used to analyze elements like arsenic and lead that have a lower concentration in fish samples. The calibration with higher concentration is 5 µg/L, and 10 µg/L, 20 µg/L, 30 µg/L, 40 µg/L, and 50 µg/L were used to analyze

elements like aluminium and zinc that have a higher concentration in fish samples. Since zinc has a higher occurrence in fish, we diluted the samples further, according to the zinc concentration, to fit in the calibration curve and repeated the analysis to obtain the concentration.

The 5 µg/L standard was used as a quality control to check the instrument drifting for the lower concentration calibration curve, and the 30 µg/L standard was used for the higher concentration calibration curve. The quality control solution was analyzed every 12–15 samples. The standard reference material was 1643f trace elements in water and was used to ensure the accuracy of daily instrument analysis.

Table 2.1. ICP-MS Operation Conditions

ICP-MS parameters	
RF power	1550 W
Octupole bias	-18 V
Quadrupole bias	-15 V
Ar gas	
Carrier gas flow rate	0.98 L/min
Makeup gas flow rate	0.1 L/min
Spray chamber temperature	2 °C
Collision gas	
He gas	3.2 mL/min

2.3 Results and Discussion

2.3.1 Determination of Trace Elements in Fish Using ICP-MS

We used highly sensitive ICP-MS to perform trace element analysis. The method detection limit was determined using the United States Environmental Protection Agency (USEPA) method [7]. At least seven spiked samples (concentration three to five signal-to-noise ratio) were prepared and went through all sample preparation steps. The standard deviation was

calculated and multiplied by the student's *t*-value for a single-tailed 99th percentile, which is the method detection limit. The limit of detection (LOD) of each trace element is shown in Table 2.2. A low LOD is necessary for determining the trace amount of elements present in fish samples.

To assess the accuracy of this method, we determined the concentrations of trace elements in a standard reference material, SRM 1566b oyster tissue. We compared the experimental values with the certified values of this standard reference material. Our results (Table 2.3) show good agreements (deviations of less than 10%) between the measured values and the certified values for most of the 20 elements, except for Al (deviation by 32%–36%), Se^{77} (14–19%), and Se^{82} (34–40%). The high standard deviation for these three elements may result from polyatomic ions interferences.

Table 2.2 Limit of Detection (LOD) for Trace Elements in Fish Sample Using ICP-MS

Element	LOD (μg/g)	monitor
Be	0.003	m/z 9
Al	0.8	m/z 27
V	0.001	m/z 51
Cr	0.003	m/z 53
Mn	0.003	m/z 55
Co	0.01	m/z 59
Ni	0.003	m/z 60
Cu	0.9	m/z 63
Zn	0.9	m/z 66
As	0.001	m/z 75
Se	0.002	m/z 77
Mo	0.001	m/z 95
Ag	0.005	m/z 107
Cd	0.001	m/z 111
Sb	0.002	m/z 121
Ba	0.03	m/z 137
Tl	0.002	m/z 205
Pb	0.3	m/z 208
Th	0.007	m/z 232
U	0.003	m/z 238

Table 2.3. Comparison of the Certified Values and Measured Values of Trace Elements in Standard Reference Materials (SRM 1566b Oyster Tissue).

		2021 (*n* = 10)		2022 (*n* = 15)	
Element	Certified Value	Determined value	Deviation	Determined value	Deviation
	mg/kg	mg/kg	%	mg/kg	%
Al	197.2 ± 6.0	133 ± 32	-32.3	126 ± 38	-35.9
As	7.65 ± 0.65	8.0 ± 0.8	4.8	7.7 ± 0.3	0.3
Cd	2.48 ± 0.08	2.7 ± 0.1	9.4	2.5 ± 0.1	1.9
Cd	2.48 ± 0.08	2.7 ± 0.1	9.2	2.6 ± 0.1	2.9
Co	0.371 ± 0.009	0.41 ± 0.02	11.8	0.38 ± 0.02	3.2
Cu 63	71.6 ± 1.6	75 ± 3	5.1	73 ± 3	1.9
Cu 65	71.6 ± 1.6	73 ± 5	-7.8	73 ± 3	2.4
Pb	0.308 ± 0.009	0.34 ± 0.01	11.8	0.32 ± 0.02	3.1
Mn	18.5 ± 0.2	19.1 ± 0.8	3.3	18.6 ± 0.9	0.7
Ni	1.04 ± 0.09	1.1 ± 0.1	6.4	1.0 ± 0.1	–3.6
Se77	2.06 ± 0.15	2.5 ± 0.1	19.1	2.3 ± 0.1	13.4
Se82	2.06 ± 0.15	2.9 ± 0.3	39.7	2.8 ± 0.1	34.4
Ag	0.666 ± 0.009	0.72 ± 0.02	8.5	0.66 ± 0.03	–0.6
V	0.577 ± 0.023	0.61 ± 0.04	6.6	0.56 ± 0.04	–2.8
Zn	1424 ± 46	1478 ± 103	3.8	1398 ± 82	–1.8

2.3.2 Concentration Profiles of Trace Elements in Different Types of Freshwater Fish

From 2020 to 2022, I analyzed 266 freshwater fish that were captured from 2014 to 2020. Twenty elements were monitored in 266 freshwater fish samples. Beryllium (Be), silver

(Ag), antimony (Sb), lead (Pb), thorium (Th), and uranium (U) were not detectable (below detection limits) in any of these 266 freshwater fish samples analyzed.

Table 2.4 shows Al, V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Ba, and TI in all fish samples and in common fish species: northern pike (NRPK), lake whitefish (LKWH), walleye (WALL), lake trout (LKTR), and rainbow trout (RNTR). The number of detected trace elements in fish samples, detection rate, average, standard deviation, and median are shown in the table. Mn, Zn, As, and Se were detected in every fish sample. V, Cr, and Ni were detected in 72%, 90%, and 76% of the fish samples, respectively. Overall, Al was detected only in 18 samples out of 266; however, it was detected in 13 out of 20 lake trout samples. Tl was detected only in 30 out of 266 fish samples, while it was detected in 95% of lake trout. Co was detected only in 15 samples out of 266, while it was detected in 14 out of 18 rainbow trout samples. Future analysis of more fish samples from different lakes may help test whether the concentrations of Al and Tl are higher in lake trout, and Co is higher in rainbow trout.

Figure 2.1 shows the trace elements in all fish, and Figure 2.2 (a-e) shows the concentration profiles of trace elements in the five main fish species. The average Zn concentration for all fish was 5 ± 2.1 mg/kg, but the Zn concentration in rainbow trout was 9.1 ± 4.2 mg/kg. Both lake trout and rainbow trout have a higher As concentration than other fish species. These results indicate that the concentrations of many trace elements vary depending on the different fish species. Cu and Al, as part of the higher concentration elements, did not seem to show any trend among the fish samples.

Except for lake trout and rainbow trout, the other three fish species were collected from multiple lakes. The trace elements concentration could change with the location of sampling and its environment. The higher concentrations of several elements from the three main fish species might be confounded by the fact that they came from one lake. Section 2.2.3 will discuss the same trace elements in different water bodies, and the trace elements variation of three main fish species in the same lakes.

Table 2.4. Summary of the Concentration of 14 Detectable Elements in All Fish and Different Fish Species.

		Al	V	Cr	Mn	Co	Ni	Cu	Zn	As	Se	Mo	Cd	Ba	Tl
all fish	**Detectable samples**	18	191	240	266	15	201	4	266	266	266	132	2	67	30
n=266	**Detectable rate**	7%	72%	90%	100%	6%	76%	2%	100%	100%	100%	50%	1%	25%	11%
	Average ± SD	1.6 ± 1.2	0.002 ± 0.001	0.021 ± 0.029	0.15 ± 0.12	0.01 ± 0.006	0.013 ± 0.017	2.4 ± 2	5 ± 2.1	0.11 ± 0.16	0.21 ± 0.14	0.009 ± 0.034	0.001	0.081 ± 0.071	0.0034 ± 0.0014
	Median	1.2	0.002	0.01	0.1	0.01	0.009	1.6	4.5	0.06	0.2	0.002	0.001	0.05	0.003
NRPK	**Detectable samples**	2	53	61	79	-	53	-	79	79	79	39	-	25	1
n=79	**Detectable rate**	3%	67%	77%	100%	-	67%	-	100%	100%	100%	49%	-	32%	1%
	Average ± SD	4.2	0.0014 ± 0.0006	0.023 ± 0.045	0.19 ± 0.15	-	0.012 ±0.014	-	5.7 ± 1.2	0.03 ± 0.02	0.10 ± 0.09	0.0029 ± 0.026	-	0.086 ± 0.051	0.002
	Median	4.2	0.001	0.01	0.1	-	0.009	-	5.6	0.02	0.09	0.002	-	0.06	0.002
LKWH	**Detectable samples**	2	61	65	66	-	44	3	66	66	66	36	1	11	2
n=66	**Detectable rate**	3%	92%	98%	100%	-	67%	5%	100%	100%	100%	55%	2%	17%	3%
	Average ± SD	1.5	0.002 ± 0.001	0.016 ± 0.013	0.12 ± 0.06	-	0.016 ± 0.030	2.8 ± 2.2	4.3 ± 1.1	0.16 ± 0.23	0.24 ± 0.16	0.0030 ± 0.0024	0.001	0.072 ± 0.050	0.003
	Median	1.5	0.002	0.01	0.1	-	0.01	1.9	4.15	0.08	0.2	0.002	0.001	0.05	0.003
WALL	**Detectable samples**	-	42	74	76	-	62	-	76	76	76	15	-	18	8
n=76	**Detectable rate**	-	55%	97%	100%	-	82%	-	100%	100%	100%	20%	-	24%	11%
	Average ± SD	-	0.0017 ± 0.0006	0.018 ± 0.013	0.078 ± 0.04	-	0.0077 ± 0.005	-	4.1 ± 1.0	0.064 ± 0.02	0.21 ± 0.10	0.054 ± 0.091	-	0.12 ± 0.10	0.0025 ± 0.0005
	Median	-	0.002	0.01	0.07	-	0.007	-	4	0.06	0.2	0.002	-	0.08	0.003
LKTR	**Detectable samples**	13	13	15	20	1	18	-	20	20	20	17	-	3	19
n=20	**Detectable rate**	65%	65%	75%	100%	5%	90%	-	100%	100%	100%	85%	-	15%	95%
	Average ± SD	1.3 ± 0.4	0.0016 ± 0.0009	0.011 ± 0.007	0.13 ± 0.09	0.01	0.011 ± 0.006	-	3.6 ± 0.4	0.38 ± 0.14	0.29 ± 0.08	0.0025 ± 0.0009	-	0.04 ± 0.01	0.0028 ± 0.0016
	Median	1.1	0.001	0.01	0.1	0.01	0.008	-	3.4	0.4	0.3	0.003	-	0.04	0.003
RNTR	**Detectable samples**	1	15	18	18	14	18	1	18	18	18	18	1	7	-
n=18	**detection rate**	6%	83%	100%	100%	78%	100%	6%	100%	100%	100%	100%	6%	39%	-
	Average ± SD	1.1	0.0035 ± 0.0016	0.026 ± 0.013	0.27 ± 0.17	0.01 ± 0.007	0.019 ± 0.012	1	9.1 ± 4.2	0.16 ± 0.09	0.44 ± 0.09	0.0044 ± 0.0025	0.001	0.041 ± 0.015	-
	Median	1.1	0.003	0.02	0.2	0.01	0.02	1	6.9	0.2	0.4	0.004	0.001	0.03	-

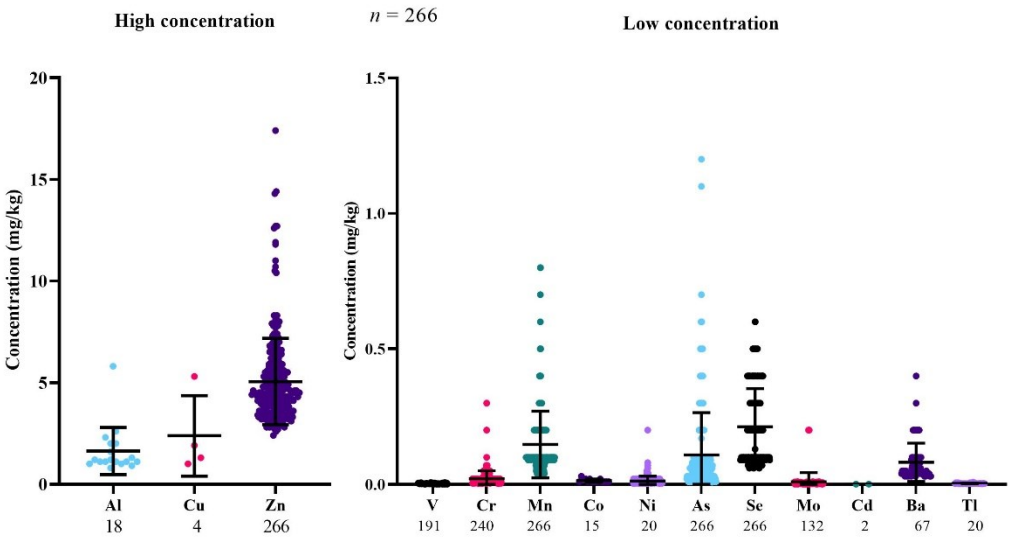

Figure 2.1. Trace elements in all fish, regardless of the fish species and the year that they were captured. The numbers below each trace element indicate the number of fish samples that had concentrations higher than the detection limit. The black bars in the graph indicate the mean value and standard deviation.

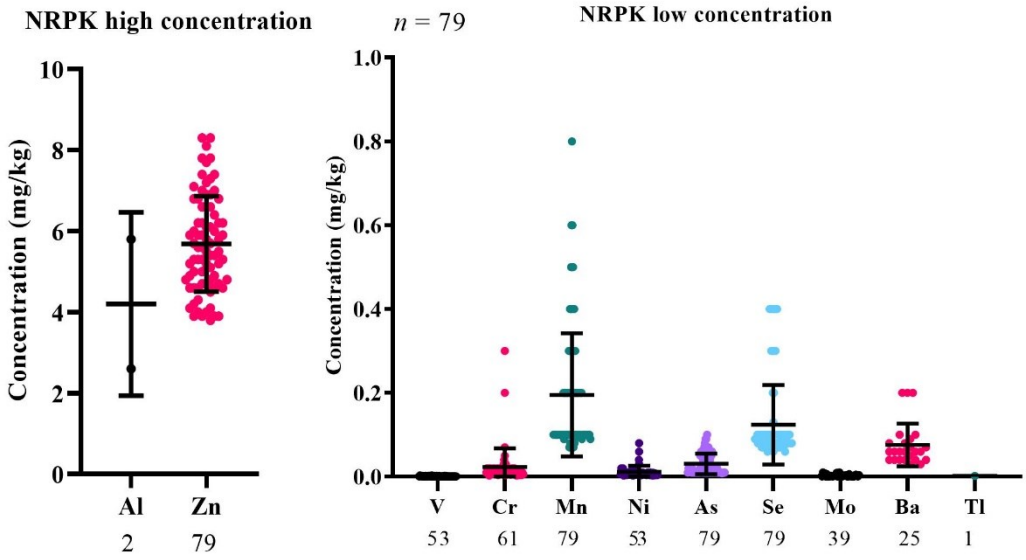

Figure 2.2. (a) Trace elements concentrations in all northern pike, regardless of the years that they were captured. The numbers below each trace element indicate the number of fish samples that had concentrations higher than the detection limit. The black bars in the graph indicate the mean value and standard deviation.

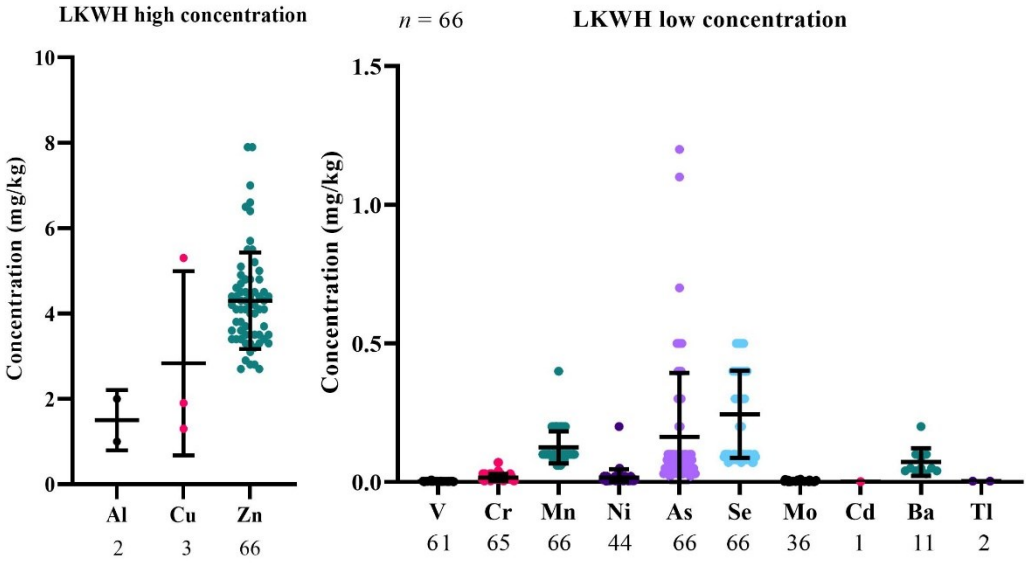

Figure 2.2. (b) Trace element concentrations in all lake whitefish, regardless of the years that they were captured. The numbers below each trace element indicate the number of fish samples that had concentrations higher than the detection limit. The black bars in the graph indicate the mean value and standard deviation.

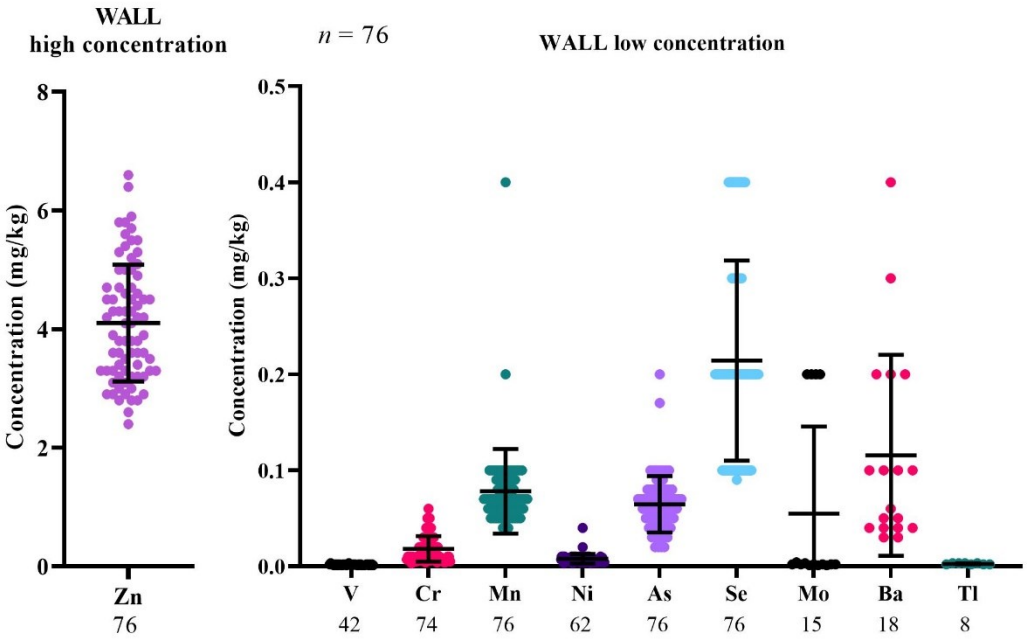

Figure 2.2. (c) Trace elements concentrations in all walleyes, regardless of the years that they were captured. The numbers below each trace element indicate the number of fish samples that had concentrations higher than the detection limit. The black bars in the graph indicate the mean value and standard deviation.

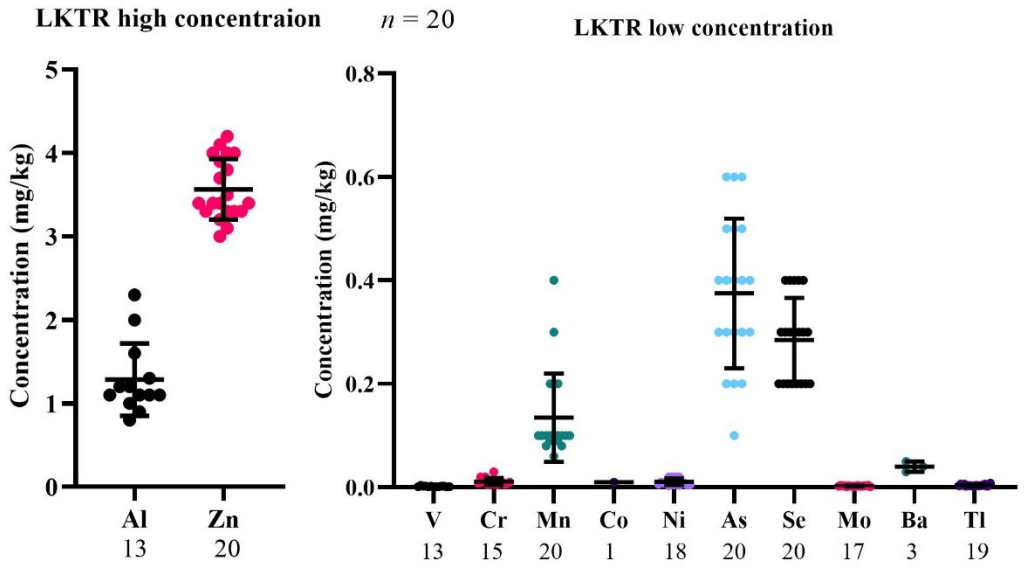

Figure 2.2. (d) Trace elements concentrations in all lake trout, regardless of the years that they were captured. The numbers below each trace element indicate the number of fish samples that had concentrations higher than the detection limit. The black bars in the graph indicate the mean value and standard deviation.

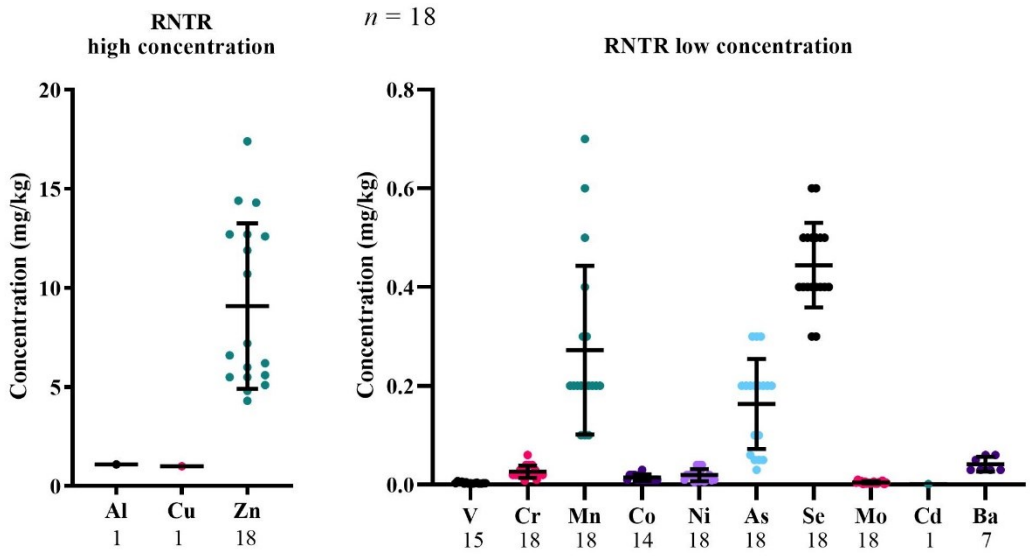

Figure 2.2. (e) Trace elements concentrations in all rainbow trout, regardless of the years that they were captured. The numbers below each trace element indicate the number of fish samples that had concentrations higher than the detection limit. The black bars in the graph indicate the mean value and standard deviation.

2.3.3 Concentration Profiles of Trace Elements in Fish Samples Collected from Different Water Bodies

This study analyzed fish collected from seven lakes: Beaver Lake (BR), Christina Lake (CL), Cold Lake (CO), Fork Lake (FL), Square Lake (SQ), Sylvan Lake (SV), and Whitefish Lake (WF), and a storm-water catchment pond: County Sportsplex Pond (CX). Samples from these water bodies were collected from 2014 to 2020. Because the number of each fish species from each water body is small, I combined all the fish species in each lake for comparing trace element concentrations among different water bodies. I selected several frequently detected elements and compared their concentrations in fish of different water bodies.

Table 2.5 shows p values comparing total arsenic concentrations in fish between water bodies. Figure 2.3 shows the concentrations of total arsenic in fish samples collected from eight water bodies and comparisons of arsenic concentrations between water bodies. The arsenic concentration of fish from Cold Lake (CO) was the highest, and it had no significant difference from County Sportsplex Pond (CX). The second highest arsenic concentration was in fish of Whitefish Lake (WF), Sylvan Lake (SV), and Christina Lake

(CL). Beaver Lake (BR) was the next. The remaining lakes had the lowest arsenic concentration in fish: 0.008–1.2 mg/kg.

Table 2.5. The p Values for the Concentration of Total Arsenic in Fish Samples Collected from Eight Different Water Bodies.

	FL	WF	BR	SQ	CL	CO	CX	SV
FL	-	0.0017	ns	ns	<0.0001	<0.0001	<0.0001	<0.0001
WF	0.0017	-	ns	<0.0001	ns	<0.0001	0.023	ns
BR	ns	ns	-	ns	<0.01	<0.0001	0.0001	<0.01
SQ	ns	<0.0001	ns	-	<0.0001	<0.0001	<0.0001	<0.0001
CL	<0.0001	ns	<0.01	<0.0001	-	<0.0001	ns	ns
CO	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-	ns	<0.0001
CX	<0.0001	0.023	0.0001	<0.0001	ns	ns	-	ns
SV	<0.0001	ns	<0.01	<0.0001	ns	<0.0001	ns	-

ns: Not Significant.

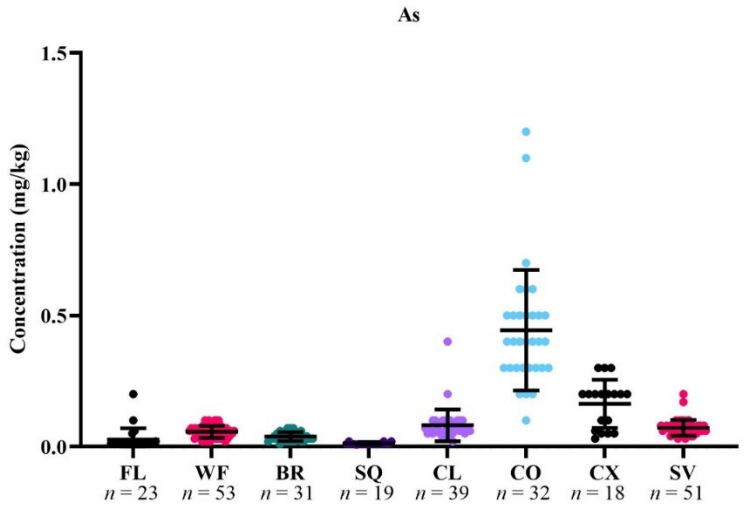

Figure 2. 3. The concentration of total arsenic in fish samples collected from eight different water bodies. All the fish species were combined in one lake. The numbers below each lake indicate the number of fish samples (all fish species) that had concentrations higher than the detection limit. The black bars in the graph indicate the mean value and standard deviation.

Figure 2.4 shows concentrations of the total selenium in fish samples collected from eight water bodies. The selenium concentration in different water bodies ranged from 0.06 to 0.6 mg/kg. Fish in Cold Lake, County Sportsplex Pond, and Sylvan Lake contained the highest concentration of selenium, while Whitefish Lake, Beaver Lake, Square Lake, and Christina Lake contained the second highest concentration, and Fork Lake contained the lowest concentration.

Table 2.6. The p Values for the Concentration of Total Selenium in Fish Samples Collected from Eight Different Water Bodies.

	FL	WF	BR	SQ	CL	CO	CX	SV
FL	-	0.017	ns	ns	<0.01	<0.0001	<0.0001	<0.0001
WF	0.017	-	ns	ns	ns	<0.0001	<0.0001	<0.0001
BR	ns	ns	-	ns	ns	<0.0001	<0.0001	<0.0001
SQ	ns	ns	ns	-	ns	<0.0001	<0.0001	<0.0001
CL	<0.01	ns	ns	ns	-	<0.001	<0.0001	<0.0001
CO	<0.0001	<0.0001	<0.0001	<0.0001	<0.001	-	ns	ns
CX	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	ns	-	ns
SV	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	ns	ns	-

ns: Not Significant.

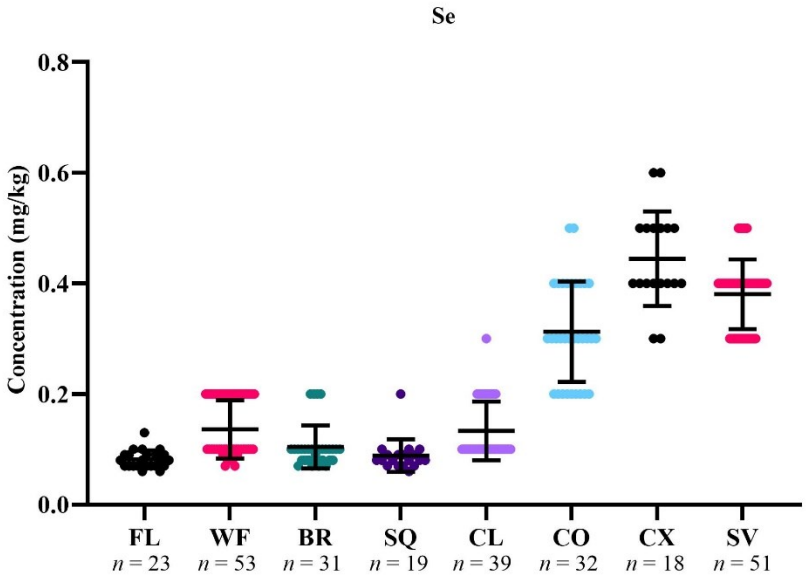

Figure 2. 4. The concentration of total selenium in fish samples collected from eight different water bodies. All fish species were combined in one lake. The numbers labelled below each lake indicate the number of fish samples (all fish species) that had concentrations higher than the detection limit. The black bars in the graph indicate the mean value and standard deviation.

Table 2.7 shows p values comparing vanadium concentrations in fish between water bodies. Figure 2.5 shows concentrations of total vanadium in fish samples collected from eight water bodies and comparisons between water bodies. The concentration of

vanadium in different water bodies ranged from 0.001 to 0.006 mg/kg. The concentration variation is relatively small compared to that of selenium and arsenic. County Sportsplex Pond seems to have the highest vanadium concentration, but there is no significant difference between Whitefish Lake and County Sportsplex Pond. Overall, the vanadium concentration in different water bodies changed slightly within the concentration range.

Table 2.7. The p Values for the Concentration of Total Vanadium in Fish Samples Collected from Eight Different Water Bodies.

	FL	WF	BR	SQ	CL	CO	CX	SV
FL	-	ns	ns	ns	ns	ns	<0.01	ns
WF	ns	-	0.026	<0.001	ns	ns	ns	ns
BR	ns	0.026	-	ns	0.022	ns	<0.0001	ns
SQ	ns	<0.001	ns	-	<0.001	<0.01	<0.0001	ns
CL	ns	ns	0.022	<0.001	-	ns	ns	ns
CO	ns	ns	ns	<0.01	ns	-	ns	ns
CX	<0.01	ns	<0.0001	<0.0001	ns	ns	-	<0.01
SV	ns	ns	ns	ns	ns	ns	<0.01	-

ns: Not Significant.

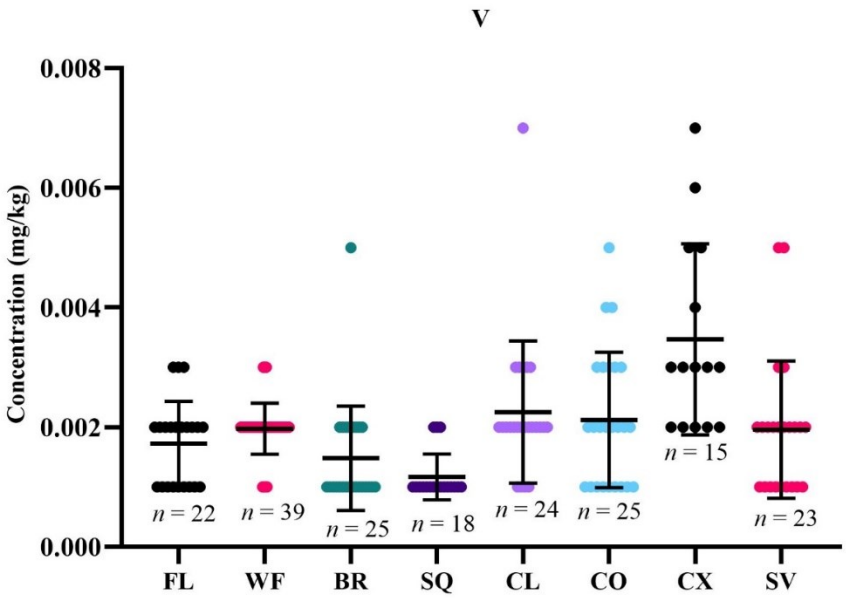

Figure 2. 5. The concentration of total vanadium in fish samples collected from eight different water bodies. All fish species were combined in one lake. The numbers below each lake indicate the number of fish samples (all fish species) that had concentrations higher than the detection limit. The black bars in the graph indicate the mean value and standard deviation.

Table 2.8 shows p values comparing chromium concentrations in fish between water bodies. Figure 2.6 shows concentration of total chromium in fish samples collected from eight water bodies and comparisons of chromium concentrations between water bodies. The chromium concentration in different lake ranged from 0.003 to 0.3 mg/kg. Fork Lake and Christina Lake had the highest chromium concentration. Overall, most fish contained chromium that was less than 0.04 mg/kg.

Table 2.8. The p Values for the Concentration of Total Chromium in Fish Samples Collected from Eight Different water bodies.

	FL	WF	BR	SQ	CL	CO	CX	SV
FL	-	<0.01	<0.0001	<0.0001	ns	ns	ns	ns
WF	<0.01	-	ns	0.017	<0.001	ns	<0.01	ns
BR	<0.0001	ns	-	ns	<0.0001	ns	<0.0001	<0.0001
SQ	<0.0001	0.017	ns	-	<0.0001	0.015	<0.0001	<0.0001
CL	ns	<0.001	<0.0001	<0.0001	-	<0.01	ns	ns
CO	ns	ns	ns	0.015	<0.01	-	ns	ns
CX	ns	<0.01	<0.0001	<0.0001	ns	ns	-	ns
SV	ns	ns	<0.0001	<0.0001	ns	ns	ns	-

ns: Not Significant.

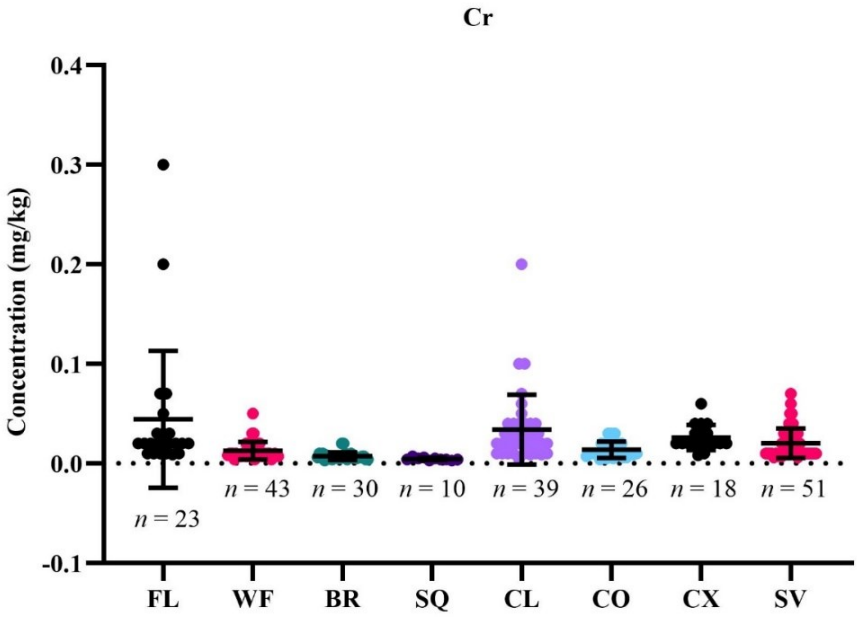

Figure 2. 6. The concentration of total chromium in fish samples collected from eight different water bodies. All fish species were combined in one lake. The numbers below each lake indicate the number of fish samples (all fish species) that had concentrations higher than the detection limit. The black bars in the graph indicate the mean value and standard deviation.

