The Impacts of Legacy Mining Operation on Inorganic Arsenic Bioaccumulation and Exposure in Yellowknife, Northwest Territories, Canada

By:

Claudia Tanamal, BSc. (Hons)

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Supervisors:

Dr. Jules Blais (University of Ottawa)

Dr. Laurie Chan (University of Ottawa)

Thesis Advisory Committee:

Dr. Alexandre Poulain (University of Ottawa)

Dr. Vance Trudeau (University of Ottawa)

Dr. John Chételat (Carleton University)

Department of Biology

Faculty of Science

University of Ottawa

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Abstract

Arsenic transfers and toxicology are important topics of research and a public health concern because arsenicosis affects millions of people worldwide every year. One of the most significant sources of arsenic in the environment is industrial wastes, such as by-products of mining operation. In Yellowknife, Northwest Territories, Canada, there were two large gold mines—Giant Mine and Con Mine, along with dozens of small-scale mines. The combined by-product of emission from these roasters might have contributed to high concentrations of arsenic found in the city. This thesis presents the results of two related studies to address the environmental health concern: (1) to investigate the arsenic transfers and arsenic species accumulation in freshwater food webs near large legacy mining operations in Yellowknife, and (2) to assess the long-term health risk of inorganic arsenic exposure from the consumption of fish in Yellowknife among the general residents and the Yellowknives Dene First Nation. We found that inorganic arsenic is biominified in food webs (i.e. inorganic arsenic concentrations diminish at higher trophic positions relative to lower trophic positions). Higher-trophic organisms have low inorganic arsenic concentrations in tissue due to biotransformation of inorganic arsenic to non-toxic organic arsenobetaine, and effective elimination of arsenic from their tissue. The trophic positions of freshwater organisms can be used to predict the range of arsenic concentrations and its species composition, accounting for more than 80% of variance. Dietary study results show that the Yellowknives Dene First Nation consumed significantly more fish in their diets (adults: 19 g/day, children: 9 g/day) compared to the general residents of Yellowknife (adults: 9 g/day, children: 5 g/day). Our probabilistic risk assessments showed no significant long-term non-carcinogenic and carcinogenic health risks of inorganic arsenic exposure from fish consumption for the majority of Yellowknife residents, but elevated cancer risks among the adult heavy fish consumers in Yellowknife. However, our data suggested that the residents of Yellowknife were not exposed to higher cancer risks of inorganic arsenic exposure compared to the general population in Canada. Therefore, due to fish health benefits and the values associated with its consumption, fish should continue to be a major source of sustenance in Yellowknife.

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List of Abbreviations

AsB	Arsenobetaine
AsC	Arsenocholine
As_2S_3	Orpiment
As_2S_4	Realgar
As(III)	Inorganic arsenic in trivalent form
As(V)	Inorganic arsenic in pentavalent form
ATSDR	Agency for Toxic Substances and Disease Registry
$BMDL_{0.5}$	Lower 95% confidence limit on the benchmark dose for 0.5% response
BMF_{TL}	Biomagnification Factor from prey to consumer
CCME	Canadian Council of Ministers of the Environment
CI	Confidence Interval
DMA	Dimethylarsinic acid
d.w.	dry weight
ENR	Environmental and Natural Resources
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization
FeAsS	Arsenopyrite
FFQ	Food Frequency Questionnaire
GMRP	Giant Mine Remediation Project
GNWT	Government of Northwest Territories
GSH	Glutathione
HEMP	Health Effects Monitoring Program
HPLC	High Performance Liquid Chromatography
HQ	Hazard Quotient
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
JECFA	Joint (FAO/WHO) Expert Committee on Food Additives
MMA	Monomethylarsonic Acid
NOAEL	No Observed Adverse Effect Level
NRC	National Research Council
NT	Northwest Territories
PTDI	Provisional Tolerable Daily Intake
RfD	Reference dose
SAHC	S-adenosylhomocysteine
SAM	S-adenosylmethionine
TRV	Toxicity Reference Value
WHO	World Health Organization
w.w.	wet weight

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Chapter 1: Introduction

1.1. General Introduction

Canada has one of the largest mining industries in the world, producing more than 60 minerals and metals, and contributing $57.6 billion to the country's Gross Domestic Product in 2016 (Marshall, 2017). The gold industry boom started in Yellowknife in 1935, when the Giant Yellowknife Gold Mines Ltd was formed (INAC, 2018). This gold rush boosted the economic growth of the city and its communities by providing jobs and business opportunities. Yellowknife housed Giant Mine, one of the biggest and most productive gold mines in the country. Together with its smaller neighbouring Con Mine, the roasters emitted an estimated 10 tonnes of arsenic trioxide dust daily in their initial years of operation (Keeling & Sandlos, 2012). Arsenic persists in the environment and is transferred through food webs (ATSDR, 2007). The inorganic arsenic emitted from the roasters was deposited to sediments (van den Berghe, 2016) and lake water (Houben et al., 2016; Palmer et al., 2015). It accumulates in plankton through water and dietary assimilation (Caumette et al., 2011; Wang et al., 2018), is transferred to fish (Zhang et al., 2012), and eventually enters human food chains through fish consumption. Other exposure pathways include exposure via ingestion and inhalation of airborne dust, especially among mine workers (Gomez-Caminero et al., 2001). Acute exposure of arsenic in humans at high dose could lead to instantaneous death, while chronic low-dose exposure to arsenic could manifest in different health outcomes, including skin problems, cardiovascular diseases, injury to the nervous system and cancers in numerous organs (Health Canada, 2006). The reports of fatal arsenic poisoning in livestock and people, and arsenic-contaminated drinking water raised concerns over the scale of arsenic contamination in Yellowknife (Keeling & Sandlos, 2012), leading the local residents to question the magnitude of the impacts of the past ore-roasting activities. A prior study has shown

arsenic concentrations in lake water exceeding 135 µg/L in lakes within 4-km of the Giant Mine (Houben et al., 2016), 14 times allowable concentrations in drinking water (Health Canada, 2006). This finding is significant because many of these lakes were used by the locals for recreational activities and fishing. Children and infants are the most vulnerable sub-populations to health effects associated with arsenic exposure, along with the Indigenous communities who rely heavily on fishing and hunting locally for food. The lakes in Yellowknife are regularly being monitored and studied for arsenic and its species, however, little is known about how arsenic and its species are transferred in aquatic food webs, and whether fish consumption in Yellowknife poses any long-term health hazard.

This research project covers the environmental fate of arsenic in freshwater food webs, as well as a population risk assessment to arsenic exposure to address the impacts of the legacy mining activities in Yellowknife and surroundings to freshwater biota and local communities. The goals of this study are to characterize the factors that determine the proportions and concentrations of inorganic arsenic in freshwater organisms and to evaluate the risk of exposure to inorganic arsenic through the consumption of fish caught around Yellowknife. I focus on the inorganic species of arsenic because these are Class 1 carcinogenic agents to humans (IARC, 2012). Most studies to date have looked only at total arsenic, which is not as informative because arsenobetaine is non-toxic (EFSA, 2010; Molin et al., 2015). It is important to know arsenic speciation for exposure studies. The specific objectives of this thesis are to (1) explain the dynamics of inorganic arsenic transfer in littoral (near-shore) and pelagic (off-shore) freshwater food chains of aquatic biota from different trophic positions using arsenic speciation and natural isotopic ratio analyses; (2) measure the concentration of arsenic species in freshwater fish samples collected from nine different lakes in Northwest Territories; and (3) determine whether there is a potential health

concern associated with inorganic arsenic exposure in the human population, based on self-reported fish intakes.

This research project involved partnerships with the Health Effect Monitoring Program (HEMP) in Yellowknife, initiated in 2016, and the Government of Northwest Territories to monitor and provide a risk assessment on arsenic exposures in Yellowknife's population. The arsenic speciation work in fish across lakes in Yellowknife was supported by the Department of Environmental and Natural Resources, the Government of Northwest Territories, before HEMP. Through HEMP, we obtained the data on the frequencies of fish consumption and the sources of fish, which are used to evaluate the associated exposure to inorganic arsenic. The results of this project will (1) contribute to the understanding of the pathway that leads to inorganic arsenic exposure in humans through diets; (2) fill in the knowledge gap in arsenic species transfers in aquatic food webs, from arsenic bioconcentration by algae to higher trophic-level fish species; and (3) inform the on-going Giant Mine Remediation Project (GMRP) and Yellowknife communities of the probabilistic long-term health risks associated with inorganic arsenic exposure from fish consumption.

1.2. Research Objectives and Hypotheses

There are three main research objectives of this project. Firstly, I aim to explain the dynamics of arsenic species transfer in littoral (near-shore) and pelagic (off-shore) freshwater food webs over a range of trophic positions, from primary producers to piscivorous fish. Carbon and nitrogen stable isotopic ratio analyses are used to describe the trophic position and feeding in benthic vs pelagic environments. The first hypothesis is that there is a higher transfer of inorganic arsenic in littoral food webs compared to pelagic food webs because organisms from littoral food

webs consist of more benthic organisms that are more exposed to lake sediments which are the source of inorganic arsenic in lake ecosystems. I also expect to see organisms from the bottom of a food chain accumulating the highest inorganic arsenic in their tissues, and a decreasing concentration of inorganic arsenic at higher trophic positions because more complex, higher-trophic organisms are more effective in metabolizing and eliminating inorganic arsenic species. Therefore, the highest inorganic arsenic bioconcentration should be observed at the bottom of the littoral food chain in a lake ecosystem.

Secondly, I measured the concentration of various arsenic species in tissues of freshwater fish of two species: northern pike (*Esox lucius*) and lake whitefish (*Coregonus clupeaformis*), collected from nine different lakes in Northwest Territories with varying distances and directions from Giant Mine. I hypothesize that the fish samples harvested from lakes closer to Giant Mine are the most affected by the high arsenic found near the roaster. Therefore, I predict that fish caught from lakes within 5 km of Giant Mine would contain higher concentrations of inorganic arsenic, than the fish caught from lakes that are further away from the mining area.

The third objective of this project is to determine the potential long-term health risk from arsenic exposure in two Yellowknife population groups: the general resident group and the Dene First Nation group, from consuming locally-caught fish. I calculated the Hazard quotient (HQ) and Cancer Risk (CR) and compared these between average and high-frequency fish consumers based on the HEMP food frequency questionnaire, taking into consideration the concentrations of inorganic arsenic measured in fish species from the mentioned lake sources. I hypothesized that higher fish consumption rates in Yellowknife contribute to elevated exposure to inorganic arsenic. My prediction was that the Yellowknives Dene First Nation group was exposed to higher inorganic arsenic than the general residents of Yellowknife because First Nations communities generally

consume more locally-caught fish. Therefore, I predicted the HQ and CR indices would be higher among the First Nations residents.

1.3. Gold Mining in Yellowknife

Canada ranks among the top five countries worldwide in the production of gold (Marshall, 2017). One of the largest and most productive mines in the history of gold mining was Giant Mine (62°29′59″N 114°21′31″W), located in Yellowknife, Northwest Territories. The mine was operative for 50 years since 1948 until its management, the Royal Oak Mines Inc., went into receivership in 1999. Giant Mine was reopened in 2000 under the ownership of Miramar Mining Corporation with its limited ore processing transferred to Con Mine. Giant Mine was permanently closed in 2004. It is currently storing 237,000 tonnes of arsenic trioxide in 15 underground chambers along with four tailing ponds (Canadian Public Health Association, 1977; Keeling & Sandlos, 2012). It is now undergoing a one billion dollar remediation project, led by Indigenous and Northern Affairs Canada (Keeling and Sandlos, 2012; Silke, 2009).

During the first ten years of the Giant Mine operation, around 20,000 tonnes of arsenic trioxide dust were released unfiltered from the burning of arsenopyrite ores (Jamieson, 2014; Keeling and Sandlos, 2012). The roasting of ores was necessary to extract gold particles that are tightly bound to sulphides (INAC and GNWT, 2010). Baghouse filtration system was eventually installed in 1958 to filter the dust by-products (Canadian Public Health Association, 1977; Keeling & Sandlos, 2012). After the baghouse installation, the emission of arsenic trioxide dust was reduced to an estimate of 3.3 tonnes per day (Keeling & Sandlos, 2012).

$$2\text{FeAsS} + 5\,O_2 \xrightarrow{\Delta} Fe_2O_3 + As_2O_3 + 2SO_2$$

Equation 1.1. Chemical reaction from the combustion of arsenopyrite ores producing toxic arsenic trioxide and sulfur dioxide by-products.

Sediment core analysis from Pocket Lake, situated beside Giant Mine, revealed a striking increase of roughly 1,700% in total arsenic concentration to over 30,000 μg/g concentration in sediment, coinciding with the timeline of Giant Mine operation (Thienpont et al., 2016) and well beyond the probable effect levels for arsenic of 17 μg/g (Canadian Council of Ministers of the Environment, 1999).

Recent lake surveys by the Government of Northwest Territories (GNWT) and Houben et al. (2016) found arsenic concentrations to be elevated in lakes within a 17-km radius of the Giant Mine, ranging from 10 to 646 μg/L of total arsenic concentration in surface water. The mean total arsenic concentration in surface water from lakes located within a 30-km radius of the mine was 40 μg/L, exceeding the Health Canada drinking water guideline of 10 μg/L (Health Canada, 2006; Houben et al., 2016; Palmer et al., 2015). Arsenic was generally highest in smaller lakes, located downwind from the roaster (northwest direction) and those situated in proximity (<5 km) to the mining area (Houben et al., 2016; Palmer et al., 2015).

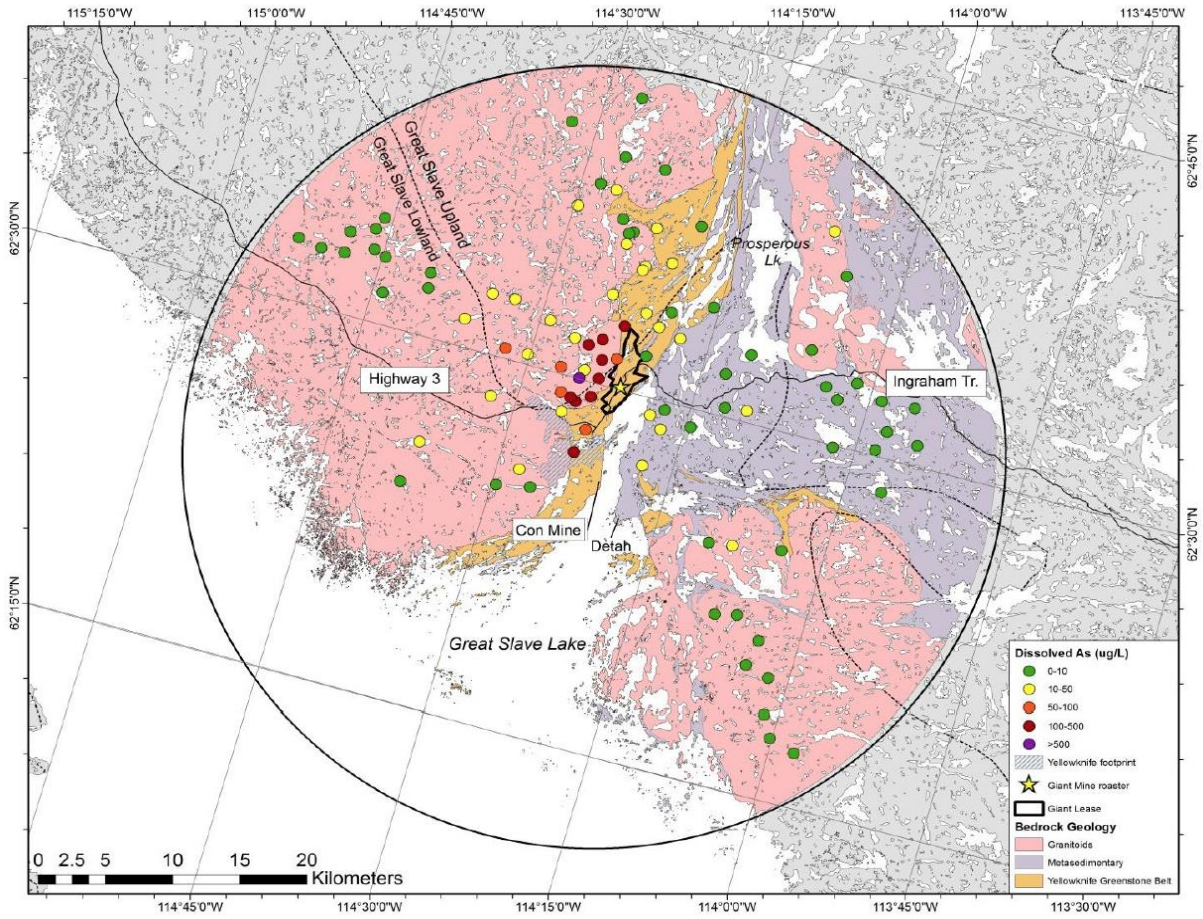

Figure 1.1. A summary map of total dissolved arsenic levels in the surface water of 98 surveyed lakes in Yellowknife, NT. Obtained from Palmer et al. (2015).

Con Mine (62°26′20″N 114°22′18″W) was the first gold mine constructed in Yellowknife and was smaller than Giant Mine. It was managed by Cominco between 1943 until 1993. The management of the mine was then taken over by Miramar Mining Corporation in 1993. Con Mine was demolished in 2016, 13 years after it was closed (Mosher, 2016; Silke, 2009). An estimated total of 10 tonnes of arsenic trioxide dust was emitted every day from the production in Con Mine, together with Giant Mine during the early 1950s (Keeling & Sandlos, 2012).

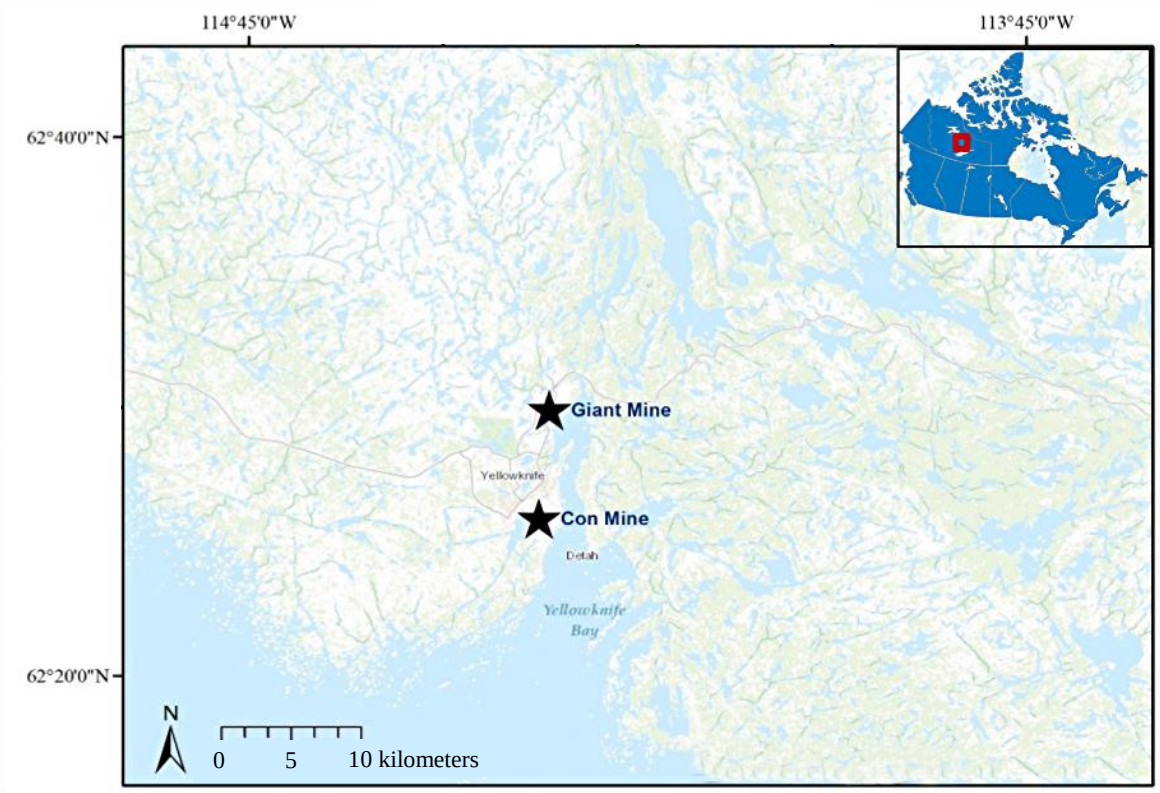

Figure 1.2. The locations of Giant Mine and Con Mine near Yellowknife, Northwest Territories. The inset shows Canada with the red square marking the location of Yellowknife and region.

1.4. Arsenic in the Environment

Arsenic is a natural metalloid widespread in earth's bedrock and is the 20th most abundant element on earth (Mandal & Suzuki, 2002). It is naturally released through bedrock weathering processes and volcanic activity, as well as from human activity through ore smelting operations (ATSDR, 2007). On the periodic table, arsenic belongs to Group VA, Period 4, atomic number 33, with a stable isotope of ^{75}As. Arsenic occurs in the environment in valence states of -3, 0, +3 and +5.

Arsenic has more than 200 known mineral forms. Roughly 60% of arsenic is in the form of arsenate conjugates, 20% is bound with sulfides and sulfosalts, and the remainder is present as

arsenites, arsenides, bound with oxides and silicates, and its elemental form (IARC, 2012; Mandal & Suzuki, 2002). The most common mineral form of arsenic is arsenopyrite (FeAsS), followed by orpiment (As_2S_3) and realgar (As_4S_4) (IARC, 2012; Mandal & Suzuki, 2002). The mineral forms are relatively stable in the environment.

The soluble forms of arsenic include arsenite (As(III)), usually found in a reducing environment, and the oxidized form arsenate (As(V)), typically found in oxic conditions (Zhang et al., 2017). Monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and organic arsenic species have been found in the environment as products of biotransformation by microorganisms (Mandal & Suzuki, 2002; Rahman & Hassler, 2014). The toxicity of arsenic compounds has been reported as follows: As(III) > As(V) > MMA > DMA > Organic arsenic species (ATSDR, 2007; NRC, 2001). Inorganic arsenic species: As(III) and As(V), are classified by the International Agency for Research on Cancer as Class I carcinogens, i.e. carcinogenic to humans based on extensive human data; while MMA and DMA species are classified as class IIB chemicals, possibly carcinogenic to humans based on *in-vitro* evidence (Escudero-Lourdes et al., 2012; IARC, 2012; Wnek et al., 2011). More recent studies have found the trivalent form of MMA to be more cytotoxic to human cells than As(III) by inhibiting DNA repair processes, disrupting enzymatic activities and inducing chromosomal mutations (Escudero-Lourdes et al., 2012; Kligerman et al., 2003; Mass et al., 2001; Wnek et al., 2011). Complex organic arsenicals, like arsenobetaine (AsB), are among the non-toxic species of arsenic and are readily eliminated from the human body (Sharma & Sohn, 2009; Spayd et al., 2012). Other complex organic arsenic species, such as arsenocholine and arsenosugar, are metabolized and excreted as primarily DMA in urine (Thomas & Bradham, 2016). The biochemical pathways to the formation of these complex organic arsenicals are not well understood (Thomas & Bradham, 2016).

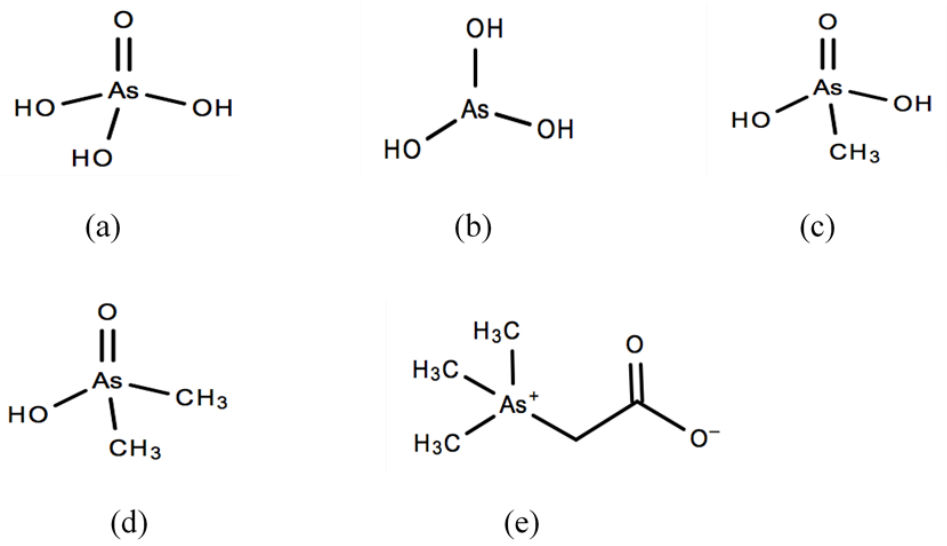

Figure 1.3. Chemical structures of soluble arsenic forms: (a) arsenate (As(V)), (b) arsenite (As(III)), (c) monomethylarsonic acid (MMA), (d) dimethylarsinic acid (DMA) and (e) organic arsenic arsenobetaine (AsB).