Table 2.9 shows p values comparing zinc concentrations in fish between waterbodies. Figure 2.7 shows concentrations of total zinc in fish samples collected from eight waterbodies and comparisons of zinc concentration between waterbodies. The zinc concentration is scattered largely and ranges from 2.4 to 17 mg/kg. County Sportsplex Pond had the highest zinc concentration, and Beaver Lake and Cold Lake had the lowest.

Table 2.9. The p Values for the Concentration of Total Zinc in Fish Samples Collected from Eight Different water bodies.

	FL	WF	BR	SQ	CL	CO	CX	SV
FL	-	ns	<0.0001	ns	ns	<0.0001	ns	0.017
WF	ns	-	<0.01	0.047	ns	0.012	<0.01	ns
BR	<0.0001	<0.01	-	<0.0001	0.02	ns	<0.0001	ns
SQ	ns	0.047	<0.0001	-	ns	<0.0001	ns	<0.01
CL	ns	ns	0.02	ns	-	0.042	<0.01	ns
CO	<0.0001	0.012	ns	<0.0001	0.042	-	<0.0001	ns
CX	ns	<0.01	<0.0001	ns	<0.01	<0.0001	-	<0.0001
SV	0.017	ns	ns	<0.01	ns	ns	<0.0001	-

ns: Not Significant

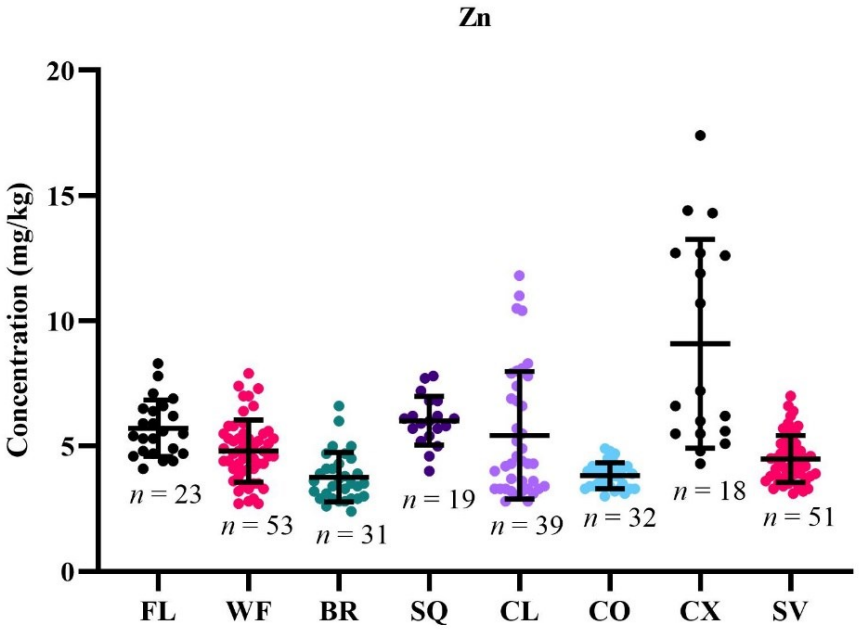

Figure 2. 7. The concentration of total zinc in fish samples collected from eight different water bodies. All fish species were combined in one lake. The numbers below each lake indicate the number of fish samples (all fish species) that had concentrations higher than the detection limit. The black bars in the graph indicate the mean value and standard deviation.

Table 2.10 shows p values comparing nickel concentrations in fish between water bodies. Figure 2.8 shows concentrations of total nickel in fish samples collected from eight water bodies and comparisons of nickel concentrations between water bodies. The nickel concentration ranged from 0.003 to 0.2 mg/kg, with one outlier in Cold Lake. There is no significant difference in Fork Lake, Cold Lake, and County Sportsplex Pond, there is no significant difference among Whitefish Lake, Christina Lake, and Cold Lake, and there is no significant difference between Fork Lake and Whitefish Lake. The scatter of Ni in different water bodies shows the center scatter was about the same. The outliers in different water bodies were more likely to determine the significant difference.

Table 2.10. The p Values for the Concentration of Total Nickel in Fish Samples Collected from Eight Different water bodies.

	FL	WF	BR	SQ	CL	CO	CX	SV
FL	-	<0.01	<0.001	<0.0001	<0.01	ns	ns	<0.0001
WF	<0.01	-	ns	0.027	ns	ns	ns	ns
BR	<0.001	ns	-	ns	ns	ns	<0.01	ns
SQ	<0.0001	0.027	ns	-	ns	<0.0001	<0.0001	ns
CL	<0.01	ns	ns	ns	-	ns	0.046	ns
CO	ns	ns	ns	<0.0001	ns	-	ns	ns
CX	ns	ns	<0.01	<0.0001	0.046	ns	-	<0.001
SV	<0.0001	ns	ns	ns	ns	ns	<0.001	-

ns: Not Significant.

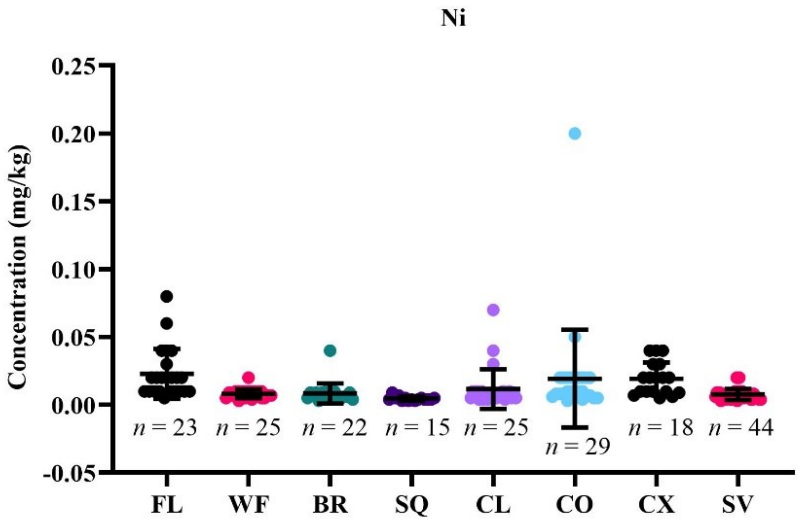

Figure 2. 8. The concentration of total nickel in fish samples collected from eight different water bodies. All fish species were combined in one lake. The numbers below each lake indicate the number of fish samples (all fish species) that had concentrations higher than the detection limit. The black bars in the graph indicate the mean value and standard deviation.

Figure 2.9 shows the overall concentrations of total manganese in fish samples collected from eight water bodies and comparisons of manganese concentrations between water bodies. Manganese had a certain scatter range in different water bodies, and the concentration ranged from 0.04 to 0.8 mg/kg. There is no significant difference for Fork Lake, Square Lake, Christina Lake, Cold Lake, and County Sportsplex Pond, which have a higher manganese concentration. Sylvan Lake and Whitefish Lake contained a lower manganese concentration.

Table 2.11. The p Values for the Concentration of Total Manganese in Fish Samples Collected from Eight Different Water Bodies.

	FL	WF	BR	SQ	CL	CO	CX	SV
FL	-	<0.0001	<0.001	ns	ns	ns	ns	<0.0001
WF	<0.0001	-	ns	<0.0001	<0.01	0.016	<0.0001	ns
BR	<0.001	ns	-	<0.001	ns	ns	<0.0001	ns
SQ	ns	<0.0001	<0.001	-	ns	ns	ns	<0.0001
CL	ns	<0.01	ns	ns	-	ns	ns	<0.01
CO	ns	0.016	ns	ns	ns	-	ns	<0.01
CX	ns	<0.0001	<0.0001	ns	ns	ns	-	<0.0001
SV	<0.0001	ns	ns	<0.0001	<0.01	<0.01	<0.0001	-

ns: Not Significant.

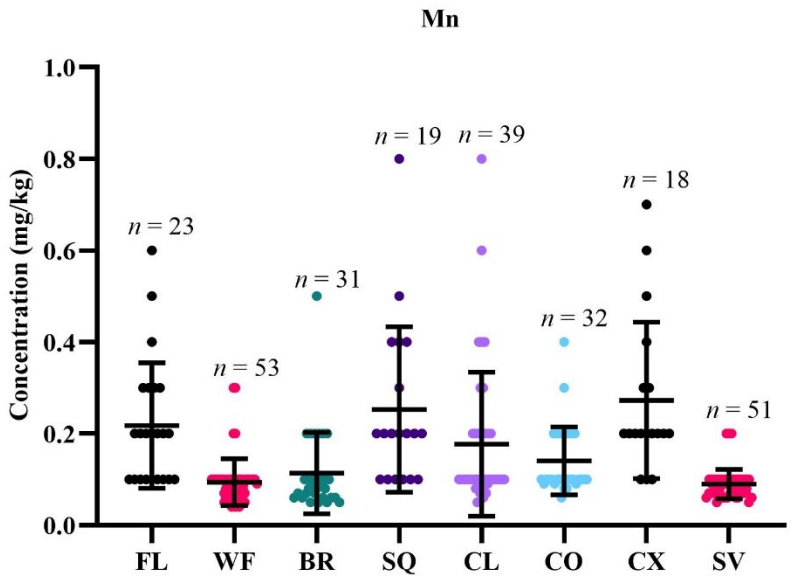

Figure 2. 9. The concentration of total manganese in fish samples collected from eight different water bodies. All fish species were combined in one lake. The numbers below each lake indicate the number of fish samples (all fish species) that had concentrations higher than the detection limit. The black bars in the graph indicate the mean value and standard deviation.

From the results of Tables 2.5−2.11 and Figures 2.3−2.9, every element had a certain range in fish of different water bodies. These results suggest that the lake environment might contribute to the observed differences in the concentrations of trace elements in fish.

I further compared three fish species, northern pike (n = 14), lake whitefish (n = 18), and walleye (n = 21), from the same lake (Whitefish Lake). I did not compare other types of fish because the number of each fish species from Whitefish Lake was too low for meaningful statistical analysis. Figures 2.10−2.12 show the arsenic, selenium, and zinc comparison in northern pike (NRPK), lake whitefish (LKWH), and walleye (WALL). These three elements were chosen due to the high detection rate (100%) in all fish samples.

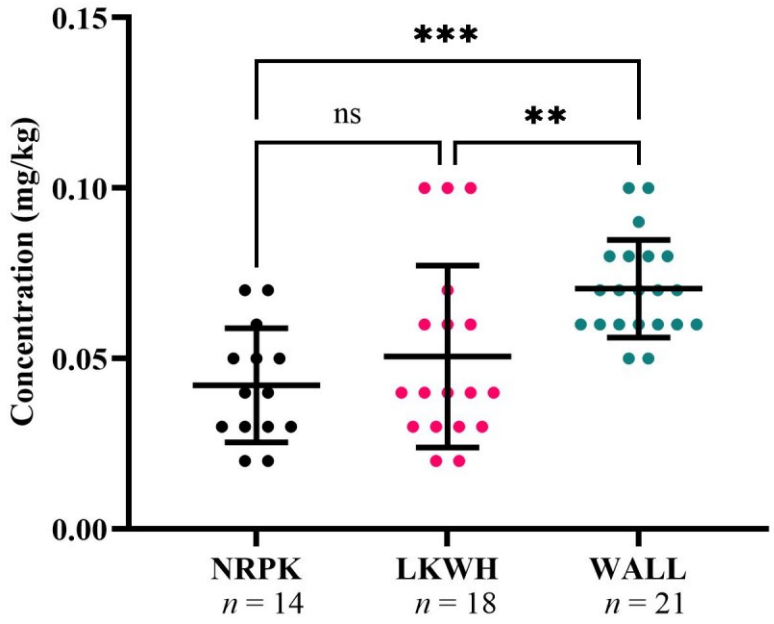

Figure 2. 10. The concentration of total arsenic in three main fish species collected from Whitefish Lake (2016). The numbers below each fish species indicate the number of fish samples that had concentrations higher than the detection limit. The labels, ns, **, and ***, indicate not significant (ns) and statistically significant with p values of <0.01 and <0.001, respectively. The black bars in the graph indicate the mean value and standard deviation.

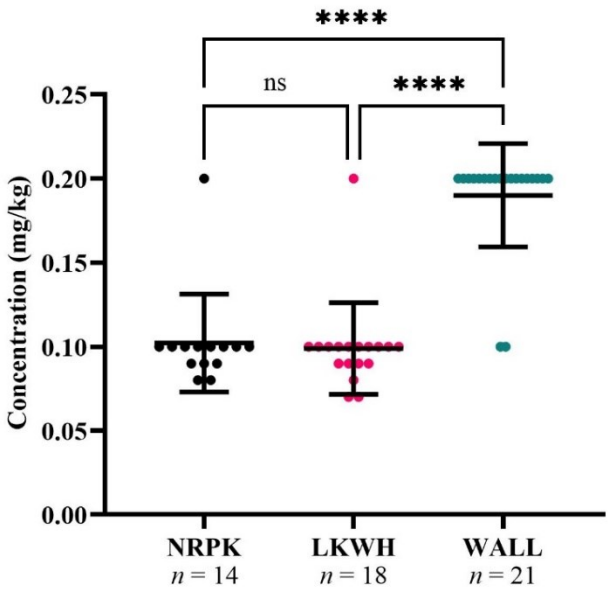

Figure 2. 11. The concentration of total selenium in three main fish species collected from Whitefish Lake (2016). The numbers below each fish species indicate the number of fish samples that had concentrations higher than the detection limit. The labels, ns and ****, indicate not significant (ns) and statistically significant with p value of <0.0001, respectively. The black bars in the graph indicate the mean value and standard deviation.

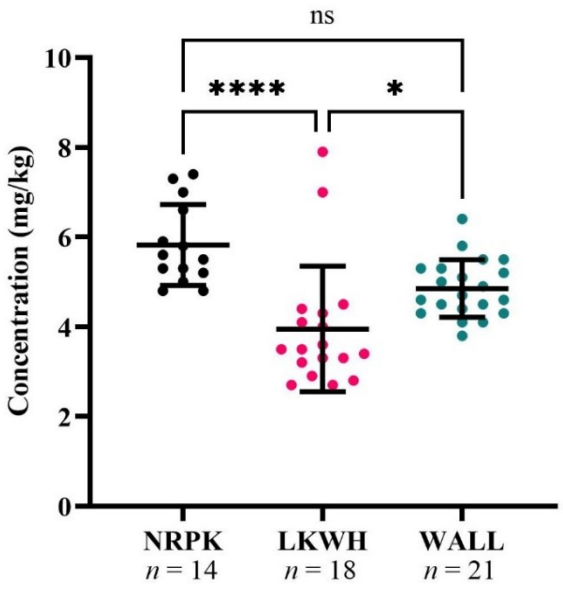

Figure 2. 12. The concentration of total zinc in three main fish species collected from Whitefish Lake (2016). The numbers below each fish species indicate the number of fish samples that had concentrations higher than the detection limit. The labels, ns, *, and ****, indicate not significant (ns) and statistically significant with p values of <0.05 and <0.0001, respectively. The black bars in the graph indicate the mean value and standard deviation.

The arsenic concentration in walleye was higher than in the other two fish species, and there is no significant difference between northern pike and lake whitefish. This is the same as the selenium distribution pattern, while the zinc concentration was the highest in northern pike, the second highest in walleye, and the lowest in lake whitefish.

The concentration of different elements in fish is more likely affected by the lake environment and circumstances. Section 2.3.4 will discuss the trace elements concentrations in fish samples collected between 2016 and 2019 from one lake .

2.3.4 Concentration Profiles of Trace Elements in Fish Samples Collected from Cold Lake Between 2016 and 2019

Because my results showed that fish samples from Cold Lake had higher arsenic concentrations than fish from other water bodies, I investigated whether this trend was consistent over time. In this section, I compared fish in Cold Lake collected between 2016 and 2019. Ms. Xiufen Lu in our lab performed the analysis of fished collected in in 2016 from Cold Lake.

First, I combined lake trout and lake whitefish to compare between 2016 and 2019. Figures 2.13−17 show several elements patterns in both fish species in Cold Lake between 2016 and 2019. There was no significant difference in vanadium and selenium concentration between these two years (Figures 2.13 and 2.14). The concentration of both chromium and nickel in 2019 was higher than in 2016, while that of zinc was lower in 2019 than in 2016 (Figures 2.15, 2.16, and 2.17).

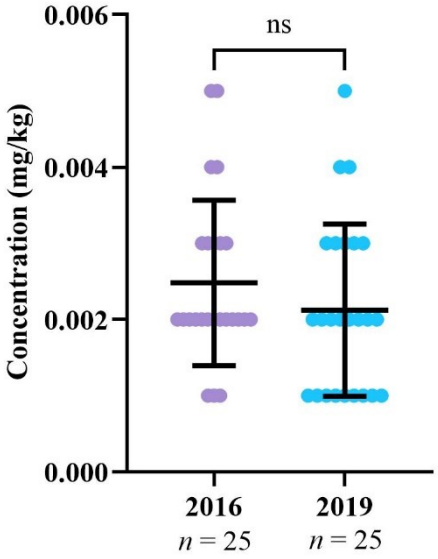

Figure 2. 13. The concentration of total vanadium in fish samples collected from Cold Lake between 2016 and 2019. All fish species were combined in one lake. The numbers below each year indicate the number of fish samples that had concentrations higher than the detection limit. The label ns indicates not significant (ns). The black bars in the graph indicate the mean value and standard deviation.

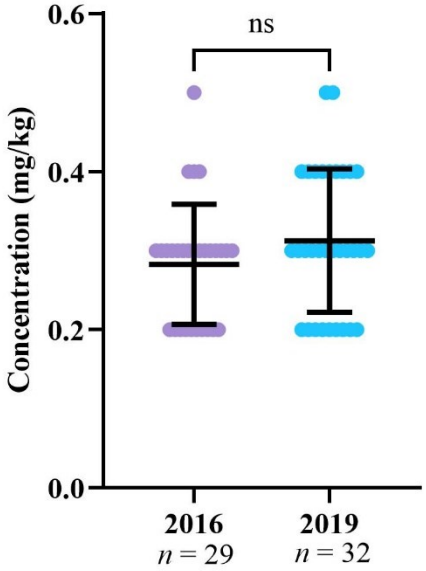

Figure 2. 14. The concentration of total selenium in fish samples collected from Cold Lake between 2016 and 2019. All fish species were combined in one lake. The numbers below each year indicate the number of fish samples that had concentrations higher than the detection limit. The label ns indicates not significant (ns). The black bars in the graph indicate the mean value and standard deviation.

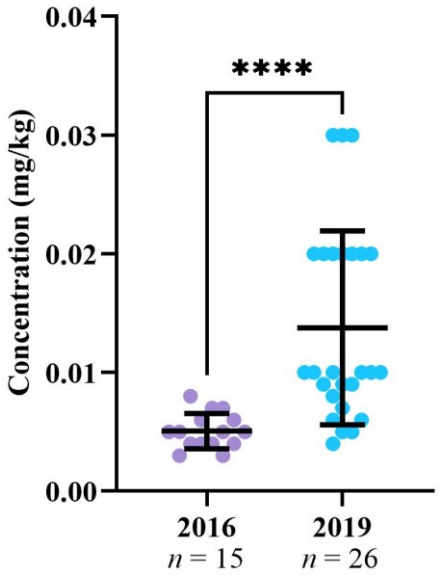

Figure 2. 15. The concentration of total chromium in fish samples collected from Cold Lake between 2016 and 2019. All fish species were combined in one lake. The numbers below each year indicate the number of fish samples that had concentrations higher than the detection limit. The label **** indicates statistically significant with p value of <0.0001. The black bars in the graph indicate the mean value and standard deviation.

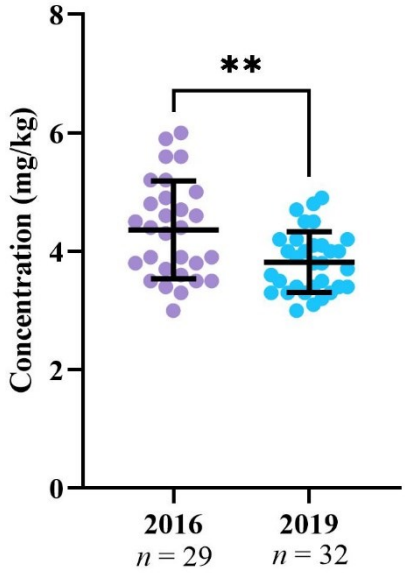

Figure 2. 16. The concentration of total zinc in fish samples collected from Cold Lake between 2016 and 2019. All fish species were combined in one lake. The numbers below each year indicate the number of fish samples that had concentrations higher than the detection limit. The label ** indicates statistically significant with p value of <0.01. The black bars in the graph indicate the mean value and standard deviation.

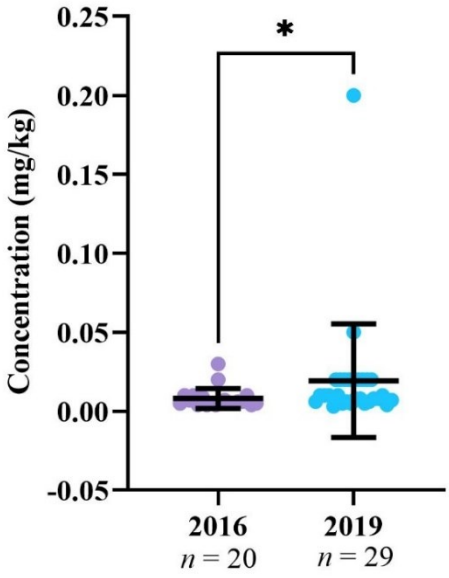

Figure 2. 17. The concentration of total nickel in fish samples collected from Cold Lake between 2016 and 2019. All fish species combined in one lake. The numbers below each year indicate the number of fish samples that had concentrations higher than the detection limit. The label * indicates statistically significant with a p value of <0.05. The black bars in the graph indicate the mean value and standard deviation.

After looking at both fish combined between two years, I compared the elements separately in lake whitefish and lake trout. The selenium concentration in lake whitefish increased from 2016 to 2019 (Figure 2.18), but in lake trout it did not change significantly (Figure 2.19). The vanadium concentration decreased significantly in lake trout from 2016 to 2019 (Figure 2.20). There was a significant difference for selenium (Figure 2.21) and vanadium concentrations in one fish species but no significant difference for both fish combined. The zinc concentration in lake whitefish was lower in 2019 than in 2016 (Figure 2.22). The arsenic concentration in lake trout was higher in 2019 than in 2016 (Figure 2.20). Other than the elements that were detected mostly, I summarized the less detectable elements. Aluminium in 2019 appeared in 15 fish samples (13 out of 15 came from lake trout) and only appeared in two fish samples in 2016. Cobalt appeared in one lake trout sample in 2019, copper was detected in two lake whitefish samples in 2019, and barium appeared in four out of 31 fish samples in 2019 and 9 out of 29 in 2016, showing a decreasing trend.

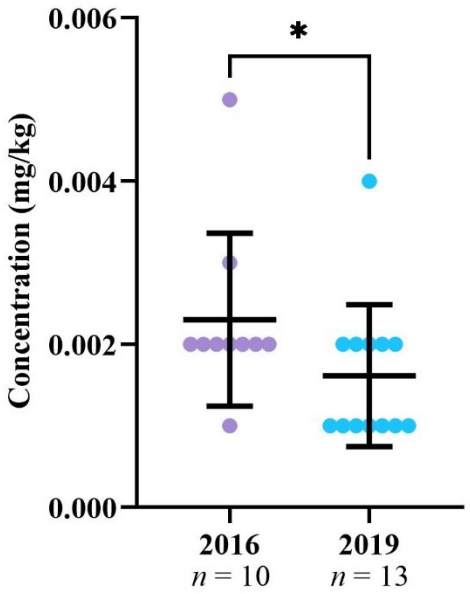

Figure 2. 18. The concentration of total vanadium in lake trout samples collected from Cold Lake between 2016 and 2019. The numbers below each year indicate the number of fish samples that had concentrations higher than the detection limit. The label * indicates statistically significant with p value of <0.05. The black bars in the graph indicate the mean value and standard deviation.

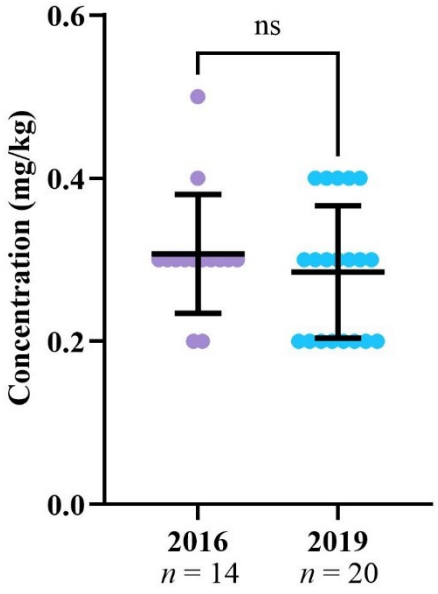

Figure 2. 19. The concentration of total selenium in lake trout samples collected from Cold Lake between 2016 and 2019. The numbers below each year indicate the number of fish samples that had concentrations higher than the detection limit. The label ns indicates not significant. The black bars in the graph indicate the mean value and standard deviation.

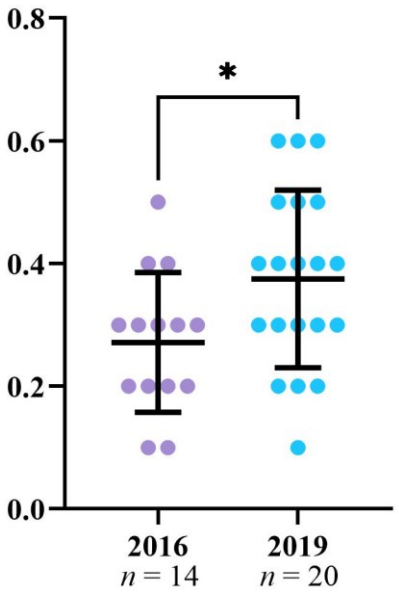

Figure 2. 20. The concentration of total arsenic in lake trout samples collected from Cold Lake between 2016 and 2019. The numbers below each year indicate the number of fish samples that had concentrations higher than the detection limit. The label * indicates statistically significant with p value of <0.05. The black bars in the graph indicate the mean value and standard deviation.

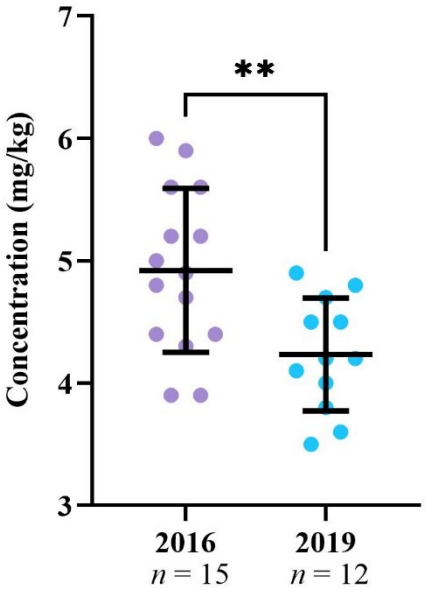

Figure 2. 21. The concentration of total zinc in lake whitefish samples collected from Cold Lake between 2016 and 2019. The numbers below each year indicate the number of fish samples that had concentrations higher than the detection limit. The label ** indicates statistically significant with p value of <0.01. The black bars in the graph indicate the mean value and standard deviation.

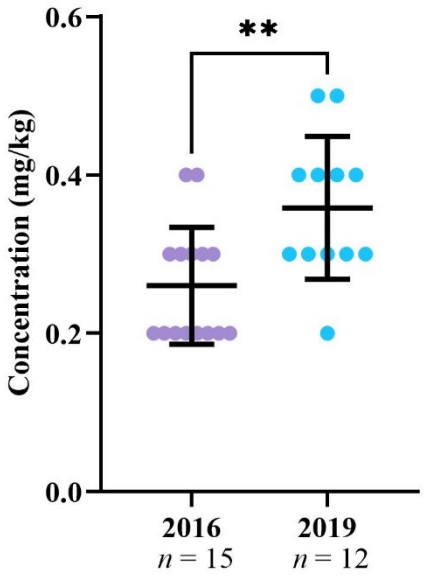

Figure 2. 22. The concentration of total selenium in lake whitefish samples collected from Cold Lake between 2016 and 2019. The numbers below each year indicate the number of fish samples that had concentrations higher than the detection limit. The label ** indicates statistically significant with p value of <0.01. The black bars in the graph indicate the mean value and standard deviation.

The concentration of both chromium and nickel increased between 2016 and 2019, while that of zinc decreased between 2016 and 2019 for two fish species combined. Chromium was detected in 15 out of 29 samples in 2016 and was detected in 25 out of 31 samples in 2019. Nickel was detected in 20 out of 29 samples in 2016 and was detected in 28 out of 31 samples in 2019. The chromium and nickel concentration differences largely result from the detectable number between two years. For lake trout, the vanadium concentration decreased, and that of arsenic increased between 2016 and 2019. For lake whitefish, the zinc concentration decreased, and that of selenium increased between 2016 and 2019.

2.4 Conclusion

A method of acid digestion and ICP-MS was successfully used for the determination of multiple elements in freshwater fish. Six out of 20 elements (Be, Ag, Sb, Pb, Th, and U) were not detectable in all fish. Some elements (Tl, Co, and Al) almost were detected in one kind of fish and completely not detectable in other fish species. Elements that were 100%

detectable or most detectable in all fish varied among different fish species. It was hard to find a correlation between fish species and element concentration.

Trace element concentrations in fish were dependent on fish captured from different water bodies. Different water bodies that were investigated in this study are in different regions in Alberta. Both the surrounding environment and lake sediment conditions might cause differences in the concentration of trace elements among different water bodies. Different trace elements have different distributions among the water bodies that were investigated.

For Cold Lake, the chromium and nickel concentrations both increased, while that of zinc decreased between 2016 and 2019 for the two fish species combined. This might indicate subtle environmental changes. However, the data from two years were not enough to conclude. Alberta Health may capture more lake whitefish and lake trout in the following years for more investigation.

2.5 Reference

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5. Wilschefski, S.C. and M.R. Baxter, *Inductively coupled plasma mass spectrometry: introduction to analytical aspects.* The Clinical Biochemist Reviews, 2019, *40(3)*, 115.
6. Darrouzès, J., et al., *Optimisation of ICPMS collision/reaction cell conditions for the simultaneous removal of argon based interferences of arsenic and selenium in water samples.* Talanta, 2007, *71(5)*, 2080-2084.
7. (EPA), *U.S.E.P.A., Definition and Procedure for the Determination of the Method Detection limit, Revision 2.* 2016.

CHAPTER 3

Arsenic Speciation in Freshwater Fish

3.1 Introduction

Arsenic exposure affected about 200 million people worldwide, whose drinking water contains arsenic higher than the WHO guideline of 10 μg/L. The major route of human exposure to arsenic is food and water ingestion. Chronic arsenic exposure is associated with an increasing risk of bladder, lung, skin, and liver cancers, diabetes, and cardiovascular diseases [1]. There are many arsenic forms present in the natural environment and in biological systems. Arsenic toxicity varies greatly depending on the chemical species [2]. Inorganic arsenic, such as arsenate (As^{V}) and arsenite (As^{III}), is considered as carcinogenic to humans (Group 1). Monomethylarsonic acid (MMA^{V}) and dimethylarsinic acid (DMA^{V}) are classified as possible human carcinogens (Group 2B). The LD_{50} of As^{III} and As^{V} in rats is 10–20 mg/kg [3-5], and LD_{50} of MMA^{V} and DMA^{V} in rats are 700–1,600 mg/kg and 700–2,600 mg/kg, respectively [6,7]. Arsenobetaine (AsB) is the most prevalent arsenic species in seafood product [8,9]. AsB is considered nontoxic to humans, with an $LD_{50} > 10{,}000$ mg/kg in rats [9,10]. The total arsenic concentration does not provide enough information for the toxicity of arsenic. It is necessary to separate, identify, and quantify arsenic species in the environment and in food.

Currently, arsenic species in marine fish have been studied extensively, while not much research has been done on arsenic speciation in freshwater fish. Arsenic concentration in marine fish usually is high, and the dominant species is AsB. Unlike marine fish, the freshwater fish arsenic profile varies greatly. Soeroes et al. found that the dominant species in some freshwater fish is arsenosugars [11], while Zheng and Hintelmann reported that inorganic arsenic As^{V} and As^{III} predominated in freshwater fish [12]. The dominant arsenic species in freshwater fish lacks consensus. Alberta lakes hold various freshwater fish and serve as a food source for local people. Therefore, a large-scale survey of Albertan freshwater fish is necessary.

Currently, high performance liquid chromatography (HPLC) with inductively coupled plasma mass spectrometry (ICP-MS) technique is commonly used for arsenic

speciation analysis. The arsenic speciation profile was reported much less in freshwater fish than in marine fish. Detection of arsenic species in freshwater fish requires high sensitivity and a low detection limit. The limit of detection of ICP-MS could be as low as 0.01 µg/L [13]. However, ICP-MS alone cannot differentiate arsenic species. When HPLC separation and ICP-MS detection are combined, detection of different arsenic species can be achieved. Thus, we developed a HPLC-ICP-MS method for arsenic speciation analysis.

The object of this chapter was to carry out arsenic speciation analysis in freshwater fish using HPLC-ICP-MS. By using this analytical method, we investigated freshwater fish arsenic speciation patterns. We also explored arsenic speciation patterns among different types of fish and fish collected from various Alberta lakes over the years.

3.2 Materials and Methods

3.2.1 Reagents and Standards

Arsenobetaine (98%, Tri Chemical Laboratories Inc., Japan), sodium m-arsenite (97%, Sigma, US), sodium arsenate (99.4%, Sigma, US), cacodylic acid (98%, Sigma, US), and monosodium acid methane arsonate (99%, Chem Service, West Chester, PA, US) were dissolved in 18.2 MΩ·cm deionized water to prepare AsB, As^{III}, As^{V}, DMA, and MMA 1000 mg As/L arsenic stock solutions. The concentrations of these arsenic species were standardized against a calibration constructed high purity (99.999%) inorganic arsenic standard (Agilent). Standard reference material (SRM) 2669 from the National Institute of Standards technology (NIST, Gaithersburg, US), and certified reference material (CRM) 18 from the National Institute for Environmental Studies (NIES, Japan) were used as quality control measures of arsenic speciation analysis. Ammonium bicarbonate (Honeywell-Fluka), and HPLC grade methanol (Fisher, US) were used for the preparation of HPLC mobile phase. All arsenic standard stock solutions were kept at 4 °C. Arsenic speciation standard solutions were diluted in deionized water daily from the above stock solutions.

3.2.2 Sample Collection and Extraction

Fish samples from seven Alberta lakes and a storm-water catchment pond were collected by Alberta Health, and fish fillet meat were sealed by the Alberta Centre for

Toxicology (University of Calgary, AB). Samples were shipped with dry ice to the University of Alberta and stored at –80 °C.