1.5. Arsenic in Freshwater

The most common source of elevated arsenic in water and soil in Canada is anthropogenic activities (e.g. wood preservatives and mining industries), and most arsenic particles end up in the sediment or soil (ATSDR, 2007). The average arsenic concentration in surface sediments of lakes around the city of Yellowknife exceeds the CCME arsenic guideline concentration of 5.9 µg/g (Canadian Council of Ministers of the Environment, 1999) with a mean of 27.7 µg/g, ranging between 5.00 µg/g to 155 µg/g in lakes closer and downwind to the mine, according to a survey by Galloway et al. (2012) from 19 lakes along the Ingraham Trail. These arsenic concentrations in the sediment samples are elevated compared to those outside the city (5-38 µg/g) (Wagemann et

al., 1978), and before the mining era in 1947 (15-25 μg/g in Back Bay and Great Slave Lake) (Murdoch et al. 1989 qtd in Galloway et al., 2012). The observed high concentrations of arsenic in sediment have been credited to the early aerial deposition of arsenic trioxide during Giant Mine operations and local bedrock geological output (Andrade et al., 2010; Galloway et al., 2012).

Van Den Berghe (2016) identified lake sediments to be both a sink and a source of arsenic in a freshwater environment. Elevated arsenic concentrations in reducing sediment porewaters result in the *in-situ* precipitation of arsenic-bearing sulphides and the upward diffusion of arsenic from sediment porewaters to lake waters. Another factor affecting arsenic concentration in lake water is lake residence time (van den Berghe, 2016; van den Berghe et al., 2018).

1.5.1. Arsenic Bioaccumulation in Food Webs

Arsenic has been found to bioconcentrate at the base of food webs. In plankton, As(V) is taken up through the phosphate-carrier pathway because of the similarity in chemical structure between phosphate and As(V), and As(III) is taken up through aquaporins (Wang et al., 2015). Arsenic may then be transferred to higher trophic levels, where it is metabolized to methylated and organic arsenic species (Kumari et al., 2017; U.S. EPA, 2015). Some studies have also found small concentrations of arsenoribose in algae that could serve as the intermediate to AsB formation in higher-trophic animals (Duncan et al., 2015; Thomas & Bradham, 2016).

In Rat Lake near Giant Mine tailings, where the water arsenic concentration was 0.25 μg/g, the total arsenic concentrations were 800 μg/g and 35 μg/g in phytoplankton and zooplankton (*Daphnia pulex*), respectively (Caumette et al., 2011). The higher concentrations of arsenic in phyto- and zooplankton relative to the lake water indicate that arsenic is bioconcentrated in the plankton. Arsenic species in phytoplankton were largely present as As(V), and more than half of

the arsenic detected in *D. pulex* was in the form of inorganic As(V) with roughly 10% as As(III) and the rest (34%) as organic arsenic (Caumette et al., 2011). The study suggested that the sources of arsenic in *D. pulex* were from sediments, phytoplankton and other ingested food and that organic arsenic species in *D. pulex* were a product of biotransformation of inorganic arsenic within the cell (Caumette et al. 2011).

Wang et al. (2018) showed that As(III) exposure from algal diet is more toxic to the zooplankton species, *D. magna*, than As(V), causing a death response at 48-hour LC_{50} (As(III)) of 1.91 mg/L, compared to LC_{50} of 3.51 mg/L for As(V)). Arsenic speciation analysis of *D. magna* after algal dietary exposure revealed 12-28% of DMA in its tissue. Since As(V) and As(III) were the only species detected in the algal diet during the acute exposure experiment, the speciation results support the hypothesis that the source of DMA in *Daphnia* was a product of the biotransformation of inorganic arsenic (Wang et al. 2018).

1.5.2. Arsenic in Fish

Fish have been used as an indicator of environmental pollutants because fish are continuously exposed to these chemicals via their gills, skin, and contaminated food sources (Kumari et al., 2017). Inorganic arsenic is metabolized in fish through repetitive reductive-methylation processes to As(III) and then to MMA and DMA metabolites (Kumari et al., 2017) (Figure 1.5). An organic arsenic species, AsB, is the dominant arsenic species found in fish tissues and is thought to be a final product of arsenic metabolism in fish (Ackley et al., 1999; ATSDR, 2007; Kumari et al., 2017; Šlejkovec et al., 2004; Zhang et al., 2012; Zhang et al., 2016). AsB accumulated through diet in fish is excreted unchanged (Amlund et al., 2006). The highest

concentrations of arsenic are usually found in arsenic metabolizing organs in fish, such as liver and kidney (Kumari et al., 2017).

Earlier studies discovered that fish species that live or feed close to the sediment had higher bioaccumulation of arsenic in their tissues compared to open-water living fish, regardless of their diets (Alamdar et al., 2017; Perera et al., 2016). A study by Cott et al. (2016) also discovered that the concentrations of arsenic in muscle and liver tissues in predatory and sediment-burrowing burbot from Yellowknife Bay to be higher than those in lake whitefish from a lower trophic position. These data suggested that sediment exposure and ingestion could be a significant source of arsenic in fish.

Given that porewater diffusion from bottom sediment is the predominating source of arsenic in lake water, and that sediment ingestion is an important source of arsenic in benthic organisms, it is plausible that littoral food webs, with high exposure to lake sediment and feeding largely on benthic food sources, accumulate higher total and inorganic arsenic than pelagic food webs.

1.6. Human Exposure to Arsenic

1.6.1. Sources of Arsenic

Human exposure to arsenic from the environment is primarily through the oral route in drinking water and arsenic-contaminated food, such as fish, seafood, rice, and other agricultural products (Gomez-Caminero et al., 2001; Schoof & Yager, 2007; Schoof et al., 1999; Williams et al., 2005). Arsenic exposure by inhalation is relevant among some mine and industrial workers, usually as arsenic trioxide (As_2O_3) bound to dust and air particles (Gomez-Caminero et al., 2001;

Yager et al., 1997). Arsenic waste through industrial activities and arsenical pesticide use can undergo oxidation, reduction, precipitation with Fe-oxyhydroxide, and biomethylation by microorganisms in water and soil, eventually making its way to the human food chain (Roy & Saha, 2002).

Arsenic in drinking water is a concern because of the prevalence of inorganic arsenic species in water. As(III) is the major species under reducing conditions, while As(V) is the most common form in oxidizing water, usually near the surface water interface (Van den Berghe, 2016; Zhang et al., 2017). High groundwater arsenic has been reported worldwide, including in Bangladesh, Japan, China, Cambodia, Vietnam, India, Iran, Romania, Argentina, Nepal, Taiwan, Hungary, Finland, Greece, the United States and Canada (Amini et al., 2008; Berg et al. 2007; Das et al., 1996; Meranger et al., 1984; Nasrabadi & Bidabadi, 2013; NRC, 2001; Shimada, 1996; Smith et al., 2000; Zhang et al., 2017).

Industrial wastes, the use of arsenic-contaminated groundwater and persistent arsenical pesticides could result in high arsenic concentrations in fish, shellfish, paddy rice plants and agricultural produce. Roughly 25% of arsenic ingested through food is in the inorganic forms. However, the inorganic arsenic proportions may vary depending on the type of food (Gomez-Caminero et al., 2001; Health Canada, 2006). Arsenic species found in rice from most countries is 75-90% inorganic arsenic: arsenite (As(III)) and arsenate (As(V)), followed by minor concentrations of DMA (Sun et al., 2009; Williams et al., 2006).

Inorganic arsenic makes up less than 7.5% of the total arsenic concentration in fish, with the majority being non-toxic organic species, such as arsenobetaine (de Rosemond et al., 2008; Lorenzana et al., 2009; Özcan et al., 2016). Plants from contaminated soil (e.g., berries, Labrador

tea) contain mostly toxic inorganic species, while mushrooms and wild game meats contain predominantly DMA and arsenobetaine (Koch et al., 2000 & 2013).

The Provisional Tolerable Daily Intake (PTDI) of inorganic arsenic of 2.0 μg/kg body weight for adults was adopted in 1988 by the joint Food and Agricultural Organization (FAO) / World Health Organization (WHO) committee and was recently withdrawn as it is no longer health-protective (FAO/WHO, 2011). The benchmark dose for inorganic arsenic was set during the seventy-second FAO/WHO meeting on Food Additives in 2011 to be $BMDL_{0.5}$ = 3.0 μg/kg body weight/day (range: 2-7 μg/kg body weight/day) based on epidemiological studies on the incidences of lung cancer (JECFA, 2011).

Arsenic exposure through food ingestion is usually higher among Indigenous populations in contaminated lands because they rely greatly on fishing locally for nourishment rather than on market food, compared to general populations (Receveur et al., 1998). However, these practices are not only culturally and financially significant to Indigenous people, but also essential for their nutritional value. Fish is an important source of protein, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in diet, and its consumption has been linked to many health benefits, including improved cardiovascular health and neural development, and reduced obesity and diabetes (Butler et al., 2017; Mozaffarian & Rimm, 2006; Nkondjock & Receveur, 2003).

1.6.2. Arsenic Distribution in Humans

The elemental form of arsenic is poorly absorbed in the body and is eliminated unchanged (Health Canada, 2006). The soluble forms of arsenic (As(III) and As(V)) are readily absorbed, with around 95% absorption rate in the gastrointestinal tract following oral ingestion (Tam et al., 1979; Zheng et al., 2002), and to a much lesser extent in the lungs (30-34%) via inhalation among

industrial workers and cigarette smokers (ATSDR, 2007). AsB, is obtained primarily through fish and seafood, is non-toxic to humans, and is excreted unchanged (Molin et al., 2015). Arsenic is poorly absorbed through the skin because it is not lipophilic enough to cross the skin barrier (ATSDR, 2007; Lew et al., 2010).

Arsenic is widely distributed in different tissues in the body and is retained in keratin-rich tissues, such as skin, nails, and hair. It is reduced to its trivalent form in the blood and binds to sulfhydryl groups (-SH) in red blood cells (ATSDR, 2007).

Inorganic arsenic biotransformation happens predominantly in the liver and to a lesser extent in the kidney (Yu, 1999). Arsenic is metabolized through a repetitive reduction with GSH-conjugation, and oxidative methylation processes, using S-adenosylmethionine (SAM) as the methyl donor to MMA and then to DMA metabolites, which are more easily excreted from the body (Aposhian et al., 2000; Vahter, 2002).

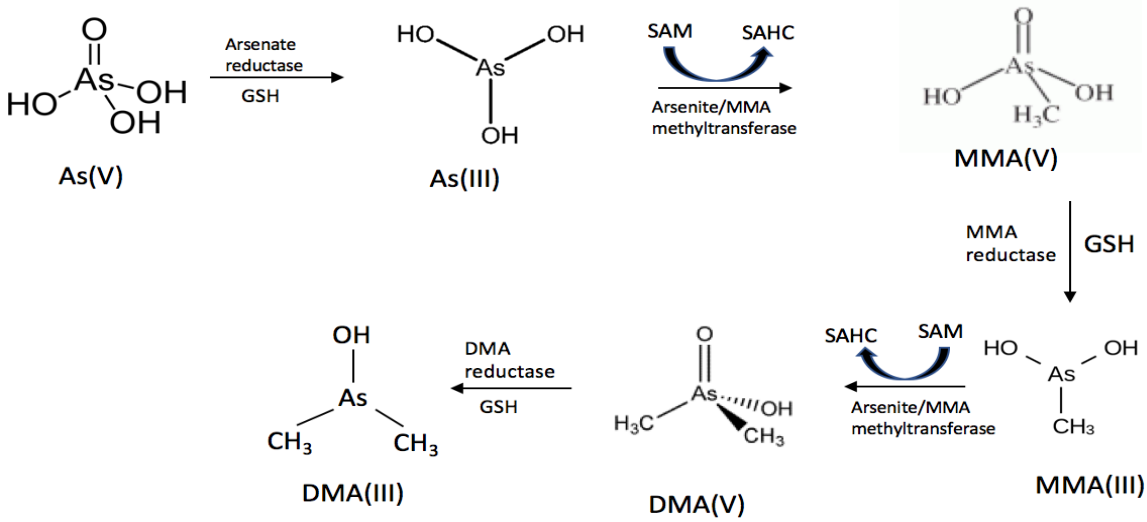

Figure 1.4. Metabolism of arsenic (modified from Aposhian et al. (2000)). GSH: glutathione, SAM: S-adenosylmethionine, SAHC: S-adenosylhomocysteine.

Arsenic is eliminated in the body through first-order elimination, primarily (~60% of intake) through the kidney in urine in a mixture of metabolites and minor concentrations through the bile in the form of As(V) (ATSDR, 2007; Buchet et al., 1981; Tam et al., 1979; Yu, 1999). Inorganic arsenic has a half-life of approximately 30 hours in the human body (Buchet et al., 1981). Around 10-30% of urinary arsenic is excreted in the form of inorganic arsenic, 10-20% in MMA, and 60-80% in DMA (Buchet et al., 1981; Loffredo et al., 2003; Molin et al., 2015; Tam et al., 1979).

1.6.3. Human Health Effects

Acute arsenic poisoning in an oral dose as low as 1 mg/kg body weight of inorganic arsenic can lead to instantaneous death (Canadian Public Health Association, 1977). Most reported cases of acute poisoning were caused by incidental ingestion of arsenical pesticides (Ratnaike, 2003). Long-term arsenic exposure in a dose as little as 0.05 mg/kg body weight has a systemic effect on the human body (ATSDR, 2007).

Arsenicosis manifests in the skin through the formation of hyperpigmentation and hyperkeratosis, usually on the palms of hands and soles of feet, as well as skin lesions. These symptoms are due to the retention of arsenic in keratin-rich tissues, primarily As(III), as it has an affinity to sulfhydryl groups of keratin (IARC, 2012).

Chronic exposure to arsenic has also been reported to affect cardiovascular and circulatory systems through the incidence of Black Foot Disease (BFD), acute myocardial infarction, and linkage to hypertension (Abhyankar et al., 2011; Dastgiri et al., 2010; Tseng, 1977; Yuan et al., 2007). Arsenic has been linked to incidences of diabetes mellitus based on epidemiological studies conducted in Taiwan (Lai et al., 1994; Tseng et al., 2012; Wang et al., 2002), Bangladesh (Rahman

et al., 1998; Rahman et al., 1999), Unites States (Zierold et al., 2004), and Denmark (Jensen & Hansen, 1998).

Continuing arsenic exposure, even at low doses, leads to cancer in multiple organs, especially the skin, liver, and lung (Chen et al., 1986; Hopenhayn-Richet al., 1998; Saúde et al., 1998; Tseng, 1977; Welch et al., 1982). The lung is a sensitive organ to arsenic toxicity because it is a high blood perfused organ. Pulmonary complications and lung cancer are also prevalent among industrial workers inhaling arsenic from fumes, as well as residents living near smelters (Milham & Strong, 1974; Welch et al., 1982). Tseng et al. (1968) found a dose-response relationship between arsenic level in drinking water and the incidence of skin cancer in men and women by conducting physical examinations on 40,421 participants in the BFD-endemic area in Taiwan. This study was then used as a standard by Agency of Toxic Substances and Disease Registry (ATSDR) and Environmental Protection Agency (EPA) to set the chronic oral reference dose (RfD) of 0.3 μg/kg body weight/day, using the No Observed Adverse Effect Level (NOAEL) on dermal effects of 0.8 μg/kg body weight/day (ATSDR, 2007; EPA, 2010).

The thesis has three chapters. The first chapter covers a general introduction of the research topic and arsenic as the chemical of interest in this research. The second chapter explores the characteristics of arsenic and its species concentrations and distributions across trophic positions of freshwater food webs near the large historical mining area. The third chapter describes arsenic speciation in various fish species from nine lakes in the Yellowknife area and covers human health risk assessment of inorganic arsenic exposure from fish consumption in the general residents of Yellowknife and the Yellowknives Dene First Nation.

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Chapter 2: Foodweb Biominification Reduces Toxic Arsenic Species in Predator Fish from a Contaminated Environment

Claudia Tanamal, Laurie H.M. Chan, Emmanuel Yumvihoze, Jules M. Blais
Biology Department, University of Ottawa, Ottawa, Ontario K1N 6N5, Canada

Significance Statement

More than 200 million people worldwide are exposed to higher than recommended levels of arsenic every day[1]. Communities are struggling to make an informed decision to maintain healthy diets from the environment (i.e. consuming fish) while protecting their health from potential chemicals of concern in the environment. Dusts produced by mining operations often contain high concentrations of inorganic arsenic that are harmful and carcinogenic to humans[2]. However, very limited scientific information is known about arsenic dynamics in food webs. Here we examine transfer of different arsenic species in freshwater food webs, spanning five trophic levels in lakes, near large mining operations that released arsenic trioxide from roaster stacks. We found that food web biominification significantly diminishes the concentrations of carcinogenic inorganic arsenic species in predatory fish.

Graphical Abstract

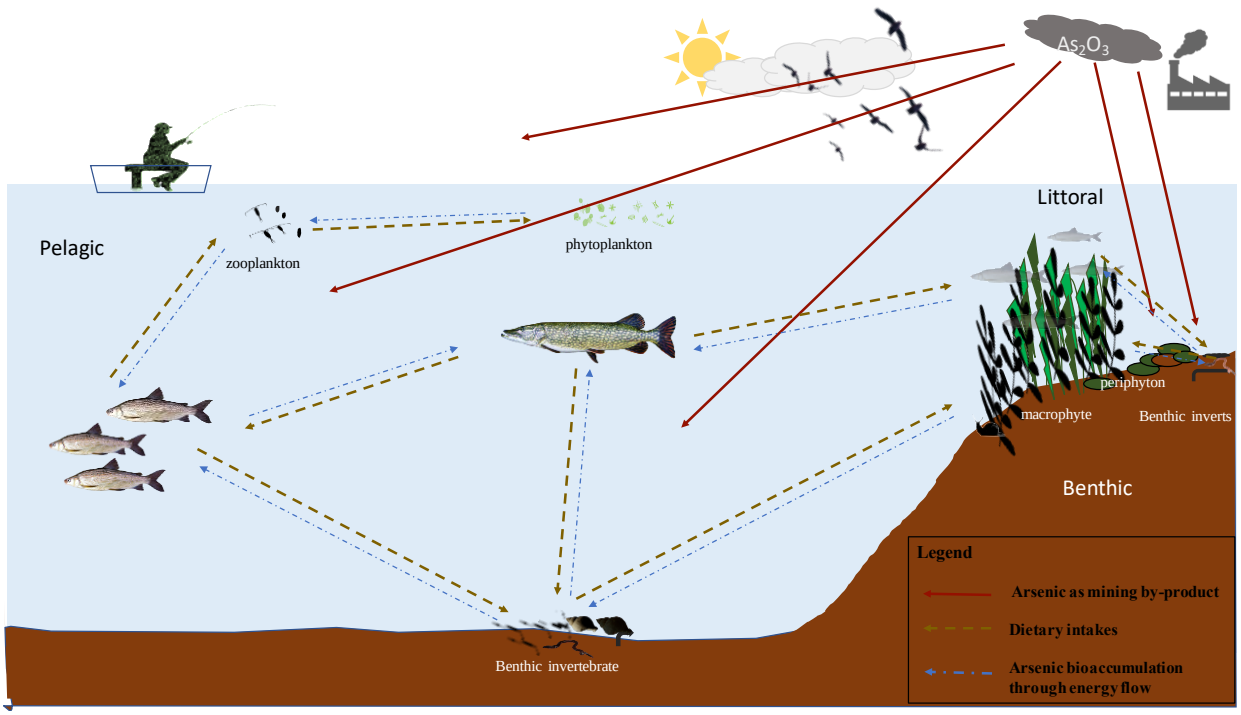

Diagram depicting the fate of arsenic trioxide from mining roasters to freshwater food webs. Red arrows indicate the deposition of arsenic trioxide from mine to freshwater. Brown arrows indicate the exposure of inorganic arsenic in biota through dietary intake. Blue arrows indicate the flow of inorganic arsenic in freshwater food webs.

Abstract

Despite the scale and magnitude of arsenic poisoning around the world, arsenic speciation and transfers in aquatic food webs are not well understood. Here we investigated arsenic species bioaccumulation in freshwater food webs near large historical mining operations in the Northwest Territories, Canada. In 2017-2018, we collected water, sediment, and the following food web biotic samples: macrophytes, periphyton, phytoplankton, zooplankton, benthic invertebrates, and small- and large-bodied fish from three lakes. Two of the lakes were within a 5-km radius of mine roasters (Long Lake and Lower Martin Lake), and a third lake (Small Lake) was situated 27 km away from the mine roaster. We characterized the food webs by feeding zones (benthic or pelagic) using $\delta^{13}C$ and trophic position based on $\delta^{15}N$, and measured the concentrations of arsenic species (As(III), As(V), MMA, DMA and organic arsenobetaine) to better understand arsenic cycling in these food webs. The total arsenic and inorganic arsenic species (As(III) and As(V)) biominified in these three food webs, meaning top carnivores had much lower concentrations of these species than their prey items. Our data suggest that higher-trophic level organisms have low inorganic arsenic concentrations in tissue due to biotransformation of inorganic arsenic to organic arsenobetaine in their tissue, and efficient elimination of arsenic. Our findings indicate that an organism's trophic position alone determines arsenic species bioaccumulation in these food webs. Benthic and pelagic connectivity does not appear to influence bioaccumulation of arsenic species in these freshwater biotas.

Keywords: Inorganic arsenic; arsenobetaine; arsenic cycle; freshwater foodwebs; mining

2.1. Introduction

Arsenic is a global contaminant affecting hundreds of millions of people worldwide[3,4]. It is a metalloid naturally released to the environment through bedrock weathering. One of the most significant anthropogenic sources of arsenic is the smelting of ores in mining operations[3]. Chronic exposure to inorganic arsenic compounds (As(III) and As(V)) in humans has been linked to cancer of various organs, cardiovascular diseases, and diabetes[4,5]. The products of inorganic arsenic biotransformation in organisms have been found to be less toxic to humans, and include methylated arsenic species (monomethylated arsenous acid (MMA) and dimethylated arsinic acid (DMA)), organic arsenobetaine (AsB) and other organoarsenicals[6–8]. However, the trivalent forms of MMA and DMA have been recently found to interfere with protein and cellular functions *in-vitro*[9–11]. Organic AsB is non-toxic to humans, non-biotransformable, and is rapidly eliminated from the body within days of ingestion[6,12,13].

Here we investigated the impact of the legacy mining operations on inorganic arsenic bioaccumulation in freshwater food webs in Yellowknife, Northwest Territories, Canada, particularly how arsenic species are transferred in food webs and the factors related to arsenic species bioaccumulation in freshwater organisms. The two major gold mines in Yellowknife were Giant Mine (62°29′59″N, 114°21′31″W) and Con Mine (62°26′20″N, 114°22′18″W), along with dozens of small-scale gold mines within and near the City that were eventually shut down[14]. Giant Mine (1948-2004) was the largest and most productive gold mine in the Northwest Territories, which produced over 220,000 kg of gold during its lifetime, and in the process released thousands of tonnes of toxic arsenic trioxide dust to the surrounding area[14–16]. Con Mine (1938-2003) was a neighboring mine, located 7 km south of Giant Mine, and an estimated 12 million tons of ores were milled from Con Mine during its active years[14]. Arsenic trioxide as a by-product of ore

roasting in these mines could leach to nearby lands and waters. Inorganic arsenic in aquatic organisms has become a public health concern due to the potential exposure to bioaccumulation through fish and seafood consumption and the retention of inorganic arsenic in humans.

Elevated arsenic around Giant Mine was still detected nearly two decades after the mine shut down. Arsenic concentration in lake surface waters around Giant Mine ranged from 10 to 646 μg/L within a 4-km radius of the roaster, and averaged 40 μg/L in a 30-km radius[17,18], well above the Canadian water quality guideline of 5 μg/L for protection of aquatic life[19]. Earlier studies showed that unicellular organisms contain predominantly inorganic arsenic species (38% to 98% of total arsenic) and less than 8% of total arsenic in zooplankton was organic arsenobetaine[20,21]. In fish, inorganic arsenic makes up less than 10% of total arsenic, and the majority is in the form of non-toxic organic arsenobetaine[22–24]. There is a lack of information on arsenic species transfer in aquatic food webs, especially in the cycling of arsenic species across trophic levels. Furthermore, the source of organic AsB in food webs is unclear, whether it may be attributed to trophic transfer or a product of biotransformation occurring in fish[25,26].

We used the natural isotopic ratios of carbon and nitrogen to characterize the freshwater food webs based on benthic and pelagic connections as determined by $\delta^{13}C$, and trophic positions of organisms based on $\delta^{15}N$. We examined the food webs in Long Lake and Lower Martin Lake (each within 5 km from Giant Mine), and Small Lake (located approximately 27 km east of the mine). We measured the concentrations of arsenic species (As(III), As(V), MMA, DMA and AsB) in water, sediment, and organism samples from each lake to examine the distribution of arsenic species across freshwater food webs affected by mining activities.

The soluble arsenic species found in water and sediment porewater from lakes are primarily in inorganic arsenic forms: As(III) and As(V)[27–29], and these are sources of inorganic arsenic to

freshwater organisms. An earlier study also indicated that sediments are the major sink and source of arsenic in aquatic environments[29]. Furthermore, arsenic concentration has been observed to be higher in tissues of sediment-burrowing fish species than other fish species from the same lake[30,31], suggesting sediment to be a major source of arsenic to aquatic biota. We hypothesized that (1) the transfers of inorganic arsenic from preys to consumers in littoral (near-shore) food webs are higher compared to those in pelagic (off-shore, open water) food webs because the littoral is more connected to inorganic arsenic species in lake sediment and consists of more benthic organisms that bioaccumulate inorganic arsenic species to higher concentrations than pelagic species; (2) higher-trophic organisms have lower inorganic arsenic concentrations in their tissue because they eliminate and biotransform inorganic arsenic species to less toxic organic species than their dietary sources[25,32,33]; and (3) aquatic biota from lakes closer to the mining area are more affected by arsenic trioxide than those from lakes further away, and therefore accumulate higher total arsenic and inorganic arsenic in their tissues.

2.2. Results

Arsenic concentrations were elevated in water and sediment samples from lakes close to the mining area. Stark contrast in total arsenic concentrations in both sediment and water samples were observed between the lakes in proximity to the mine roasters (Long Lake and Lower Martin Lake) and the reference (Small Lake) (Figure 2.1), with up to 165-fold higher total arsenic concentration in sediment and around 10-fold higher total arsenic concentration in water samples in Long Lake (water: 51 µg/L, sediment: 1421 µg/g) and Lower Martin Lake (water: 51 µg/L, sediment: 1021 µg/g), compared to Small Lake (water: 5.9 µg/L, sediment: 8.6 µg/g) (Supplementary Fig. 2.1).

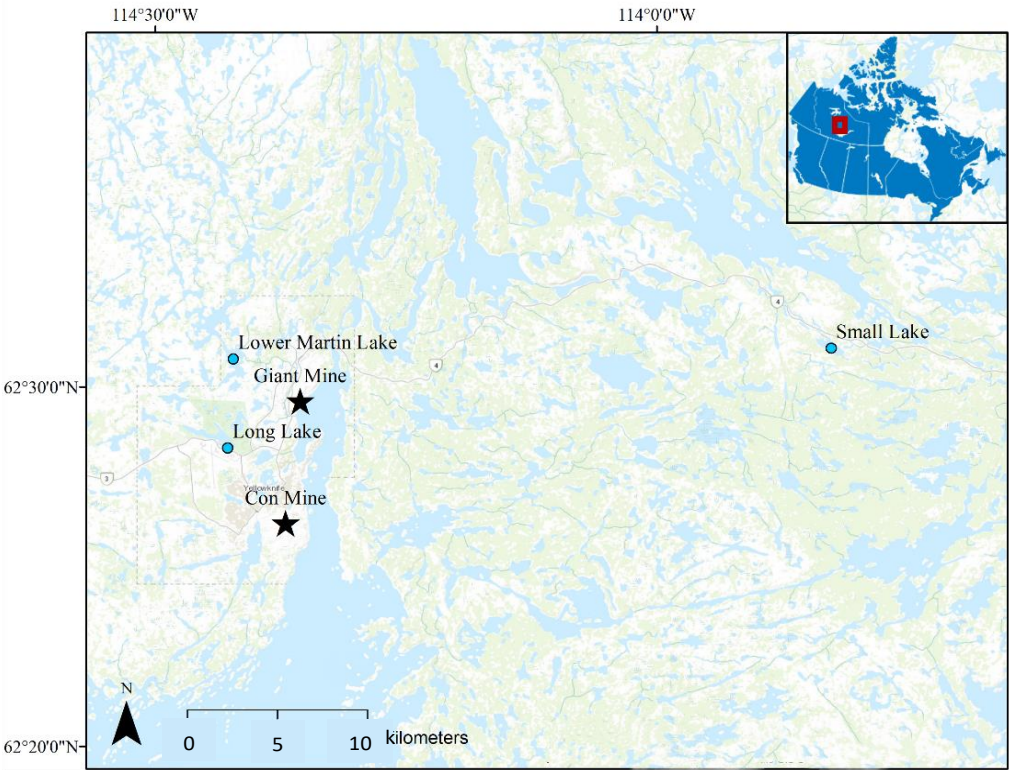

Figure 2.1. Map showing the location of the three studied lakes: Long Lake (62°28'41.30"N, 114°26'3.91"W), Lower Martin Lake (62°30'47.21"N, 114°25'16.04"W), and Small Lake (62°31'3.96"N, 113°49'35.36"W), and the neighboring mines: Giant Mine and Con Mine in Yellowknife, Northwest Territories, Canada.

The majority (58% to 85%) of the soluble arsenic species in sediment was in the form of inorganic arsenic (As(III) and As(V)) (Supplementary Fig. 2.1). Lower Martin Lake had the highest proportion of As(III) detected in sediment with 34%, while other lakes had none. The dissolved arsenic concentrations in water samples between Lower Martin Lake and Long Lake were similar on average (at 51 µg/L) even though the sediment-arsenic concentration in Long Lake was higher (at 1421 µg/g) than Lower Martin Lake (at 1021 µg/g), indicating higher mobility of arsenic from sediment to overlying water in Lower Martin Lake. Nearly all of the dissolved arsenic

species in the water column are inorganic arsenic with 89% to 98% of it being As(V) species (Supplementary Fig. 2.1). This finding is consistent with a recent survey of lake waters from 17 lakes near Yellowknife where ~99% of arsenic species in lake waters was As(V)[28].

Arsenic concentration and speciation in food webs. Total arsenic concentrations in freshwater biota ranged from 734 µg/g to 2.65 µg/g in Long Lake, 973 µg/g to 2.80 µg/g in Lower Martin Lake, and 9.90 µg/g to 0.32 µg/g in Small Lake (Supplementary Table 2.3). The highest total arsenic concentration was at the bottom of the littoral food webs: periphyton, in all lakes (Long Lake: 734 µg/g; Lower Martin Lake: 973 µg/g; and Small Lake: 9.90 µg/g), and the dominant arsenic species present was inorganic As(V) (30-88%). Nevertheless, the dietary assimilation of arsenic from periphyton in invertebrates was low, with only 7.69 µg/g of total arsenic (0.71 µg/g of inorganic arsenic) bioaccumulated in benthic invertebrates from Lower Martin Lake, and 1.87 µg/g total arsenic (0.78 µg/g of inorganic arsenic) in invertebrates from Small Lake. Biotransformation of inorganic arsenic to AsB was observed in benthic invertebrates (Long Lake: 0.19 µg/g AsB; Lower Martin Lake: 2.22 µg/g AsB; Small Lake: 0.56 µg/g AsB). The large-bodied fish species: lake whitefish and northern pike, had the lowest average total arsenic concentrations in the food webs of the three lakes, ranging from 2.65 µg/g to 3.97 µg/g in Long Lake, 3.67 µg/g to 5.97 µg/g in Lower Martin Lake, and 0.32 µg/g to 0.43 µg/g in Small Lake. We found no significant difference in the sum of inorganic arsenic species in these fish between the mining-impacted lakes and the reference lake (Kruskal-Wallis test, ANOVA; $p>0.05$). These results indicated that although high arsenic concentration accumulated at the base of food webs, very little inorganic arsenic reached higher trophic levels.

A prominent transition in the proportions of detected arsenic speciation was seen across trophic levels, from ~100% inorganic arsenic in primary producers in the food webs to a majority of AsB (up to 90%) in large-bodied fish species, especially in lake whitefish (Supplementary Fig. 2.2). Arsenic speciation in the autotrophs of both the littoral and pelagic food chains (macrophytes, periphyton, phytoplankton) consisted almost entirely of inorganic arsenic species (As(III) and As(V)). Small-bodied fish species, such as spottail shiner, ninespine stickleback and juvenile pike, had a mix of arsenic metabolites: 11%-65% inorganic and 3%-35% organic AsB species in whole-body tissues. Large-bodied fish species, such as lake whitefish had AsB (56% to 90%) predominantly in muscle tissue, while northern pike had mostly AsB (25% to 53%) and DMA (21% to 47%) in muscle tissue. We also found significantly higher inorganic arsenic in fish liver tissue, compared to muscle tissue (Paired-sample t-test; $p \leq 0.05$) in both lake whitefish (muscle As: 0.04 ± 0.02 µg/g, liver As: 0.10 ± 0.03 µg/g) and northern pike (muscle As: 0.04 ± 0.02 µg/g, liver As: 0.08 ± 0.03 µg/g) from Small Lake. Muscle tissue contained higher AsB concentrations than liver tissue (Paired-sample t-test; $p \leq 0.05$) in lake whitefish (muscle AsB: 0.29 ± 0.20 µg/g, liver AsB: 0.04 ± 0.03 µg/g) and northern pike (muscle AsB: 0.18 ± 0.06 µg/g, liver AsB: 0.04 ± 0.02 µg/g).

The total arsenic concentrations in biotas from Long Lake and Lower Martin Lake were significantly elevated compared to the concentrations in the reference Small Lake (Kruskal-Wallis test, *post-hoc* Nemenyi test; $p \leq 0.05$). Nonetheless, the concentrations of inorganic arsenic in organisms across lakes did not differ significantly between the mining-impacted lakes and the reference lake (Kruskal-Wallis test, ANOVA; $p > 0.05$).

Trophic position as a significant factor defining the bioaccumulation of inorganic arsenic and total arsenic in freshwater biota. Regression models showed that inorganic arsenic concentrations in freshwater organisms were inversely correlated to trophic position: Long Lake

(R^2= 0.89, p<0.001), Lower Martin Lake (R^2= 0.55, p<0.05), and Small Lake (R^2= 0.74, p<0.005) (Figure 2.2 A-C). Our analyses revealed that 55% to 89% of the variances observed in inorganic arsenic concentrations in freshwater biota could be predicted using the organism's trophic position alone. On average, only 20% to 39% of inorganic arsenic concentration accumulated in organisms of the next trophic level. Our data showed no correlations between arsenobetaine concentrations in organisms and trophic position (p>0.05) (Figure 2.2 D-F). The highest arsenobetaine concentrations were found in phytoplankton (3.69 µg/g dw) in Long Lake, lake whitefish (5.40 ± 1.32 µg/g dw) in Lower Martin Lake, and zooplankton (2.31 µg/g dw) in Small Lake (Supplementary Table 2.3).

Log-transformed total arsenic concentrations in freshwater biota were inversely related to trophic position in Long Lake (R^2= 0.55, p<0.05) and Small Lake (R^2= 0.74, p<0.005), though not significantly in Lower Martin Lake (R^2= 0.23, p =0.13) (Figure 2G-I). Regression model analyses showed that 23-74% of the variance in the total arsenic concentrations in aquatic biota could be explained by trophic position. The total arsenic concentration in biota was biominified in these food webs by a factor of 0.43-0.56 per trophic position. Although the regression model for Lower Martin Lake did not show significance, the diminution of total arsenic concentration across trophic positions was apparent. The variance of the data in this model might have been affected by the

broad range of total arsenic concentrations in the primary producers of Lower Martin Lake.

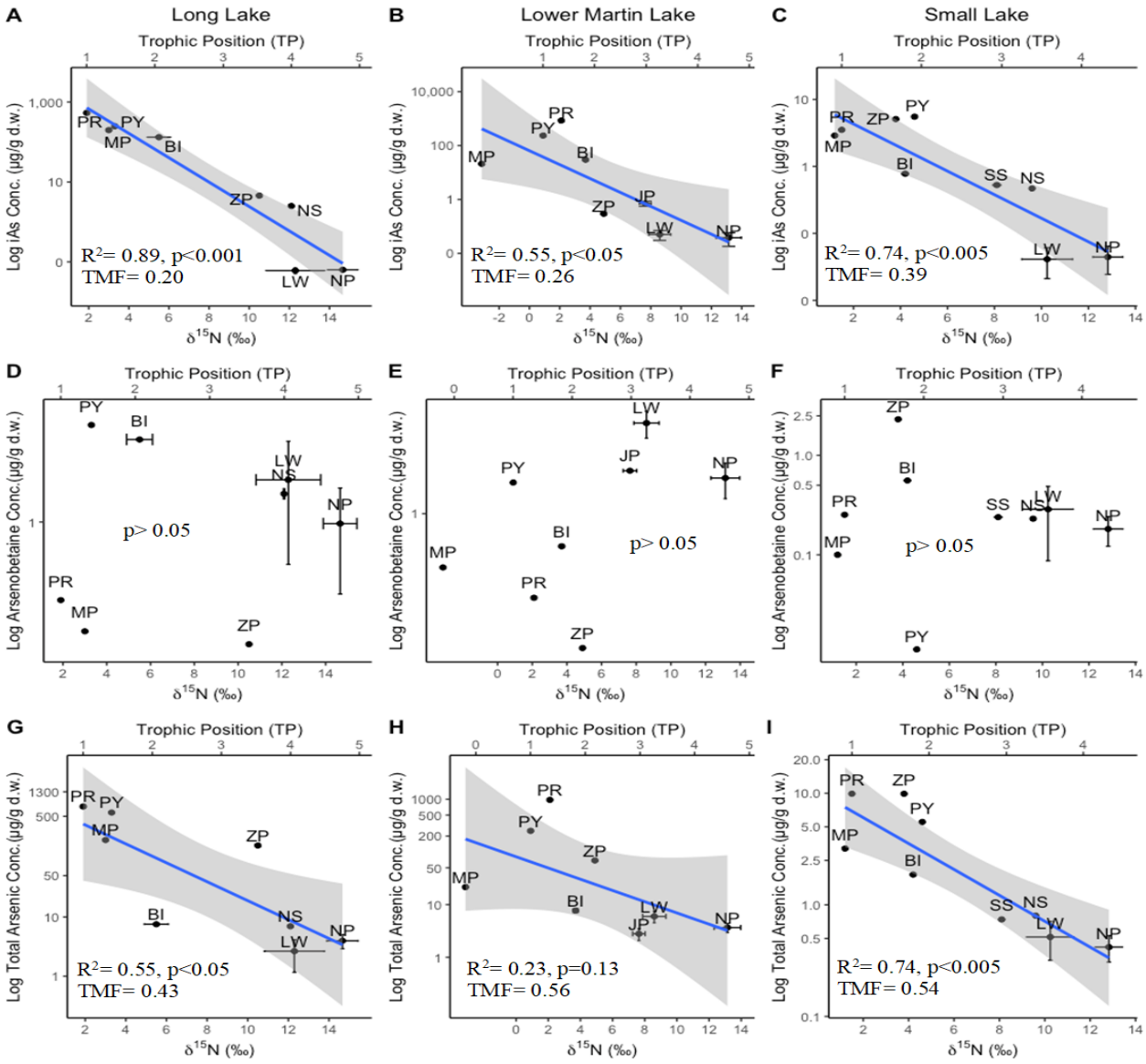

Figure 2.2. Inorganic arsenic (top panels), arsenobetaine (middle panels), and total arsenic (bottom panels) concentrations across trophic positions (TP) of aquatic biota in (A, D, G) Long Lake, (B, E, H) Lower Martin Lake and (C, F, I) Small Lake, presented as linear regression models. Trophic Magnification Factor (TMF) < 1.0 indicates trophic diminution of chemical. Data are presented as means ± SD. PR: periphyton, PY: phytoplankton, MP: macrophyte, ZP: zooplankton, BI: benthic invertebrates, SS: spottail shiners, NS: ninespine stickleback, JP: juvenile northern pike, LW: lake whitefish, NP: northern pike.

Trophic positions characterize the distribution of arsenic species in freshwater biota. The proportions of inorganic arsenic to total arsenic in freshwater biota were inversely correlated to $\delta^{15}N$ indicative of trophic position (biominification) as follows: Lower Martin Lake: $R^2=0.74$, $p<0.01$; Long Lake: $R^2=0.56$, $p<0.05$; Small Lake: $R^2=0.18$, $p>0.05$. Conversely, the proportions of organic AsB to total arsenic were positively correlated with trophic position as measured by $\delta^{15}N$ (Lower Martin Lake: $R^2=0.45$, $p<0.05$; Long Lake: $R^2=0.35$, $p=0.07$; Small Lake: $R^2=0.65$, $p<0.01$) (Figure 2.3). The inverse relationships of inorganic arsenic proportions and trophic positions are especially prominent in food webs of lakes with high arsenic content (Long Lake and Lower Martin Lake), explaining 56-74% of variances in the inorganic arsenic proportions observed in freshwater biota in the two lakes. The decrease in inorganic arsenic proportion in the tissues of aquatic organisms was accompanied by the increase in the proportion of non-toxic organic AsB in the food webs.

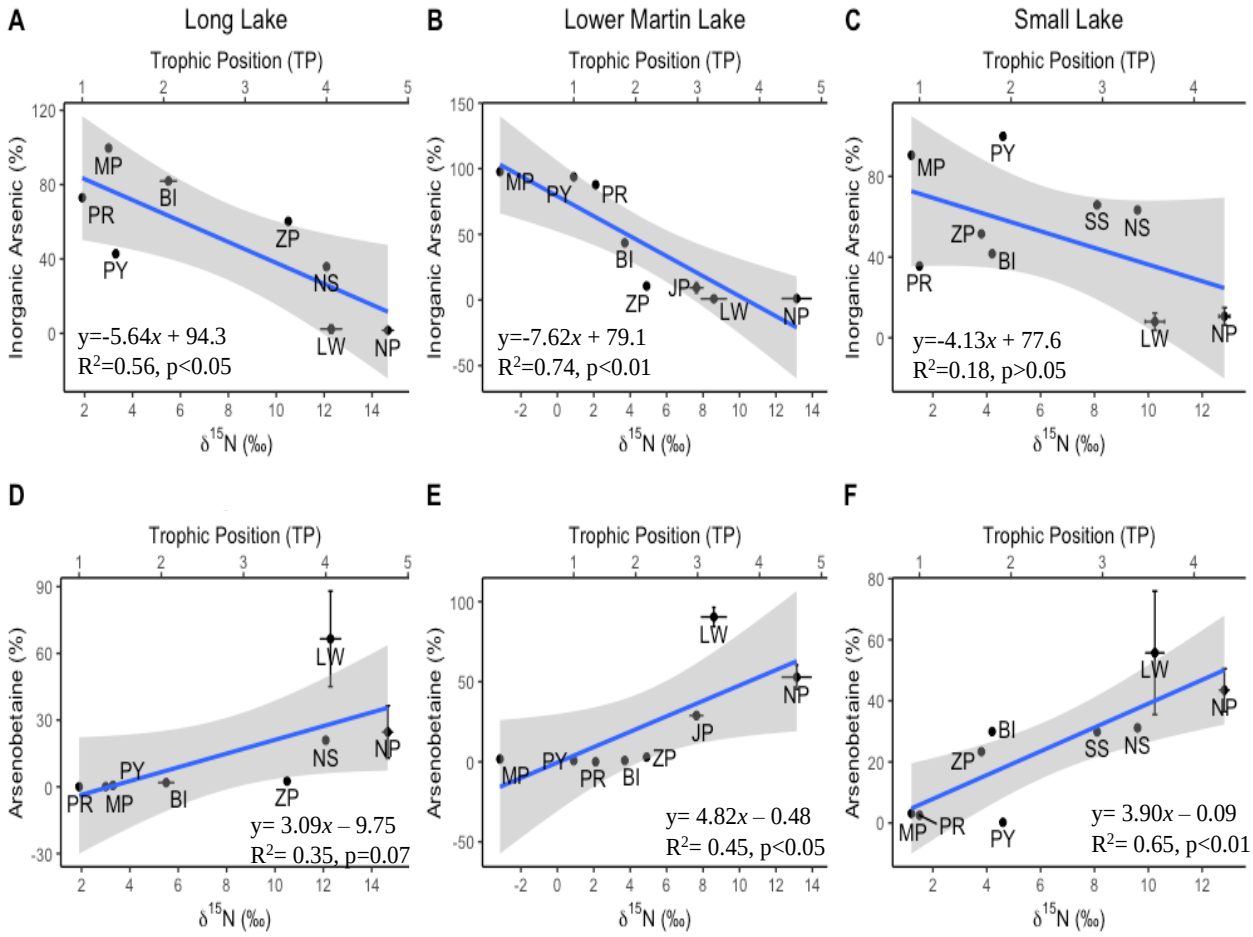

Figure 2.3. Proportions of inorganic arsenic (top panels) and arsenobetaine (bottom panels) across trophic positions (TP) as determined by $\delta^{15}N$ in aquatic biota from the (A, D) Long Lake, (B, E) Lower Martin Lake and (C, F) Small Lake, presented as linear regression models. Data are presented as means ± SD. PR: periphyton, PY: phytoplankton, MP: macrophyte, ZP: zooplankton, BI: benthic invertebrates, SS: spottail shiners, NS: ninespine stickleback, JP: juvenile northern pike, LW: lake whitefish, NP: northern pike.

There were significantly higher proportions of AsB in muscle tissue of lake whitefish of a lower trophic position than in those of northern pike in lakes with high arsenic: Long Lake and Lower Martin Lake (Two-sample T-test, $p<0.0001$), while the proportions of DMA in muscle tissues of northern pike were significantly higher than those in lake whitefish in all lakes (Two-sample T-test, $p<0.0001$). These results suggest that DMA accumulates preferably in northern pike,

while AsB is the preferred metabolite in lake whitefish. Higher accumulation of DMA than AsB in the muscle tissues of some fish species has also been reported in several papers[23,24].

Arsenic distribution in freshwater organisms is unrelated to their habitat preference.

Spearman's rank correlation analyses showed that the biomagnification factors (BMF) of the total arsenic and the inorganic arsenic in food webs were unrelated to organism feeding habitat, as indicated by $\delta^{13}C$ ($p>0.05$) (Figure 2.4). Benthic-invertebrate diet contributed to relatively small retention of inorganic arsenic to its consumers, accounting for less than 0.15 assimilation factor of inorganic arsenic to the consumers across the studied lakes. In Lower Martin Lake, the benthic-invertebrate diet in juvenile pike of the littoral food web contributed to 0.04 assimilation factor of inorganic arsenic, compared to the zooplankton-diet in lake whitefish, of the pelagic food web in the same trophic level, which accounted for 0.195 assimilation factor. Zooplankton diets contributed to nearly 5-fold of inorganic arsenic assimilation in the consumers compared to benthic invertebrate diets in Lower Martin Lake.

Our results showed that periphyton accumulates higher total and inorganic arsenic at the bottom of littoral (benthic) food webs, however, benthic diets and littoral feeding zone did not contribute to higher assimilation of total and inorganic arsenic in biota at higher trophic positions. Despite the high concentrations of total arsenic and As(V) in periphyton across lakes, low assimilation of total and inorganic arsenic from periphyton to its consumers was observed (BMF_{TP} (Total As) $=3.42 \times 10^{-5}$ to 0.549; BMF_{TP} (iAs) =0.001 to 0.152) (Figure 2.4).

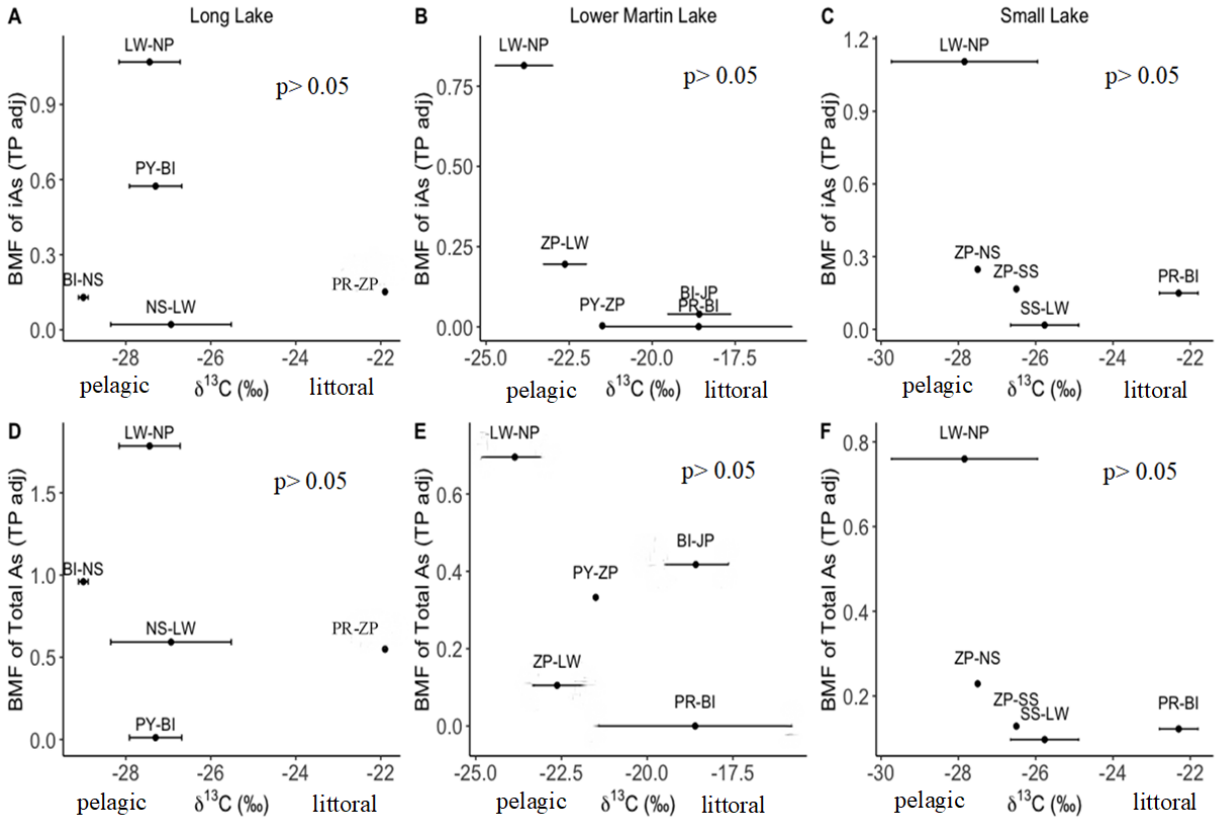

Figure 2.4. Relationships between trophic-position-adjusted Biomagnification Factor (BMF_{TP}) of inorganic arsenic (top) and total arsenic (bottom) from prey to predator, and the feeding zones ($\delta^{13}C$) of freshwater organisms from (A, D) Long Lake, (B, E) Lower Martin Lake and (C, F) Small Lake. BMF < 1.0 indicates diminution of chemical. Data are presented as means ± SD. PR: periphyton, PY: phytoplankton, ZP: zooplankton, BI: benthic invertebrates, SS: spottail shiners, NS: ninespine stickleback, JP: juvenile northern pike, LW: lake whitefish, NP: northern pike.