Fish meat samples were thawed at room temperature overnight prior to analysis. After thawing, they were ground in a blender (Kitchen Aid) till the meat texture is uniform. A 2 ± 0.2 g sample of the fish meat was weighed into a 15 mL polypropylene centrifuge tube (Fisher, US) and the exact weight was recorded. A 10 mL mixture of methanol-water (v/v 1:1) was added to the tube and homogenized by a Powergen 125 homogenizer (Fisher, US) until no big chunks of fish meat were seen. The sample solution was sonicated for 60 min and then centrifuged at 4000 rpm for 40 min. Another three extractions with 5 mL of methanol-water (v/v 1:1) were conducted. The supernatant was combined in a 50 mL beaker and heated at 60 °C on a hot plate until dryness. Then, the concentrated supernatant was diluted with deionized water to 5 mL by using a volumetric flask and poured into a 15 mL tube. The solution was centrifuged at 4000 rpm for 20 min and then aspirated into a 1 mL syringe (BD Biosciences, US), filtered by a 0.45 μm membrane (Mandel scientific, CA). The filtered solution was ready for arsenic speciation analysis using HPLC and ICP-MS. A 0.1g sample of SRM 1568b rice flour and DORM-4 fish protein were weighted and went through the whole extraction process. These standard reference materials were used for the quality control purpose. The extraction of fish samples and method blank samples were carried out in duplicate.

The rest of the supernatant and residues were stored at –20 °C until total arsenic analysis by using the method described in Section 2.2.2.

3.2.3 Arsenic Speciation Analysis by HPLC and ICP-MS

Arsenic speciation analysis in fish extracts was performed on a PRP-X100S anion exchange column (5μm × 4.1 mm ID ×150 mm, Hamilton, US) with a guard column (PRP-X100S, Hamilton, US) by an Agilent 1100 series HPLC system (Agilent Technologies, US). The mobile phase was prepared as 5% MeOH with 35 mM ammonium bicarbonate (NH_4HCO_3) (Sigma-Aldrich), and the pH was adjusted to 8.25; NH_4HCO_3 is compatible with HPLC-ICP-MS. A small percentage of MeOH in the mobile phase solution could increase the signal of arsenic detection. Next, the mobile phase solution was filtered through a 0.45 μm membrane and put into a sonication bath for 15 min before HPLC

separation. The HPLC conditions are shown in Table 3.1. The injection volume is 50 µL each time for every standard and sample. The ICP-MS condition were the same as those for the trace element analysis (Chapter 2).

Table 3.1. Gradient Elution Conditions for HPLC Separation and HPLC Conditions. The Mobile Phase Remained Constant, and the Flow Sate Started at 0.8 mL/min from 0 to 4 min. From 4.01 min to 20 min, the Flow Rate Increased to 1.5 mL/min and Kept There to the End of the Analysis

	HPLC Parameters
Column	PRP-X100 anion exchange, 5µm × 4.1 mm ID × 150 mm
Mobile Phase	5% MeOH, 35 mM Ammonium bicarbonate, pH adjusted to 8.25
Flow Rate	
0 ~ 4 (min)	0.8 mL/min
4.01 ~ 20 (min)	1.5 mL/min

After HPLC separation, the column was connected to the nebulizer pump of ICP-MS (Agilent 7500cs Octopole, Japan) to be detected. The arsenic species were monitored at m/z = 75.0 (As^{+}) by ICP-MS. Quantification of each arsenic species was determined through calibration of arsenic stock solutions. A mixture of five arsenic standard species, AsB, As^{III}, DMA, MMA, and As^{V}, were running in freshwater fish arsenic speciation. A calibration curve from individual arsenic stock solutions was prepared on a daily basis with concentrations ranging from 0.1 µg to 20 µg As/L prior to fish analysis. Identification of arsenic species in freshwater fish was achieved by matching the retention time and arsenic standards spiking to the sample. An extra 5 µg As/L standard solution was used to check instrument drifting every four to six samples. SRM 1568b rice flour and DORM-4 fish protein were analyzed for method quality control. SRM 2669 LI was used to check the accuracy of AsB, DMA, and MMA of the calibration curve, and LII was used to check As^{V}. CRM 18 was used to check the accuracy of AsB and DMA of the calibration curve.

3.3 Results and Discussion

3.3.1 Arsenic Species in Fish by HPLC and ICP-MS

3.3.1.1 Determination of Arsenic Species using HPLC-ICP-MS

Figure 3.1 (a) shows a typical HPLC-ICP-MS chromatogram of five arsenic species in a mixed standard solution. A PRP-X100 anion exchange column was used for separation. All five arsenic species (AsB, As^{III}, DMA, MMA, and As^{V}) were well baseline-resolved within 20 min. Figure 3.1 (b) shows a typical arsenic speciation chromatogram of lake whitefish that was obtained from HPLC-ICP-MS analysis. Lake whitefish is one of the most common fish that are consumed and we have analyzed. The pK_a of these five arsenic species are shown in Table 3.2. The elution order was as follows: AsB, As^{III}, DMA, MMA, and As^{V}. AsB elutes first due to its zwitterion characteristics.

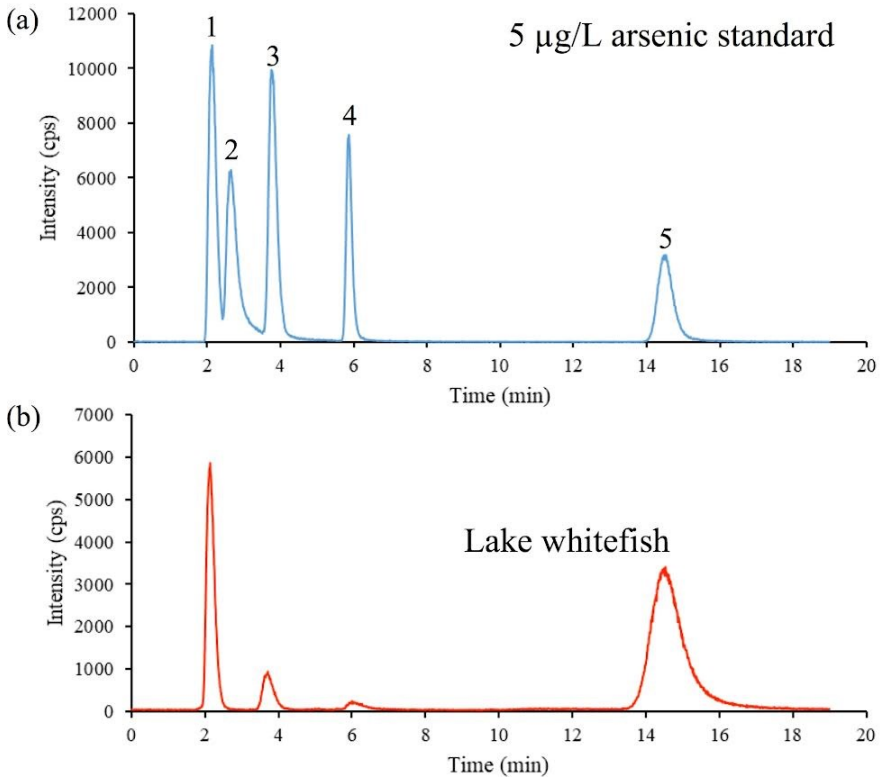
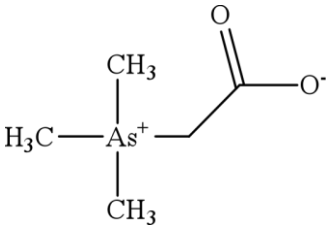
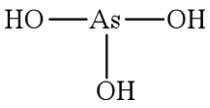
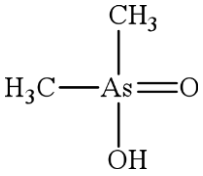
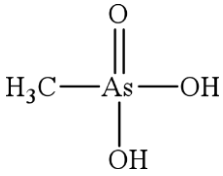
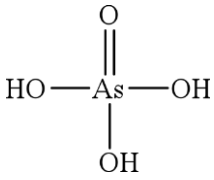


Figure 3. 1. Chromatograms obtained from HPLC-ICP-MS analyses of (a) 5 µg/L arsenic standards, (b) Lake whitefish. An anion exchange column (PRP-X100, 4 µm × 4.6 mm ID × 15 cm) was used for separation. The mobile phase contained 5% methanol in water with 35 mM ammonium bicarbonate (pH adjusted to 8.25). The peaks labelled 1-5 are AsB, As^{III}, DMA, MMA, and As^{V}, respectively.

Table 3.2. Arsenic Standard Species and Their pK_a Value in This Study

Abbrev.	Structure	pK_a
AsB		pK_a = 2.18
As^{III}		pK_{a1} = 9.23 pK_{a2} = 12.1 pK_{a3} = 13.4
DMA		pK_a = 6.2
MMA		pK_{a1} = 4.1 pK_{a2} = 8.7
As^{V}		pK_{a1} = 2.19 pK_{a2} = 6.98 pK_{a3} = 11.53

On the basis of retention time match between the peaks in the standards and in the sample, Figure 3.2 (b) suggested the suspect presence of AsB, DMA, MMA, and As^{V} in the lake whitefish sample. To check whether the fish sample matrix affects the retention time of arsenic species, I added arsenic standards to the fish sample extract and repeated the analysis of the spiked sample. Five arsenic standards (AsB, As^{III}, DMA, MMA, and As^{V}) were spiked individually to lake whitefish samples and analyzed using HPLC-ICP-

MS, as shown in Figure 3.2 (a-e). The chromatograms were compared with the original sample and standard chromatograms. If the suspected peak in fish sample increases obviously after the corresponding standard arsenic spike, the identity of the suspected arsenic species could be confirmed as the spiked arsenic standard.

The spike experiments supported the identity of AsB, DMA, and As^V. In Figure 3.2 (d), the MMA peak retention time is different from the suspected peak. This peak is not due to MMA; but is an unknown arsenic species. We denote this peak as Unknown 5. A small flat peak after Unknown 5 before As^V was denoted as Unknown 1. These two unknown arsenic species remain to be identified. Their retention times are similar to those of arsenosugars reported in marine organisms [14].

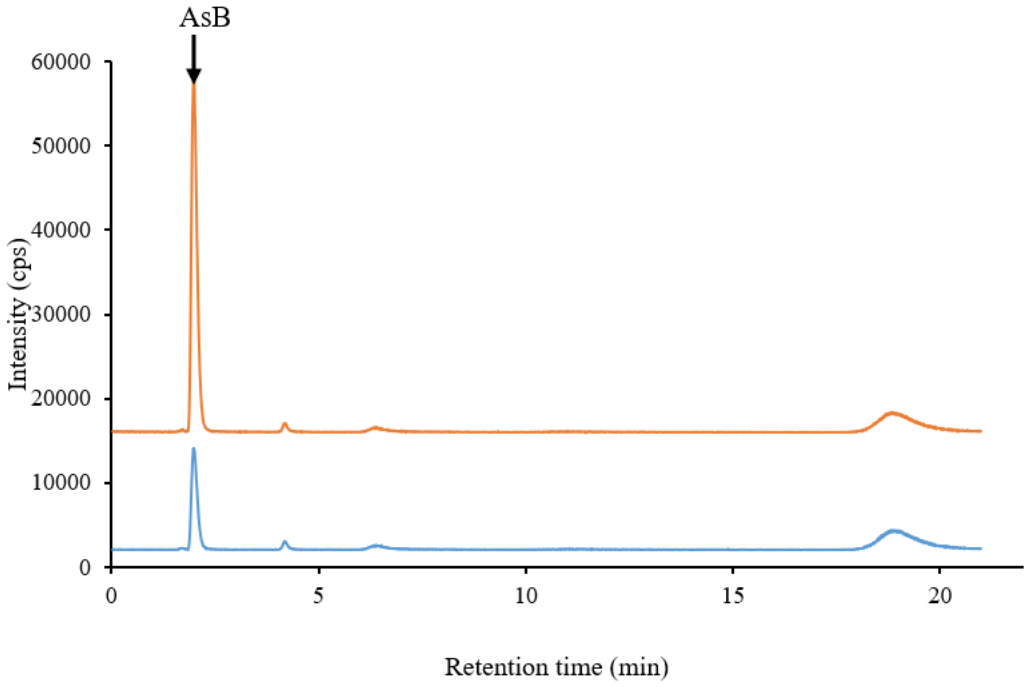


Figure 3.2. (a) Chromatograms obtained from HPLC-ICP-MS analysis of arsenic in a lake whitefish sample (bottom chromatogram) and the lake whitefish sample spiked with 15 µg/L AsB standard (top chromatogram).

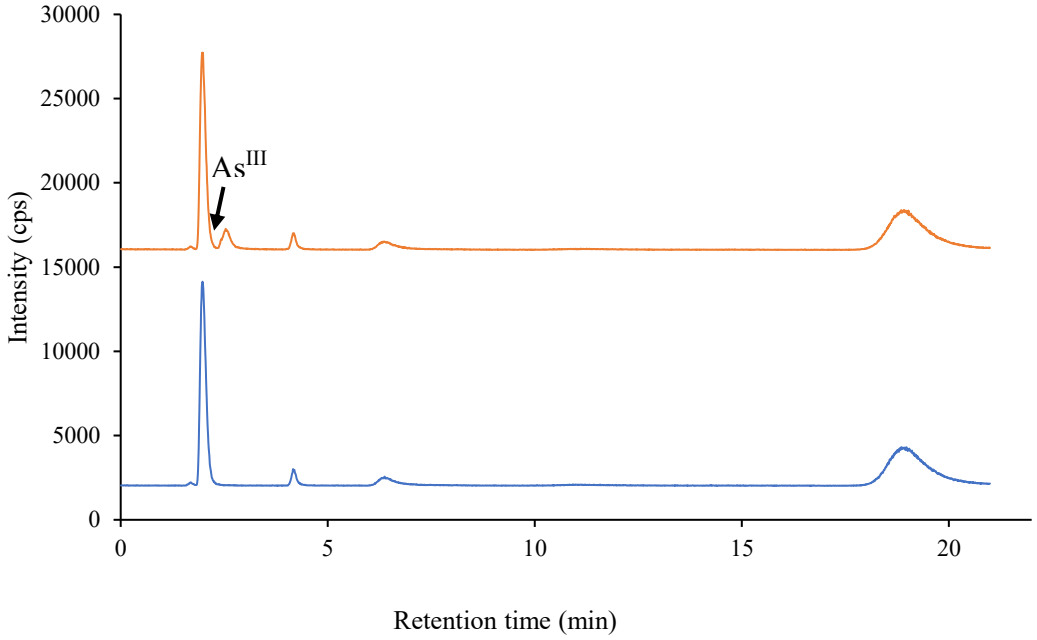


Figure 3.2. (b) Chromatograms obtained from HPLC-ICP-MS analysis of arsenic in a lake whitefish sample and the lake whitefish sample spiked with 1 µg/L As^{III} standard.

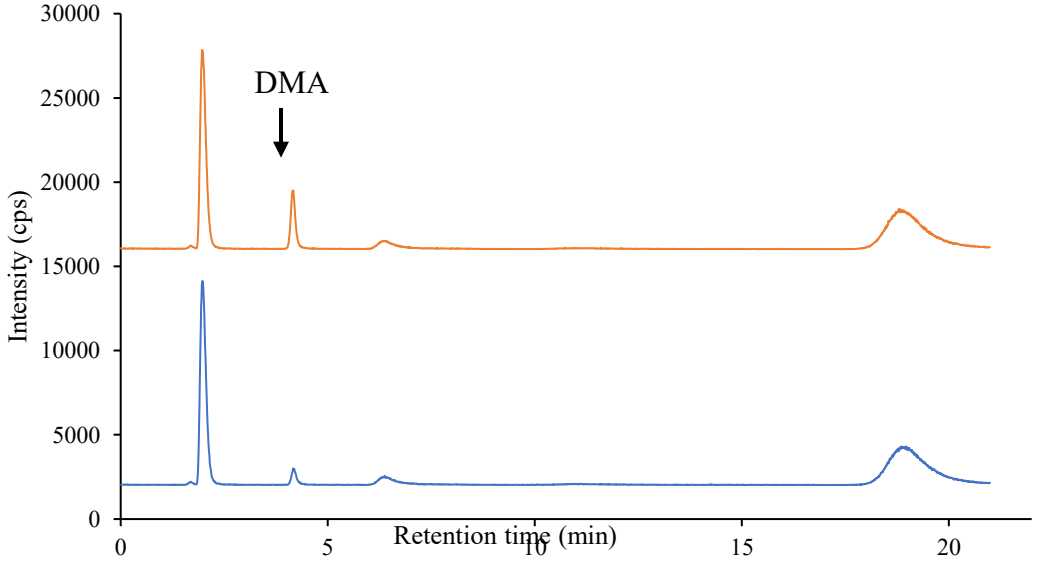


Figure 3.2. (c) Chromatograms obtained from HPLC-ICP-MS analysis of arsenic in a lake whitefish sample and the lake whitefish sample spiked with 3 µg/L DMA standard.

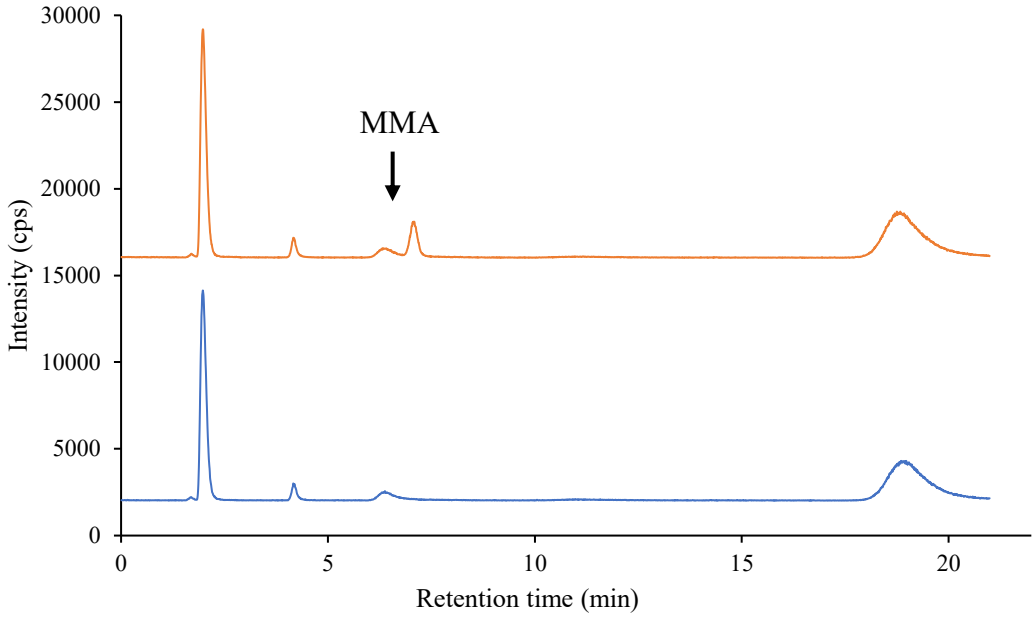


Figure 3.2. (d) Chromatograms obtained from HPLC-ICP-MS analysis of arsenic in a lake whitefish sample and the walleye sample spiked with 2 µg/L MMA standard.

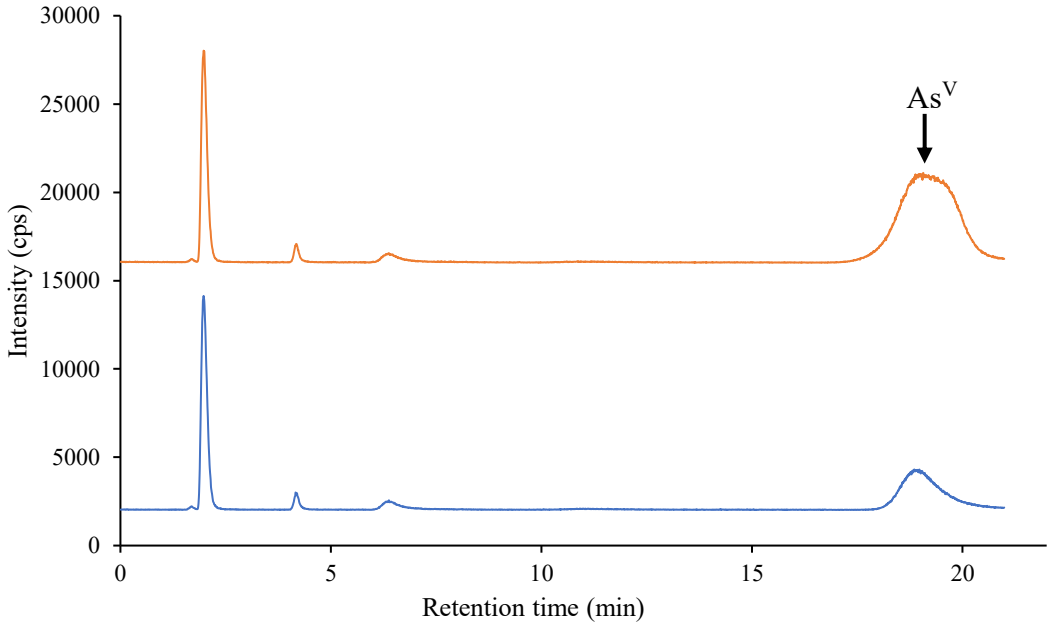


Figure 3.2. (e) Chromatograms obtained from HPLC-ICP-MS analysis of arsenic in a lake whitefish sample and the lake whitefish sample spiked with 20 µg/L As^{V} standard.

In addition to lake whitefish, the other two main fish species analyzed in the project are northern pike and walleye. Spike experiments for northern pike and walleye were

performed similarly to those for lake whitefish samples. Results are shown in Figure 3.3 (a-e) and Figure 3.4 (a-e). These results indicate that northern pike contained AsB and DMA (Figure 3.3 (a) and (c)). There is no unidentified arsenic species present in northern pike. The walleye contained AsB, DMA, and As^V. Similar to the observations of lake whitefish, a small peak between DMA and As^V in Figure 3.4 (d) was not MMA, but the Unknown 5. A small peak before AsB was denoted as Unknown 2. Unknown 2 elutes earlier than AsB, suggesting that it is probably positively charged. Arsenocholine (AsC) and tetramethylarsonium ion (TETRA), both positively charged, have been found in marine organisms.

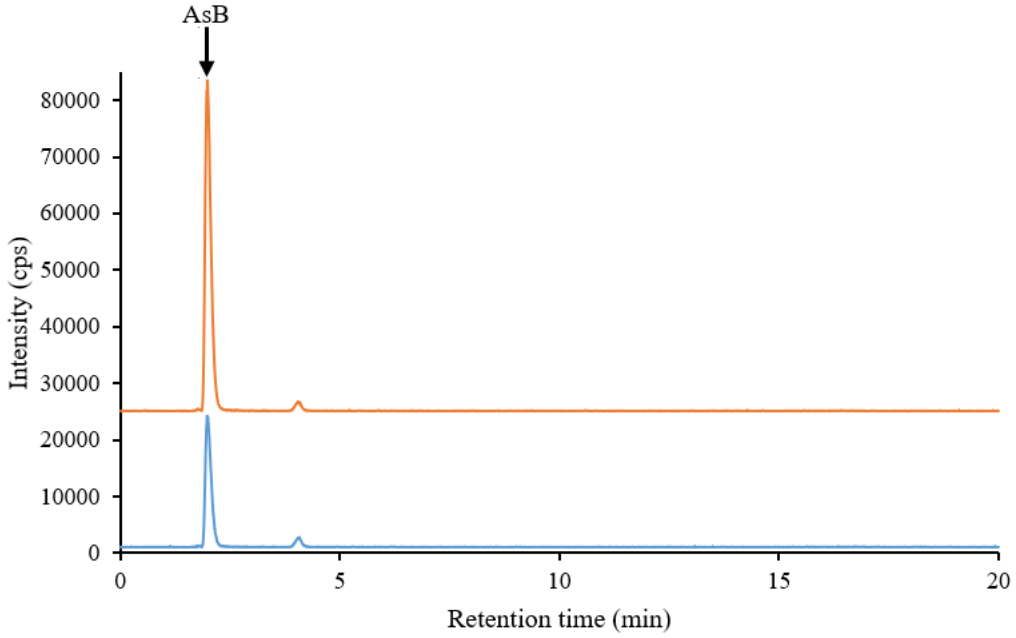


Figure 3.3. (a) Chromatograms obtained from HPLC-ICP-MS analysis of arsenic in a northern pike sample and the northern pike sample spiked with 20 µg/L AsB standard.

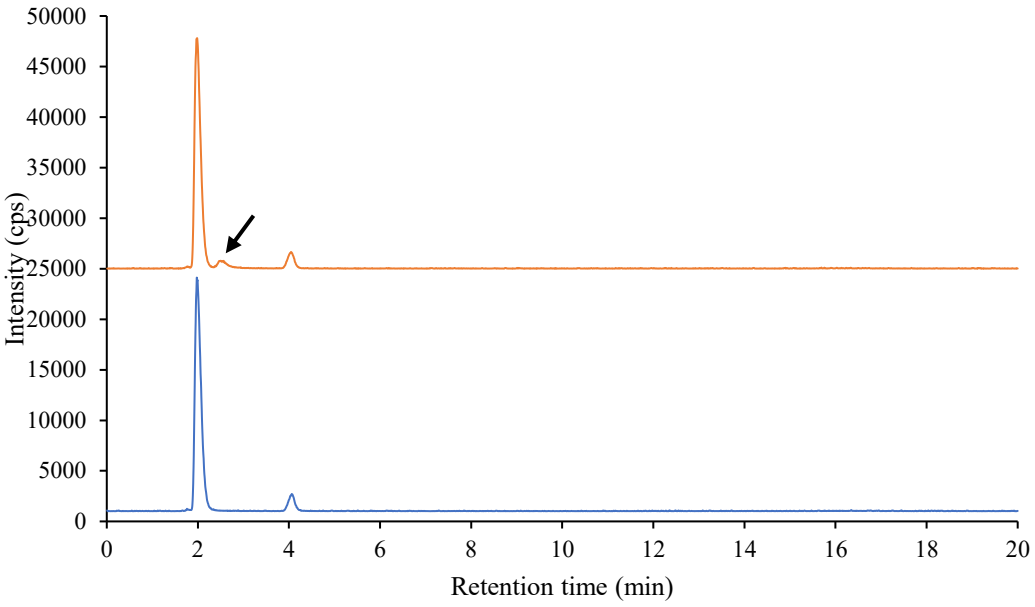


Figure 3.3. (b) Chromatograms obtained from HPLC-ICP-MS analysis of arsenic in a northern pike sample and the northern pike sample spiked with 1 µg/L As^{III} standard.

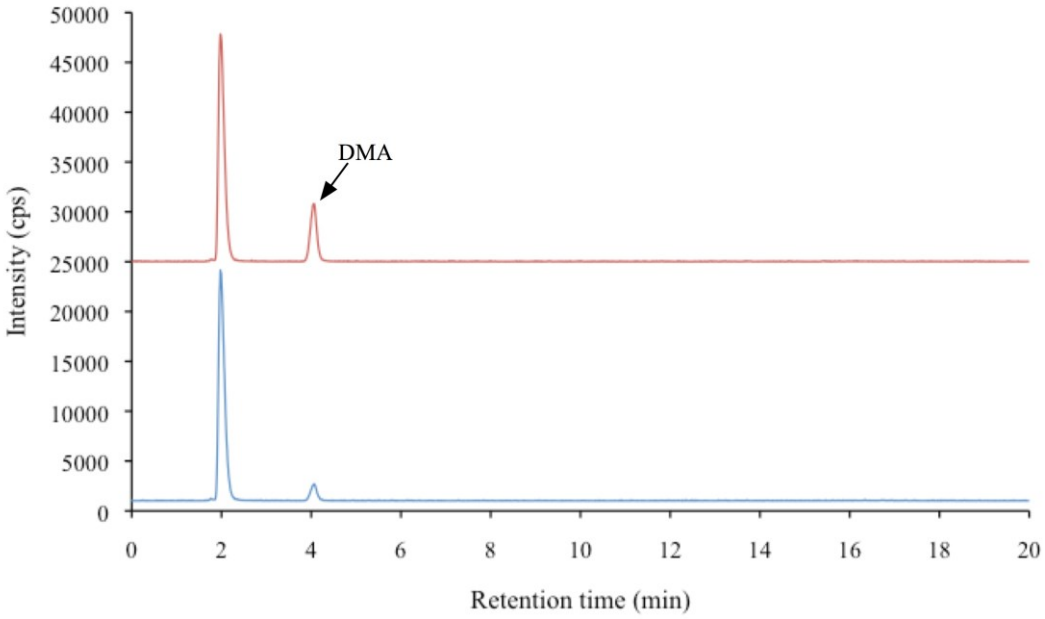


Figure 3.3. (c) Chromatograms obtained from HPLC-ICP-MS analysis of arsenic in a northern pike sample and the northern pike sample spiked with 3 µg/L DMA standard.

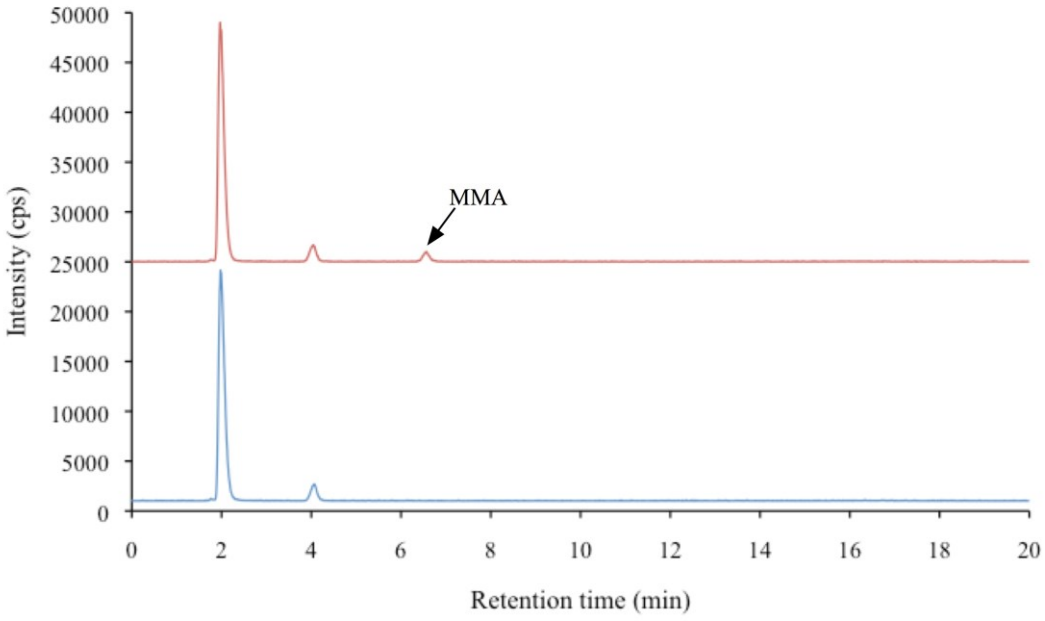


Figure 3.3. (d) Chromatograms obtained from HPLC-ICP-MS analysis of arsenic in a northern pike sample and the northern pike sample spiked with 1 µg/L MMA standard.

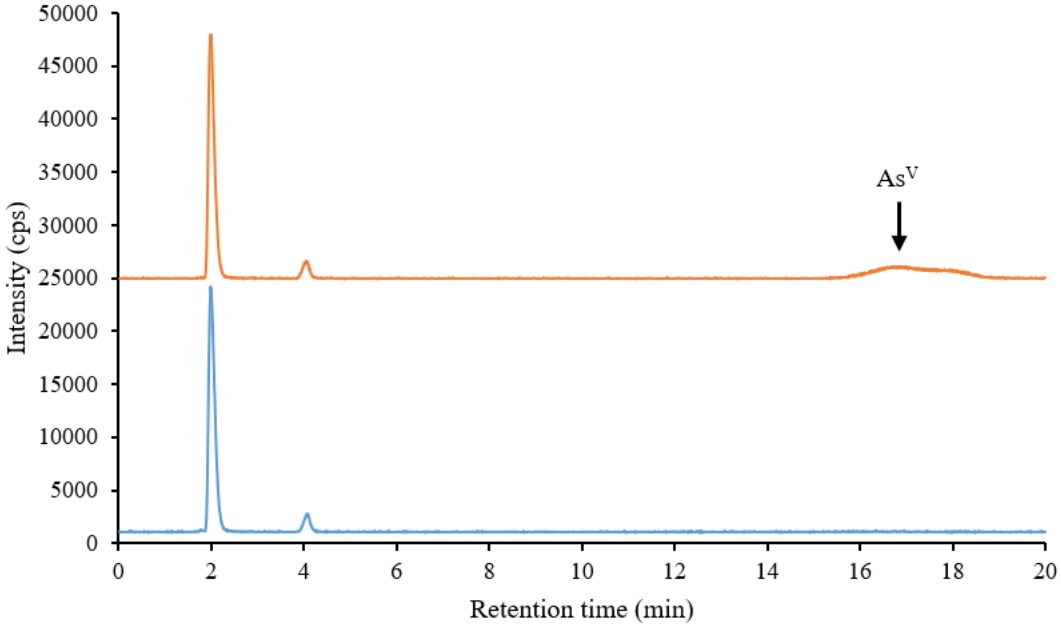


Figure 3.3. (e) Chromatograms obtained from HPLC-ICP-MS analysis of arsenic in a northern pike sample and a northern pike sample with 10 µg As^V standard spike for identification.

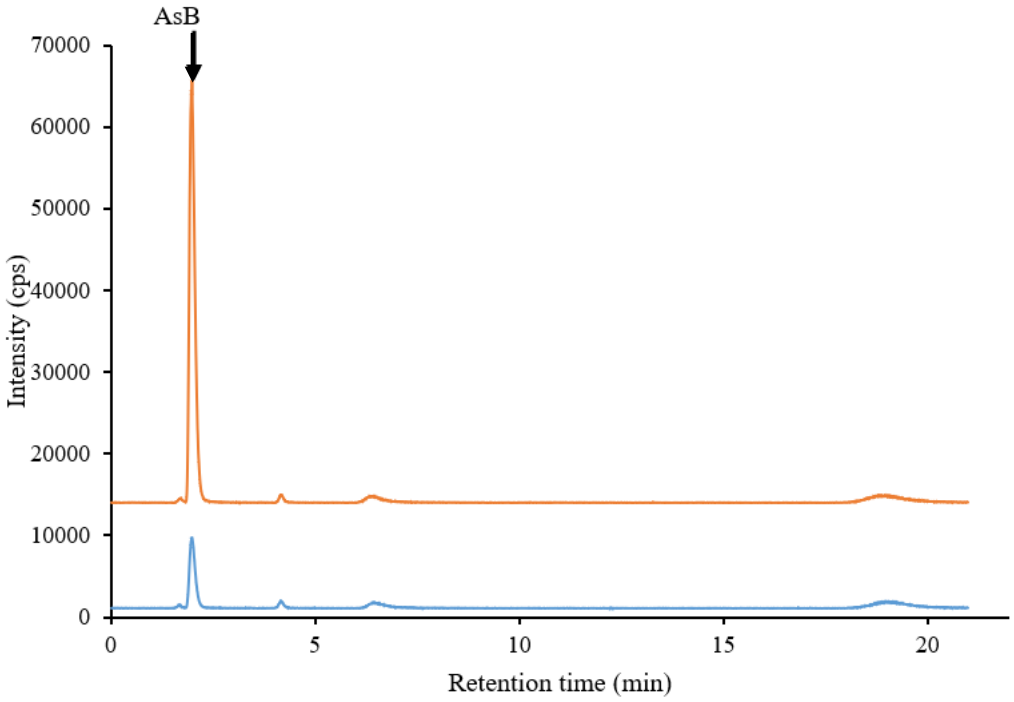


Figure 3.4. (a) Chromatograms obtained from HPLC-ICP-MS analysis of arsenic in a walleye sample and the walleye sample spiked with 20 µg/L AsB standard.