2.3. Discussion

Total arsenic concentrations in water, sediment and biota samples were elevated in lakes near (<5 km) the large-scale legacy mining area in Yellowknife, Northwest Territories, Canada, compared to the reference lake (Small Lake) far from the mines, supporting our hypothesis that lakes closer to the mining area were more impacted by the legacy arsenic contaminants. Supplementary to our finding, ample literature has reported evidence of higher arsenic

concentrations in lakes near Giant Mine[17,18]. The higher total arsenic concentrations in sediment samples than lake water samples also suggested that most of the arsenic released from the mine's early atmospheric deposition settled at the bottom sediment of lakes. The higher As(III) proportion in the sediment of Lower Martin Lake compared to Long Lake, and the elevated dissolved arsenic in the water samples from Lower Martin Lake was likely influenced by the higher organic matter concentration in Lower Martin Lake that enhances arsenic mobilization from the sediment to the lake water through the release of iron-oxide bound arsenic and the competition between arsenic and organic anions for binding sites[28,34,35].

We found that inorganic arsenic was biominified in the food webs in all lakes. The proportions of organic arsenobetaine to total arsenic were positively correlated to organism trophic position indicating an increased biotransformation efficiency of inorganic arsenic to organic AsB in higher-trophic organisms. However, our data showed low AsB in food webs, suggesting that AsB, as a final product of inorganic arsenic biotransformation in tissue[25], was readily eliminated by organisms. Only 20%-38% of inorganic arsenic was transferred in the organism to the next trophic level. In addition to the low dietary assimilation of inorganic arsenic in the food webs, the biotransformation of inorganic arsenic to organic AsB in organisms of higher trophic positions in the food webs contributes to the lower concentration of inorganic species retained in tissues at the top of food chains.

Total arsenic concentrations were the highest among periphyton in all lakes, with 30%-88% of the species as inorganic As(V). An earlier study has also shown that benthic periphyton bioconcentrates arsenic up to 9,700 fold greater than concentrations in water, oxidizing most of it to As(V) in a laboratory simulation[36,37]. Therefore, periphyton biofilm, a major energy source at the bottom of littoral food webs, could serve as a significant source of arsenate in the food chains

in lakes with high arsenic. Inorganic arsenic compounds, As(III) and As(V), in lake sediment and water are the primary forms of arsenic exposure in abiotic environments in freshwater ecosystems. We found the majority of dissolved arsenic species present in lake water (89%-98%) and in lake sediment (58%-85%) to be inorganic arsenic compounds, suggesting that the source of organic arsenic species in freshwater organisms is likely the product of biotransformation. However, the concentrations of inorganic arsenic in freshwater organisms did not differ significantly between impacted and non-impacted lakes, indicating that inorganic arsenic is effectively biotransformed and eliminated from tissues of higher trophic level organisms. In the food webs of all lakes, arsenic speciation in autotrophs at the base of food webs was dominated by inorganic arsenic forms, while organic AsB was predominant in high-trophic organisms. These results corroborated our hypothesis that inorganic arsenic is more effectively metabolized into organic AsB in more complex, higher-trophic level organisms. However, the retention of AsB in tissues through dietary intake could also contribute to the high proportion of AsB found in fish tissues. A laboratory experiment by Amlund et al. (2006) has shown some retention of arsenobetaine in fish dosed with [^{14}C]-AsB: 2-38% of the administered dose was retained in the tissues after 14 days. Although the biotransformation of inorganic arsenic to organic AsB is still unclear, the metabolism of arsenic in fish could be caused by species-related physiology. Here, we observed more retention of AsB in the muscle tissue of lake whitefish and more DMA in the muscle tissue of northern pike. The assimilation efficiency of dietary AsB among fish species could also differ significantly, as shown by a previous study where Atlantic Cod (absorption efficiency= 15±1%, $k_{elimination}$= 0.009 ± 0.002 day^{-1}) exhibited twice the assimilation efficiency of dietary AsB than that of Atlantic Salmon (absorption efficiency= 8±1%, $k_{elimination}$= 0.019 ± 0.003 day^{-1}), following the identical dosing of 25 µg/g of AsB in feed for three months, followed by a three-month depuration period[39]. These

observations suggest that species differences could determine both the biotransformation of arsenic in fish and dietary assimilation of AsB. Nevertheless, further studies are needed to elucidate biochemical pathways of inorganic arsenic biotransformation in high-trophic fish species. Previous toxicokinetics studies of arsenic have reported the following ranges of uptake and elimination constants of total arsenic in multiple tissues of various fish species: k_{uptake} (0.06-1.53 mL/g.day) and $k_{elimination}$ (0.06-1.15 day^{-1}), representing large ranges in uptake and depuration kinetics among species. An earlier study on arsenic species distribution in an open seagrass ecosystem concluded that a variety of factors, including exposure, diet and physiology, could affect arsenic speciation in freshwater organisms[40]. Here we showed that trophic position is inversely related to total and inorganic arsenic in food webs, explaining up to 89% of the variance within the foodweb. Higher-trophic organisms have lower total arsenic and inorganic arsenic concentrations because they eliminate and biotransform inorganic arsenic into organic AsB.

Here we tested whether littoral food webs accumulate higher total and inorganic arsenic than pelagic food webs by surmising that a preference for a benthic diet and littoral feeding contribute to higher total and inorganic arsenic assimilation in food webs. However, our results proved otherwise. Despite living in sediment, benthic invertebrates transfer little inorganic arsenic to their consumers. Habitat preference was unrelated to the concentrations of total arsenic and inorganic arsenic in freshwater biota. We concluded that benthic periphyton serves as a sink of inorganic arsenic, particularly arsenate, but not a significant source to the food webs because the bound arsenate may be unavailable to consumers. Lopez et al. (2016) suggested that iron oxides produced by periphyton biofilms tended to attract As(V) from the surrounding environment and bioconcentrated As(V) in the biofilms[37]. However, the bioavailability of As(V) to consumers

tended to be low due to the strong bonds of As(V) to iron oxides[41]. Due to these characteristics, periphyton biofilms have been utilized in arsenic wastewater removal technology[41].

Our findings provide a characterization of arsenic species cycling in aquatic food webs in subarctic lakes near large gold mining operations, particularly how arsenic speciation changes from the abiotic environment to the biota spanning a range of trophic positions. Further studies are needed to elucidate the metabolic mechanisms of inorganic arsenic biotransformation in aquatic organisms and whether the pathways are specific to fish species. We showed that high-trophic predatory fish species are able to reduce toxic inorganic arsenic from their tissues through foodweb biominification.

Methods

Study sites

We collected complete food web samples from three lakes in August-September 2017: periphyton, phytoplankton, zooplankton, benthic invertebrates and small-bodied fish. Lakes consisted of two medium-sized lakes (~100 ha) located within 5 km of Giant Mine: Long Lake and Lower Martin Lake, and a reference lake: Small Lake (~100 ha) situated outside the mining influence (Figure 2.1). Additional biotic samples, such as submerged macrophytes, zooplankton and large-bodied fish from Small Lake, were collected throughout 2018 with the help of Dr. Pete A. Cott and Michael Palmer. Long Lake is situated beside the Yellowknife airport, 5 km southwest from Giant Mine. Its beach shoreline is used by residents for recreational purposes. Lower Martin Lake is situated 3.5 km northwest and downwind of Giant Mine's roaster stack. It is connected on the upper side to Martin Lake, where fish migrate during the winter season when Lower Martin

Lake freezes to the bottom. Both Long Lake and Lower Martin Lake have granitoid bedrock geology[18], which has previously been associated with low natural arsenic mobilization in water due to its low carbonate mineral content[42,43]. The reference lake in this study, Small Lake, is located alongside a quiet cabin area, 27 km east of the roaster. Small Lake has metasedimentary bedrock[18], which tends to have higher natural arsenic content in the bedrock and favours arsenic release to water due to the higher pH of the water and the reducing environment[43]. Since the reference lake is situated outside mining areas, the main source of arsenic in this lake is bedrock arsenic weathering. The pH of water from all lakes was weakly alkaline (7.9-8.2) and was similar among all lakes. Long Lake and Small Lake are classified as oligotrophic lakes, with low dissolved organic concentration (DOC), total nitrogen, and total phosphorus concentrations indicating low productivity in these lakes, while Lower Martin Lake is a meso-eutrophic lake with intermediate autochthonous production, higher DOC, total nitrogen and phosphorus concentrations in the water, but has high oxygen at the bottom water (Supplementary Table 2.1 & 2.2).

Lake and food-web sampling

In-Situ Water Measurements: Vertical profiles of water every 0.5-m or 1-m interval for dissolved oxygen (DO) in % and mg/L, temperature in °C, conductivity and specific conductivity in µS/cm, and salinity in ppt were measured from lake centers using a YSI Multiprobe meter Handheld device on a Zodiac boat (Supplementary Table 2.2).

Water: We collected surface water samples from the center of each lake for physicochemical analysis: physics (alkalinity, color, solids, pH), inorganic nutrients (nitrogen, dissolved organic carbon and phosphorous), organic nutrient (chlorophyll a), major ions (calcium, chloride, fluoride, nitrate, nitrite, magnesium, potassium, sodium, sulphate) and trace metals, at

the Taiga Laboratory in Yellowknife, as well as 15-mL mid-depth water samples at the same locations using a 2-L Van Dorn Sampler on a Zodiac boat for arsenic speciation analysis. All tubes and bottles were rinsed with lake water at least three times before sample collection. Water was overfilled into the sample containers to avoid any air residue.

Surface Sediment: Sediment cores were collected using a UWITEC Gravity corer in the lake center from the side of an inflatable Zodiac. The top 5-cm sediment samples were sliced from the cores under ultra-high purity (UHP) nitrogen in a polyethylene glove chamber (Cole Parmer, USA) and then transferred to double-bagged WhirlPaks.

Macrophytes: Submerged macrophyte samples were collected close to the shore of each lake using an inflatable Zodiac. Whole samples, including the leaves and stems, were stored frozen in Ziploc bags. Samples were rinsed using deionized water before freeze-drying.

Periphyton: 8 palm-sized rocks were collected in random along the shore of each lake by wading using a chest wader and bagged into WhirlPak bags. Rocks were scrubbed by hand and rinsed using lake water. Scrubbed residues were transferred into 1-L Nalgene bottles for filtration using 0.7-μm Whatman GF/F filters (GE Life Sciences, USA).

Phytoplankton: 6 L of surface water were collected in the center of each lake on an inflatable Zodiac by surface water grab into six 1-L Nalgene bottles. All bottles were rinsed at least three times using lake water before water collection. A series of filtrations were conducted to retain phytoplankton from the collected water, following Caumette et al. (2011)'s protocol[20]. Water was first filtered through a 63-μm mesh to remove large particles and zooplankton, followed by a second filtration with 0.45-μm Whatman GF/C mesh filter (GE Life Sciences, USA) to retain phytoplankton. Filters containing phytoplankton samples were then air-dried for 72 hours and stored inside small Petri dishes covered with aluminum foil.

Zooplankton: Samples were collected in the center of each lake on an inflatable Zodiac using a 63-µm plankton net and transferred into sterile 100-mL urine cup.

Benthic Invertebrates: Invertebrates from surface sediment were obtained by kicking the top sediment of lake littoral zone into a kick net. Collected materials were sifted, and invertebrates were picked and sorted into small WhirlPak bags containing lake water. Samples were then moved into a 16-oz Ziploc plastic container and refrigerated for 24 to 48 hours to allow invertebrates to empty stomach contents. Invertebrates were finally transferred into 50-mL polypropylene tubes (Corning, USA) and refrigerated at 4ºC until analyzed.

Fish Species: small-bodied fish were caught using a small seine net near the shore of each lake and euthanized in the field by pithing the head using a jackknife. Samples were transferred into WhirlPak bags and kept frozen to be analyzed whole. Large-bodied fish species consisted primarily of northern pike (*Esox lucius*) and lake whitefish (*Coregonus clupeaformis*). Muscle and liver tissue samples of the two species from Long Lake (n= 10 per species / lake) and Lower Martin Lake (n= 10 per species / lake) were provided from the archive samples collected during the 2016 spring season by the Government of Northwest Territories, Environmental and Natural Resources. Fish from Small Lake (n=8 northern pike and n=8 lake whitefish) were collected by Dr. Pete A. Cott and Michael Palmer using a seine net in 2018. Both muscle and liver tissues were harvested in the field and sent in a cooler to the Laboratory for the Analysis of Natural and Synthetic Environmental Toxicants (LANSET) at the University of Ottawa.

Laboratory analysis

Abiotic Sample Preparation: water samples for arsenic analysis were preserved by adding 0.28 M HNO_3 and stored in a refrigerator at 4ºC. The collected sediment samples were freeze-dried using commercial SuperModulyo lyophilizer (Thermo Scientific, USA) for 36 hours and then ground using mortar and pestle into powder form.

Biotic Sample Preparation: All biotic samples were freeze-dried using commercial SuperModulyo lyophilizer (Thermo Scientific, USA) for 48 hours and homogenized using a Magic Bullet or mortar and pestle. Small-bodied fish and invertebrate samples were processed as whole-body samples, while large-bodied fish were separated into muscle and liver sub-samples. The freeze-drying method was chosen as the most appropriate and effective storing method to preserve arsenic species in biotic samples based on previous studies[44,45].

Stable isotope analysis: The primary carbon energy source and trophic position in the food webs are determined based on the natural stable isotopic ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$, respectively. Stable isotopes $\delta^{13}C$ and $\delta^{15}N$ were measured in all biotic samples collected from the three lakes (Long Lake, Lower Martin Lake and Small Lake) to separate littoral and pelagic end members, as well as to discriminate each level of the trophic position in aquatic organisms from each lake.

Dried biotic samples (2 to 10 mg weighed in tin capsules) were submitted to Ján Veizer Stable Isotope Laboratory (previously GG Hatch Laboratory) for stable isotopes $\delta^{13}C$ and $\delta^{15}N$ analyses. Samples were first loaded to a Vario El Cube elemental analyzer (Elementar, Germany) to quantify their %C and %N, and then coupled to a Delta Advantage Isotopic-ratio Mass Spectrometer (Thermo, Germany) to measure the ratios of heavier to lighter isotopes (R). The ratios R is expressed in ‰ (per mil) and calculated as the following:

$\delta X(‰) = (R_{sample}/R_{standard} - 1) \times 1000$, where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$ ratio

Equation 2.1. $\delta^{13}C$ and $\delta^{15}N$ equation for stable isotope analyses.

Vienna PeeDee Belemnite (vPDB) and atmospheric nitrogen (N_2) were used as standard reference materials, which were normalized per the internal standards used. These standards included C-51 Nicotinamide, C-52 mix of ammonium sulphate and sucrose, C-54 caffeine and blind standard C-55: glutamic acid. All isotopic ratio measurements were calibrated using international standards for quality control, and duplicate samples were used. Analytical precision of isotopic measurements is around 0.2‰.

The ratios of carbon stable isotope (${}^{13}C/{}^{12}C$) are generally conserved and reflective of carbon source among littoral and pelagic endpoints[46]. Littoral communities having higher $\delta^{13}C$ values compared to pelagic organisms[47]. Trophic Position (TP) of each species determined through $\delta^{15}N$ can be calculated using the following formula:

$$TP = \lambda + \delta^{15}N_{adjusted}/\Delta_n$$

Equation 2.2. Trophic Position (TP) equation.

, where: λ = trophic position of the primary producer or 1

$$\delta^{15}N_{adjusted} = \delta^{15}N_{organism} - \delta^{15}N_{primaryproducer}$$

Δ_n = isotopic enrichment per trophic position or 3.4 ‰[48]

Trophic Magnification Factor (TMF) can be determined as a slope of $\delta^{15}N$ and arsenic concentration, following Ouédraogo et al. (2015)'s method. TMF < 1.0 indicates a biodiminution

of metalloid transfer across trophic positions[47].

Total arsenic and speciation analyses: For total arsenic analysis, 0.1-0.5 g of homogenized samples were digested with 2.5 mL of 70%v/v OmniTrace HNO_3 (EMD Millipore, USA) on an SCP Science model DigiPREP block digestion at 100°C for 180 minutes, and then 1.5 mL of 30% v/v H_2O_2 (Fisher Chemical, USA) was added to each tube on the hotplate and run for 45 minutes at 95°C. The extracts were diluted with Millipore-Q (MQ) deionized water to 10 mL and then filtered and vortexed before analysis. For arsenic speciation analysis, dried sediment (0.05 g) and food web samples (0.01-0.1 g) (with the exception of fish) were digested using 4 mL of 0.28 M HNO_3. Approximately 0.1 g of freeze-dried fish sample was extracted using 4 mL of 1:1 Methanol: MQ-water at 100°C for 180 minutes on DigiPREP block and diluted to 10 mL using MQ deionized water. Whole filters (47-mm Whatman GF/C) of phytoplankton samples were used for total arsenic and arsenic speciation analyses, and a quarter of giant filters (110-mm Whatman GF/F) of periphyton samples were used in the analyses. In both digestion methods for speciation works, extracts were centrifuged at 4000 rpm for 15 minutes to obtain the supernatants, which were syringe-filtered using 0.2 µm Whatman PVDF filter media (GE Life Sciences, USA) before analyses. All samples (water, sediment, biota) were analyzed for total arsenic using ICP-MS and arsenic species: As(III), As(V), MMA, DMA and AsB, using HPLC-ICP-MS technique in accordance to FDA standards (Elemental Analysis Manual Section 4.11). Mass balances for all reference materials and samples were verified to be within the range of 90-120% for accuracy. Method blanks, calibration blanks and standards, and various standard reference material, DORM-4 and DOLT-5 (NRC, Canada) were used for quality assurance. The concentrations of arsenic were measured in µg/g dry weight for solid samples and µg/L for water samples. The method limit of

detection was 0.002 μg/kg for AsB, 0.05 μg/kg for As(III), 0.06 μg/kg for DMA, 0.02 μg/kg for MMA, and 0.1 μg/kg for As(V).

The biomagnification factors (BMF) of total arsenic and inorganic species from the diet to the consumer in food chains within a one unit increase in trophic position were calculated using the following equation:

$$BMF_{TP} = 10^{\frac{\log(C_{consumer}/C_{diet})}{TP_{consumer}-TP_{diet}}}$$

Equation 2.3. Trophic-position-normalized Biomagnification Factor from the diet to the consumer (BMF_{TP}) equation.

, where: $C_{consumer}$ = concentration of chemical in the consumer

C_{diet} = concentration of chemical in the diet

$TP_{consumer}$ = trophic position of the consumer in the food web

TP_{diet} = trophic position of the diet in the food web

Statistical analysis

All figures and tables are presented in means ± standard deviation. All plots and statistical computations were made using R open-source software version 3.5.2 for Mac OS X. We used non-parametric Kruskal-Wallis rank sum test (One-way ANOVA) and *post-hoc* Tukey and Kramer (Nemenyi) test to compare the carbon source ($\delta^{13}C$), trophic positions ($\delta^{15}N$), total arsenic and inorganic arsenic (As(III) and As(V)) values in organisms across lakes. Two-sample t-tests were used to compare arsenic species concentrations and proportions in fish samples. Shapiro-Wilk normality test and F variance test were used to check for assumptions prior to T-tests. The relationships between arsenic species concentration and their proportions and trophic positions presented using $\delta^{15}N$, were measured as linear regression models. The data from each variable

were first tested using the Shapiro-Wilk normality test before plotting. Variables that failed the normality test were log-transformed to gain normality. Afterward, the linear models were examined with the studentized Breusch-Pagan test for heteroscedasticity, the Ramsey's RESET test for correctness of functional form, and Shapiro-Wilk test for normality in the residual values. The relationships between BMF and $\delta^{13}C$ were measured using non-parametric Spearman's correlation tests. Statistical significance was set at 95% confidence in all analyses.

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Chapter 3: Health Risk Assessment of Inorganic Arsenic Exposure Through Fish Consumption in Yellowknife, Northwest Territories, Canada

Claudia Tanamal, Jules M. Blais, Emmanuel Yumvihoze, Laurie H.M. Chan
Biology Department, University of Ottawa, Ottawa, ON K1N 6N6, Canada

Abstract

Yellowknife served as a major hub for gold mining industries in the Northwest Territories, Canada during the 1900s. The Giant Mine was the largest gold mine in Yellowknife, operating adjacent to the Con Mine, a smaller scale gold mine, until the early 2000s. An estimated 10,000 kg arsenic trioxide dust were produced every day as by-products from the smelting operations. Elevated arsenic concentrations reported in fish caught near the mines are a public health concern. We collected 180 samples of three species of commonly consumed fish; lake whitefish (*Coregonus clupeaformis*), northern pike (*Esox lucius*), and burbot (*Lota lota*) from nine lakes around Yellowknife in 2013-2018. Arsenic species analyzed include As(III), As(V), Monomethylarsonate (MMA), Dimethylarsinic acid (DMA) and arsenobetaine. Concentrations were measured using High-Performance Liquid Chromatography and Inductively Coupled Plasma-Mass Spectrometry. The average concentration of total arsenic in fish muscle tissues was 2.30 ± 1.72 µg/g dry weight and 3.16 ± 2.49 µg/g dry weight in burbot liver tissues. Most of the arsenic species found in fish muscle were non-toxic arsenobetaine (mean= 58.6 ± 34.5%), while in the fish liver tissues, species were predominantly DMA (mean = 76.6 ± 21.6%). Inorganic arsenic species (As(III) and As(V)) accounted for less than 20% of the arsenic detected in fish. Data on the consumption of locally-caught fish were collected from 1,611 residents in Yellowknife during 2017-2018 from 1,417 general residents of Yellowknife (1,150 adults and 267 children) and 194 members from the

Yellowknives Dene First Nation (123 adults and 71 children). The mean consumption rates of the three fish species of interest were higher among the Yellowknives Dene First Nation at 19±29 g/day (N= 120 consumers) in adults and 7±11 g/day (N=68 consumers) in children, compared to the general residents at 9±15 g/day (N= 1,055 consumers) in adults and 5±8 g/day (N= 246 consumers) in children. We evaluated the long-term non-carcinogenic and cancer health risks from inorganic arsenic exposure (Class 1 chemical) through local fish consumption using Monte-Carlo simulations. Our results indicated that there were no significant non-cancer health risks in all groups, but elevated lifetime cancer risks among adults of the two population groups at the 95th percentile. However, the lifetime cancer risks of inorganic arsenic exposure in Yellowknife were lower to those of the general population in Canada.

Keywords: arsenic exposure, fish, inorganic arsenic, mining, probabilistic risk assessment

Key Findings

- Fish from some lakes geographically closer to the mining area had a significantly higher total arsenic concentration in tissues compared to the regional reference lake away from the mines.
- The important factors determining inorganic arsenic concentrations in fish are the location and fish species.
- Arsenic compounds found in fish muscle tissue were predominantly organic arsenobetaine, and DMA in burbot liver tissue.
- The Yellowknives Dene First Nation had higher fish consumption rates than the general residents in 2016-2018.

- Probabilistic long-term cancer risks associated with fish consumption were found to be elevated among the adults, heavy consumers in Yellowknife.