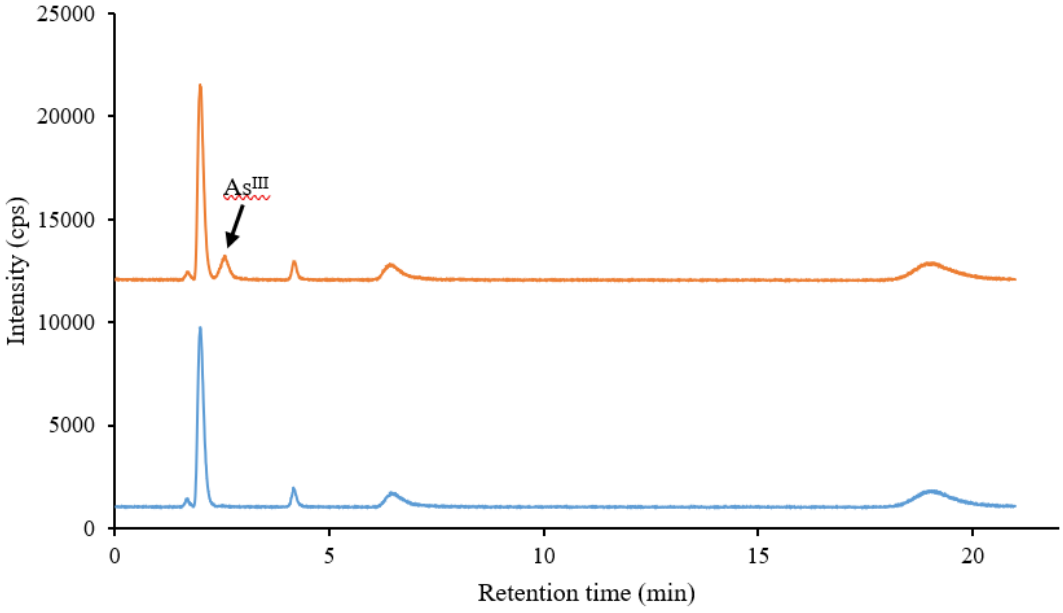


Figure 3.4. (b) Chromatograms obtained from HPLC-ICP-MS analysis of arsenic in a walleye sample and the walleye sample with 2 µg/L As^{III} standard.

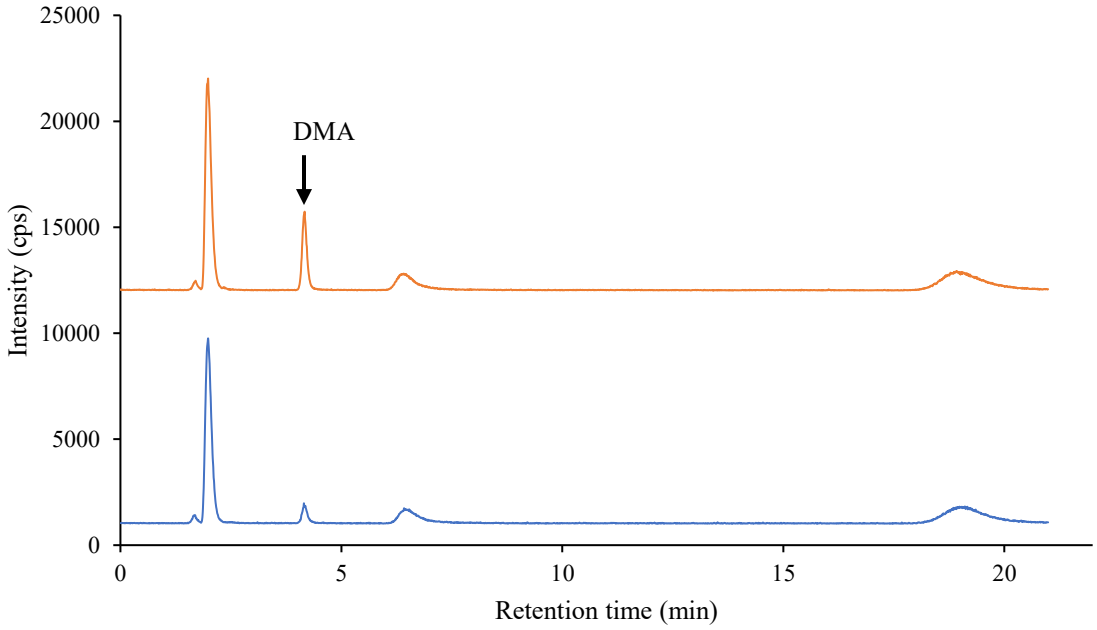


Figure 3.4. (c) Chromatograms obtained from HPLC-ICP-MS analysis of arsenic in a walleye sample and the walleye sample with 3 µg/L DMA standard.

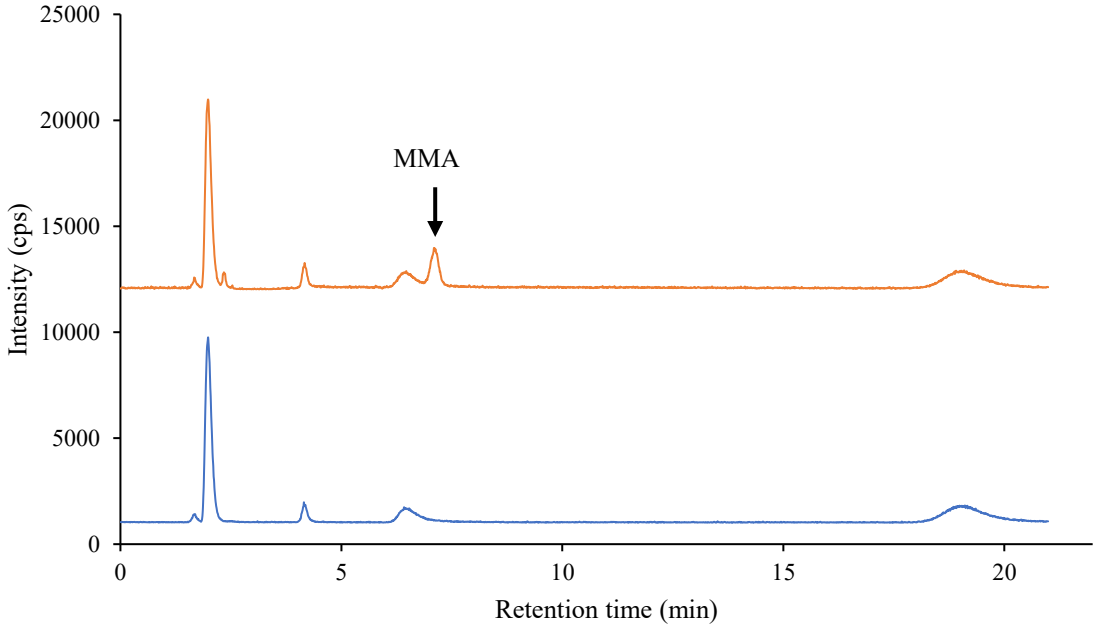


Figure 3.4. (d) Chromatograms obtained from HPLC-ICP-MS analysis of arsenic in a walleye sample and the walleye sample spiked with 2 µg/L MMA.

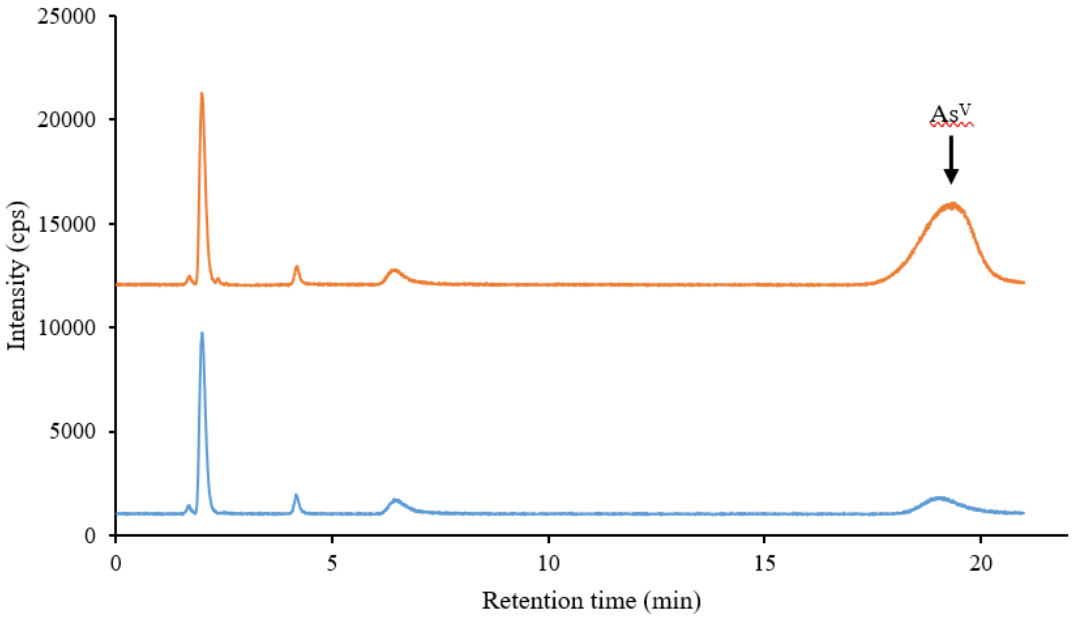


Figure 3.4. (e). Chromatograms obtained from HPLC-ICP-MS analysis of arsenic in a walleye sample and the walleye sample with 20 µg/L As^V standard.

3.3.1.2 Quantification of Arsenic Species using HPLC-ICP-MS

HPLC-ICP-MS was also used for quantifying the concentration of individual arsenic species. Typical calibration curves of five arsenic standards, at concentrations of 0.1, 0.2, 0.5, 1, 5, 10, 20 µg/L, from HPLC-ICP-MS analysis, are shown in Figure 3.5 (a-e). The calibrations were linear and correlation coefficient was close to 1 or equal to 1. The limit of detection (LOD) was 0.00025 µg/g (wet weight of fish filet) for all arsenic species and is shown in Table 3.3. A low LOD allowed us to detect and quantify trace arsenic species in fish meat.

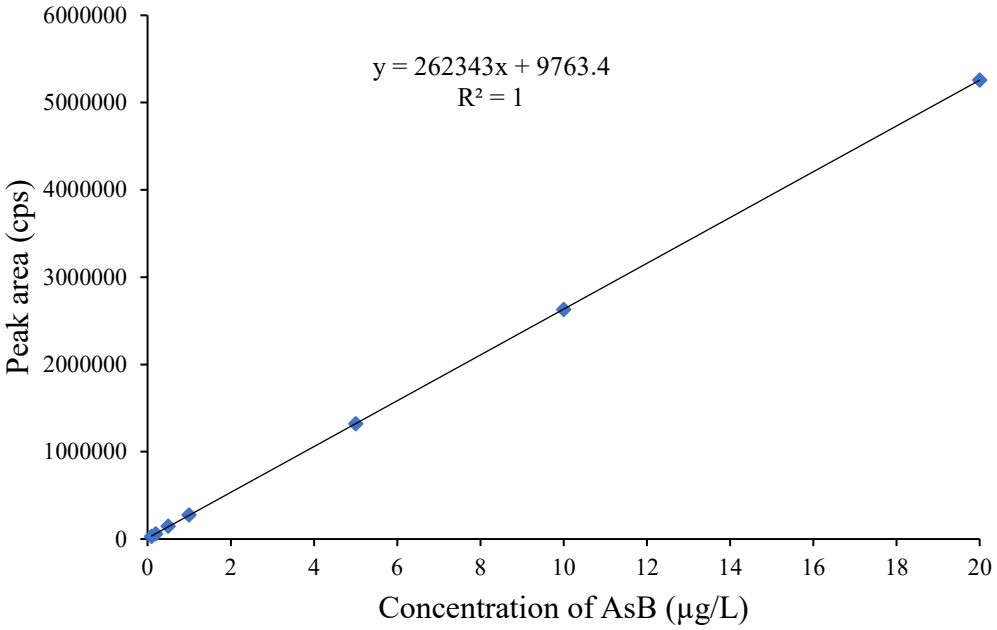


Figure 3.5. (a) A calibration curve generated from the HPLC-ICP-MS analyses of 0.1, 0.2, 0.5, 1, 5, 10, and 20 µg/L AsB standards.

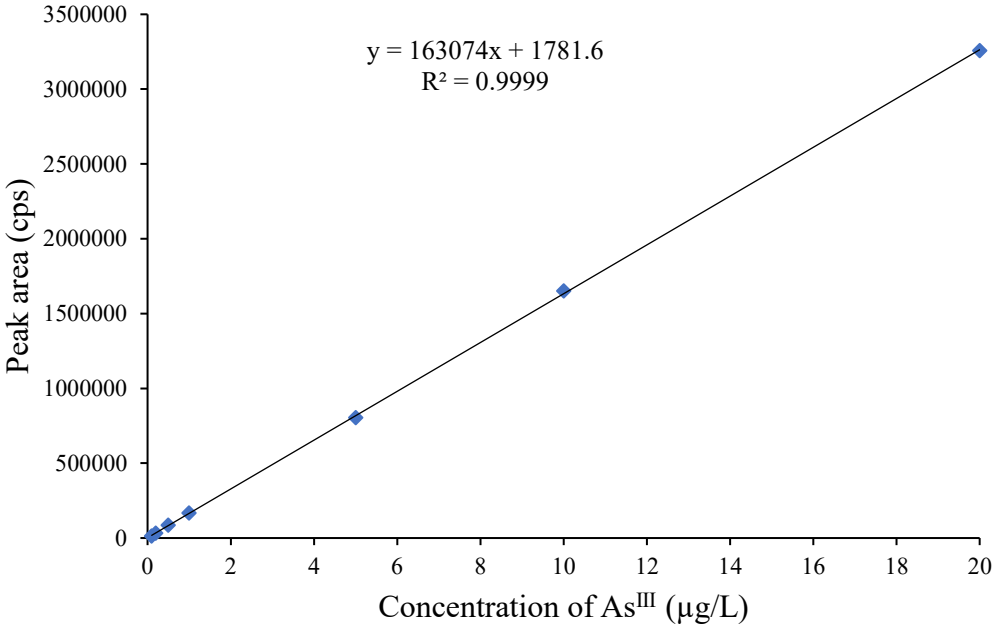


Figure 3.5. (b) A calibration curve generated from the HPLC-ICP-MS analyses of 0.1, 0.2, 0.5, 1, 5, 10, and 20 µg/L As^{III} standards.

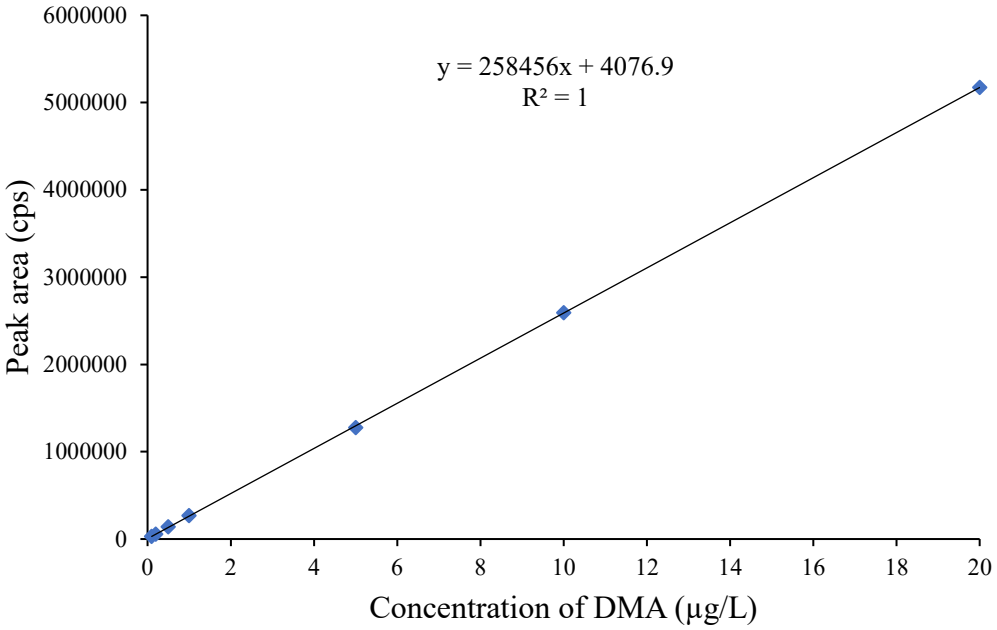


Figure 3.5. (c) A calibration curve generated from the HPLC-ICP-MS analyses of 0.1, 0.2, 0.5, 1, 5, 10, and 20 µg/L DMA standards.

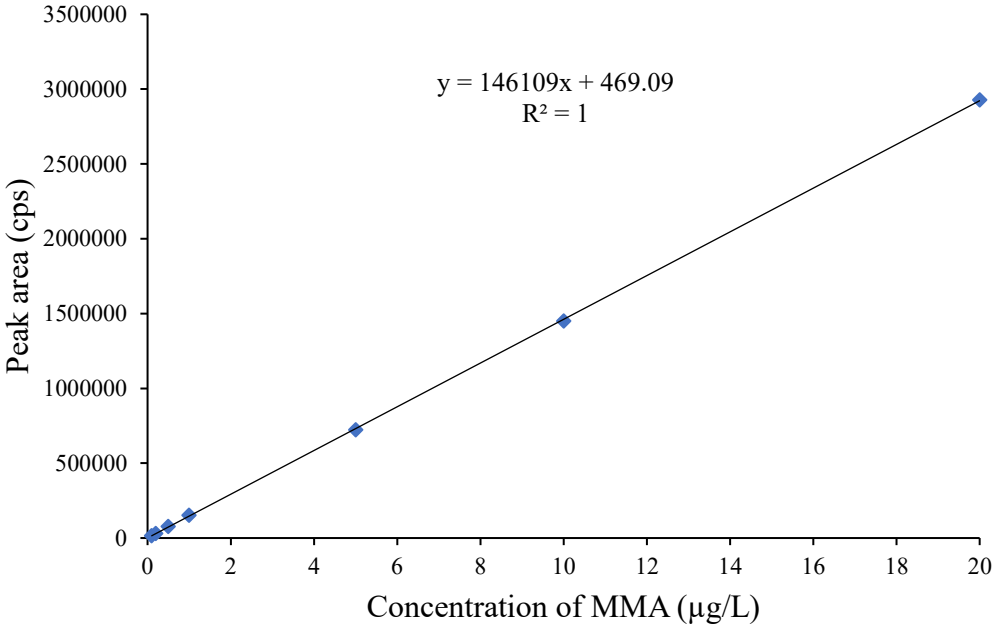


Figure 3.5. (d) A calibration curve generated from the HPLC-ICP-MS analyses of 0.1, 0.2, 0.5, 1, 5, 10, and 20 µg/L MMA standards.

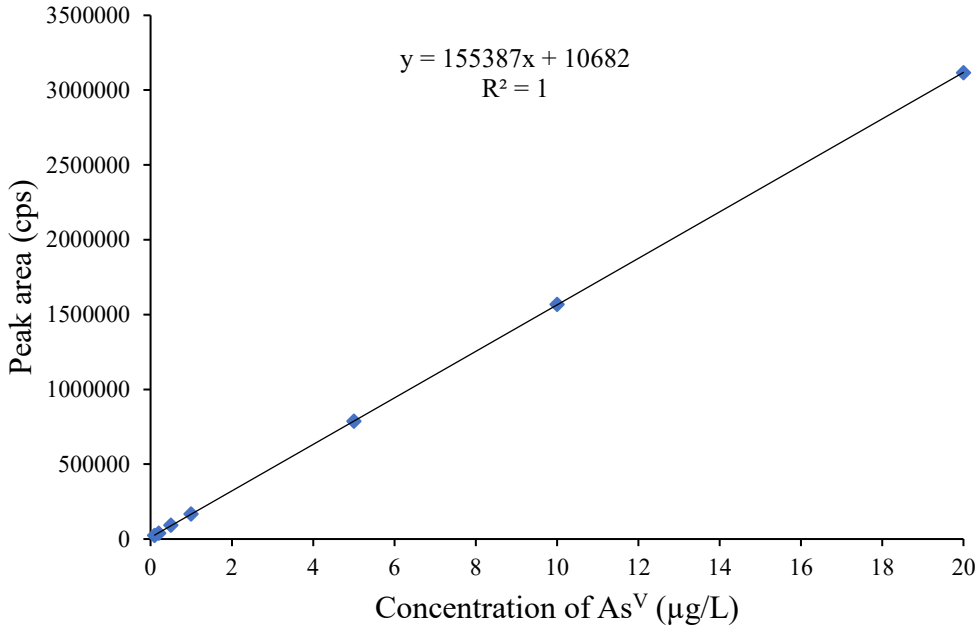


Figure 3.5. (e) A calibration curve generated from the HPLC-ICP-MS analyses of 0.1, 0.2, 0.5, 1, 5, 10, and 20 µg/L As^V standards.

SRM 2669 Level I and CRM 18 were used for checking the accuracy of analysis during 2020 and 2021. SRM Level I, Level II, and CRM 18 were used for checking the accuracy later in 2021 and 2022. SRM 2669 Level I has certified values for AsB, DMA, MMA, and Level II has certified value for As^V. CRM 18 has certified values for AsB and DMA. Comparisons of the certified values with the measured values from our analyses in three years (2020 to 2022) are summarized in Table 3.4. Our measured results are in good agreement with the certified values.

Table 3.3. Limit of Detection (LOD) for Arsenic Species in Fish Samples Using HPLC-ICP-MS

Arsenic species	LOD (µg/g)
AsB	0.00025
As^{III}	0.00025
DMA	0.00025
MMA	0.00025
As^{V}	0.00025

Table 3.4. Certified and Measured Arsenic Concentrations of Standard Reference Materials

			Measured in 2020−2021 (n = 7)		Measured in 2021−2022 (n = 13)	
Reference material	As species	Certified value	Determined value	Accuracy	Determined value	Accuracy
		µg/L	µg/L	%	µg/L	%
SRM 2669 Level I	AsB	12.4 ± 1.9	12.6 ± 1.0	98.9	12.8 ± 0.9	99.7
	DMA	3.47 ± 1.9	3.3 ± 0.4	97.5	3.6 ± 0.7	97.6
	MMA	1.87 ± 0.39	2.0 ± 0.2	93.2	1.93 ± 0.07	96.8
SRM 2669 Level II	As^{V}	6.16 ± 0.95	NA	NA	5.7 ± 0.3	91.9
CRM 18	AsB	69 ± 12	72 ± 4	95.8	71 ± 4	96.5
	DMA	36 ± 9	39 ± 4	91.7	40 ± 3	89

NA indicates not analysed.

SRM 1568b rice flour and DORM-4 fish protein were analysed in duplicate and were used for quality controls of the whole method, from extraction to the final HPLC-ICP-MS analysis. We show four different chromatograms from the analysis of two SRMs on four different days (Figure 3.6 (a-b)). Fish samples were analyzed together on the same day when SRMs were analyzed, These chromatograms show excellent repeatability. These reference materials only have the total arsenic concentration certified. There was no certified value for individual arsenic species in these materials. We added the measured concentrations of individual arsenic species and compared the sum with the certified total arsenic value. Results are summarized in Table 3.5.

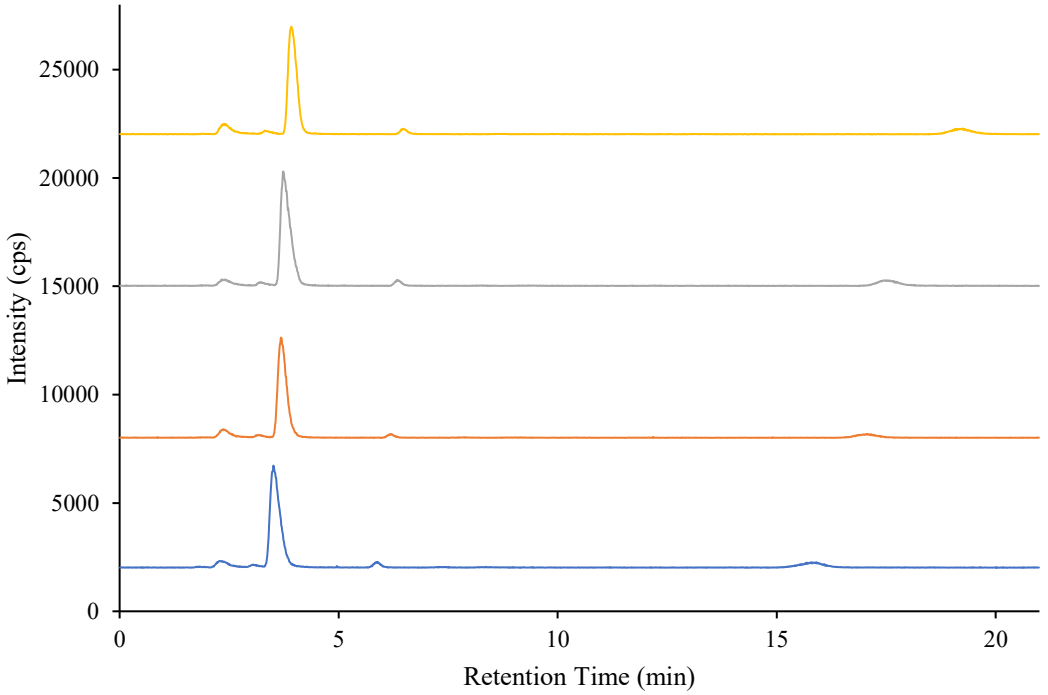


Figure 3.6. (a) Chromatograms obtained from HPLC-ICP-MS of SRM 1568b rice flour on four different days when fish samples were analyzed**.**

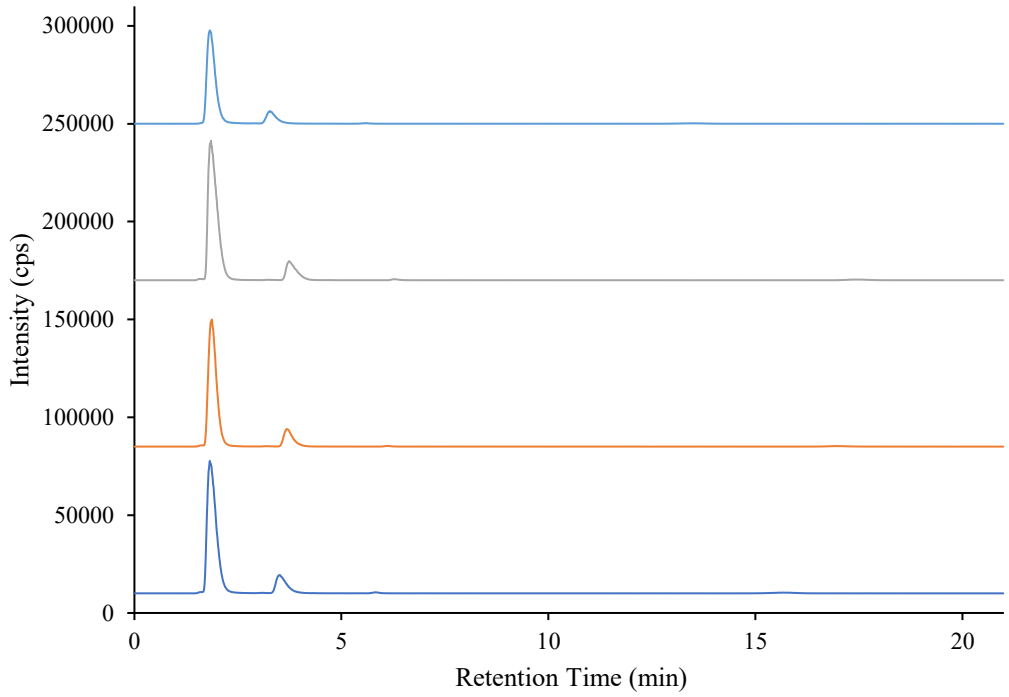


Figure 3.6. (b) Chromatograms obtained from HPLC-ICP-MS of DORM-4 fish tissue on four different days when fish samples were analysed.

Table 3.5. Certified and Measured Arsenic Concentration in Standard Reference Materials Analysed Between 2020 and 2022

		2020−2021 (n = 7)		2021−2022 (n =13)	
	Certified value	Determined value	Recovery	Determined value	Recovery
	mg/kg	mg/kg	%	mg/kg	%
DORM4	6.8	5.0 ± 0.4	73	4.9 ± 0.3	71
SRM1568b	0.285	0.19 ± 0.01	68	0.21 ± 0.02	75

3.3.2 Extraction Efficiency of Arsenic Species in Freshwater Fish

Figure 3.7. illustrates a schematic to check for mass balance in freshwater fish by HPLC-ICP-MS. The supernatant is the extracts after a four-time MeOH-water extraction. The residue is the fish meat after extraction. The supernatant and residue were acid digested for

total arsenic concentration analysis by ICP-MS. The results were compared with the total arsenic concentration described in Chapter 2.

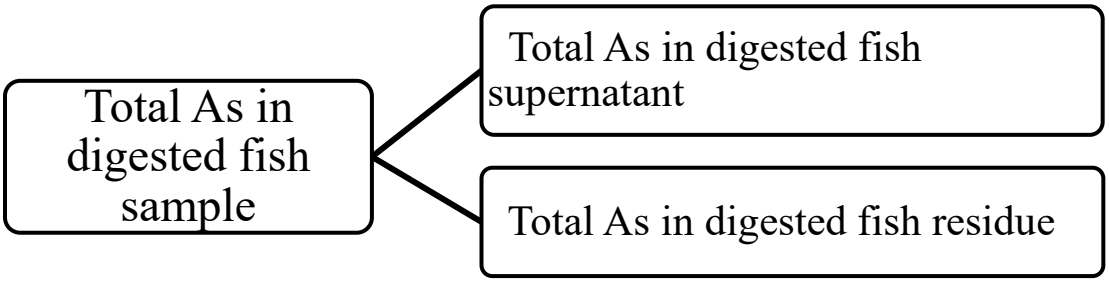


Figure 3.7. Mass balance schematic. Total arsenic concentration in digested fish residue and digested fish supernatant after extraction should be similar to or the same as the total arsenic concentration in digested fish samples.

We analyzed the three most common fish species present in our study, northern pike (NRPK), lake whitefish (LKWH), and walleye (WALL). Methanol-water could not extract non-polar arsenolipids from fish [15]. The different fillet meat of fish species has different fat contents and might have a different extraction efficiency.

Further, we were wondering what arsenic species can be in the residue that methanol-water extraction could not extract. From the literature, other organic solvents, such as dichloromethane (DCM) and methyl-*tert*-butylether (MTBE) coul extract non-polar arsenolipids [16, 17]. Various enzymes, including protease type XIV, pancreatin, pepsin, and trypsin could break the arsenic bond to protein and thus improve the extraction efficiency [18-21].

3.3.2.1 Mass Balance

To evaluate the mass balance, I separately digested the supernatant and residue after methanol-water extraction, and determined arsenic concentration using ICP-MS. In addition, another aliquot of the original fish samples went through acid digestion directly and were analyzed using ICP-MS. The digestion method was described in Chapter 2. Individual fish samples, including the supernatant and residue were prepared in triplicate. I repeated the experiments with two Lake Whitefish, three Northern Pike, and three Walleye samples.

The results of mass balance, expressed as percentage of sum of arsenic in the supernatant and residue over the total arsenic concentration in fish, are summarized in Table 3.6. The overall percentages are 91−97% for northern pike, 88−90% for lake whitefish, and 97−99% for walleye. The sample preparation process, such as extraction, transferring the solution, evaporation, and pipetting, might cause sample loss. These factors might contribute to the mass balance of lower than 100%.

Table 3.6. Recovery for Arsenic Species in Northern Pike, Lake Whitefish, and Walleye

Fish samples	sum/total
Lake whitefish-01	88%
Lake whitefish-02	90%
Northern pike-01	97%
Northern pike-02	94%
Northern pike-03	91%
Walleye-01	97%
Walleye-02	99%
Walleye-03	98%

3.3.2.2 Extraction Efficiency of Arsenic Species from Freshwater Fish Samples

Extraction efficiency was determined as the percent of total arsenic concentration in the supernatant from the methanol-water extraction in fish over the total arsenic concentration in fish. The extraction efficiency of northern pike, lake whitefish, and walleye is given in Table 3.7.

Table 3.7. Extraction Efficiency for Arsenic Species from Northern Pike, Lake Whitefish, and Walleye

Fish samples	Extraction efficiency
Lake whitefish-01	44%
Lake whitefish-02	50%
Northern pike-01	49%
Northern pike-02	48%
Northern pike-03	51%
Walleye-01	41%
Walleye-02	43%
Walleye-03	44%

For methanol-water extraction, the extraction efficiency of arsenic species from northern pike is 48−51%, from lake whitefish is 44−50%, from walleye is 41−44%. The results consistent with published studies regarding the extraction efficiency of freshwater fish [12, 22, 23]. Overall, extraction using methanol-water has a lower efficiency than extraction with acid and enzyme digestion [18, 24, 25]. A mixture of methanol and water may not be able to extract strongly bound and non-polar arsenic species.

We further compared the sum of the arsenic species and total arsenic (from Chapter 2) in 266 freshwater fish samples, shown in Figure 3.8 (a). The sum of the arsenic species included five common arsenic species and unknown species that were detected by HPLC-ICP-MS. The unknown species concentration was estimated by the nearest known standard. Figure 3.8 (b) shows the comparison in the sum of the arsenic species and total arsenic concentration from 79 northern pike, 66 lake whitefish, and 76 walleye samples. The regression lines for northern pike and walleye were similar, while the regression line for lake whitefish was drawn with significant outliers, causing a significant difference. The outlier can be lake whitefish in Cold Lake, which will be discussed in Section 3.3.5.

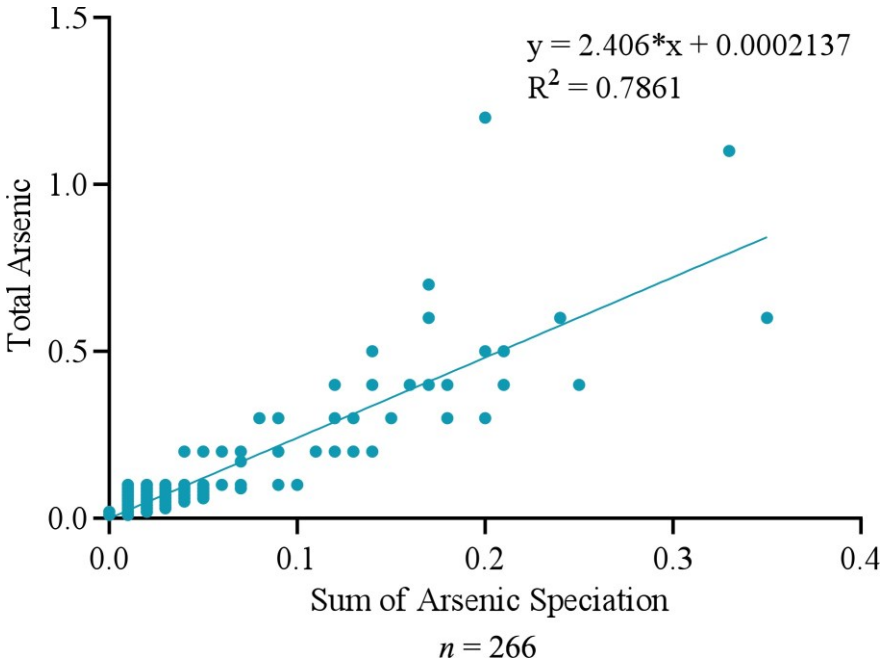


Figure 3.8. (a) Comparing the sum of the arsenic speciation results and total arsenic results in 266 freshwater fish analyzed.

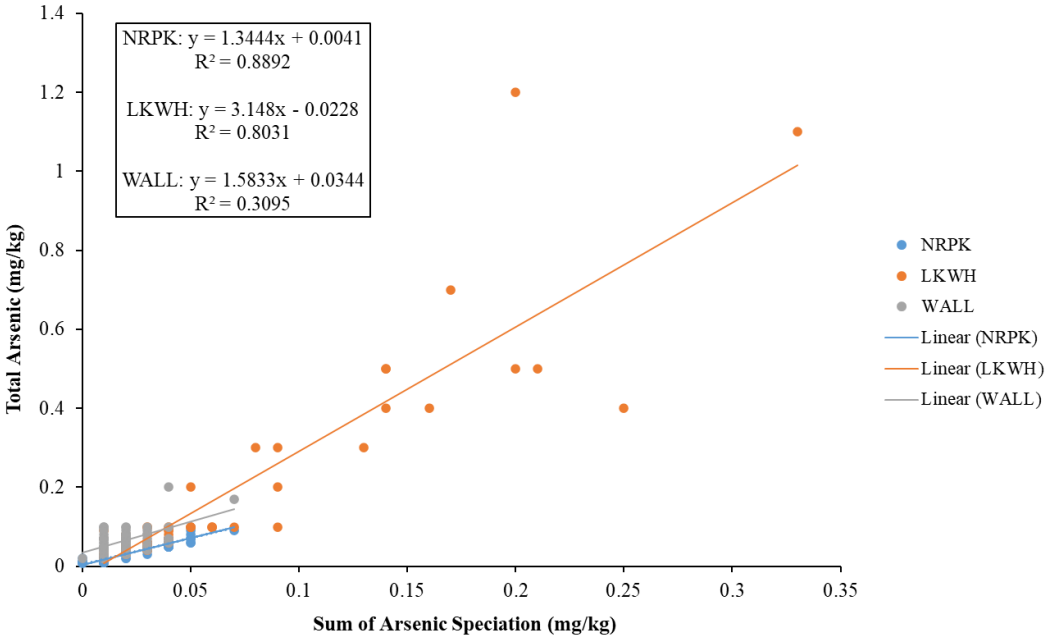


Figure 3.8. (b) Comparing the sum of the arsenic speciation results and total arsenic results in 79 northern pike (NRPK), 66 lake whitefish (LKWH), and 76 walleye (WALL) samples analyzed.

3.3.3 Arsenic Speciation Patterns of Different Fish Species

I have studied 266 freshwater fish that Alberta Health collected from 2014 to 2020. The overall concentrations of arsenic species are summarized in Table 3.8, including median

and meant ± standard deviation from all fish samples that have detectable arsenic species. Northern pike (NRPK), lake whitefish (LKWH), and walleye (WALL) are three major fish that were collected from every lake from 2014 to 2020. The arsenic speciation patterns for rainbow trout (RNTR) and lake trout (LKTR) also were studied since the fish numbers are over 10 and statistically meaningful. Figure 3.9 (a-e) shows typical chromatograms from HPLC-ICP-MS analyses of arsenic standards, blank, and five types of freshwater fish samples. Four out of five arsenic species, including AsB, DMA, MMA, As^{V}, and some unknown arsenic species were detected. As^{III} was not detected in any freshwater fish in this study.

Table 3. 8. Overall Concentrations of Arsenic Species from All Fish Samples and Different Fish species that have Detectable Arsenic Species.

	lake	AsB	DMA	MMA	AsV	UN1	UN2	UN3	UN4	UN5	UN7	sum As Species	sum of DMA,MMA,ASV	Exclude AsB
all fish	Detectable sample	265	259	2	183	50	15	3	4	177	1			
n= 266	Detectable rate	100%	97%	1%	69%	19%	6%	1%	2%	67%	0.40%			
	Mean	0.033 ± 0.055	0.0035 ± 0.0057	0.0003	0.0093 ± 0.0080	0.0054 ± 0.0052	0.0011 ± 0.0008	0.0018 ± 0.0012	0.00072 ± 0.000531	0.0023 ± 0.0021	0.00037	0.045 ± 0.57	0.0098 ± 0.0092	0.012 ± 0.0094
	Median	0.0081	0.002	0.0003	0.0075	0.004	0.0008	0.002	0.001	0.002	0	0.023	0.007	0.0096
NRPK	Detection rate	78	79		23		2	1	1					
n= 79	Detected sample	99%	100%		29%		3%	0.01%	0.01%					
	Mean	0.013 ± 0.014	0.0071 ± 0.0076		0.00096 ± 0.00082		0.0005 ± 0.0007	0.001	0.003			0.020 ± 0.17	0.0073 ± 0.0077	0.007 ± 0.007
	Median	0.0055	0.004		0.001		0.0005	0.001	0.003			0.011	0.005	0.005
LKWH	Detected sample	66	64	2	66	7	3		2	59	1			
n = 66	Detection rate	100%	97%	3%	100%	11%	5%		3%	89%	2%			
	Mean	0.041 ± 0.061	0.0025 ± 0.0035	0.00031 ± 0.00004	0.015 ± 0.009	0.00065 ± 0.00016	0.00062 ± 0.00051		0.0023 ± 0.0011	0.0012 ± 0.00082	0.00037	0.059 ± 0.066	0.017 ± 0.011	0.018 ± 0.012
	Median	0.014	0.0011	0.00031	0.013	0.0007	0.00033		0.0023	0.00099	0.00037	0.029	0.016	0.017
WALL	Detected sample	76	72		76		4			75				
n = 76	Detection rate	100%	95%		100%		5%			99%				
	Mean	0.0065 ± 0.0075	0.0014 ± 0.0010		0.0082 ± 0.0046		0.0006 ± 0.0003			0.0033 ± 0.0023		0.019 ± 0.009	0.0097 ± 0.0049	0.013 ± 0.006
	Median	0.005	0.0013		0.0075		0.00062			0.0026		0.017	0.009	0.013
RNTR	Detected sample	18	17		12	18				18				
n = 18	Detection rate	100%	94%		67%	100%				100%				
	Mean	0.088 ± 0.055	0.00048 ± 0.00022		0.0010 ± 0.0005	0.0097 ± 0.0047				0.0034 ± 0.0023		0.10 ± 0.06	0.0012 ± 0.0007	0.014 ± 0.005
	Median	0.11	0.00042		0.001	0.0099				0.0023		0.12	0.001	0.014
LKTR	Detected sample	20	20			19			1	18				
n = 20	Detection rate	100%	100%			95%			5%	90%				
	Mean	0.15 ± 0.07	0.0015 ± 0.0004			0.0046 ± 0.0045			0.00052	0.00091 ± 0.00062		0.15 ± 0.07	0.0015 ± 0.0004	0.0015 ± 0.0004
	Median	0.14	0.0016			0.0037			0.00052	0.00078		0.15	0.0016	0.0055

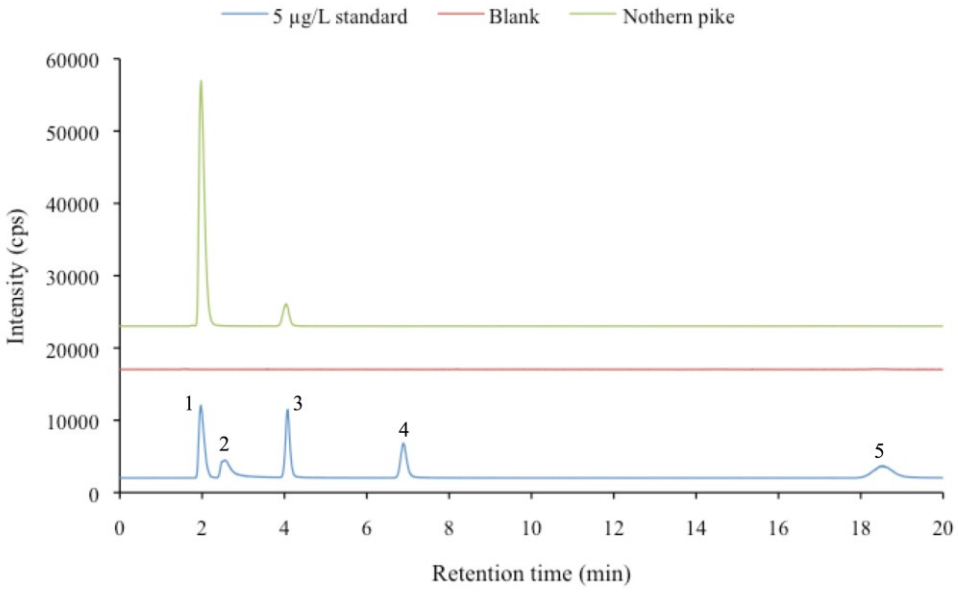


Figure 3.9. (a) Chromatograms obtained from HPLC-ICP-MS analyses of 5 µg/L arsenic standards, blank, and a northern pike sample. An anion exchange column (PRP-X100, 4 µm × 4.6 mm ID × 15 cm) was used for separation. The mobile phase contained 5% methanol in water with 35 mM ammonium bicarbonate (pH adjusted to 8.25). The peaks labelled 1-5 are AsB, As^{III}, DMA, MMA, and As^{V}, respectively.

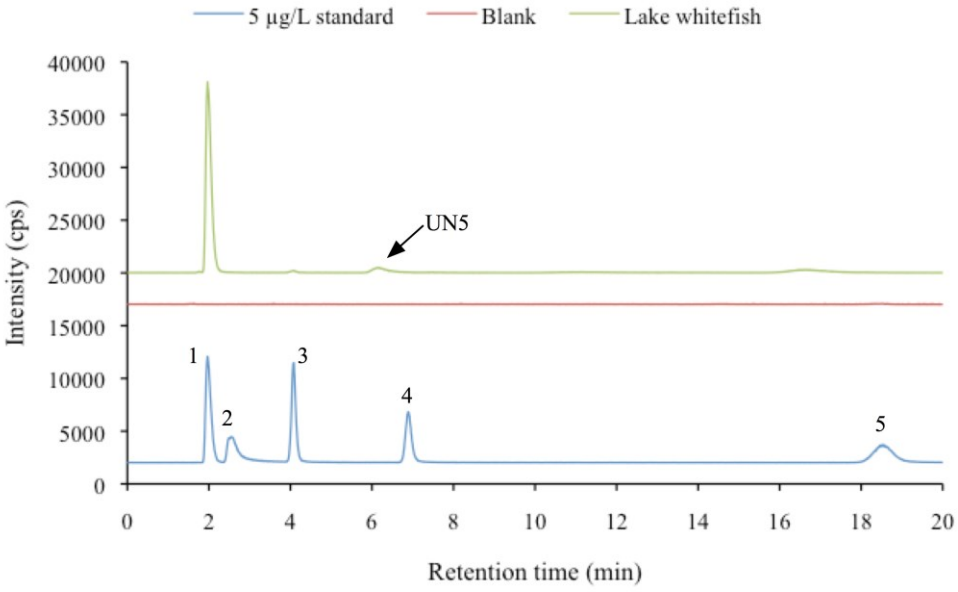


Figure 3.9. (b) Chromatograms obtained from HPLC-ICP-MS analyses of 5 µg/L arsenic standards, blank, and a lake whitefish sample. An anion exchange column (PRP-X100, 4 µm × 4.6 mm ID × 15 cm) was used for separation. The mobile phase contained 5% methanol in water with 35 mM ammonium bicarbonate (pH adjusted to 8.25). The peaks labelled 1-5 are AsB, As^{III}, DMA, MMA, and As^{V}, respectively.

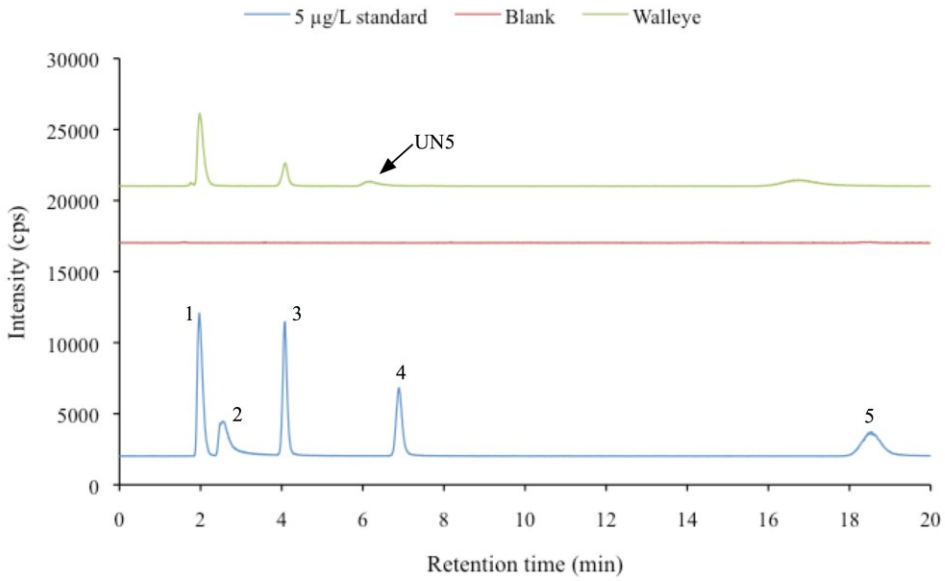


Figure 3.9. (c) Chromatograms obtained from HPLC-ICP-MS analyses of 5 µg/L arsenic standards, blank, and a walleye sample. An anion exchange column (PRP-X100, 4 µm × 4.6 mm ID × 15 cm) was used for separation. The mobile phase contained 5% methanol in water with 35 mM ammonium bicarbonate (pH adjusted to 8.25). The peaks labelled 1-5 are AsB, As^{III}, DMA, MMA, and As^{V}, respectively.

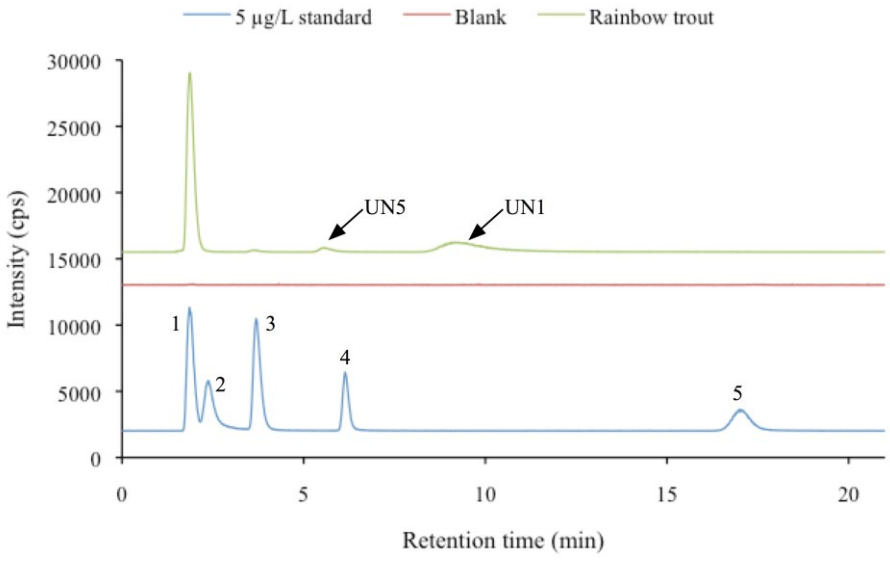


Figure 3.9. (d) Chromatograms obtained from HPLC-ICP-MS analyses of 5 µg/L arsenic standards, blank, and a rainbow trout sample. An anion exchange column (PRP-X100, 4 µm × 4.6 mm ID × 15 cm) was used for separation. The mobile phase contained 5% methanol in water with 35 mM ammonium bicarbonate (pH adjusted to 8.25). The peaks labelled 1-5 are AsB, As^{III}, DMA, MMA, and As^{V}, respectively.

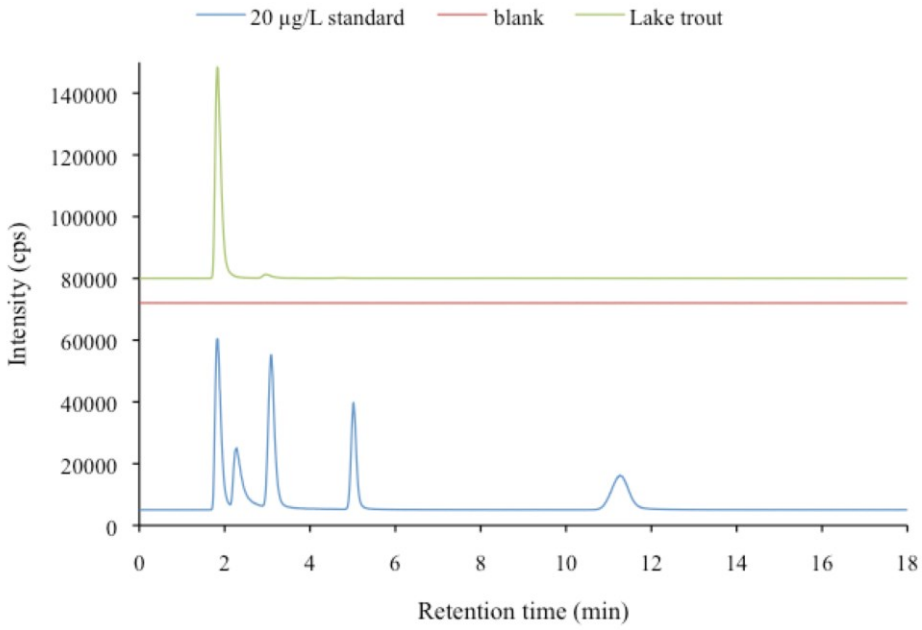


Figure 3.9. (e) Chromatograms obtained from HPLC-ICP-MS analyses of 20 µg/L arsenic standards, blank, and a lake trout sample. An anion exchange column (PRP-X100, 4 µm × 4.6 mm ID × 15 cm) was used for separation. The mobile phase contained 5% methanol in water with 35 mM ammonium bicarbonate (pH adjusted to 8.25). The peaks labelled 1-5 are AsB, As^{III}, DMA, MMA, and As^{V}, respectively.

As described in Chapter 3.3.1, different freshwater fish have different arsenic speciation patterns. The main arsenic species of northern pike are AsB and DMA. The dominant arsenic species in lake whitefish are AsB, DMA, AsV, and Unknown 5 (UN5). In walleye AsB, DMA, As^{V} and Unknown 5 are dominant. The dominant arsenic species in rainbow trout are AsB, DMA, As^{V}, Unknown 1 (UN1), and Unknown 5 (UN5). Unknown 1 is the peak between MMA and As^{V}. The major arsenic species in lake trout is AsB with a few DMA.

Figure 3.10 (a) shows the arsenic species concentrations in all analyzed freshwater fish (n = 266) captured from 2014 to 2020. AsB was detected most in freshwater fish, that is, in 265 out of 266 samples, with a detection rate of 99.6%. DMA was detected in 259 out of 266 freshwater fish samples, with a detection rate of 97.4%. As^{V} was detected in 183 out of 266 freshwater fish samples, corresponding to a detection rate of 68.8%. Unknown 5 (UN5) was detected in 177 out of 266 freshwater fish samples, representing a detection rate of 66.5%. These four arsenic species are detected most frequently in freshwater fish. MMA, Unknown1 (UN1), Unknown 2 (UN2), Unknown 3 (UN3),

Unknown 4 (UN4), and Unknown 7 (UN7) were detected in 2, 50, 15, 3, 4, and 1, out of 266 samples, corresponding to detection rates of 0.8%, 18.8%, 5.6%, 1.1%, 1.5%, and 0.4%, respectively.

The mean concentration and standard deviation of each arsenic species were calculated separately. The concentration of unknown arsenic species was estimated by calibrating against the nearest arsenic standard peaks. The summed concentration of all As species is 0.045 ± 0.057 mg/kg (Figure 3.10 (b)). Since AsB is non-toxic to humans, we excluded AsB and obtained the sum of DMA, MMA, and As^V concentration, which is 0.0098 ± 0.0092 mg/kg. The concentration of AsB is 0.033 ± 0.056 mg/kg. The concentrations of DMA, MMA (n = 2), As^V, UN1, UN2, UN3, UN4, UN5, and UN7 (n = 1) are 0.0035 ± 0.0057, 0.0003, 0.0093 ± 0.008, 0.0054 ± 0.0053, 0.001 ± 0.0008, 0.0018 ± 0.0012, 0.00072 ± 0.00053, 0.0023 ± 0.0021, and 0.00037 mg/kg, respectively.

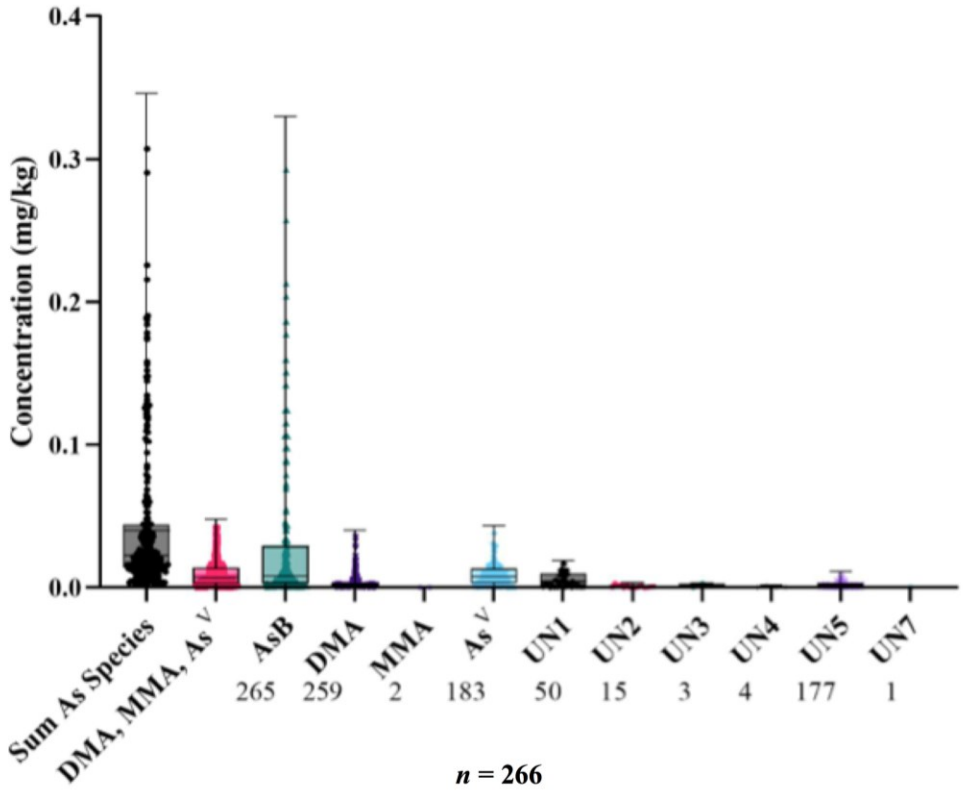


Figure 3.10. (a) Concentrations of detected arsenic species in all 266 freshwater fish samples. The concentration is expressed as mg of arsenic per kg of fish tissue in wet weight. The number below each arsenic species indicates the number of fish samples that had detectable arsenic species (above the detection limit of 0.00025 mg/kg). The boxes in the graph range from 25th percentile to 75th percentile of the

distribution and the range indicate the interquartile range. The median is indicated by a line across the box. The whiskers on box indicate 1.5 interquartile range.

It is not easy to see the concentrations with only one axis due to the high AsB concentrations, the sum of the As concentrations, and the sum of DMA, MMA, and As^{V}. I have separated the sum of As and the sum of DMA, MMA, As^{V}, and AsB in one high As concentration axis (Figure 3.10 (b)) and the rest of the As species in a low As concentration axis (Figure 3.10 (c)). By comparing the axis range for the high As concentration figure and the low As concentration figure, there is one magnitude difference between the two figures. This shows that the other arsenic species concentrations are 0.04 mg/kg and one magnitude less than the 0.4 mg concentration of AsB. Figure 3.10 (d) shows the combined high concentration axis and low concentration axis together to have a better view of overall arsenic concentration.

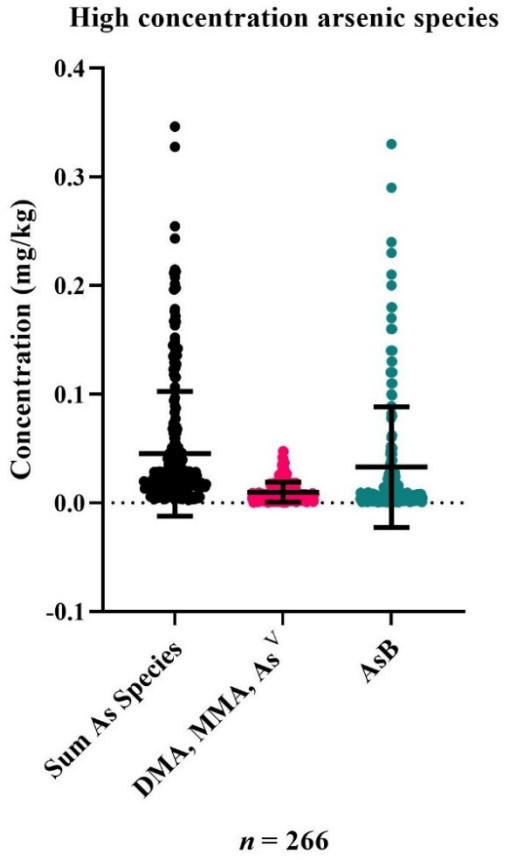


Figure 3.10. (b) Concentrations of all detected arsenic species, the sum of DMA, MMA, and As^V, and AsB in all 266 fish samples analysed. The concentration unit is mg of arsenic per kg of fish tissue in wet weight. The black bars in the graph indicate the mean value and standard deviation.

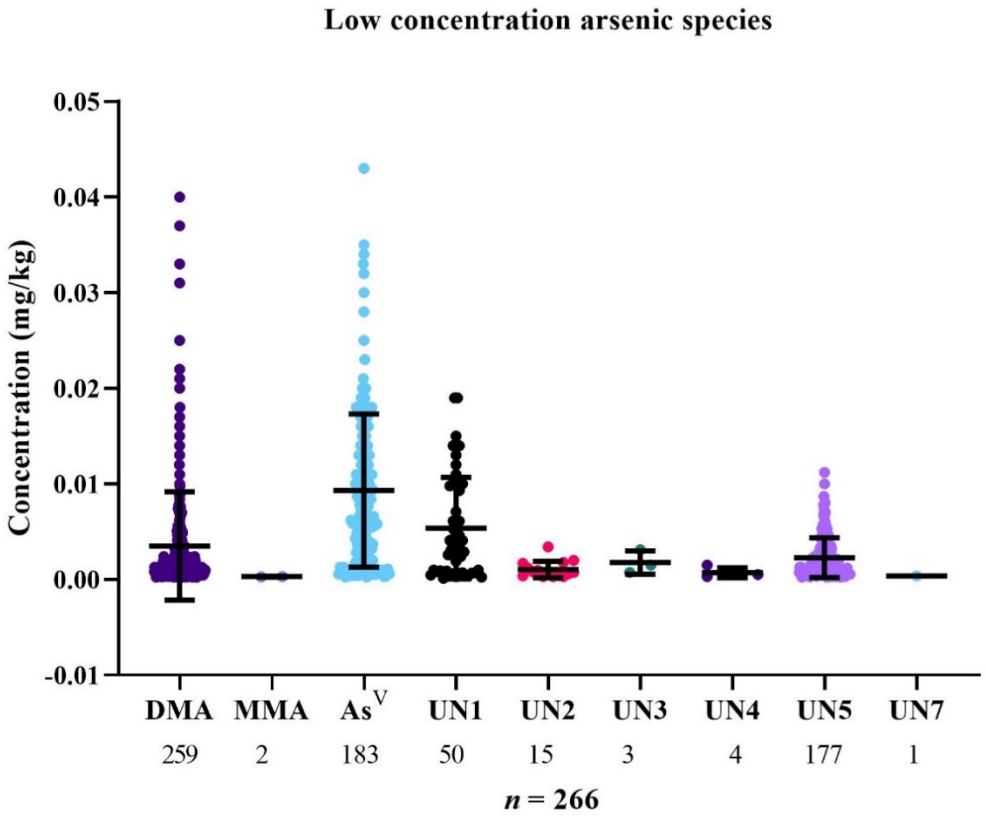


Figure 3.10. (c) Concentrations of DMA, MMA, As^V, and unidentified (unknown, UN) arsenic species in 266 fish samples analysed. The concentration unit is mg of arsenic per kg of fish tissue in wet weight. The number below each arsenic species indicates the number of fish samples that had detectable arsenic species (above the detection limit of 0.00025 mg/kg). The black bars in the graph indicate the mean value and standard deviation.

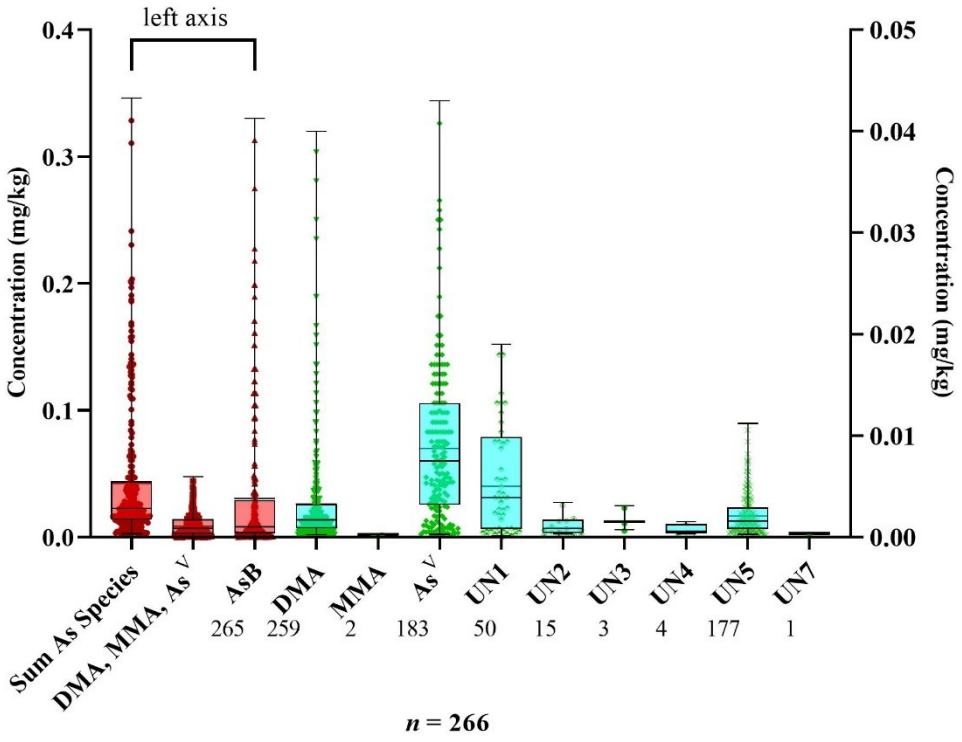


Figure 3.10. (d) Concentrations of all detected arsenic species in 266 fish samples. The red bars correspond to the left axis and the green bars correspond to the right axis. The concentrations are expressed as mg of arsenic per kg of fish tissue in wet weight. The number below each arsenic species indicates the number of fish samples that had detectable arsenic species (above the detection limit of 0.00025 mg/kg). The boxes in the graph range from 25th percentile to 75th percentile of the distribution and the range indicate the interquartile range. The median is indicated by a line across the box. The whiskers on box indicate 1.5 interquartile range.

To analyze freshwater fish further, we looked at arsenic in different fish species, including northern pike, lake whitefish, walleye, rainbow trout, and lake trout, regardless of lake. We analyzed 79 northern pike, 66 lake whitefish, 76 walleye, 18 rainbow trout, and 20 lake trout samples. I have compared the sum of the As species and the sum of DMA, MMA, As^V for different fish species. The sample normality was checked and was not distributed normally. The Mann–Whiteney U test was conducted between two different fish species.

The sum of all As species comparison is shown in Figure 3.11 (a). The mean concentrations of all arsenic species in northern pike, lake whitefish, walleye, rainbow trout, and lake trout were 0.02 ± 0.017, 0.059 ± 0.066, 0.019 ± 0.01, 0.1 ± 0.055, and

0.15 ± 0.07 mg/kg, respectively. There is a significant difference between northern pike and lake whitefish ($p < 0.0001$) and between walleye and lake whitefish ($p < 0.0001$). The sum of As is lake trout is significantly higher than that in lake whitefish ($p < 0.01$). The Sum of As in rainbow trout is significantly higher than that in northern pike and lake whitefish ($p < 0.0001$).

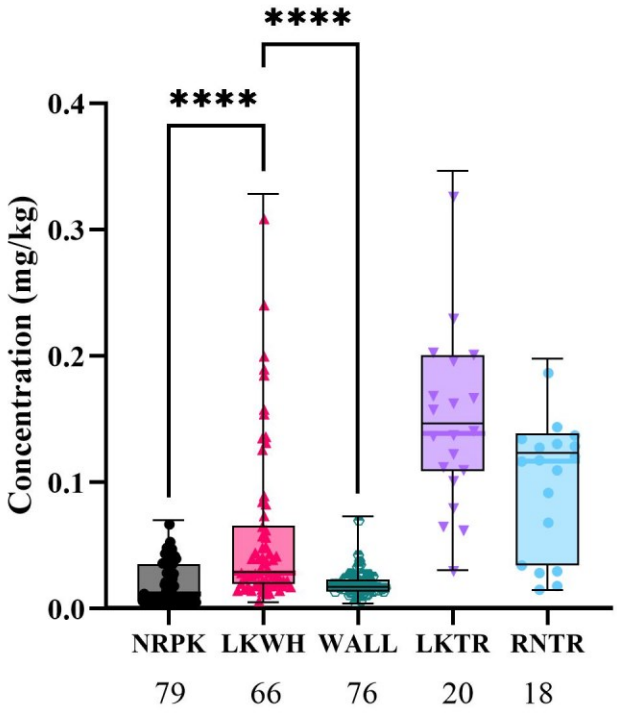


Figure 3.11. (a) The sum concentration of all detected arsenic species in different fish species from all water bodies. Fish from different water bodies of the same fish species were combined for this data analysis. The number of samples containing detectable arsenic are shown below the name of each fish species. The label, ****, indicates statistically significant with p value of < 0.0001. The boxes in the graph range from 25th percentile to 75th percentile of the distribution and the range indicate the interquartile range. The median is indicated by a line across the box. The whiskers on box indicate 1.5 interquartile range.

The sum of DMA, MMA, and As^V is shown in Figure 3.11 (b). The average concentrations of these arsenic species in northern pike, lake whitefish, walleye, rainbow trout, and lake trout were 0.0073 ± 0.0078, 0.017 ± 0.011, 0.0097 ± 0.0049, 0.0012 ± 0.00072, and 0.0015 ± 0.00044 mg/kg, respectively. There is a significant difference between northern pike and lake whitefish ($p < 0.0001$), between walleye and lake whitefish

($p < 0.05$), and between northern pike and walleye ($p < 0.05$). No significant difference was determined between lake trout and rainbow trout ($p > 0.99$).

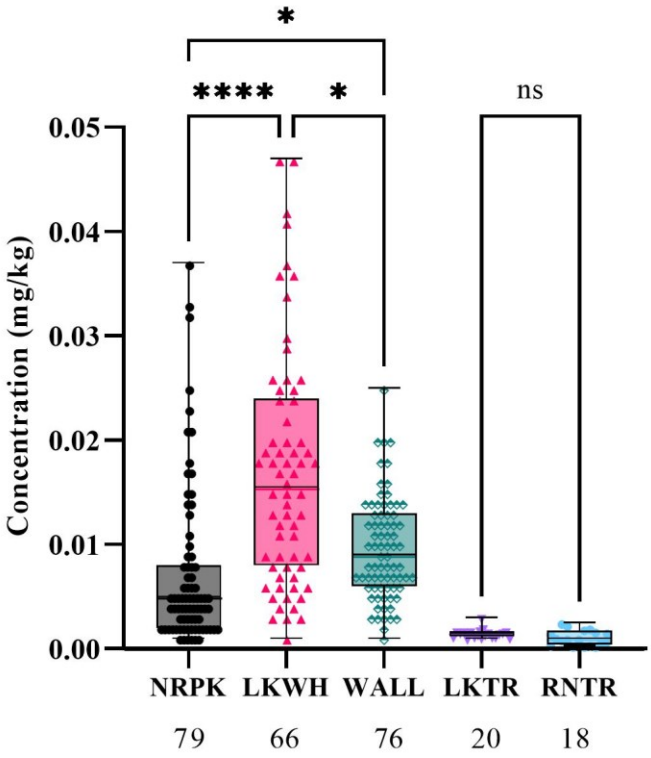


Figure 3.11. (b) The summed concentration of DMA, MMA, and As^V in different fish species from all lake. The number below each fish species indicates the number of samples that had DMA, MMA, and As^V concentration above the detection limit of 0.00025 mg/kg. The labels, ns, *, and ****, indicate not significant (ns) and statistically significant with p values of <0.05 and <0.0001, respectively. The boxes in the graph range from 25th percentile to 75th percentile of the distribution and the range indicate the interquartile range. The median is indicated by a line across the box. The whiskers on box indicate 1.5 interquartile range.