3.1. Introduction

Arsenic is a ubiquitous trace element that is naturally present in the earth's crust, mainly in the form of arsenopyrite (Mandal & Suzuki, 2002). Exposure to elevated levels of arsenic has been reported in different parts of the world: Bangladesh (Smith, Lingas, & Rahman, 2000), Taiwan (Lai et al., 1994) and South America (Biggs et al., 1997; Concha, Vogler, Lezcano, Nermell, & Vahter, 1998; Mazumder, 2007). The source of exposure is most commonly through drinking well water containing high naturally-occurring inorganic arsenic (NRC, 2001). In Canada, high arsenic in drinking water is relatively uncommon; high arsenic exposure is usually from anthropogenic sources, such as wood preservative industries and mining activities (Wang & Mulligan, 2006). The Giant Mine in Yellowknife, Northwest Territories, Canada, is one of the largest and most productive gold mines in Canadian history (Keeling & Sandlos, 2012), yielding more than 20,000 kg of gold over its lifetime. It was rigorously operating from 1948 to 1999 and on limited production from 1999 to 2004. Together with its neighbouring Con Mine (1938-2003), it released an estimated 10,000 kg of arsenic trioxide dust daily through the roasting of arsenopyrite ores to extract gold particles (Keeling & Sandlos, 2012). Currently, there are 237,000 tonnes of arsenic trioxide by-product stored in 15 underground chambers on the Giant Mine property, along with three large tailing ponds that drain into the Baker Creek and eventually to the Yellowknife Bay. Giant Mine is recognized as one of the most contaminated sites in Canada, with a projected $1 billion required for remediation costs and up to 15 years to clean up (INAC, 2018). Although both mines are no longer operational, transport of arsenic and other metals by surface runoff and

groundwater migration is still possible. The Giant Mine Remediation Project proposed to artificially freeze the underground arsenic trioxide blocks in 2015 to prevent the drainage of arsenic from underground chambers (AANDC, 2015).

Fish is a good source of protein and essential fatty acids and its consumption has been linked to reduced risk of cardiovascular diseases, myocardial infarction, inflammatory-related diseases and other health benefits (Daviglus et al., 2002). The Yellowknives Dene First Nation are the Indigenous peoples living in Yellowknife. Based on the data from the NWT Labour Force Surveys conducted in 1998, 2003, 2008, and 2013, about 40% of people residing in the Northwest Territories hunted or fished their own food resources (GNWT, 2015). The NWT Community Survey revealed that 282 households (4% households) in Yellowknife consumed 75% or more of their fish or meat in 2014 from fishing or hunting (GNWT, 2014). The Yellowknives Dene First Nation were exposed to the legacy mining contaminants because of their dependence on land and water as their main food resources (AFN, 2009). On average, 43% of Indigenous residents in the Northwest Territories hunted or fished for subsistence and recreational purposes, as compared to only 33% in non-Indigenous communities (GNWT, 2015). Fish consumption could be a significant source of arsenic to Yellowknife residents. Fish consumption rates have been shown to correlate with arsenic concentrations in various biomarkers of exposure: blood, cord blood and breast milk (Miklavčič et al., 2013), as well as urine (Navas-Acien et al., 2011), among consumers.

Chronic arsenic exposure at a dose as little as 0.05 mg/kg body weight has a systemic effect on the human body: cardiovascular, integumentary, pulmonary and endocrinal effects, and can lead to cancer in multiple organs (ATSDR, 2007), while acute arsenic exposure at an oral dose of 1-3 mg/kg is lethal (ATSDR, 2007). The toxicity of arsenic compounds has been reported as: As(III) > As(V) > MMA > DMA > Organic arsenic species (ATSDR, 2007; NRC, 2001). Inorganic

arsenic species, As(III) and As(V), have been classified by the International Agency for Research on Cancer as Class I chemicals, carcinogenic to humans; while MMA and DMA species are classified as Class IIB chemicals, possibly carcinogenic to humans based on *in-vitro* evidences (Escudero-Lourdes et al., 2012; IARC, 2012; Wnek et al., 2011). Several *in-vitro* studies have revealed the trivalent form of MMA to be more toxic to human cells than As(III) by inhibiting DNA repair processes, disrupting enzymatic activities and inducing chromosomal mutations (Escudero-Lourdes et al., 2012; Kligerman et al., 2003; Mass et al., 2001; Wnek et al., 2011). Health effects from arsenic exposure were generally associated with exposure to the inorganic species (As(III) and As(V)). Upon ingestion, inorganic arsenic is metabolized via a series of reduction and methylation processes to As(V), Monomethylarsonic acid (MMA) and Dimethylarsinic acid (DMA).

Previous arsenic speciation studies revealed that most arsenic species in fish muscle is organic arsenobetaine, which is non-toxic in humans and is rapidly excreted in the urine after ingestion (ATSDR, 2007; Ozcan et al., 2016). Inorganic arsenic usually makes up less than 10% of the total arsenic in fish muscle (de Rosemond et al., 2008; Schoof & Yager, 2007). Nevertheless, the pathway of arsenic species biotransformation in fish remains unclear. Previous studies have proposed that fish transform inorganic arsenic into organic arsenic species, marked by the high concentration of organic arsenic species in tissue (Lunde, 1972; Zhang et al., 2012, 2016).

The aims of this study were (1) to measure the concentration of arsenic and its species in fish around Yellowknife, and (2) to evaluate the potential health risks from inorganic arsenic exposure through fish consumption. We hypothesize that (1) the distance between lakes and the mining area is negatively associated with total arsenic and inorganic arsenic concentrations in fish because fish from lakes closer to the mining area have higher arsenic in the food web due to

historical As deposition onto the lake; (2) The Yellowknives Dene First Nation have elevated health risks from inorganic arsenic exposure compared to the general population because they consume more fish that have higher inorganic arsenic concentrations.

3.2. Materials and Methods

Sample Collection and Sites

We collected a total of 180 fish samples from nine lakes around Yellowknife, Northwest Territories (Figure 3.1). A total of ten dorsal fish muscle samples were collected from each of the following species: lake whitefish (*Coregonus clupeaformis*) and northern pike (*Esox lucius*), with the exception of eight northern pike dorsal muscle samples from Grace Lake. Samples were obtained from the following lakes by Dr. Mark Poesch of the University of Alberta through the Environment and Natural Resources in 2017: Long Lake (62°28'41.30"N, 114°26'3.91"W), Grace Lake (62°25'10.37"N, 114°26'37.90"W), Kam Lake (62°25'19.10"N, 114°24'17.54"W), Lower Martin Lake (62°30'47.21"N, 114°25'16.04"W), Walsh Lake (62°34'54.53"N, 114°16'15.10"W) and Banting Lake (62°38'16.05"N, 114°17'22.61"W). Dr. John Chételat (Environment and Climate Change Canada) also provided a total of eight to ten fish muscle tissue from the 2013-2015 sampling season of the two fish species, in addition to five burbot liver tissues (*Lota lota*) each, from Yellowknife Bay (62°29'4.40"N, 114°20'13.00"W) and Great Slave Lake (62°20'56.68"N, 114°21'40.33"W). Additional fish samples of lake whitefish and northern pike from Small Lake (62°31'3.96"N, 113°49'35.36"W), were collected by Dr. Pete Cott and Mike Palmer in 2018. The captured fish were euthanized by pithing the head with a sharp knife. Fish were skinned and dissected in the field, and shipped to the Laboratory for the Analysis of Natural and Synthetic Environmental Toxicants at the University of Ottawa using ice coolers. The fork length (in mm),

and the total weight (in grams), of all fish, were recorded (Table 3.1). Our fish collection protocol was in accordance with the Canadian Council on Animal Care's *Guidelines on: the care and use of fish in research, teaching and testing* (CCAC, 2005), and was approved by the University of Ottawa's Animal Care Committee under Protocol BL-2894, Fisheries and Oceans Canada for the use of fish for scientific purposes under License S-17/18-3032-YK-A2, and Aurora Research Institute under License #16043 (Refer to *Supporting Documents*).

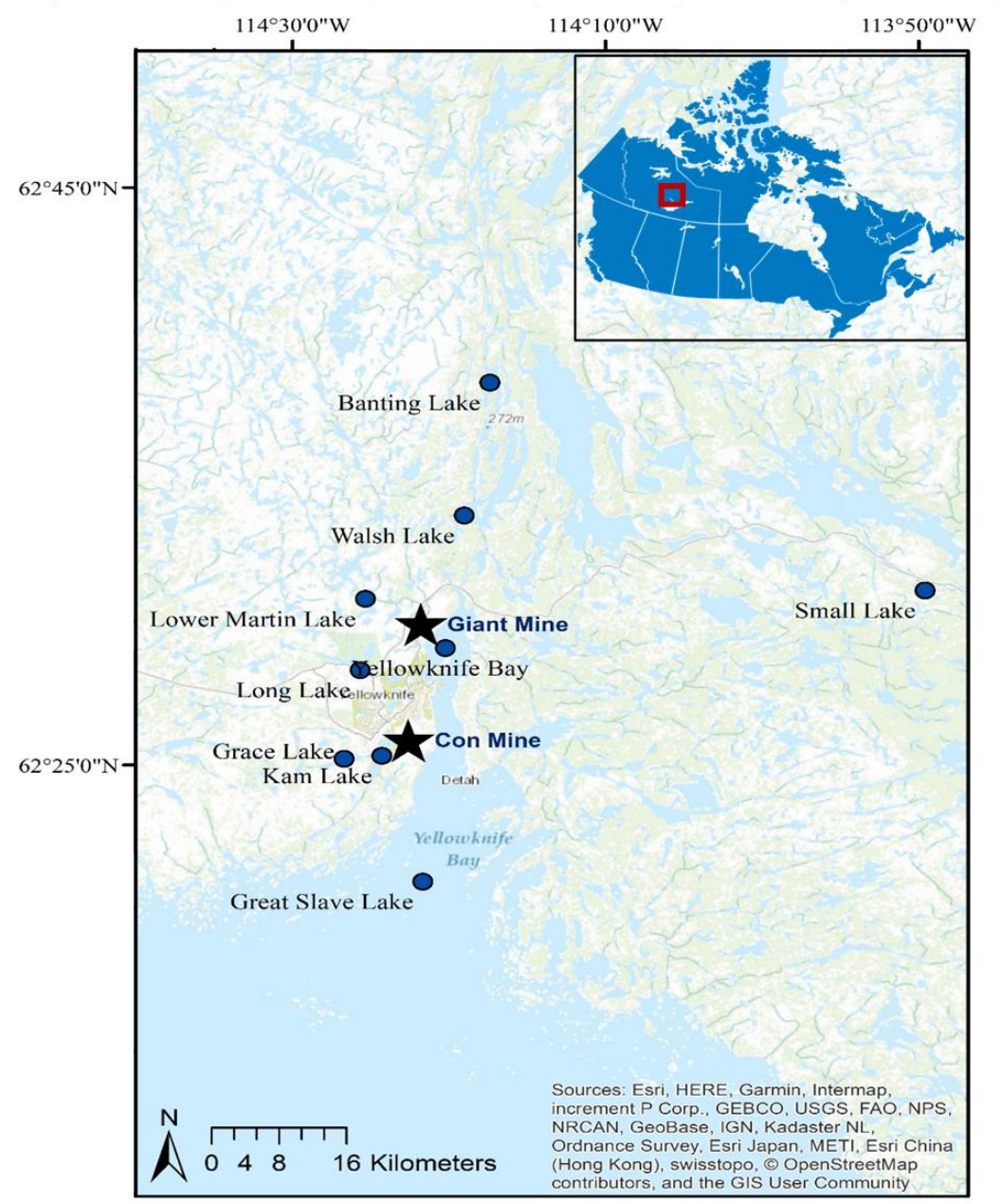

Figure 3.1. Fish sampling locations in Yellowknife, Northwest Territories.

Table 3.1. Biometrics of the collected fish samples from the nine lakes around Yellowknife.

Location	Species	N	Tissue	Fork Length (mm) ± std	Total Weight (g) ± std
Yellowknife Bay	Lake whitefish	8	Muscle	413.2 ± 31.9	951.9 ± 225.1
	Northern pike	9	Muscle	580.2 ± 105.6	1399.4 ± 672.7
	Burbot	5	Liver	593.2 ± 181.9	1658.0 ± 1014.1
Great Slave Lake	Lake whitefish	10	Muscle	352.4 ± 35.2	512.0 ± 214.2
	Northern pike	9	Muscle	559.0 ± 92.4	1050.0 ± 400.8
	Burbot	5	Liver	567.8 ± 98.7	1112.6 ± 462.6
Lower Martin Lake	Lake whitefish	10	Muscle	406.8 ± 14.9	921.2 ± 164.2
	Northern pike	10	Muscle	549.1 ± 32.7	1139.7 ± 273.0
Long Lake	Lake whitefish	10	Muscle	379.4 ± 39.1	874.0 ± 243.8
	Northern pike	10	Muscle	555.7 ± 25.3	1240.7 ± 132.6
Kam Lake	Lake whitefish	10	Muscle	421.1 ± 20.9	1312.9 ± 190.6
	Northern pike	10	Muscle	557.4 ± 10.1	1402.7 ± 146.9
Grace Lake	Lake whitefish	10	Muscle	413.1 ± 12.4	1248.4 ± 167.3
	Northern pike	8	Muscle	555.4 ± 62.5	1160.0 ± 442.6
Banting Lake	Lake whitefish	10	Muscle	425.3 ± 15.1	1117.5 ± 180.3
	Northern pike	10	Muscle	562.2 ± 15.5	1028.9 ± 221.4
Walsh Lake	Lake whitefish	10	Muscle	408.6 ± 6.7	903.0 ± 57.2
	Northern pike	10	Muscle	543.9 ± 23.2	1098.0 ± 141.1
Small Lake	Lake whitefish	8	Muscle	446.5 ± 21.2	1280.0 ± 375.4
	Northern pike	8	Muscle	597.1 ± 91.8	1556.1 ± 744.7

Sample preparation and Arsenic Analysis

Sample Preparation: Fish tissue samples were freeze-dried using a commercial SuperModulyo lyophilizer (Thermo Scientific, USA) for 24 to 36 hours, and then homogenized using a Magic Bullet processor before arsenic analysis. The sample weights before and after lyophilization were used to determine % moisture.

Total Arsenic analysis: 0.1 to 0.5 g of homogenized samples were digested with 2.5 mL of 70%v/v OmniTrace HNO_3 (EMD Millipore, USA) on an SCP Science model DigiPREP block digestion at

100°C for 180 minutes, and 1.5 mL of 30% v/v certified ACS H_2O_2 (Fisher Chemical, USA) was later added to each tube on the hotplate and heated for an additional 45 minutes at 95°C. The extracts were cooled to room temperature and diluted with Milli-Q deionized water to 10 mL. The digested solutions were then filtered using 0.45-micron DigiFILTERs and vortexed before analysis. The concentration of total arsenic was determined using inductively coupled plasma-mass spectrometry (ICP-MS: 7700x, Agilent Technologies, USA). Reference materials used were IAEA-407 (IAEA, Monaco) and DOLT-5 (NRC, Canada) for fish tissues. Total arsenic concentrations were within 95-125% of certified values, with a mean average of 104±7%.

Arsenic Speciation Analysis: 0.1 g of dry samples were extracted using 4 mL of 1:1 Methanol:MilliQ-water at 100°C for 180 minutes on DigiPREP block and diluted to 10 mL using Milli-Q deionized water. Extracts were centrifuged at 4000 rpm for 15 minutes and syringe-filtered using 0.2 µm PVDF filter media (Whatman, USA) before analysis. The concentration of various arsenic species: As(III), As(V), MMA, DMA and arsenobetaine, in samples were measured using high-performance liquid chromatography (HPLC: 1200, Agilent Technologies, USA) with inductively coupled plasma-mass spectrometry (ICP-MS: 7700x, Agilent Technologies, USA), in accordance to the FDA standards, Elemental Analysis Manual Section 4.11. The method limits of detection were 0.002 µg/g for arsenobetaine, 0.06 µg/g for As(III), 0.07 µg/g for DMA, 0.004 µg/g for MMA and 0.1 µg/g for As(V). Method blanks, calibration blanks and standards, and various standard reference material: DORM-4 and DOLT-5 (NRC, Canada), were used for quality assurance. Mass balances for all reference materials and samples were tested to be within the range of 95-130% of certified values (mean= 115±10%) for accuracy.

Human Health Risk Assessment

Data on the frequencies and amounts of various fish species consumed among the adult (aged 18 to 65) and child residents (aged 3 to 17) in Yellowknife were obtained from the Food Frequency Questionnaire (FFQ: Refer to *Supporting Documents*) collected by the Yellowknife Health Effects Monitoring Program for risk assessment studies (Chan et al., *in prep*). Information on the portion of fish meals was gathered using food visual models. All data used in this study were provided by consenting participants, recruited through random selection and on a voluntary basis. A copy of the consent form is attached in the *Supporting Documents*. Participants comprised of the general residents of Yellowknife and the Yellowknives Dene First Nation living in Yellowknife for at least twelve months. The Yellowknife Health Effect Monitoring Program applied the First Nations principles of Ownership, Control, Access and Possession of data throughout the entire process (Schnarch, 2004). The information collected for the research was strictly confidential, and participants could choose to withdraw their data from the study at any time. The protocol used by the research program was approved by University of Ottawa Research Ethics Board under file #H05-17-07, Aurora Research Institute license #16497 and Aurora College Research Ethics Committee under protocol #20180401 (Refer to *Supporting Documents*).

Daily fish consumption rates among participants were calculated by adding the total amount of fish consumed in a year (grams) divided by the total days of fish meals in a year (days). The survey was conducted in two waves: fall 2017 and spring 2018, with a total of 1,611 participants: 1,417 general residents (1,150 adults and 267 children) and 194 members of the Yellowknives Dene First Nation (123 adults and 71 children). The daily fish consumption rates (g/day) of lake whitefish, northern pike and burbot liver from various lakes around the city were used to estimate the potential non-carcinogenic and carcinogenic health risks related to long-term arsenic exposure

from fish consumption among reporting consumers. Only the inorganic forms of arsenic (As(III) and As(V)) in fish were taken into account for this risk assessment as these are the most toxic and carcinogenic species to humans (ATSDR, 2007). At the time this manuscript was prepared, the US EPA was revising their arsenic risk assessment under IRIS. Therefore, the methodology adopted in this paper was based on the latest available guidelines at the time. The chronic non-cancer Hazard Quotient (HQ) and the Incremental Lifetime Cancer Risk (ILCR) were evaluated using the following equations (Health Canada, 2010a; US EPA, 2000):

$$LADD = \frac{Cf_{iAs} \times IR \times EF \times ED}{BW \times 365\,days/year \times LE}$$

Equation 3.1. Lifetime Average Daily Dose (LADD) equation.

$$HQ_{iAs} = \frac{\text{LADD}}{RfD_{iAs}}$$

Equation 3.2. Non-carcinogenic Hazard Quotient (HQ) equation.

$$ILCR_{iAs} = \text{LADD} \times CSF_{iAs}$$

Equation 3.3. Incremental Lifetime Carcinogenic Risk (ILCR) equation.

, where: Cf_{iAs} = concentration of inorganic arsenic in fish (µg/g wet weight)

IR = daily ingestion rate of fish (g/day)

EF = exposure frequency (365 days/year)

ED = exposure duration (adult= 80 years (Health Canada, 2010), child= 10 years (US EPA, 2019))

BW = body weight of Yellowknife inhabitant (kg BW)

LE = life expectancy (80 years)

RfD_{iAs} = reference dose of inorganic arsenic (3×10^{-4} mg/kg BW.day) (US EPA, 2000)

CSF_{iAs} = cancer slope factor of inorganic arsenic exposure (1.8 kg BW.day/mg) (Health Canada, 2010b)

An HQ value greater than 0.2 indicates an elevated health risk associated with *de Minimus* lifetime risk when not all sources of exposure are accounted in the assessment (Health Canada, 2010a). An ILCR value less than 1×10^{-5} indicates negligible carcinogenic health risks (Health Canada, 2010a). Self-reported body weight was used to estimate daily exposure. The measured inorganic arsenic concentration in fish in dry weight was converted to the wet weight based on the corresponding % moisture, with the average conversion factors of 0.2 in fish muscle tissues and 0.4 in fish liver tissues.

We used Monte-Carlo simulation to calculate the non-carcinogenic and carcinogenic health risks (HQ and ILCR indices) from inorganic arsenic exposure through fish consumption among Yellowknife residents. These simulation tests were generated through the Crystal Ball software version 11.1 for Windows PC, to take into account the uncertainty distributions of all variables in HQ and CR computations using N= 10,000 trials.

Statistical Analysis

All data are presented in average value ± one standard deviation. The concentration of arsenic in fish samples was measured in µg/g dry weight (dw). All figures and statistical analyses were generated in R open-source software version 3.5.2 for Mac OS X. We used two-way ANOVA and *post-hoc* Tukey's multiple comparisons of means tests to compare the total arsenic and

inorganic arsenic concentrations in fish samples between the two fish species: lake whitefish and northern pike, and to compare fish total arsenic and inorganic species concentrations in various lakes around Yellowknife. Two-sample T-test was used to compare arsenic species concentration in lake whitefish and northern pike within the lakes. The relationships between the concentrations of different arsenic species detected in fish tissues were measured using Pearson's correlation tests. Statistical significance for all analyses was set at 0.05.

3.3. Results and Discussion

Total and Inorganic Arsenic Concentrations in Fish

The average total arsenic concentration in fish muscle tissues from the nine lakes in the Yellowknife area was 2.30 ± 1.72 µg/g dw, with a range of 0.42 to 5.97 µg/g dw (Figure 3.2), which is comparable to other published results on fish-arsenic concentration in the Yellowknife area: 0.05 to 2.80 µg/g dw (Cott et al., 2016), 0.57 to 1.15 µg/g dw (de Rosemond et al., 2008), and <0.05 to 6.90 µg/g dw (Stantec, 2014). Location was a significant factor determining total arsenic concentration in fish (two-way ANOVA; $p<0.05$) and differences in total arsenic concentration in fish were not species-related. Conversely, both fish species and location were significant factors determining the concentration of inorganic arsenic in fish (Two-way ANOVA; $p<0.05$).

The concentrations of arsenic in fish muscle tissue from Yellowknife Bay were not significantly different from those in its big lake reference further south of the roasters (Great Slave Lake) (*post-hoc* Tukey's; $p>0.05$). The concentrations of arsenic in fish from Grace Lake and Lower Martin Lake were significantly higher than the reference Small Lake (Grace Lake: mean difference= 4.44 µg/g dw, $p<0.05$; Lower Martin Lake: mean difference= 4.35 µg/g dw, $p<0.05$). Fish from Lower Martin Lake and Grace Lake had the highest total arsenic concentration in muscle tissue, with 5.97 ± 1.46 µg/g dw and 5.68 ± 5.89 µg/g dw, respectively. Fish from the reference Small Lake, located 27 km east of the mining area, had the lowest average total arsenic concentration in the muscle tissue: 0.46 ± 0.16 µg/g dw. Our results suggest that fish from in-land lakes near the mine roasters were more affected by legacy arsenic from mining, as compared to fish from lakes further away from mine roasters. Exception to the preceding statement was seen in Kam Lake located close to the mining area but had relatively low total arsenic concentrations in fish. However, Tukey's tests showed that fish from Kam Lake had a significantly higher inorganic arsenic concentrations, compared to fish from the reference Small Lake (mean difference =0.06 ug/g, $p<0.05$) (Figure 3.2). Although the concentration of total arsenic in fish from the Kam Lake was low, the inorganic arsenic concentration in these fish was the highest across the lakes, especially in lake whitefish (0.131 ± 0.101 µg/g dw). This contradiction indicates that total arsenic concentration in tissues alone is not sufficient for risk assessment for human health.