The comparison of AsB in different fish is shown in Figure 3.11 (c). The average concentrations of AsB in northern pike, lake whitefish, walleye, rainbow trout, and lake trout were 0.013 ± 0.014, 0.041 ± 0.061, 0.0065 ± 0.0075, 0.0088 ± 0.00055, and 0.0088 ± 0.0055 mg/kg, respectively. There is a significant difference between northern pike and lake whitefish ($p < 0.001$) and between walleye and lake whitefish ($p < 0.0001$). No significant difference was determined between lake trout and rainbow trout ($p > 0.99$).

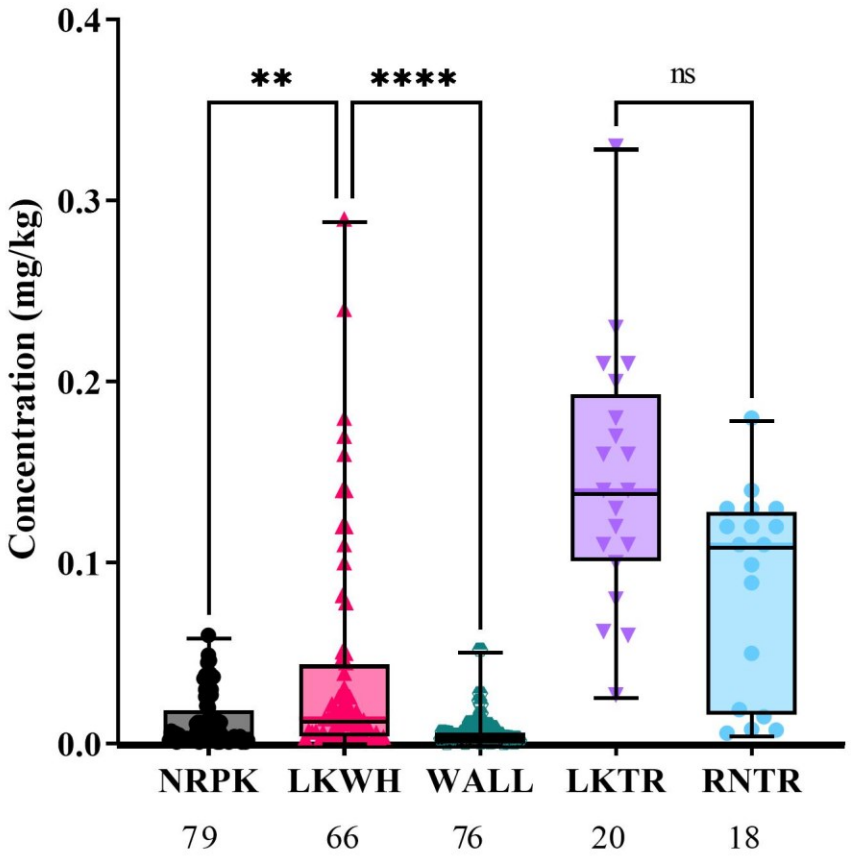


Figure 3.11. (c) Concentration of AsB in different fish species. Fish from different water bodies of the same fish species were combined for this data analysis. The number below each fish species indicates the number of samples containing detectable AsB (above the detection limit of 0.00025 mg/kg). The labels, ns, **, and ****, indicate not significant (ns) and statistically significant with p values of <0.01 and <0.0001, respectively. The boxes in the graph range from 25th percentile to 75th percentile of the distribution and the range indicate the interquartile range. The median is indicated by a line across the box. The whiskers on box indicate 1.5 interquartile range.

Figure 3.11 (d) shows the individual arsenic species concentrations in northern pike. AsB was detected in 78 northern pike samples, and the average was 0.013 ± 0.014 mg/kg. DMA was detected in all 79 northern pike, and the average was 0.0071 ± 0.0076 mg/kg, while As^{V} was detected only in 23 northern pike, and the average was 0.00096 ± 0.0082 mg/kg. Unknown 2, Unknown 3, and Unknown 4 were detected only in two, one, and one northern pike sample(s).

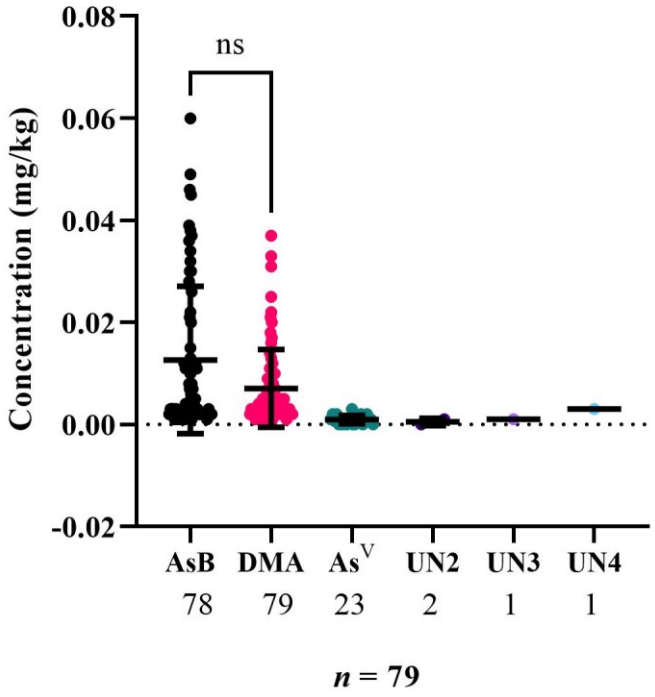


Figure 3.11. (d) Concentrations of individual arsenic species in northern pike. Results of all northern pike samples (n=79) collected from all lakes were analyzed. The number below each arsenic species indicates the number of samples containing detectable arsenic species. There is no significant difference between AsB and DMA concentrations. The concentrations of AsB and DMA are significantly ($p<0.001$) higher than the concentrations of other arsenic species. The black bars in the graph indicate the mean value and standard deviation.

Figure 3.11 (e) shows the individual arsenic species concentrations in lake whitefish. AsB and As^V were detected in all 66 lake whitefish samples, and the average was 0.041 ± 0.061 mg/kg and 0.015 ± 0.0093 mg/kg, respectively. DMA and Unknown 5 were detected in 64 and 59 lake whitefish, and the averages were 0.0025 ± 0.0035 and 0.0012 ± 0.00082 mg/kg, respectively. MMA, Unknown 1, Unknown 2, Unknown 4, and Unknown 7 were detected only in two, seven, three two, one lake whitefish.

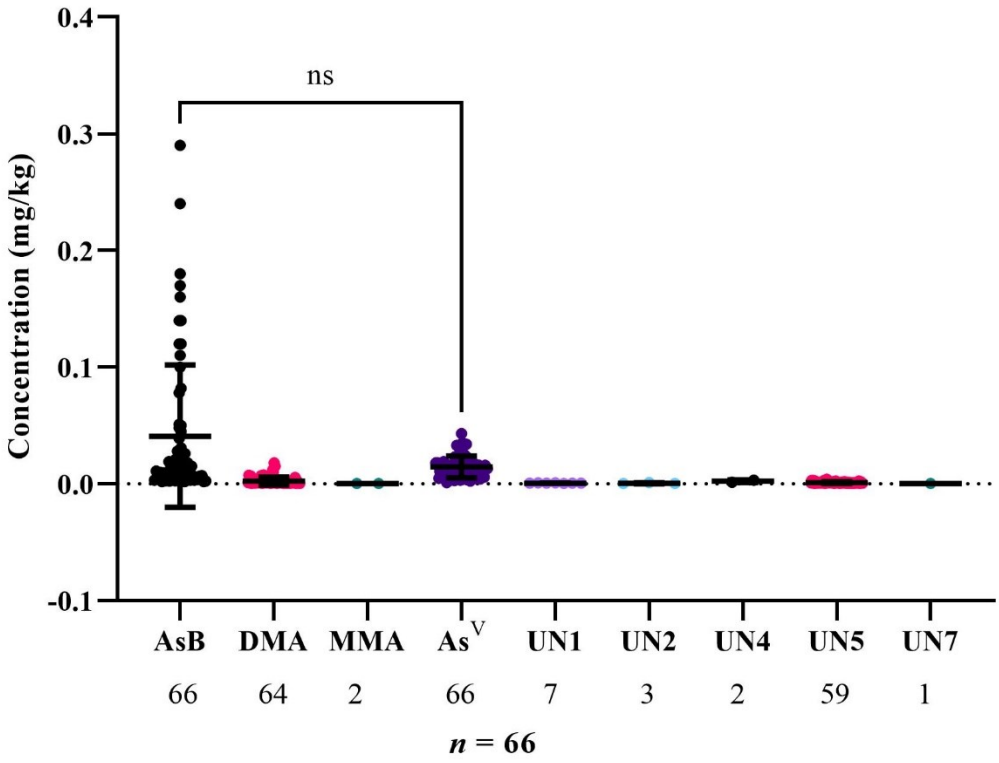


Figure 3.11. (e) Concentrations of arsenic species in lake whitefish from all lakes. Results of all whitefish samples (n=66) collected from all lakes were analyzed. The number below each arsenic species indicates the number of samples containing detectable arsenic species. There is no significant difference between AsB and As^V concentrations. The concentrations of AsB and As^V are significantly ($p<0.001$) higher than the concentrations of other arsenic species. The black bars in the graph indicate the mean value and standard deviation.

Figure 3.11 (f) shows the individual arsenic species concentrations in walleye. AsB and As^V were detected in all 76 walleye samples, and the averages were 0.0065 ± 0.0075 and 0.0082 ± 0.0046 mg/kg, respectively. DMA and Unknown 5 were detected in 72 and 75 walleye, and the averages were 0.0014 ± 0.001 and 0.0033 ± 0.0023 mg/kg, respectively. Unknown 2 was detected only in four walleyes.

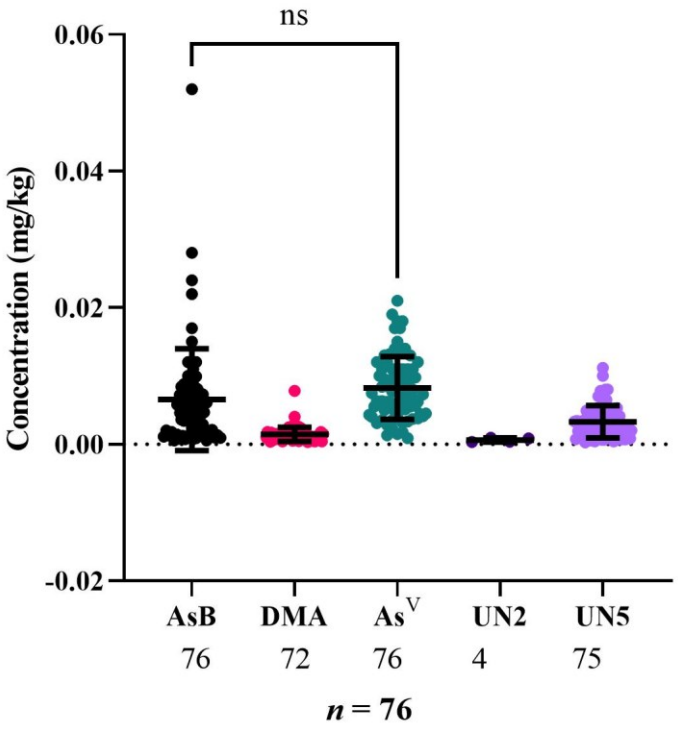


Figure 3.11. (f) Concentrations of arsenic species in walleye from all lakes. Results of all walleye samples (n=76) collected from all lakes were analyzed. The number below each arsenic species indicates the number of samples containing detectable arsenic species. There is no significant difference between AsB and As^V concentrations. The concentrations of AsB and As^V are significantly ($p<0.001$) higher than the concentrations of other arsenic species. The black bars in the graph indicate the mean value and standard deviation.

Figure 3.11 (g) shows the individual arsenic species concentrations in rainbow trout in County Sportsplex Pond (CX). AsB, UN1, and UN5 were detected in all 18 rainbow trout, and the average was 0.088 ± 0.055, 0.0097 ± 0.0047, and 0.0034 ± 0.0023 mg/kg, respectively. DMA and As^V were detected in 17 and 12 rainbow trout, and the average was 0.00048 ± 0.00022 and 0.001 ± 0.00045 mg/kg, respectively.

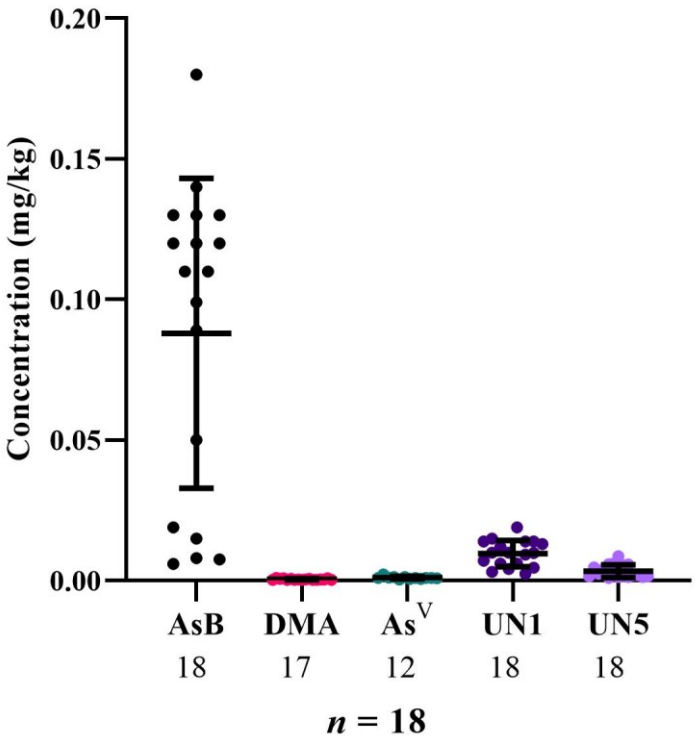


Figure 3.11. (g) Concentrations of arsenic species in rainbow trout (n=18) collected from County Sportsplex Pond (CX). The number below each arsenic species indicates the number of rainbow trout fish containing detectable arsenic species. The concentration of AsB is significantly ($p<0.001$) higher than the concentrations of other arsenic species. The black bars in the graph indicate the mean value and standard deviation.

Figure 3.11 (h) shows the individual arsenic species concentrations in lake trout from Cold Lake (CO). AsB and DMA were detected in all 20 lake trout, and the average was 0.15 ± 0.07 mg/kg and 0.0015 ± 0.00044 mg/kg, respectively. Unknown 1 and Unknown 5 were detected in 19 and 18 lake trout, and the average was 0.0046 ± 0.0045 and 0.00091 ± 0.00062 mg/kg, respectively. Unknown 4 was detected only in one lake trout.

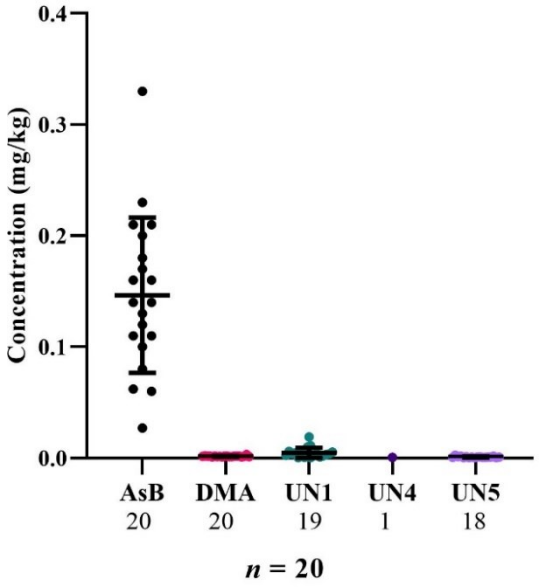


Figure 3.11. (h) Concentrations of arsenic species in lake trout (n=20) collected from Cold Lake (CO). The number below each arsenic species indicates the number of lake trout fish containing detectable arsenic species. The concentration of AsB is significantly ($p<0.001$) higher than the concentrations of other arsenic species. The black bars in the graph indicate the mean value and standard deviation.

I further looked at the major arsenic species in these five fish species and summarized them in Table 3.9. From Table 3.9 and Figure 3.11 (d-f), both AsB and DMA are dominant arsenic species in northern pike ($p > 0.05$). For lake whitefish, both AsB and As^V are the main arsenic species ($p > 0.05$). Both AsB and As^V are predominant in walleye ($p > 0.05$). Both rainbow trout and lake trout have AsB as the dominant arsenic species. The percentage of AsB, DMA, and As^V are different among the five types of fish. Three unknown arsenic species are also substantial in walleye and rainbow trout. UN5 accounts for 17% of all detectable arsenic species in walleye. UN1 and UN5 is account for 9% and 3% of all detectable arsenic species in rainbow trout.

Table 3. 9. Major Arsenic Species and their relative concentrations (Proportions) in Five Different types of Freshwater Fish

As species / Fish species	AsB	DMA	As^V
Northern pike	65%	36%	
Lake whitefish	69%	4%	25%
Walleye	34%	7%	42%
Rainbow trout	86%		
Lake trout	95%	1%	

3.3.4 Arsenic Speciation in Fish between Water Bodies

I investigated arsenic speciation in the same fish species from different water bodies. Figure 3.12. shows locations of the water bodies in Alberta, from which fish were caught for this study. I investigated seven lakes: Beaver Lake (BR), Christina Lake (CL), Cold Lake (CO), Fork Lake (FL), Square Lake (SQ), Sylvan Lake (SV), and Whitefish Lake (WF), and a storm-water catchment pond: County Sportsplex Pond (CX).

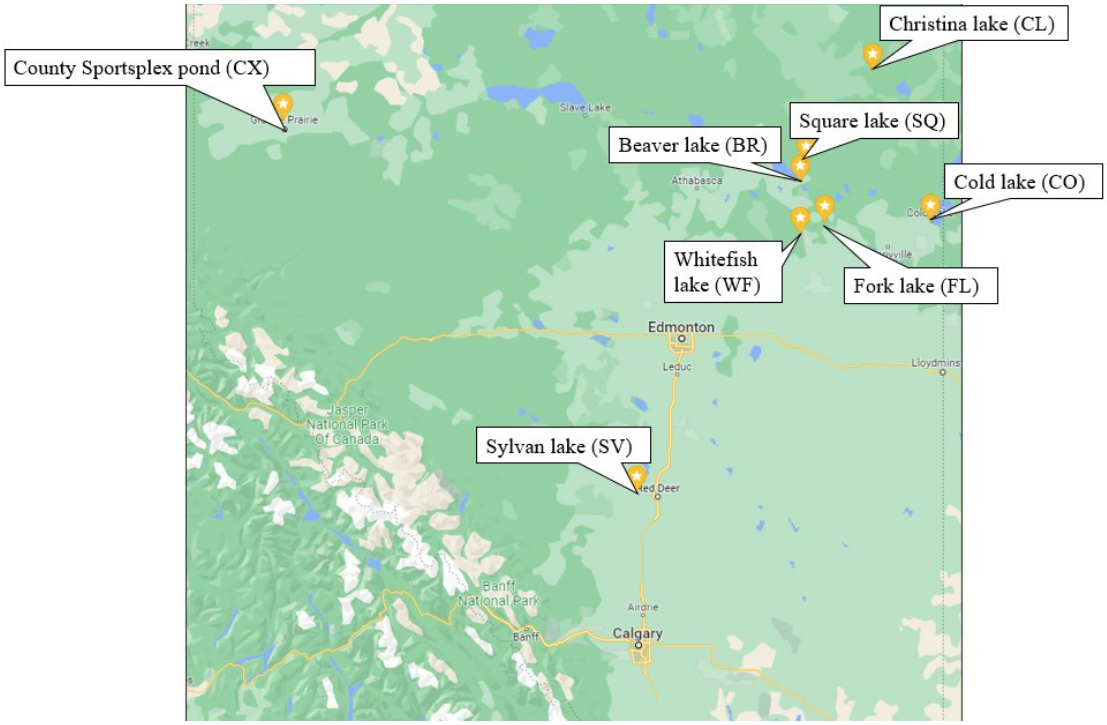


Figure 3.12. Locations of eight Alberta water bodies from which fish samples were collected and analyzed in this study.

Sylvan lake is located about 19 km northeast of Red Deer, Alberta, and is mainly supplied by ground water [26]. Frequent human activity occurs in Sylvan Lake. Beaver Lake and Square Lake are located close to each other as are Whitefish Lake and Fork Lake. Cold Lake is in northeast Alberta, close to the border of Saskatchewan. Christina Lake is located further north of Cold Lake. I compared the arsenic species in the same fish species between lakes, and the p values for the comparisons are summarized in Table 3.10.

Table 3. 10. The p Values from comparisons of Arsenic Species in the Same Fish Species from Different Lakes, regardless of the Year of collection.

		AsB	DMA	As^V	UN5
NRPK	Kruskal–Wallis test	<0.0001	<0.0001		
	SV vs CL	<0.05	<0.001		
	SV vs SQ	<0.0001	ns		
	SV vs WF	<0.001	<0.0001		
	SV vs FL	<0.0001	<0.001		
	CL vs SQ	<0.0001	<0.0001		
	CL vs WF	<0.005	ns		
	CL vs FL	<0.0001	<0.0001		
	SQ vs WF	<0.0001	<0.0001		
	SQ vs FL	<0.05	<0.0001		
	WF vs FL	<0.0001	<0.0001		
LKWH	Kruskal–Wallis test	<0.0001	<0.0001	<0.0001	<0.0001
	SV vs CO	<0.0001	<0.0001	<0.05	<0.01
	SV vs WF	ns	ns	ns	<0.01
	WF vs CO	<0.0001	<0.0001	<0.01	ns
WALL	Kruskal–Wallis test	<0.001	<0.0001	<0.001	<0.0001
	SV vs CL	ns	ns	<0.05	<0.0001
	SV vs BR	ns	<0.001	ns	<0.05
	SV vs WF	<0.01	<0.05	ns	<0.05
	CL vs BR	ns	<0.01	ns	<0.0001
	CL vs WF	<0.0001	ns	<0.001	<0.0001
	BR vs WF	<0.0001	<0.0001	<0.01	ns

ns: Not significant ($p>0.05$).

Figure 3.13 (a) shows arsenic speciation patterns and concentrations in northern pike from Sylvan Lake (2020), Christina Lake (2019), Square Lake (2018), Whitefish Lake (2016), and Fork Lake (2014). AsB in Sylvan Lake was higher than in any other lakes, and

DMA was lower than in other lakes, except for Square Lake. AsB in Christina Lake was higher than in Square Lake, Whitefish Lake, and Fork Lake, and DMA was higher than in Square Lake and Fork Lake. Both AsB and DMA in Whitefish Lake were higher than in Square lake and Fork Lake. Northern pike in different lakes have small differences in arsenic concentration. The main arsenic species in northern pike are AsB and DMA, with small variations in arsenic species among different lakes. There is no detectable As^V in fish from Square Lake. Different unknown arsenic species were detected in fish from different lakes.

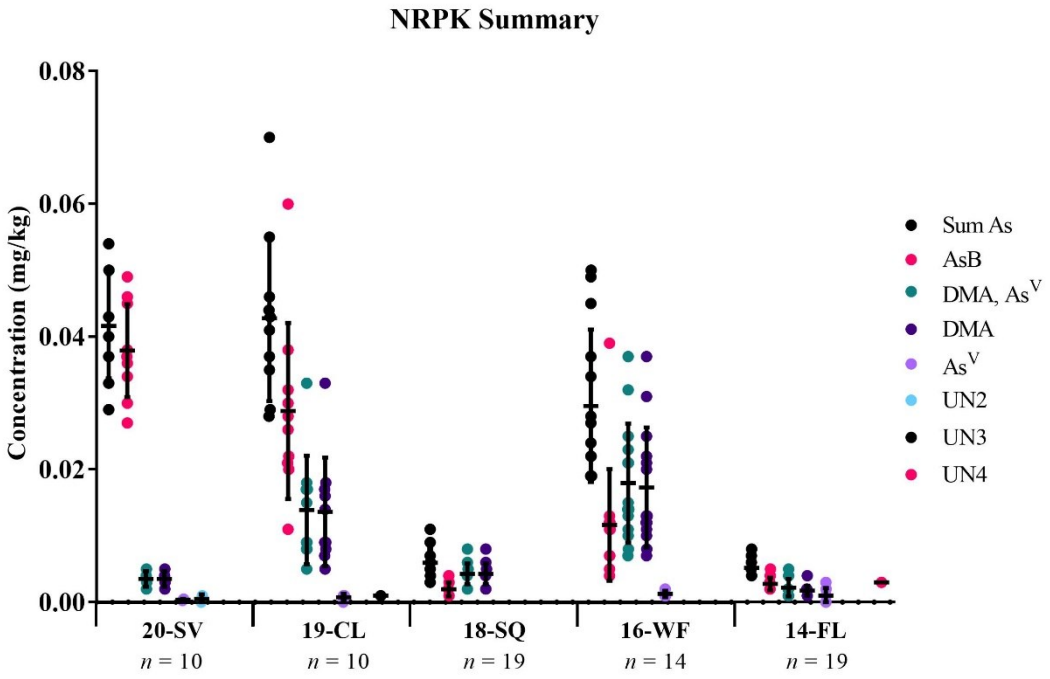


Figure 3.13 (a) Concentrations of arsenic species in northern pike collected from five different lakes. The two-digit number before the lake name indicate the year of fish sample collection, e.g., 2020 and 2019. The number below the name of each lake indicates the number of northern pike fish analyzed. The label DMA, As^V represents the sum of DMA and As^V concentrations. The black bars in the graph indicate the mean value and standard deviation.

Figure 3.13 (b) shows arsenic speciation patterns and concentrations in the lake whitefish from Sylvan Lake (2020), Cold Lake (2019), and Whitefish Lake (2016). AsB, DMA, and As^V in Cold Lake were higher than in both Sylvan Lake and Whitefish Lake. Unknown 5 in Sylvan Lake was higher than in Cold Lake and Whitefish Lake. AsB in Cold Lake was one order of magnitude higher than in other lakes. I will discuss Cold Lake

further in Section 3.3.5. Except for Cold Lake, there was little difference in arsenic concentrations between lake whitefish from Sylvan Lake and Whitefish Lake, and they were below 0.05 mg/kg. The main arsenic species were AsB, DMA, As^V, and Unknown 5. Unknown 1 and Unknown 7 were present in Sylvan Lake, while MMA and Unknown 1, 2, and 4 were present in Whitefish Lake

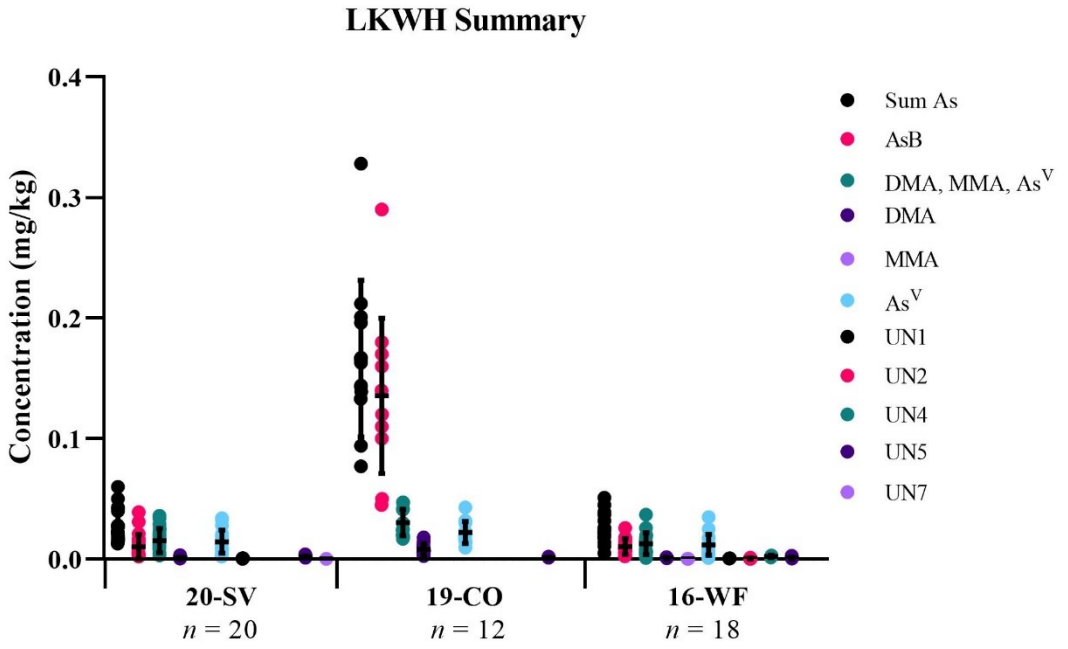


Figure 3.13. (b) Concentrations of arsenic species in lake whitefish collected from three lakes. The number before the lake name indicate the year of fish samples collection. The number below the name of each lake indicates the number of lake whitefish analyzed. DMA, MMA, As^V represents the sum of DMA, MMA, and As^V concentrations. The black bars in the graph indicate the mean value and standard deviation.

Figure 3.13 (c) shows arsenic speciation patterns and concentrations in walleye from Sylvan Lake (2020), Christina Lake (2019), Beaver Lake (2018), and Whitefish Lake (2016). AsB in Whitefish Lake was lower, and DMA was higher than in any other lakes. DMA in Christina Lake was higher than in Beaver Lake. As^V was higher in Whitefish Lake than in Beaver Lake and Christina Lake, and higher in Sylvan Lake than in Christina Lake. Unknown 5 in Sylvan Lake was higher than in any other lakes. In Christina Lake, Unknown 5 was higher than in Beaver Lake and Whitefish Lake. The arsenic concentration in walleye in different lakes varies a little, and most were below 0.04 mg/kg. The main arsenic species

in walleye were AsB, DMA, As^V, and Unknown 5. Unknown 2 is only present in Sylvan Lake.

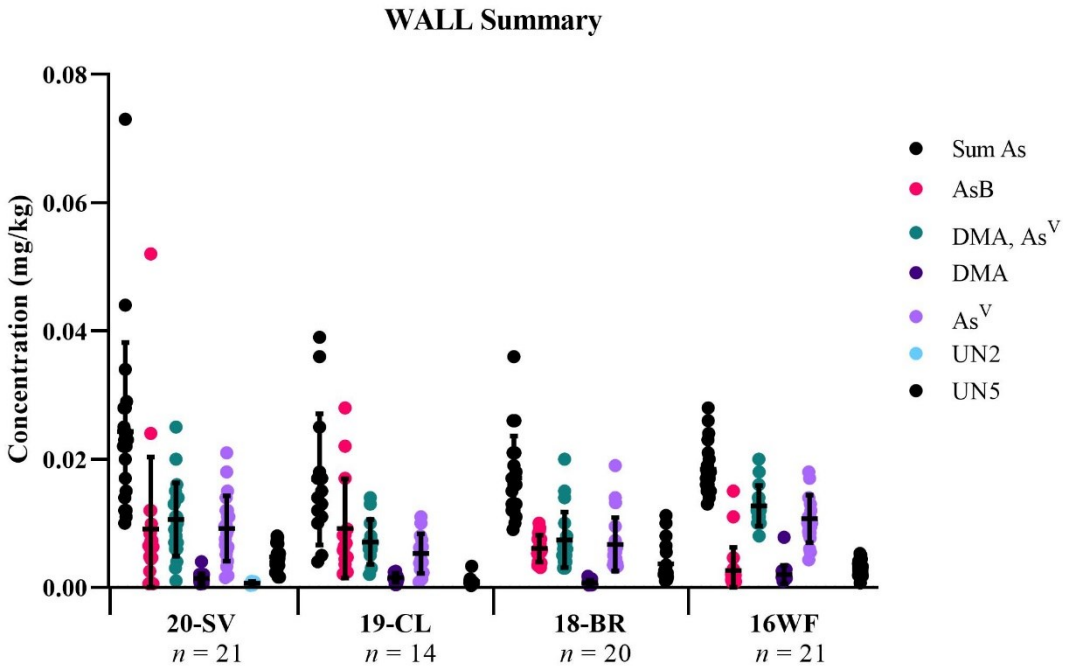


Figure 3.13. (c) Concentrations of arsenic species in walleye collected from four different lakes. The number before the lake name indicate the year of fish sample collection. The number below the name of each lake indicates the number of walleye fish analyzed. DMA, As^V represents the sum of DMA and As^V concentrations. The black bars in the graph indicate the mean value and standard deviation.

Since all three major fish, northern pike, lake whitefish, and walleye are present in Sylvan Lake, Christina Lake, and Whitefish Lake, I compared the arsenic concentrations in these three fish types within the same lake. Table 3.11 shows the p values for individual arsenic concentrations compared between different fish. As^V is not the major arsenic species, and Unknown 5 was not detectable in northern pike; only lake whitefish and walleye were compared.

Table 3. 11. The p values for Arsenic Species in Fish Species (Northern Pike, Lake Whitefish, and Walleye) from the Same Lake

		AsB	DMA	As^V	UN5
16-WF	Kruskal–Wallis test	<0.0001	<0.0001	-	-
	NRPK vs LKWH	ns	<0.0001	-	-
	NRPK vs WALL	<0.0001	<0.0001	-	-
	LKWH vs WALL	<0.0001	<0.001	ns	<0.0001
19-CL	Kruskal–Wallis test	<0.0001	<0.0001	-	-
	NRPK vs LKWH	ns	<0.001	-	-
	NRPK vs WALL	<0.001	<0.0001	-	-
	LKWH vs WALL	<0.0001	ns	<0.05	ns
20-SV	Kruskal–Wallis test	<0.0001	<0.0001	-	-
	NRPK vs LKWH	<0.0001	<0.0001	-	-
	NRPK vs WALL	<0.0001	<0.0001	-	-
	LKWH vs WALL	ns	<0.05	ns	<0.0001

ns: Not significant.

Figure 3.14 (a) shows a comparison of three types of fish collected from Whitefish Lake in 2016. Overall arsenic species in three major fish species are mostly under 0.05 mg/kg. The AsB concentration is higher in northern pike and lake whitefish, and walleye has the lowest AsB amount. The DMA concentration is lower than 0.03 mg/kg, and the concentration order in the three fish is northern pike >> walleye > lake whitefish. There is no significant difference for As^V in lake whitefish and walleye. Unknown 5 is higher in walleye than in lake whitefish. Unknown 1, 2, and 4 were detected in lake whitefish but not in walleye and northern pike.

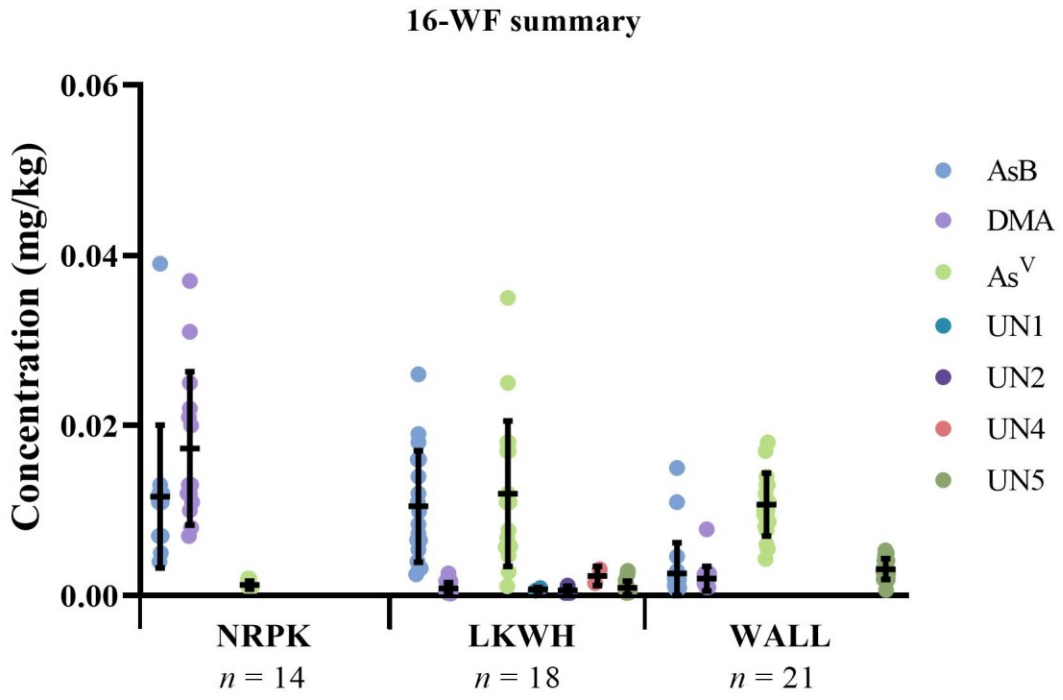


Figure 3.14. (a) Concentrations of arsenic species in three major types of fish collected from Whitefish Lake in 2016 (16-WF). The number below the name of fish indicates the number of fish analyzed. Fourteen northern pike, 18 lake whitefish, and 21 walleye fish were collected and analyzed for arsenic speciation. The label DMA, MMA, As^V represents the sum of DMA, MMA, and As^V concentrations. The black bars in the graph indicate the mean value and standard deviation.

Figure 3.14 (b) shows three fish in Christina Lake in 2019. Except that AsB in one lake whitefish was 0.24 mg/kg, other arsenic species are under 0.1 mg/kg. There is no significant difference between AsB in northern pike and lake whitefish. AsB in walleye is lower than northern pike and lake whitefish. The DMA concentration is lower than 0.02 mg/kg, and the concentration order in the three fish is northern pike >> walleye or lake whitefish. As^V in lake whitefish was higher than in walleye, and there was no difference for Unknown 5. Unknown 1 and 3 were detected only in lake whitefish.

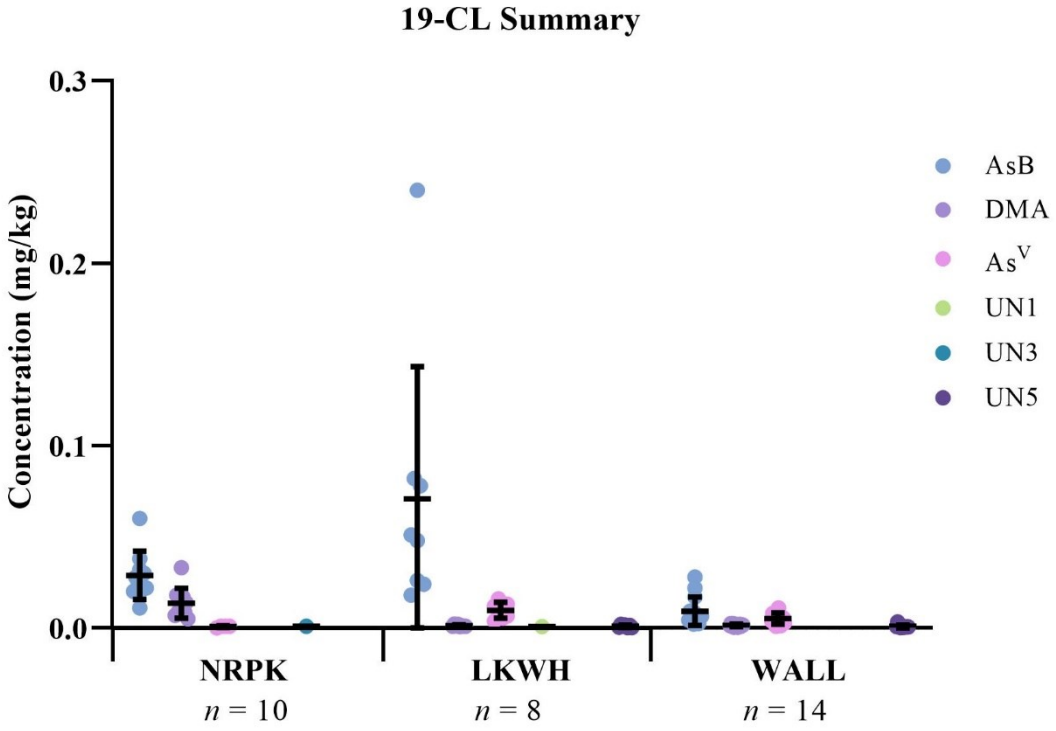


Figure 3.14. (b) Concentrations of arsenic species in three types of fish collected from Christina Lake in 2019 (19-CL). The number below the name of fish indicates the number of fish analyzed. Ten northern pike, eight lake whitefish, and 14 walleye fish were collected and analyzed for arsenic speciation. DMA, As^V represents the sum of DMA and As^V concentrations. The black bars in the graph indicate the mean value and standard deviation.

Figure 3.14 (c) shows a comparison of arsenic species in three types of fish collected from Sylvan Lake in 2020. The arsenic species concentration was generally under 0.05 mg/kg. AsB in northern pike was significantly higher than in lake whitefish and walleye. The DMA concentration was lower than 0.005 mg/kg, and DMA in northern pike was higher than in lake whitefish and walleye. There is no difference for As^V, and Unknown 5 in walleye is significantly higher than in lake whitefish. Unknown 1 was detected in lake whitefish and Unknown 2 was detected in walleye and northern pike.

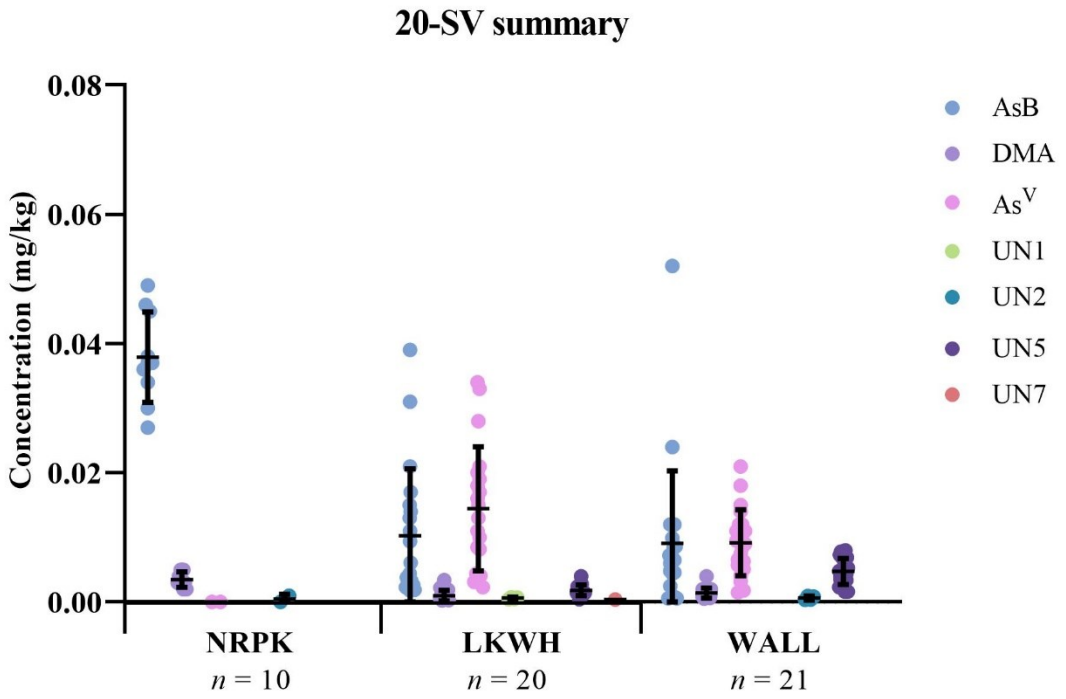


Figure 3.14. (c) All three major fish Concentrations of arsenic species in three types of fish collected from Sylvan Lake in 2020 (20-SV). The number below the name of fish indicates the number of fish analyzed. DMA, As^V represents the sum of DMA and As^V concentrations. The black bars in the graph indicate the mean value and standard deviation.

Overall, except for AsB in lake whitefish in Christina Lake, the concentrations of arsenic species were below 0.05 mg/kg. There is a higher chance that unknown species were detected in lake whitefish. Variations existed in three major fish species within the same lakes.

3.3.5 Arsenic Speciation in Fish of the Same Lake Between Two Years

We obtained fish of the same species from Sylvan Lake and Cold Lake between two years. Fish from Cold Lake represented those containing high concentrations of arsenic, and fish from Sylvan Lake contained lower concentrations of arsenic species. To see if there was any temporal change in the concentrations of arsenic species, I compared the same fish species from the same lake between two years.

Alberta Health visited Sylvan Lake in 2012 and 2020, and sampled three main types of fish, northern pike, lake whitefish, and walleye. Alberta Health also collected lake whitefish and lake trout from Cold Lake in 2016 and 2019. Ms. Xiufen Lu performed the

analysis of fish collected from Sylvan Lake in 2012 and from Cold Lake in 2016. I used these data to compare with my analysis data of fish samples collected from Sylvan Lake in 2020 and from Cold Lake in 2019. Table 3.12 summarizes p values for the comparisons of fish samples from Sylvan Lake.

Table 3. 12. The p Values of the Arsenic Species comparing in Northern Pike, Lake Whitefish, and Walleye in Sylvan Lake Between 2012 and 2020. First, Regardless of the Year, a Comparison of the Arsenic Concentrations Among Three Fish species. Second, a Comparison of the Arsenic Species Among Three Fish Species in 2012 and 2020. Finally, a Comparison of the Arsenic Species in Three Fish Species Between 2012 and 2020 (ns: Not Significant)

		sum As	DMA, As^V	AsB	DMA	As^V	UN5
2012 & 2020	Kruskal_Wallis test	<0.01	<0.01	<0.0001	<0.0001		
	NRPK vs LKWH	<0.01	<0.01	<0.0001	<0.001	-	-
	NRPK vs WALL	<0.001	<0.001	<0.0001	<0.01	-	-
	LKWH vs WALL	ns	ns	ns	<0.01	<0.05	<0.001
2012	Kruskal–Wallis test	ns	ns	ns	ns	ns	ns
2020	Kruskal–Wallis test	<0.001	<0.001	<0.0001	<0.0001	-	-
	NRPK vs LKWH	<0.001	<0.001	<0.0001	<0.0001	-	-
	NRPK vs WALL	<0.0001	<0.0001	<0.0001	<0.0001		
	LKWH vs WALL	ns	ns	ns	<0.05	ns	<0.0001
2012 vs 2020	all fish combined	<0.05	<0.01	ns	<0.01	ns	<0.05
2012 vs 2020	NRPK	ns	<0.05	ns	<0.05	-	-
	LKWH	ns	<0.05	ns	ns	ns	<0.05
	WALL	ns	ns	ns	ns	ns	ns

Figure 3.15 (a-b) shows a comparison of northern pike, lake whitefish, and walleye collected from Sylvan Lake. The sum of all As species in northern pike was higher than in lake whitefish and walleye, while the sum of DMA and As^V in northern pike was lower than in lake whitefish and walleye. Figure 3.15 (c) shows the individual arsenic concentrations in northern pike, lake whitefish, and walleye. AsB in northern pike was higher than in lake whitefish and walleye, with DMA concentrations in the order northern pike > walleye > lake whitefish. As^V in lake whitefish was higher than in walleye, and Unknown 5 was lower in lake whitefish

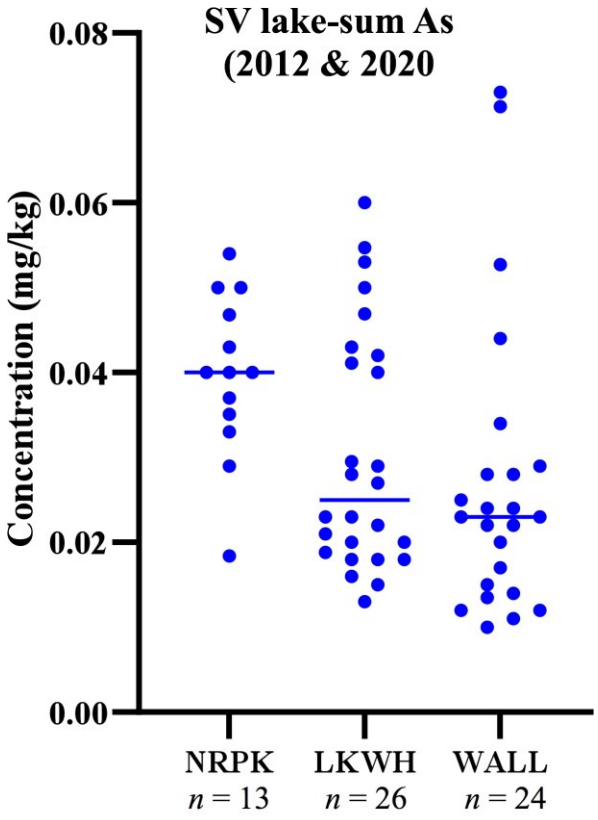


Figure 3.15. (a) A comparison of the sum As concentrations in northern pike, lake whitefish, and walleye collected from Sylvan Lake. Fish samples collected both in 2012 and 2020 were combined for this comparison.

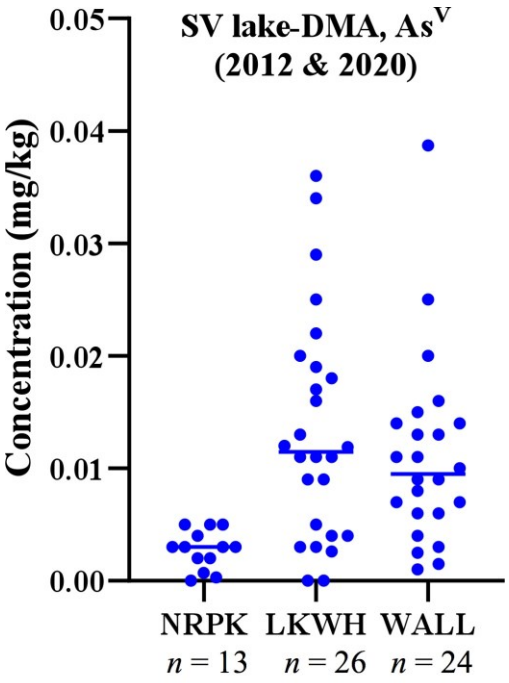


Figure 3.15. (b) A comparison of the sum of DMA and As^V concentrations in northern pike, lake whitefish, and walleye collected from Sylvan Lake in 2012 and 2020. Fish samples collected from both years were combined for this comparison.

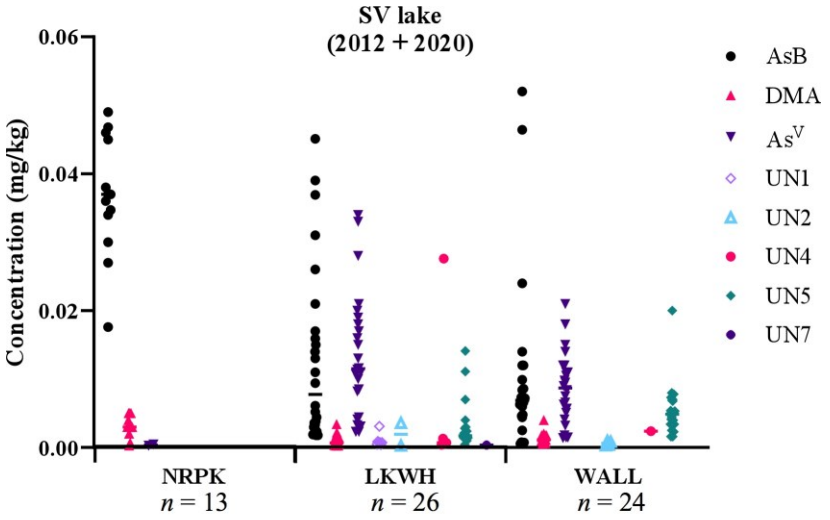


Figure 3.15. (c) A comparison of individual arsenic species concentrations in northern pike, lake whitefish, and walleye collected from Sylvan Lake in 2012 and 2020. Fish samples collected from both years were combined for this comparison.

Next, I looked at the three main fish species in 2012 and 2020 separately (Figure 3.15(d)). For Sylvan Lake in 2012, there was no significant difference for any of the arsenic

species among northern pike, lake whitefish, and walleye. The arsenic species comparison in Sylvan Lake in 2020 was described in Section 3.3.4.

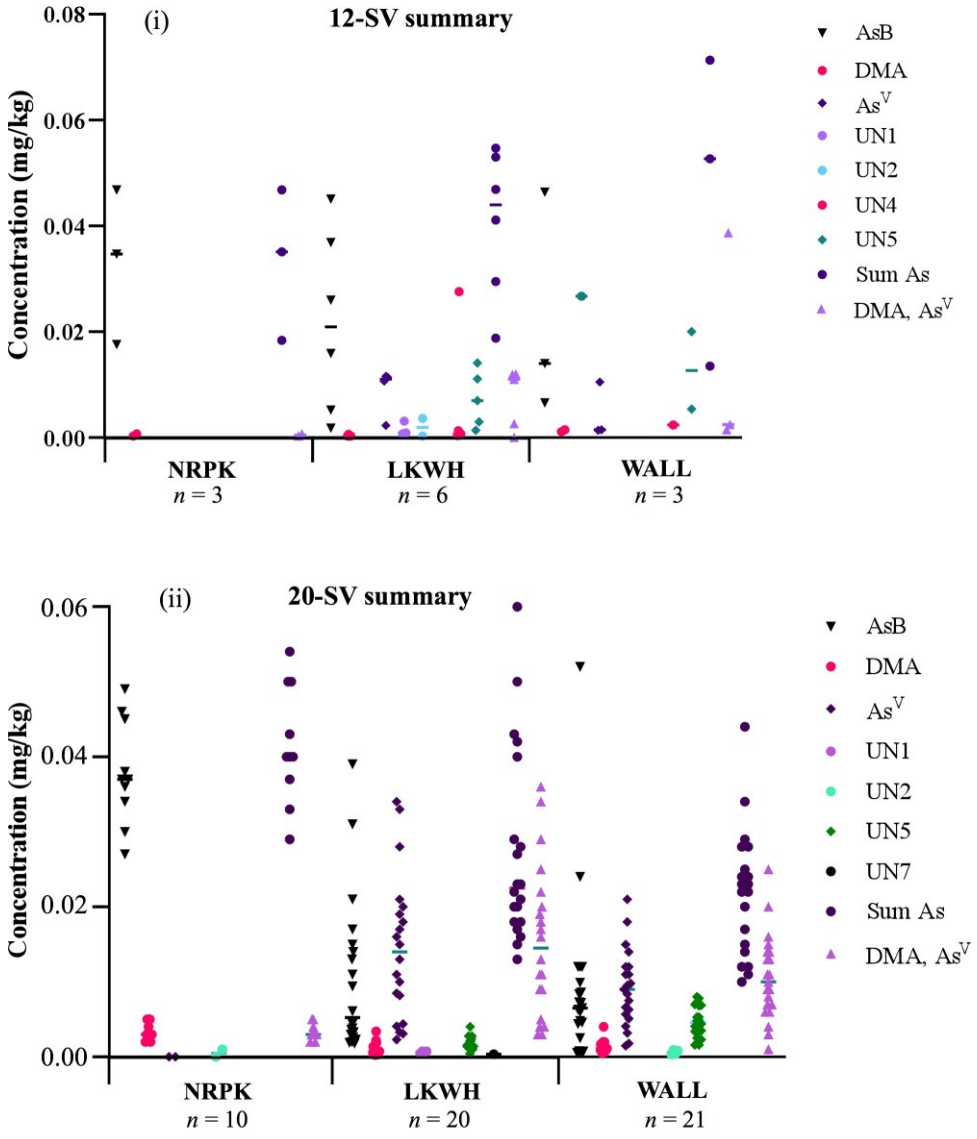


Figure 3.15. (d) Concentrations of arsenic species in northern pike, lake whitefish, and walleye collected from Sylvan Lake in 2012 (top figure) and 2020 (bottom figure). The number below the name of fish indicates the number of fish analyzed.

Figure 3.15 (e) shows the combined three major fish for each year, compared between 2012 and 2020. The sum of As was lower ($p<0.05$) in 2020 than in 2012, while DMA and the sum of DMA and As^V were higher in 2020 than in 2012. Unknown 5 in 2020 was lower compared to 2012. There was no significant difference for other arsenic species between 2012 and 2020.

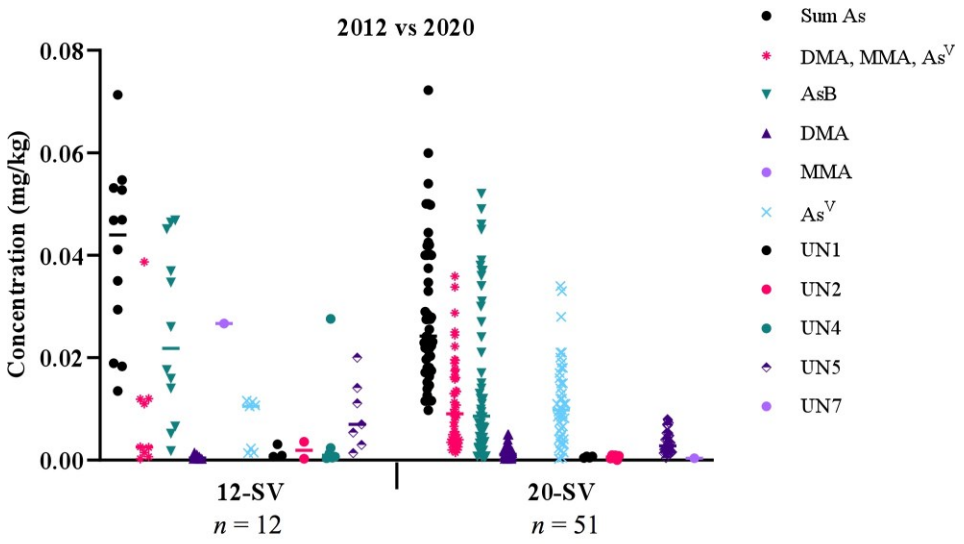


Figure 3.15. (e) A comparison of arsenic species concentrations in fish collected in 2012 (12-SV) and 2020 (20-SV) from Sylvan Lake. Total numbers of northern pike, lake whitefish, and walleye collected from Sylvan Lake in 2012 (n=12) and 2020 (n=51) were used in this comparison.

Individual fish species were compared between 2012 and 2020, respectively (Figure 3.15 (f-h)). Figure 3.15 (f) shows the northern pike in 2012 and 2020. There was no significant difference in the sum of As and AsB. The sum of DMA and As^V, and DMA were significantly higher in 2020 than 2012. As^V and Unknown 2 were detected in two different fish samples. Figure 3.15 (g) shows lake whitefish in 2012 and 2020. There was no significant difference in the sum of As, AsB, DMA, and As^V. The sum of DMA and As^V was higher in 2020 than in 2012, and Unknown 5 was higher in 2012 than in 2020. Unknown 1, 2, and 4 were detected in three, two, and five fish samples, respectively, in 2012, while only Unknown 1 was detected in four fish samples in 2020. Figure 3.15 (h) shows the walleye in 2012 and 2020. There was no significant difference in the sum of As, the sum of DMA and As^V, AsB, DMA, As^V, and Unknown 5. Unknown 2 appeared in four samples in 2020 and Unknown 4 appeared in one sample in 2012.

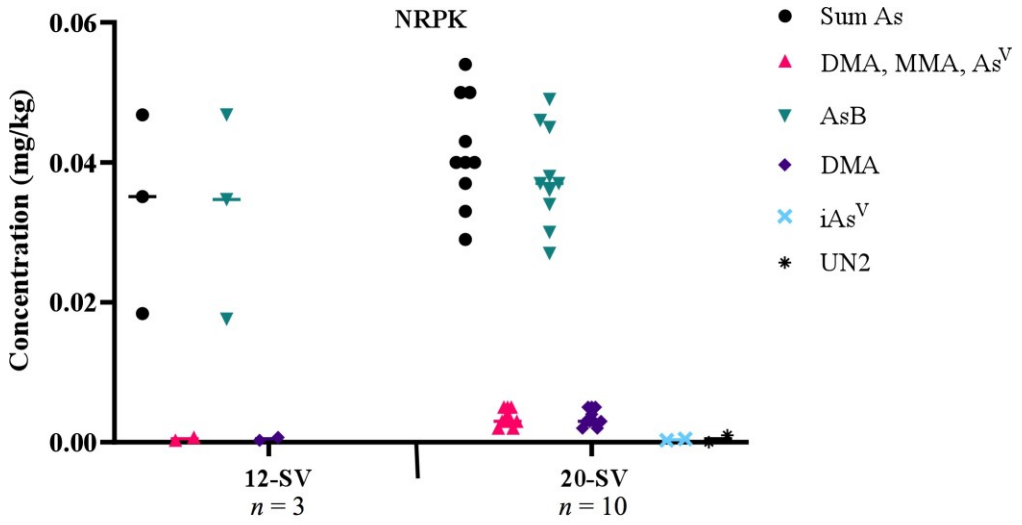


Figure 3.15. (f) A comparison of arsenic species concentrations in three northern pike collected in 2012 and 10 northern pike collected in 2020, both from Sylvan Lake.

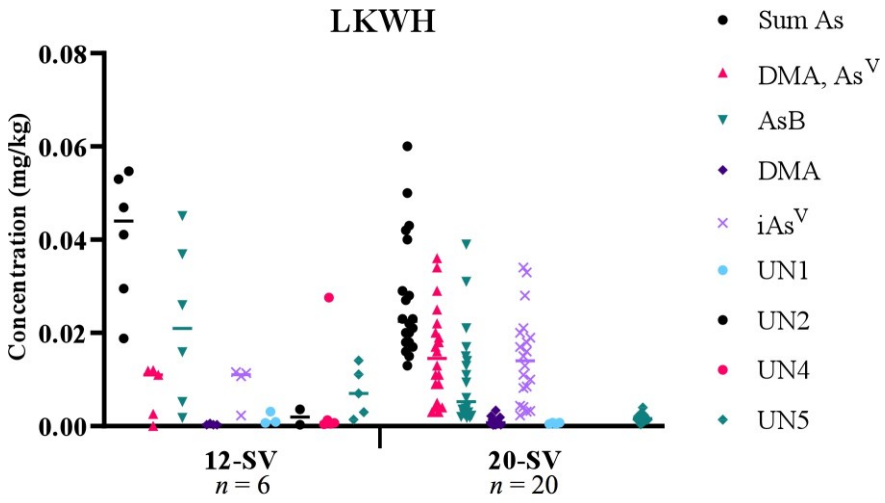


Figure 3.15. (g) A comparison of arsenic species concentrations in 6 lake whitefish collected in 2012 and 20 lake whitefish collected in 2020, from Sylvan Lake.

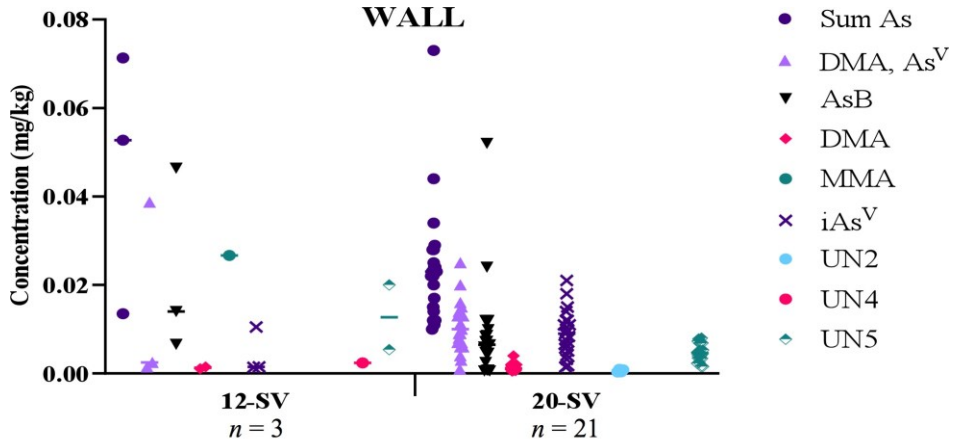


Figure 3.15. (h) A comparison of arsenic species concentrations in 3 walleye collected in 2012 and 21 walleye collected in 2020, from Sylvan Lake.

Sylvan Lake was supplied mainly by ground water, and the arsenic concentration in the lake was two orders magnitude lower than the Health Canada guideline. Regardless, in 2012 or 2020, the arsenic concentration in Sylvan Lake was lower than 0.08 mg/kg. To focus further on individual fish species, the sum of As for northern pike was higher than in the two other fish species, while the sum of DMA was lower for northern pike than for the other two fish species. As^V was highest and DMA was lowest in lake whitefish.

After investigation of normal arsenic levels in lake (Sylvan Lake), I looked at the high arsenic level lake, Cold Lake, between 2016 and 2019. The Cold Lake soil contains about 10 times higher As compared to the background arsenic (~5 µg/g) [27]. Pyrite (FeS_2) or arsenopyrite (FeAsS) were believed to be the source of the elevated levels of arsenic. Higher As (>10 µg/L) is present in ground water. It is related to bedrock formations containing marine shale, which contains pyrite [28]. Cold Lake was located at part of the Athabasca oil sands; the Imperil Oil plant was mining oil in this region. Rich oil (bitumen) deposits were located up to 400 m in depth. The oil plants need to heat bitumen to decrease the viscosity in order to be collected, which further increases the arsenic desorption to groundwater from the soil [29]. It is meaningful to focus on the arsenic concentration variation in Cold Lake between two years.

Table 3.13 summarizes comparisons of arsenic species in fish from Cold Lake. Figure 3.16 (a-b) shows the sum of As and the sum of DMA and As^V concentrations in lake whitefish and lake trout from 2016 and 2019 together. A Kruskal–Wallis test showed

that there was no significant difference for the sum of As. The sum of DMA and As^V in lake whitefish was higher than in lake trout. Figure 3.16 (c) shows the individual arsenic compounds in lake whitefish and lake trout. There was no significant difference for AsB and Unknown 5 between lake whitefish and lake trout. DMA in lake whitefish was higher than in lake trout.

Table 3.13. The p Values of Arsenic Species comparing in Lake Whitefish and Lake Trout between 2016 and 2019.

		sum As	DMA, As^V	AsB	DMA	As^V	UN5
2016 & 2019	LKWH vs LKTR	ns	<0.0001	ns	<0.0001	ns	ns
2016	LKWH vs LKTR	ns	<0.01	ns	<0.0001	ns	<0.01
2019	LKWH vs LKTR	ns	<0.0001	ns	<0.0001	-	ns
2016 vs 2019	LKWH & LKTR	<0.05	ns	<0.05	ns	ns	ns
2016 vs 2019	LKWH	ns	ns	ns	ns	ns	ns
	LKTR	<0.05	ns	ns	ns	-	<0.05

ns: Not significant.
First, Regardless of the Year, a Comparison of the Arsenic Concentration in Lake Whitefish and Lake Trout. Second, a Comparison of the Arsenic Species in Lake Whitefish and Lake Trout in 2016 and 2019 Respectively. Then, a Comparison of the Arsenic species in Lake Whitefish and Lake Trout Together, Between 2016 and 2019. Finally, a Comparison of the Arsenic Species in Lake Whitefish and Lake Trout Between 2016 and 2019

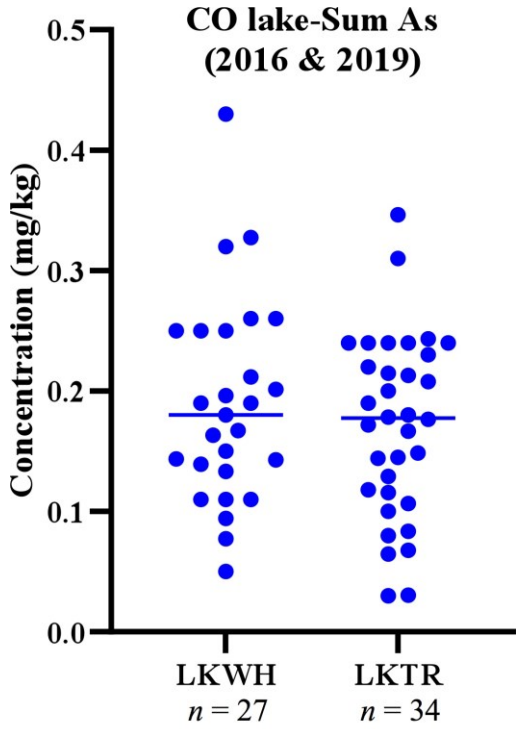


Figure 3.16. (a) A comparison of the sum As concentrations in lake whitefish and lake trout collected from Cold Lake. Results of fish collected in 2016 and 2019 were combined for this comparison.

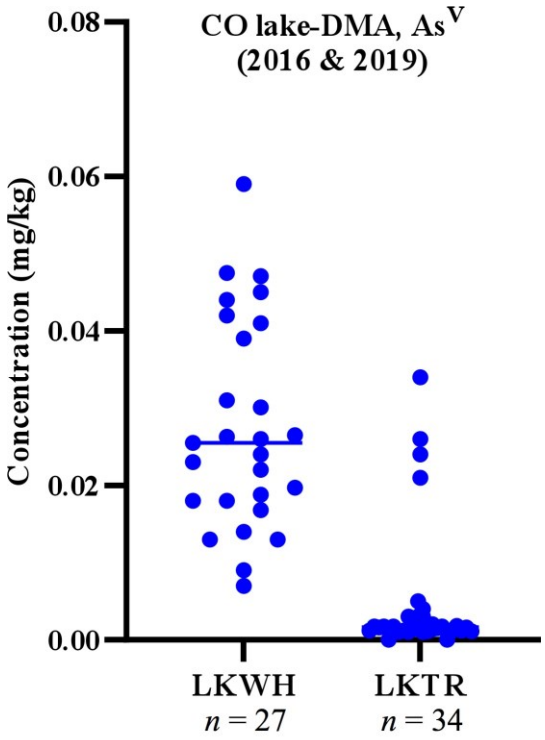


Figure 3.16. (b) A comparison of DMA and As^V concentrations in lake whitefish and lake trout collected from Cold Lake. Results of fish collected in 2016 and 2019 were combined for this analysis.

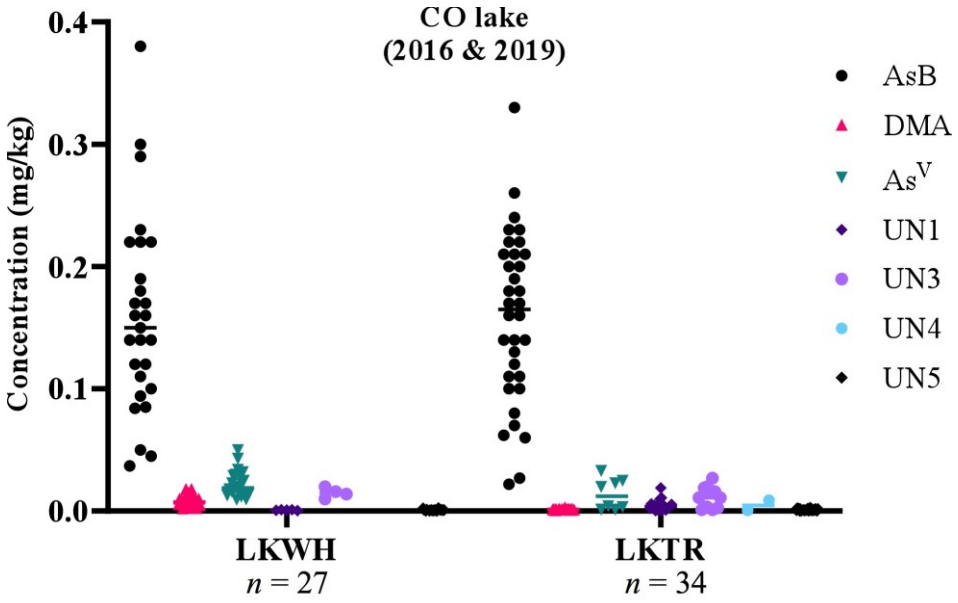


Figure 3.16. (c) Concentrations of individual arsenic species in lake whitefish and lake trout collected from Cold Lake. Results of fish collected in 2016 and 2019 were combined for this analysis.