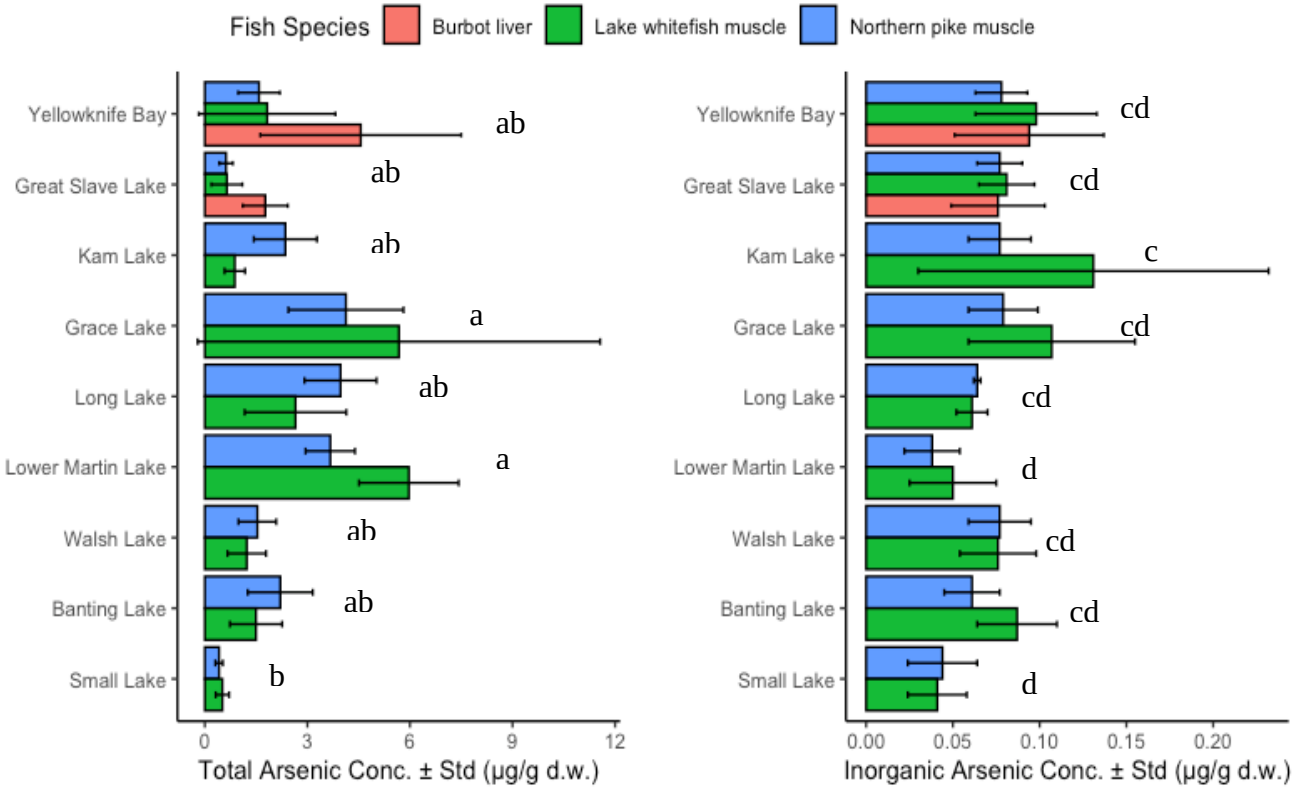

Figure 3.2. Total Arsenic and inorganic arsenic concentrations in fish tissue from lakes around Yellowknife. Same letter labels (a,b,c,d) means no significant differences (Two-way ANOVA, *Post-hoc* Tukey's; $p>0.05$) in the mean arsenic concentrations in fish from the lakes.

The average arsenic concentration in burbot liver was 3.16 ± 2.49 µg/g dw. Total arsenic concentrations in burbot liver were higher than those in fish muscle tissue from the two sites where liver was analyzed (Figure 3.2), and were also higher in sampling site closer to the mines: 4.56 ± 2.94 µg/g dw in Yellowknife Bay, compared to 1.77 ± 0.66 µg/g dw in Great Slave Lake. Liver tissue accumulates higher arsenic than muscle tissue as it is the main biotransformation organ of arsenic (Lunde, 1972). Previous studies have reported arsenic bioconcentration in fish organs as the following: Gastrointestinal tract > liver > muscle (de Rosemond et al., 2008; Foata et al., 2009).

Arsenic Speciation in Fish

Arsenic species in fish muscle were predominantly in the form of organic arsenobetaine (mean = 58.6 ± 34.5 %) (Figure 3.3). Inorganic arsenic comprised less than 20% of total arsenic in fish tissues (Table 3.2). Lake whitefish had a higher inorganic arsenic concentration in muscle compared to northern pike (mean difference = 0.02 ug/g, $p<0.05$), as well as the proportion of inorganic arsenic to total arsenic (mean difference = 2.96%, $p<0.05$). The theory explaining the higher inorganic arsenic levels found in lake whitefish is two-fold: (1) Adult lake whitefish feed primarily on benthic invertebrates at the lake bottom (COSEWIC, 2005), which might have exposed these fish to high inorganic arsenic through sediment ingestion, contributing to the higher inorganic arsenic concentration in muscle tissue, as opposed to northern pike that feed nearly exclusively on midwater fish (Harvey, 2009); and (2) Lake whitefish occupy a lower trophic position in the food webs than northern pike (Cott et al., 2011; *Chapter 2*), which has been associated with higher arsenic concentration and proportion of inorganic arsenic to total arsenic in freshwater organisms (*Chapter 2*). Most arsenic species in burbot liver was in the form of DMA (mean = 76.6 ± 21.6 %), and only less than 5% of total arsenic was in the form of inorganic arsenic (mean = 3.9 ± 2.7 %). Since the predominant species of soluble arsenic species in lake water and sediment are inorganic As(III) and As(V) (Pothier et al., 2018), we conclude that the organic arsenicals in fish tissues are products of inorganic arsenic biotransformation and retention through dietary exposure. Inorganic arsenic is taken up by fish through gills and ingestion (Fonseca et al., 2017; Zhang et al., 2012). Northern pike had significantly higher DMA to total arsenic proportion in tissue compared to that in lake whitefish (Two-sample T-test, mean difference = 34.5%, $p<0.0001$), indicating that inorganic arsenic species are preferably bio-transformed to DMA rather than arsenobetaine in northern pike. Conversely, lake whitefish metabolizes most arsenic into

arsenobetaine (mean arsenobetaine proportion= 68.7 ± 36.4%). These findings suggest species-specific retention of arsenic compounds in fish species that could be related to differences in arsenic biotransformation pathways and diets.

Table 3.2. Summary of total arsenic and inorganic arsenic concentration in various fish species across lakes in Yellowknife.

Location	Species	N	Tissue	Total As (µg/g d.w.) ± std	Total iAs (µg/g d.w.) ± std	%iAs ± std
Yellowknife Bay	Lake whitefish	8	Muscle	1.82 ± 2.00	0.098 ± 0.035	9.3 ± 6.7
	Northern pike	9	Muscle	1.59 ± 0.61	0.078 ± 0.015	6.1 ± 3.7
	Burbot	5	Liver	4.56 ± 2.94	0.094 ± 0.043	3.5 ± 3.9
Great Slave Lake	Lake whitefish	10	Muscle	0.65 ± 0.45	0.081 ± 0.016	19.6 ± 14.9
	Northern pike	9	Muscle	0.60 ± 0.18	0.077 ± 0.013	14.1 ± 5.5
	Burbot	5	Liver	1.77 ± 0.66	0.076 ± 0.027	4.4 ± 0.9
Lower Martin Lake	Lake whitefish	10	Muscle	5.97 ± 1.46	0.050 ± 0.025	0.9 ± 0.4
	Northern pike	10	Muscle	3.67 ± 0.72	0.038 ± 0.016	1.1 ± 0.5
Long Lake	Lake whitefish	10	Muscle	2.65 ± 1.49	0.061 ± 0.009	3.0 ± 2.0
	Northern pike	10	Muscle	3.97 ± 1.06	0.064 ± 0.002	1.7 ± 0.5
Kam Lake	Lake whitefish	10	Muscle	0.88 ± 0.30	0.131 ± 0.101	15.1 ± 10.3
	Northern pike	10	Muscle	2.36 ± 0.92	0.077 ± 0.018	3.7 ± 1.5
Grace Lake	Lake whitefish	10	Muscle	5.68 ± 5.89	0.107 ± 0.048	3.2 ± 2.7
	Northern pike	8	Muscle	4.13 ± 1.68	0.079 ± 0.020	2.2 ± 1.0
Banting Lake	Lake whitefish	10	Muscle	1.50 ± 0.76	0.087 ± 0.023	6.9 ± 3.6
	Northern pike	10	Muscle	2.21 ± 0.95	0.061 ± 0.016	3.1 ± 1.4
Walsh Lake	Lake whitefish	10	Muscle	1.23 ± 0.56	0.076 ± 0.022	7.7 ± 4.8
	Northern pike	10	Muscle	1.54 ± 0.55	0.077 ± 0.018	5.6 ± 2.3
Small Lake	Lake whitefish	8	Muscle	0.52 ± 0.20	0.041 ± 0.017	8.9 ± 4.2
	Northern pike	8	Muscle	0.42 ± 0.11	0.044 ± 0.020	10.4 ± 4.1

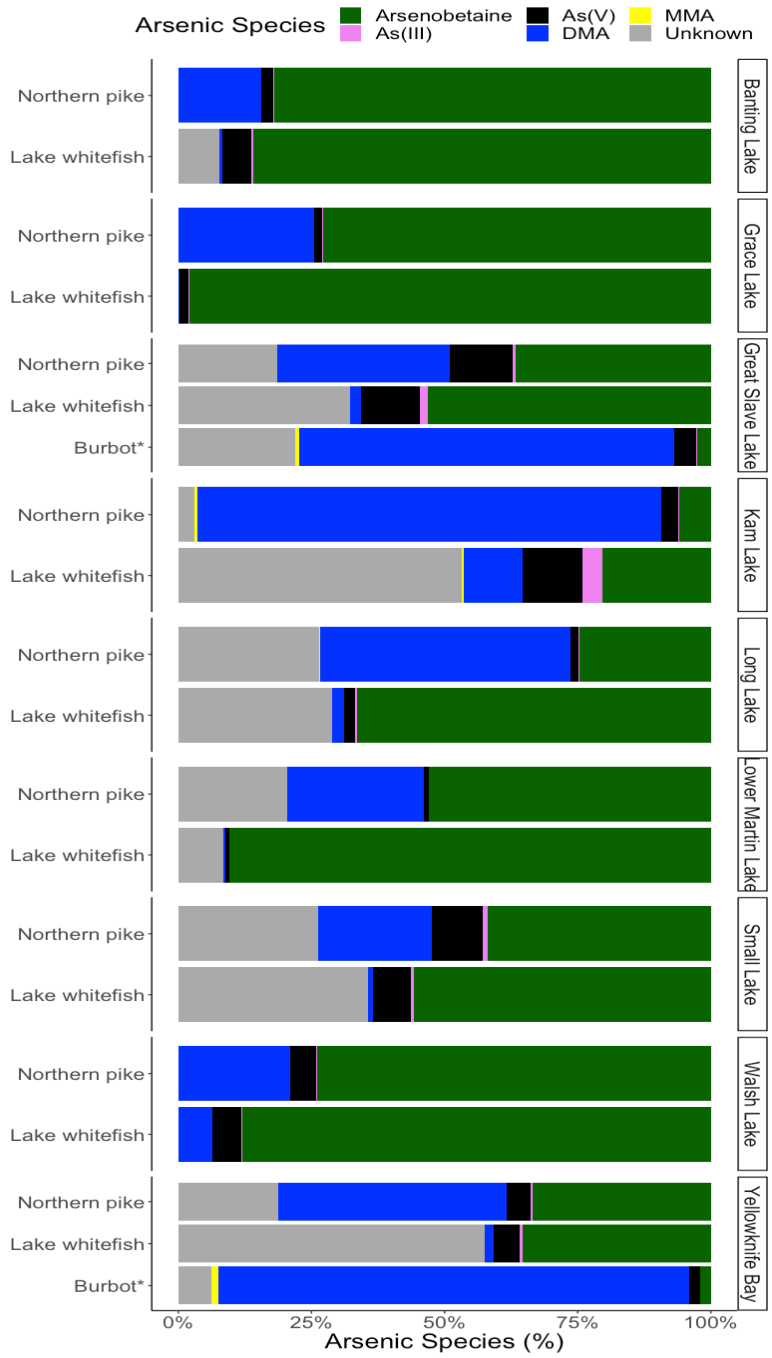

Figure 3.3. The proportion of detected arsenic species in the muscle tissue of fish across lakes. *Burbot arsenic concentration was measured in liver tissue.

Correlation of Arsenic Species in Fish Tissues

The correlation matrices of arsenic species concentrations in the three fish species are presented in Table 3.3. In lake whitefish muscle, total arsenic concentration was significantly correlated with concentration of As(V) ($r=0.236$, $p<0.05$) and arsenobetaine ($r=0.960$, $p<0.01$). In northern pike, high total arsenic in muscle was significantly correlated to high MMA ($r=0.480$, $p<0.01$), DMA ($r=0.624$, $p<0.01$) and arsenobetaine ($r=0.721$, $p<0.01$), while in burbot liver tissue, high arsenic levels were strongly correlated to high levels of MMA ($r=0.827$, $p<0.01$), DMA ($r=0.967$, $p<0.01$) and arsenobetaine ($r=0.869$, $p<0.01$). Our results indicated that upon exposure, inorganic arsenic in fish is transformed into predominantly arsenobetaine in lake whitefish; and to MMA, DMA and arsenobetaine in northern pike and burbot liver tissue.

The proportions of inorganic arsenic to total arsenic in fish was inversely related to total arsenic concentration in all three fish species (lake whitefish: $r=-0.434$, $p<0.01$; northern pike: $r=-0.727$, $p<0.01$; burbot liver: $r=-0.655$, $p<0.05$), suggesting that inorganic arsenic concentration in fish does not increase proportionally to total arsenic concentration in tissues. The accumulation of toxic inorganic arsenic in tissues is restricted with the increasing total arsenic in tissues. Similar to our finding, another study has also shown a decline in the retention of inorganic arsenic with an increasing arsenic concentration in fish (Jia et al., 2018), which outcome has been attributed to arsenic biotransformation to organic forms and arsenic excretion in fish.

In this study, we observed two possible pathways of arsenic biotransformation in fish species: (1) biotransformation to arsenobetaine, as in lake whitefish, and (2) biotransformation to methylated arsenic species (MMA and DMA), as in northern pike and burbot liver. These pathways could be specific to the fish species.

Table 3.3. Pearson correlation matrices of arsenic concentration and arsenic species in (A) lake whitefish muscle, (B) northern pike muscle, and (C) burbot liver.

A	Total Arsenic	As(III)	DMA	MMA	As(V)	iAs	%iAs	AsB
Total Arsenic	1	-0.063	-0.126	0.060	0.236*	0.131	-0.434**	0.960**
As(III)	-0.063	1	0.058	0.489**	-0.045	0.668**	0.456**	-0.082
DMA	-0.126	0.058	1	0.175	0.208	0.196	0.055	-0.128
MMA	0.060	0.489**	0.175	1	0.277**	0.549**	0.199	0.022
As(V)	0.236*	-0.045	0.208	0.277**	1	0.713**	0.149	0.228*
iAs	0.131	0.668**	0.196	0.549**	0.713	1	0.431**	0.112
%iAs	-0.434**	0.456**	0.055	0.199	0.149	0.431**	1	-0.387**
AsB	0.960**	-0.082	-0.128	0.022	0.228*	0.112	-0.387**	1

B	Total Arsenic	As(III)	DMA	MMA	As(V)	iAs	%iAs	AsB
Total Arsenic	1	0.016	0.624**	0.480**	-0.017	-0.013	-0.727**	0.721**
As(III)	0.016	1	0.058	0.124	-0.074	0.139	0.071	-0.067
DMA	0.624**	0.058	1	0.803**	0.209	0.220*	-0.497**	0.000
MMA	0.480**	0.124	0.803**	1	0.053	0.079	-0.347**	-0.087
As(V)	-0.017	-0.074	0.209	0.053	1	0.977**	0.247*	-0.008
iAs	-0.013	0.139	0.220*	0.079	0.977**	1	0.260*	-0.022
%iAs	-0.727**	0.071	-0.497**	-0.347**	0.247*	0.260*	1	-0.466**
AsB	0.721**	-0.067	0.000	-0.087	-0.008	-0.022	-0.466**	1

C	Total Arsenic	As(III)	DMA	MMA	As(V)	iAs	%iAs	AsB
Total Arsenic	1	-0.037	0.967**	0.827**	0.598	0.612	-0.655*	0.869**
As(III)	-0.037	1	0.063	-0.005	-0.273	-0.081	0.099	0.009
DMA	0.967**	0.063	1	0.870**	0.643*	0.679*	-0.547	0.918**
MMA	0.827**	-0.005	0.870**	1	0.545	0.564	-0.525	0.774**
As(V)	0.598	-0.273	0.643*	0.545	1	0.981**	-0.074	0.703*
iAs	0.612	-0.081	0.679*	0.564	0.981**	1	-0.057	0.731*
%iAs	-0.655*	0.099	-0.547	-0.525	-0.074	-0.057	1	-0.427
AsB	0.869**	0.009	0.918**	0.774**	0.703*	0.731*	-0.427	1

Significance levels at *p<0.05 and **p<0.01.

Fish Consumption in Yellowknife

We collected daily fish consumption data from a total of 1,611 participants in the Yellowknife Health Effects Monitoring Program's FFQ survey, which included 1,417 residents from the

general population (1,150 adults and 267 children) and 194 members of the Yellowknives Dene First Nation (123 adults and 71 children) (Table 3.4). Out of 1,417 participants from the general population group, 1,409 participants (99%) provided their consumption information for each of the fish species in the FFQ. All 194 participants from the Yellowknives Dene First Nation provided their consumption data for all fish species indicated in the FFQ. The most consumed fish species in both groups were lake whitefish (89-98% consumers), lake trout (49-71% consumers), walleye (21-43% consumers) and northern pike (10-34% consumers) (Table 3.4). Similar to the prior Dene Dietary Survey in 1998, whitefish and trout remained as the two most commonly consumed fish species (83-97% consumers) among the Yellowknives Dene First Nation, however the number of pike consumers had declined significantly (1998: 26-50% consumers; 2017-2018: 10-12% consumers) (Receveur et al., 1998). The daily consumption rates of lake whitefish, northern pike and burbot liver documented in our risk assessment covered approximately 60% of total fish consumption among Yellowknife residents.

We included the most common sources of locally-caught fish among Yellowknife residents in our exposure assessment: Yellowknife Bay, Great Slave Lake, Long Lake, Grace Lake, Kam Lake, Martin Lake, Walsh Lake, Banting Lake and Small Lake. Out of 1,073 participants who reported fishing in Yellowknife, 89% of them reported consuming locally harvested fish (N= 960). More than half of participants (63%) reported fishing in the Great Slave Lake area, and 46% of them reported fishing in Yellowknife Bay and Back Bay area. 15% of participants reported fishing in Walsh Lake, 8% in Long Lake, and around 5% or less of participants reported fishing in each of the following locations: Banting Lake, Kam Lake, Grace Lake and Small Lake. Other lakes that were frequently mentioned by the participants but were not covered in this study were Prosperous Lake, Prelude Lake and Pontoon Lake. These three lakes are large-scale lakes measuring over 300

ha in surface area, situated at least 10 km away from the mining area, with water arsenic concentrations below 10 µg/L based on a previous survey (Palmer et al., 2015). Based on these characteristics, we expect that these lakes will probably have similar or lower arsenic and inorganic arsenic concentrations in fish compared to the range of concentrations that we reported. This risk assessment serves as an overall assessment of inorganic arsenic exposure through fish intake from the most commonly fished lakes in the area, however, it is not exhaustive.

Daily fish consumption rates of various fish species were the highest among the Yellowknives Dene First Nation adult consumers with a mean average of 6±7 g/day (range= 1-19 g/day), than the general adult consumers with an average of 4±2 g/day (range= 0.8-8 g/day). Children in Yellowknife generally consumed smaller portions of various fish species than adults, with a mean average of 2±1 g/day (range= 0.5-5 g/day) among the general population and 4±5 g/day (range= 0-14 g/day) among the Yellowknives Dene First Nation. Northern pike and burbot were more consumed among the general residents (northern pike: 2-4 g/day; burbot: 2-3 g/day, burbot liver: 0.5-2 g/day, inconnu: 3 g/day), compared to the Yellowknives Dene First Nation residents (northern pike: 1-3 g/day; burbot: 0.3-2 g/day, burbot liver: 0-1 g/day). The recommended fish intake proposed by Health Canada's *Food Guide for Healthy Eating* is at least 150 grams (2 servings) per week (Health Canada, 2007) or equivalent to 21 g/day. The average general resident group in Yellowknife had lower than the recommended fish intake at 15 g/day, while the Yellowknives Dene First Nation had higher fish intake at 32 g/day.

Table 3.4. Summary table of daily fish consumption (g/day) of various fish species among the general residents (N=1,417 participants) and Yellowknives Dene First Nation (N=194 participants).

Fish Species	Group	Variable		YK General Population		YK Dene First Nation	
				Adult	Child	Adult	Child
Lake Whitefish	All Participants	n		1148	267	123	71
		Consumption rate (g/day)	Average	7	4	18	7
			50th percentile (median)	3	2	8	3
			95th percentile	28	16	72	24
	Consumers Only	n (%)		1022 (89)	238 (89)	120 (98)	68 (96)
		Consumption rate (g/day)	Average	8	5	19	7
			50th percentile (median)	4	2	8	4
			95th percentile	29	16	62	19
Lake Trout	All Participants	n		1147	267	123	71
		Consumption rate (g/day)	Average	3	2	10	3
			50th percentile (median)	0.5	0.1	1	0
			95th percentile	16	8	48	23
	Consumers Only	n (%)		678 (59)	135 (51)	87 (71)	35 (49)
		Consumption rate (g/day)	Average	5	3	13	6
			50th percentile (median)	2	1	6	2
			95th percentile	23	10	52	27
Northern Pike	All Participants	n		1143	266	123	71
		Consumption rate (g/day)	Average	1	0.7	0.4	0.1
			50th percentile (median)	0	0	0	0
			95th percentile	7	4	1.3	1
	Consumers Only	n (%)		389 (34)	84 (32)	15 (12)	7 (10)
		Consumption rate (g/day)	Average	4	2	3	1
			50th percentile (median)	2	1	0.7	1

			95th percentile	15	11	13	3
Burbot Liver	All Participants		n	1144	264	123	71
		Consumption rate (g/day)	Average	0.06	0.002	0.1	0
			50th percentile (median)	0	0	0	0
			95th percentile	0	0	0.4	0
	Consumers Only		n (%)	26 (3)	1 (0.3)	14 (11)	0 (0)
		Consumption rate (g/day)	Average	2	0.5	1	0
			50th percentile (median)	0.5	0.5	0.3	0
			95th percentile	11	0.5	4	0
Burbot	All Participants		n	1145	264	123	71
		Consumption rate (g/day)	Average	0.6	0.4	0.2	0.02
			50th percentile (median)	0	0	0	0
			95th percentile	3	1	1	0.3
	Consumers Only		n (%)	233 (21)	39 (15)	18 (15)	4 (6)
		Consumption rate (g/day)	Average	3	2	2	0.3
			50th percentile (median)	1	0.7	0.7	0.3
			95th percentile	12	6	4	0.3
Inconnu (Connie)	All Participants		n	1147	266	123	71
		Consumption rate (g/day)	Average	0.7	0.2	0.6	0.5
			50th percentile (median)	0	0	0	0
			95th percentile	4	1	3	5
	Consumers Only		n (%)	250 (22)	47 (18)	34 (28)	7 (10)
		Consumption rate (g/day)	Average	3	1	2	5
			50th percentile (median)	1	0.7	1	3
			95th percentile	11	5	8	10
Walleye (Pickerel)	All Participants		n	1146	265	123	71
			Average	1	0.7	3	3

		Consumption rate (g/day)	50th percentile (median)	0	0	0	0
			95th percentile	5	3	8	21
	Consumers Only		n (%)	496 (43)	81 (31)	36 (29)	15 (21)
		Consumption rate (g/day)	Average	3	2	10	14
			50th percentile (median)	1	0.7	2	3
			95th percentile	10	8	72	62
Grayling (Bluefish)	All Participants		n	1147	266	123	71
		Consumption rate (g/day)	Average	0.04	0.05	0.04	0.02
			50th percentile (median)	0	0	0	0
			95th percentile	0.1	0	0	0
	Consumers Only		n (%)	64 (6)	9 (3)	5 (4)	1 (1)
		Consumption rate (g/day)	Average	0.8	1	1	1
			50th percentile (median)	0.5	0.8	0.2	1
			95th percentile	2	4	3	1

Risk Assessment

We assessed the potential chronic health risks to inorganic arsenic exposure from fish consumption using two indices (HQ and ILCR) using Monte-Carlo simulation at 10,000 repetitions to estimate risks with the uncertainty distributions of body weight and the daily fish consumption rate in the two consumer groups, as well as the inorganic arsenic concentration measured in fish (Table 3.5). Body weight (BW) and daily fish consumption (IR) data were taken from the consumers of whitefish, pike and burbot liver: N= 1,055 adult consumers and 267 child consumers from the general resident group and N= 120 adult consumers and 68 child consumers from the Yellowknives Dene First Nation.

Table 3.5. Variables used for Monte-Carlo simulation on non-carcinogenic health risk (HQ) and carcinogenic risk (ILCR) on inorganic arsenic exposure from the reported fish consumption among general residents and the Yellowknives Dene First Nation.

Variables	**General population**		**Yellowknives Dene First Nation**	
	Adult (N= 1,055)	**Child (N= 246)**	**Adult (N= 120)**	**Child (N= 68)**
Body weight, BW (kg)	Normal (Mean= 79.7, Std = 19.7)	Normal (Mean= 37.0, Std= 18.1)	Normal (Mean= 86.0, Std= 23.3)	Normal (Mean= 52.9, Std= 22.0)
Daily fish consumption, IR (g/day)	Lognormal (Mean = 9.3, Std = 15.1)	Lognormal (Mean = 5.2, Std = 7.8)	Lognormal (Mean = 19.3, Std = 29.0)	Lognormal (Mean = 7.1, Std = 10.9)
Fish iAs conc., Cf_iAs (µg/g w.w.)	Lognormal (Mean = 0.017, Std = 0.010)			

The probabilistic distributions of non-cancer health risk (HQ) and lifetime cancer risk (ILCR) in the two resident groups are shown in Table 3.6. Among the adult consumers from the general population, the HQ values ranged from 95%CI: 0.00-0.03, with a mean value of 0.007, while in the Yellowknives Dene First Nation adults, HQ values ranged from 95%CI: 0.00-0.05, with a mean average of 0.01. Although the Yellowknives Dene First Nation adults had higher HQ values compared to the adult general residents, the values at 95th percentile were still much lower than the value associated with *de Minimus* lifetime risk at HQ>0.2 (Health Canada, 2010a), indicating that there were no appreciable long-term non-carcinogenic health risks related to fish consumption in adults of both groups at 95th percentile. Since the children in the two resident groups consumed much less fish than the adults, the ranges of probabilistic HQ in children were lower than those in adults (General residents: mean= 0.001, 95%CI: 0.00-0.005; Yellowknives Dene First Nation: mean= 0.001, 95%CI: 0.00-0.005). In cases where exposures from all sources are not considered, standard risk assessment practice estimates potential hazards against a hazard benchmark of 0.2. This ensures that site-related exposures do not exceed twenty percent (20%) of the toxicity reference value on a daily basis. Even with the more conservative setting of the hazard benchmark of 0.2, the estimated ranges were all below, suggesting the risk of inorganic arsenic exposure from consuming local fish at the current level is acceptable.

The probabilistic ILCR values for the average adult residents were within the acceptable level at 3.8 x 10^{-6} and 7.5 x 10^{-6} in general resident group and the Yellowknives Dene First nation, respectively. However, the ILCR values among adults at the 95th percentile exceeded the limit of negligible cancer risk proposed by Health Canada of 1 x10^{-5} (Health Canada, 2010a) with ILCR (general residents)= 1.4 x 10^{-5} and ILCR (Yellowknives Dene first Nation)= 2.7 x 10^{-5}, suggesting that there was elevated cancer risk associated with fish intakes at 95th percentiles among the adult

population in Yellowknife. In children, the probabilistic ILCR values at 95th percentile were within the acceptable value (ILCR<10^{-5}) at 3.0 x 10^{-6} among the general residents and 2.4 x 10^{-6} among the Yellowknives Dene First Nation. Although our probabilistic ILCR values in adults were higher at the 95th percentile, these values were lower than the ILCR values at the 5th percentile among the general population in Canada (ILCR= 1.4 x 10^{-4}) (Faure et al., 2019), indicating that Yellowknife residents were not exposed to higher cancer risks of inorganic arsenic exposure compared to Canadian general population.

Table 3.6. Monte-Carlo simulation (N=10,000) of non-carcinogenic health risk (HQ) and carcinogenic risk (ILCR) of inorganic arsenic exposure based on the reported fish consumption rates among the general population and Yellowknives Dene First Nation.

Population Group	**Age Group**	**HQ**			**ILCR**		
		Mean	**50th percentile**	**95th percentile**	**Mean**	**50th percentile**	**95th percentile**
General population	Adult (18-79)	0.007	0.003	0.03	3.8 x 10^{-6}	1.7 x 10^{-6}	1.4 x 10^{-5}
	Child (3-17)	0.001	0.0005	0.005	9.8 x 10^{-7}	2.8 x 10^{-7}	3.0 x 10^{-6}
Yellowknives Dene First Nation	Adult (18-79)	0.01	0.006	0.05	7.5 x 10^{-6}	3.4 x 10^{-6}	2.7 x 10^{-5}
	Child (3-17)	0.001	0.0005	0.005	1.1 x 10^{-6}	2.6 x 10^{-7}	2.4 x 10^{-6}

In summary, our risk assessment concluded that fish consumption in Yellowknife did not pose any substantial chronic health risks to the average residents, but slightly elevated cancer risk among the heavy fish consumers in adults. However, there are a number of weaknesses in the design of the study that might result in an underestimation of health risks. We did not consider arsenic exposure from store-bought fish, other local fish species consumed, or fish caught in other

lakes in the area. We covered approximately 60% of reported fish consumption in Yellowknife population using the consumption data for lake whitefish, northern pike and burbot liver in our risk assessment. Assuming that other fish species had similar inorganic arsenic concentrations, the estimated daily exposure rate of inorganic arsenic from fish consumption was 0.26 µg/day among the general resident adults, 0.02 µg/day among the general resident children, 0.54 µg/day among the Yellowknives Dene First Nation adults and 0.03 µg/day among the Yellowknives Dene First Nation children. Using the distribution of body weights, we estimated that the daily doses of inorganic arsenic exposure from fish consumption in Yellowknife encompassed 1-2% of the US EPA's reference dose of 3×10^{-4} mg/kg.day (US EPA, 2000).

Fish consumption serves as an essential source of nutrition, especially for eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), among other nutrients that are irreplaceable by other food substitutes. Fishing also has a significant cultural value in the Indigenous communities that rely on fishing for nourishment. A Yellowknife Dene Dietary Survey in 1998 showed that ~70% of Dene households reported fishing and 30% of participants revealed that they could not afford to buy all their food from the store if traditional sources of food were not available (Receveur et al., 1998). Fish consumption has been linked to many health benefits, such as reduced cardiovascular-related mortality (Mozaffarian & Rimm, 2006), reduced obesity and diabetes (Nkondjock & Receveur, 2003), and improved neuropsychological performances in children and adolescents (Butler et al., 2017). Incorporation of sufficient seafood in maternal diets of more than 340 grams per week has been correlated to developmental benefits in children, according to an Avon Longitudinal Study of Parents and Children involving 11,875 pregnant women, and limiting this source of nutrients could be detrimental to children (Hibbeln et al., 2007). Our findings show that the risk of lost nutrients from fish outweighs the health risks associated with inorganic arsenic

exposure from fish consumption in the majority of Yellowknife population. Therefore, we support incorporating sufficient fish in diets, in accordance to Health Canada's recommendation of at least 150 grams of fish per week and following site-specific fish consumption advisories posted by the Health and Social Services in Yellowknife. However, our results suggest that fish consumption from Kam Lake might be discouraged as it had a significantly higher concentration of inorganic arsenic species, compared to fish from the regional reference lake. Large-bodied fish occupying a higher trophic position in food webs generally accumulate less inorganic arsenic in the tissues (*Chapter 2*), posing little in the way of consumption issues. However, large-bodied fish that were found to have low inorganic arsenic concentrations in tissue could still have high concentrations of methylmercury or other chemicals of potential concern.

We calculated the allowable daily intake and weekly servings for each of the fish species in each location without exceeding 20% of the RfD (Table 3.7). As the results show, there is negligible risk of inorganic arsenic exposure even with consumption of multiple servings per day.

Table 3.7. The allowable daily intake (g) and weekly servings for each fish species in each location without exceeding 20% of the RfD. One serving = 150 gram.

Location	Fish Species	Allowable intake per day (g)	Allowable servings per week
Yellowknife Bay	Lake whitefish	245	11
	Northern pike	308	14
	Burbot liver	128	6
Great Slave Lake	Lake whitefish	296	14
	Northern pike	312	14
	Burbot liver	128	7
Lower Martin Lake	Lake whitefish	480	22
	Northern pike	631	29
Long Lake	Lake whitefish	393	18
	Northern pike	375	17
Kam Lake	Lake whitefish	183	8
	Northern pike	312	14
Grace Lake	Lake whitefish	224	10
	Northern pike	304	14
Banting Lake	Lake whitefish	276	13
	Northern pike	393	18
Walsh Lake	Lake whitefish	316	15
	Northern pike	312	14
Small Lake	Lake whitefish	585	27
	Northern pike	545	25

3.4. Conclusion

The average total arsenic concentration in fish muscle across the nine lakes in Yellowknife was 2.30 ± 1.72 µg/g dw, ranging from 0.42 to 5.97 µg/g dw. Higher concentrations of arsenic were detected in the liver tissues of burbot: 4.56 ± 2.94 µg/g dw in Yellowknife Bay and 1.77 ± 0.66 µg/g dw in Great Slave Lake. Elevated concentrations of total arsenic in fish were still seen after almost two decades after the closing of both Giant Mine and Con Mine. An important factor determining the variability in total arsenic concentrations in fish around Yellowknife is the location; the proximity of the lakes to the legacy mining operations, while the speciation of arsenic in fish is influenced by both fish species and the location of lakes. Arsenic species in fish muscle was predominantly arsenobetaine, with less than 20% of total arsenic in inorganic arsenic forms. Burbot liver consisted of primarily DMA, and less than 5% of total arsenic was inorganic arsenicals. Inorganic arsenic concentration in fish is inversely related to the total arsenic concentration in the tissues, indicating that the inorganic arsenic concentration in fish tissue does not increase proportionally with the total arsenic concentration in tissue. Biotransformation pathways of inorganic arsenic in fish are species-specific. Most of the arsenic in lake whitefish is transformed to arsenobetaine, while higher proportion was being transformed to DMA in northern pike than in lake whitefish. The Yellowknives Dene First Nation consumed more fish, in general, compared to the general residents, and adults in Yellowknife consumed much more fish than children. The results of health risk assessments, using HQ index for non-carcinogenic health risks and ILCR index for cancer risks, show that there were no significant health risks of inorganic arsenic exposure among average consumers based on the reported consumption of locally-caught fish, but cancer risks could be elevated among heavy fish consumers. It is important to note that this study does not address the potential long-term effects of legacy arsenic exposure of the populations in

Yellowknife when Giant Mine was still in operation. Also, the Yellowknives Dene First Nation may have taken special precautions to lower their arsenic exposure, e.g. by avoiding fish from lakes with are known to have higher arsenic, and reducing their local fish consumption. This study also does not address other indirect health risks associated with changes in their traditional diet and lifestyle as a result of the mining operations.

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Chapter 4: Conclusion

This thesis assesses the impact of historical mining operations to arsenic species bioaccumulation in freshwater food webs, and inorganic arsenic exposure through fish consumption in local communities in Yellowknife, Northwest Territories, Canada. In the first research chapter (*Chapter 2*), I explained the dynamics of arsenic species accumulation in freshwater food webs and compared the species accumulation between littoral (near-shore) and pelagic (off-shore) food webs near large legacy mining area in Yellowknife, Northwest Territories, Canada. In the second research chapter (*Chapter 3*), I measured the concentrations of various arsenic species in tissues of freshwater fish from nine different lakes of varying distances and directions from mine roasters and evaluated the long-term health risks of carcinogenic inorganic arsenic exposure from fish consumption among Yellowknife population groups.

4.1. Main Findings and Stated Hypotheses

My first hypothesis was that there is more transfer of inorganic arsenic in littoral food webs than in pelagic food webs because the littoral food webs consist of more benthic organisms that are more exposed to lake sediments. However, we found that feeding zones are an irrelevant factor to total and inorganic arsenic bioaccumulation in freshwater organisms. Therefore, I reject my first hypothesis. Trophic positions in food webs are the determining factor to total and inorganic arsenic concentrations and proportions in freshwater organisms, explaining up to 89% of variances seen in the data. The total and inorganic arsenic concentrations were bio-minified in food webs. The proportions of arsenobetaine to total arsenic in freshwater biota increased with trophic position, indicating that there is more biotransformation to organic arsenobetaine at high trophic level. The biomininfication of inorganic and total arsenic bioaccumulation in food webs could be due to the

biotransformation of inorganic arsenic to organic species in biotic tissues, low dietary assimilation of inorganic arsenic from preys to consumers and elimination of arsenic from tissue.

My second hypothesis was that fish from lakes closer to Giant Mine is the most affected by the high arsenic found near the mine. The concentrations of total and inorganic arsenic were measured in fish muscle tissue of two fish species—lake whitefish (*Coregonus clupeaformis*) and northern pike (*Esox lucius*) caught from nine different lakes around Giant Mine. A significant factor determining the total arsenic concentrations in fish was the location or source of fish; meanwhile, both the location and the fish species were relevant factors explaining the inorganic arsenic concentrations in fish. Total arsenic concentrations in fish were elevated for lakes situated closer to the mining area. However, the carcinogenic inorganic arsenic concentrations in fish were similar across lakes. The predominant arsenic species in fish tissue was the non-toxic organic arsenobetaine. Lake whitefish had higher inorganic arsenic concentrations on average, compared to northern pike. Moreover, species-specific accumulation of arsenic was observed between the two fish species. Northern pike accumulated more DMA in its muscle tissue, while lake whitefish retained more organic arsenobetaine in its muscle. Species-specific accumulation of arsenic in fish species could be due to differences in fish diets and physiology.

My third hypothesis was that higher fish consumption rates in Yellowknife contribute to elevated exposure to inorganic arsenic. I hypothesized that the Yellowknives Dene First Nation have elevated health risks associated with higher inorganic arsenic exposure compared to the general residents of Yellowknife because the First Nation group consumes more locally-caught fish. Although the Yellowknives Dene First Nation consumed more fish than the general resident group in 2016-2018 and children consumed less fish than adults, the inorganic arsenic concentrations detected in fish tissues were low. Our analysis using Monte-Carlo simulation

models of Cancer Risk (CR) and Hazard Quotient (HQ) indices concluded that there were no substantial long-term cancer and non-cancer health risks from fish consumption for the majority of Yellowknife residents, but elevated cancer risks among the adults from the two population groups. However, these cancer risks, as indicated by CR index at the 95th percentile, were still below the CR of inorganic arsenic exposure among the general population in Canada at the 5th percentile, indicating that Yellowknife residents were not exposed to higher cancer risks of inorganic arsenic exposure than the general population in Canada.

4.2. Concluding Remarks

This thesis research contributes to the understanding of arsenic cycle in freshwater food webs near large mining operations and the health risk estimation of inorganic arsenic exposure through fish consumption in the general residents of Yellowknife and the Yellowknives Dene First Nation group. The literature on arsenic species cycling in freshwater food webs has been especially limited. Past papers studying arsenic transfers in aquatic food webs have only looked at relatively short food chains (≤3 trophic levels) from one sampling site (Foust et al., 2016; Kirby & Maher, 2002; Tu et al., 2011), and there was only one study looking at the transfer of arsenic in freshwater food webs in Yellowknife back in 1978 and most recently in 2019, however with no speciation work (Chetelat et al., 2019; Wagemann et al., 1978). Chetelat et al. (2019) found food web biodilution of total arsenic in freshwater organisms, spanning the primary producers, aquatic invertebrates and fish from Yellowknife Bay, supporting the findings of this study. This manuscript is the first to offer clear evidence of food web bio-minification of inorganic arsenic in food webs and arsenic species cycling spanning five trophic levels in two in-land lakes affected by the mining and one reference in-land lake. Although we established the patterns of arsenic

species bioaccumulation in freshwater food webs, the biochemical pathway of arsenic biotransformation in fish is still unclear. Further studies should also investigate the contribution of fish diets to arsenic species bioaccumulation in fish tissue. Our risk assessment study emphasized on the importance of arsenic speciation in estimating risk and calculating the exposure of chemicals because different species of arsenic pose different levels of carcinogenicity to humans. Inorganic arsenic compounds are class I carcinogens, while organic arsenobetaine is non-toxic to humans. In our paper, I found that the health benefits of consuming fish in Yellowknife outweigh the risk of inorganic arsenic exposure. Therefore, my recommendation is to incorporate sufficient fish in daily diets, especially for children.

The findings of this study will redound to the benefits of the general public considering the challenge many communities face to achieve the beneficial level of nutrients from the environment while protecting human and ecosystem health. The take-away from this study is that food web bio-minification delimits the concentrations of carcinogenic, inorganic arsenic in large-bodied fish. Thus, restricting the exposure to these chemicals from fish consumption. The outcomes of this research contribute to the scientific information essential for the development of well-informed policies by the health sectors in Yellowknife and worldwide facing similar issues, and improve our understanding of the fate of arsenic by-product from mining in the environment (i.e. how arsenic trioxide from mines is transferred from the environment to freshwater food webs, the pattern of food web bio-minification, and trophic positions as identified to be a major determinant of inorganic arsenic bioaccumulation in freshwater organisms). Hence, this paper addresses an emerging issue in arsenic research and delivers new insight into the understanding of the fate of environmental pollutants.

Reference

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Supplementary Materials

Supplementary Table 2.1. Lake characteristics and surface water chemistry of the three lakes. DOC: dissolved organic carbon.

Location	Distance from roaster	Area	Bedrock geology(Palmer et al., 2015)	pH	DOC (mg/L)	Total P (mg/L)	Total N (mg/L)	Na	SO_4 (mg/L)	As
Long Lake	9.0 km SW	116 ha	Granitoid	8.2	8.1	0.018	0.47	30	19	50.3
Lower Martin Lake	6.5 km NW	126 ha	Granitoid	7.9	23.5	0.032	1.51	4.6	4	54.0
Small Lake	55 km E	90 ha	Meta-sedimentary	7.9	13.8	0.005	0.58	4.9	5	1.4

Supplementary Table 2.2. Vertical water profile measurements from the center of the three studied lakes using YSI Multiprobe Handheld.

Location (depth)	Distance from surface (m)	Dissolved oxygen (mg/L)	Dissolved oxygen (%)	Temperature (°C)	Conductivity (µS)	Specific Conducti-vity (µS)	Salinity (ppt)
Small Lake (2 m)	0	9.72	101.5	17.4	127.9	151	0.1
	0.5	8.67	90.5	17.4	134.9	157.6	0.1
	1.0	8.43	87.6	17.4	134.6	157.5	0.1
	1.5	8.74	91.2	17.4	134.7	157.6	0.1
Lower Martin Lake (1.6 m)	0	8.92	87.5	15.9	128.8	154.2	0.1
	0.5	8.64	65.1	15.7	130.8	159.3	0.1
	1.0	8.83	89.9	15.7	131.1	159.5	0.1
	1.25	9.48	95.8	15.7	131.1	159.5	0.1
Long Lake (6.5 m)	0	9.63	98.2	16	357.5	432	0.2
	1	9.41	95.5	16	358.5	432.7	0.2
	2	9.31	95.9	16	358.2	432.9	0.2
	3	9.36	95.8	15.9	356.8	432.7	0.2
	4	9.27	94.5	15.7	355.4	432.4	0.2
	5	9.23	94.6	15.4	352.9	432.3	0.2
	6	2.85	38.3	15.4	352.9	432.3	0.2

Supplementary Table 2.3. Arsenic and its species concentrations (± SD) in aquatic organisms from the three studied lakes.

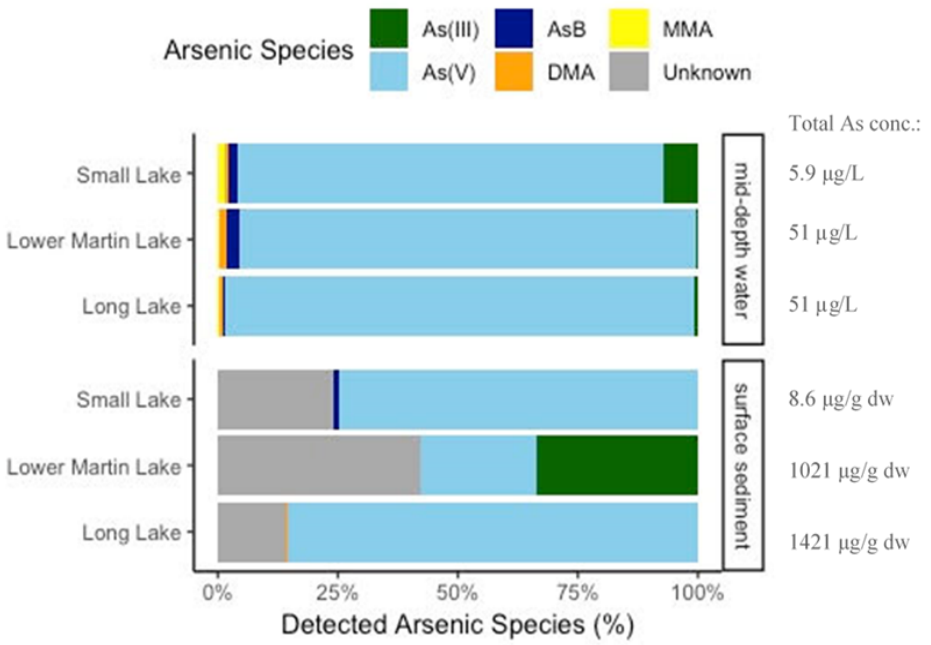
N: sample sizes, l: liver tissue, m: muscle tissue, juv: juvenile, <DL: Below detection limit, iAs: inorganic arsenic species (As(III) + As(V)).

Organism	N	Total As (µg/g dw)	As(III) (µg/g dw)	As(V) (µg/g dw)	MMA (µg/g dw)	DMA (µg/g dw)	AsB (µg/g dw)	Total iAs (µg/g dw)
Long Lake								
Macrophyte	whole	199.33	<DL	198.66	0.12	0.31	0.23	198.66
Periphyton	whole	733.61	46.96	488.41	0.13	0.43	0.35	535.37
Phytoplankton	whole	579.87	<DL	248.09	<DL	76.32	3.69	248.09
Zooplankton	whole	161.14	0.69	131.37	0.35	0.94	3.04	4.55
Invertebrates	whole	7.55	0.26	4.29	0.04	0.08	0.19	132.06
Ninespine Stickleback	6	6.98	0.06	2.44	0.09	0.08	1.46	2.50
Lake Whitefish	10(m)	2.65 ± 1.49[a]	<DL	<DL	<DL	0.06 ± 0.06	1.77 ± 1.21[b]	0.06 ± 0.01
Northern Pike	10(m)	3.97 ± 1.06[a]	<DL	<DL	0.01	1.87 ± 0.41	0.98 ± 0.60[b]	0.06 ± 0.002
Lower Martin Lake								
Macrophyte	whole	21.53	<DL	21.03	0.06	0.07	0.37	21.03

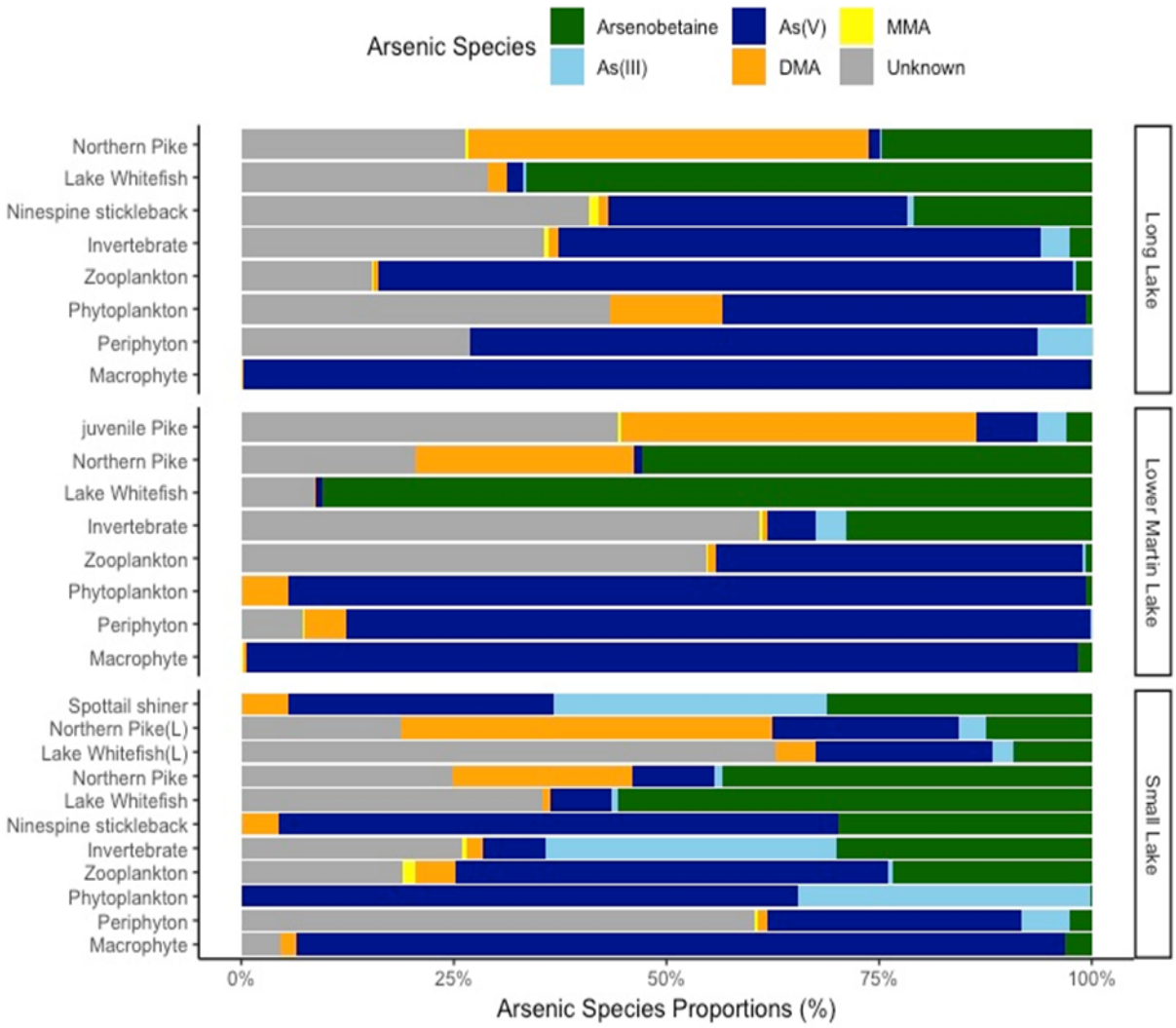
Periphyton	whole	972.80	0.64	852.57	0.59	47.70	0.21	853.21
Phytoplankton	whole	251.09	<DL	235.33	<DL	13.97	1.78	235.33
Zooplankton	whole	68.79	0.17	29.72	0.13	0.66	0.54	29.89
Invertebrates	whole	7.69	0.28	0.43	0.02	<DL	2.22	0.71
Northern Pike (juv)	11	2.80 ± 0.72	0.12 ± 0.11	0.19 ± 0.07	0.01	0.11 ± 0.36	0.08 ± 0.07	0.31 ± 0.17
Lake Whitefish	10(m)	5.97 ± 1.46[c]	<DL	<DL	<DL	<DL	5.40 ± 1.32[d]	0.05 ± 0.02
Northern Pike	10(m)	3.67 ± 0.72[c]	<DL	<DL	<DL	0.94 ± 0.46	1.94 ± 0.62[d]	0.04 ± 0.02
Small Lake								
Macrophyte	whole	3.19	<DL	2.89	<DL	0.06	0.10	2.89
Periphyton	whole	9.90	0.57	2.95	0.03	0.12	0.25	3.52
Phytoplankton	whole	5.53	1.91	3.62	<DL	<DL	0.01	5.52
Zooplankton	whole	9.89	0.06	5.03	0.14	0.47	2.31	5.09
Invertebrates	whole	1.87	0.64	0.14	0.01	<DL	0.56	0.78
Ninespine Stickleback	5	0.80	<DL	0.53	<DL	<DL	0.24	0.47
Spottail Shiner	23	0.74	0.24	0.23	<DL	<DL	0.23	0.53