Figure 3.16 (d) shows arsenic concentrations in lake whitefish and lake trout collected in 2016 and 2019 from Cold Lake. For both years, there was no significant difference for the sum of As and AsB. Both the sum of DMA and As^V, and DMA in lake whitefish were higher than in lake trout between 2016 and 2019. Figure 3.16 (c) shows the individual arsenic compounds in lake whitefish and lake trout in 2016 and 2019 together. There was no significant difference between lake whitefish and lake trout for AsB, As^V, and Unknown 5. DMA in lake whitefish was higher than in lake trout.

Figure 3.16 (d) compares the arsenic compounds in lake whitefish and lake trout from 2016 and 2019 separately. In both 2016 and 2019, there was no significant difference between lake whitefish and lake trout for the sum of As and AsB. The sum of DMA and As^V was higher in lake whitefish than in lake trout. Unknown 5 in 2016 from lake trout was higher than from lake whitefish.

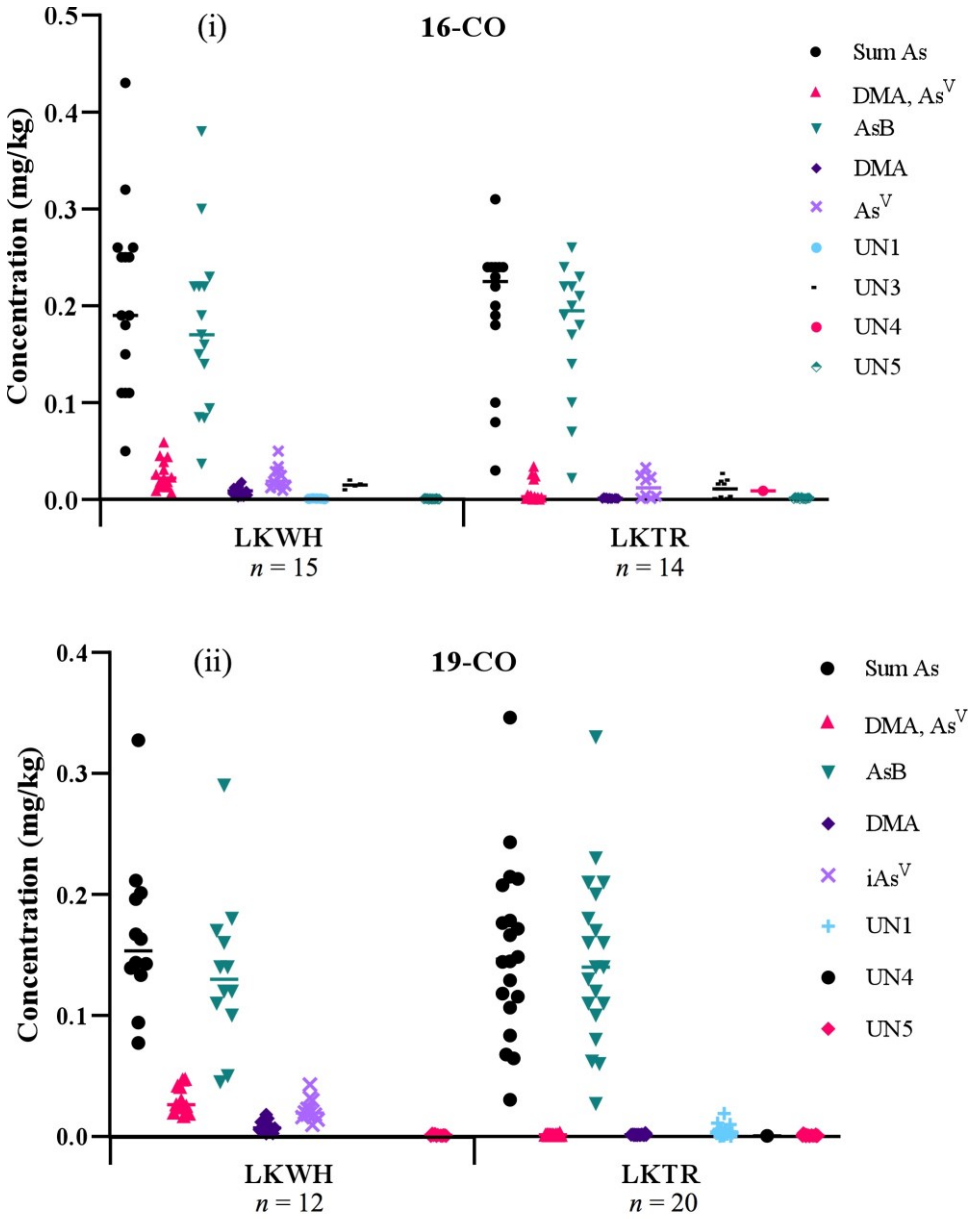


Figure 3.16. (d) Concentrations of arsenic species in lake whitefish and lake trout collected from Cold Lake in 2016 (top figure) and 2019 (bottom figure).

Figure 3.16 (e) shows a comparison of 2016 and 2019 collections of lake whitefish and lake trout from Cold Lake. The sum of As and AsB was lower in 2019 than in 2016. There is no significant difference in other arsenic concentrations between 2016 and 2019.

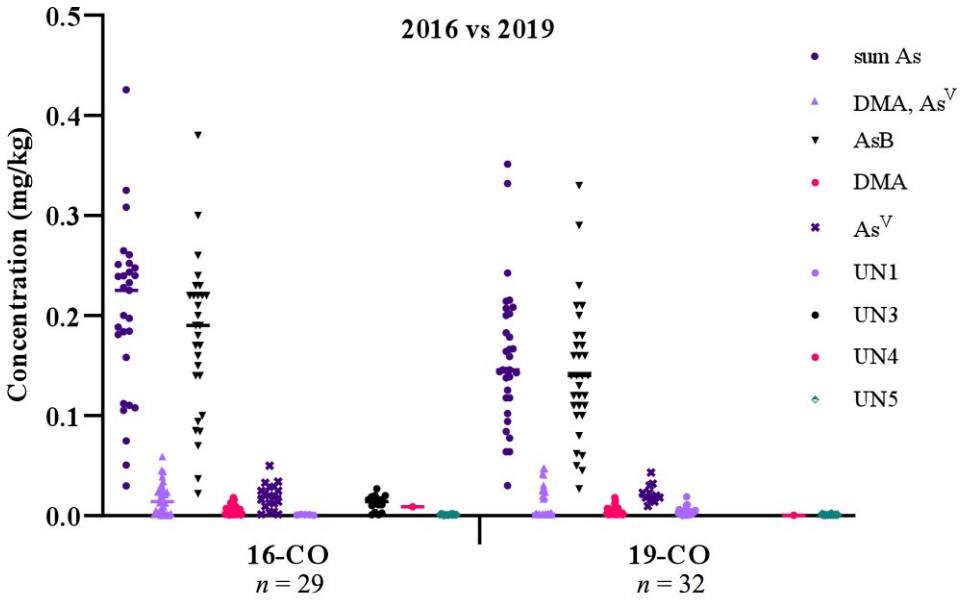


Figure 3.16. (e) A comparison of arsenic species concentrations in lake whitefish and lake trout collected from Cold Lake between two collection years, 2016 and 2019. Results of lake whitefish and lake trout were used together for this comparison.

Figure 3.16 (f-g) further shows comparisons between two years in arsenic concentrations of lake whitefish (f) and lake trout (g). For lake whitefish, there was no significant difference for any arsenic species between 2016 and 2019. Unknown 1 and 3 were detected in 2016 and were not detected in 2019. For lake trout, there were no significant differences for AsB and DMA between 2016 and 2019. The sum of As in lake trout of 2016 was higher than in 2019. Unknown 5 in lake trout of 2019 was lower than in 2016.

Overall, there was little change in arsenic concentrations between two sample years. The higher arsenic concentrations in fish of Cold Lake mostly resulted from AsB. Excluding AsB, the other arsenic species concentrations were generally under 0.04 mg/kg.

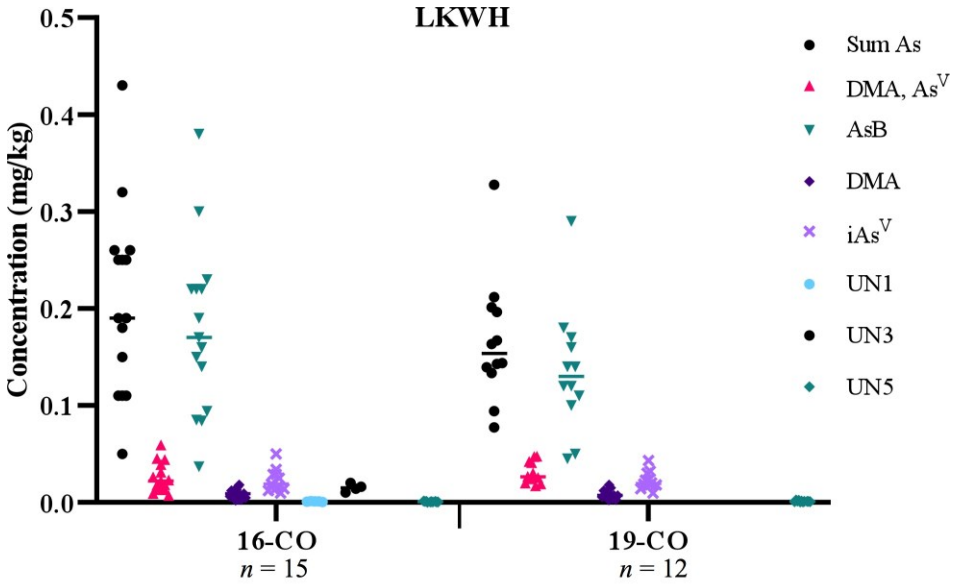


Figure 3.16. (f) A comparison of arsenic species concentrations in lake whitefish collected in 2016 (n=15) and 2019 (n=12) from Cold Lake.

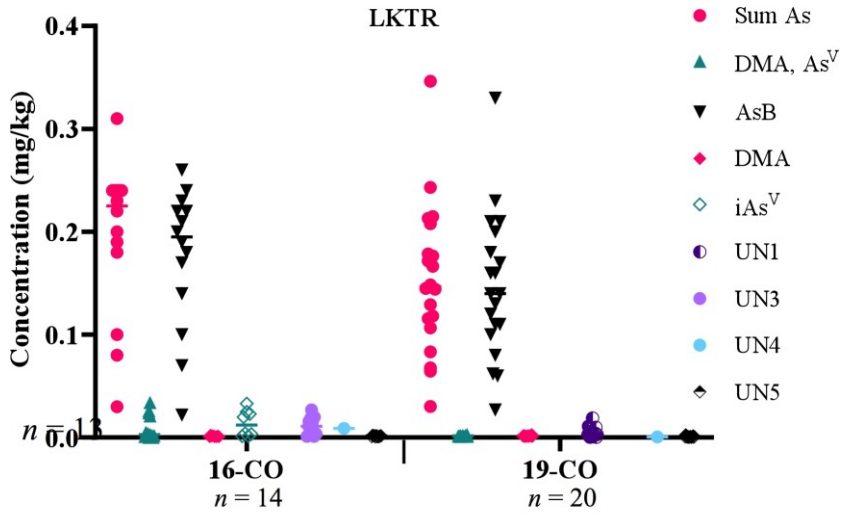


Figure 3.16. (g) A comparison of Arsenic species concentrations in lake trout collected in 2016 (n=14) and 2019 (n=20) from Cold Lake.

3.3.6 Comparison of Arsenic Species in Fish of Cold Lake with Fish from other Water Bodies

To explore why the concentrations of arsenic in fish of Cold Lake were higher than from other water bodies, I compared AsB, sum of all arsenic species, and arsenic species excluding AsB. I hypothesized that a main difference was due to AsB.

Table 3.14 and Figure 3.17 (a) show that the total concentration of all detectable arsenic species in 61 fish of Cold Lake (0.18 ± 0.08 mg/kg) were approximately 6 times higher than that of 234 fish from other seven water bodies (0.030 ± 0.033 mg/kg). The majority of the higher concentration was due to AsB, which was 0.16 ± 0.07 mg/kg in 61 fish of Cold Lake and 0.018 ± 0.032 mg/kg in 234 fish from other water bodies (Table 3.13 and Figure 3.17 (b)). Excluding AsB, the sum of other arsenic species had a smaller difference between the fish from Cold Lake and fish from other seven water bodies (Figure 3.17 (c)). The summed concentrations of other arsenic species, including DMA, MMA, As^V, and unknowns, were 0.021 ± 0.016 mg/kg in 61 fish of Cold Lake and 0.012 ± 0.0084 mg/kg in 234 fish of other seven water bodies(Table 3.14).

Table 3. 14. Comparison of Arsenic Species in Fish of Cold Lake with Fish of Other Water Bodies

all fish	Cold Lake *n* = 61 Con (mg/kg)	Other water bodies *n* = 234 Conc (mg/kg)	p value
Sum of all As			
Median	0.18	0.02	
Mean ± SD	0.18 ± 0.08	0.030 ± 0.033	$< 2.2 \times 10^{-16}$
AsB			
Median	0.16	0.0065	
Mean ± SD	0.16 ± 0.07	0.018 ± 0.032	$< 2.2 \times 10^{-16}$
Exclude AsB			
Median	0.02	0.0097	
Mean ± SD	0.021 ± 0.016	0.012 ± 0.0084	0.0009
Lake whitefish	Cold Lake *n* = 27 Conc (mg/kg)	Other lakes *n* = 54 Conc (mg/kg)	p value
Sum of all As			
Median	0.18	0.026	
Mean ± SD	0.19 ± 0.08	0.035 ± 0.036	2.8×10^{-12}
AsB			
Median	0.15	0.011	
Mean ± SD	0.16 ± 0.08	0.020 ± 0.035	3.9×10^{-12}
Exclude AsB			
Median	0.03	0.015	
Mean ± SD	0.031 ± 0.012	0.015 ± 0.0097	1.4×10^{-7}

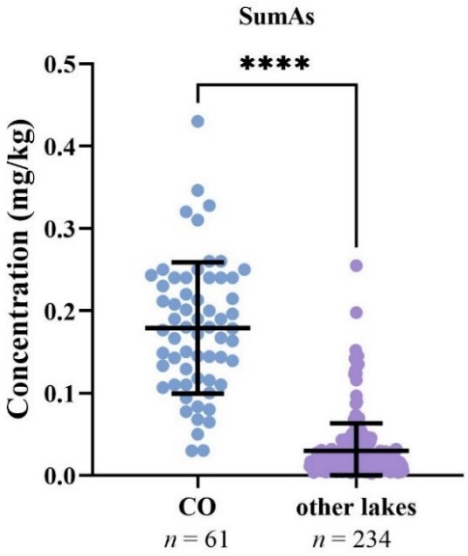


Figure 3.17. (a) Sum arsenic concentrations in fish from Cold Lake (n=61) compared with those from all other seven water bodies (n=234). The total concentrations of all detected arsenic species are significantly higher ($p<0.0001$) in the fish from Cold Lake than in the fish from other water bodies. The black bar in the graph indicated mean with standard deviation.

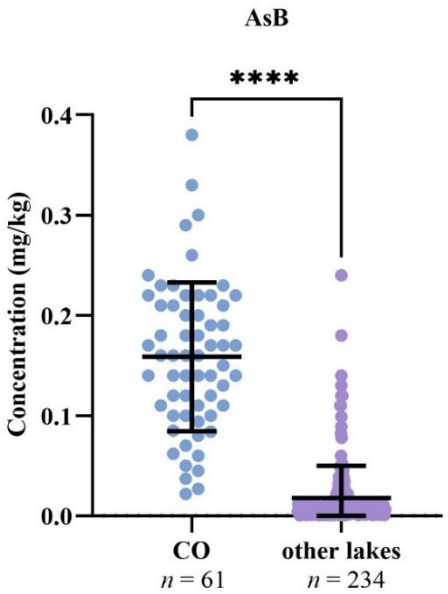


Figure 3.17. (b) A comparison of AsB concentrations in fish from Cold Lake (n=61) and fish from all other seven water bodies (n=234). The AsB concentrations are significantly higher ($p<0.0001$) in the fish from Cold Lake than in the fish from other water bodies. The black bar in the graph indicated mean with standard deviation.

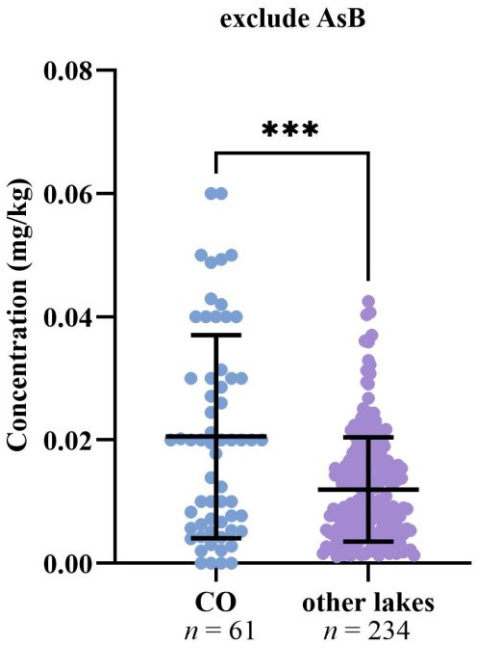


Figure 3.17. (c) Summed arsenic concentrations, *excluding AsB*, in fish from Cold Lake (n=61) compared with fish from all other seven water bodies (n=234). The difference is small, but statistically significant (p=0.0009). The black bar in the graph indicated mean with standard deviation.

Figure 3.18 and Table 3.13 also show comparisons of lake whitefish only between Cold Lake and other six lakes. The total concentration of all detectable arsenic species in 27 lake whitefish of Cold Lake (0.19 ± 0.08 mg/kg) was approximately 5 times higher than that of 54 lake whitefish from other six lakes (0.035 ± 0.036 mg/kg) (Figure 3.18 (a)). Among all arsenic species, AsB concentration was approximately 8 times higher in lake whitefish of Cold Lake (0.16 ± 0.08 mg/kg) than lake whitefish of other six lakes (0.020 ± 0.035 mg/kg) (Figure 3.18 (b)). Excluding AsB, the sum of other arsenic species had a smaller difference between the lake whitefish from Cold Lake and lake whitefish from other six lakes (Figure 3.18 (c)). The summed concentrations of other arsenic species, including DMA, MMA, As^{V}, and unknowns, were 0.031 ± 0.012 mg/kg in 27 lake whitefish of Cold Lake and 0.015 ± 0.0097 mg/kg in 54 lake whitefish of other six lakes (Table 3.13).

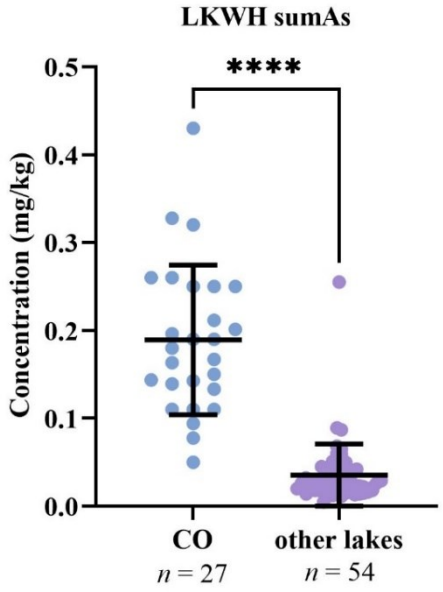


Figure 3.18. (a) Total concentrations of all detectable arsenic species in lake whitefish from Cold Lake (n=27) compared with those from other six lakes (n=54). The total concentrations of all detected arsenic species are significantly higher ($p<0.0001$) in the lake whitefish from Cold Lake than in lake whitefish from other lakes. The black bar in the graph indicated mean with standard deviation.

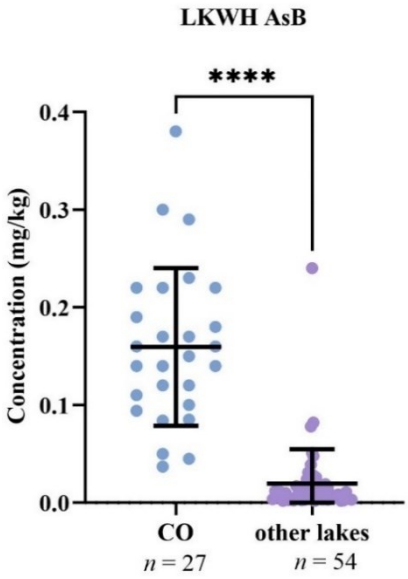


Figure 3.18. (b) A comparison of AsB concentrations in lake whitefish from Cold Lake (n=27) and lake whitefish from all other six lakes (n=54). The AsB concentrations are significantly higher ($p<0.0001$) in the lake whitefish from Cold Lake than in the lake whitefish from other lakes. The black bar in the graph indicated mean with standard deviation

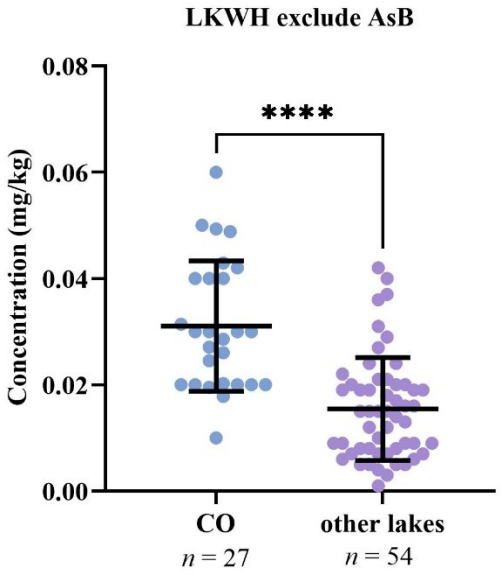


Figure 3.18. (c) Summed arsenic concentrations, *excluding AsB*, in lake whitefish from Cold Lake (n=27) compared with lake whitefish from all other six lakes (n=54). The difference is statistically significant ($p<0.0001$). The black bar in the graph indicated mean with standard deviation

3.4 CONCLUSION

A method of HPLC-ICP-MS was successfully used for the determination of arsenic species in 266 freshwater fish collected from eight Alberta water bodies over several years. A predominant arsenic species in the fish is AsB, ranging from 34% in walleye to 95% in lake trout. DMA and As^{V} are the second most commonly detectable arsenic species in these freshwater fish samples. Up to five unidentified arsenic species are also detectable but their chemical nature is unknown. Future research is needed to identify these new arsenic species.

In fish samples from seven of the eight waterbodies, the concentrations of arsenic species vary little from lake to lake. One exception is fish from Cold Lake, which has approximately 6 times higher total arsenic concentration than fish from other seven waterbodies. Most of this difference is attributed to AsB, which is approximately 8 times higher in fish from Cold Lake than fish from other waterbodies. Reasons for the higher concentrations of AsB and total arsenic in fish of Cold Lake are not known.

Comparisons between two years of fish sample collection from two lakes show similar concentrations of arsenic species between two years. Additional research with

multiple years of sample collection is needed to determine any changes of arsenic concentration in fish over time.

There are several limitations in this study. For the extraction procedure, I only used methanol-water extraction, and the extraction efficiency is low. A multiple-step extraction can be performed to extract more arsenic species and increase the extraction efficiency. Several unknown arsenic species were detected. Usually present at low concentrations these unknown arsenic species are hard to identify. ESI-MS could be used to help identify these unknown arsenic species.

There are hundreds of lakes in Alberta, and most lakes were only sampled once. So far, Alberta Health has collected different fish species from different lakes. There has not been sufficient number of resampling from the same lake. Therefore, it is hard to tell the arsenic concentration trend over years. In addition, there is not a large enough number of fish from each lake to compare and correlate arsenic concentration with fish size, age, or sex.

This Alberta freshwater fish surveillance project supported by Alberta Health has been undergoing for over a decade, and over 2000 freshwater fish were analyzed by our laboratory. My research contributes to an Alberta freshwater fish dataset. A student who continues with the project will use my data and the entire fish dataset to carry out additional statistical analysis.

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CHAPTER 4

General Discussion and Conclusions

4.1 Review of Thesis Objective

Human exposure to excessive trace elements could result in adverse health outcomes. Among trace elements, arsenic adversely affects 200 million people worldwide. Humans are primarily exposed to trace elements through food and water ingestion. Freshwater fish is one of the major food sources for local people. It is important to measure the concentrations of trace elements in local fish for assessments of the overall dietary intake of trace elements.

The World Health Organization and Health Canada have set the drinking water arsenic guideline as 10 μg/L (maximum contaminant level, MCL). There is no regulatory guideline of arsenic in fish. One of the reasons for a lack of guideline is that many arsenic species may be present in fish and these arsenic species have very different toxicities. While inorganic arsenite (As^{III}) and arsenate (As^{V}) are highly toxic, with median lethal dose (LD_{50}) of 10-20 mg/kg (tested in rats), arsenobetaine (AsB) is much less toxic, with LD_{50} (~10,000 mg/kg) similar to that of sodium chloride (LD_{50} ~4,000 mg/kg). Therefore, any guidelines and health risk assessment must be based on arsenic species, not the total arsenic concentration. It is important to determine arsenic speciation in fish.

Marine organisms generally contain high concentrations of arsenic, and extensive research has been carried out to determine arsenic speciation in seafood. However, because of analytical challenges for the determination of various arsenic species present at much lower concentrations in freshwater fish, much less is known about arsenic species in freshwater fish.

The primary objective of this thesis research was to determine the concentrations of trace elements and arsenic species in freshwater fish, with a focus on fish commonly captured from lakes in Alberta, including northern pike, lake whitefish, walleye, lake trout, and rainbow trout. This research was in collaboration with Alberta Health. The research team selected lakes across Alberta for fish sample collection, and Alberta Health provided

our laboratory with fish filet for analysis. My thesis research focused on analyses of 266 fish samples, collected from eight water bodies in Alberta between 2012 and 2020.

4.2 Summary of Results

In Chapter 2, I discussed the determination of trace element concentrations in 266 fish, using inductively coupled plasma mass spectrometry (ICP-MS). Among the 20 elements that were chosen for detection using ICP-MS, the concentrations of Ag, Be, Pb, Sb, Th, and U were below the detection limits of 0.003, 0.3, 0.002, 0.007, and 0.003 mg/kg (mg of the element per kg of fish tissue in wet weight), respectively. As, Mn, Se, and Zn were detected in all 266 fish samples, and their mean concentrations and standard deviations were and 0.10 ± 0.16 mg/kg for As, 0.2 ± 0.1 mg/kg for Mn, 0.2 ± 0.1 mg/kg for Se, and 5 ± 2 mg/kg for Zn. Cr, Ni, and V were detected in 90%, 76%, and 72% of all the fish samples, respectively. Al was detectable (above the detection limit of 0.8 mg/kg) only in 18 samples out of 266; however, it was detectable in 13 out of 20 lake trout samples. Co was detectable (above the detection limit of 0.01 mg/kg) only in 15 samples out of 266, while it was detectable in 14 out of 18 rainbow trout samples. Tl was detectable (above the detection limit of 0.002 mg/kg) only in 30 out of 266 fish samples, while it was detectable in 95% of lake trout. Future analysis of more fish samples from different lakes may help test whether the concentrations of Al and Tl are higher in lake trout, and Co is higher in rainbow trout.

In Chapter 3, I described the determination of arsenic species in fish, using high-performance liquid chromatography coupled with inductively coupled plasma mass spectrometry (HPLC-ICP-MS). I further investigated arsenic speciation patterns in different fish species, the same fish species from different water bodies, and the same fish species collected between two years from the same lakes.

The HPLC-ICP-MS method was able to separate and quantify five main arsenic species commonly found in food: arsenobetaine (AsB), arsenite (As^{III}), dimethylarsinic acid (DMA^{V}), monomethylarsonic acid (MMA^{V}), and arsenate (As^{V}). These arsenic species were baseline separated within 20 min, using an anion exchange column (PRP-X100). The detection limit of these five arsenic species was 0.00025 mg As per kg of fish tissue in wet weight. The sum of arsenic concentrations in the methanol-water extract and

in the residue agreed well with the total arsenic concentration in the acid digest. Analysis of standard reference materials showed good agreements between the measured values (sum of arsenic species) and the certified values (total arsenic).

The predominant arsenic species in the 266 fish samples is arsenobetaine (AsB), accounting for 34% (walleye), 65% (northern pike), 69% (lake whitefish), 86% (rainbow trout), and 95% (lake trout) of all arsenic species detected in each of these five main types of fish. Five arsenic species, whose HPLC retention times did not match with any of the available arsenic standards, were also detected. The identity of these five arsenic species remained unknown. Comparisons among the major fish types, including northern pike, lake whitefish, walleye, lake trout, and rainbow trout, showed differences in arsenic speciation patterns. For example, 36% of all northern pike had detectable dimethylarsinic acid (DMA), 25% of all lake whitefish and 42% of all walleye had detectable inorganic arsenate (As^V), and lake trout and rainbow trout did not have detectable DMA or As^V.

Comparisons between two years of fish sample collection from two lakes, Sylvan Lake and Cold Lake, showed similar concentrations of arsenic species between two years. Fish from Sylvan Lake had lower concentrations of arsenic than fish from Cold Lake.

Comparisons of arsenic speciation results among fish from eight water bodies showed that fish from Cold Lake had approximately 6 times higher total arsenic concentration than fish from other seven water bodies. The higher arsenic concentration in the fish of Cold Lake was mostly attributed to AsB, which was approximately 8 times higher in fish from Cold Lake than fish from other water bodies. Reasons for the higher concentrations of AsB and total arsenic in fish of Cold Lake remained not clear.

4.3 Discussions and Perspectives

The sum of As species concentrations in the 266 fish samples was 0.045 ± 0.057 mg/kg (median 0.023 mg/kg, range 0.00025−0.35 mg/kg). These results are comparable to the results reported from other countries. For example, in Shandong Province, China, the average concentration of total arsenic in freshwater fish was 0.075 mg/kg (0.007–0.49 mg/kg) [1]. In France, the average concentration of total arsenic in freshwater fish was 0.119 ± 0.076 mg/kg (0.029–0.233 mg/kg) [2]. In Keban Dam Reservoir, Turkey, the

concentration of total arsenic in freshwater fish was 0.0983 ± 0.0482 mg/kg (0.038–0.191 mg/kg) [3].

Because AsB is a major arsenic species in the fish analysed in this study (0.033 ± 0.055 mg/kg, median 0.0081 mg/kg, range 0.00056−0.33 mg/kg) and because AsB is much less toxic (almost non-toxic) than other arsenic species, I excluded the concentration of AsB from the following consideration. It puts the detected concentration of arsenic species into perspective of human exposure.

The sum of all other arsenic species, excluding AsB, was 0.012 ± 0.009 mg/kg, (median 0.0096 mg/kg, and range 0.0011−0.049 mg/kg). These included estimated concentrations of the unidentified arsenic species (UN1 through to UN7). Because the chemical natures and toxicological properties of these unidentified arsenic species are not known, I included them along with the toxic arsenic species for the purpose of conservative assessment. According to the WHO and Health Canada drinking water arsenic guideline of 10 µg/L, the maximum amount allowable from consuming 2 L of drinking water per day would be 20 µg arsenic (10 µg/L × 2 L = 20 µg). With a typical ingestion rate of 100 g (0.1 kg) of fish filet per day, the average amount of arsenic intake would be 1 µg (0.01 mg/kg × 0.1 kg = 0.001 mg or 1 µg). This amount of toxic arsenic species (1 µg) from the consumption of 100 g fish is much lower than the maximum allowable amount of arsenic (20 µg per day) from drinking water. At the upper concentration range (0.049 mg/kg) of toxic arsenic species, the amount of arsenic intake from the consumption of 100 g (0.1 kg) fish filet would be 4.9 µg (0.1 mg/kg × 0.049 kg = 0.0049 mg or 4.9 µg). This amount of toxic arsenic species (4.9 µg) from fish consumption is still lower than the maximum allowable amount of arsenic (20 µg) from drinking water.

Health Canada has a guideline of 3.5 mg total arsenic per kg fish protein (3.5 pm or mg/kg). For comparing to this guideline, the measured concentrations of arsenic species in fish tissue (in wet weight) would need to be converted by taking into account the moisture and protein contents. The Joint FAO (Food and Agriculture Organization) and WHO Expert Committee on Food Additives (JECFA) recommended 3.0 µg arsenic per kg body weight per day (µg/kg·d) as the maximum daily intake of arsenic. Our results of

average 1 µg arsenic intake from the consumption of 100 g fish by a 70-kg person would equal to 0.0016 µg arsenic per kg body weight per day (1 µg As ÷ 70 kg body weight = 0.014 µg As per kg body weight per day). This daily intake value of toxic arsenic species (0.014 µg/kg·d) is much lower than the JECFA recommended maximum value of 3.0 µg/kg·d. The sum of all arsenic species excluding AsB in Cold Lake is 0.002 to 0.049 mg/kg and in other seven water bodies is 0.0011 to 0.043 mg/kg. If individuals ingest 100 g of fish from Cold Lake containing the upper concentration (0.049 mg/kg) of toxic arsenic species, the daily arsenic intake (49 µg) from the consumption of 100 g fish filet would be 0.07 µg/kg·d (49 µg As per day ÷ 70 kg body weight = 0.07 µg As per kg body weight per day). If individuals ingest 100g of fish from other seven water bodies containing the upper concentration (0.043 mg/kg) of toxic arsenic species, the daily arsenic intake (43 µg) from the consumption of 100g fish filet would be 0.061 µg/kg·d (43 µg As per day ÷ 70 kg body weight = 0.061 µg As per kg body weight per day). Both upper end values (0.07 µg/kg·d and 0.061 µg/kg·d) from Cold Lake and other seven water bodies are lower than the JECFA recommended maximum of 3.0 µg/kg·d. These estimates suggest that moderate consumption of common fish captured in Alberta lakes does not exceed the recommended values of maximum daily intake of arsenic.

4.4 Future Research

Previous studies have shown the methanol-water extraction process is mild and is suitable for arsenic speciation analysis. In this study, I extracted arsenic species from freshwater fish samples using a mixture of methanol and water. Methanol-water is efficient for extracting most water-soluble arsenic species. However, it is not efficient for extracting lipid-soluble and tightly bound arsenic species. Lipid-soluble arsenic species are not able to be extracted using methanol-water alone. An organic solvent, such as dichloromethane (DCM), would help extract nonpolar arsenic species such as arsenolipids [4]. In addition, the methanol-water mixture is not able to extract arsenic species tightly bound, e.g., to proteins [5]. Various enzymes, such as proteases, pepsin, α-amylase, and a combination of enzymes, could help release the bound arsenic species and increase the extraction efficiency [6, 7]. In this study, I extracted arsenic species from freshwater fish only using methanol-water extraction with sonication. In the future, a sequential extraction method,

including the extraction of arsenic species first with methanol-water, enzyme digestion, and then with DCM, could be optimized. The sequential extraction process would help extract water soluble as well as lipid soluble arsenic species.

I detected six unknown arsenic species. Their retention times on the strong anion exchange chromatography did not match with those of any arsenic standards available to us. Although the concentrations of unknown arsenic species are low, it is important to identify these arsenic species for future studies of their toxicity. On the basis of what is known from marine organisms, I hypothesize that these unknown arsenic species could be different arsenosugars and arsenic-containing hydrocarbons and lipids. Future research using HPLC separation with simultaneous ESI-MS and ICP-MS detection could be used to identify some of these unknown arsenic species.

In this study, I analysed 266 freshwater fish samples. This number is not sufficient for detailed statistical analysis to understand any differences in arsenic speciation as affected by the type, size, age, and sex of fish, the water conditions, lake environment, the year and season of fish sampling. Alberta Health sampled from different lakes in different years over the past decade. Future repeated sampling of more fish samples from the same lakes in multiple years would be useful. If possible, a larger number of fish from one lake would be analysed to test any correlation of arsenic speciation with fish weight, size, age, or sex.

Future research will also benefit from incorporating the results from this study into a comprehensive dataset containing detailed information of arsenic species in fish samples representing all Alberta lakes. The dataset and resources will be useful for assessing daily intake and for setting evidence-based guidelines on fish consumption.

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