Lake Whitefish	8(m)	0.52 ± 0.20[e]	<DL	<DL	<DL	<DL	0.29 ± 0.20[i]	0.04 ± 0.02[g]
Northern Pike	8(m)	0.42 ± 0.11[f]	<DL	<DL	<DL	0.09 ± 0.02	0.18 ± 0.06[j]	0.04 ± 0.02[h]
Lake Whitefish	8(l)	0.43 ± 0.19[e]	<DL	<DL	<DL	<DL	0.04 ± 0.03[i]	0.10 ± 0.03[g]
Northern Pike	8(l)	0.32 ± 0.09[f]	<DL	<DL	<DL	0.14 ± 0.04	0.04 ± 0.02[j]	0.08 ± 0.03[h]

[a]Significantly higher total As concentration in muscle tissues of Northern Pike than Lake Whitefish (Two-sample T-test, $p < 0.05$)
[b]Significantly higher AsB concentration in muscle tissues of Lake Whitefish than Northern Pike (Two-sample T-test, $p < 0.05$)
[c]Significantly higher total As concentration in muscle tissues of Lake Whitefish than Northern Pike (Two-sample T-test, $p < 0.001$)
[d]Significantly higher AsB concentration in muscle tissues of Lake Whitefish than Northern Pike (Two-sample T-test, $p < 0.0001$)
[e]No significant difference (Paired T-test, $p > 0.05$) found in total As concentration in muscle and liver tissues within the species
[f]No significant difference (Paired T-test, $p > 0.05$) found in total As concentration in muscle and liver tissues within the species
[g]Significantly higher total iAs found in the liver tissues than the muscle tissues of the species (Paired T-test, $p \leq 0.001$)
[h]Significantly higher total iAs found in the liver tissues than the muscle tissues of the species (Paired T-test, $p \leq 0.05$)
[i]Significantly higher AsB found in the muscle tissues than the liver tissues of the species (Paired T-test, $p \leq 0.05$)
[j]Significantly higher AsB found in the muscle tissues than the liver tissues of the species (Paired T-test, $p \leq 0.001$)



Supplementary Fig. 2.1. Proportions of detected arsenic species in mid-depth water samples (top) and surface sediment samples (bottom) of the three lakes. Total arsenic concentration of water (in µg/L) and sediment (in µg/g dry weight) are shown to the right of the bar plots.



Supplementary Fig. 2.2. Proportions of various arsenic species in freshwater biota from the three studied lakes. (L) represents arsenic species proportions in liver tissues of indicated fish species.

Supporting Documents

A. University of Ottawa Animal Care Protocol Approval

BL-2894_Yellowknife.pdf

B. Northwest Territories Scientific Research License (ARI) and Amendments

ARI license.pdf

ARI Amendment.pdf

ARI date1 amendment.pdf

C. License to Fish for Scientific Purposes (DFO)

AUP 2017-59 Letter of Approval.pdf

DFO license.pdf

D. Research Ethics Approvals for Yellowknife Health Effects Monitoring Program

YKHEMP-AuroraResearchInstitute_application3773_May2017.pdf

YKhemp-Research ethics license.pdf

H05-17-07 uOttawa ethics Approval CHAN_Jun 26 2018.pdf

E. Food Frequency Questionnaire

Food Frequency Questionnaire

WILD FISH CONSUMPTION

1. a) In the past 12 months, have you eaten any Whitefish? ☐ Yes ☐No
   If answered Yes above, please answer the following:
   b) In the Summer (June-Aug), how many days did you eat Whitefish? _____

   In the Spring (Apr-May), how many days did you eat Whitefish? _____

   In the Winter (Nov-Mar), how many days did you eat Whitefish? _____

   In the Fall (Sept-Oct), how many days did you eat Whitefish? _____

   c) On the days when you ate Whitefish, how much did you usually eat? (*Refer to visual guide*)
   (i) Flat size: ☐OV-XS ☐ OV-S ☐ OV-M ☐ OV-L ☐ OV-XL ☐OV-J
   (ii) Thickness: ☐T01 ☐T02 ☐T03 ☐T04 ☐T05 ☐T06 ☐T07 ☐T08 ☐T09 ☐T10 ☐T11 ☐T12 ☐T13 ☐T14 ☐T15 ☐T16

2. a) In the past 12 months, have you eaten any Lake Trout? ☐ Yes ☐No
   If answered Yes above, please answer the following:
   b) In the Summer (June-Aug), how many days did you eat Lake Trout? _____

   In the Spring (Apr-May), how many days did you eat Lake Trout? _____

   In the Winter (Nov-Mar), how many days did you eat Lake Trout? _____

   In the Fall (Sept-Oct), how many days did you eat Lake Trout? _____

   c) On the days when you ate Lake Trout, how much did you usually eat? (*Refer to visual guide*)
   (i) Flat size: ☐OV-XS ☐ OV-S ☐ OV-M ☐ OV-L ☐ OV-XL ☐OV-J
   (ii) Thickness: ☐T01 ☐T02 ☐T03 ☐T04 ☐T05 ☐T06 ☐T07 ☐T08 ☐T09 ☐T10 ☐T11 ☐T12 ☐T13 ☐T14 ☐T15 ☐T16

3. a) In the past 12 months, have you eaten any Northern Pike (Jackfish)? ☐ Yes ☐No
   If answered Yes above, please answer the following:
   b) In the Summer (June-Aug), how many days did you eat Northern Pike?
   _____

   In the Spring (Apr-May), how many days did you eat Northern Pike? _____

In the Winter (Nov-Mar), how many days did you eat Northern Pike? _____

In the Fall (Sept-Oct), how many days did you eat Northern Pike? _____

c) On the days when you ate Northern Pike (Jackfish), how much did you usually eat? (*Refer to visual guide*)

(i) Flat size: ☐OV-XS ☐ OV-S ☐ OV-M ☐ OV-L ☐ OV-XL ☐OV-J

(ii) Thickness: ☐T01 ☐T02 ☐T03 ☐T04 ☐T05 ☐T06 ☐T07 ☐T08 ☐T09 ☐T10 ☐T11 ☐T12 ☐T13 ☐T14 ☐T15 ☐T16

4. a) In the past 12 months, have you eaten any Burbot (Louche) liver? ☐ Yes ☐ No

If answered Yes above, please answer the following:

b) In the Summer (June-Aug), how many days did you eat Burbot liver? _____

In the Spring (Apr-May), how many days did you eat Burbot liver? _____

In the Winter (Nov-Mar), how many days did you eat Burbot liver? _____

In the Fall (Sept-Oct), how many days did you eat Burbot liver? _____

c) On the days when you ate Burbot liver, how much did you usually eat? (*Refer to visual guide*)

(i) Flat size: ☐OV-XS ☐ OV-S ☐ OV-M ☐ OV-L ☐ OV-XL ☐OV-J

(ii) Thickness: ☐T01 ☐T02 ☐T03 ☐T04 ☐T05 ☐T06 ☐T07 ☐T08 ☐T09 ☐T10 ☐T11 ☐T12 ☐T13 ☐T14 ☐T15 ☐T16

5. a) In the past 12 months, have you eaten any Burbot (Louche)? ☐ Yes ☐ No

If answered Yes above, please answer the following:

b) In the Summer (June-Aug), how many days did you eat Burbot? _____

In the Spring (Apr-May), how many days did you eat Burbot? _____

In the Winter (Nov-Mar), how many days did you eat Burbot? _____

In the Fall (Sept-Oct), how many days did you eat Burbot? _____

c) On the days when you ate Burbot, how much did you usually eat? (*Refer to visual guide*)

(i) Flat size: ☐OV-XS ☐ OV-S ☐ OV-M ☐ OV-L ☐ OV-XL ☐OV-J

(ii) Thickness: ☐T01 ☐T02 ☐T03 ☐T04 ☐T05 ☐T06 ☐T07 ☐T08 ☐T09 ☐T10 ☐T11 ☐T12 ☐T13 ☐T14 ☐T15 ☐T16

a) In the past 12 months, have you eaten any Connie (Inconnu)? ☐ Yes ☐ No

If answered Yes above, please answer the following:

b) In the Summer (June-Aug), how many days did you eat Connie? _____

In the Spring (Apr-May), how many days did you eat Connie? _____

In the Winter (Nov-Mar), how many days did you eat Connie? _____

In the Fall (Sept-Oct), how many days did you eat Connie? _____

c) On the days when you ate Connie (Inconnu), how much did you usually eat? (*Refer to visual guide*)

(i) Flat size: ☐OV-XS ☐ OV-S ☐ OV-M ☐ OV-L ☐ OV-XL ☐OV-J

(ii) Thickness: ☐T01 ☐T02 ☐T03 ☐T04 ☐T05 ☐T06 ☐T07 ☐T08 ☐T09 ☐T10 ☐T11 ☐T12 ☐T13 ☐T14 ☐T15 ☐T16

6. a) In the past 12 months, have you eaten any Pickerel (Walleye)? ☐ Yes ☐No

If answered Yes above, please answer the following:

b) In the Summer (June-Aug), how many days did you eat Pickerel? _____

In the Spring (Apr-May), how many days did you eat Pickerel? _____

In the Winter (Nov-Mar), how many days did you eat Pickerel? _____

In the Fall (Sept-Oct), how many days did you eat Pickerel? _____

c) On the days when you ate Pickerel (Walleye), how much did you usually eat? (*Refer to visual guide*)

(i) Flat size: ☐OV-XS ☐ OV-S ☐ OV-M ☐ OV-L ☐ OV-XL ☐OV-J

(ii) Thickness: ☐T01 ☐T02 ☐T03 ☐T04 ☐T05 ☐T06 ☐T07 ☐T08 ☐T09 ☐T10 ☐T11 ☐T12 ☐T13 ☐T14 ☐T15 ☐T16

7. a) In the past 12 months, have you eaten any Grayling (Bluefish)? ☐ Yes ☐No

If answered Yes above, please answer the following:

b) In the Summer (June-Aug), how many days did you eat Grayling? _____

In the Spring (Apr-May), how many days did you eat Grayling? _____

In the Winter (Nov-Mar), how many days did you eat Grayling? _____

In the Fall (Sept-Oct), how many days did you eat Grayling? _____

c) On the days when you ate Grayling (Bluefish), how much did you usually eat? (*Refer to visual guide*)

(i) Flat size: ☐OV-XS ☐ OV-S ☐ OV-M ☐ OV-L ☐ OV-XL ☐OV-J

(ii) Thickness: ☐T01 ☐T02 ☐T03 ☐T04 ☐T05 ☐T06 ☐T07 ☐T08 ☐T09 ☐T10 ☐T11 ☐T12 ☐T13 ☐T14 ☐T15 ☐T16

8. a) In the past 12 months, have you eaten any Longnose Sucker? ☐ Yes ☐No

If answered Yes above, please answer the following:

b) In the Summer (June-Aug), how many days did you eat Longnose Sucker?

_____

In the Spring (Apr-May), how many days did you eat Longnose Sucker?

_____

In the Winter (Nov-Mar), how many days did you eat Longnose Sucker?

_____

In the Fall (Sept-Oct), how many days did you eat Longnose Sucker? _____

c) On the days when you ate Longnose Sucker, how much did you usually eat? (*Refer to visual guide*)

(i) Flat size: ☐OV-XS ☐ OV-S ☐ OV-M ☐ OV-L ☐ OV-XL ☐OV-J

(ii) Thickness: ☐T01 ☐T02 ☐T03 ☐T04 ☐T05 ☐T06 ☐T07 ☐T08 ☐T09 ☐T10 ☐T11 ☐T12 ☐T13 ☐T14 ☐T15 ☐T16

F. Consent Forms

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Université d'Ottawa | University of Ottawa
Département de Biologie | **Department of Biology**
30 Marie Curie, Ottawa, ON K1N 6N5
ON Canada K1N 6N5
Tel: (613) 562-5800 x6349

Consent Form
(Yellowknives Dene First Nation, 12+ years)

Title of study: Health Effects Monitoring Program

Invitation to Participate: You are invited to participate in the Health Effects Monitoring Program as part of the Giant Mine Remediation Project. This study is led by Dr. Laurie Chan of the University of Ottawa. Funding is provided through Indigenous and Northern Affairs Canada.

Purpose of the Study: The purpose of the Health Effects Monitoring Program is to establish current baseline levels of contaminants, and examine possible health effects among residents in Ndilo, Dettah, and Yellowknife in the Northwest Territories, before remediation work begins. Then, during remediation, new monitoring results will be compared to the baseline to ensure participants' arsenic levels are not increasing because of work being done at Giant Mine. The monitoring program will focus on arsenic, and other Contaminants of Potential Concern (COPC) such as cadmium, lead, manganese, antimony and vanadium which may be released as a result of the remediation project.

Participation: If you agree to participate, a nurse will conduct a 60–minute interview to complete a short lifestyle questionnaire, and a food frequency questionnaire on a variety of wild fish, animals, birds, and berries consumed. In addition, the nurse will complete a medical history questionnaire, and conduct a brief medical examination that will include weighing, measuring height, and taking blood pressure. We will ask you to provide some toenail samples, a urine sample collected in the morning, and a saliva sample taken with a buccal swab from the inside of your cheek. Toenail and urine samples will be sent to the laboratory to test for arsenic and other metals of concern. The buccal swab will be used to test whether you have or do not have 20 specific genes that can help you to get rid of arsenic in your body more easily.

Risks: There is no physical harm anticipated for participating in the monitoring program. Some of the questions in the Lifestyle Questionnaire are sensitive and personal, and you may feel uncomfortable. You don't have to answer all questions. You may also feel anxious about the type and amount of contaminants we may find in your body. You will receive your results with interpretation in a personal letter within a few months of data collection. A nurse of the research team will also be available to meet with you to explain your result, in case you had elevated levels

of contaminants, the nurse will work with you to lower your exposure, and conduct further testing if necessary (i.e. blood test to confirm high exceedance).

Benefits: You will have the opportunity to find out whether you have been exposed to arsenic and other metals of concern. At the same time your participation will contribute to the understanding of arsenic exposure and its health effects in Ndilo, Dettah and Yellowknife.

Confidentiality and anonymity: All information you provide will be kept strictly confidential and will never be publicly attached to your name. You will receive your results with interpretation in a personal letter.
Conservation of data: The data collected (questionnaires, toenails, urine and saliva) will be kept in a secure manner (in a computer in a secure room at the University of Ottawa) until completion of the program. The Principal Investigator, along with research students, Janet Cheung and Dr. Rajendra Parajuli, will have access to the data. The data will only be used for the purpose of this study. A copy of the master database shall be provided to the Institute for Circumpolar Health Research, and kept in a secure manner, once data collection is complete.

Compensation: A gift card for grocery in the amount of $50 will be provided to thank you for participating in the program.

Voluntary Participation: Your participation is voluntary. You are under no obligation to participate. If you choose to participate, you can withdraw from the study at any time and/or refuse to answer any questions without suffering any negative consequences. If you choose to withdraw, all information and data you have provided will be destroyed or returned to you on request. No samples of toenails, urine or saliva will be collected without your permission.

Who can I talk to if I have questions or problems?
The local research assistant will answer any questions you may have about this program or you may contact the following project team member at any time in the future.

Collect calls will be accepted.

Research Supervisor:
Dr. Laurie Chan
Professor and Canada Research Chair in Toxicology and Environmental Health
University of Ottawa, Faculty of Biology
Tel: 613-562-5800 ext. 7116
Email: laurie.chan@uottawa.ca

Yellowknife Contact:
Stacey Sundberg
Assistant Coordinator
Work: 867-873-9337
Email: stacey.sundberg@ichr.ca

If you have any questions regarding the ethical conduct of this study, you may contact:

Protocol Officer for Ethics in Research,
University of Ottawa, Tabaret Hall,
550 Cumberland Street, Room 154,
Ottawa, ON K1N 6N5
Tel: (613) 562-5387
Email: ethics@uottawa.ca

There are two copies of the consent form of which one will be kept by Dr. Chan.
Your decision to participate in the Health Effects Monitoring Program is completely up to you. You are free to withdraw from the program at any time, and you can choose not to answer any questions you don't feel comfortable answering.

By signing this form, I agree that:

1.	I understand that I am being asked to participate in a Health Effects Monitoring Program that will focus on Arsenic and other contaminants of primary concern for the Giant Mine Remediation Project.	Yes	No
2.	I understand that I have the right to not participate, to refuse to answer a question and the right to stop at any time.	Yes	No
3.	I understand that I can ask any questions related to the study at any time.	Yes	No
4.	I understand that my personal information will be kept confidential.	Yes	No
5.	I agree to give urine sample and be informed of the result.	Yes	No
6.	I agree to give toenail sample and be informed of the result.	Yes	No
7.	I agree to give saliva sample and be informed of the result.	Yes	No
8.	I agree to complete a medical history and undergo a brief medical examination by a nurse.	Yes	No
9.	A follow-up study is planned in 5 to 10 years. I agree to be contacted again to be invited to participate in the follow up study.	Yes	No
10.	I agree to keep my samples in a biobank until the end of the study.	Yes	No
11.	I hereby consent to participate in the study.	Yes	No

Name of participant _____

Date of Birth (day/month/year) _____

_____
_____
Signature Date
(day/month/year)

Telephone number _____

Participant's mailing address (for returning results of sample analysis):

_____
_

_____
_

Name of person who obtained consent

_____
_____
Signature Date
(day/month/year)

uOttawa

Université d'Ottawa | University of Ottawa
Département de Biologie | **Department of Biology**
30 Marie Curie, Ottawa, ON K1N 6N5
ON Canada K1N 6N5
Tel: (613) 562-5800 x6349

Consent Form
(Yellowknife residents, 12 -79 years)

Title of study: Health Effects Monitoring Program

Invitation to Participate: You have been randomly selected to participate in the Health Effects Monitoring Program as part of the Giant Mine Remediation Project. This study is led by Dr. Laurie Chan of the University of Ottawa. Funding is provided through Indigenous and Northern Affairs Canada.

Purpose of the Study: The purpose of the Health Effects Monitoring Program is to establish current baseline levels of contaminants, and examine possible health effects among residents in Ndilo, Dettah, and Yellowknife in the Northwest Territories, before remediation work begins. Then, during remediation, new monitoring results will be compared to the baseline to ensure participants' arsenic levels are not increasing because of work being done at Giant Mine. The monitoring program will focus on arsenic, and other Contaminants of Potential Concern (COPC) such as cadmium, lead, manganese, antimony and vanadium which may be released as a result of the remediation project.

Participation: If you agree to participate, we will conduct a 30–minute interview to complete a short lifestyle questionnaire, and a food frequency questionnaire on a variety of wild fish consumed. We will ask you to provide some toenail samples, a urine sample collected in the morning, and a saliva sample taken with a buccal swab from the inside of your cheek. Toenail and urine samples will be sent to the laboratory to test for arsenic and other metals of concern. The buccal swab will be used to test whether you have or do not have 20 specific genes that can help you to get rid of arsenic in your body more easily.

You will also be asked for permission to access your medical files for the past 5 years. We will investigate whether you have experienced symptoms related to arsenic or other contaminant exposure. This information will be coded with our study ID number.

Risks: There is no physical harm anticipated for participating in the monitoring program. Some of the questions in the Lifestyle Questionnaire are sensitive and personal, and you may feel uncomfortable. You don't have to answer all questions. You may also feel anxious about the type and amount of contaminants we may find in your body. You will receive your results with interpretation in a personal letter within a few months of data collection. A nurse of the research team will also be available to meet with you to explain your result, in case you had elevated levels

of contaminants, the nurse will work with you to lower your exposure, and conduct further testing if necessary (i.e. blood test to confirm high exceedance).

Benefits: You will have the opportunity to find out whether you have been exposed to arsenic and other metals of concern. At the same time your participation will contribute to the understanding of arsenic exposure and its health effects in Yellowknife, Ndilo and Dettah.

Confidentiality and anonymity: All information you provide will be kept strictly confidential and will never be publicly attached to your name. You will receive your results with interpretation in a personal letter.

Conservation of data: The data collected (questionnaires, toenails, urine and saliva) will be kept in a secure manner (in a computer in a secure room at the University of Ottawa) until completion of the program. The Principal Investigator, along with research students, Janet Cheung and Dr. Rajendra Parajuli, will have access to the data. The data will only be used for the purpose of this study. A copy of the master database shall be provided to the Institute for Circumpolar Health Research, and kept in a secure manner, once data collection is complete.

Compensation: A gift card for grocery in the amount of $50 will be provided to thank you for participating in the program.

Voluntary Participation: Your participation is voluntary. You are under no obligation to participate. If you choose to participate, you can withdraw from the study at any time and/or refuse to answer any questions without suffering any negative consequences. If you choose to withdraw, all information and data you have provided will be destroyed or returned to you on request. No samples of toenails, urine or saliva will be collected without your permission.

Who can I talk to if I have questions or problems?
The local research assistant will answer any questions you may have about this program or you may contact the following project team member at any time in the future.

Collect calls will be accepted.

Research Supervisor:
Dr. Laurie Chan
Professor and Canada Research Chair in Toxicology and Environmental Health
University of Ottawa, Faculty of Biology
Tel: 613-562-5800 ext. 7116
Email: laurie.chan@uottawa.ca

Yellowknife Contact:
Stacey Sundberg
Assistant Coordinator
Work: 867-873-9337
Email: stacey.sundberg@ichr.ca

If you have any questions regarding the ethical conduct of this study, you may contact:

Protocol Officer for Ethics in Research,
University of Ottawa, Tabaret Hall,
550 Cumberland Street, Room 154,
Ottawa, ON K1N 6N5
Tel: (613) 562-5387
Email: ethics@uottawa.ca

There are two copies of the consent form of which one will be kept by Dr. Chan.
Your decision to participate in the Health Effects Monitoring Program is completely up to you. You are free to withdraw from the program at any time, and you can choose not to answer any questions you don't feel comfortable answering.

By signing this form, I agree that:

1.	I understand that I am being asked to participate in a Health Effects Monitoring Program that will focus on Arsenic and other contaminants of primary concern for the Giant Mine Remediation Project.	Yes	No
2.	I understand that I have the right to not participate, to refuse to answer a question and the right to stop at any time.	Yes	No
3.	I understand that I can ask any questions related to the study at any time.	Yes	No
4.	I understand that my personal information will be kept confidential.	Yes	No
5.	I agree to give urine sample and be informed of the result.	Yes	No
6.	I agree to give toenail sample and be informed of the result.	Yes	No
7.	I agree to give saliva sample and be informed of the result.	Yes	No
8.	I agree to complete a medical history and undergo a brief medical examination by a nurse.	Yes	No
9.	A follow-up study is planned in 5 to 10 years. I agree to be contacted again to be invited to participate in the follow up study.	Yes	No
10.	I agree to keep my samples in a biobank until the end of the study.	Yes	No
11.	I hereby consent to participate in the study.	Yes	No

Name of participant _____

Date of Birth (day/month/year) _____

_____
_____
Signature Date
(day/month/year)

Telephone number _____

Participant's mailing address (for returning results of sample analysis):

_____
_

_____
_

Name of person who obtained consent

_____
_____
Signature Date
(day/month/year)